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THE LABORATORY TEXT-BOOK OF PUBLIC HEALTH.

ONE OF THE LABORATORIES OF STATE MEDICINE IN KING'S COLLEGE

THE

LABORATORY TEXT-BOOK

OF

PUBLIC HEALTH.

BY

WILLIAM R. SMITH, M.D., D.Sc., F.R.S. EDIN.

FELLOW OF THE INSTITUTE OF CHEMISTRY;
DIPLOMATE IN PUBLIC HEALTH OF THE UNIVERSITY OF CAMBRIDGE;
BARRISTER-AT-LAW;
PROFESSOR OF FORENSIC MEDICINE AND DIRECTOR OF THE LABORATORIES OF STATE MEDICINE IN KING'S COLLEGE, LONDON;
MEDICAL OFFICER OF THE SCHOOL BOARD FOR LONDON;
MEDICAL OFFICER OF HEALTH AND PUBLIC ANALYST FOR WOOLWICH;
PRESIDENT OF THE BRITISH INSTITUTE OF PUBLIC HEALTH.

HENRY RENSHAW
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TO

THE MOST HONOURABLE

THE MARQUIS OF LONDONDERRY, K.G.,

CHAIRMAN OF THE SCHOOL BOARD FOR LONDON

THIS BOOK IS DEDICATED AS A MARK OF RESPECT FOR THE VALUABLE WORK DONE DURING THE PAST TWENTY-FIVE YEARS BY

THE SCHOOL BOARD FOR LONDON,

AND AS A TRIBUTE OF APPRECIATION OF HIS LORDSHIP'S MANY ACTS OF KINDNESS AND CONSIDERATION.

PREFACE.

The necessity of a Laboratory Text-book for Public Health work has been forcibly impressed upon my notice during the past few years in connection with the Laboratory training of Candidates for Public Health Diplomas. I have therefore decided to place in a book form various sheets of instruction which from time to time have been placed at the disposal of the students in the State Medicine Laboratories of King's College.

The methods employed are those I have found most suited to the requirements of Candidates for Public Health Diplomas. Some of these give only roughly approximate results, but are intended to familiarise students with various pieces of apparatus, such as the nitrometer.

I have to acknowledge assistance from many quarters in the production of the illustrations, and particularly to Dr. E. Klein for his kindness in allowing me to reproduce various diagrams from his Reports to the

Local Government Board, and to Mr. Griffiths for his excellent photographs of the starches.

I am indebted to my former Demonstrator, Mr. G. N. Huntly, for much valuable assistance in Parts I. to IV.; to my present Demonstrator Mr. C. G. Moor for assistance in the Food Section, and to Dr. Cartwright Wood for the revision of the Section on Bacteriology.

My acknowledgments are more especially due to Dr. F. Drew Harris, Assistant Demonstrator in the Laboratories of State Medicine at King's College, for his able assistance throughout and general supervision of the work whilst passing through the press.

WILLIAM R. SMITH.

KING'S COLLEGE,
20th June, 1896.

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THE LABORATORY TEXT-BOOK OF PUBLIC HEALTH.

PART I.

PHYSICS AND METEOROLOGY.

CHAPTER I.

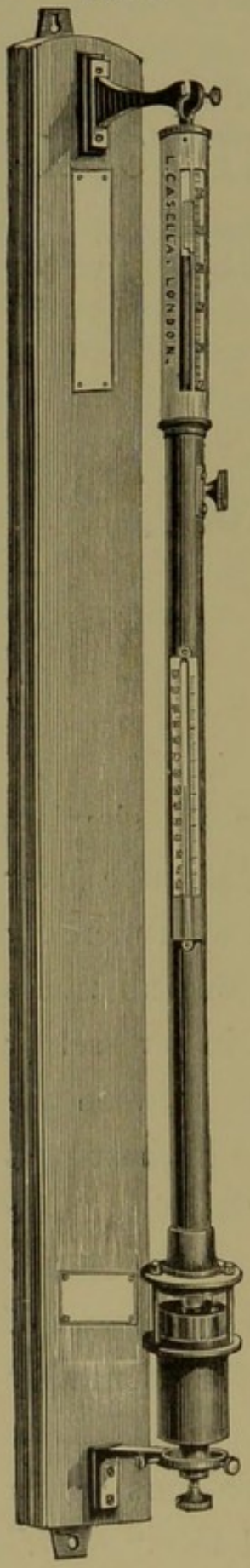
THE BAROMETER.

§ 1. **CONSTRUCTION.**—The barometer, in its simplest and most accurate form, consists of a wide glass tube (over one inch in width) which, after being filled with mercury in such a manner as to completely drive out all traces both of air and moisture, is then inverted in a vessel or cistern of clean dry mercury. The distance between the level of the mercury in the cistern and the highest part of the meniscus in the tube is called the "height of the barometer," and in a standard instrument of the dimensions given above, this height is measured by means of a cathetometer, which is an instrument for accurately measuring vertical heights.

Two patterns of portable standard barometers are in common use, known as the "Fortin" and the "Kew."

In both of these the glass tube is encased in a brass tube, in the upper part of which two wide vertical slits are made, so as to enable the position of the mercury column to be seen; and since the height of the barometer varies at most but a few inches, it is only the upper part that has a scale engraved upon it either in inches or in millimetres. As the mercury in the tube rises, the level of that in the cistern falls, and *vice versa*, and the mode of allowing for this change in the cistern level constitutes the sole difference between the two patterns.

FIG. 1.

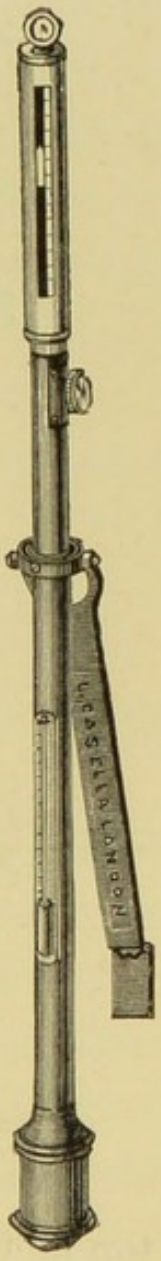


In the Fortin barometer (Fig. 1) the cistern level is itself adjustable, a screw underneath acting upon a leather bag. In the cistern lid, which is rigidly attached to the brass tube, is screwed an ivory pointer, and this is so placed by the maker that its point is exactly 30·000 inches below the "30" inch mark on the scale. If now the level of the mercury in the cistern is gradually raised to this point by means of the screw underneath, that is, until the ivory point appears to just touch its own image, the height of the mercury in the tube as read off on the brass scale

must give the true difference of level between the mercury in the tube and that in the cistern.

In the Kew barometer (Fig. 2), which is the one generally adopted for marine work, the cistern level is not capable of adjustment, but the divisions on the scale are contracted instead, so that, although the "inches" of the scale in this pattern are not true inches, yet they give the true height of the barometer and will agree exactly with a good Fortin barometer if properly made and adjusted.

FIG. 2.



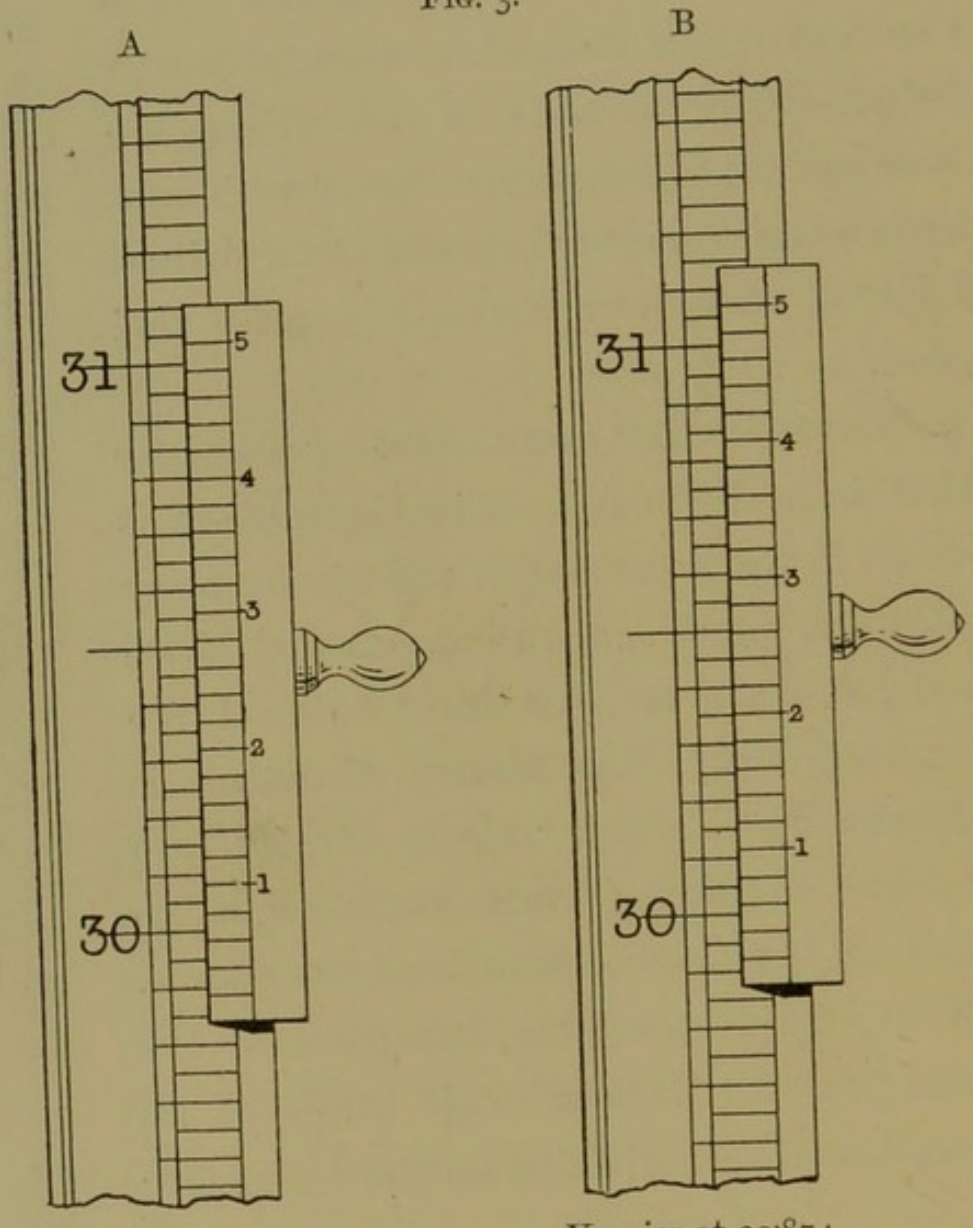
§ 2. **METHOD OF READING** (barometer assumed to be a Fortin).—*The Vernier.*—It is preferable to learn the use of the vernier away from the barometer on an ivory model twice the natural size. At first learn to read the vernier empirically, the theory of which is as follows: The vernier scale is divided into twenty-five divisions; this corresponds to twenty-four divisions on the barometer scale. It will be evident, therefore, that each of the divisions on the vernier scale is $\frac{1}{25}$ of its own size less than a division on the barometer scale.

Now, the divisions on the barometer scale are 0·05 $(\frac{1}{20})$ in. in length. By the use of the vernier, therefore, it is possible to show differences of the $\frac{1}{25}$ of the $\frac{1}{20}$ of an inch = 0·002 inch. Note, then, that there are two scales, one which is fixed, the barometer scale, and the other sliding upon this—the vernier scale. Each

inch on the barometer scale is divided into ten parts (0·1″), and each of these again into two (0·05″). There are two cases possible.

1*st Case.*—The bottom end of the vernier (*i.e.*, the

FIG. 3.



Vernier at 29·838. Vernier at 29·874.

top of the mercury column after adjustment) falls in the *lower* half of any given tenth. (Fig. 3, A.)

Read and write down at once the first place of decimals, thus 29·8. The figure engraved on the vernier next below the coincidence, *i.e.*, the point where a division on the scale coincides with a division on the

vernier (·3), is the second place of decimals; the number of vernier divisions between the ·3 and the coincidence (4), multiplied by two, gives the third place of decimals (in this case 8). Hence, in the present case the complete reading is 29·838 inches.

2nd Case.—The lower end of the vernier falls in the *upper* half of any particular tenth. (Fig. 3, B.) Here the first reading is not 29·8, but 29·85, the remainder of the reading being carried out exactly as above. Hence the rule: Add 5 to the figure on the vernier for the second place of decimals. In the present case, therefore, the complete reading is 29·874.

The following order must be observed in reading a standard Fortin barometer.

(1) Read the attached thermometer.

(2) Slowly adjust the level of the cistern.

(3) Gently tap the tube near the top of the column with the finger.

(4) Adjust the vernier till its two lower edges (back and front) form a tangent to the convex surface of the mercury, so that the back edge of the vernier, the top of the mercury, the front edge of the vernier, and the eye of the observer are all in the same straight line. Read the vernier and write down the result.

(5) Carefully lower the level of the cistern until the mercury is just clear of the ivory pointer and shut the case.

This last should on no account be omitted, as if the ivory pointer is left in contact with the mercury, dirt collects

on the surface round the pointer, forming a black scum, which, by destroying the mirror surface, renders an accurate adjustment of the cistern level impossible. The operations are carried out in exactly the same order for a "Kew pattern" instrument, except that directions (2) and (5) are obviously omitted.

§ 3. **CORRECTIONS.**—The object of reading the barometer is to find the actual pressure of the atmosphere at any given time, say in pounds weight per square inch. To get this, the crude reading requires to be corrected for:

(1) Error due to capillarity.

(2) Errors due to incorrect adjustment of ivory pointer by the maker, and to badly graduated scale.

(3) Temperature.

After these corrections have been applied the resulting figure will be the true height of the barometer in the room in which the reading is made; and when a gas has been measured at atmospheric pressure this height is the figure required for its correction. (*See* "Determination of Nitrates by Crum's Method," § 36.) But barometer readings made for meteorological purposes have to be compared with each other, and in this case it is clear that the height of the cistern above sea level must be taken into account. This can be accurately done by levelling from the nearest Ordnance bench mark, or approximately, with a good aneroid. This correction is usually applied from a table.

The correction for the error due to capillarity depends on the size of the tube; the smaller the tube the greater

will be the correction, which is always a positive one, since mercury being a liquid which does not wet the tube, the column is depressed. The accuracy of scales engraved on brass by good makers is usually greater than that required for barometer readings, the errors being less than ·001″. The correction for index error in a Fortin pattern barometer is obviously constant, and may be either positive or negative, depending only upon the adjustment of the ivory pointer. In the Kew pattern the index error may be variable, and must be determined at several points by comparison with a standard and corrections applied accordingly.

In practice, these two corrections are not taken separately. Every meteorological barometer that has any pretensions to accuracy is sent to Kew Observatory to be compared with the Kew standard instrument, and the algebraical sum of these two errors (index + capillarity) is given on the certificate as "Correction." In such a certificate "Correction = ·000 in." does not necessarily mean that the tip of the ivory pointer is exactly 30·000 in. below the mark 30 in. on the scale. It only means that the maker has succeeded in making the index error (say − ·005) exactly equal and opposite to the capillarity error (say + ·005), and the sum of these corrections being + ·005 − ·005 = ·000 the Kew certificate will read: Correction = ·000 in.

The following is the Kew certificate of a standard Fortin barometer belonging to the State Medicine Department of King's College, London:

CERTIFICATE OF EXAMINATION ISSUED BY THE KEW OBSERVATORY, RICHMOND, SURREY.

STANDARD BAROMETER, No. 973, by J. HICKS, London.
Compared with the STANDARD BAROMETER *of the Kew Observatory.*

CORRECTION (including capillary action) = ·000 in.
,, ,, *metrical scale* = 0·00 mm.
Scales examined and found correct.

CORRECTIONS to Attached Thermometer. No. 651493, 91.

At 32°	At 42°	At 52°	At 62°	At 72°	At 82°	At 92°
−0·0°	−0·0°	−0·0°	−0 0°	−0·0°	−0·0°	+0·1°

Note.—I. When the sign of the correction is +, the quantity is to be *added* to the observed scale reading, and when − to be *subtracted* from it.

II. As mercurial thermometers are liable to read too high through age, this thermometer ought to be again tested at some future date, at the melting point of ice, and if its reading at that point be found different from the one now given, an appropriate correction should be applied to all the above points.

G. M. WHIPPLE,
Superintendent.

KEW OBSERVATORY,
August 1891.

The density of mercury decreases regularly as the temperature rises. Hence if the temperature rises the barometer will rise, even although the actual pressure of the air has remained absolutely the same. For this reason some standard temperature must be chosen to which the observations must be reduced. The temperature chosen is the ice-point (0° C.). Now the co-efficient of expansion of mercury is ·000181 for 1° C., thus, if the barometer reads 760 mm., at 15° C., the height of the equivalent column at 0° is $760\,[1-(\cdot000181\times15^\circ)]$ or

757·94 mm., and this is the true height so far as this part of the correction is concerned.

But the brass scale is also longer at 15° C. than at 0° C., hence, on that account, the height of the column will appear to be too short. Hence, the co-efficient of linear expansion of brass being ·000019 per 1° C., the brass scale is

$$(760 \times \cdot000019 \times 15^\circ \text{ mm.}) = \cdot22 \text{ mm. too long,}$$

and this has to be added to the result before obtained, giving 758·2 mm. as the corrected height of the barometer.

In general, if l is the observed height in mm. t° C., the temperature, the complete correction is

$-(\cdot000181 - \cdot000019)\,lt$. and the true height: $l-(\cdot000181-\cdot000019)\,lt$, that is, $l-\cdot000162\,lt$.*

From this formula correction tables have been compiled, from which in practice the temperature correction is always obtained. (*See* "Hints to Meteorological Observers," prepared by William Marriott, F.R.Met.Soc.).

* It should be noted that the metre scale is *correct at* 0°, and hence the correction is simple and easy to follow.

The English scale is correct at 62° F., and hence the above formula has to be modified to include this and also the change of the temperature unit (1° F.).

The total correction is made up of two parts, exactly as above: 1. A correction to be added for the expansion of the scale $l \times \cdot000104\,(t-62^\circ)$; 2. A correction to be subtracted for the expansion of the mercury, $l \times (1-\cdot001t^\circ)$. These two combined give the convenient formula,

$$\text{Corrected height} = l - l\,\frac{(\cdot09t^\circ - 2\cdot56)}{1000}$$

EXAMPLES.

Height of Barometer.

1. Reading of attached thermometer . . .	13·9° C.
Correction from Kew certificate . . .	+0·1
Corrected temperature	14·0 C.
Barometer reading	749·1 mm.
Correction (capillarity + index error) from Kew certificate	+0·1
	749·2 mm.
Temperature correction	−1·7
Corrected barometer height	747·5 mm.
2. Reading of attached thermometer	59·3° F.
Correction from Kew certificate . . .	−0·2
Corrected temperature	59·1° F.
Barometer reading	29·937 in.
Correction from Kew certificate . . .	+0·003
	29·940 in.
Temperature correction	−0·083
Corrected barometer height	= 29·857 in.

CHAPTER II.

THERMOMETRY AND HYGROMETRY.

§ 4. **THERMOMETERS IN USE.**—A complete set of thermometers for meteorological purposes comprises :

1. Dry Bulb, kept in a properly constructed "screen."
2. Wet Bulb „ „ „ „
3. Maximum „ „ „ „
4. Minimum „ „ „ „
5. Grass Minimum (Terrestrial Radiation).
6. Solar Radiation (Black Bulb *in vacuo*).
7. „ „ (Bright Bulb *in vacuo*).

Of these the first four are essential, the grass minimum generally being added ; the solar radiation thermometers, owing to the great difficulty of making two similarly constructed instruments give similar readings, are frequently dispensed with.

Since the whole value of meteorological observations depends upon comparisons, a strictly uniform method must be followed in all cases. The chief points to be observed are : the thermometers are to be divided *on the stem*, to be furnished with a Kew certificate, and so far as the first four are concerned, then placed in a suitably constructed screen, so that the bulbs are shielded

both from the direct rays of the sun and from air currents caused by surrounding buildings. (Fig. 4.)

FIG. 4.

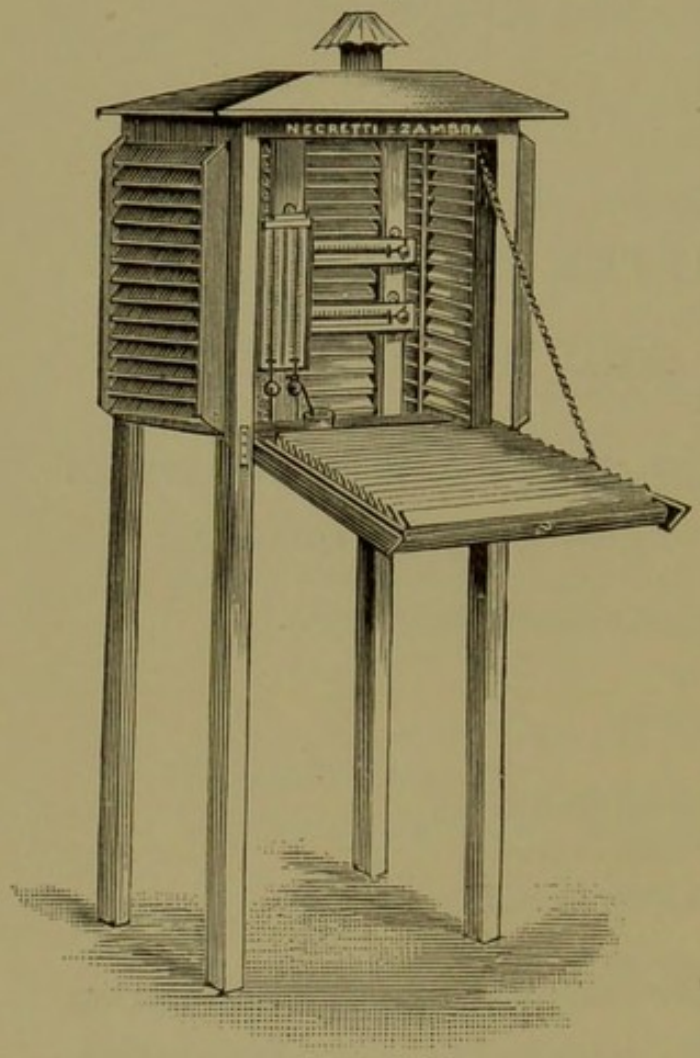
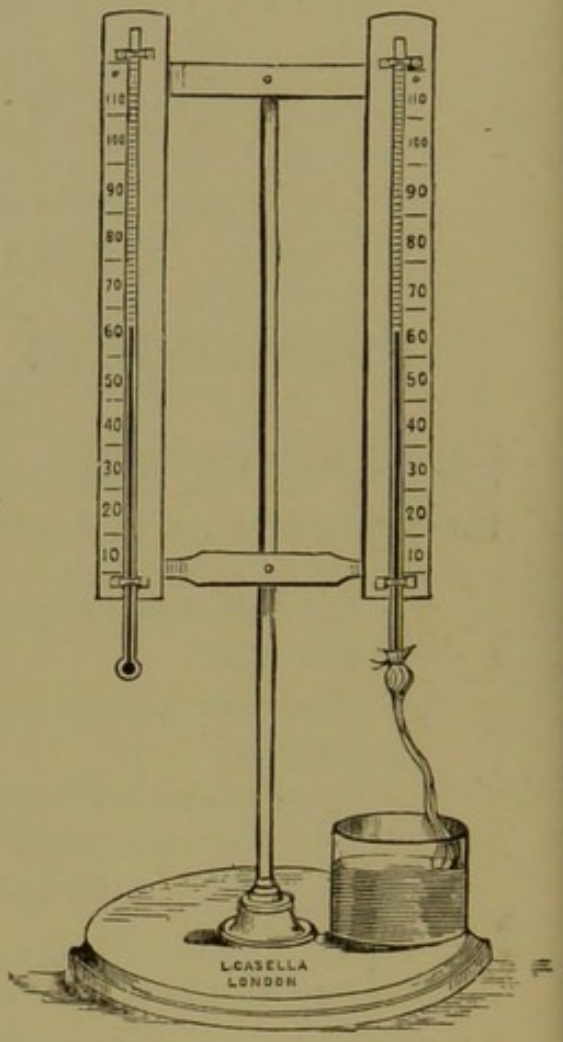


FIG. 5.

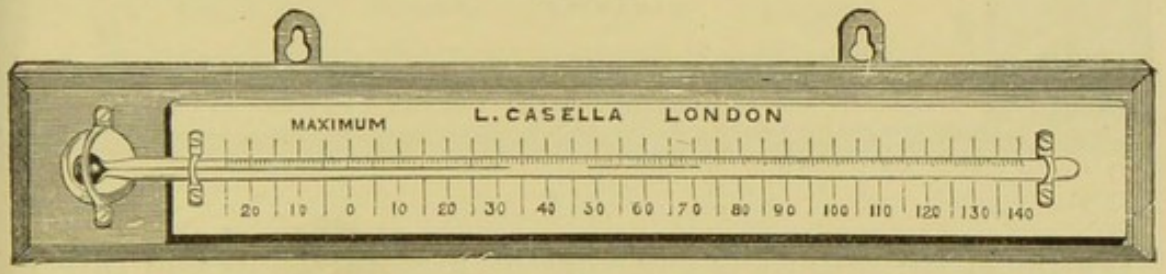


§ 5. **Dry and Wet Bulb Thermometers.**—The dry and wet bulb thermometers are two thermometers of identical construction. (Fig. 5.) The bulb of the wet thermometer is enclosed in a little bag of muslin, and round the neck of the bulb and over the muslin is twisted loosely a thread of lamp wick or darning cotton. The other end of this is placed in a little copper vessel of distilled water, which is placed on one side of, and a little beneath, the wet bulb (the conducting thread is about three inches long), so that the evaporation from

the water in the reservoir may not affect the reading of the dry bulb. The screen is so constructed that the bulbs are four feet from the ground, and as far away as possible from trees or buildings.

§ 6. **Maximum Thermometers.**—The maximum thermometer (Fig. 6) is a mercury thermometer having a constriction in the bore of the tube just above the bulb. While the temperature is rising the mercury forces its way through this constriction easily enough ; on cooling,

FIG. 6.

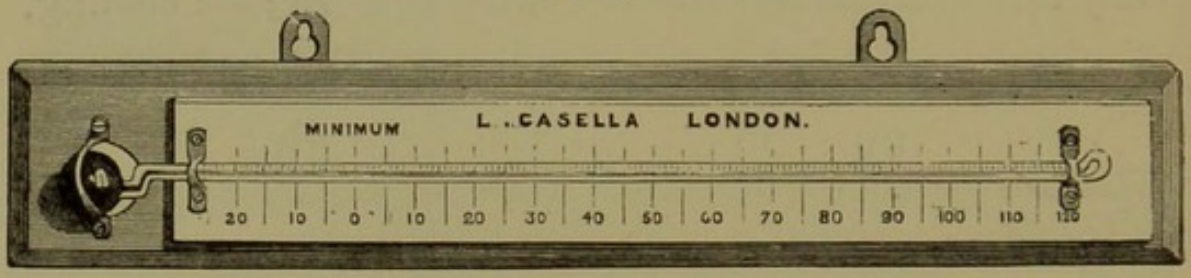


however, instead of the mercury being dragged back through this hole, the thread breaks just below the constriction, leaving the forced out thread stationary in the tube. Hence the top end of this thread shows the highest temperature the thermometer has attained since its last setting. This setting is done very simply, a quick jerk easily reuniting the two threads. The maximum thermometer, for obvious reasons, should always be read before lifting it out of the screen to reset.

§ 7. **Minimum Thermometers.** — The minimum thermometer (Fig. 7) has alcohol for the expanding liquid. Owing to the surface tension of liquids, a free liquid surface is stretched and behaves in a different manner to the body of the liquid, somewhat like a stretched rubber-sheet. Hence a little piece of glass or metal

will move freely up or down the tube inside the alcohol but cannot break through the surface skin unless considerable force is used. This fact is utilised in the minimum thermometer, a light dumb-bell shaped index of blackened glass being used; the instrument having been originally set by tilting up the tube till the little dumb-bell touches the surface of the alcohol. If the

FIG. 7.



temperature falls, the contracting alcohol drags the index with it; with a rising temperature, on the other hand, the alcohol flows freely past the index, which remains fixed. Hence the position of the upper end of the index indicates the lowest temperature attained since the last setting.

The "grass minimum" or "terrestrial radiation minimum," thermometer is of exactly similar build, but is enclosed in a stout glass cover to protect it from accidental breakage.

§ 8. **HYGROMETRY.**—*Definition.*—The relative humidity of the atmosphere is the ratio of the actual amount of aqueous vapour present at any given temperature to the maximum possible amount for the same temperature. If we call the actual amount present e, the maximum possible amount of vapour for the same temperature, f, then the

Relative Humidity $= \frac{100e}{f}$. (The only reason for the factor 100 is to avoid fractions).

The first question that naturally arises out of this definition is how can the "amount," e, be measured. Practically this is always determined by the pressure it exerts. The next point that arises is, is there really a maximum possible pressure for each temperature, in other words, at a given temperature will a fixed quantity of air take up a fixed quantity of water as vapour and no more? The following experiment shows that this is really the case.

Take three clean glass tubes of about the same diameter and a yard long. Fill them carefully with mercury through a long thistle funnel to avoid air bubbles, and invert them side by side in a glass mercury trough. If quite free from air, these will all stand at the same height; the empty space above the mercury in each will be vacuous, and hence the pressure exerted on the upper surface of the mercury will be *nil*. They will be in fact three simple barometers. Now let a small bubble of air into one of these and by means of a steel metre scale read the difference in level produced. If this equals say 127·5 mm. of mercury, then the pressure exerted by the air upon the top surface of the mercury is clearly equal to this. Now let up into this and also into the third tube a few drops of recently boiled and cooled distilled water, leaving the first tube untouched. Leave the three tubes for about half an hour together, and again read, and also note the temperature of a thermometer hanging between them. If care has been

taken not to touch the tops of the tubes with the hand, the results will be somewhat as follows:

TEMPERATURE 65·2° F.

1. Original height in the three tubes . . .	767·5 mm.
2. Height after introducing dry air . . .	640·0 „
3. Height after introducing water . . .	624·5 „
4. Height in 3rd tube after introducing water only	752·0 „

Now it is seen that in (4), although the water is in excess, that at 65·2° F., it cannot exert a greater pressure on the top surface of the mercury than 15·5 mm. (or ·622 inches), hence the pressure exerted by saturated water vapour is fixed when the temperature is fixed.

From (1) and (2) it is seen that the pressure of the air introduced is 137·5 mm., and since the difference between (2) and (3) is 15·5 mm., it follows that the pressure exerted by saturated aqueous vapour for a fixed temperature is the same whether air is present or not.

By the comparison of (1) and (4) then you have determined the pressure of saturated aqueous vapour at 65·2° F., and found it to be ·622 in. or 15·5 mm. of mercury. By a long series of experiments conducted on precisely the same plan, but with greater attention to details, Regnault determined the saturation-pressure at points from −40° F. and upwards, and the following table (which will be afterwards referred to as Regnault's table) gives the results of his experiments, as far as will be necessary for present purposes. The values for

tenths of a degree can either be got by interpolation as with logarithms or by reference to the complete table in Glaisher's Hygrometrical Tables, or similar works.

TABLE I.

Table shewing the Pressure of Saturated Aqueous Vapour, in Inches of Mercury, from 32° to 100° F., calculated from the experiments of Regnault.

Temp. F.	Pressure of Vapour.	Temp. F.	Pressure of Vapour.	Temp. F.	Pressure of Vapour.
32°	·181 in.	55°	·433 in.	78°	·958 in.
33	·188	56	·449	79	·990
34	·196	57	·465	80	1·023
35	·204	58	·482	81	1·057
36	·212	59	·500	82	1·092
37	·220	60	·518	83	1·128
38	·229	61	·537	84	1·165
39	·238	62	·556	85	1·203
40	·247	63	·576	86	1·242
41	·257	64	·596	87	1·282
42	·267	65	·617	88	1·323
43	·277	66	·639	89	1·366
44	·288	67	·661	90	1·410
45	·299	68	·684	91	1·455
46	·311	69	·708	92	1·501
47	·323	70	·733	93	1·548
48	·335	71	·759	94	1·596
49	·348	72	·785	95	1·646
50	·361	73	·812	96	1·697
51	·374	74	·840	97	1·751
52	·388	75	·868	98	1·806
53	·403	76	·897	99	1·862
54	·418	77	·927	100	1·918

Now take the third tube containing water only. At 65·2 it contains (1) saturated water-gas, or saturated aqueous vapour, exerting a pressure of 15·5 mm. of mercury, and (2), a drop of liquid water. If the tube is gently warmed the column of mercury will slowly fall, that is, the pressure of the saturated vapour increases

with the temperature. At some particular temperature, say 80° F., in this case the last drop of liquid water will disappear, and if the whole be still further heated, the water-gas, now no longer saturated, will go on expanding like any other gas. If now this gas is slowly cooled to the same temperature at which the last droplet disappeared (80° F.), water will again appear on the glass, and there will be a difference of 1·023 in. between the levels of this tube and the barometer tube. This temperature at which the water just begins to deposit, is called the "dew-point," and if by any means for any given sample of air this "dew-point" can be determined, a reference to the above table clearly gives the actual pressure of the aqueous vapour in the air at the time; in other words, it gives the e in the formula $\frac{100e}{f}$. But a second reference to the same table for the actual temperature of the room also gives f. Hence, if the dew-point can be determined in any way, this table is sufficient to give the relative humidity $\frac{100e}{f}$. In practice the dew-point is determined in two ways.

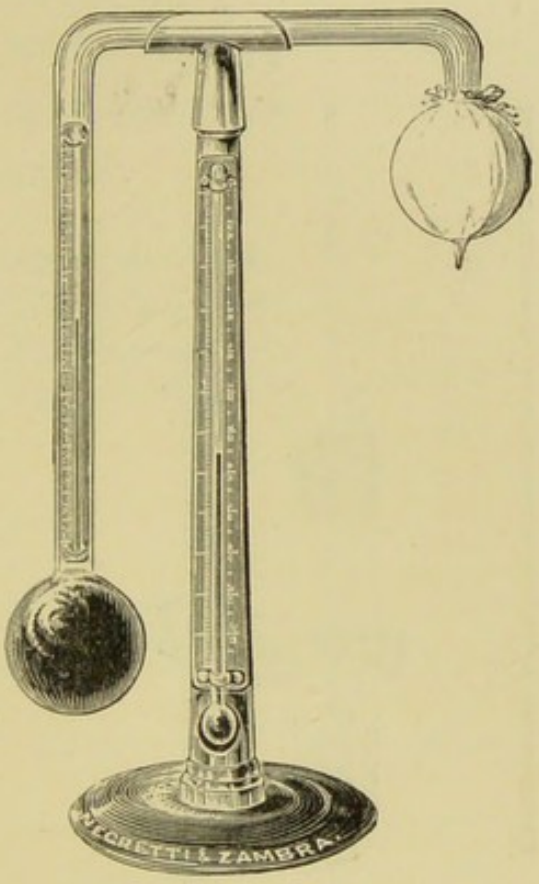
(1) Directly, with a dew-point hygrometer.

(2) Indirectly, with the wet and dry bulb thermometers.

§ 9. **Dew-Point Hygrometers.**—The chief dew-point hygrometers in order of date are those of Daniell, Regnault, and Dines. They all depend upon the same principle. A polished surface is slowly cooled down until the first signs of deposition of dew appear upon it; the temperature at which this occurs is noted, the instrument is then allowed to gradually warm again, the

temperature at which the last speck of dew disappears also noted, and the mean of these two readings, which should not differ by more than 0·2° F., is taken as the dew point.

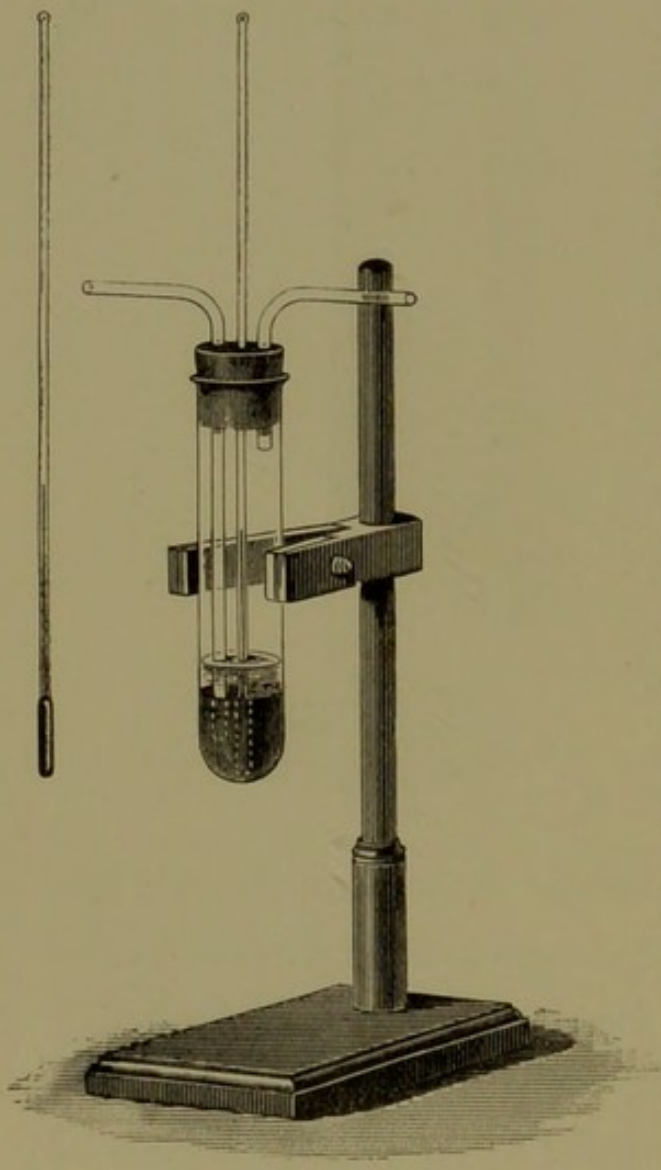
Fig. 8.



Daniell's dew-point hygrometer (Fig. 8) consists of two glass bulbs connected by a tube bent twice at right angles. The left-hand bulb consists of black glass, and contains a delicate thermometer, the bulb of which is plunged in some ether, the stem being visible in the tube above. The right-hand bulb is covered with some muslin, and also contains ether. A little ether is now dropped on the muslin, which, evaporating, causes a considerable lowering of the temperature of this bulb. As a consequence, the ether in the left-hand bulb distils over into the right-hand one. The temperature of the left-hand or blackened bulb is thus lowered, and as soon as the dew-point is reached, the blackened surface becomes covered with moisture. The reading of the contained thermometer is at once taken. After a time the moisture disappears, when the temperature is again read. The mean of these two readings is then found, and constitutes the dew-point.

Regnault's hygrometer is similar to the preceding, but polished silver is used instead of the black bulb. Distillation is brought about by means of an aspirator.

FIG. 9.



A simple dew-point hygrometer for laboratory use is made as follows. (Fig. 9.) A wide test-tube ("boiling tube") is silvered on the outside up to about 1½ inch from the bottom, and is fitted with a trebly perforated cork, one hole carrying a delicate thermometer, which dips into some ether, and the other two glass tubes bent wash-bottle fashion. Another thermometer is hung by the side to give the temperature of the air. By gently blowing through a long rubber tube, the desired lowering of the temperature is obtained; the readings being taken through a telescope placed about six feet away, in order to avoid the heating effect of the body and the moisture in the breath having any disturbing effect. The two thermometers must be carefully compared before use.

§ 10. **Use of Glaisher's Factors.**—The other method

of determining the dew-point is an indirect one. A reading is taken of the dry- and wet-bulb thermometers respectively; the excess of the dry- over the wet-bulb is multiplied by the corresponding "Glaisher's factor," taken from the following table; and the resulting product is the excess of the temperature of the air over the dew-point. Or, in symbols, if:

D° = reading of Dry Bulb Thermometer.
W° = reading of Wet Bulb Thermometer.
G_D = Glaisher's factor for temperature D.
Then Dew-Point = $D - G_D (D - W)$.

TABLE II.

Table of Glaisher's Factors.

Reading of Dry Bulb Therm.	Factor.	Reading of Dry Bulb Therm.	Factor.	Reading of Dry Bulb Therm.	Factor.
32°	3·32	55°	1·96	78°	1·69
33	3·01	56	1·94	79	1·69
34	2·77	57	1·92	80	1·68
35	2·60	58	1·90	81	1·68
36	2·50	59	1·89	82	1·67
37	2·42	60	1·88	83	1·67
38	2·36	61	1·87	84	1·66
39	2·32	62	1·86	85	1·65
40	2·29	63	1·85	86	1·65
41	2·26	64	1·83	87	1·64
42	2·23	65	1·82	88	1·64
43	2·20	66	1·81	89	1·63
44	2·18	67	1·80	90	1·63
45	2·16	68	1·79	91	1·62
46	2·14	69	1·78	92	1·62
47	2·12	70	1·77	93	1·61
48	2·10	71	1·76	94	1·60
49	2·08	72	1·75	95	1·60
50	2·06	73	1·74	96	1·59
51	2·04	74	1·73	97	1·59
52	2·00	75	1·72	98	1·58
53	1·98	76	1·71	99	1·58
54	1·96	77	1·70	100	1·57

Experiments:

(1) Take a set of readings of all the outdoor meteorological instruments, namely wet- and dry-bulb, maximum and minimum thermometers in screen, grass minimum, and rain gauge, and correct from Kew certificates.

(2) Carry out experiment with three barometers as described in text.

(3) Determine the dew-point directly by above-described condensing hygrometer, and compare with the dew-point obtained by the use of Glaisher's factors upon the readings in 1.

(4) From the dew-point, dry-bulb temperature, and Regnault's table find the relative humidity.

CHAPTER III.

PHYSICAL PROPERTIES OF GASES.

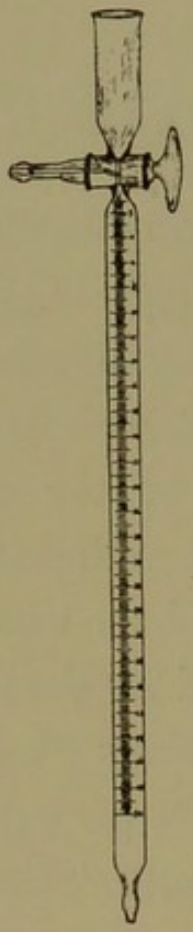
§ 11. **EFFECT OF PRESSURE.**—For a gas to be confined in any space pressure requires to be exerted; a given weight of gas introduced into a vacuous globe, however large, instantly fills every part of it and exerts a definite normal pressure on the walls of the containing vessel, owing to the ceaseless bombardment of the walls by the molecules of the gas, and an exactly equal and opposite pressure must be exerted by the containing vessel upon the gas to keep things steady. Pressure may be measured in pounds weight per square inch, or, more conveniently, in inches or millimetres of mercury, since a column of mercury, one inch high, at 32° F., exerts a pressure of ·4917 lb. per sq. in. in London.

To find the law connecting pressure and volume of a fixed weight of gas when the temperature is constant, the following arrangement may conveniently be used.

Fig. 10 is a gas burette of the form generally known as a "nitrometer." It contains 50 cc., and is divided into tenths of a cubic centimetre and is provided with a

suitable levelling tube,* both being held by a double clamp. The tap is opened freely to the air, and the levelling tube lowered until exactly 25 c.c. of air have been drawn in and the mercury is at the same level in both limbs. The tap is shut; the volume (25 c.c.) of air is now at the pressure of the atmosphere, found by reading the barometer (in mm.). The right-hand tube is now raised so as to compress the air in the other, especial care being taken here and subsequently that the hands do not touch the graduated limb, and the volume is read off as exactly as possible; at the same time, by means of the steel metre scale, the difference of levels is read off in millimetres. About six readings are taken in precisely the same manner, with gradually increasing pressures ranging up to two atmospheres. Then going back to the atmospheric pressure, about six more observations are taken at pressures *less* than atmospheric. In the first set the actual pressure *on* the gas at any one reading, and hence the pressure exerted *by* the gas on the containing walls is evidently the height of the barometer *plus* the difference of levels in the two tubes; in the second set the actual pressure is given by the height of the barometer *minus* the difference of levels.

FIG. 10.



* For this measurement the ordinary levelling tube is removed and replaced by another at least 40 in. long. It is not shown in the figure.

The results of the experiments should be entered in tabular form.

The nitrometer must have a good tap, which must be carefully cleaned and regreased before each set of experiments with a mixture of india-rubber and vaseline, and then tested in position to make sure that it is air-tight. Better results are obtained when the graduated limb is surrounded by a glass cylinder filled with water.

10/4/94. EFFECT OF PRESSURE ON AIR. Bar. 750 mm.

(I.) Volume in c.c. = v.	(II.) Difference of levels in mm.	True pressure on gas = p. / Barometer ± (II.)	p × v.
12·0 c.c.	+812	1562	18744
14·0	+593	1343	18802
16·0	+419	1169	18704
18·0	+292	1042	18756
20·0	+191	941	18820
22·0	+100	850	18700
25·0	0	750	18750
27·0	− 55	695	18765
30·0	−121	629	18870
32·0	−161	589	18848
34·0	−200	550	18700
36·0	−230	520	18720

From these experiments it follows at once that the pressure (p) multiplied by the volume (v) of any fixed weight of air is a constant quantity (provided always that the temperature remains the same) or, in symbols $p\,v = \text{Const.}$

This relation, which was discovered in 1662 by Boyle, and seventeen years afterwards independently by Mariotte, is commonly known in England as "Boyle's Law," in France as "Mariotte's Law."

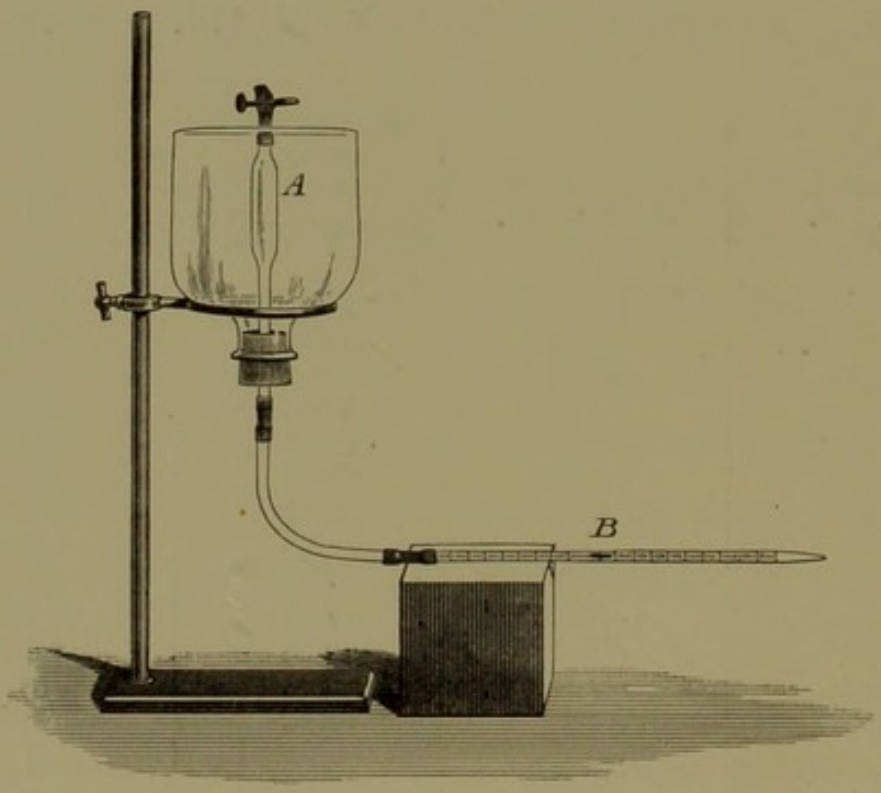
Experiments.—(1) Carry out the experiment as above described with 25 c.c. of air.

(2) Repeat, using 25 c.c. of coal-gas.

(3) Plot out these results on squared paper.

§ 12. **EFFECT OF TEMPERATURE.**—To study the effect of temperature on a gas the arrangement shown in Fig. 11, which is easily put together in any laboratory,

FIG. 11.



is used : a cylindrical glass bulb, *A*, holding about 20 c.c. (a broken pipette answers very well) is fitted up as shown above, and to this is joined a glass tube *B*, divided into hundredths of a cubic centimetre. The capacity of *A* down to the first graduation of *B* has been determined by filling with mercury and weighing, and is written on the tube. To make a measurement, dry air is drawn through the whole, a little mercury drawn into the graduated tube

until it stands at the starting-point of the graduation, and the jacketing tube apparatus filled with crushed ice, the pinchcock being left open at the top. After half an hour, when the air in *A* may be assumed to be at 0° C., the pinchcock is closed, and the exact position of the mercury thread read off. Then the ice is removed from the jacket and replaced by water at about 13° C., the whole well stirred, and the thermometer and the new position of the mercury thread read off as nearly as possible simultaneously.

The mode of working out the results will be best seen from the following example.

Capacity of bulb down to first mark	20·34 c.c.
Temperature of water	13·1° C.
Hence rise of temperature	13·1° C.
Increase of volume read off on B	·975 c.c.

Therefore increase of volume for 1° C. = ·0744 c.c. on 20·34 c.c., that is ·00366 c.c. on 1 c.c.

But the increase of volume on the unit of volume per degree is called the "coefficient of expansion," and since the apparatus is so arranged that the pressure on the gas remains the same (atmospheric) during the whole time of the experiment, it follows that the coefficient of expansion of air at constant pressure as given by this experiment is ·00366.

Exact experiments have shown that all gases have nearly the same coefficient of expansion, namely $\frac{1}{273}$ of their bulk at 0° C. A convenient way of remembering this is,

273 volumes at 0° C. become
274 „ 1° C.
275 „ 2° C.
288 „ 15° C., or
(273+t) „ t° C., or
(273−t) − t° C.

This is usually known as "Charles' law."

If instead of taking 0° C., that is, the melting-point of ice, as a starting-point for temperature, degrees are counted from −273° C., and if these are called degrees in absolute temperature, Charles' law takes the very simple form: "At constant pressure, the volume of a gas is proportional to its absolute temperature," or in symbols $V = A\,T$.

§ 13. **THE LAW OF GASES.**—Both Boyle's and Charles' laws are combined in the very convenient expression $\frac{PV}{T}$ = Constant for a given weight of gas.

To exemplify the use of this formula an example is appended.

A water residue, shaken with strong sulphuric acid and mercury, gives 15·1 c.c. of nitric oxide, measured at a temperature of 16·5° C., and at atmospheric pressure (barometer = 745 mm.).

What will be the volume of this gas, if its temperature be reduced to 0° C., and its pressure altered to 760 mm.?

Here—Old pressure = $P_1 = 745$.
Old temperature = $T_1 = (16{\cdot}5^\circ + 273^\circ) = 289{\cdot}5^\circ$.
Old volume = $V_1 = 15{\cdot}1$ cc.
and—New pressure is to be = $P_2 = 760$.
New temperature is = $T_2 = (0+273^\circ) = 273^\circ$.
New volume = $V_2 = x$.

But, from the above formula, for the same weight of gas,

$$\frac{P_1V_1}{T_1} = \text{Constant} = \frac{P_2V_2}{T_2}; \text{ or, putting in the above values:}$$

$$\frac{745 \times 15{\cdot}1}{289{\cdot}5} = \frac{760 \times x}{273}; \text{ which gives directly}$$

$$x = \left(\frac{745}{760} \times \frac{273}{289{\cdot}5} \times 15{\cdot}1\right) \text{c.c.} = 13{\cdot}96 \text{ c.c.}$$

Experiments.—(1) Find the coefficient of expansion at constant pressure of

(*a*) Dry air,

(*b*) Coal gas,

with the apparatus above described.

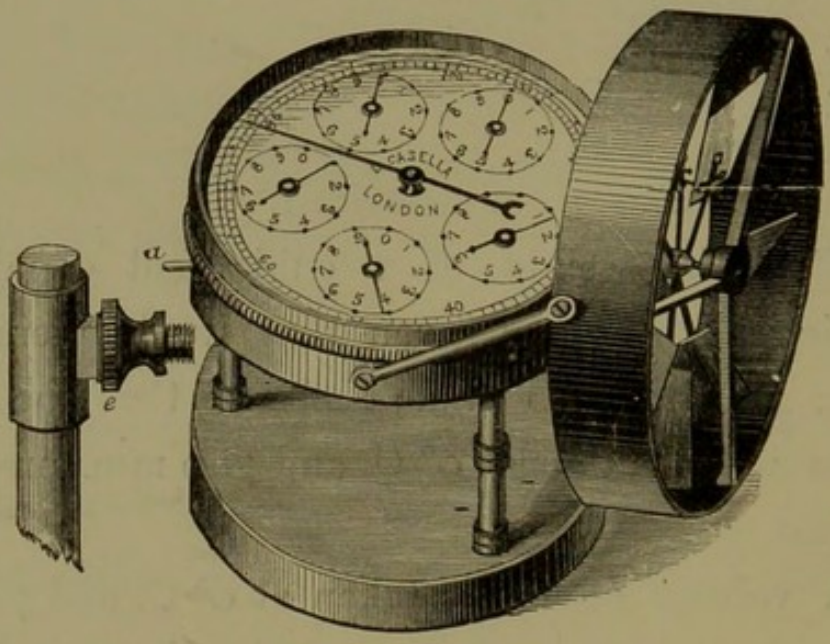
(2) Find the weight of 1 cubic foot of air in a ventilating shaft, being given that its temperature is 200° C.; pressure, 760 mm.; and also that the weight of one cubic foot of air at 0° C. and 760 mm. pressure is 567 grains.

(3) A volume of gas is taken at 0° C. and 760 mm. pressure; if it is warmed to 15·° C., what must be the pressure upon it, if it is to have exactly its original volume?

§ 14. **USE OF ANEMOMETERS.**—An anemometer is an instrument for measuring the velocity of an air current. In ventilation studies, the quantity of air passing a given point in a shaft is often required. This quantity (Q, cubic feet) cannot conveniently be measured directly, but since Q is equal to the product of the mean velocity of the air (V), and the area (A) at that point ($Q = A\,V$), and the area is easily measured; an anemometer is used which gives V the mean velocity. Two

types of instrument may be used for this purpose; the anemometer with vanes, and Fletcher's anemometer. The first of these (Fig. 12) has light aluminium vanes fixed to a spindle; this spindle by means of a little (*a*) stud can be thrown in gear or out of gear with a counting arrangement. The vanes are turned by the direct action of the current of air, and the number of revolutions recorded

FIG. 12.



by the counter during the minute it is geared to the spindle, suitably corrected by the constant supplied with the instrument, gives the velocity. The value of these revolutions has to be ascertained by the maker by direct experiment; that is, by forcing a known volume of air, at a uniform rate, through a channel of given size, and ascertaining the number of revolutions made by the vanes. Owing to the friction caused by the spindle and gear, anemometers of this pattern do not register velocities lower than a certain figure. Hence the formula giving the velocity is of the form:

$$V = a + bN.$$

Where V = velocity of air, in linear feet per minute,

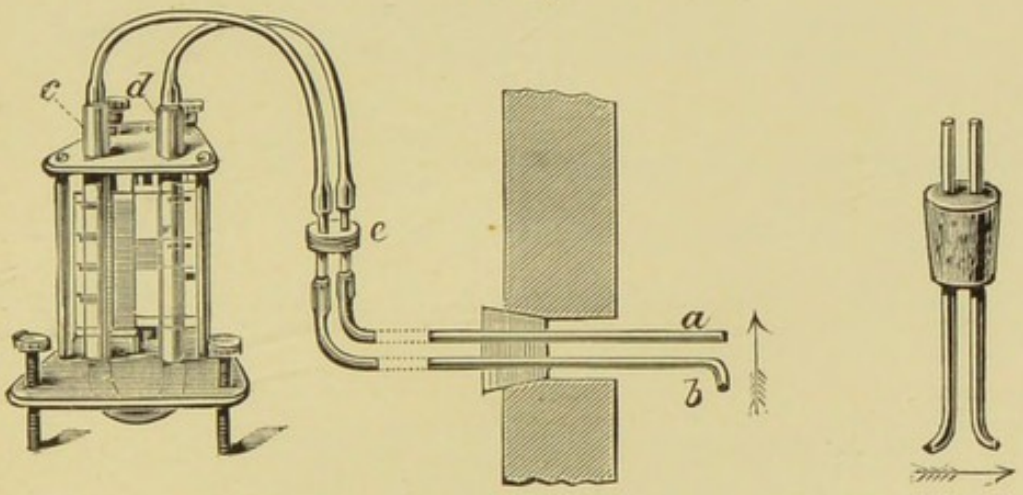
a = a constant (supplied by the maker) showing the minimum velocity of current which will move the vanes;

b = a constant (introduced into the counting gear by the maker);

N = number of turns of spindle in one minute.

Fletcher's anemometer (Fig. 13) is chiefly convenient for determining the velocities in heated flues, and consists of a part introduced into the flue, two metal tubes (*a*, *b*), about 0·3 inches diameter, one bent at a right

FIG. 13.



angle (*b*), the other straight, the two free ends of which are connected with a U-shaped manometer containing ether, between which is a sliding disc (*e*) for reversing the ether in tube. The greater the velocity, the greater is the difference of level between the two limbs of the manometer (*c*, *d*), the velocity varying as the square root of the pressure, or $v = 28{\cdot}55\sqrt{p}$. A full table of corrections, taking temperature also into account, is supplied with each instrument. The manometer may be placed at any convenient distance away from the flue.

Experiments.—(1) Determine the velocity of the up-going air in the given shaft with the vane anemometer, from which deduce the quantity of air per hour passing through it.

(2) Repeat the experiment with a Fletcher anemometer and compare the two results.

CHAPTER IV.

PHYSICAL PROPERTIES OF LIQUIDS.

§ 15. **DENSITY, SPECIFIC GRAVITY.**—*Definition:*

$$\text{Specific gravity} = \frac{\text{weight of any volume of a substance.}}{\text{weight of the same volume of water.}}$$

The notation S. G. $\frac{100^\circ\ \text{C.}}{15^\circ\ \text{C.}}$, meaning the specific gravity of a substance at a temperature of 100° C., compared with water at 15° C. is a convenient one. Thus, from the definition

$$\text{S. G. } \frac{100^\circ}{15^\circ} = \frac{\text{weight of any volume of a substance at } 100^\circ.}{\text{weight of same volume of water at } 15^\circ.}$$

It should be noticed that it does not matter what units of weight are employed, whether pounds, ounces, grams, or grains, the number which expresses the specific gravity comes out the same in all.

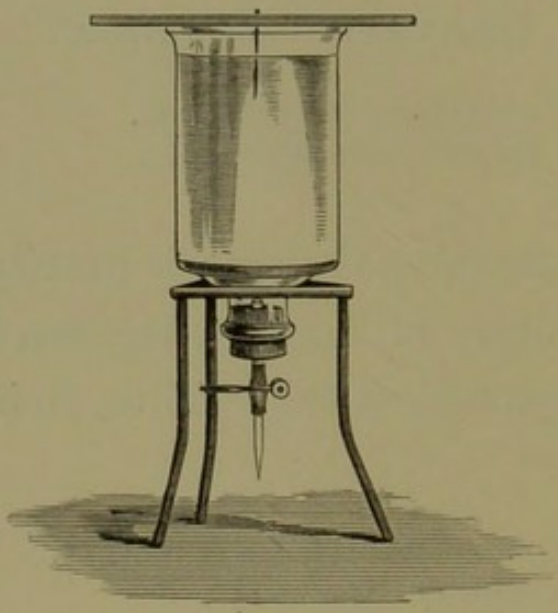
Definition.—Density is the number of units of weight in the unit volume. Here the number obviously depends upon the unit chosen; thus, in English measure, the density of water is the number of pounds per cubic foot; in the metric system, the number of grams per cubic centimetre. But since one gram has been taken to

be the weight of one cubic centimetre of water measured at 4° C., it follows that the specific gravity of any substance referred to water at 4° C. will be represented by the same number as the density in grams per c.c.

Thus, for water, $D^{15°} = \cdot 99912$ grams per c.c., and its S. G. $\frac{15°}{4°} = \cdot 99912$.

If the exact meaning of the word "density" be thoroughly grasped, it will be found much simpler to work with than the phrase "specific gravity;" so many meanings having been assigned to this latter as to render it ambiguous, unless both temperatures are explicitly stated.

FIG. 14.



§ 16. **Density of a Solid.** — The method described below, although not capable of a very high degree of accuracy, illustrates the definition very well, and is especially suitable for the determination of such substances as soils, or rocks which are difficult to sample.

An inverted glass bell jar has its lower end closed with a singly bored india-rubber cork, carrying a glass tube joined to a glass jet by a piece of india-rubber tubing provided with a pinchcock. Across the top of the bell jar is a piece of wood carrying a needle (Fig. 14). Suppose that it be now required to find the specific gravity of some sand. A glass beaker is hung by means of a piece of wire or

fine string to the wooden crosspiece ; water is then poured in till the beaker is completely immersed, and the needle point just wetted. By carefully opening the pinchcock the water is allowed to run out drop by drop until the needle just touches its own reflection, that is, is just level with the surface of the water. The sand (about 500 grams) is now weighed out and carefully poured into the submerged beaker : the level of the water in the belljar will consequently be raised. If now, by running out water into a tared glass beaker the original level of the water is restored, the volume run out can be at once determined by reweighing the small beaker, and this will obviously be the same as the volume of sand introduced. Thus, suppose the weight of the dry sand = 500 grams, and the water run out = 200 grams or 200 c.c (since the effect of temperature may, in this case, be neglected). Then 200 c.c. of sand weigh 500 grams, or 1 c.c. of sand weighs 2·50 grams,* that is (*Cp.* definition), 2·50 is the density of sand as determined in this experiment.

§ 17. **Density of a Liquid.**—There are several ways of taking the density of a liquid, the one adopted in any particular case depending upon the amount of accuracy which is required.

(*a*) *Hydrometer.*—If a greater accuracy than one in a thousand is not required, and there is sufficient of the liquid at hand, a hydrometer is always used. Floating bodies displace exactly their own weight of the liquid in which they float. Now, suppose the weight of a given hydrometer to be 30 grams. If this is to float in water

* See § 24 for the distinction between 2·5 and 2·50.

(at 4° C.) it must displace exactly 30 c.c., that is, the part immersed has a volume of 30 c.c. Now, for any liquid of density ρ, by definition, 1 c.c. weighs ρ grams, and the amount displaced must be $\left(\frac{30}{\rho}\right)$ c.c. Hence theoretically a hydrometer can be graduated by knowing the volume at each point on its stem. Practically, however, it is graduated empirically by means of liquids whose densities have been determined by the more accurate methods detailed below.

(*b*) *Plummet.*—In the hydrometer the weight of the substance immersed is kept constant and its volume varied; in the plummet method of taking densities the volume is kept the same, and the alteration of weight noted. Consider a glass plummet hanging from a balance beam by a very fine platinum wire, and having a volume of 5 c.c. If it is completely sunk in a liquid of density 1, it will displace 5 c.c., that is, 5 grams of liquid. Hence the plummet will weigh 5 grams less than it did in air. In a liquid of density ρ grams per c.c., 5 c.c. will still be displaced, *i.e.*, a weight of 5 ρ grams. Hence the plummet will lose in weight 5 ρ grams. It is not necessary to make the glass plummet any particular size. If weighed first in air, and then in water at 4° C., the number of grams it loses in weight represents the weight of the water displaced by it, that is, the number of cubic centimetres displaced. Hence the rule.

$$\text{Density} = \frac{\text{loss of weight in liquid.}}{\text{loss of weight in water.}}$$

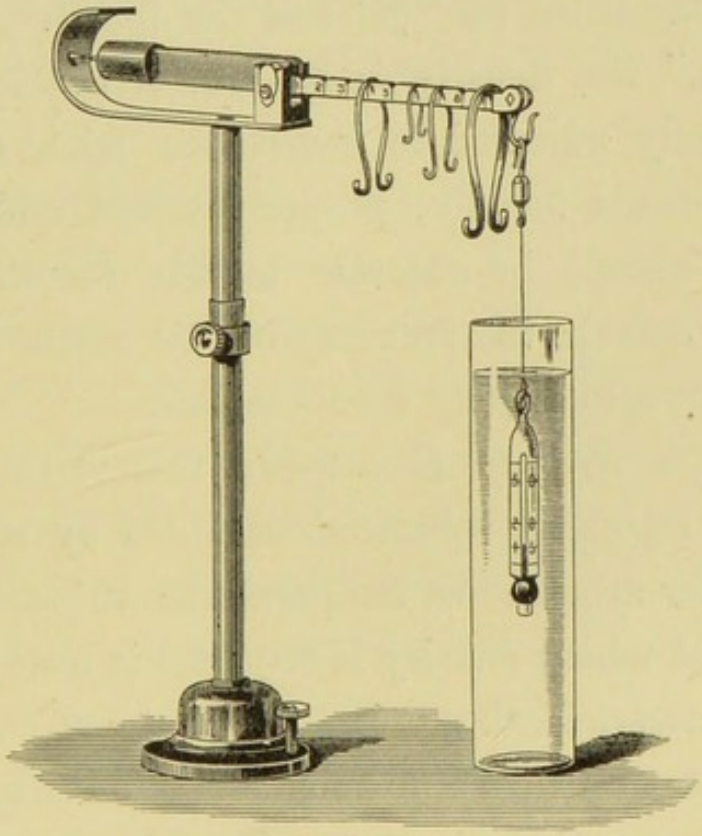
To allow for temperature, if both water and liquid are

at the same temperature, the above ratio multiplied by the true density of water at that temperature gives the true density. Thus at 15°, the density of water is ·99912, or 1 c.c. weighs ·99912 grams; for any other liquid.

$$D^{15} = \cdot 99912 \times \frac{\text{loss of weight in liquid at } 15^\circ.}{\text{loss of weight in water at } 15^\circ.}$$

Strictly speaking, since the plummet has been weighed

FIG. 15.



Westphal Balance (set at ·9476).

in air, a fluid whose density is about ·0012 grams per c.c., a correction ought to be introduced for this. But for liquids whose density is near that of water (as milk, 1·03) this correction is too small to take into account.

To economise time in taking densities with the

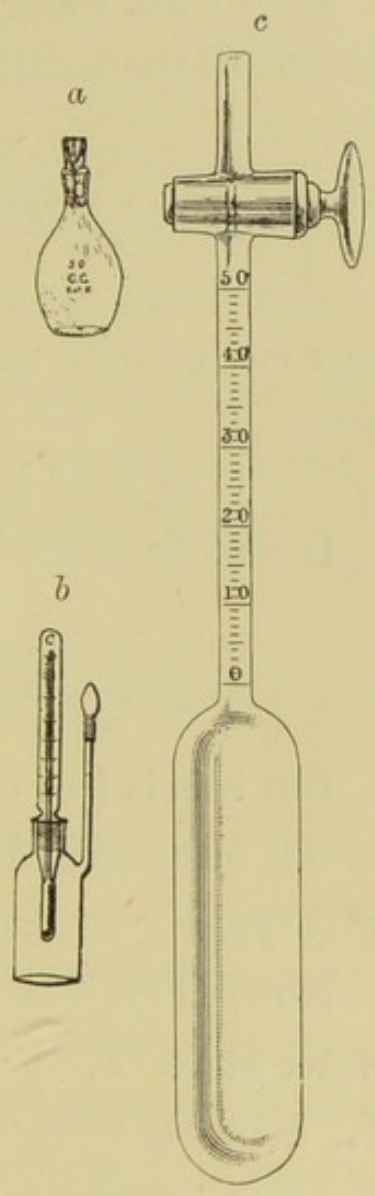
plummet a special balance (Westphal's balance) has been designed. This balance, which is a modified steel-yard, is furnished with four sizes of riders, each exactly one-tenth the weight, of the next larger size. These are placed in little notches on the beam, numbered 1 to 10, and in actually measuring a density are shifted about until the two points on the left of the knife-edge—one on the beam, and the other fixed to the stand—are brought absolutely into line. The density is read off directly. Thus, if the largest rider is on the nick "9," the next on nick "4," the third on "7," and the fourth or smallest on the "6," the density is 0·9476, if the balance has been properly adjusted to commence with (Fig. 15). In a well-made balance, properly tested and handled, the result should be accurate to the fourth place of decimals (·0001); the accuracy of this method is amply sufficient for all ordinary food analysis.

(*c*) *Specific Gravity Bottle.*—In this method a glass bottle has its exact volume determined by weighing it full of water at a known temperature; the same volume of the liquid whose density is required is now taken and its weight ascertained.

The bottle may take many shapes, the most usual being a light glass vessel of the shape shown in Fig. 16*a*, carrying a well ground in stopper of thermometer tube. Such a bottle usually has engraved on it "25 c.c. at 60° F.," or "50 c.c. at 15·5° C.," the exact capacity, however, must *always* be determined before use by weighing with distilled water. Suppose for example it is required to determine the density of a sample of milk at 60° F.

The milk must be thoroughly shaken, so that the cream may be uniformly diffused, and then allowed to stand for about fifteen minutes to clear it from air bubbles. Some of the milk, which must have been previously cooled below 60° F., is gently stirred with a thermometer (if the temperature of the laboratory is below 60° F., heating must be resorted to) until the temperature is just below 60° F. (say 59·0°–59·5°) and then poured into the clean dry bottle, which has been previously tared, till nearly full.

FIG. 16.



The thermometer is now put into the bottle and used as a stirrer. When the temperature is exactly 60° F., the bottle, without further handling, is filled up as quickly as possible from the beaker, and the stopper, previously wetted with milk to avoid air-bubbles, quickly pressed in with a slight turn, the excess is driven through the stopper and at once wiped off. The bottle is now wiped with a clean cloth and weighed. If cooling goes on and the liquid contracts during weighing it will obviously not affect the results. The capacity of the vessel must have been previously determined with water in a precisely similar manner.

The following example will show how the results are calculated.

Weight of the dry bottle	13·1832
Weight of bottle, + water at 60° F. . .	38·1705
„ „ + milk at 60° F. . . .	38·9645

Also 1 c.c. water at 60° F. = ·99908 grams. Hence at 60° F. the bottle holds

$$\frac{(38{\cdot}1705 - 13{\cdot}1832)}{{\cdot}99908} \text{ or } \frac{24{\cdot}9873}{{\cdot}99908} = 25{\cdot}0103 \text{ true c.c.}$$

Hence 25·0103 c.c. of milk at 60° F. = 25·7813 grams, or 1 c.c. of milk at 60° F. weighs 1·03083 grams, that is—

D $^{60°\ F.}$ of milk = 1·03083 (grams per c.c.).

It will be noticed that

$$\frac{\text{Weight of bottle full of milk at } 60°}{\text{Weight of bottle full of water at } 60°} = \frac{25{\cdot}7813}{24{\cdot}9873} = 1{\cdot}03177.$$

But this is (see definition) the specific gravity of milk at 60° F. compared with water at 60° F., or S. G. $\frac{60°}{60°}$ = 1·03177.

Now, D $^{60°\ F.}$ is really S. G. $\frac{60°}{39{\cdot}2}$, and since 1 c.c. of water at 60° F. = ·99908 grams = ·99908 c.c. at 39°·2 F., hence D $^{60°}$ = ·99908 S. G. $\frac{60}{60}$ = ·99908 × 1·03177 = 1·0308 grams per c.c. as before.

CHAPTER V.

RELATIONS BETWEEN PRESSURE AND HEAD.

§ 18. **Hydrostatic Pressure.**—It is easily shown by theoretical reasoning that the pressure exerted at any point by a column of liquid above it depends upon:

(1) The height of the column.

(2) The density of the liquid.

(3) The value of the acceleration due to gravity, commonly denoted by "g."

Since, however, g is absolutely constant at a given place, and practically constant over such an area as the United Kingdom ($g = 32{\cdot}2$ feet per second, per second), the first two points need only to be considered. The most important fact to remember is that the shape of the column has absolutely no effect on the pressure it exerts.

For the ordinary proofs the student is referred to text-books on elementary hydrostatics. It should, however, be noted that the word "pressure" is commonly used in two different senses, and much confusion consequently arises. The proper use of the word "pressure" is a weight of so many pounds per unit of area,

thus, the pressure in a boiler is spoken of as 120 lb. per square inch, or a wind pressure as 2 lb. per square foot. The unit of area, if not directly expressed, is always implied. In the other sense, "the pressure of the atmosphere on the body is 15 tons," the total pressure exerted over the whole surface is meant. If the word "total" is used all ambiguity is removed.

§ 19. **Experimental proof that the pressure exerted by a column of liquid is proportional to its height.**—To show that in a given liquid the pressure in any layer is proportional to the length of the liquid column above it, a cylindrical brass bucket, carrying a millimetre scale outside, is weighed, and then small shot slowly added (from a tared beaker) until it just floats upright. The total weight of the bucket and shot is then noted, and also the depth of the bottom of the bucket below the surface of the water. More shot is now added, and a fresh reading of the new position taken, and so on, arranging the results as in the following example:

Weight, bucket, and shot. (W.)	Depth immersed. (H.)	$\left(\frac{W.}{H.}\right)$	Area of bottom.	Upward pressure in grams. per sq. cm.
grms.	cm.		sq. cm.	
106	5·0	21·2	21·23	4·99
127	6·0	21·1	,,	5·99
147	7·0	21·0	,,	6·92
169	8·0	21·1	,,	7·96
193	9·0	21·4	,,	9·09
213	10·0	21·3	,,	10·03
320	15·0	21·3	,,	15·08
	Mean . .	21·2		

The results may also with advantage be expressed graphically upon squared paper, where the distances measured vertically represent the weight, and the distances measured horizontally the corresponding heights.

The question which now arises is that seeing the bucket is floating steadily in a vertical position, what are the forces acting upon it? By the fundamental property of a fluid the water is everywhere pressing upon it at right angles to its surface. It is easy to see that any horizontal thrust on one side is exactly balanced by an equal thrust in the opposite direction on the other side, and the fact that the bucket remains steady and shows no tendency to move in any particular direction also proves that these horizontal forces are exactly balanced. The water also exerts an upward thrust on the bottom—say p grams weight per square centimetre. If the area of the bottom is A square centimetres, the total upward push $P = pA$. But this is balanced by an equal and opposite push due to the weight of the whole, W.

Hence $W = pA$.

But it has been found by the above experiment that $W = HC$ (where C is constant) and equal here to 21·2. $\therefore p = H \times \left(\frac{C}{A}\right)$ where $\frac{C}{A}$ is constant;

that is to say, the pressure per unit area is proportional to the depth.

In the above case the radius of the cylinder was 2·6 cm., hence the area $= 3\frac{1}{7} \times (\text{radius})^2 = 21{\cdot}23$ sq. cm. At a depth of 10 cm. the weight of the whole was 213 grams, hence the pressure at 10 cm. depth is 10·03

grams per sq. cm., or at 1 cm., 1·003 gram per sq. cm., or almost exactly 1 gram per sq. cm.

If now the experiment be repeated with alcohol (of density ·85 grams per c.c.) in an exactly similar manner, it is found again that the pressure varies directly as the depth, and for 1 cm. is ·85 grams per sq. cm. Hence, at the same depth, on the same area in the two liquids, the pressure is proportional to the density.

§ 20. **Experimental proof that the shape of a column of liquid has no influence upon the pressure it exerts.**—The simple apparatus described below gives a further experimental proof of the effect of shape of the column and density of the fluid.

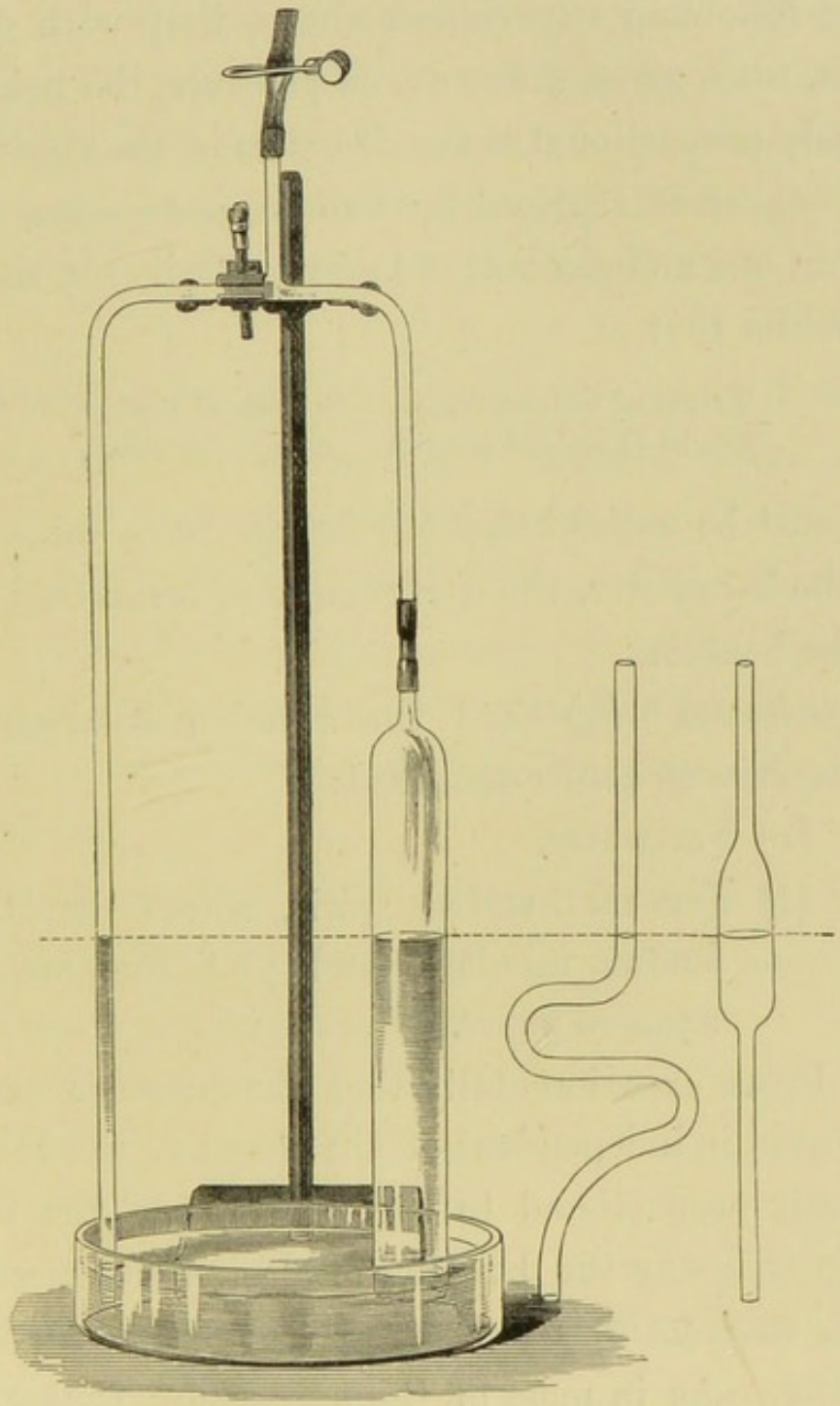
It consists of a glass T-tube (Fig. 17), furnished with a piece of rubber tubing and a pinchcock. A straight piece of tubing about a foot long is joined to one arm of the T (the left in Fig. 17). To the other is fastened a shorter piece, fitted either with rubber tubing or cork, so that glass tubes of varying shape and size can be joined on. The arrrangement will be easily understood from the figure.

To show that with the same liquid the height to which the fluid will rise in the tube is independent of the shape of the tube and depends only upon the pressure, the following experiment may be made.

The two halves of the right-hand limb are first joined together with a piece of tubing of any size. One large beaker filled with water is then placed under both. A mark is made on the undivided tube, and by sucking at the top tube of the T the water is raised to this point. The

water is found to rise to exactly the same height in the other leg, even if this should happen to fall in the middle of the bulb, and this holds when the various

FIG. 17.



connecting pieces are put in, bulbous or otherwise. Now the pressure on the surface of the water in the beaker is that of the atmosphere, the pressures in the tops of the

water columns must obviously be identical, since there is free communication through the T-piece and everything is steady; hence with a given liquid a given difference of pressure means a fixed height of column.

The following experiment shows that with different liquids, for a given difference of pressure, the heights are inversely proportional to the densities of the liquids. The large beaker is replaced by two separate ones, having water in one and alcohol (of known density) in the other. It is found that

$$\frac{\text{Height of water column}}{\text{Height of alcohol column}} = \frac{\text{density of alcohol.}}{\text{density of water.}}$$

It must be noticed that the height to be measured is, as in the barometer, the difference of levels in the tube and the beaker.

Experiments.—(1) Find the relation between depth and pressure as above described:

(*a*) For water,

(*b*) For methylated spirit, whose density you know, tabulate, and plot the results on squared paper.

(2) Prove experimentally that the pressure exercised by a liquid is independent of the shape of the tube, and is directly proportional to the densities of the liquids. Find in this way the densities of alcohol and mercury.

§ 21. **REMARKS ON THE USE OF GRADUATED VESSELS.**—As in most of the succeeding chapters the volumetric method of analysis is largely used, it will be as well to give here a few general rules regarding standard solutions. No water or liquid of any kind is to be

added to the solutions, and the bottles must be carefully restoppered and replaced after use. It is very important to make sure that the burette or the pipette is free from grease. A vigorous use of a clean burette brush and the free use of tap-water, followed by a rinse with distilled water, will usually ensure this for the burette. Immediately after using, the burette or pipette is rinsed well with distilled water and inverted to drain. On no account must a solution be left overnight in a measuring vessel.

In general, a burette on commencing to work will be clean but wet. If the standard solution were poured into this and used at once, the water previously in the burette would make the solution too weak. Hence the burette or pipette is always rinsed out with a few cubic centimetres of the standard solution, this is drained out, but not returned into the bottle, and the burette is then filled up.

On the completion of the analysis, if all the solution is not required, it is run back into the bottle, the burette rinsed out, and inverted in its stand.

In preliminary work, where the object is not so much to arrive at a knowledge of the exact amount of the various constituents, but rather to become acquainted with the principles of the methods, the burettes, pipettes, flasks, etc., may be assumed to contain exactly the amounts engraved on them.

§ 22. **Calibration of Measuring Vessels.** — The burettes, pipettes, and flasks in use in the laboratory are marked by the maker to hold certain volumes. For

rough work, where a greater accuracy than 1 in 100 is not required, these values may be assumed to be correct. For accurate work, however, it is necessary to "calibrate" these vessels, that is, to find out by experiment what the capacity up to the marks really is.* This is done by weighing them filled with distilled water. At 4° C., 1 gram of water measures 1 c.c. (by definition of 1 gram). The following table gives the relation at other temperatures.

The weight of 1000 c.c. of water at t° C., when determined by means of brass weights in air of t° (and average pressure) is equal to (1000 - x) grams.

t°	10°	11°	12°	13°	14°	15°	16°	17°
x·	1·34	1·43	1·52	1·63	1·76	1·89	2·04	2·20

t°	18°	19°	20°	21°	22°	23°	24°	25°
x·	2·37	2·55	2·74	2·95	3·17	3·39	3·63	3·88

Thus, if a flask holds 99·98 grams of water at 17° C., from the above table 1 c.c. at 17° weighs ·9978 grams. Hence—

$$99{\cdot}98 \text{ grams} = \frac{99{\cdot}98}{99{\cdot}78} \text{ or } 100{\cdot}2 \text{ c.c. at } 17°.$$

Flasks.—The flask is first thoroughly cleaned, then rinsed with distilled water, and after drying in a current

* Since this chapter was written, graduated vessels have been put upon the market whose accuracy can be relied on (without calibration by the user) to 1 part in 1000 (0·1 per cent.). These vessels have been verified and stamped at the Reichanstalt, an institution supported by the German Government, where every kind of measuring instrument can be verified.

of hot air, weighed. If the capacity is 100 c.c. or less, it is weighed to a milligram on an ordinary chemical balance. If more (250 c.c. 500 c.c., or a litre), the weighings are made on a large rough balance to ·02 gram.

The filling is done from a large beaker of distilled water (the temperature of which is taken after well stirring with a thermometer) through a funnel to avoid wetting the neck, the last few drops being added from a pipette. If the eye is on the proper level, the ring round the neck appears as a single line, not as a flat ellipse; and the lowest point of the meniscus should just touch this line. The capacity, after correction, is scratched on the flask with a writing diamond.

Burettes: (*a*) *Without a float.*—The effect of lighting upon the reading of the burette should first be noticed, also the necessity of getting the eye on a level with the meniscus. A piece of white paper or porcelain held at an angle of 45° to the burette gives a sharp, definite line.

The results will be quite worthless, unless the tube is clean and free from grease. After filling with distilled water at the known temperature of the laboratory, successive portions of 10 c.c. are drawn off with the utmost care into a weighed stoppered flask. As a check, on repeating, 5 c.c. are first drawn out, and then 10 c.c. as before, so that whilst the first set gives the capacity down to 10, 20, 30, 40, 50 c.c., the second set gives 5, 15, 25, 35, 45, 50 c.c.

After correcting for temperature from the above table, the results of the two sets are plotted upon squared

paper. The two curves should not differ at any point by more than ·04 c.c. If a larger discrepancy appears, the calibration must be repeated.

(β) *With a float.*—This is more troublesome in some respects, but gives more accurate results; the error of reading should not exceed ·01 c.c. If a burette has been calibrated with one particular float, it must afterwards be used with the same float. Hence both burette and float should carry the same number.

Pipettes must be thoroughly cleaned (if necessary, with strong HNO_3). The best test of a glass vessel being clean and free from grease is, that if rinsed out with distilled water and drained, no drops are visible on the glass; it does not, in fact, appear to be wet at all. Before proceeding to calibrate, it is as well to practise filling and delivering the pipette exactly from the mark. A light stoppered glass flask is now weighed to 1 mgr. The pipette is filled above the mark with distilled water of a known temperature, and the point of the pipette is wiped with a cloth. By holding a white tile (or piece of paper) at the back of the pipette, a sharp reading is obtained, and it is easy by slightly relaxing the finger to bring the meniscus down to the mark. The hanging drop is taken off with the tile, the pipette held vertically over the weighed flask, emptied, and allowed to drain for (about) 15 seconds after the main bulk of the fluid has run out. The surface of the liquid is then just touched with the point of the pipette, the flask quickly stoppered, and reweighed.

The contents of a pipette should never be blown out.

The mean of some five observations, duly corrected for temperature, may be taken as giving the true volume.

An excellent way of checking the results is to carefully calibrate a 100 c.c. flask, and then, after well drying it, to fill it with the pipette, using always the same mode of emptying. As flasks are usually too large, the small amount required to fill the flask to the mark is then run in from a burette. The calculation is obvious. The following is an example.

Exact capacity of flask in true c.c. = 100·25 c.c. A 10 c.c. pipette, 10 times filled, + ·50 c.c. from a burette, exactly fills the flask. Therefore 10 × volume of pipette = 100·25 − 0·5 = 99·75.

Hence, true volume of pipette = 9·975 c.c. The mean of five separate weighings gave a practically identical result (9·976 c.c.).

PART II.

THE ANALYSIS OF POTABLE WATERS.

CHAPTER I.

TOTAL SOLID RESIDUE.

§ 23. **USE OF THE BALANCE.**—To find the number of grains per gallon of solid matter in tap-water.

This requires the use of a chemical balance and weights.

Fig. 18 shows one of a type constructed to carry 50 grams, and "turn" with about a quarter of a milligram, although with careful weighing it will indicate differences of $\frac{1}{10}$ mgr.

Before handling a balance, the operation of weighing should be watched once or twice. The following points must be attended to in weighing.

Precautions in Weighing.

(1) See that the "rider" is in its place on the rider-arm, and that the latter is out of the way of the beam.

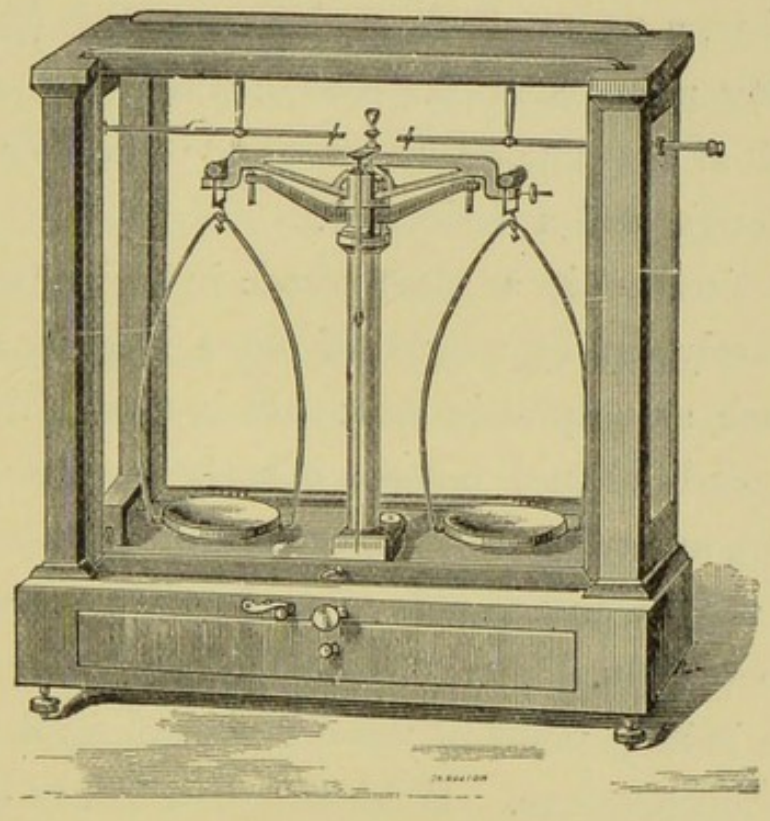
(2) Do not stop the swinging of the balance with a jerk; it is best to stop it when the pointer is in its central position.

(3) Stop the swinging of the balance, *i.e.*, lift the beam, when weights are to be added or taken away.

(4) The position of the observer should be central, so that there may be no parallax in observing the position of the pointer.

(5) Final weighings must be made with the balance

FIG. 18.



case closed, and care must be taken that the pans do not swing.

(6) Do not weigh a body when hot; the heat causes air currents, which affect the weighing.

(7) All substances liable to injure the pans must be weighed in appropriate vessels.

(8) Remove the weights from the pans, the rider from the beam, and close the balance.

It is desirable to see a weighing performed before you touch the balance.

The determination of the total solids is a very simple matter. It is only necessary to evaporate to dryness a known quantity of water in a weighed plantinum dish and re-weigh. The difference between the two weighings represents the solid matter in the quantity of water taken.

The following apparatus will be required: a balance and weights, a 70 c.c. flask, a platinum dish (preferably one holding more than 70 c.c.), a water bath, a drying oven kept at a temperature of 120° C., a pair of iron crucible tongs, and a desiccator.

The desiccator is a glass vessel, in which the air is kept perfectly dry by means of some hygroscopic substance, such as sulphuric acid. It is essential that the platinum dish should possess exactly the same weight both before and after the evaporation of the water; and the only way to ensure this is to have it perfectly dry on both occasions. This is secured by heating the dish above the boiling point of water and cooling it in a perfectly dry atmosphere. Hold the dish firmly in a pair of iron (not brass) crucible tongs over a Bunsen burner, until every part has been heated to redness; put while still hot into a dessicator on a clay tile, clay triangle, or asbestos pad, and allow it to cool down to the temperature of the room, which will take at least ten minutes. Carry to the balance in the desiccator and weigh it as quickly as possible to the tenth of a milligram. Repeat this several times, at least four, until the weight is constant, and the use of the balance becomes fairly easy.

Measure out 70 c.c. of the water requiring analysis

into the dish after placing it on the water bath, using the copper one fixed in the laboratory, or extemporising one out of a glass beaker which contains boiling water, and is of such a size that the platinum dish may fit conveniently into its mouth, small pieces of paper being interposed between the dish and the beaker to allow of the escape of the steam; when the contents of the dish are apparently dry, place in the air oven at 120° C. for half an hour, cool in the desiccator and weigh, and repeat this till a constant weight is obtained.

Since the quantity of water taken (70 c.c.) contains 70,000 milligrams, the number of milligrams of residue represents parts per 70,000, but "grains per gallon" are parts per 70,000 (1 gallon = 10 lb. Av. = 10 × 7000 grains). Hence, by taking 70 c.c. of water for evaporation, the number of milligrams gives at once, without any calculation, the number of grains per gallon; 70 c.c. of water is therefore known as a miniature gallon.

The results should appear in a note-book as in the following example taken from an actual analysis.

(Date) .

Determination of total solid matter in tap water.

Dish No. P_2 = 1st weighing 35·5570 grms.
" " 2nd " 35·5565 "
" " 3rd " 35·5560 "
" " 4th " 35·5560 "

Dish No. P_2 + Residue from 70 c.c. water = 1st weighing 35·5790 grms.
" " " " 2nd " 35·5780 "
" " " " 3rd " 35·5780 "
" " " " 4th " 35·5780 "

Dish + dry residue = 35·5780 grms.
Dish = 35·5560 "

Residue ·0220

= 22·0 mgr. on 70 c.c. or 22·0 grains per gallon.

§ 24. **EXPERIMENTAL ERROR.** — A few words as to the importance of taking into account the various unavoidable errors of experiment may not be out of place here. Assuming the weights are correct, and that they have been correctly counted, there is still a certain error owing to the fact that it takes a small though definite weight to "turn" the balance. Suppose that with the balance used in the above case, this is 0·1 mgr. Then, saying that the weight of the dish is 29·8317 grms. simply means that it is between 29·8318 grms. and 29·8316 grms.; and similarly for the second weighing. Hence the true weight of the residue may have been anything between 24·1 and 24·3 mgr. This may be definitely expressed thus $24{\cdot}2 \pm 0{\cdot}1$ mgr. or simply 24·2, if it be clearly understood that the last decimal place given is the one liable to uncertainty. Thus, suppose that 700 c.c. had been measured out, taken to dryness, with complete exclusion of dust, dried, and weighed with the same degree of accuracy. Suppose the weight of the residue were 241·3 mgr., this would give 24·13 grains per gallon, with the uncertainty in the last figure. If the residue had weighed 241·0 mgr., then the result would, in this case, have been expressed as 24·10 grains per gallon. Hence, 24·1 and 24·10 have distinct and separate meanings in work of this kind.

Now, returning to the first case, suppose it is necessary to turn this into "parts per 100,000."

Since 1 in 70,000, *i.e.*, 1 grain per gallon;

$\frac{1}{7}$ in 10,000;

$\frac{7}{10}$ in 100,000

the number in grains per gallon, here 24·1, multiplied by 10 and divided by 7 gives parts per 100,000. Now, $24{\cdot}1 \times \frac{10}{7} = 34{\cdot}428571$ parts per 100,000. But it is obviously absurd to keep in all these figures. The point is to reject just so many that the last figure left in shall be the uncertain one. In this case the uncertainty is 0·1 part in 24·1 parts, or 1 in 240, this is about 0·4 per cent. Now 0·4 per cent. is 0·14 on 34·4, hence it is seen that the 34·428571 may be anything between 34·54 and 34·26, so that the first decimal place is the last significant figure. The result of the conversion then is 34·4 parts per 100,000.

In all transformations of this kind, and in fact in giving the results of any experimental work, great care must be taken not to leave in any more figures than the accuracy of the experiment justifies.

Experiment.—Find the number of grains per gallon of solid residue in tap water.

CHAPTER II.

ESTIMATION OF CHLORINE (as Chlorides).

§ 25. **ESTIMATION OF CHLORINE.** — To find the number of grains per gallon of chlorine in tap water.

Before commencing the quantitative test, try the following experiments:

(1) To a solution of a chloride in a test tube, add some silver nitrate solution.

(2) In another tube to a solution of potassium chromate (yellow) add silver nitrate solution.

(3) To the red precipitate obtained in the second experiment add a few drops of ammonium chloride solution and shake.

(4) Add slowly more silver nitrate solution to the last tube until the red colour reappears.

In the first experiment silver chloride is formed according to the equation,

$$AgNO_3 + NaCl = AgCl + NaNO_3$$

which, being insoluble in water, separates as a curdy white precipitate.

In the second tube silver chromate is formed thus :

$$2\,AgNO_3 + K_2CrO_4 = Ag_2CrO_4 + 2\,KNO_3.$$

which, being also insoluble in water, separates as a crimson-red precipitate.

The result of the third experiment is to prove that a chloride in solution is able to attack the silver chromate with formation of silver chloride, thus :

$$Ag_2CrO_4 + 2\,NH_4Cl = 2\,AgCl + (NH_4)_2\,CrO_4.$$

On adding gradually more silver nitrate it is found that the liquid remains colourless so long as silver chloride is being formed, that is, so long as there is any chloride in solution for the silver nitrate to react with, and the production of a red colour (silver chromate) taken with the result of the third experiment is a proof that the whole of the chloride has just been precipitated. As the chromate solution *indicates* when all the chloride has been thrown out of solution, it is called an "indicator."

The apparatus required will be two deep porcelain dishes, two glass stirring rods, a 70 c.c. flask, a burette with glass tap holding 25 c.c. and divided into $\frac{1}{10}$ ths of a c.c.; a standard solution of silver nitrate containing 4·79 grams to the litre, and a solution of potassium chromate. The latter must have been freed from all traces of chlorides, not infrequently present, by treating with sufficient silver nitrate to form a red precipitate, and filtering.

Then into one basin measure 70 c.c. of distilled water, and into the other 70 c.c. of the sample of water in which

the chlorine is to be estimated, and which must be neutral or faintly alkaline. Colour both to the same tint with chromate, and then see how much of the standard silver nitrate solution the distilled water takes to give it a perceptibly red tint when compared with the other. Note this amount. Now add the silver solution drop by drop, with constant stirring, to the other basin, until it acquires the same redness as the distilled water has. Subtract the amount required to give a colour in the absence of chlorides previously found, and the number of cubic centimetres remaining represents the number of milligrams of chlorine in the 70 c.c. of water taken, that is, the number of grains per gallon. Repeat this at least three times on the same water.

A slightly more accurate but more tedious method is to evaporate the 70 c.c. nearly to dryness, leaving say from 3 to 5 c.c., add one drop of chromate and titrate. The change of colour is here perfectly sharp, half a drop (0·03 c.c.) being quite sufficient to distinctly turn the colour.

The reason for taking 4·79 grams of silver nitrate to the litre is as follows:

Since $NaCl + AgNO_3 = AgCl + NaNO_3$.

$(23.00 + 35.37) + (107{\cdot}66 + 14{\cdot}01 + 47{\cdot}88) = (107{\cdot}66 + 35{\cdot}37) + (23 + 14{\cdot}01 + 47{\cdot}88)$

so that from 169·55 parts of $AgNO_3$ 107·66 parts of Ag always combine with 35·37 parts of Cl.

Hence	Cl		$AgNO_3$
	35·37	=	169·55.
Or,	1	=	4·79.

that is 4·79 parts of $AgNO_3$ dissolved in water will exactly precipitate 1 part of Cl. Hence, to obtain a solution containing sufficient $AgNO_3$ per cubic centimetre to throw down 1 mgr. Cl., 4·79 mgrs. $AgNO_3$ per c.c. should be taken, that is 4·79 grams to the litre.

Experiments.—(1) Find the number of grains per gallon of chlorine in the samples of water submitted to you.

Questions.

1. It is required to estimate the total solids in a water with an accuracy of 1 in 1000. How much water must be evaporated—(1) If the residue can be weighed with a possible error of + ·25 mgr.; (2) With a possible error of + ·1 mgr.?

2. If results are required in parts per 100,000, how much water must be taken to give the results without further calculation?

3. It is required to make up a standard solution of silver nitrate of which 1 c.c. will exactly precipitate 1 mgr. of common salt. Show exactly how to calculate this.

4. A rough test on 70 c.c. of water shows that about 1·0 grain of chlorine per gallon is present. If an accuracy of 1 in 500 is required how much water must be evaporated down, other conditions remaining the same?

CHAPTER III.

THE ESTIMATION OF AMMONIA.

§ 26. **NESSLERISING.** — The determination of the amount of ammonia in a very weak aqueous solution.

This is done by the help of a re-agent called "Nessler's re-agent," * the operation being known as Nesslerising. Dilute solutions of ammonia, or ammonia salts treated with Nessler solution, that is, an alkaline solution of the double iodide of mercury and potassium, give a yellowish-brown precipitate, or, in the case of extreme dilution, yellow tints only. (Try this with a little ammonium chloride solution.) The depth of the tint thus produced depends upon the concentration, that is to say, upon the number of grams of ammonia in the litre of water; and the determination of the ammonia by the comparison of these tints with the tints produced in solutions of known concentration is called Nesslerising. A solution strong enough to give a precipitate, or, indeed, one containing more than ·05 mgr. NH_3 in 50 c.c., cannot be accurately Nesslerised. Stronger

* For mode of preparation, see Appendix.

solutions must be diluted down with ammonia-free water, the degree of dilution necessary being carefully noted at the time. The depth of tint obtained by adding the Nessler re-agent to an aqueous solution of ammonia (*note particularly that this order must not be reversed*) depends upon :

(1) The quantity of ammonia.

(2) The height of the column looked through.

(3) The temperature.

(4) The time that the solution is allowed to stand after adding the Nessler re-agent.

(5) The quantity of Nessler solution added.

As regards the temperature the effect is slight though quite distinct. For a given quantity of ammonia the higher the temperature the deeper the tint.

In ordinary work, where great accuracy is not required, this may be neglected. In a rapidly conducted distillation, however, with an inefficient condenser, if the estimation is carried out on each tube-full as it comes over, the error from this cause may become appreciable. It tends to make the amount of ammonia found too large.

In connection with this try the following experiments :

1. Distil some water rapidly through a small glass condenser ; note the temperatures of the room and of the distillate.
2. Take two clean Nessler glasses, add to each ·03 mgr. NH_3, and fill up to the mark with ammonia free water. Raise one of them to about 10° C. above the other, add Nessler and compare their tints by the second method of Nesslerising described below.

   Express the results as apparent increase of NH_3 per degree C. Repeat with different quantities of ammonia.

   As regards the effect of time, it will be seen that for about

minutes after adding the Nessler re-agent the tint deepens. Hence the two tubes, the one containing the unknown amount ("test") and the other containing the known amount ("standard"), should always be started (*i.e.*, have the Nessler solution added) as nearly together as possible.

Experiment.—Take four tubes. Add to each the same quantity of ammonia, say, ·03 mgr., and fill up to the mark with distilled water. Add to one 2 c.c. of Nessler, then after a minute 2 c.c. to the second, and so on. Immediately after starting the last tube compare the tints of the four. Then allow to stand for five minutes and compare again.

In the first method of Nesslerising, the height of the column is kept the same, and the concentration of the known solution is varied.

Thus, suppose it is required to find the amount of ammonia in a solution containing only ammonium chloride in distilled water, the following will be necessary: Nessler glasses, made of colourless glass and having a mark at 50 c.c., thoroughly cleaned by the vigorous application of a clean test-tube brush and free use of tap water; a standard solution of ammonia containing ·01 mgr. NH_3 in 1 c.c., a 100 c.c. flask, a white tile, some Nessler reagent, a 2 c.c. pipette, a burette divided into $\frac{1}{10}$ths of a cubic centimetre to hold the standard ammonia solution, and some distilled water free from ammonia.

Take in a Nessler glass 50 c.c. of the solution in which the ammonia is to be estimated, and which may be called for shortness the "test" solution; add 2 c.c. of the Nessler re-agent, and stir vigorously with the pipette; make up another tube with 5 c.c. standard ammonia solution (·05 mgr. NH_3), fill up to the mark with ammonia free water and add the same amount of

Nessler. If the tint in the first case is darker than in the second, sufficient time having been allowed for the full development of the colour, from what has been said before, the original test solution must be diluted. The most advantageous way of doing this is to measure out 50 c.c. into the 100 c.c. flask, fill up to the mark with the ammonia free water, shake thoroughly, pour out 50 c.c. and treat as before. If this is still too strong, again fill up the flask and so on, taking especial care to note down each dilution at the time. When 50 c.c. of the diluted solution contains a suitable amount of ammonia the straw-coloured liquid obtained after adding the Nessler solution has to be matched by repeated trials with tubes containing known quantities of ammonia.

Perhaps this may be best seen from one or two actual examples.

First case.—The test solution did not require diluting.

To 50 c.c. were added 2 c.c. of Nessler re-agent. This tint was imitated in another tube by 5 c.c. standard ammonia and 45 c.c. ammonia free water, but this developed too strong a colour. Hence the ammonia present in the "test" was less than ·05 mgr. NH_3 per 50 c.c. Another tube was treated exactly as above but with only ·03 mgr. NH_3 added (that is 3 c.c. of the standard ammonia from the burette). This proved to be too little. A third trial showed that ·04 mgr. NH_3 was too much. In a fourth attempt 3·5 c.c. (·035 mgr. NH_3) gave a tint exactly the same as the test solution. To make sure of this a fresh lot of the

"test" solution was taken, and also a fresh tube containing ·035 mgr. NH_3; both were started together as nearly as possible and compared after five minutes. The tints were equal. Hence 50 c.c. of the test solution contain ·035 mgr. of NH_3 or 0·7 mgr. per litre.

Second case.—The test solution required diluting.

50 c.c. was poured into a 100 c.c. stoppered flask, which was filled to the mark with distilled water free from ammonia, and the whole thoroughly shaken. Half this (50 c.c.) was poured out into a Nessler glass, and the Nessler re-agent added. The colour was obviously greater than that produced by ·05 mgr. NH_3; the 100 c.c. flask containing the remaining 50 c.c. was again filled up to the mark and shaken as before. 50 c.c. of this was compared with ·04 mgr. NH_3, which latter proved to be too great. A second trial showed it to be equal to ·036 mgr., and this result was confirmed by the 50 c.c. remaining in the flask.

Since 50 c.c. of the original ammonia solution was put into the flask in the first place, after first dilution 50 c.c. = 25 c.c. of original solution.

After second dilution 50 c.c. = 12·5 c.c. of original solution.

Hence 12·5 cc. = ·036 mgr. NH_3; or 1 c.c. = ·00288 mgr. NH_3; or the concentration is ·00288 grams. NH_3 per litre.

Here the assumption is made, that if all other conditions are the same in both cases, such as temperature, height of column, quantity of Nessler solution, and time of standing, the same quantity of ammonia (NH_3) will

always develop the same tint. This can be tried in two ways.

1. Add the same quantity of ammonia (say ·025 mgr.) to two glasses, fill up and start together. Here the tints must obviously be the same.
2. Prepare a solution of ammonium sulphate, containing ·01 mgr. NH_3 per c.c., and compare the tints given by, say, 2 c.c. of this, with 2 c.c. of the ordinary ammonium chloride solution. The equality of tint shows that the acid with which the ammonia happens to be combined does not interfere with the reaction.

Practise this mode of Nesslerising until you can match any tint readily in two, or at most three trials. Do not go on to the second mode of Nesslerising until quite certain of this.

In this second method, instead of altering the concentration (c), the height (h) is altered. First prove experimentally that, within certain limits, the depth of tint is proportional to height × concentration, or hc. Take five clean Nessler glasses on a white tile, and add 1·5, 2·0, 2·5, 3·0, 3·5 c.c. of the standard solution of ammonia to each respectively; fill each to the mark with ammonia free water, and start with Nessler as nearly as possible together. Number the tubes from 1 to 5, calling that tube No. 1 with ·015 mgr. NH_3. Take numbers 3 and 4. By pouring out some of the contents of the latter into a clean beaker, it will be found possible to make the tint equal to that of No. 3, of course looking down the tube.

Measure the height of the column remaining in the tube. Pour back some of this liquid and repeat once or twice. The closeness with which the results agree measures roughly the accuracy of the work.

It will be found that when the height in 4 is $\frac{5}{6}$ of its original height the depth of tint equals that in 3.

Treat pairs 1 and 5, 2 and 4 similarly.

It will be found that reduced to

$$\frac{3}{7} - \text{No. } 5 = \text{No. } 1.$$

$$\frac{2}{3} - \text{No. } 4 = \text{No. } 2.$$

Now take No. 1, and reduce all the others by pouring out to the same tint, tabulating the results as in the following example:

No.	1. Height (h) in $\frac{1}{10}$ of an inch.	2. Concentration in mgr. per litre.	3. h × c.	4. Volume remaining.	5. Volume × c.
1	32	·30	9·6	52	15·6
2	24	·40	9·6	39	15·6
3	19	·50	9·5	31	15·5
4	16	·60	9 6	26	15 6
5	13·5	·70	9·45	22	15·4

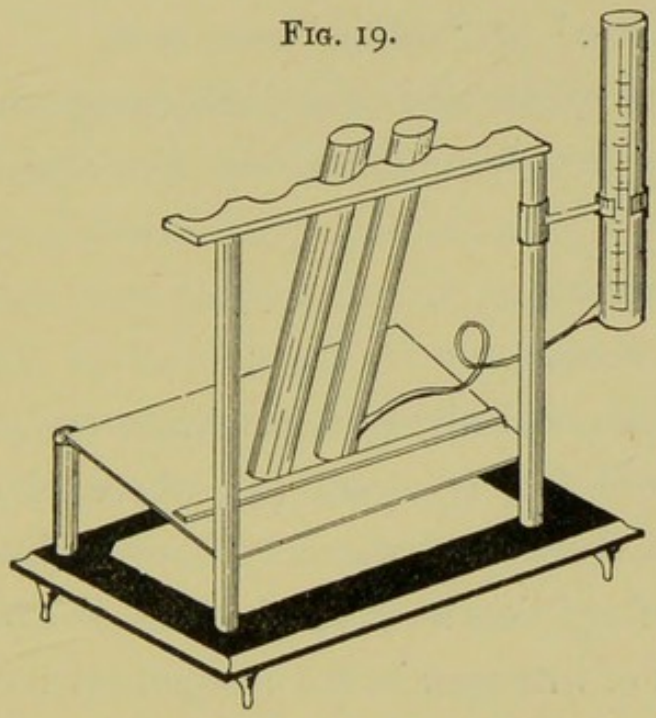
Since the volume of a cylinder is directly proportional to its height, it follows that the volume remaining in the Nessler glass after adjusting the tint can be measured instead of the height. Thus, in the above example each glass to start with contained 52 c.c.; the amount poured out of each was measured in a measuring cylinder, and this subtracted from 52, the original volume, gave the numbers in the fourth column. The fifth column shows

that the product of this multiplied by the concentration, was here again practically constant.

Several instruments have been devised to effect the alteration in height and measure the volume run out conveniently. One of the simplest of these colorimeters is that devised by Hehner and shown in Fig. 19.

Here the "standard" Nessler glass is furnished with

FIG. 19.



a side tube communicating with a graduated glass, which latter can be easily raised or lowered and left in any desired position. Its use in the second method of Nesslerising will be obvious. It not only is more speedy and convenient than the pouring out method above described, but since it permits of the comparison being rapidly repeated several times and the mean reading being taken, greater accuracy is obtained.

Experiments.—1. Determine, by the first method of

Nesslerising, the amount of ammonia in the solutions given to you.

2. Repeat the estimations by the second method, upon the same solutions and compare the results.

§ 27. **FREE AND ALBUMINOID AMMONIA.**—The element most characteristic of sewage contamination is nitrogen. In a water which has been polluted with sewage, nitrogen may be present:

(1) As "free" or "saline" ammonia.

(2) As indefinite, albuminoid-like compounds. (Total nitrogen in this form constitutes Frankland's "organic nitrogen.")

(3) As nitrites.

(4) As nitrates.

(5) As dissolved nitrogen gas (from the air).

As regards (1), (3), and (4), there is little or no difficulty in their estimation. There is, however, great difference of opinion as to the best mode of determining the amount of nitrogen in the compounds included under the second heading. Since no definite composition can be assigned to these albuminoid substances, it is impossible to say that in a given water there are so many grains per gallon of these nitrogen compounds. If in two waters, one contains just twice as much of these compounds as the other, any process which will bring out this fact must be regarded as satisfactory, and, in such empirical work as water analysis, the process of Wanklyn, Chapman, and Smith gives results sufficient for most practical purposes.

Since, like all other methods of determining organic

matter in drinking water, this is essentially an empirical process, in order that the standards laid down by the originators may be of any use at all, their method must be rigidly followed in every detail. Any "improvement" on an empirical method of this kind is absolutely valueless until either the new results have been experimentally interpreted in terms of the old, or until the new process has been tried upon a large number of waters of known quality.

Freed from detail, the process is briefly this. Some of the water is rendered just alkaline with Na_2CO_3, and is then rapidly distilled from a retort until four quantities of 50 c.c. each have been collected in Nessler glasses.

The lamp is now removed for an instant, some potassium permanganate solution* rendered strongly alkaline with caustic potash is added, the distillation recommenced, and some three to five more Nessler glasses distilled over. The ammonia in the latter is the "albuminoid ammonia" of Wanklyn. It must be remembered that the whole of the nitrogen present other than as ammonia salts, nitrates, and nitrites, is not given off in this way. The authors only claim that a constant proportion of this whole amount is given off under these conditions. Recent experiments tend to show that the nitrogen in the albuminoid ammonia is about one-half of the total organic nitrogen.

In addition to the apparatus and re-agents required for estimating the amounts of ammonia in the distillate there will be required the alkaline solution of perman-

* See Appendix on re-agents.

ganate, some sodium carbonate, a stoppered retort and a condenser in suitable stands (the retort must hold at least one litre), a 500 c.c. measuring flask (and smaller ones if highly polluted waters are to be examined), a glass funnel, and a stock of capillary glass tubes closed at one end.

It is assumed that the student is thoroughly acquainted with the previous exercise, so that the problem of determining an unknown amount of ammonia in 50 c.c. of water is perfectly familiar to him. Moreover, remembering that larger quantities of ammonia than ·05 mgr. in 50 c.c. cannot be accurately estimated without previous dilution, it is desirable to so adjust the quantity of water taken for distillation that the ammonia in the first Nessler glass shall not exceed this amount. This can be easily done by making a preliminary trial with 2 c.c. of Nessler's solution upon 50 c.c. of the natural water.

If no appreciable tint develops in five minutes, 500 c.c. is taken for the distillation. If a yellow tint is obtained, a smaller quantity is measured out and made up to 500 c.c. with pure ammonia free distilled water.

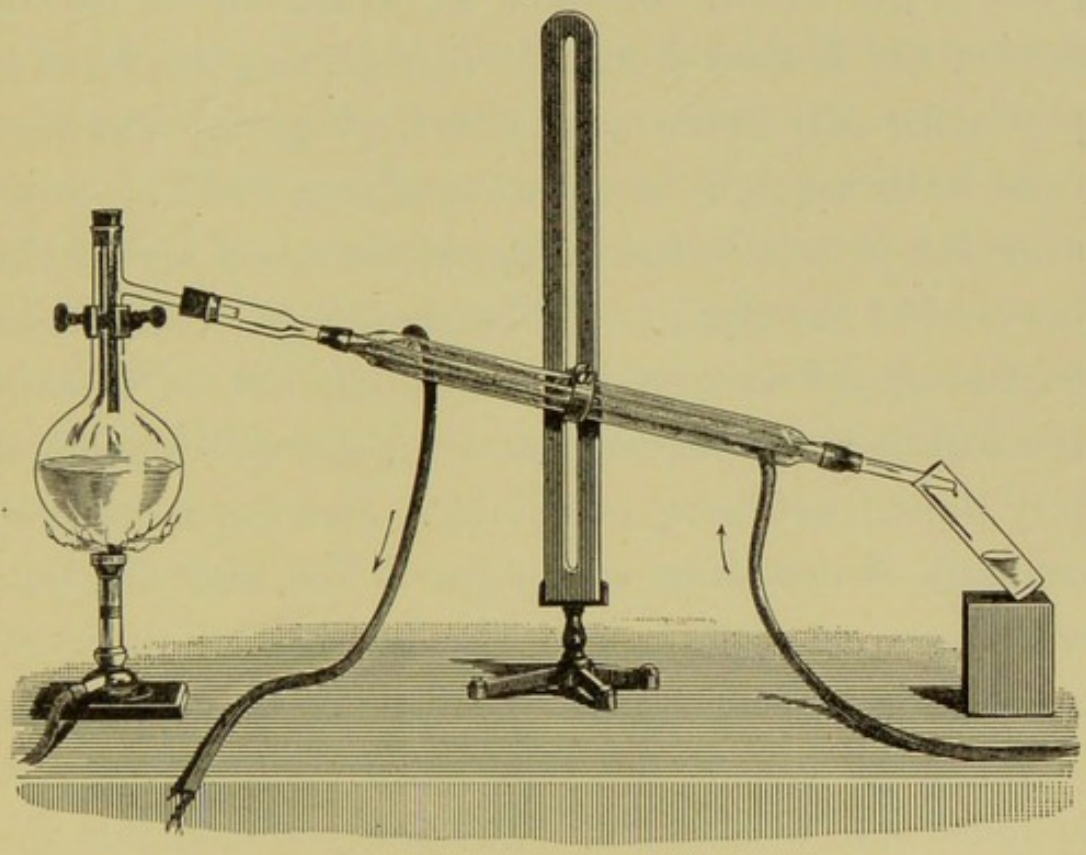
Although a rough guess from the tint obtained in this way will usually serve as sufficient guide to the necessary quantity, yet it is not difficult to calculate out the amount required.

Let the amount of ammonia in 50 c.c. of the original water be p mgr., as found by a rough experiment. Let x be the quantity of water sought, containing y mgr. of free NH_3, then the first 50 c.c. of distillate will contain $\frac{3}{4}y$ mgr. NH_3 (see below); and this is not to exceed ·05 mgr., hence $\frac{3}{4}y =$ or $<$ ·05, or $y =$ or $<$ ·066. But in original water 1 c.c. $= \frac{y}{x}$ mgr. NH_3, or $50 \times \frac{y}{x}$ in 50 c.c. $= p$ (by preliminary trial); $\therefore x = \frac{50y}{p} =$

$\frac{50 \times \cdot 066}{p} = \frac{3 \cdot 3}{p}$ or less, thus in a sewage effluent, p = ·04 mgr., ∴ quantity of water to be taken $= \frac{3 \cdot 3}{\cdot 04} = 82\frac{1}{2}$ c.c. or less. This simple calculation is chiefly useful for sewage effluents and badly polluted surface wells.

§ 28. **DETAILS OF WANKLYN'S PROCESS.**—The following description is practically in Wanklyn's own

FIG. 20.



words.* The retort having been washed out with a little strong acid (either hydrochloric or sulphuric acid), is thoroughly rinsed with good tap water, until the few drops which drain out do not taste acid. It is then mounted in its holder, and properly connected with the Liebig's condenser, either by means of a wide india-rubber tube, or else it is just packed into the condenser

* Wanklyn, "Water Analysis."

with a little writing paper or tinfoil. Half a litre of the sample of water (or, if necessary, a smaller quantity as above explained) is next measured in the half-litre flask, and poured into the retort through a funnel kept specially for the purpose. If the water is not alkaline, a little freshly ignited Na_2CO_3 is added. Then the stopper, which must be kept scrupulously clean, is put into its place in the retort, the water is turned on to the condenser, the Bunsen burner is lighted, and the flame applied externally to the naked retort. (Fig. 20.) The retort must be thrust right down into the flame, which, however, must not play upon the surface of the retort higher than the level of the liquid. If a large-sized Fletcher Bunsen burner capped with gauze be used, any risk of breaking the flask is almost entirely avoided, the ordinary laboratory Bunsen not being so suitable. In a few minutes the contents of the retort will begin to boil, and the water will begin to distil over. The distillate is to be collected in well-cleaned glass cylinders for the Nessler test.

When 50 c.c. of distillate have distilled over, the cylinder is to be changed. The first 50 c.c. should then be Nesslerised as in the last chapter. The distillation is to be continued until 150 c.c. more have come over, and the 150 c.c. of distillate are to be thrown away. Having done so, and thereby reduced the contents of the retort from 500 c.c. to 300 c.c., the lamp is removed for a moment.

Fifty cubic centimetres of the solution of potash and potassium permanganate are then to be poured into the retort through the funnel, some half-dozen capillary glass tubes, recently made and closed at one end, are added,

the stopper replaced, and the distillation proceeded with. The presence of the capillary glass tubes completely avoids any "bumping." Three tubes-full of 50 c.c. each are collected (very bad waters may require four, or even five), distillation stopped, and the whole left standing until required for another analysis. The amount of ammonia in each of the Nessler glasses is then determined as usual.

It has been found by experience that it is unnecessary to determine separately each 50 c.c. of the free ammonia, since the first 50 c.c. always contains $\frac{3}{4}$ of the whole amount of free ammonia present in the water taken for distillation; and since one-third of $\frac{3}{4}$ gives the remaining quarter, it is sufficient to accurately determine the amount in the first glass, and add one-third of this to find the total free ammonia.

Experiments.—Determine the amount of free and albuminoid ammonia,

(*a*) In tap-water,
(*b*) In unfiltered Thames water,
(*c*) In water from a deep chalk well,
(*d*) In shallow well water (unpolluted),
(*e*) In shallow well water (polluted),
(*f*) In sewage effluents obtained by the following processes.
    (1) The A. B. C. process.
    (2) The lime and ferruginous alum process.
    (3) A sewage farm,

and from any other sewage works visited during the course.

Enter the results of analysis as in the following example. If the form is written out, with spaces just left for the figures, prior to commencing work, any liability to error is much reduced.

Afterwards tabulate the whole set in parallel columns, comparing each with the standards given in Appendix 3.

(Date) 20th *October* 1895. Water No. 14 B.

Preliminary test on 50 c.c. gives no appreciable tint. Hence 500 c.c. taken for analysis.

Free Ammonia.

1st 50 c.c. = ·003 mgr. NH_3.
(Add $\frac{1}{3}$) = ·001

·004 mgr. NH_3.

= ·008 parts per million.
= ·0006 grains per gallon.

Albuminoid Ammonia.

1st 50 c.c. = ·008 mgr. NH_3.
2nd „ = ·003
3rd „ = ·000
4th „ = ·000

·011 mgr. NH_3.

= ·022 parts per million.
= ·0015 grains per gallon.

CHAPTER IV.

FRANKLAND AND ARMSTRONG'S COMBUSTION PROCESS.

§ 29. **GENERAL DESCRIPTION.** — This process aims at finding out the absolute amounts of carbon and nitrogen due to organic pollution. Since the exact composition of the sewage when it enters the water is unknown, so far as the nitrogen is concerned, it matters little whether the total amount is estimated, as in the present case, or a constant fraction of it as in Wanklyn's method.

But whilst the latter entirely neglects the organic carbon, Frankland estimates both the organic carbon and the organic nitrogen simultaneously, and from the ratio of these two, important conclusions can be drawn as to the nature of the pollution. Whilst, however, it is possible with two or three months' training to get reasonably good results by the method of Wanklyn, Chapman, and Smith, a training of some years in water analysis is required to get even approximate results by the Frankland method, which indeed only yields perfectly good results in the hands of a specialist constantly

performing such analyses. But assuming these conditions, the results of consecutive determinations exhibit a higher degree of concordance than is attained by any other method; and although one single analysis will extend over two or three days, a considerable number can be easily kept going on together with great economy of time; hence the combustion process is particularly serviceable in such inquiries as the constancy of composition of a water supply, or the variation in the quality of a river-water at different points, but is quite unsuited to the wants of the medical officer of health.

A general description only is given here, so that reports of water analyses in which this method of measuring organic pollution is adopted may be utilised. In the last chapter (§ 27) a list of the forms in which nitrogen may appear in drinking water was given. Carbon may appear (1) as carbonates as $CaCO_3$, (2) as dissolved CO_2, (3) as organic carbon.

§ 30. **DETAILS OF THE PROCESS.**—(1) A litre of the water (or less if the free ammonia suggests pollution) is mixed with 20 c.c. of a saturated solution of SO_2 in pure water, and the mixture briskly boiled for a few seconds. This turns the carbonates into sulphites, and thus eliminates the CO_2 from this source. A drop of ferrous chloride is added, and the whole evaporated to dryness on a water bath, with special precautions to exclude dust. The only carbon now left in the residue is the organic carbon, the only nitrogen that originally present as free ammonia (fixed by the sulphurous acid) and the organic nitrogen. The CO_2 from the

carbonates and the nitrogen from the nitrites and nitrates will have been completely expelled.

(2) A "combustion" is now made of this residue by heating it to redness in vacuo with oxide of copper, as though it were an organic substance. The resulting gases are pumped out and collected. The mixture of CO_2, NO, and N_2 is analysed in an apparatus specially designed to measure accurately the small quantity of gas produced, and from this the weights of "organic carbon" and "organic nitrogen" are obtained, allowance having been made for the nitrogen arising from the previously determined free ammonia.

In the gaseous mixture the total volume is first measured at a known volume, pressure, and temperature. Then the CO_2 is removed by a little potash solution, and the gas remeasured. A slight excess of pure oxygen turns the colourless gas NO into the red oxides N_2O_3, N_2O_4, which are both instantly absorbed by the potash. Then a drop of pyrogallic acid solution is allowed to run up and mix with the potash, and the alkaline solution of potassium pyrogallate thus formed, on shaking, completely absorbs the excess of oxygen. The gas remaining, which should be pure nitrogen, is finally measured. The reductions to weight are of precisely the same type as in the estimation of nitrogen by Crum's method (§ 36).

CHAPTER V.

THE OXYGEN PROCESS.

§ 31. **GENERAL DESCRIPTION.**—This process, first suggested by Forchammer, and since subjected to numerous modifications, depends upon the estimation of the amount of oxygen which a known volume of the water abstracts from a standard solution of potassium permanganate.

The amount of "oxygen absorbed" can be ascertained with great exactitude and little trouble. It was soon found that dropping in the permanganate solution up to the appearance of a pink colour did not give reliable results. The permanganate must be in excess, and must act for a considerable time. To secure comparable results the following points must be fixed:

(1) Is the permanganate to act in acid or in alkaline solution? If in acid, what acid shall be used?

(2) How long is the reaction to go on?

(3) At what temperature should the water be kept?

(4) How is the excess of potassium permanganate (*i.e.*, the amount of oxygen remaining over) to be ascertained?

Since different answers can be given to each of these

questions, it will easily be understood that many methods are in use, viz., those of Tidy, Kubel, Wanklyn, the Society of Public Analysts, &c.

In England practically only two are used, that of Tidy and the process agreed upon by the Society of Public Analysts, and these differ only in two details.

§ 32. **THE TIDY-FORCHAMMER PROCESS.**—Here the reaction takes place in presence of sulphuric acid, the reaction going on for three hours at the temperature of the room, and the excess of permanganate being determined by the employment of sodium thiosulphate, and the iodine and starch reaction. Details of the re-agents required will be found in Appendix 1.

Two white glass stoppered bottles each holding about 500 c.c. are first cleaned with strong sulphuric acid, and then with water. In the one is placed 250 c.c. of the water under examination, and in the other the same volume of pure, organically-free distilled water. To each is then added 10 c.c. of dilute (1 in 4) sulphuric acid, and 10 c.c. (= 1 mgr. of available oxygen) of the standard potassium permanganate solution; the bottles are stoppered, shaken, and allowed to stand together for three hours. At the end of that time, the amount of permanganate still undecomposed is determined by adding to each bottle a small crystal of pure potassium iodide, when an amount of iodine will be liberated exactly equivalent to the quantity of permanganate still present in the bottle. Now from a burette*

* For accurate work, the burette should carry a float, and have been calibrated. See § 22.

the solution of sodium thiosulphate is run in until the yellow colour of the dissolved iodine is nearly, but not quite removed. A few drops of clear, freshly made starch solution added to this gives a blue colour, and on further additions of the thiosulphate, drop by drop, the exact moment when the whole of the iodine is removed is marked by the disappearance of the blue colour. Just a little practice is required to recognise the exact point; if the blue colour does not return in a quarter of an hour, too much thiosulphate has been added.

If the amount of thiosulphate solution be A c.c. with pure distilled water, B c.c. with the water under examination, A evidently expresses the amount of permanganate added to the water, B the excess of permanganate over that destroyed by the organic matter in the water, and (A − B) the amount actually consumed after three hours' contact. Since the amount of available oxygen in the 10 c.c. of permanganate added is 1 mgr., the oxygen consumed by the quantity of water taken (250 c.c.) will be:

$$\frac{(A-B)}{A} \times 1 \text{ mgr.}$$

For very bad waters, smaller quantities than 250 c.c. must be taken (*e.g.*, 70 c.c.), and for sewage effluents the permanganate solution may contain 10 mgr. of O in 10 cc.

§ 33. **Modifications of the Oxygen Process.**—The following reactions take place here:

(1) $K_2Mn_2O_8 + 3H_2SO_4 = K_2SO_4 + 2MnSO_4 + 3H_2O + 5O$ (given up to the organic matter).

When the excess of permanganate is destroyed by the addition of KI the reaction is:

(2) $K_2Mn_2O_8 + 8H_2SO_4 + 10KI = 6K_2SO_4 + 2MnSO_4 + 8H_2O + 5\ I_2$

and this iodine with the sodium thiosulphate reacts thus:

(3) $2Na_2S_2O_3 + I_2 = 2NaI + Na_2S_4O_6$.

In the modification adopted by the Society of Public Analysts, the bottles, with their contents, are raised to 80° F., and kept at that temperature during the whole course of the reaction; and instead of one test stopped after three hours, two are made, one stopped after fifteen minutes, the other after four hours. Each bottle is titrated side by side with its own special blank, containing pure water. In other respects the manipulation is precisely as above.

Experiment.—Determine the amount of oxygen absorbed by tap water and distilled water in three hours, and enter the results as in the following example:

	Tap water. 17/4/94.	Ordinary distilled water.	Pure distilled water.
Amount of water taken .	250 c.c.	250 c.c.	250 c.c.
Amount dil. H_2SO_4 added	10 c.c.	10 c.c.	10 c.c.
Amount of permanganate added (10 c.c. = 1 mgr. O).	10 c.c.	10 c.c.	10 c.c.
Sodium thiosulphate added . . .	27·83	29·95	30·17
$\left(\frac{A - B}{A}\right) \times 1$ mgr. . .	·0776 mgr.	·0073 mgr.	
Oxygen absorbed in grains per gallon . . .	·0217	·0020	

CHAPTER VI.

NITROGEN AS NITRATES AND NITRITES.

§ 34. **CHIEF METHODS USED.**—Very many methods have been proposed for estimating nitrogen existing as nitrates in potable waters.

Practically, however, they all fall into one of three groups.

(1) Methods in which the nitrate is reduced by nascent hydrogen to ammonia, and this latter estimated either alkalimetrically or by Nesslerising. Here the reaction is a perfectly definite one:

$$HNO_3 + 8H = NH_3 + 3H_2O,$$

and the great delicacy of the Nessler re-agent renders it applicable to very minute amounts of nitrates.

(2) Methods in which the nitrogen of the nitrate is converted into nitric oxide (NO), and this gas measured. Here again the reaction is well defined:

$$2HNO_3 = H_2O + 2NO + 3O \text{ (given up to some reducing agent).}$$

(3) Methods in which advantage is taken of the oxidising power of HNO_3 to oxidise some organic sub-

stance to a colouring matter, comparing the colour so produced with a standard colour solution; or to remove the colour from some colouring matter. These reactions cannot usually be expressed by equations.

As examples of the first group, soda and metallic aluminium, and the "zinc-copper couple" are both used as reducing agents in water analysis. The latter is the easier in practice, and is carried out as follows.

§ 35. **Use of the Zinc-Copper Couple.**—Take a clean, 4 oz. wide mouthed, stoppered bottle, cut a strip of zinc foil of such a height as to just come up to the shoulder of the bottle, and about 6 to 8 inches long. Corrugate this by doubling it repeatedly on itself, and then pulling it out so that when inserted into the bottle it will spring out and fill the bottle.

The zinc must now be cleaned by shaking it with the following re-agents (1) Dilute potash solution to remove grease; (2) Water to remove potash; (3) Dilute H_2SO_4 solution (if all the grease has been properly removed hydrogen gas will develop evenly all over the surface); (4) Water to remove the acid. Now fill up the bottle with a three per cent. solution of $CuSO_4.5H_2O$ (30 grams of crystallised copper sulphate in a litre of distilled water). In about three minutes the zinc foil will be uniformly coated with a black deposit of metallic copper. The copper solution is then poured off, the "couple" rinsed with water, which is finally displaced with the water under examination. The bottle is left in a warm place (25° to 30° C.) for twenty-four hours; at the end of this time, unless the amount of oxidised nitrogen is very

large indeed, the whole of the nitrates will have been reduced to ammonia.

It has been proved that in this reduction a nitrite is first produced, and that the ammonia is formed from this. Hence, as soon as the nitrites disappear it is quite certain that no nitrates are left.

An extremely minute quantity of a nitrite can be detected qualitatively; in the present instance add KI starch solution and acetic acid; if nitrites are present a blue colour will appear.

The ammonia formed is Nesslerised directly in the ordinary way, except that in making up the "standard" glass the original (unreduced) water is used instead of distilled water. This serves two purposes: (1) The amount of ammonia originally present, as such, in the water is the same in each glass, and consequently need not be corrected for; and (2) if the water is very hard a similar turbidity is produced in each glass. In the case where the amount of ammonia produced is too great to be directly Nesslerised, and accordingly has to be diluted with ammonia-free distilled water, the standard glass must be made up with the similarly diluted unreduced water.

In the case of a water originally containing nitrites, the ammonia found $\times \frac{14}{17}$ = nitrogen as nitrates and nitrites.

If the amount of N as nitrate is required separately, the only way of arriving at this is to determine the N as nitrite (§ 38) and subtract it from the figure just found. This remark applies generally to the other methods given

below. It must be remembered that whilst nitrites have plenty of distinctive reactions and can be always easily detected, nitrates cannot be qualitatively detected in the presence of nitrites, at any rate in potable waters.

§ 36. **Crum's Method.**—As an example of the second type, methods in which NO is finally obtained and measured, that due to Crum is perhaps the most convenient. It depends upon the fact that a nitrate (or a nitrite) shaken up with strong sulphuric acid and mercury gives up all its N as NO, according to the equations :

$$RNO_3 + H_2SO_4 = RHSO_4 + HNO_3 \text{ and}$$
$$2HNO_3 + 3Hg + 3H_2SO_4 = 4H_2O + 3HgSO_4 + 2NO.$$

A given quantity of the water, varying from one litre in the case of a good river supply down to 100 c.c. for a shallow surface well, is evaporated down to dryness on the water bath, and the residue carefully extracted with small quantities of boiling distilled water, decanting through a small filter, not more than 1 to 1½ inch in diameter.

The filtrate, which contains the whole of the nitrates, is received in a small lipped basin of platinum or porcelain, and again evaporated on the water bath until the whole is reduced to 2 c.c. or less. The nitrometer (Fig. 10), which is practically an inverted burette with a cup above the tap, is graduated so that the readings begin at the tap (see § 11.) The nitrometer is completely filled with mercury up to the tap and the concentrated filtrate

carefully transferred with the help of a thin glass rod to the cup; by cautiously opening the tap this is sucked into the tube. 1 c.c. of pure concentrated sulphuric acid (the purity as regards nitrous compounds must have been previously ascertained by a blank experiment) is now run round the dish, then down the glass rod into the cup, and sucked in as before. This operation is repeated three times, making in all four cubic centimetres of strong acid.

By adding the acid slowly in this way undue heating of the liquid under the tap is avoided, and the dish is thoroughly washed.

If any gas is given off at this stage (CO_2 or perhaps HCl) it is removed by raising the pressure tube and slowly opening the tap. The nitrometer is now taken out of the stand and regularly shaken for ten minutes in such a manner that the mercury is thrown up into the acid mixture, forming a kind of emulsion; at the end of this time practically all the NO is evolved; the nitrometer is replaced in the stand and allowed to remain until the froth has completely subsided and until the whole has attained the temperature of the room. A column of sulphuric acid of the same strength and height is put into the pressure tube, the levels equalised, and the temperature taken. The mode of working out results will be best shown by an actual example.

500 c.c. of water treated as above gave 2·8 c.c. of NO at 16°·7, barometer 755 mm. The volume found has finally to be expressed as so many grains per gallon, *i.e.*, as the weight. Now the weights of 1 c.c. of the various

gases have all been determined at the fixed temperature 0° C. and the fixed pressure 760 mm., hence the first step is to "reduce" this volume, 2·8 c.c., to the volume it would occupy if its temperature were 0° C. and its pressure 760 mm. of mercury. This done as in § 13, and we have

$$2{\cdot}8 \times \frac{273}{273 + 16°{\cdot}7} \times \frac{755}{760} = 2{\cdot}62 \text{ c.c.}$$

The results have to be expressed as so much nitrogen. If NO is treated with some re-agent, such as red hot copper, which will take away all its oxygen, the nitrogen gas remaining will be just half the volume originally occupied by the NO. Hence, if the volume 2·62 c.c. is halved it may be treated as though it were 1·31 c.c. of nitrogen. But 1 c.c. of nitrogen at 0° C. and 760 mm. weighs ·001256 grams, and 1·31 × ·001256 = ·00164 grams nitrogen which were contained in 500 c.c., *i.e.*, 0·235 mgr. in 70 c.c. or 0·235 grains per gallon of N as nitrate.

In practice, the whole equation would be combined in one formula thus:

$$2{\cdot}8 \times \frac{273}{(273 + 16{\cdot}7)} \times \frac{755}{760} \times \tfrac{1}{2} \times 1{\cdot}256 \text{ mgr.} \times \frac{70}{500} = {\cdot}235 \text{ grains per gallon.}$$

The accuracy of the process may be increased if necessary, either by using Frankland's gas apparatus for measuring the resulting NO, and thus measuring it to ·001 c.c. or by taking larger quantities of water for evaporation. There appears, however, to be always a slight loss of nitric acid on evaporation.

If the water contains much organic matter, the results

will not be exact unless the chlorine is removed with silver sulphate before evaporation.

§ 37. **A COLOUR METHOD OF ESTIMATING NITRATES.**—In this process two solutions are required—a standard solution of potassium nitrate, of which 1 c.c. = ·0001 gram of N, and a solution of phenol-sulphonic acid.*

A measured volume of the water is evaporated just to dryness in a platinum or porcelain dish. 1 c.c. of the phenol-sulphonic acid is added and thoroughly mixed with the residue by means of a glass rod. 1 c.c. of water is added, three drops of sulphuric acid, and the dish gently warmed over the water-bath. The liquid is then diluted with about 25 c.c. of water, ammonium hydrate added in excess, and the solution made up to 100 c.c.

The nitrate converts the phenol-sulphonic acid into a mixture of nitro-derivatives, which, by the action of the ammonium hydrate, are converted into the ammonium salts; these impart to the solution a yellow colour, the intensity of which is proportional to the amount present.

1 c.c. of the standard KNO_3 solution is now similarly evaporated in a platinum basin, treated as above, and made up to 100 c.c. This coloured solution is now used as a standard to "Nesslerise" the yellow solution obtained from the water, exactly as in the process of estimating ammonia.

The volume of the water to be taken for evaporation

* For preparation, *see* Appendix.

should be between 10 and 25 c.c., according to the amount of nitrates expected to be present.

§ 38. **Estimation of Nitrites.**—Owing to the facility with which free nitrous acid forms colouring matters of high tinctorial power with amido-derivatives of benzene and allied compounds, there are many qualitative tests for nitrites of extreme delicacy. Only one of this type need be mentioned here: a solution of any salt of meta-phenylene-diamine (m)-$C_6H_4(NH_2)_2$, in an acid solution gives a brown precipitate (Bismarck brown) with a strong solution of a nitrite, or a reddish yellow colour in solutions of that strength likely to be found in natural waters. The test is best carried out in a Nessler glass on a white porcelain slab, and time must be given for the colour to develop. A test of another type is potassium iodide, starch solution, and acetic acid. If nitrous acid is present there is an immediate blue colour. A colour developing after some ten minutes or so is no indication of a nitrite.

As the section on the estimation of ammonia shows, it is not a very difficult matter to turn such a qualitative colour-test as is given by meta-phenylene-diamine into a quantitative method for the estimation of minute quantities. The general method is precisely as in Nesslerising. There is a standard solution of a nitrite of which 1 c.c. = ·01 mgr. N as NO_2 (*see* Appendix), a solution of meta-phenylene-diamine, and one of sulphuric acid.

100 c.c. of the water are placed in a Nessler glass, and 1 c.c. each of meta-phenylene-diamine and dilute acid added. If colour is rapidly produced the water

must be diluted with distilled water free from nitrous acid, and other trials made. The dilution is sufficient when colour is plainly seen at the end of one minute. A rough shot with the standard is started at the same time, and after twenty minutes the two are compared by the second method of Nesslerising (§ 26).

It may be here pointed out that although the KI and starch test is most serviceable as a qualitative test for nitrous acid, yet, contrary to the statements made in some text-books, this cannot be developed into a quantitative method, unless elaborate precautions are taken to eliminate all dissolved oxygen, the latter exercising a disturbing influence.

CHAPTER VII.

HARDNESS AND ALKALINITY.

§ 39. **MEANING OF "HARDNESS."**—The ordinary method of estimating the "hardness" of water is by determining how many cubic centimetres of soap solution a given volume takes to form a lather. In ordinary drinking water the only two metals which need be considered as producers of hardness are calcium and magnesium. Both of these form insoluble salts with the fatty acids in soap (oleic, stearic, &c.) So long as any salt of calcium or magnesium remains in solution it will be impossible to make a lather with the water, that is, to wash with it. Magnesian waters are rare round London. Calcium (or magnesium) may occur in water as the chloride, $CaCl_2$; sulphate, $CaSO_4$; nitrate, $Ca(NO_3)_2$; and bicarbonate, $CaH_2(CO_3)_2$. It is very important to notice that it is the calcium alone (or magnesium) that settles the hardness, and that the acid with which it is combined has no effect. Thus, if 10 mgr. $CaCO_3$ are dissolved in dilute HClAq, the superfluous HCl removed by repeated evaporation on the water-bath with water, and another portion of 10 mgr.

$CaCO_3$, treated in the same way with dilute HNO_3Aq, each of these solutions will use up the same amount of soap solution. Similarly, if the chalk is suspended in water, and dissolved by a stream of CO_2, the resulting solution has the same hardness so far as the soap solution can measure it. The hardness of the tap water in the laboratory is chiefly due to calcium bicarbonate, $CaH_2(CO_3)_2$, but there are inconveniences in the use of a standard solution of this substance. It tends to lose CO_2 and deposit chalk on keeping; and as it is a primary necessity of a standard solution that it shall keep its strength indefinitely, the chloride is used for preference.

The Soap Solution.—This can be made up in two ways.

(1) By dissolving about 10 grammes of Castile soap in about a litre of weak alcohol (35 per cent.), and filtering. (Wanklyn.)

(2) By grinding up "lead plaster" (150 gr.) (a lead soap) with K_2CO_3 (40 gr.) and alcohol in a mortar, and diluting the strong soap solution thus obtained with weak alcohol.* It is made of such a strength that 1 c.c. will roughly precipitate the calcium from 1 mgr. of $CaCO_3$, dissolved in any convenient acid.

It is very important to notice that such a soap solution is not a standard solution, and ought not to be used as such. It fails to satisfy the primary necessity of a

* If methylated spirit be used it must not contain petroleum. This interferes with the formation of the lather, and is also prejudicial to the keeping qualities of the solution.

standard solution, as it alters in strength from day to day. This change (the solution grows weaker) is very capricious; sometimes it is very slight, almost nothing, for a fortnight, and then the solution suddenly decomposes. Hence it has to be checked against a standard calcium solution (that is, against a water of standard hardness) at every test; or at least once a day if continually used.

The Standard Calcium Solution is made by dissolving ·2 gr. (200 mgr.) of chalk (pure, dry, precipitated $CaCO_3$), in any suitable acid, generally HClAq, removing the excess of acid by four or five evaporations to dryness on the water-bath with small quantities of distilled water, and making up to 1000 c.c.; 50 c.c. of this solution contain 10 mgr. $CaCO_3$, or, more strictly, Ca equivalent in soap-destroying power to 10 mgr. $CaCO_3$.

§ 40. **DETAILS OF METHOD AND MODE OF CALCULATION.**—The test is made as follows:

(1) 50 c.c. of distilled water are measured into a clean stoppered bottle of about 300 c.c. capacity and the volume of soap solution it requires to form a lather permanent for two minutes, when the bottle is laid on its side, is determined.

(2) The volume of soap solution used by 50 c.c. of the standard hardness solution (containing Ca = 10 mgr. $CaCO_3$) is next determined in the same way.

(3) The experiment is now repeated on 50 c.c. of the water under examination. If, as is commonly the case, the bulk of the calcium is present as bicarbonate, CO_2 will be set free during the reaction. This must be

sucked out of the bottle from time to time with a glass tube, as it affects the production of froth.

In no case must more than 1 c.c. of soap solution be added between each shake, even where the amount to be added is approximately known; and, as the end of the reaction is approached, not more than two drops at a time ought to be run out of the burette.

As regards the recognition of the exact point when the lather becomes permanent, it is usual in most text-books to give five minutes as the time. As, however, this, if faithfully followed out, would mean spending about an hour over each careful determination, that is, three hours in all, two minutes appears to be the more practical time. Moreover, after a little practice, the time is hardly taken into account, for the feel of the bottle on shaking is a better guide to the approaching termination. As will be proved below, if two observers have quite different ideas as to what constitutes a lather, and one regularly adds, say 0·5 c.c. more soap than the other, by working as described here, they will get identical results for the hardness of the water.

Although, as stated above, the calcium may be present as $CaH_2(CO_3)_2$, $CaSO_4$, $CaCl_2$, $Ca(NO_3)_2$ etc., yet it is convenient to express the results as though all the hardness were due to chalk, $CaCO_3$, and this is always done even although magnesium may have had a share in producing the hardness of the water.

The calculation is simple, and is as follows. Remembering that 50 c.c. of tap-water may be looked upon as

50 c.c. of distilled water, together with certain salts, the three experiments give:

	No. of c.c. soap solution.
(1) 50 c.c. distilled water . . . =	a
(2) 50 c.c. distilled water + 10 mgr. $CaCO_3$ =	A
(3) 50 c.c. distilled water + x mgr.* $CaCO_3$ =	B

(Where x is the unknown number of mgr. of chalk in 50 c.c. of the water.)

Hence, from (1) and (2):

The hardness due to 10 mgr. $CaCO_3$ = (A − a) c.c. of soap, or

$$1 \text{ c.c. of soap solution} = \frac{10}{(A - a)} \text{ mgr. } CaCO_3.$$

From (1) and (3):

The hardness due to x mgr. $CaCO_3$ = (B − a) c.c. soap =

$$(B - a) \times \frac{10}{A - a} \text{ mgr. } CaCO_3 = x.$$

But 70 c.c. contains $\frac{7}{5}$ times as many mgr. as 50 c.c., hence there are:

$$(\frac{7}{5} \times \frac{B - a}{A - a} \times 10) \text{ or } \frac{14\,(B - a)}{A - a} \text{ grains of "} CaCO_3 \text{" per gallon.}$$

It should be mentioned that if the water is very hard, that is, if B is very much larger than A, the results are not good, unless the water is diluted down with distilled water. If dilution is necessary the best results are obtained when the B of the diluted water is as near A as possible.

* It should be remembered that this "x mgr. $CaCO_3$" literally means that if all the magnesium were replaced by its equivalent of calcium, and then all the calcium salts turned into the carbonate the amount would be x mgr.

The following numerical example may make this clearer:

(1) 50 c.c. distilled water took 3·1 c.c. soap solution.
(2) 50 c.c. standard $CaCO_3$ took 15·7 c.c. soap;

$$\therefore \text{ 1 c.c. of soap} = \frac{10}{12{\cdot}6} = {\cdot}794 \text{ mgr. } CaCO_3.$$

(3) 50 c.c. of tap water took 17·1 c.c. soap.

But of this the water, as such, was responsible for 3·1 c.c., hence the calcium salts, expressed conventionally as $CaCO_3$, took 14·0 c.c. soap.

$$= 14 \times {\cdot}794 \text{ mgr. or } 11{\cdot}12 \text{ mgr. } CaCO_3 \text{ in 50 c.c.;}$$

$$i.e., \frac{7}{5} \times 11{\cdot}12 = 15{\cdot}6 \text{ mgr. on 70 c.c.;}$$

i.e., total hardness = 15·6 grains $CaCO_3$ per gallon.

Using the formula above (B − a) = 14·0 (A − a) = 12·6,

$$\therefore \frac{14\,(B - a)}{A - a} = \frac{14 \times 14{\cdot}0}{12{\cdot}6} = 15{\cdot}6 \text{ as before.}$$

Now, suppose the same water is tested by some one who habitually adds 0·7 c.c. more soap to 50 c.c. of liquid before he is satisfied that a permanent lather is produced.

Here a = 3·8 (B − a) = 14·0.
A = 16·4 (A − a) = 12·6.
B = 17·8.

and the total hardness is $\frac{14 \times 14{\cdot}0}{12{\cdot}6} = 15{\cdot}6$ grains $CaCO_3$ per gallon as before.

Hence, by taking the little extra trouble to work in this way, three errors are completely eliminated.

(1) The error due to the fact that even a pure chalk-free water uses a certain amount of soap.
(2) The error due to differences in opinion as to what constitutes a permanent lather, and

(3) The error due to variation in strength of the soap solution.

Water containing calcium and magnesium bicarbonates deposits a large proportion of these on prolonged boiling, the CO_2 being expelled with the steam, and the neutral carbonates being more or less completely precipitated. If $CaSO_4$ is present, some of this may be deposited too. Hence the hardness of such waters is diminished after boiling, and this reduced hardness is technically known as the "permanent hardness"; the difference between this and the "total hardness" as determined above, being called the "temporary hardness." It is customary to determine this, although the result is of doubtful value.

100 c.c. of the water is measured out into a wide-mouthed conical flask, and boiled vigorously until reduced to about half its bulk, then cooled, and made up to the original volume with distilled water. After shaking well, 50 c.c. of this are withdrawn, and the hardness determined exactly as above described.

§ 41. **ACIDIMETRY AND ALKALIMETRY.**—If to an aqueous solution of hydrochloric acid some solution of litmus is added, the whole becomes red. If now a weak solution of an alkali is slowly added drop by drop, the acidity of the solution becomes less and less, until, when the acid is just neutralised, the red changes to a purple. A drop more alkali and the whole becomes bright blue. The litmus here, like the potassium chromate in the chlorine estimation, acts as an indicator, showing the exact point when there is excess of neither acid nor

alkali, *i.e.*, the point of neutralisation. From the equation:

$$\underset{\text{alkaline.}}{NaHO} + \underset{\text{acid.}}{HCl} = \underset{\text{neutral.}}{NaCl} + \underset{\text{neutral.}}{H_2O}.$$
$$(23 + 1 + 16) + (1 + 35{\cdot}47) = (23 + 35{\cdot}47) + (2 + 16)$$

it is clear that 40 grams of sodium hydroxide (caustic soda) are exactly neutralised by an aqueous solution of hydrochloric acid containing 36·47 grams of anhydrous HCl. The molecular weight of NaHO being 40, this taken in grams (40 grm.) is called a "gram-molecule" of NaHO. Hence, if one solution be taken containing a gram-molecule of caustic soda in the litre, and another containing a gram-molecule of hydrochloric acid in the litre, and if these two litres be mixed together the resulting solution will be exactly neutral, and also, obviously, the same will be the case if any volume of the one be mixed with an equal volume of the other.

A solution of hydrochloric acid containing a gram-molecule or 36·47 grams of HCl to the litre is called "normal" or $\frac{N}{1}$ solution. Any solution of an alkali which neutralises the above, volume for volume, is also a normal solution. Solutions of $\frac{1}{10}$ and $\frac{1}{100}$ normal strength are called decinormal, $\left(\frac{N}{10}\right)$, and centinormal, $\left(\frac{N}{100}\right)$, respectively. Any other acid which neutralises the normal solution of an alkali as above defined, volume for volume, is a normal acid. Thus for sulphuric acid, the equation

$$H_2SO_4 + 2NaHO = Na_2SO_4 + 2H_2O.$$
$$98 + (2 \times 40) = 142 + (2 \times 18)$$

shows that $\frac{98}{2}$ or 49 grams of acid to the litre will give a

normal solution, that is, one capable of neutralising, volume for volume, a solution of caustic soda containing 40 grams to the litre. Thus for an acid containing two atoms of replaceable hydrogen in the molecule, instead of taking a gram-molecule per litre to form a normal solution, a $\frac{\text{gram-molecule}}{2}$ is taken. This is also the case for a base containing two replaceable hydroxyl (OH) groups like $Ba(OH)_2$. Thus the molecular weight of barium hydroxide being 170·8, $\frac{170{\cdot}8}{2}$ or 65·4 grams $Ba(OH)_2$ to the litre gives a normal solution of baryta.

None of the acids or alkalies hitherto mentioned (H_2SO_4, HCl, NaHO, $Ba(OH)_2$) can be obtained commercially in a sufficiently pure state to be definitely weighed out; they all contain an unknown quantity of water. From what has been said above, it is clear that if only one normal solution can be prepared, any other normal alkali or acid solution can be prepared from it.

Normal sulphuric acid is a convenient starting-point. This is prepared by diluting about 60 c.c. of pure concentrated sulphuric acid with five or six times its volume of water, allowing the mixture to cool and making it up to two litres. Such a solution will now contain rather more than 49 grams of sulphuric acid to the litre. To determine its exact strength, a platinum basin is carefully dried and weighed, as in § 23; and to it about 2 gr. of pure Na_2CO_3 is added, it is then gently heated over a rose burner, cooled in a desiccator and weighed quickly.

The heating, &c., is repeated until the weight is constant. The salt is then dissolved in a small quantity of water, and covered with a large watch-glass, then drawing

the cover slightly aside, 25 c.c. of the acid are added from a pipette.* The dish and contents are placed on the water-bath, and as soon as the evolution of gas has ceased, the cover is removed, and its under surface rinsed with a fine jet from a wash-bottle into the dish, and then the liquid evaporated to complete dryness. The residue is dried to constant weight in the air-bath, exactly like the water residue (§ 23), except that the oven is kept at 180° instead of at 120°. The residue must contain an excess of Na_2CO_3.

Here the sulphuric acid has turned the CO_2 out of the Na_2CO_3, and 106 grams of Na_2CO_3 giving 142 grams of Na_2SO_4, 98 grams (one molecule weight) of sulphuric acid will cause an increase of weight of $(142-106)=36$ grams. Hence, 1 grm. increase of weight represents $\frac{98}{36}$ or 2·722 grams H_2SO_4.

Thus, in the following example, a roughly normal acid was prepared as above described.

Dish P_2 = 35·5562 grms.
Dish $P_2 + Na_2CO_3$.
After 1st heating 37·8731 grms.,
„ 2nd „ 37·8665 „
„ 3rd „ 37.8664 „
Hence 2·3102 grams Na_2CO_3 taken.

Then, 25 c.c. of the acid were added, evaporated to complete dryness, and heated to constant weight at 180° C. and

Dish + Na_2CO_3 + Na_2SO_4 = (1) 38·3310 grms.,
(2) 38·3279 „
(3) 38·3224 „
(4) 38·3224 „
Hence gain in weight = ·456 grams.

* This should be a calibrated one if accuracy is required.

since 1 grm. increase in weight corresponds to 2·722 grms. H_2SO_4, the amount of sulphuric acid present in the 25 c.c. is $2{\cdot}722 \times {\cdot}456 = 1{\cdot}241$ grms. and in 1 c.c. $= \frac{1{\cdot}241}{25}$ or ·04965 gr. But a normal solution contains ·049 grams, hence this is $\frac{{\cdot}04965}{{\cdot}049}$ or 1·013 times the strength of a true normal solution, or, as it is usually written $\frac{N}{1} \times 1{\cdot}013$.

Two courses are now open, either every 1000 c.c. can be diluted to 1013, when obviously a normal solution is obtained, or, as is sometimes more convenient in accurate working, the acid is simply labelled $\frac{N}{1} \times 1{\cdot}013$ H_2SO_4, and if at any time, say 10 c.c. is measured out, this is entered as $10{\cdot}13$ $\frac{N}{1}$ H_2SO_4 (*i.e.*, $10 \times 1{\cdot}013$).

Having now a $\frac{N}{1}$ acid, a $\frac{N}{1}$ NaOH solution is made to correspond with it by first making one slightly over strength, finding its exact strength by the $\frac{N}{1}$ H_2SO_4 and litmus, diluting down to be exactly $\frac{N}{1}$, and finally checking against the acid.

Experiments.—(1) Find the exact strength of the approximately normal H_2SO_4 given to you, expressing the result as:

(*a*) The number of grams of H_2SO_4 per c.c.

(*b*) As $\frac{N}{1}$ solution × a factor.

(*c*) The number of cubic centimetres of water which must be added to a litre to make it exactly normal ($\frac{N}{1} \times 1{\cdot}000$).

(2) Find the percentage of Na_2CO_3 in the pure, but not specially dried carbonate in the laboratory, and compare your results with percentage of water found in the same sample of carbonate in the first part of (1).

§ 42. **HARDNESS BY ALKALIMETRY.** — In this method, instead of directly measuring the soap-destroying power of the calcium and magnesium salts, advantage is taken of the fact that a fixed amount of calcium carbonate requires a fixed amount of acid to neutralise it; the carbonates of calcium and magnesium, in fact, are measured by their alkalinity. The process for carrying out this is practically that worked out by Mr. O. Hehner.

Two standard solutions are required.

(1) $\frac{N}{50}$ H_2SO_4, made by diluting 20 c.c. $\frac{N}{1}$ H_2SO_4 to a litre with distilled water.

(2) $\frac{N}{50}$ Na_2CO_3, made by dissolving 1·06 gr. pure, freshly ignited Na_2CO_3 in a litre of distilled water.

1 c.c. of $\frac{N}{50}$ acid = * 1 mgr. $CaCO_3$, whilst 1 c.c. of $\frac{N}{50}$ Na_2CO_3 solution precipitates a like amount of $CaCO_3$ from any soluble lime salt and an equivalent amount of $MgCO_3$. Equal volumes of the two solutions neutralise each other, and this should be proved by experiment.

Two equal conical flasks are taken. Into each is measured 70 c.c. of the water to be tested, and two drops of methyl-orange† solution are added. The two will of course have exactly the same yellow tint. To one of them the standard acid is now carefully added until the colour changes; by thus having a comparison flask the slightest change of colour is readily detected. With

* This is purely accidental, the molecular weight of $CaCO_3$ being 100.

† Several other "indicators" are in common use besides litmus, such as phenacetoline, cochineal, phenol-phthaleïn, &c. Methyl-orange is used here because it is unaffected by CO_2.

excess of acid, the methyl-orange becomes a bright pink, but this point must not be reached here, a slight darkening to orange being sufficient to show that the right point has been reached. Each cubic centimetre indicates one grain per gallon of temporary hardness, reckoned as $CaCO_3$.

To the other 70 c.c. of the water a measured quantity of the sodium carbonate solution is added, more than enough to decompose the whole of the soluble salts of lime and magnesia. Generally an amount in c.c. equal to about the number of grains of total solids per gallon is sufficient.

The mixed solutions are then evaporated in a platinum* dish to dryness. The residue is taken up in a little recently boiled distilled water, the solution then filtered through a very small filter (as in the preliminary to Crum's method of estimating nitrates, § 36,) and the residue washed three or four times with small quantities of hot distilled water. The alkalinity of the clear solution is titrated by means of the standard acid. The alkali added, minus the acid used, indicates the permanent hardness calculated as $CaCO_3$.

§ 43. **Reactions.**—The reaction in the first direct titration is :

$$\underset{\text{(neutral to methyl-orange.)}}{CaH_2(CO_3)_2 + H_2SO_4} = \underset{\text{(neutral.)}}{CaSO_4} + \underset{\text{(neutral to methyl-orange.)}}{H_2O + 2CO_2}.$$

Hence so long as any carbonate is left unattacked, the methyl-orange is unaffected. Directly all the carbonates

* Glass or porcelain cannot be used, but a nickel dish does very well if a platinum one be not at hand.

are turned into sulphates, the next drop of H_2SO_4 turns the solution orange-pink.

In the second titration, the reactions during the evaporation are these. First, the calcium bicarbonate is decomposed, thus :

$$CaH_2(CO_3)_2 = CaCO_3 + H_2O + CO_2,$$

and as the chalk thus produced is insoluble in distilled water, the whole of the temporary hardness is removed by the evaporation without using up any of the Na_2CO_3. If other soluble salts of calcium and magnesium are present, such as $CaSO_4$, $Ca(NO_3)_2$, $MgCl_2$,&c., they react with the Na_2CO_3 thus :

$$CaSO_4 + Na_2CO_3 = Na_2SO_4 + CaCO_3.$$
neutral, alkaline, neutral, insoluble;

and hence on extracting with hot water there will be a loss of alkalinity, and the amount of Na_2CO_3 used up, this is directly measured by the acid, and is directly proportional to the amount of the soluble salts of lime and magnesia existing in the water, that is, of the permanent hardness.

Experiment.—Determine by this method the temporary and permanent hardness of any three waters, and compare the results with the figures given by the soap test.

Enter up as in the following example.

Temporary Hardness :

$$70 \text{ c.c.} = 12{\cdot}7 \text{ c.c.} \frac{N}{50} H_2SO_4.$$

70 c.c. = 12·7 grains $CaCO_3$ per gallon.

Permanent Hardness:

70 c.c. + 25 c.c. $\frac{N}{50}$ Na_2CO_3 evaporated to dryness in platinum, and extracted with hot distilled water.

$$Na_2CO_3 \text{ left} = 18{\cdot}6 \text{ c.c. } \frac{N}{50} \text{ acid,}$$

$$\text{Hence } 25{\cdot}0 - 18{\cdot}6 = 6{\cdot}4 \text{ c.c } \frac{N}{50} Na_2CO_3 \text{ used up,}$$

or, permanent hardness = 6·4 grains $CaCO_3$ per gallon.

Total hardnesss = 12·7 + 6.4 = 19·1 grains per gallon.

CHAPTER VIII.

COMPOSITION OF THE WATER RESIDUE.

§ 44. **GENERAL DESCRIPTION.** — Water which has been uncontaminated by manufacturing operations, and which is not in a metallurgical district, will give a residue consisting chiefly of calcium, magnesium, and sodium, as chlorides, carbonates, nitrates, or sulphates.

In the neighbourhood of mines many poisonous metals may be found in the water. Thus, in some streams in the neighbourhood of copper mines there may be sufficient copper in solution to give a visible deposit on a knife-blade. A complete qualitative analysis of such waters can only be made by evaporating a large quantity(some 20 to 30 litres) to dryness and examining the solid residue according to the methods given in text-books of qualitative analysis, and the scheme must include the search for many of the rare metals, since rubidium, cæsium, strontium, &c., are generally present in very minute traces. Such a treatment, however, is quite outside the range of this book, and it will be sufficient to look for lead, iron, zinc, copper, calcium, magnesium, sodium; and the acid radicles of

carbonic, sulphuric, hydrochloric, nitric, nitrous, phosphoric, and silicic acids.

The presence of one of the heavy metals is roughly indicated by taking 70 c.c. of the water in a porcelain dish, and stirring it with a glass rod previously dipped in sulphide of ammonium; if lead, copper, or iron be present, a blackish coloration of the water arises; if on the addition of hydrochloric acid the black coloration disappears, iron is indicated, and, if not, copper or lead; a concentrated portion of a water containing lead would give a yellow precipitate, with bichromate of potassium, whilst a similar portion of a water containing copper would give a claret-coloured precipitate with ferrocyanide of potassium.

§ 45. **Lead** is liable to be taken up from pipes and cisterns, and, being a cumulative poison, is not permissible in any quantity in a drinking water.*

For a qualitative test, two clean Nessler glasses are taken, and quantities of 50 c.c. of the water are placed in each. Both are acidified with dilute HClAq, and to one of them 5 c.c. of a saturated aqueous solution of H_2S is added. If, after well stirring, there is no appreciable difference between the two glasses, the water is for all practical purposes free from lead (and copper). If, however, lead is present, owing to the formation of the black lead sulphide the glass to which the H_2S water has been added will assume a dark tint, easily detected by comparison with the other glass. In a rough examination this is sufficient to condemn the water.

* Some authorities allow up to 0·1 grain per gallon.

If, however, some idea of the amount is required, a standard solution of lead, containing 0·1 mgr. lead per c.c. is taken, and the colour matched exactly as in Nesslerising. The accurate estimation of small quantities of lead (or other metals) does not fall within the scope of this book, since, if the student has sufficient manipulative skill to carry them out, he must necessarily be familiar with the larger works on the subject.

A standard solution is made by dissolving 0·1 grm. lead in a little HNO_3Aq, evaporating to dryness, dissolving in distilled water and making up to a litre.

Or it may be made by dissolving 1·66 gram of crystallised acetate of lead in one litre of distilled water. This solution contains one milligram of lead in one c.c.

§ 46. **Iron**, unless present in sufficient quantity to give a perceptibly inky taste to the water, is not objectionable. It may be tested for in precisely the same way as lead, using as the test the blue colour developed by potassium ferrocyanide, after 1 c.c. of concentrated HNO_3 has been added to the water. The rough quantitative estimation runs on precisely similar lines.

A standard solution of iron is made by dissolving 4·96 grams of crystallised protosulphate of iron in one litre of water. Such a solution will contain one milligram of iron in every c.c.

§ 47. **Copper.**—As in the qualitative test for lead, two clean Nessler glasses are taken, and 50 c.c. of the water are placed in each. Both are acidulated with

HClAq, and to one, a few drops of a saturated solution of H_2S is added. Stir well, and if copper is present, there will be noted a perceptible darkening of the water. To distinguish this darkening from that due to lead, take another Nessler glass and add two or three drops of potassium ferrocyanide, when, if copper be present, a claret colour will be developed. The quantitative estimation is on the same lines as that of lead.

A standard solution of copper is made by dissolving 3·93 grams of crystallised sulphate of copper in one litre of water, when one milligram of copper will be present in every c.c.

§ 48. **Zinc and Silica.**—Zinc is liable to be present from galvanised cisterns. At least a litre of the water is acidified with HClAq, and evaporated to dryness in a platinum dish, the residue moistened with HClAq, is filtered through a small ash-free filter, and the insoluble residue, which is SiO_2, well washed with small quantities of hot water.

The filter and its contents, burnt in a weighed platinum dish, gives the amount of **silica** present. The filtrate is warmed in a large platinum dish with a little NH_4Cl and NH_4HO solutions, filtered if necessary, and then some H_2S water added. If the liquid after standing two days remains clear, zinc is absent. A flocculent white precipitate denotes zinc as sulphide. To prove this, the liquid is decanted away, the precipitate dissolved in dilute HClAq, and ammonia and ammonium carbonate solution added in excess, the white precipitate filtered off, washed down into the point of the filter, and

dried in the air oven at 120°. If this is now scraped up on a platinum spatula or a piece of platinum foil and ignited, a residue is obtained, which is yellow when hot, and turns white on cooling. This is a good confirmatory test for zinc.

§ 49. **Calcium and Magnesium.**—The sum of the calcium and magnesium, reckoned as $CaCO_3$ has already been obtained by Hehner's method of taking hardness. Calcium is precipitated as calcium oxalate by ammonium oxalate, and advantage is taken of this fact to separate it from magnesium. The following method, although by no means the best available, is sufficiently good for most purposes of water analysis.

The total amount of Ca and Mg reckoned as $CaCO_3$ is determined as in § 42. Now 250 c.c. of the water is shaken with a few crystals of neutral ammonium oxalate and allowed to stand overnight. The temporary and permanent hardness, redetermined by § 42, gives the amount of magnesium.

Magnesium may be tested for qualitatively by adding a slight excess of ammonium oxalate solution to about 500 c.c. of water, and allowing to stand for 24 hours, then decanting the clear liquid, free from calcium, through a filter. On now concentrating this to about 50 c.c., and then adding to the solution ammonium chloride, ammonia, and sodium phosphate respectively, a white crystalline precipitate of $(NH_4)MgPO_4$ will be deposited; this occurs at once if there is much magnesium present, or after twenty-four hours if there are only traces.

§ 50. **Sodium and Acid Radicles.**—Sodium Salts are

nearly always present in water. About 500 c.c. evaporated to dryness, and extracted with about 2 c.c. of distilled water, will generally give a vivid sodium flame if a drop on a clean platinum wire is introduced into a Bunsen flame. Repeating the experiment on another drop, looking at the flame through a stout piece of blue glass, a transient crimson flash is noticed if potassium is present. To make sure of the crimson flash, it should be compared with the colour given by a potassium salt; the end of a cigarette gives a very good potassium flame.

Chlorine (Cl) has been already dealt with (§ 25).

Sulphuric Acid (SO_4). Acidify the water with HCl, and add $BaCl_2$. An immediate turbidity denotes sulphates. For smaller quantities, standing for a day will be necessary.

Phosphoric Acid (PO_4). The ordinary test is a nitric acid solution of ammonium molybdate, but its use requires care. Two test-tubes are taken, and each is one-fifth filled with ammonium molybdate solution. To one of them is added some of the water to be tested, concentrated if necessary, and both tubes are gently warmed together to a temperature not exceeding 90° C., well below the boiling point. The one containing a phosphate will have a yellow precipitate, or with very small quantities simply a yellow colour, which becomes obvious on comparing with the companion tube.

Carbonate (CO_3). Carbonic acid exists in two forms in natural waters—(1) Free; and (2) Combined.

If a water is vigorously boiled and the escaping gases

passed into clear lime water, the latter will become turbid owing to the escaping CO_2. The liquid remaining behind, which in the case of water containing bicarbonate of calcium will also be turbid from precipitated chalk, after a time ceases to give off CO_2. If now the liquid is acidified with dilute HClAq, a fresh supply of CO_2 will be obtained on further boiling, and this should be generally proved before entering carbonic acid as present, since all distilled water, and most natural waters, will contain a certain amount of dissolved CO_2.

Nitrite and Nitrate.—These have been already dealt with in §§ 34–38. It should be noted, however, that whereas there are plenty of qualitative tests for nitrites which are equally available whether nitrates are present or not, yet there is no reliable qualitative test for small quantities of a nitrate, unless nitrites are absent.

CHAPTER IX.

THE GASES IN WATER.

§ 51. **GENERAL DESCRIPTION.** — Since water will dissolve with greater or less ease any gases with which it comes in contact, a great variety of gases may be found in natural waters : *e.g.*, CO_2, O_2, N_2, SO_2, H_2S, CH_4, etc.

Practically an ordinary potable water on boiling gives off three gases only, oxygen, nitrogen, and carbon dioxide ; and it is for the most part to the last gas that the pleasant taste and sparkling appearance are due. Since, however, the water from a highly polluted well and that from a deep chalk well may both contain plenty of CO_2, from quite different causes, the chief interest lies in the oxygen and nitrogen. Pure water shaken with air, owing to the much greater solubility of oxygen, always dissolves about 35 volumes of oxygen to 65 volumes of nitrogen. If, however, the water is polluted with sewage, the oxidation which converts the ammonia compounds into nitrites and nitrates, goes on at the expense of the dissolved oxygen. Hence, in an unpolluted water, the gases boiled out, freed from CO_2 give oxygen to nitrogen as 1 to 2 or thereabouts. In a

polluted water, on the other hand, where oxidation is actively going on, the ratio may be much lower. Thus, Miller found for Thames water the ratio of oxygen to nitrogen at Kingston to be 1 to 2, at Hammersmith 1 to 3·7, at Somerset House 1 to 10·5, at Greenwich 1 to 60, at Woolwich 1 to 52, and at Erith 1 to 8·1, showing clearly the effect of London sewage. Several processes have been devised for determining the amount of dissolved oxygen in water, of which the best are Roscoe and Lunt's modification of Schützenberger's process, and Thresh's process.

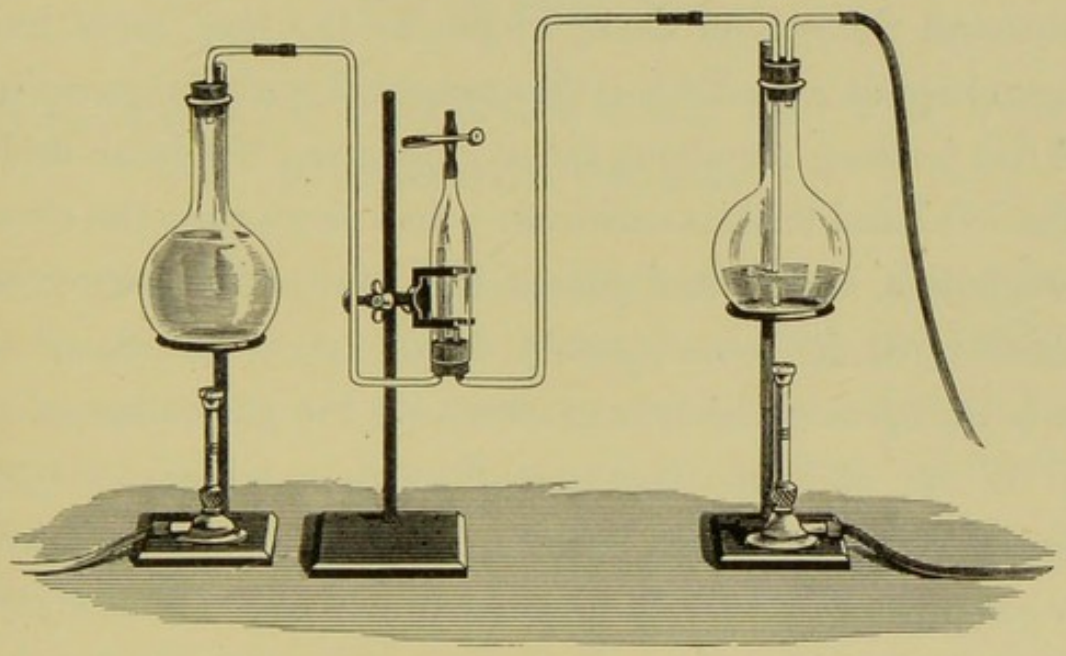
In those methods where the gases are actually boiled out and measured, the most rapid is that of Dibdin, who uses a modified mercury pump. The modification of Reichardt's apparatus, shown below, due to Tiemann and Preusse has the advantage that it can be readily set up in any laboratory.

§ 52. **Reichardt's Process for Estimating the Gases Dissolved in Water.**—Two large flasks, each holding about a litre, are connected by tubes to the gas-collector in the middle. All the stoppers are of good soft rubber: the whole arrangement will be clear from the diagram (Fig. 21).

The flask with the doubly bored stopper is filled rather more than half full of boiled distilled water, and the other flask and stopper removed; then, by blowing into the long rubber tube, water is driven over into the gas-collector and the adjoining tubes until the air is wholly displaced. The rubber tubes on the top of the gas collector and that from which the other flask was removed are closed by pinchcocks. The flask that was

removed is now filled to the brim with distilled water, the stopper inserted, the excess of water filling the short glass tube, and the flask again connected up, the pinchcock being opened. The water in the half filled flask is now heated to gentle boiling, and that in the full flask allowed to boil somewhat more rapidly. The absorbed air is thus driven out and any dissolved gases collect in the upper

FIG. 21.



part of the gas-collector, from which they are removed by occasionally opening the pinchcock and blowing into the long rubber tube.

When, upon cooling the apparatus, the gases which have collected disappear, the left hand flask is removed (its lamp having been previously removed, and pinch-cock closed) and emptied. Everything is now ready for a determination.

The cooled flask, whose capacity has been previously determined, is filled with the water to be examined, and

the stopper pressed in so far that the air in the short glass tube is completely driven out. This tube is now slipped into its rubber connecting tube, care being taken that in so doing no air bubbles are enclosed. The pinchcock being opened, and the water gently heated to boiling point, the dissolved gases come over with the steam and remain in the upper half of the gas-collector, care being taken that the latter is never more than half emptied of its water; otherwise there is danger of loss through the second flask, which, by the way, must have been kept at a gentle boil throughout the whole operation. After heating for about twenty minutes, the flame under the left hand flask is removed. In a short time the steam condenses, and water passes back into the boiling-out flask until it is completely filled. Any bubble of air remaining must be driven over into the gas-collector.

While still hot the gas-collector is joined up by a capillary tube to a gas burette, precisely as though it were a gas pipette (see § 73), the gases drawn over and analysed, after cooling, by Hempel pipettes, as in § 73.

The CO_2 is first absorbed in the potash pipette, and then the O_2 by phosphorus; the residual gas is called nitrogen in the results. It may in certain cases contain argon and helium.

PART III.

ANALYSIS OF AIR.

CHAPTER I.

QUALITATIVE EXAMINATION OF AIR FOR POISONOUS GASES.

§ 53. **FEATURES OF THE PROBLEM.** — Since in the neighbourhood of chemical works, smelting works, etc., traces of poisonous gases are liable to be present in air, a qualitative examination of air for such gases has been included in the examinations for a Diploma in Public Health. To enable the candidate to find out the impurity, it is usual to give him some air in a gas jar containing a considerable proportion of the gas in question, and the whole of the present chapter has been arranged to meet this special case. In actual practice, methods similar in character to that adopted for the determination of sulphur in air (see chap. iii. part 3) would have to be adopted.

The following is a short résumé of the chief reactions of such common gases as may be considered to come within the scope of an examination in hygiene.

§ 54. **CHLORINE,** Cl_2.—This is a greenish-yellow gas,

having an irritating and characteristic smell. Moistened blue litmus paper is first reddened and then bleached by this gas. It dissolves in cold water; the solution liberates iodine from potassium iodide, which is shown in the presence of starch by the blue colour of iodide of starch, and precipitates AgCl as a curdy white precipitate, from a nitric acid solution of $AgNO_3$. The iodine reaction is demonstrated by holding in the gas a piece of starch paper moistened with potassium iodide solution.

§ 55. **HYDROCHLORIC ACID**, HCl.—This is a colourless gas, possessing a pungent, acid smell, and which fumes in moist air. Blue litmus paper is immediately reddened by it; but it does not affect potassium iodide and starch paper. Hydrochloric acid gas is very soluble in water, giving a strongly acid solution, from which $AgNO_3$ precipitates white AgCl.

§ 56. **NITRIC ACID**, HNO_3. — This is a liquid at ordinary temperature, but air shaken with the warm liquid acid and transferred to a clean dry jar gives the following reactions; it has a strong acid smell, reddens blue litmus, and is soluble in water; a paper moistened with potassium iodide and starch is unaffected if the acid is free from the lower oxides. An aqueous solution evaporated down gives with brucine a red colour.

§ 57. **NITROUS FUMES**, N_2O_3, NO_2.—This is the red gas obtained by admitting air (that is oxygen) to the colourless nitric oxide, NO, or by the action of nitric acid on organic substances such as sugar. It has an acid smell, reddens litmus paper, liberates iodine from potassium iodide and hence gives a blue colour with KI and

starch paper. The reaction with brucine is similar to that obtained with nitric acid.

§ 58. **SULPHUR DIOXIDE**, SO_2 (Sulphurous acid).—This is the gas given off when sulphur is burned in air; and is a product of many smelting operations. It is a colourless gas with a well known smell, difficult, however, to recognise when greatly diluted with air. It reddens litmus; a drop of $K_2Cr_2O_7$ solution (the "red chromate of potash") on filter paper immersed in the gas goes green owing to the reduction of the dichromate to chromium sulphate. This reaction is uncertain when the quantity of SO_2 is small; but the aqueous solution warmed with a little bromine water, and nitric acid added, and then $BaCl_2$ never fails to give a white precipitate of $BaSO_4$.

§ 59. **AMMONIA**, NH_3.—This is a colourless gas with a pungent, characteristic smell, it turns red litmus paper blue, is very soluble in water, and an aqueous solution gives the usual reaction with Nessler.

§ 60. **HYDROGEN SULPHIDE**, H_2S (sulphuretted hydrogen).—This is a colourless gas possessing the well known smell of rotten eggs. It turns blue litmus paper a very pale red, so pale as to be hardly noticeable. Lead paper (filter paper moistened with a solution of lead acetate) is turned black, owing to the formation of black lead sulphide. The gas is soluble in cold water.

The vapour of ammonium sulphydrate, NH_4HS, consists of a mixture of NH_3 and H_2S, and gives the reactions of both gases.

§ 61. **CARBON DISULPHIDE**, CS_2.—This is a liquid at

ordinary temperatures. The vapour has a characteristic smell, somewhat resembling that of hydrogen sulphide, and is without action upon either litmus or lead paper. If a light is applied to a mixture of the vapour and air, a flame runs down the jar, and the residue gives abundant evidence of SO_2.

§ 62. **COAL GAS.**—This is a complicated mixture of gases (H_2, CH_4, C_2H_4, C_3H_6, CO, O_2, CO_2, N_2, C_6H_6, and sometimes higher paraffins).

For the present purpose it is sufficiently characterised by its smell, neutrality to test papers, by its burning, and by the CO_2 thereby formed.

§ 63. **CARBON DIOXIDE,** CO_2 (carbonic acid).—This is a heavy colourless and odourless gas; without action on test papers (except that if a good portion is present blue litmus is turned faintly red), it gives a turbidity (chalk) with clear lime water.

Carbon monoxide and ozone will be mentioned later.

It will be noticed that all these gases have a more or less distinctive smell with the exception of CO and CO_2.

The following table of the chief reactions of the above gases will be found useful.

Name.	Formula.	Blue Litmus.	Red Litmus.	$K_2Cr_2O_7$.	Lead Acetate.	KI and Starch.	Confirmatory Test.
Chlorine	Cl_2	Red, bleached	Bleached	—	—	Blue	$AgNO_3$
Hydrochloric acid . .	HCl	Red	—	—	—	—	$AgNO_3$
Nitric acid	HNO_3	Red	—	—	—	—	Brucine
Nitrous fumes . . .	NO, N_2O_3, NO_2	Red	—	—	—	Blue	Brucine
Sulphur dioxide . .	SO_2	Red	—	Green	—	—	Br_2 and $BaCl_2$
Ammonia	NH_3	—	Blue	—	—	—	Nessler
Hydrogen sulphide . .	H_2S	? Red	—	—	Black	—	Smell
Ammonium sulphydrate .	NH_4HS	—	Blue	—	Black	—	Smell
Carbon bisulphide . .	CS_2	—	—	—	—	—	Ignite & test residue
Coal gas	—	—	—	—	—	—	Smell and ignite
Carbon dioxide . . .	CO_2	? Red	—	—	—	—	Lime water
Carbon monoxide . .	CO	—	—	—	—	—	Blood reaction

§ 64. **SCHEME FOR IDENTIFYING ONE GAS.**— From this table it is easy to construct the following scheme, working on the assumption that only one gas is present (ammonium sulphydrate excepted).

(1) The colour, if any, is noted.

(2) Smell. On removing stopper, the gas jar is immediately covered with the palm of the hand, and the stopper smelt; then, on replacing the stopper, the palm is smelt.

(3) After moistening with neutral distilled water the following test-papers, red litmus, blue litmus, potassium iodide and starch, potassium dichromate and lead acetate (the two last being extemporised from filter paper) are introduced one by one into the jar, just lifting the stopper far enough to allow of their insertion. The papers are not allowed to fall down to the bottom of the jar, as that would interfere with subsequent tests but are held up by the stopper.

Too much reliance must not be placed on the smell, as this alone would not be acccepted as sufficient evidence by an examiner. The following detailed scheme will perhaps be found the most useful.

Either chlorine, hydrogen chloride, hydrogen nitrate, "nitrous fumes," sulphur dioxide, ammonia, hydrogen sulphide, carbon bisulphide, coal gas, carbon dioxide, or carbon monoxide may be present.

I. Blue Litmus Reddened.	II. Red Litmus turned Blue.	III. Neutral to Litmus.
CL_2	NH_3	CS_2
HCl	—	Coal gas
HNO_3	—	(CO_2)
N_2O_3, &c.	—	CO
SO_2	—	(H_2S)
(H_2S)	—	—
(CO_2)	—	—

Further Separation of Group I.

(H_2S and CO_2 will be considered in Group III.)

Either Cl_2, HCl, HNO_3, N_2O_3, or SO_2 may be present.

I.a. Blue Colour with KI and Starch.	I.b. Turns $K_2Cr_2O_7$ Green.	I.c. Affects neither KI nor $K_2Cr_2O_7$.
Cl_2	SO_2	HCl
N_2O_3, &c.	—	HNO_3

Both sub-groups **I.a, and I.b,** are dealt with in the same manner, namely, shaken with half a test tube full of distilled water, this divided into two parts, one evaporated down with brucine solution and the other treated with silver nitrate. Further confirmatory tests, if necessary, have already been given under the respective gases.

The confirmatory test for sub-group **I.b,** is bromine water, dilute nitric acid, and barium chloride, and this test should be applied even if the chromate test fails. The reactions are

$$SO_2 + 2\,H_2O + Br_2 = H_2SO_4 + 2\,HBr. \text{ and}$$
$$H_2SO_4 + Ba\,Cl_2 = Ba\,SO_4 + 2\,HCl.$$

Group II.

NH_3.

Since ammonia is the only common gas with an alkaline reaction, this together with its smell are conclusive. The Nessler test gives confirmation, but will fail if H_2S is also present (vapour of ammonium sulphide), owing to the latter forming black mercuric sulphide. But in this case the smell and alkaline reaction are sufficient.

Group III.

CS_2, Coal gas, CO_2, CO, H_2S.

III.a. Blackens Lead Paper.	III.b. Have no Smell.	III.c. Burn on applying a light.
H_2S	CO	CS_2
—	CO_2	Coal gas

III.a. H_2S requires no comment.

III.b. CO, on account of its extremely poisonous nature, can scarcely be given in a gas-jar at an examination.

§ 65. **DETECTION OF CARBON MONOXIDE.**—Small quantities of carbon monoxide are always detected by means of blood. H. W. Vogel was the first to use the well known spectrum of blood impregnated with carbon monoxide as a means of detecting small quantities of the gas. This reaction is especially valuable, because the carbon monoxide can hardly be confounded with any other gas; a fact which, on account of its highly poisonous character, is of great importance in analyses undertaken from a sanitary standpoint.

To detect carbon monoxide, Vogel directs that a 100 c.c. bottle, filled with water, be emptied in the room containing the gas, and that 2 to 3 c.m. of a highly diluted aqueous solution of blood be poured into the bottle and shaken for a few minutes. The blood solution must be of such a strength as to show only a very faint red colour, yet still giving the well known absorption bands in a column as thick as a test-tube. When carbon monoxide is present, the blood at once takes on a rose colour, and upon the addition of a few drops of strong ammonium sulphide the absorption bands do not disappear. In blood free from carbon monoxide in the reaction with ammonium sulphide the absorption bands are replaced by a broad and weakly defined band. A small pocket direct-vision spectroscope, one of the kind sold as a "rain-band spectroscope," does very well for this work. Vogel states that amounts down to 0·25 per cent. can be clearly detected.

Still smaller amounts can be detected by the use of live animals. A mouse, allowed to breathe for some time the air of a room supposed to contain a trace of carbon monoxide, and killed by immersing the trap in water, yields a considerable quantity of blood by cutting it in two in the region of the heart. Using proper precautions this test will shew down to 0·03 per cent. of carbon monoxide. Further particulars will be found in Hempel's Gas Analysis. It has been found that 0·05 per cent. and upwards of carbon monoxide gives decided symptoms of poisoning in the case of a mouse, thus showing the extremely poisonous action of this gas.

Carbon monoxide, then, being practically inadmissible for the purposes of examinations, a gas that is without smell and giving no reaction with the various test-papers, may be at once shaken with lime water for CO_2. No reaction here indicates pure air.

III.c. contains CS_2 and Coal Gas.

Here the smell is a sufficient guide. For confirmation after igniting the mixture, CS_2 gives a mixture of CO_2 and SO_2, of which the latter only may be easily proved. (It should be remembered that lime water gives a white precipitate with both CO_2 and SO_2.)

Coal gas after ignition gives only CO_2 and water.

§ **66. Ozone**, O_3, is an allotropic form of oxygen containing three atoms of oxygen in the molecule, ordinary oxygen containing but two. This extra atom is very readily yielded up to bodies capable of oxidation; hence ozone acts as a powerful oxidiser and disinfectant.

In quantity it is poisonous; but the presence of a minute trace in air is regarded as desirable, because ozone and certain putrescible matters cannot exist together. Thus ozone is seldom found in crowded cities. Red litmus paper, upon which potassium iodide has been dried, turns blue with as little as ·0003 mgr. of ozone. Clean, dry mercury, shaken up with air containing a trace of ozone, undergoes an extraordinary change, becoming black and dirty and clinging to the bottle. The quantitative estimation of ozone by noting the depth of shade attained by potassium iodide and starch papers has been attempted, but the results are not very definite.

CHAPTER II.

REVIEW OF METHODS OF ESTIMATING CARBON DIOXIDE.

§ 67. **REVIEW OF CHIEF METHODS.**—The methods used for making this determination will be described in considerable detail, because of its great importance, in connection with the many questions of practical hygiene, such as the ventilation of buildings, factories, etc. The processes in use may be divided into absolute and empirical methods; the former standing by themselves, self-contained, the latter requiring to be standardised against an "absolute" method.

Four typical methods are described, due respectively to Pettenkofer, Hesse, Lunge and Zeckendorf, Pettersson and Palmqvist; and it may be as well to specify the conditions under which the use of each becomes suitable. Where accuracy is the primary object, and time and trouble secondary, then Pettenkofer's method, with large bottles, would be used. Hesse's modification gives for a slight sacrifice in accuracy a considerable gain in speed and convenience. For rough work, at the minimum of cost, the Lunge and Zeckendorf process works admirably,

and without previous technical training. Lastly, if an absolute method be preferred, and if a very large number of air samples have to be examined in a limited time, and only moderate accuracy is required, Pettersson and Palmqvist's apparatus leaves nothing to be desired. Some practice and manipulative skill, however, are required before satisfactory results can be obtained by this last process.

§ 68. **PETTENKOFER'S METHOD.**—This method of determining the volume of carbon dioxide in air depends upon finding the amount of calcium or barium hydrate which is precipitated as carbonate from solution, when lime or baryta water in excess is well shaken with a known volume of the air.

This is effected by using a known volume of the baryta water, the value of which is determined by titration against a standard oxalic acid solution.* After exposure to a measured volume of the air, the baryta water is again titrated with the acid. The difference between the two results corresponds to the amount of carbon dioxide present.

The following apparatus will be required: (1) A standard acid of such strength that 1 c.c. of it is equivalent to 1 c.c. of CO_2 at 0° and 760 mm. (2) Baryta water, not necessarily standardised, but approximately equal in strength to the acid. (3) A large bottle, holding from three to six litres, with a hollow stopper (preferably of exactly 50 c.c. capacity). (4) A bellows

* Unless the student has worked through the exercises on acidimetry (§ 41), this chapter should be read over again.

(either ordinary or suction bellows) to fill the bottle with the air of the room. (5) A bottle for the baryta water, so arranged that the 60 c.c. pipette can be filled without access of CO_2. (6) A 60 c.c. pipette. (7) A 50 c.c. burette, divided into tenths, fitted with a float. This holds the standard acid, and for anything save the roughest work, must have been calibrated. (8) Small conical flasks for titration, phenol-phthaleïn as an indicator; an aneroid barometer, and a thermometer.

Theory of the Method.—The alkalinity of the baryta water is measured by the number of cubic centimetres (say A) of the standard acid required to neutralise a given volume of it. If this same volume of baryta water is shaken with air containing CO_2, the latter is removed with formation of $BaCO_3$. This baryta, so far as the acid (oxalic) is concerned, is lost, since $BaCO_3$ is not attacked by a weak solution of oxalic acid, and the solution remaining will require less acid (say B c.c.) to neutralise it, and the difference (A–B) c.c. represents the quantity of CO_2 present in the bottle.

Now the strength of the acid solution is obtained as follows. With oxalic acid the reaction is:

$$\underset{\text{(In solution)}}{H_2C_2O_4} + \underset{\text{(In solution)}}{Ba(OH)_2} = \underset{\text{(Neutral insoluble pp.)}}{BaC_2O_4} + 2H_2O.$$

With the carbonic acid in the air the reaction is similar:

$$\underset{\text{(In air)}}{CO_2} + \underset{\text{(In solution)}}{Ba(OH)_2} = \underset{\text{(Neutral insoluble pp.)}}{BaCO_3} + H_2O.$$

Hence, so far as the power of neutralising baryta

is concerned, one gram-molecule of $H_2C_2O_4$, and one gram-molecule of CO_2 are exactly equal, that is, both neutralise the same weight of baryta. But

$H_2 = 2 \times 1 = 2$		$C = 12.$
$C_2 = 2 \times 12 = 24$	and	$O_2 = 32.$
$O_4 = 4 \times 16 = 64$		—
—		44
90		

Hence 90 grams of dry oxalic acid will have the same neutralising power as 44 grams of carbonic acid. But since the ordinary crystallised oxalic acid is not anhydrous $H_2C_2O_4$, but is $H_2C_2O_4 + 2H_2O$, it follows that 90 + 36 or 126 grams of the crystals will be equivalent to 44 grams of CO_2.

It will obviously simplify the subsequent calculations if the acid solution is made of such a strength that 1 c.c. of it corresponds to 1 c.c. of gaseous CO_2. To do this, since 1 c.c. CO_2 at 0° and 760 weighs ·001971 grams, the strength of the oxalic acid solution must be such that 1 c.c. of it is equivalent to ·001971 grams CO_2.

Now

126 grams of crystallised oxalic acid = 44 grams CO_2.

Hence $\frac{126}{44}$ „ „ „ = 1 gram CO_2.

or, $\frac{126}{44} \times$ ·001971 „ „ = ·001971 grams CO_2.

Hence 1 c.c. of oxalic acid solution must contain $\frac{126}{44} \times$ ·001971 = ·005645 grams of crystallised oxalic acid, or 5·645 grams to the litre of recently boiled and cooled distilled water gives the desired solution.

The Process.—The actual determination consists of three parts:

(1) The collection of the gas sample, and the addition of 60 c.c. of clear baryta water.

(2) The titration of the baryta solution.

(3) The titration of the residual baryta.

The most accurate way to titrate the baryta is to make a preliminary trial without any special precautions to exclude extraneous carbonic acid; suppose, for example, 60 c.c. baryta require 58·0 c.c. of acid, to just decolorise the phenol-phthaleïn.

This will probably be a little too low, as during the time necessarily spent over the titration, carbonic acid will be absorbed from the air, especially from the breath. Now, into a clean flask nearly as much acid is run out as in the first trial, say 55·0 c.c.; 60 c.c. of bartya is added to this, and the titration carefully completed. Since the bulk of the baryta is neutralised before it comes into contact with the air at all, the time necessary to secure accuracy may be spent over the completion without any appreciable absorption of carbonic acid taking place, the result being say 58·50 c.c. of acid.

Now, the capacity of the flask is taken (say 6080 c.c. including the stopper). By means of the bellows the clean, dry bottle is filled with the air of the room, 60 c.c. clear baryta water quickly added, the bottle stoppered, well shaken, and laid on its side. The absorption will be complete in about an hour if the bottle is given an occasional shake, and during this time the two titrations of the baryta water ought to be carried out. For the

titration of the residual baryta after the carbonic acid has been completely absorbed, about 40 c.c. (supposing that the proportion of CO_2 is not expected to much exceed the normal) of the standard acid is carefully measured out into a clean titration flask, and the large bottle inverted so that the stopper is filled. Then, having a small ground glass plate ready to hand, the stopper is withdrawn in a vertical position over a basin; the excess of liquid (about 10 c.c.) runs away, and the top of the baryta solution in the stopper quickly sheared off with the glass plate, taking care that no air bubble is enclosed. The 50 c.c. to be titrated being now completely protected from the air, the outside of the stopper can be wiped dry with a clean cloth, then the stopperfull quickly and neatly poured into the titration flask containing the 40 c.c. standard oxalic acid and phenol-phthaleïn, and the remaining baryta rapidly neutralised by the oxalic acid from the burette.*

Suppose that the amount of acid used up by 50 c.c. of baryta after shaking with air is 45·25 c.c. It has been necessary to take an aliquot portion ($\frac{5}{6}$) of the baryta water, because of the impossibility of obtaining the whole 60 c.c.; any attempt to wash out the bottle with distilled water would introduce large errors owing to the CO_2 in the breath.

* The exact volume of the stopper is found by preliminary experiments with water, conducted in precisely the same manner. It will not usually be exactly 50·00 c.c., but the correction for the difference is easily introduced.

Since	50 c.c.	Baryta	= 45·25 c.c. acid.
Then	60 c.c.	„	= 54·30 c.c. „
But originally	60 c.c.	„	= 58·50 c.c. „

Hence the amount of carbonic acid which has combined with the baryta is equivalent to 58·50 − 54·30 = 4.20 c.c. oxalic acid, and this, from the strength of the acid is equal to 4·2 c.c. of CO_2 at 0° and 760°. The rest of the work is purely arithmetical. Results are usually expressed in parts of carbonic acid per thousand of air, or the number of cubic centimeters in the litre.

The first step is obviously to see what volume 4·2 c.c. of CO_2, measured at 0° and 700 mm. will assume at the temperature and pressure of the room at the time the air sample was enclosed. Take these as 17° C. and 780 mm. [This is always a small correction, and for rough work is usually neglected.]

As in § 13:

$$\frac{p_0 v_0}{T_0} = \frac{p_1 v_1}{T_1} \text{ and, therefore,}$$

$$\frac{760 \times 4{\cdot}2}{273} = \frac{780 \times v_1}{290}, \text{ hence}$$

$$v_1 = \frac{290}{273} \times \frac{760}{780} \times 4{\cdot}2 = 4{\cdot}35 \text{ c.c.}$$

Now, what was the volume of air which contained this 4·35 c.c.? The volume of the empty bottle, with stopper, was 6080 c.c. But as the 60 c.c. of baryta water ran out of the pipette, 60 c.c. of air were displaced, and this escaped at once without parting with its carbonic acid. Hence the volume of air acted upon is (6080 − 60) = 6020 c.c., and 4·35 c.c. in 6020 is $\frac{4{\cdot}35}{6{\cdot}02}$ in 1000, that is, ·72 parts per thousand.

§ 69. **HESSE'S MODIFICATION.***—The method above described with comparatively little practice gives concordant and reliable results. If, however, speed is an object, and if, moreover, the air to be tested is at a distance from the laboratory, Hesse's modification of the Pettenkofer process is very rapid and convenient.

The necessary apparatus may be divided into a stationary and a portable portion.

The apparatus for the laboratory comprises the following:

(1) A large bottle holding several litres, and filled with concentrated baryta water. One kilo. baryta, and 50 grms. barium chloride are put into from 4 to 5 kilograms of distilled water. As the solution is used it is replaced by water as long as there is material in excess to saturate the water.

(2) A bottle containing dilute baryta water, and fitted with a guard tube containing soda lime, for freeing the entering air from carbon dioxide. This dilute baryta water is made by adding about 30 c.c. of the concentrated solution to a litre of well boiled distilled water.

(3) The standard solution of oxalic acid, as in the original process, 1 c.c. = 1 c.c. CO_2.

(4) A solution of 1 part of phenol-phthaleïn in 250 parts of alcohol.

The portable apparatus comprises:

(1) Five thick-walled conical Erlenmeyer flasks of $\frac{1}{2}$, $\frac{1}{4}$,

* The following description of Hesse's method is due to Clemens Winkler, "Anleitung zur Untersuchung," &c.; see also Hempel's "Methods of Gas Analysis," English Edition, p. 258.

$\frac{1}{8}$, $\frac{1}{12}$, $\frac{1}{16}$ litre capacity, and supplied with well fitting doubly-bored rubber stoppers. The point to which the rubber stopper reaches is marked on the first four flasks, and their capacity up to this mark written on each flask with a diamond. The openings of these four flasks are closed with pieces of glass rod from 3 to 5 c.m. long. These rods are well rounded at the lower ends, the upper ends being widened like a button.

(2) A thick walled 10 c.c. pipette.

(3) A burette with glass tap, holding from 10 to 15 c.c., graduated in tenths, and having a tip 7 to 10 c.m. long.

(4) A 300 c.c. bottle, provided with a soda-lime guard tube, and filled with dilute baryta water. This is filled in the laboratory by connecting it with the large reserve bottle containing dilute baryta water and driving the solution over through the syphon. A few drops of phenol-phthaleïn solution are added to the bottle when filled.

(5) A 250 c.c. bottle filled with dilute oxalic acid. This is prepared by bringing 25 c.c. of the standardised oxalic acid into the 250 c.c. flask, and then filling up to the mark with recently boiled and cooled distilled water.

(6) A thermometer.

(7) A small aneroid barometer.

The amounts of solutions here given for the portable apparatus are sufficient for thirty separate determinations; in other words, at least ten analyses, including a control determination each time, and the standardising of the solution, can be made with the above quantities.

Each determination of carbon dioxide by Hesse's method is a double one, the two determinations being made with volumes of air of different size. Accordingly, flasks of $\frac{1}{2}$ and $\frac{1}{4}$, or $\frac{1}{4}$ and $\frac{1}{8}$, or $\frac{1}{4}$ and $\frac{1}{12}$ litre capacity are used for taking the samples of air, the sizes of the flasks chosen depending upon whether a smaller or a larger amount of carbon dioxide in the air is to be expected. The samples are taken by completely filling the flasks at the place where the air is to be examined with water which has the temperature of the place, then emptying the flasks, and rinsing them with distilled water free from carbonic acid. Or as an alternative the air is sucked out from the clean dry flask with a rubber suction pump, care being taken to carry on the pumping long enough to ensure complete removal of the air originally present. In either case, care must be taken that the flask is not warmed by the hand, and that no air exhaled by the operator enters the flask.

To absorb the carbon dioxide, the 10 c.c. pipette is put through one of the openings of a stopper fitting the flask; its end is inserted in the rubber tube of the supply flask, and the pipette is rinsed with a little baryta solution drawn up into it. The pipette is now filled, adjusted to the mark, and the stopper through which it passes is inserted in the neck of the flask containing the sample of air.

The baryta is now run into the flask, the second opening of the stopper being obstructed with the finger or a glass rod to such an extent that the displaced air can just escape. The glass rod is then pushed into

place, and the pipette is freed from the few drops of solution adhering to it by closing it at the top and warming it with the hand. The pipette is then drawn out of the stopper, and the second opening is closed with a glass rod. The same proceeding is repeated with a second flask of different capacity. The two flasks are allowed to stand for some time with occasional shaking, and in the meantime the strength of the baryta water is determined. The strength of the baryta water is determined by putting into the small flask of $\frac{1}{16}$ litre capacity nearly as much standardised oxalic acid solution (weak) as will be required in the titration, and then running in 10 c.c. of the baryta solution exactly as into the other flasks. The titration is then finished off as before. The baryta water which has been shaken with the air is titrated without previously removing the $BaCO_3$. The titration is made as follows:

Removing the glass rod from one of the openings in the stopper after adding a few drops of phenol-phthaleïn, the tip of the burette (already filled with dilute oxalic acid solution) is immediately inserted, the tip of the burette reaching as far as possible into the flask. By opening the burette tap the acid is allowed to enter rapidly at first, but at the last only drop by drop. If the increased pressure inside the flask checks the flow of liquid from the burette, this pressure is removed by lifting the glass stopper for a moment. When the solution is neutral, that is, when it is completely decolorised, the amount of acid run out is noted, and the contents of the second flask titrated in the same manner.

It is clear that when the amount of carbon dioxide present is small, the accuracy of the determination is increased by using larger volumes of air. For this reason Hesse uses a flask of $\frac{3}{4}$ or 1 litre capacity whenever the carbon dioxide is probably below the limit for dwelling rooms, as, for example, in the open air. He also uses these sizes when great accuracy is required. Of course, sufficient baryta solution must be taken to ensure its being present up to the end of the operation, the colour of the phenol-phthaleïn must not be discharged by the carbon dioxide.

The calculations are precisely like those already given; tables to facilitate the temperature and pressure corrections have been compiled by Hesse.

The application of this method to the examination of ground air is obvious. Further details and examples will be found in Hempel's "Gas Analysis" (translated by Dennis) from which the above description by Winkler of Hesse's method has been taken.

§ 70. **LUNGE AND ZECKENDORF'S METHOD.**—In the methods just described a fixed quantity of air is taken, and the amount of re-agent used by it determined. In minimetric methods, on the contrary, the amount of re-agent is fixed, and the quantity of air required to effect a given change is determined. Minimetric methods are all empirical, that is, the results obtained have to be interpreted by some standard process. Hence, to secure reliable results it is necessary:

(1) That the comparison with the standard process

(usually Pettenkofer's) shall be accurately carried out under well-specified conditions.

(2) That in the subsequent use of the minimetric apparatus the conditions shall be strictly parallel.

The best of the minimetric processes recently introduced is that due to Lunge and Zeckendorf. If to a solution of sodium carbonate (Na_2CO_3) phenol-phthaleïn is added, a brilliant scarlet colour is obtained. A very dilute solution of this when shaken with air containing carbon dioxide is decolorised.

A strong solution is made by dissolving 5·3 grm. pure dry sodium carbonate in about 250 c.c. of distilled water; to this is added 1 grm. phenol-phthaleïn, the whole gently warmed till the latter has completely dissolved, then allowed to cool and made up to a litre. This $\frac{N}{10}$ Na_2CO_3 will keep in a well-closed bottle for some time.

The set of apparatus* sold for this process comprises (1) An india-rubber pump, holding 70 c.c., and fitted with two rubber valves. (2) A stout glass bottle, also of 70 c.c. capacity, with a rubber cork, doubly bored, and fitted with two glass tubes wash-bottle fashion. (3) A stoppered bottle with a mark at 100 c.c. (4) A 2 c.c. pipette. (5) A 10 c.c. pipette.

To make the weak solution 2 c.c. of the decinormal solution are measured out into the 100 c.c. bottle, and the latter filled up to the mark with recently boiled and cooled distilled water; it is obviously necessary to use water free from carbon dioxide for this purpose.

* The whole set, without standard solution, and neatly put together in a case, is sold at 10*s.* 6*d.*

This $\frac{N}{500}$ solution must be made up afresh for each set of experiments, and cannot be relied upon if it has been made up for more than a day.

If the average quantity of carbonic acid in the air of a room is required, the air must be well mixed with a large sheet of cardboard or an open umbrella; if the air at a particular point is to be taken, the end of the rubber tube into which the air is drawn is placed in it, the other end joined air-tight to the longer glass tube, and the ball worked several times till both ball and bottle may be judged to be full of the air. The cork is now removed and 10 c.c. of the $\frac{N}{500}$ solution introduced (the liquid must not be blown out), the stopper replaced, and the bottle shaken rapidly for about a minute. The ball is now firmly squeezed in such a manner that it is emptied as far as possible, and bubbles gently through the pink liquid. The bottle is shaken as before, and the whole repeated until the colour is discharged. The number of times the bottle is shaken should be systematically counted, preferably by making a mark on paper immediately at the conclusion of each shake; a reference to the following table, a copy of which is supplied with each instrument, gives the percentage of carbon dioxide present. It will be found that there is an uncertainty of two or three shakes in the case of very pure air, but as this only means about ·04 parts per thousand (·004 per cent.), and, as moreover, the instrument is only intended and used for the air of rooms, this constitutes no practical difficulty. The table of course only represents the results of an experimental comparison

with Pettenkofer's method made by the inventors of the process.

Number of Pressures of India Rubber Ball.	Parts per 1000 of Carbonic Acid in the Air.	Number of Pressures of India Rubber Ball.	Parts per 1000 of Carbonic Acid in the Air.
2	3·0	16	0·71
3	2·5	17	0·69
4	2·1	18	0·66
5	1·8	19	0·64
6	1·55	20	0·62
7	1·35	22	0.58
8	1·15	24	0 54
9	1·0	26	0.51
10	0·9	28	0·49
11	0·87	30	0·48
12	0·83	35	0·42
13	0·8	40	0·38
14	0·77	48	0·30
15	0·74		

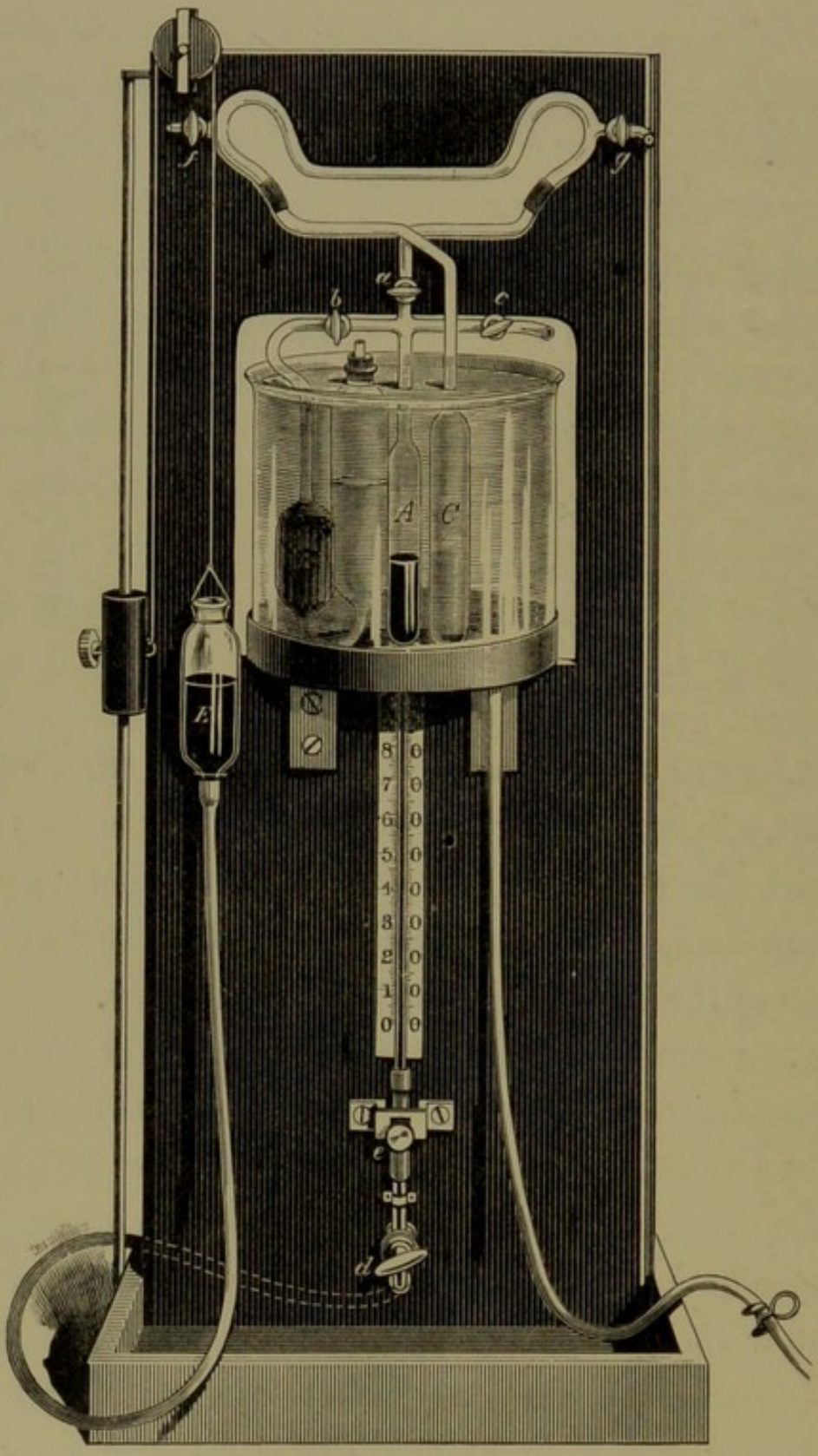
§ 71. **PETTERSON AND PALMQVIST'S METHOD.**—In this, a special apparatus is used, by the employment of which the effects of any changes of temperature and pressure during the experiment are eliminated and the volume of the carbon dioxide read off directly.

Fig. 22 shows the apparatus. The sample of air may be collected in small glass tubes having a glass tap at each end by sucking with a small india-rubber pump like that described in the last section, the air is then transferred at the side tube of the apparatus over dry mercury.* Or the whole may be taken to the place where the air is to be examined, the glass jacket filled with water, the outside air drawn in at the side tube, and by a few

* If this is done special arrangements have to be made to introduce the air into the apparatus at atmospheric pressure.

simple manipulations, which only take a few minutes,

FIG. 22.



the carbon dioxide is determined with an accuracy of about 0·01 per cent.

The description will be given in the author's own words.

"The carbon dioxide is absorbed in the Orsat potash-tube, B, and the air is measured before and after the absorption, in the pipette A and its graduated tube. The measuring pipette is filled with mercury or air, or emptied of the same, by raising or lowering the mercury reservoir E, which is joined to the lower end of the graduated tube of A by means of a wired rubber tube. There must always be a drop of water on the surface of the mercury; the air standing over the mercury is thus kept saturated with moisture. In reading the volumes, the meniscus of the mercury is each time so adjusted that the pressure in A is exactly the same as the pressure of the air in the compensation tube C.

"A differential manometer containing a drop of coloured liquid (petroleum, in which azo-benzol is dissolved), and connected by capillary glass tubes on the one side with A and on the other with C, serves as the indicator in these operations. By moving the reservoir E, and then, having closed the tap *d*, suitably turning the screw *e*, the level of the mercury in A is so adjusted that the drop of liquid in the manometer stands at zero. It is obvious that in this manner it is always possible to bring back the air in A to the same pressure as that prevailing in the compensator C. Since the air in both the compensator and pipette is, from the beginning of the experiment, separated from the external atmosphere by closing the stopcocks *f*, *g*, &c., any variations in the external atmosphere have no effect. This is also true of

changes of temperature; these eliminate themselves by acting in the same manner and to the same extent upon the pressure of the air in A and C, provided that the water in the outer vessel which surrounds the main parts of the apparatus is sufficiently stirred. For these reasons no observation of temperature or barometric pressure is necessary. The changes in volume read off on the scale give directly the amount of carbon dioxide in hundredths of per cent. by volume.

"Each analysis consists of three operations.

(1) "The air is drawn in from the outside (or from the tube as above-mentioned, with proper adjustment of pressure), the level of the mercury in the graduated tube being brought to the zero mark.

"In measuring the volume the taps *f*, *g*, *b*, *c*, and *d* must be closed.

(2) "The taps *d* and *b* are opened, *a* is closed, and the air is driven over into the potash pipette. After one or two minutes the carbon dioxide is absorbed and the air may be brought back into the measuring tube, tap *b* closed, and *a* is opened.

(3) "The mercury level is so adjusted that the index again takes its normal position. The decrease of volume is then read off on the scale."

Although this apparatus demands practice and a fair amount of skill in its manipulation, since it requires absolutely no calculations and gives very rapid results, it is invaluable when a very large number of carbonic acid determinations have to be made consecutively.

CHAPTER III.*

EXAMINATION OF AIR OF TOWNS.

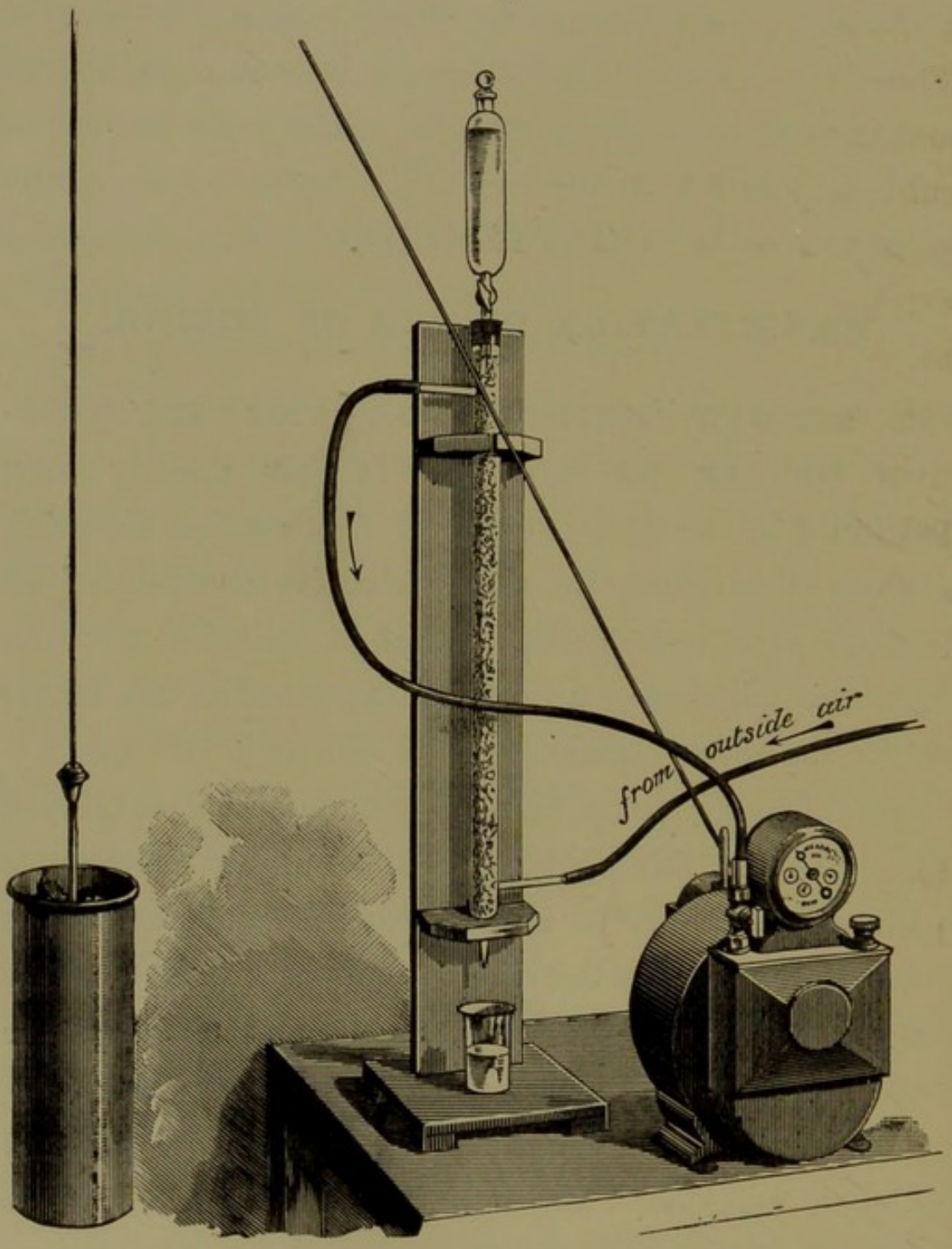
§ 72. **SPECIAL METHOD FOR THE EXAMINATION OF AIR OF TOWNS.**—It has already been mentioned in the first chapter of this section that the methods there given for the qualitative analysis of air containing noxious gases, although suitable for the artificial conditions of a practical examination are valueless for the actual examination of the air of towns.

The following description of the apparatus employed (Fig. 23) in determining the amount of sulphur compounds in the air will serve to show how the difficult problem of measuring air contamination can be attacked. The principle of the method is this: a large known volume of the air is drawn up through a column of glass beads, the latter being moistened with a weak solution of hydrogen peroxide. The sulphur compounds are thus

* This short chapter has been inserted to explain the use of the meter-aspirator and fittings in the State Medicine Class Room of King's College, London, and to draw attention to the important work done by the Air Analysis Committee of the Manchester Field Naturalists' and Archæologists' Society, the chief results of which are published in the Reports of this Society for 1891 and 1892.

oxidised to sulphuric acid, which remains in the hydrogen peroxide solution. This solution, after being concen-

FIG. 23.



trated by boiling down over a spirit lamp, is precipitated by barium chloride, and the barium sulphate ignited and weighed in the usual way.

Since the amount of sulphur in the air is extremely

small, large volumes of air have to be drawn through the apparatus, and this is most conveniently done by a meter-aspirator. This is an ordinary wet meter to which a clock-work attachment is fitted, driven by a weight. This draws the air through the column of glass beads and then measures it. In fine clear weather about 100 cubic feet should be drawn through the tower; in fog 50 cubic feet will be sufficient. The following figures extracted from a paper by Dr. G. H. Bailey will serve to show the relatively enormous increase in sulphur compounds in the air during fog.

Sulphur compounds—expressed in volumes of SO_3 per million volumes of air.

At the Owens College, Manchester.

In clear breezy weather	0·1 to 0·5
Dull hazy weather in the winter months .	2 to 5
Slight fog	2 to 10
Dense fog	10 to 20

In a very dense fog, then, the sulphur compounds may be as much as two hundred times the amount found in bright breezy weather.

CHAPTER IV.

METHODS OF GAS ANALYSIS.

§ 73. **THE SIMPLER METHODS OF GAS ANALYSIS.**—The nitrometer, already described in connection with the verification of Boyle's law (§ 11, Fig. 10), and used also in the estimation of nitrogen by Crum's method, can be very readily applied to quantitative estimation of the constituents of a gas mixture.

In gas analysis, as ordinarily carried out, a measured quantity of the gas under examination is brought into intimate contact with substances, solids or liquids, which exercise a selective absorption upon its constituents. Take, for example, the gas expired from the lungs. A measured quantity of this is brought first into contact with caustic potash solution, the volume again measured and the reduction noted; then, into contact with an alkaline solution of pyrogallol, or with water and sticks of phosphorus, and the residual gas again measured. Strictly speaking, the results of the analysis ought to be entered as gas absorbed by caustic potash, by phosphorus, and as residual gas left unabsorbed by these two re-agents. It is usual, however, to pre-suppose a knowledge of the

qualitative composition of the gas, and enter the results as carbon dioxide, oxygen, and nitrogen* respectively.

The amount of gas absorbed may be measured in various ways. Thus, it may always be brought back to the same volume by reducing the pressure, and the quantity of gas abstracted calculated from the reduction of pressure. This is the method adopted by Frankland in analysing the gases obtained from the combustion of the water residue. It is particularly suitable when very small volumes are being dealt with, but necessitates rather cumbersome apparatus.

Or, again, the gas, after treatment with an absorbent, may be always brought back to the same pressure and the reduction of volume read off. This is the method used with the nitrometer.

In the case where only one constituent of a gas has to be determined, the absorbent may be introduced through the cup into the nitrometer itself. Take, as an example, the analysis of expired air. The nitrometer is first completely filled with clean mercury, the levelling tube lowered, the tap† opened, and gas drawn in (from a gasholder) until over 50 c.c. have been taken. The tap is now shut, the rubber tubing nipped with the left hand,

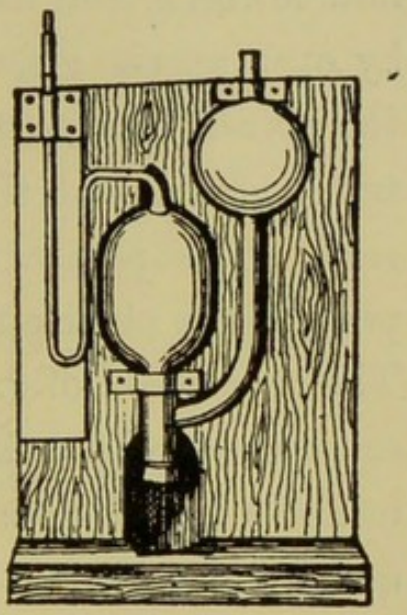
* The amount of "nitrogen" given in gas analysis by no means necessarily represents the amount of that gas actually present, for it includes, in the first place, all gases that are not soluble in the usual re-agents (argon, for instance); traces of gases left unabsorbed by these re-agents, and, in fact, includes the algebraic sum of all the errors of the analysis.

† The student should take out, wash, and regrease the tap of the nitrometer, both before and after use. He must make himself thoroughly familiar with the construction of this three-way tap, before attempting the above.

and the levelling tube raised with the right and fixed in its clip. By cautiously loosening the grip on the tube, mercury is allowed to pass in, and the contained gas slowly compressed until the meniscus exactly coincides with the lowest graduation mark, 50 c.c. If this mark should be accidently overstepped, the levelling tube must be lowered again, the tube nipped, and the whole operation repeated. Having now exactly 50 c.c. of gas confined by a pressure slightly greater than that of the atmosphere, the tap is opened to the air for a second with the right hand, and then closed again, the left hand firmly nipping the rubber tube the while. This allows the excess of gas in the tube to escape, and now exactly 50 c.c. has been confined at the atmospheric pressure for the time being. This mode of filling must always be adopted when sufficient gas is available. About 2 to 3 c.c. of a 30 to 50 per cent. potash solution is now poured into the cup, the levelling tube lowered so as to reduce the pressure of the contained gas *slightly* below that of the atmosphere (about 1 c.m.), and the tap just sufficiently opened to admit the potash in a very slow stream, a little being left in the cup, for obvious reasons. The mercury is then swirled up into the gas, to facilitate the absorption, care being taken not to handle the upper part of the tube; the whole is re-allowed to stand for five minutes, then levelled and read off. Suppose the volume reads after treatment with potash 48·1. Then 1·9 c.c. of carbon dioxide, or 3·8 per cent., is present. After use, the nitrometer must at once be well washed, every trace of potash removed, and the tap cleaned and regreased. If

several constituents have to be determined, it is more convenient to carry on the absorption in separate vessels, called gas-pipettes, the construction of which will be obvious from Fig. 24. For the determinations here described, two of these Hempel's pipettes * of the form shown will be required; one containing 50 per cent. of caustic potash solution, and packed with fine wire iron gauze to give greater absorbing surface, and the other filled with fine sticks of phosphorus and water. Taking, for example, the same gas mixture as before, the nitrometer is filled in the manner described above.

FIG. 24.



The pipette is furnished with a piece of stout rubber tubing (fine bore), of sufficient length to connect with the nitrometer, and a pinchcock is provided. The liquid in the pipette is first driven up to a mark made about an inch below the rubber tube, and kept there by closing the pinchcock; the nitrometer is now joined, the pinchcock and the glass tap opened, and the whole of the gas driven over into the pipette by gradually raising the levelling tube. (It will be noticed that a small amount of air is enclosed between the two taps when the apparatus is connected up in this way. This error falls on the "nitrogen," but is so small that

* This form of pipette is due to W. Hempel (*see* Hempel's "Gas Analysis," pp. 33, 34.)

it can be neglected by a student unaccustomed to handle gas apparatus.) In the case of carbon dioxide, absorption is complete in less than a minute. The levelling tube is now lowered, and the gas allowed to slowly stream back by opening the glass tap, great care being taken to bring the potash back to the same mark on the tube. The tap is now closed, the mercury levelled, and the volume read. The potash pipette is now removed, and the phosphorus pipette fixed on in an exactly similar manner. The absorption of oxygen is not so rapid as that of the carbon dioxide. At a temperature of 20° C., three minutes will be usually sufficient; at 14° C., more than a quarter of an hour or longer is required. Hence these operations must all be carried out in rooms at 18° to 20° C.; and, to make quite sure that all the oxygen is absorbed, the gas must be passed back again in the pipette and left there three minutes, then drawn back and remeasured. Since the whole analysis is finished in about half an hour, during which time no appreciable change in the height of the barometer can take place, it follows that the whole analysis is conducted at a constant pressure, that of the atmosphere for the time being.

If due care is taken in handling the measuring tube, the temperature also may be regarded as constant. Should there be a greater rise than 1° C. during the analysis, however, due correction must be made (see § 12). In accurate work, the temperature of the measuring tube is kept constant by a water jacket. The results should be entered as in the following example:

Air expired from Lungs (last portion).

Volume taken for analysis	50·0 c.c.
Volume after potash pipette (1) . . .	47·9
„ „ „ (2) . . .	47·9
Volume after phosphorus pipette (1) . .	39·6
„ „ „ „ (2) . .	39·55

Hence 50 c.c. is made up of :

Carbon dioxide . . .	2·1 .	4·2 per cent.
Oxygen	8·35 .	16·7
Nitrogen (by difference) .	39·55 .	79·1
		100·0

Temperature at commencement, 18·4; end, 19

Exercises.—Make analyses with the nitrometer and Hempel's pipettes of the following gas mixtures :

(1) Air.

(2) Expired air.

(3) Air boiled out of water (§ 52).

(4) Air from a sewer.

PART IV.

ANALYSIS OF FOODS.

CHAPTER I.

ANALYSIS OF MILK.

§ 74. **MILK.**—In the present chapter no attempt will be made to give an exhaustive account of milk analysis. Although there is a very large literature bearing on this subject, there is no text-book containing a full description of recent methods; the back volumes of the *Analyst*, however, give an account, either original or in abstract, of all that is required.

A complete milk analysis comprises the estimation of the percentage of water, the fat, milk, sugar, caseine, and the various mineral constituents composing the ash. The ash, milk-sugar, and caseine are usually grouped together as the "solids not fat."

Only the following determinations will be dealt with here:

1. The total solids.
2. The fat.
3. The specific gravity.
4. The ash, and the chlorine in the ash.

§ 75. (1) **Determination of the Total Solids.**—For this purpose a flat-bottomed porcelain (or platinum *) dish is taken, about 5 cm. diameter; in a dish of this size five grams of milk just covers the bottom of the dish in a thin layer.

The dish having been dried to constant weight (see § 23) at 100° C. in the water oven, about 5 c.c. of the recently shaken milk is measured out into it, and the dish reweighed as quickly as possible. The increase of weight gives the quantity of milk taken. The dish is now placed over a water-bath, and left there until the visible water of the milk has evaporated and the residue appears quite dry; this usually occurs after about two hours. The dish is now removed to the water-oven and dried to constant weight, which, for porcelain dishes, may take from four to six hours. The determination is practically identical with the determination of the solid residue in potable water (§ 23), except that the drying is not taken above 100° C., and that the milk is weighed instead of measured. The following example may make this clear:

Dish "7" + milk	= 23·987
Dish "7" .	= 18·956
Milk taken	= 5·031

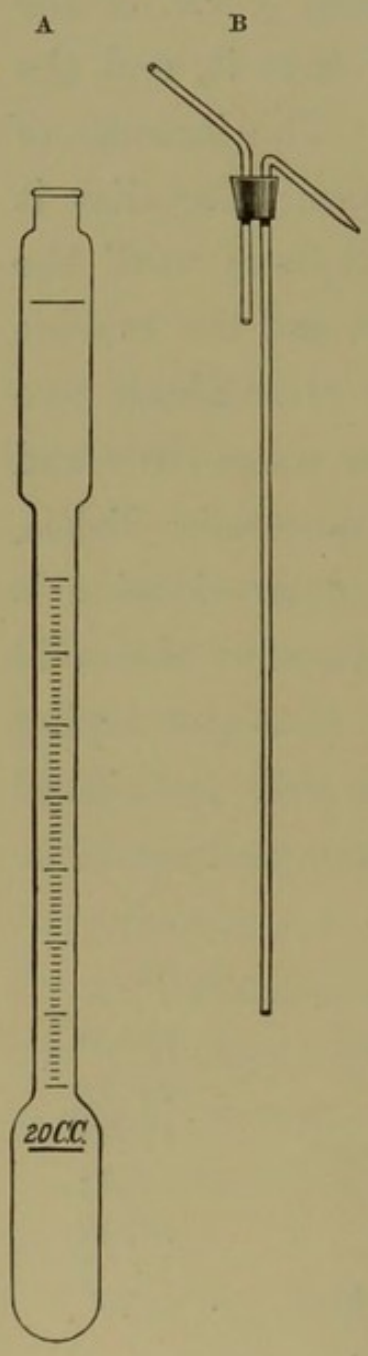
Dish + Residue.	
1st weighing . .	19·600
2nd „ . .	19·587
3rd „ . .	19·583
4th „ . .	19·583
Dish "7" . .	18·956
Residue .	·527

= 10·48 per cent. total solids.

* It is easier to dry the solid residue to constant weight in a platinum dish. The drying is more complete and takes less time. The determination of the ash is also conveniently carried out on the same portion of milk.

§ 76. (2) **Determination of the Fat.**—Whilst chemists are practically agreed upon the method of determining the total solids in milk, this is by no means the case as regards the fat. This is hardly the place to discuss the various methods that have been proposed, but it may be pointed out that, in comparing any two methods, the one yielding the higher percentage of fat is to be preferred, provided always that the fat produced has been shown to be pure butter-fat and nothing else. The two methods here described are fairly typical, giving practically identical results in skilled hands, results, however, that are higher than those obtained on the same milk by any maceration or scrubbing-out process.

FIG. 25.



(*a*) **Werner-Schmidt's Process.**—About 10 gr. of milk (the exact weight taken being of course determined by weighing) is mixed with 10 c.c. of fuming HClAq, either in a stout test-tube, or, better, in a tube of the shape shown in Fig. 25. The acid mixture is now brought to the boil over a small flame, and allowed to boil for about a minute. The tube must be well shaken during the heating, and great care taken that the liquid does not

bump, otherwise the contents will be projected from the tube. After boiling, the tube is allowed to stand for a couple of minutes until the separated fat has risen in a layer to the surface of the brown liquid, it is then cooled in water. When quite cold, about 25 c.c. of ether is added, the whole well shaken, and allowed to stand until the ethereal layer has separated. A cork carrying the little wash-bottle arrangement is now fitted on to the tube, and the ether blown over as completely as possible into a tared glass flask, great care being taken that none of the aqueous acid liquid gets blown over with the ether. The operation is repeated with a second and third quantity of ether. In this way the whole of the fat originally present in the ten grams of milk is transferred with the ether to the tared flask, and it is now only necessary to distil away the ether on a water bath (at about 60° C.), and to dry the fat remaining behind to a constant weight in the water oven, to get the actual weight of fat in the amount of milk taken.

Example.

Schmidt Tube + Milk = 65·873 grms.
Tube . . . = 55·821

Milk taken = 10·052

Flask + Fat . . = 18·437 . 1st weighing
18·433 . 2nd „

18·433 . 3rd „
Flask . . . = 18·073

·360 gr. fat.

$$\therefore \frac{\cdot 360}{10\cdot 052} \times 100 = 3\cdot 58 \text{ per cent. fat.}$$

(*b*) **Adams' Process.**—In this method a known quantity of the milk (about 5 grm.) is evenly distributed over a strip of stout blotting paper, the whole allowed to dry at the temperature of the room, coiled up, and extracted with ether in a special apparatus. Strips of paper specially made for this process can now be obtained without difficulty. They are sold as "fat-free," but except for rough work cannot be trusted, and it is better to exhaust each coil with ether before using. The process is here described with the convenient modifications introduced by Messrs. Allen and Chattaway (*Analyst*, xi. 71).

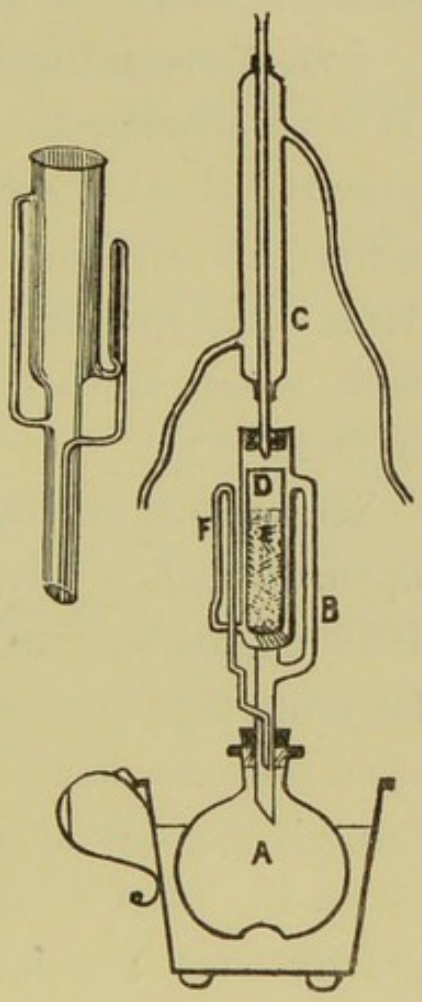
The strip of paper is threaded with string in such a manner that when wound up each concentric ring of paper is kept away from the next by a distance equal to the thickness of the string.* The same string, which has been previously boiled in a weak solution of sodium carbonate to remove resin, serves to fasten on a small cap of filter paper and to fix the whole coil together. The finished coil is then extracted with ether in a Soxhlet extractor, one form of which is shown in Fig. 26.

About 5 grm. of milk (well shaken about ten minutes previously) is then dropped on to the coil, the latter being hung up by its loop of string. If a 5 c.c. pipette is used the weight of milk used may be calculated from its specific gravity, but it is better to take the actual

* The method of threading is perfectly obvious after examining a coil already wound, or seeing one threaded. Hence a lengthy verbal explanation is omitted.

weight of the same milk delivered by the same pipette under similar conditions. The weight used for the determination of the total solids serves for this. The charged coil is hung up to dry in a place protected from dust; it is a great advantage of this method that a coil so prepared may be kept indefinitely for reference without alteration. The extraction is carried out as shown in Fig. 26.

Fig. 26.



A tared glass flask (A), into which is placed about 50 c.c. of ether, is fitted with a cork and the coil (D) is placed in the "Soxhlet" (B), the latter being surmounted by a condenser (C), which must be an efficient one.

The action of the extractor is as follows. The flask being placed in water kept at about 60° C., ether distils up the wide tube, rises into the condenser, and is there condensed to liquid, which drops on the coil. The latter soaks in this ether, parting with some of its fat until the liquid reaches the level of the top of the syphon tube. The little syphon (F) now acts, and the whole of the ether, together with the fat dissolved in it, is transferred to the flask. The ether then again distils up, and the whole operation is repeated. In this way, if the temperature of the bath be properly adjusted, and

the condenser water started, the complete extraction of fat* from any substance becomes perfectly automatic. In the present case the extraction may be considered complete in about 1½ hour, or 20 extractions. Finally, the ether is distilled away and the residue dried to constant weight as usual.

§ 77. **Determination of the Specific Gravity.** — It is unfortunate that the words "density" and "specific gravity" should have been so loosely used by analysts in connection with milk. What is usually meant by the term is the specific gravity of milk at 60° F. compared with water at the same temperature, S.G. $^{60°\ F.}/_{60°\ F.}$ in fact (§ 17).

The specific gravity of milk has been proved to depend upon two factors only, the total solid matter and the fat.† If in a given milk the total solid matter is reduced, the fat being kept constant, the specific gravity is lowered; if, on the other hand, the fat is increased, the total solids being kept constant as before, the specific gravity is also lowered. Thus, a skimmed milk has a greater specific gravity than the milk from which it was originally derived, but if this skimmed milk be gradually diluted down with water it may be brought to the same specific gravity as the original milk. The specific gravity alone then, is of no value in determining the quality of a milk.

The actual relation existing between the total solids

* It should be noted that "fat" in ordinary food analyses is usually synonymous with "substances dissolved out by ether."

† And also, very slightly, upon the time which has elapsed since leaving the cow.

(T), fat (F), and the specific gravity (G)* has been found to be [Hehner and Richmond, *Analyst*, xiii. 26].

$$F = \cdot 859\,T - \cdot 2182\,G - \cdot 05\left(\frac{G}{T} - 2\cdot 5\right) \text{ if positive.}$$

This formula is based on determinations of fat by Adams' method.

The actual determination of S.G. $\frac{60^\circ\,F.}{60^\circ\,F.}$ is carried out precisely as directed in § 17*b*, or 17*c*.

The specific gravity bottle, or a good, well-tested Westphal balance is generally used, the hydrometer not being sufficiently accurate for this purpose.

By means of this formula, which, it must be borne in mind, is a purely experimental one, the fat can be indirectly determined by means of the total solids and specific gravity. Thus, if the total solids at a given sample of milk be found to be 13·02, S.G. $\frac{60^\circ\,F.}{60^\circ\,F.} = 1\cdot 0327$, then, from the above formula

$$\text{Fat} = 4\cdot 05 \text{ per cent.}$$

and hence solids not fat $= 8\cdot 97$ per cent.

§ 78. **The Ash.**—For this purpose the residue from the total solids determination may be used, if in platinum, or some ten grams of milk may be taken down separately in a larger platinum dish. The incineration must be conducted at as low a temperature as possible; this is most conveniently done in a gas muffle or over an

* Note the G in this formula is the actual specific gravity, less one multiplied by 1000, thus if

$$\text{S. G. } \frac{60^\circ\,F}{60^\circ\,F} = 1\cdot 0312,\ G = (1\cdot 0312 - 1\cdot 0000)\ 1000 = 31\cdot 2.$$

Argand burner; if the temperature is allowed to rise to a red heat there will be some difficulty in getting a white ash, and some chloride will be volatilised.

After the ash has been weighed, about 2 c.c. of water may be added and the whole triturated with a small glass rod. The liquid should not be strongly alkaline (addition of Na_2CO_3 to the milk). It is easy to add a drop of potassium chromate solution and titrate with a standard silver solution as in § 25. This gives the percentage of chlorine.

§ 79. **Composition of Milk and Calculation from Analyses.**—It has been taken for granted that the student is acquainted with the general composition of milk so far as this is given in the theoretical text-books on Hygiene, and it has been thought outside the scope of this book to insert tables showing the composition of normal and abnormal milks.* Only just as much will be introduced here as may be necessary to calculate the amount of water added to, or fat abstracted from, a given milk.

It was soon found by the pioneers in milk analysis that the fat in samples of perfectly genuine milk was liable to vary considerably according to the breed of the cow, the mode of feeding, the time of year, the time from calving, &c. In fact, perfectly genuine milks will give figures for fat varying from 3 per cent. up to 10 per cent. or even higher. Single cows have been

* The student who wishes to pursue this side of the subject farther should consult W. Blyth's "Treatise on Foods," which contains a very complete monograph on "Milk," of considerable historical interest.

known on rare occasions to give milk containing as little as 2·75 per cent. of fat; anything below this may be taken to point to either adulteration or fat removal.

The solids not fat, on the other hand, exhibit a remarkable constancy, the mixed milk of a number of cows rarely going outside 8·9 to 9·3 per cent., and averaging 9·0 per cent. Individual cows, however, have been stated to give milk, containing as little as 8·5 per cent. of solids not fat, and as the Adulteration Acts are at present interpretated, public analysts have to make this extreme figure, instead of the average 9 per cent., the basis of their calculations.

Consider a milk giving 12·5 per cent. of total solids, 3·5 per cent of fat, and hence by difference, 9·0 per cent. of solids not fat. Let 20 grams of water be added to 100 grams of this milk; the total weight becomes 120 grams. Hence 100 grams of the mixture contains $\frac{100}{120}$=83·3 grams of the original (undiluted) milk and 16·7 grams of added water; that is, 9·0 grams of solids not fat in 120 grams of the mixture, or $\frac{100}{120} \times 9{\cdot}0 = 7{\cdot}5$ grams of solids not fat in 100 grams of the mixture.

Now, suppose these two data (9·0 grams and 7.5 grams per 100 grams found by analysis) are given, and it is required to work back to the amount of added water. Since 100 grams of the pure milk contained 9 grams of solids not fat, 1 gram of milk contained ·09 grams, and hence the 7·5 grams found represents $\frac{7{\cdot}5}{{\cdot}09} = 83{\cdot}3$ grams of pure milk in the diluted milk, and the remaining 16·7 grams the added water. After this

particular case is thoroughly understood, there will be no difficulty in proving the general case.

Take a pure milk, 100 grams of which contain p grams of solids not fat. Let y grams of water be added to this. The total weight of the mixture is $100 + y$ grams. Hence 100 grams of the mixture contain $\left(\frac{100^2}{100+y}\right)$ grams of the original milk, the remaining $\left(\frac{100y}{100+y}\right)$ grams being added water.

The solids, not fat in 100 grams of the mixture will be $\left(\frac{100p}{100+y}\right)$ grams. But this is a figure found by direct analysis of the mixture $= q$ grams per 100.

$$\text{Hence } \frac{100p}{100+y} = q, \text{ or } y = \frac{100(p-q)}{q}$$

But the amount of "added water" is:

$$\frac{100+y}{100y} = 100\left\{\frac{100\left(\frac{p}{q}-1\right)}{100+100\left(\frac{p}{q}-1\right)}\right\} = 100\left(1-\frac{q}{p}\right)$$

But in most cases the analyst does not know what value to assign to p, except that it cannot be less than 8·5. Hence, if p is put equal to 8·5, the formula becomes

$$\text{Amount of added water} = \text{or} > 100\left(1-\frac{q}{8{\cdot}5}\right)$$

and the certificate is worded, "contains at least . . . parts of added water," implying that if the milk were originally of average quality, the amount of added water would be about 5 per cent. greater.

As regards fat abstraction (which includes dilution

with skimmed milk), a lower limit of 2·75 has to be taken. In cases of fat abstraction only (without dilution with water), if the found fat is f per cent., the original fat F per cent., a process of reasoning similar to the above leads to the formula :

Percentage abstracted, calculated on fat originally present in the milk

$$= \frac{100^2\left(1 - \frac{f}{F}\right)}{100 - f}$$

or since f is small, $100 - f$ is usually taken as 100, and then this simplifies to $100\left(1 - \frac{f}{F}\right)$: thus, taking $F = 2{\cdot}75$ at least, $100\left(1 - \frac{f}{2{\cdot}75}\right)$ per cent. of the fat originally present has been abstracted.

In the case of fat abstraction followed by the addition of water, f in the above formula would not be the fat found, but the fat calculated back to the original milk by means of the found solids not fat. A numerical example will make this clear. A milk gave on analysis—

Fat . . .	= 1·70
Solids not fat	= 7·30
Total solids	= 9·00

First, the water added is not less than $100 - \frac{7{\cdot}3}{8{\cdot}5} = 14{\cdot}1$ per cent., giving 85·9 per cent. of genuine milk.

Hence, present fat : original fat :: 85·9 : 100 ;

or, original fat (on undiluted milk) $= \frac{100}{85{\cdot}9} \times 1{\cdot}7 = 1{\cdot}98\ \%$;

Hence, fat removed before dilution, is at least $100\left(1 - \frac{1{\cdot}98}{2{\cdot}75}\right) = 28\ \%$.

CHAPTER II.

ANALYSIS OF BUTTER.

§ 80. **BUTTER.**—This usually contains about 3 per cent. of curd, 12 per cent. of water, and 85 per cent. of butter-fat.

Anything above 16 per cent. of water is regarded as an adulteration. English made butters are not often found to contain an excess of water, but those of Irish manufacture frequently contain excessive amounts, which in most cases have been deliberately incorporated.

Butter-fat differs from all other oils and fats in containing a notable proportion of glycyl butyrate, and it is on this characteristic that the principal processes to detect foreign fats in butter are based.

§ 81. **ESTIMATION OF THE WATER.**—A known weight, say 5 grams, is exposed to a temperature of 103° C., till constant; the loss in weight is due to the expulsion of the water. The curd may then be determined by pouring the contents of the dish on to a small filter and washing with dry ether till all the fat is removed; the mass of curd (and salt) remaining on the filter is dried and weighed. After weighing, it is ashed in the same dish,

and the ash subtracted from the previous weight gives the curd. It is rarely necessary to estimate the curd or salt.

§ 82. **EXAMINATION FOR FOREIGN FATS.**—About 40 grams of the sample are placed in a beaker on the water-bath and melted, when the water and curd will sink, leaving the fat fairly clear. This is then poured off through a dry filter, and if it is pure butter it generally filters very readily, while it often happens that margarine filters very slowly.

The three chief processes for the examination of the fat are:

(1) The Valenta test.
(2) The Reichert process.
(3) Hehner's method.

The last of these methods is complicated and long, so only the first two methods will be described in detail.

(1) **The Valenta test.**

This test depends on the intermiscibility of butter-fat and strong acetic acid at a low temperature, whereas all other oils and fats that could be used for the purpose of adulterating butter do not dissolve till a much higher temperature is reached.

The procedure is as follows: 3 c.c. of the butter-fat and 3 c.c. of strong acetic acid (99·0 per cent.) are placed in a test-tube and warmed with gentle shaking. When the mixture has become clear a thermometer is inserted, the liquid stirred, and the thermometer reading taken when turbidity begins to appear; this generally takes place at about 35° to 40° C. in the case of genuine butter.

In the case of margarine, however, the mixture does not become clear till it is boiled, and then becomes turbid again very rapidly, usually about 85° C.

Thus a mixture of butter-fat and margarine-fat would yield the turbidity at about 60° C.

This test is a very valuable one, both on account of the short time it takes and because of the accuracy of its indications.

(2) **The Reichert process.**

This process has been modified by various workers, but the method chiefly used in this country is the Reichert-Meissdl modification, which is performed as follows:

5 grams of butter-fat are weighed into an 8-ounce oxygen flask, and saponified by an addition of 2 c.c. of 50 per cent. caustic soda and 25 c.c. of strong spirit. The saponification occupies some twenty minutes, the flask being fitted with a reflux condenser and placed on a water-bath.

After saponification is complete the alcohol is distilled off, and 100 c.c. of hot water added to dissolve the soap. When the soap is quite dissolved 40 c.c. of sulphuric acid, 25 c.c. to the litre, are added, and a small piece of pipe-clay to obviate bumping.

The liquid is then distilled, and the distillate received in a flask holding 110 c.c.

The distillate is then filtered, and 100 c.c. of it mixed with $\frac{N}{10}$ alkali and phenolphthaleïn. To allow for the 10 c.c. over the 100 c.c. we have titrated, $\frac{1}{10}$ of the number of c.c of alkali required is added and the figure so obtained is termed the "Reichert + a tenth."

In the case of pure butter this will be about 27·5 c.c., while margarine may yield a distillate which will require 1 c.c. of $\frac{N}{10}$ alkali.

Margarine is prepared by churning up melted and clarified animal fats, usually beef or mutton, with milk or skim milk. In this way the curd or casein found in margarine is produced and the mixture has more or less of the flavour of genuine butter. Any such fictitious butter can now only be legally sold in this country under the name "margarine," and must, when exposed for sale, bear this name on a conspicuous label.

CHAPTER III.

EXAMINATION OF COFFEE.

§ 83. **COFFEE.**—In the analysis of plants, or of products derived from plants, many different determinations can be carried out. Thus, in addition to the microscopical examination, estimations may be made of the following:

(1) The total ash.
(2) The ash soluble in water.
(3) The alkalinity of the soluble ash.
(4) The ash insoluble in water, but soluble in dilute acids.
(5) The insoluble ash ("sand").
(6) The constituents of the ash.
(7) Matters extracted by ether ("fat").
(8) Starch, sugar, tannin.
(9) The specific gravity of the aqueous infusion.
(10) The total nitrogen and other determinations in special cases, such as the alkaloids in tea, pepper, or the total sulphur in mustard.

The majority of these processes, however, require a

considerable amount of manipulative skill, and are quite outside the scope of this book. Fortunately, in the case of coffee, the methods in general use are fairly simple, as it is usually possible to decide upon the genuineness of a sample of coffee by a careful microscopical examination, supplemented by a determination of the specific gravity of a 10 per cent. aqueous infusion. To these, however, on account of their general application, are added the methods for determining the percentages of fat and ash.

Microscopical Examination.—For this, some of the coffee should be triturated with hot water (genuine coffee is very hard and is only crushed with some difficulty), and carefully examined, first with a low power ("1 in." or "$\frac{2}{3}$"), and afterwards with a higher power ("$\frac{1}{4}$" or "$\frac{1}{6}$"). Most of the text-books on general hygiene contain good drawings of the microscopical appearance of coffee and its chief adulterants.* These are of comparatively little use, unless supplemented by repeated examinations of the actual substances under the microscope.

The Specific Gravity of the 10 per cent. Infusion.—For this ten grams of coffee is weighed out into a conical boiling flask, 100 c.c. of water added, and the whole brought to the boil with frequent shaking. After 30 seconds' actual boiling, the whole is poured on to a dry filter. The S.G. $\frac{15{\cdot}5}{15{\cdot}5}$ of the filtrate is taken after cooling, most conveniently with the Westphal balance (see § 17 *b*). For genuine coffee, S.G. $\frac{15°{\cdot}5}{15°{\cdot}5}$ may vary between 1·0070 to 1·009, but is usually about 1·0080.

* *See* Bell's "Chemistry of Foods," Part I.

Chicory, the commonest adulterant of coffee, treated in this way gives an infusion of S.G. $\frac{15{\cdot}5}{15{\cdot}5}$ 1·0220 to 1·0240. If a careful microscopical examination has shown that chicory is the only adulterant, then this determination gives a rough approximation to the relative proportions. Thus, taking 1·023 as the S.G. $\frac{15{\cdot}5\ C.}{15{\cdot}0\ C.}$ of pure chicory, and 1·008 for pure coffee, a mixture of the two having S.G. $\frac{15{\cdot}5}{15{\cdot}5}$ of its 10 per cent. infusion 1·0168, would have 41 per cent. of coffee. For each addition of 1 per cent. chicory increases the specific gravity $\frac{1{\cdot}023 - 1{\cdot}008}{100} = {\cdot}00015$; in the above example the increase is $1{\cdot}0168 - 1{\cdot}0080 = {\cdot}0088$, hence there is $\frac{{\cdot}0088}{{\cdot}00015} = 59$ per cent. chicory present.

Owing to the natural variations in the figures for coffee and for chicory, this figure (40 per cent.) is liable to an error of at least +5 per cent.; hence all that the determination really tells us is that the mixture contains between 35 and 45 per cent. of coffee, or, as it is usually put, "at least 55 per cent. of added chicory."

Estimation of Fatty Matters.—The coffee (5 gr.) after being enclosed in a cartridge of stout filter paper, is thoroughly exhausted with ether in a Soxhlet extractor, in a precisely similar manner to that adopted for milk (§ 75 *b*). This gives the total substances extracted by ether, usually called "fat" in analyses of plant products.

Estimation of Ash.—Ten grams of coffee are weighed out into a tared platinum dish, and gently ignited until the ash is quite white. In all ash determinations it is important to keep the temperature as low as possible.

This may be done either by using an Argand burner turned down low, or, more conveniently, by using a gas muffle, the latter giving a current of air over the ash, as well as complete control over the temperature. The dish is cooled in the desiccator, and the increase in weight of the dish $\times 10$ gives the total ash per cent. This is now repeatedly extracted with small quantities of boiling water pouring through a small filter (see § 36). The filtrate is received in a weighed dish—preferably of platinum, but porcelain will do—evaporated to dryness on the water bath, very gently ignited and weighed. This gives the percentage of soluble ash. The insoluble residue is now washed with small successive portions of hot HClAq, pouring off through the same filter. Any residue remaining is washed with hot water on the filter, and the latter, which should be of the quality known as "ash-free," placed wet in the red-hot platinum dish and burnt to an ash. The residue is the insoluble ash (sand). The difference between the total ash and the sum of the sand and the soluble ash gives the percentage of ash soluble in acid.

After weighing the soluble ash it is convenient to titrate it in the dish with $\frac{N}{10}$ HCl and methyl-orange. The results may be expressed in c.c. of $\frac{N}{10}$ acid per 100 gr. coffee, or this may be calculated to KOH, and expressed as "per cent. of potash." This figure is not of much use for coffee, but it is of great service in the case of tea.

CHAPTER IV.

SPIRITS, WINES, AND BEER.

§ 84. **BRANDY, WHISKY, RUM, AND GIN.**—Brandy, whisky, and rum are required to be sold at a strength of not less than 25 degrees under proof. Gin is allowed to be 35 degrees under proof. These limits are fixed by the Sale of Food and Drugs Act, 1879.

Brandy is prepared by the distillation of wine, and it usually has a specific gravity of about ·930, the total solids being about 1·0 per cent.

Whisky is prepared from grain, mostly from that of barley both malted and raw. It is matured in sherry casks, from which it takes up traces of tannin, sugar, &c.

Rum is prepared by distilling fermented sugar-cane juice. The total solids in rum may be from ·7 to 1·5 per cent., and the specific gravity varies from about ·874 to ·926.

Gin is prepared originally from grain, and flavoured with juniper berries. It is sometimes sweetened with cane sugar.

The alcohol in all of the above spirits is most conveniently estimated by distilling 100 c.c., then making

the distillate up to 100 c.c., and taking the specific gravity, when, on reference to an alcohol table, the amount of proof spirit present by volume is at once seen.

§ 85. **ALCOHOL CALCULATIONS.**—Proof spirit is defined by Act of Parliament to be a liquid of such a density that at 51° F. 13 volumes of it shall be equal in weight to 12 volumes of water at the same temperature. Spirits stronger than this are said to be over proof, while weaker spirits are said to be under proof.

Supposing we require to ascertain the quantity of whisky at 25° U.P. in a watered sample of 30° U.P.—

$$\frac{70 \times 100}{75} = 93{\cdot}3$$

Thus the sample consists of 93·3 parts of whisky of 25° U.P. and 6·7 parts of water.

Tables showing the amount of absolute alcohol by weight, the amount of absolute alcohol by volume, and the quantity of proof spirit per cent. corresponding to the specific gravity, will be found in the appendix.

§ 86. **WINES.** — The alcoholic strength of wines is best determined by distillation, as before described. It is also customary to determine the extractive matter and the ash, together with the acidity both volatile and fixed, the former being calculated to acetic acid, the latter to tartaric acid.

The following are the figures yielded by some typical wines :

	Specific Gravity at 15·5° C.	Extract.	Volatile Acid, as Acetic.	Fixed Acid, as Tartaric.	Ash.	Alcohol by weight per cent.
Claret . .	—	2·7	·08	·51	·4	9·8
Port . .	·9974	5·4	—	·5	·3	17·5
Sherry . .	·9940	4·2	·15	·27	·4	17·2
Vin Ordinaire	—	5·0	·11	·61	·45	7·0
Champagne .	—	12·4	—	—	·3	7·9

§ 87. **BEER.** — Strictly speaking, beer should be brewed from an infusion of malt, and bittered with an infusion of hops. In actual practice, however, malt substitutes and converted starches are largely used. Two methods of fermentation are employed, which are known as the "High" and the "Low." The latter of these is largely employed abroad in the manufacture of lager beers, which beers are usually poor in alcohol and high in extractive matters. They are thus liable to undergo a secondary fermentation, and to prevent this they often contain preservatives. Porter is a rather weak liquor, coloured with roasted malt. Stout is a stronger variety of porter.

In the analysis of beers it is customary to determine the specific gravity, the amount of alcohol, the extractive matter, ash, and acidity, this last being calculated to acetic acid.

The following are the figures yielded by some typical beers:

-	Specific Gravity at 15·5° C.	Extract.	Acidity.	Ash.	Alcohol by weight per cent.
Stout . .	1·020	6·0	—	—	5·4
Lager . .	1·013	5·1	—	·2	3·5
Burton (pale)	1·010	5·1	·2	—	5·3
Bitter Ale .	—	5·4	·1	—	5·4

PART V.

MICROSCOPICAL WORK.

CHAPTER I.

ANIMAL PARASITES.—CESTODA.

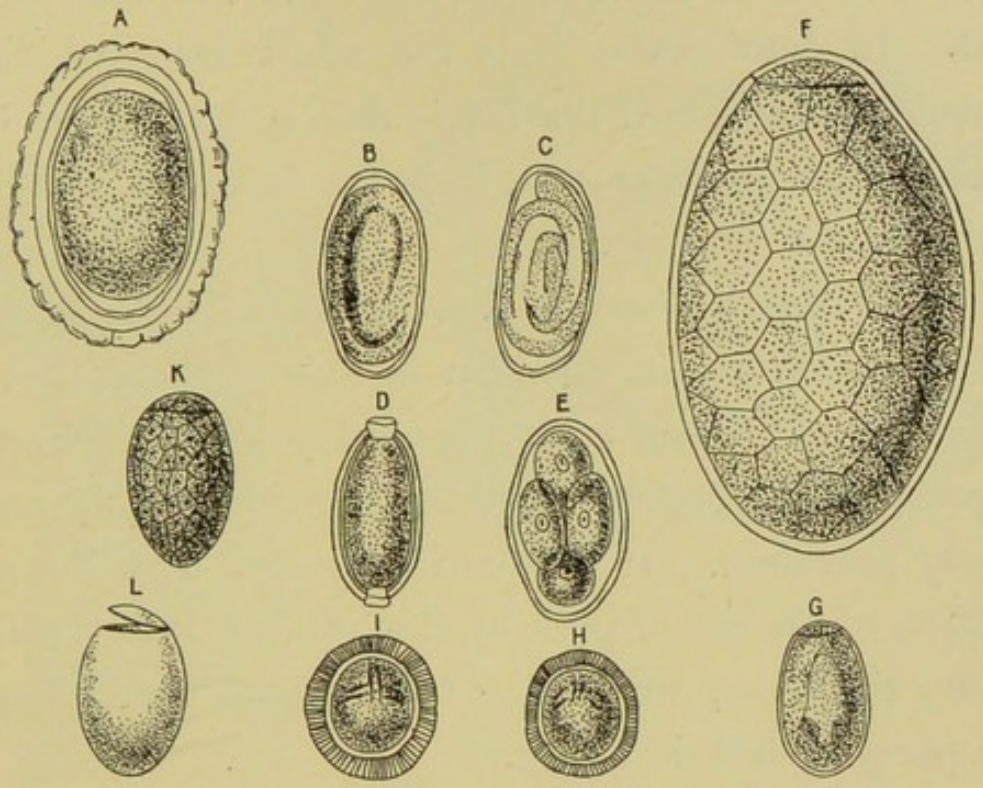
§ 88. **GENERAL CONSIDERATIONS.**—In this part of the book it is proposed to treat generally of the microscopical work necessary for students of public health. The more special branches relating to bacteriology will be found described in Part VI. It is hardly necessary in a work of this character to describe in detail the action and uses of the microscope. It will be enough to point out that, in general, a microscope having a $\frac{1}{2}$ and a $\frac{1}{6}$-inch objectives will be found amply sufficient for all purposes; and that without exception the lower power should be the first used.

§ 89. **PARASITES.**—Parasites may be broadly divided into two classes—viz., animal or zoo-parasites, and vegetable or phyto-parasites—these include all those organisms which live at the expense of their host, whether that host is itself an animal or a vegetable. Parasites are world-wide in their distribution, and include an enormous number of species. Here it is only necessary to concern

ourselves with those which infest man or the animals and vegetables which are used as the food of man.

Parasites differ greatly in the amount of their parasitism. Some appear to be entirely parasitic, *e.g.*, the

FIG. 27. (After Leuckart.)



Eggs of worms found in alimentary canal of man.

A. Ascaris Lumbricoides. B., C. Oxyuris Vermicularis. D. Tricho-cephalus Dispar. E. Dochmius Duodenalis. F. Distoma Hepaticum. G. Distoma Lanceolatum. H. Tænia Solium. I. Tænia Mediocanellata. K., L. Bothriocephalus Latus.

intestinal worms, though even in these, some short phase of their life is passed as a free-living organism. Others are only parasites in one stage of their existence, whilst a third class are only occasionally parasitic, being more often found as free-living organisms.

§ 90. **ANIMAL PARASITES.—Cestoda.**—To this class

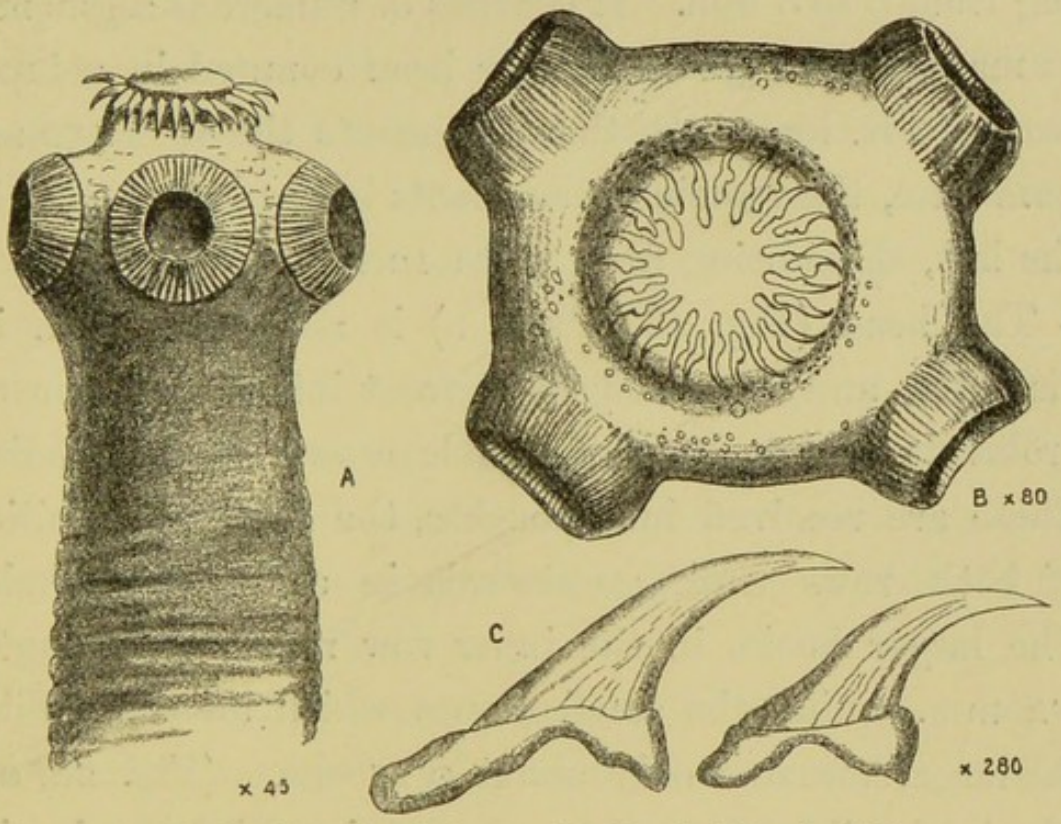
belong some of the most important parasites which infest man—viz., the tapeworms. They all possess the characteristic of passing through two distinct phases, each of which requires a different host for its full development. The adult form exists as an intestinal worm, whilst the larval form is found as a cysticercus in the flesh of its host. In most cases it is the adult form which is parasitic in man, *e.g.*, the *Tænia Solium*, but in the case of the *Tænia Ecchinococcus*, it is the larval form which is the human parasite.

§ 91. **Tænia Solium.**—*Distribution and Life History.*—This worm is not common in this country, but in Ireland, India, some of the central portions of Europe (especially in Germany), North America, and Japan, it is very prevalent. It has been suggested that the prohibition of the use of pork by the Mosaic law as a food among the Jews, was owing to the great prevalence of this parasite in Asia Minor.

Although the name suggests that the *Tænia Solium* always occurs singly, this is by no means the case, since as many as forty worms have been expelled from one man at a time. The pig in the case of this parasite plays as follows the part of the intermediate host. The eggs of the *Tænia Solium*, scattered about by winds, &c., become mixed with the pig's food and are thus swallowed; on reaching the stomach, the shells are at once dissolved and the contained six-hooked embryos set free. These immediately start boring into the tissues of the animal, until they reach the muscles and fatty parts of the trunk, where they

become encapsuled, constituting the condition known as "measly pork." It now only remains for this flesh, imperfectly cooked, to be eaten by man, for the parasite to complete its cycle of existence. The scolex then on reaching the human intestines, attaches itself to the

FIG. 28. (After Leuckart.)



A. Head of Tænia Solium. B. Head of Tænia Solium (apical surface). C. Large and small hooks of Tænia Solium.

alimentary mucous membrane and begins to grow, producing in time sexually mature proglottides, which break off, become voided, and in their turn distribute their eggs broadcast.

Occasionally the cysticercus is found in man, when, according to Leuckart, the patient is usually self-infected. It is much more usual, however, for the adult worm alone to occur as the human parasite. Perroncito

has conducted a number of experiments which prove that the cysticerci in measly pork are rendered harmless by heating to 50° C. (120° F.).

General Characters.—The *Tænia Solium* is a long, flat worm, varying in length from 2 to 3 metres, with its greatest width in the centre of its body, here measuring from 6 to 8 mm. It consists of numerous segments, as many as 800 joints having been counted in a tape-worm 10 ft. long. Of these segments the first 450 are immature, the remaining segments increasing in size to the last, these being from 10 to 12 mm. long.

The head (Fig. 28, A and B) is about 0·6 mm. in diameter, and is prolonged in front into a rostellum or proboscis, which bears a double crown of 26 hooks. These are received into pouches, the sharp extremities of both rows ending outwards in a common circle. The larger hooks in the inner row measure in length 0·2 mm., whilst the smaller ones which alternate with the larger measure only about 0·15 mm. (Fig. 28, C.) The hooks differ from those present in allied species in being compressed and somewhat thin.

The head is provided with four suckers, and is not infrequently dotted with black pigment granules. It is continued behind into a narrow neck about 1 cm. in length, in which the segmentation is represented by transverse lines. These lines become gradually more widely separated, and assume a rectangular form at about $\frac{1}{3}$ of the length of the worm from the head. The immature joints are much broader than they are long, whereas the mature segments are about twice as long as

they are wide, being about 10 mm. in length by 5 mm. in width. These latter contain a branched uterus, the branches ending dendritically, and a testis consisting of numerous sacs, from which a vas deferens passes to the sickle-shaped penis. The common sexual opening is situated at the border of the proglottis below the middle line.

There is no mouth or alimentary canal, but a well-developed water-vascular system is present, consisting of at first four and then of two main longitudinal trunks. This system was mistaken by earlier writers for an alimentary canal.

The eggs of the *Tænia Solium* (Fig. 27, H) are globular, and about 0·04 mm. in diameter, and are surrounded by a thick shell, which exhibits a number of radiating and circular lines. The six-hooked embryo is sometimes to be seen in the interior.

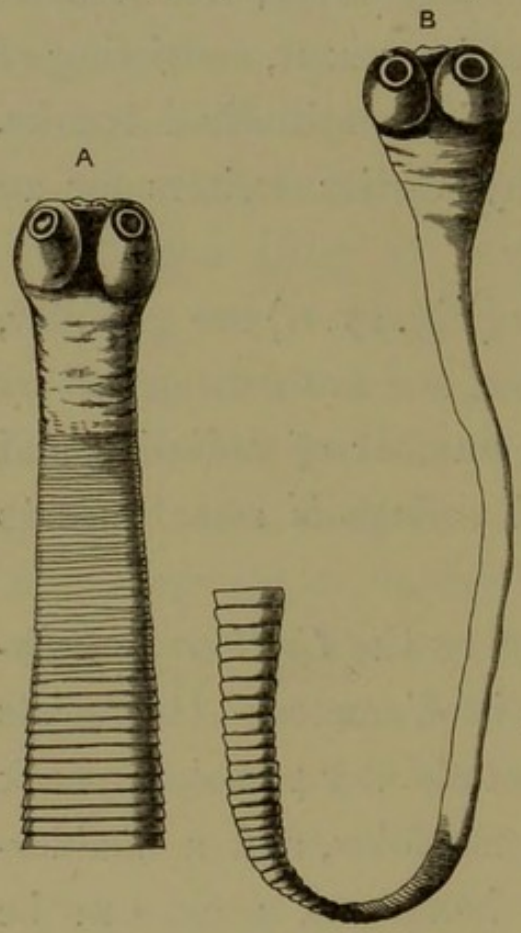
The scolex, commonly known as the *Cysticercus Cellulosæ*, varies from 8 to 13 mm. in diameter. It consists of a head with four suckers and about 24 hooks, a neck which displays regular transverse folds, and a bladder-like vesicle, into which the head and neck can be retracted. This vesicle in the muscles of the pig has usually an elliptical form, the longest diameter of which is in the same direction as that of the fibres.

§ 92. **Tænia Mediocanellata vel Saginata.**—*Distribution.*—This worm, formerly supposed to be of rare occurrence, is really more common in this country than the *Tænia Solium*. It is found largely in Africa, especially in Abyssinia, where it is stated that almost

every person is infected with it. India, Arabia, and South Russia are also much infested with it. It is more rarely found in Europe, but really no country is entirely exempt from it. In America and Australia it is also of fairly frequent occurrence.

Life History.—The ox plays the part of intermediate host to this worm, the life-history of which corresponds in almost every particular with that of the *Tænia Solium*. Perroncito affirms that a temperature of 45° C. (113° F.) is sufficient to cause the death of the cysticercus.

FIG. 29. (After Leuckart.)



Head of Tænia Mediocanellata (A, retracted; B, extended).

Appearance.—The *Tænia Mediocanellata* (Fig. 29) is the largest tapeworm which infests the human body. It is longer, broader, and thicker than the *Tænia Solium*, and attains a length of 6 to 8 metres. It consists of 1200 to 1400 segments and is broadest at the centre of the body, where it measures about 12 mm. to 14 mm.

The head is from 1 mm. to 2 mm. in diameter and possesses no rostellum or hooks. It has four large suckers, usually surrounded by numbers of dark pigment granules.

The neck is narrow and about 1 mm. to 1·5 mm. in length, and is succeeded by about 500 sexually immature segments, whose breadth greatly exceeds their length. The sexually mature segments are thus much more numerous in this worm than in the *Tænia Solium*. The ripe proglottides measure about 18 mm. to 20 mm. in length and from 5 mm. to 7 mm. in breadth. The uterus is very branched, but the branches do not end dendritically. The testes are roundish sacs, and both organs (uterus and testis) open at a common cloaca below the middle of the margin of the proglottis. The name of this worm is derived from the peculiar arrangement of the water-vascular system.

The eggs (Fig. 27, 1) are markedly oval and measure about 0·03 mm. in their short diameter. They are surrounded by a thick shell with a border of small rods.

The *Cysticercus Bovis* is smaller than the *Cysticercus Cellulosæ* and is usually roundish in shape and never exceeds 1 cm. in diameter. It contains a scolex, identical in appearance with the head of the adult worm.

§ 93. **Bothriocephalus Latus.**—*Distribution.*—This parasite has been but rarely found beyond the confines of Europe. In certain parts of Switzerland, especially on the shores of the Lakes of Geneva and Biel, it is very prevalent. It is also found in Sweden, Russia, and the coast regions of Eastern Prussia.

Life History.—The pike and perch seem to act as its intermediate hosts, but it is probable that further research will show its presence in other fish.

Unlike the tapeworms previously described, this

worm does not appear to break up into living proglottides, for the ova escape by the bursting of the over-distended uterus. After the eggs have been immersed in water for some 4 to 8 weeks, embryonic changes take place which result in the liberation of a ciliated embryo (Fig. 30), which is able to swim about with a rotatory movement. The ciliated covering is shed after a few days, and the six-hooked embryo liberated. This is swallowed by the pike and, boring into the muscles of that fish, develops into the cysticercal form of the *Bothriocephalus Latus*.

Fig. 30.

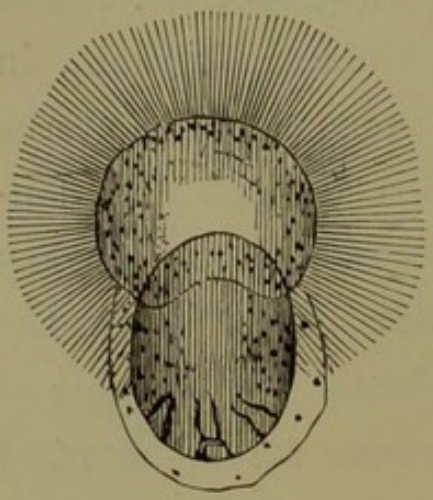
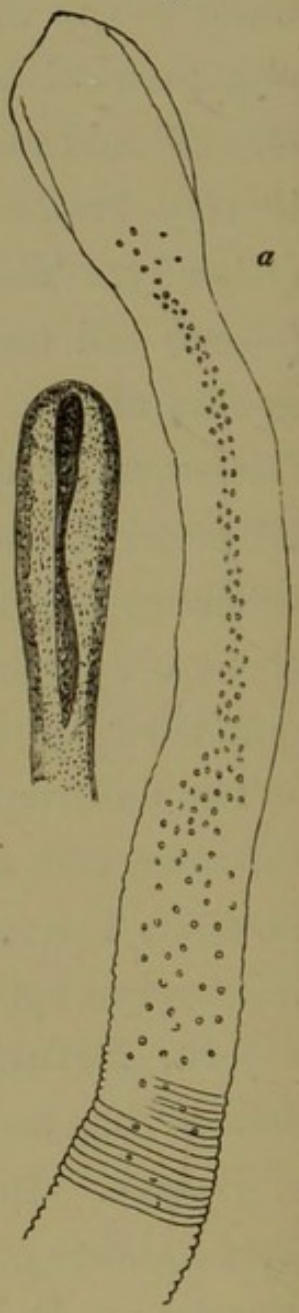


Fig. 31.



Appearance.—This worm attains a considerable length, sometimes being 8 m. long. The number of segments varies from 3000 to 4000. The head (Fig. 31) is about 2 mm. to 5 mm. in length, and 1 mm. in breadth, and is somewhat oval or club-shaped. It has two laterally disposed suckers, but possesses no hooklets. The immature segments, about 600 in number, are very narrow and only gradually increase in size. The breadth of the segments

increases gradually, reaching sometimes as much as 12 mm. to 20 mm., but the length never exceeds 4 mm. to 5 mm. Posteriorly the breadth decreases, whilst the length increases. The posterior segments are also much thicker, from the number of contained ova. The uterus consists of a coiled, ribbon-like tube, and the reproductive orifices are situated about the middle of the ventral surface, the vagina being below the penis.

The water-vascular system consists of two main longitudinal vessels, from which no transverse canals have yet been seen to pass.

The eggs (Fig. 27, K, 4) are oval in form, and average 0·05 mm. in length by 0·035 mm. in breadth. They are enveloped in a brown shell, and are provided with a lid or operculum at one end.

The larval form measures about 1 cm. to 2 cm. in length, and 2 mm. to 3 mm. in breadth. It is somewhat club-shaped and has the head usually invaginated. Otherwise it resembles the adult worm.

This worm presents a great tendency to the formation of monstrosities, *i.e.*, double segments, and double reproductive organs.

§ 94. **Tænia Nana.**—*Distribution.*—This worm is only rarely parasitic in man. It is fairly common in Egypt, where Bilharz found it in large numbers in the duodenum of a boy. Its life-history is but imperfectly known, but it is probable that the cysticercus exists in some kind of insect or snail.

Appearance.—The full grown worm measures only about 12 to 20 mm. in length, and has a maximum

breadth of about 0.5 mm. It bears 150 to 170 segments. The head has a diameter of about 0·3 mm., and is provided with four suckers and a rostellum. The rostellum is armed with a single row of hooks, which number from 22 to 28. The head is succeeded by a thread-like neck and body. Posteriorly the body enlarges greatly and for the last third has an almost uniform breadth.

The eggs are round and possess two envelopes, somewhat widely separated from each other. They measure about 0·04 mm. in diameter, and contain a six-hooked embryo.

§ 95. **Tænia Elliptica vel Cucumerina.**—*Distribution.*—This worm is also only rarely parasitic in man but is extremely common in the dog and cat, indeed, nearly all the known cases of its human occurrence are in children. The dog-louse is the intermediate host of this parasite. The dog-louse irritates the skin of the dog, who at once licks the place, and thus enables the cycle of life of the parasite to be completed.

Appearance.—The *Tænia Elliptica* is a small worm, measuring about 18 to 25 cm. in length, and in the posterior joints about 1·5 to 2 mm. in breadth. The head, about 0·3 mm. in diameter, has four suckers and a club-shaped rostellum which carries from 3 to 4 rows of hooks, numbering in all about 60. The first 40 joints are very narrow and short, but they then begin to greatly increase, especially in length. The ripe proglottides, numbering about 25, are of a red colour, due to the egg masses within. Each proglottis has a double set of

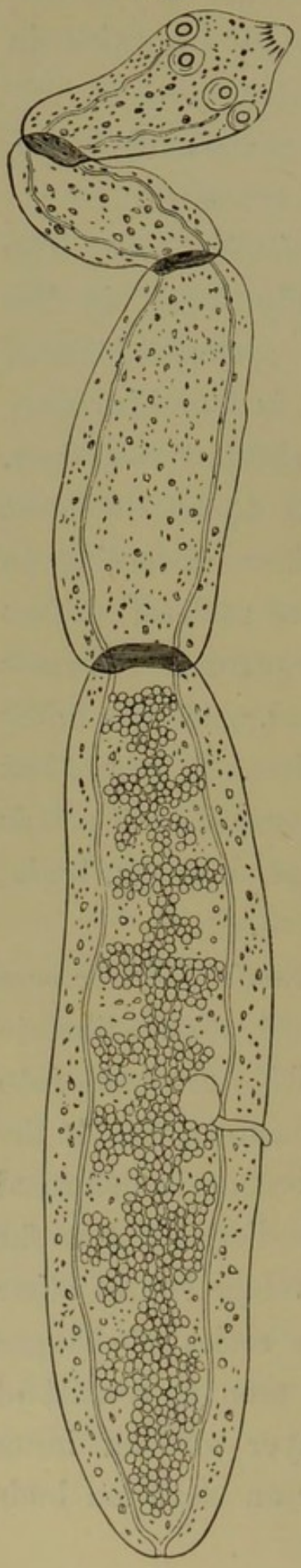
reproductive organs, which open at either side of the margin of the proglottis. The eggs are brownish in colour and measure 0·05 mm. in diameter.

The cysticercus resembles the head of the adult worm, and is usually seen with the rostellum retracted.

§ 96. **Tænia Echinococcus.** *Distribution.* — The adult form of this tapeworm is only found in the dog, the wolf, and the jackal. Its cysticercal form, under the name of hydatids, however, is found in many animals, including man, monkeys, sheep, oxen, deer, camels, and horses. Iceland appears to be the most frequent habitat of this parasite, where, according to Leuckart, it is estimated that there are at least 10,000 cases of human hydatid disease. A large number of cases also occur in Germany and Switzerland, and it is fairly common in Australia, Egypt, and Algiers. It occurs but rarely in England. The liver is the favourite seat of this parasite, after which follow the lungs, kidneys, brain, and muscular tissues in the order named.

Life History.—The eggs of the *Tænia Echinococcus* being swallowed by some susceptible host, at once liberate the contained six-hooked embryo, which, burrowing into the tissues, is carried by the blood stream and lodged in the liver, lungs, etc. Here the embryos become transformed into simple vesicular or bladder-like bodies, which so far corresponds with the cysticercal development of other tapeworms. Now, however, a unique series of changes take place. The wall of the cyst is seen to consist of an outer homogeneous cuticular layer and an inner granular one. From this inner layer an elevation buds

FIG. 32.



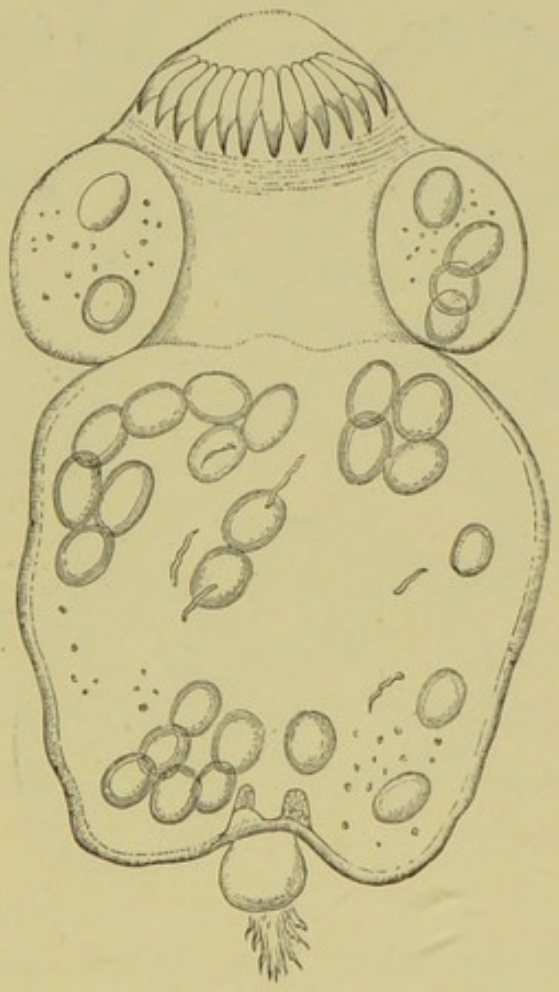
out, which becomes vacuolated and in time assumes the appearance of a true echinococcus head. This enlarges still more, and is known as a brood capsule. From the outer wall of this capsule, echinococcus heads bud, and becoming invaginated, constitute the daughter cysts. This process may be repeated with the formation of grand-daughter hydatids. These echinococcus heads on being swallowed by the dog develop into the adult tape-worm.

Appearance. — The adult worm (Fig. 32) consists of only four segments and rarely exceeds 5 mm. in length. The head possesses four suckers and a rostellum armed with a double row of large hooks, numbering in all about 40. The head is elongated behind into a narrow neck. The final segment, which alone possesses mature generative organs, equals in length the whole of the other three. It contains a central rosette-shaped uterus, which opens below the centre of the margin. The testis consists of a number of sacs, from which a tortuous vas-deferens passes to the penis. According to Leuckart, the

penis is often seen bent round and inserted into the vaginal outlet, thus performing the act of self-impregnation. Cobbold, however, throws some doubt upon this.

The eggs are globular and about ·01 mm. in diameter.

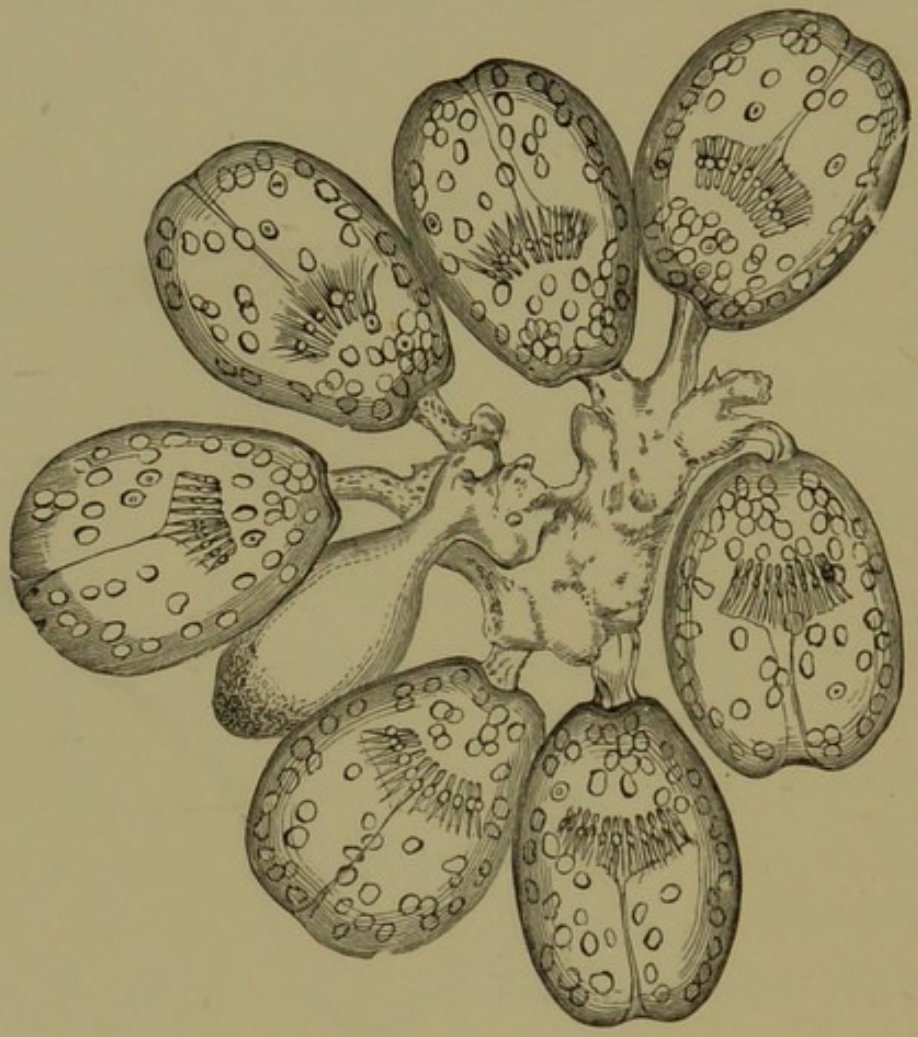
FIG. 33.



The echinococcus heads (Fig. 33) in their fully developed state are about 3 mm. in length. They are solid throughout and exhibit a more or less hour-glass constriction at the centre of the body. The head bears a rostellum, carrying a double row of hooks and also possesses four suckers. In short, the echinococcus head represents the head and neck of the adult worm.

Sometimes the echinococcus heads are found inverted (Fig. 34). In this condition they appear almost spherical, and exhibit a large depression on their anterior border,

FIG. 34.



where the invagination has taken place. At the bottom of this depression, the four suckers, together with the rostellum and circlets of hooks, can be seen.

CHAPTER II.

ANIMAL PARASITES.—TREMATODA.

§ 97. **Distoma Hepaticum.** — *Distribution.* — This parasite is very widely distributed and is of most common occurrence in sheep and oxen, causing the disease known as "the rot." In man, however, it has been but rarely found.

Life History.—The eggs of the fluke, having been voided by its host, undergo the first part of their development in water. Here, in the course of about six weeks, a ciliated embryo is developed, which escapes from the shell and swims about. After a few days, the ciliated covering is shed, and it is probable that the embryo gains access to some fresh-water mollusc. In its intermediate host, it is supposed that the embryo becomes changed into a sac, which develops new larvæ in its interior and constitute the well-known cercariæ. These cercariæ escape and, leading for a time a free existence, are swallowed by sheep, whose liver they at once penetrate and develop into the adult fluke.

Appearance.—This worm is of a pale yellow colour,

and measures from 18 to 25 mm. in length and from 12 to 15 mm. in breadth (Fig. 35). The body is very flat and displays distinct dorsal and ventral surfaces. The head and neck are somewhat pointed and furnished with two suckers, one placed terminally, the other ventrally, immediately below the root of the neck. The reproductive orifices are situated in the middle line above the ventral sucker. The skin is covered by a number of small chitinous spines. The oral sucker is perforated in the middle and communicates with the œsophagus. No anus has yet been seen.

FIG. 35.

The eggs (Fig. 27 F.) measure 0·15 mm. long and 0·09 mm. broad.

The ciliated embryo is conical in shape and displays at its anterior end a central papilla devoid of cilia. The tail is bluntly pointed. It measures about 0·13 mm. in length, and 0·05 mm. in breadth in its broadest part.

The *Distoma Lanceolatum* has been found occasionally as a parasite in man, but it is so rare as hardly to warrant description.

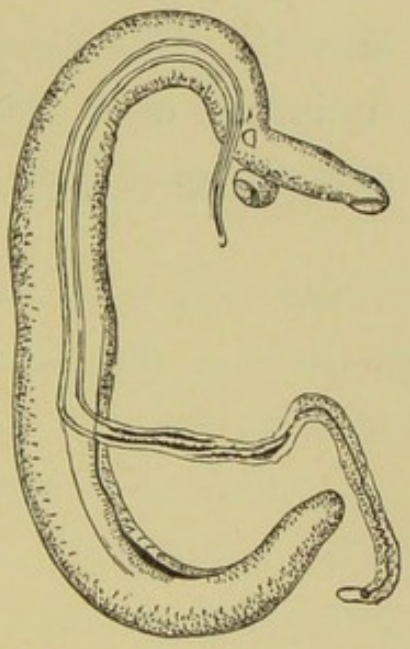
§ 98. **Bilharzia Hæmatobia.**—*Distribution.* — This parasite inhabits the portal, the splenic, and mesenteric veins, and also the inferior vena cava of men and monkeys. It is extremely common in Egypt along the banks of the Nile, in Abyssinia, Mauritius, the Cape, and Natal, and is the cause of dysentery and endemic hæmaturia in its host.

Life-History.—This fluke is peculiar in having the

sexes distinct and not combined in the same animal. The eggs develop a ciliated embryo, and it seems probable that the life-history corresponds with that of the *Distoma Hepaticum*, consisting of a sporocystic and cercarian stage passed respectively in some mollusc and as a free living organism.

Appearance.—The male worm measures about 12 to 14 mm. in length, and has a flattened body, which, from the ventral sucker to the tail, is rolled into a tube and constitutes the gynæcophoric canal. The female is filiform in shape and measures 16 to 19 mm. in length, and is lodged in the gynæcophoric canal during the copulatory act (Fig. 36). The female measures about 0·085 mm. in thickness in the anterior part, and 0·28 mm. in the posterior part. In both sexes oral and ventral suckers are provided and are placed near each other in the front of the body. The reproductive orifices are placed below the ventral sucker. The caudal end is pointed. The intestine consists of two blind canals, which unite below the ventral sucker.

FIG. 36.



Bilharzia Haemotobia, male and female, the latter enclosed in the gynæcophoric canal.

The eggs are somewhat variable in form, being either pyriform or oval. They measure about 0·15 mm. long by 0·05 mm. broad, and are furnished with a spine, which is either placed laterally or terminally. It is

stated that ova with a lateral spine occur in the intestine, whilst those with a terminal spine are to be found in the bladder. No operculum has been seen, but Bilharz describes a lateral slit near the anterior pole, through which he saw the ciliated embryos escaping. The ciliated embryo measures 0·11 mm. in length by 0.04 mm. in breadth. The anterior end is pointed, and presents the rudiments of a digestive apparatus. In the interior a quantity of sarcode globules may be seen.

CHAPTER III.

ANIMAL PARASITES.—NEMATODA.

Nearly all the parasites belonging to this class inhabit the intestines. Their life-history is but imperfectly known, and it has been stated that in the case of many of them there is no need for an intermediate host.

§ 99. **Ascaris Lumbricoides.**—*Distribution.*—This worm is particularly common in situations where there is an abundant water supply, such as the lowlands of Holland, Sweden, and other places. But the Mauritius seems to be the place where these worms most abound.

Life History.—This still requires investigation. Thousands of eggs are constantly voided by the host. It appears that the ova then undergo the first stages of their development in water. Cobbold states that after seven months' existence in water, no embryos had yet left the eggs. Whether there is an intermediate host appears doubtful, and Cobbold is of opinion that such is not the case, but that the eggs, after undergoing changes in water, are directly swallowed by man, and develop into the adult worm.

Appearance.—The *Ascaris Lumbricoides* is cylindrical in

shape, and of a pinkish brown colour. It resembles the common earth-worm. The male measures from 100 to 150 mm., the female being much larger, reaching sometimes a length of 350 mm. and being 6 mm. in diameter. The body is smooth, and marked by numerous fine rings and tapers to a point at each end. The head is furnished with a tri-papillated mouth. The caudal extremity is more or less blunt, and in the male is furnished with two chitinous spines, marking the genital orifice, it is also bent and hook-like.

The female genital orifice is situated on the under surface, near the middle of the body.

This worm is furnished with a mouth and well-developed alimentary canal, which terminates by a transverse slit near the tail.

The testis consists of a single coiled tube about a yard long. The uterus is short, but divides into two long horns.

The ova (Fig. 27 A) are very numerous, oval in shape and nodulated, and when mature possess a double shell. They measure 0·056 mm. in their shortest diameter. They are said not to be killed either by drying or freezing.

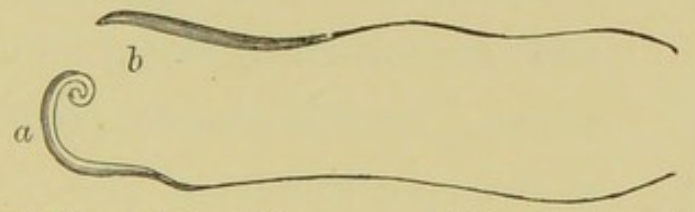
§ 100. **Trichocephalus Dispar** or **Whip worm.**—*Distribution.*—This worm is exceedingly common in France, and, according to Davaine, infests more than half of the inhabitants of Paris. In England, however, it appears to be most rare.

Life-History.—From the researches of Davaine it seems that no development takes place in the eggs till

after they have been expelled from the intestine of their host. It is probable that the eggs reach water, and that six months elapse before embryonic formation commences. The full-grown embryo is said to measure 0·076 mm. in length and to resemble the adult worm.

Appearance.—The male and female approximate each other in length, measuring from 40 to 50 mm. The anterior portion, consisting of about two-thirds of the

FIG. 37.



Trichocephalus Dispar. *a.* Male. *b.* Female.

entire length, is filiform and thread-like, but the caudal extremity is blunt and contains the genital organs. A band of papillæ runs on one side. The tail of the male is curved (Fig. 37 A), and exhibits at its extremity a short penis-sheath armed with retroverted spines. The tail of the female is straight (Fig. 37 B).

The eggs are oval and measure about 0·056 mm. in their longest diameter (Fig. 27 D).

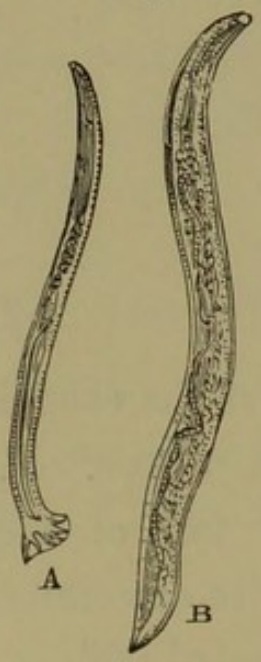
§ 101. **Sclerostoma Duodenale.**—*Distribution.*—This worm is common in Northern Italy, and greatly affected the men engaged in making the St. Gothard tunnel. In Egypt, too, it is of very frequent occurrence, where it causes a most intractable form of anæmia known as Egyptian chlorosis.

Life-History.—The earlier stages of this parasite are passed in moist earth, from which they become transferred

to well-water and thence to human beings. They are often present in large numbers in the small intestine, where they burrow into the mucous membrane, and cause the severe form of anæmia before referred to.

Appearance.—The male worm measures about 10 mm. in length, the female reaching a length of about 12 mm. The head is somewhat pointed and tapering and is furnished with a mouth, the lower lip of which contains four conical and asymmetrical teeth. The body is about $\frac{1}{80}$ inch in diameter and terminates in the male in a blunt tail. This tail is furnished with a three-lobed bursa and two well marked spines (Fig. 38 A). The female ends in a sharp-pointed tail (Fig. 38 B).

FIG. 38.



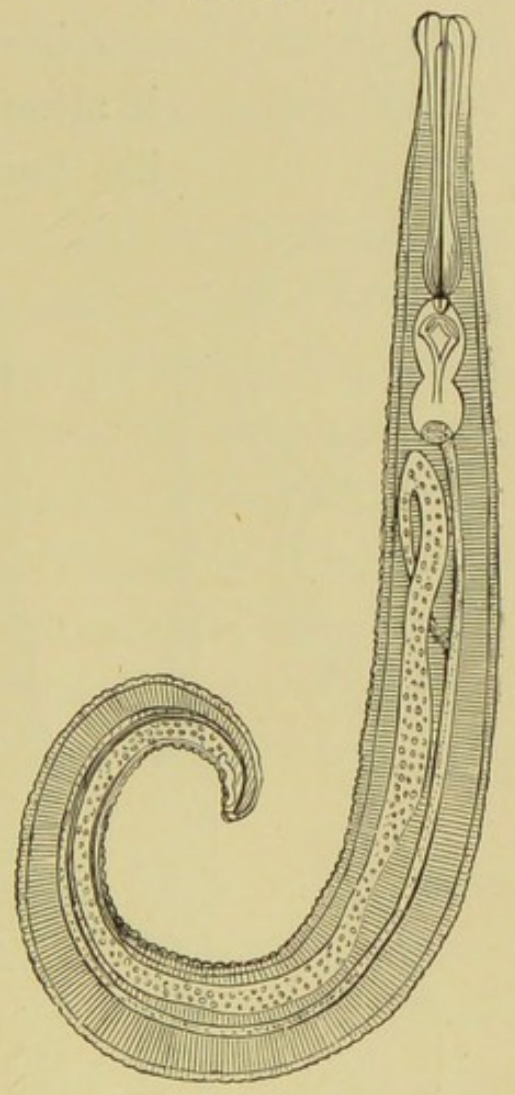
Sclerostoma Duodenale. A. Male. B. Female.

§ 102. **Oxyuris Vermicularis.**—*Distribution.*—These small worms are almost world-wide in their distribution, and form by far the most common human parasite. They inhabit, often in large numbers, the colon, whence they wander to the rectum. Their occurrence is not limited to young children, as has been sometimes stated.

Life-History.—This has not been fully worked out, but it seems probable that the eggs cannot develop before they are introduced into the stomach. They probably gain access to this by means of water or uncooked vegetables.

Appearance.—The female of this small worm measures about 10 mm. in length, whilst the male rarely exceeds 4 mm. (Fig. 39). The head appears somewhat truncated, and has a tri-papillated mouth, which leads into a somewhat triangular œsophagus. The body is fusiform and terminates in the female in a sharp-pointed extremity, which, according to Cobbold, is divided into three points. In the male the caudal extremity is blunt and is provided with a spiculum.

FIG. 39.



The testis is a simple cylindrical tube, much folded upon itself, and ends in the cloacal cavity. It is furnished with a small imperforate penis.

The uterus is two-horned and leads to the vagina, which opens near the anal orifice. The eggs are asymmetrically oval, and measure about 0·05 mm. long, and 0·03 mm. wide (Fig. 27 B, C).

§ 103. **Filaria Medinensis vel Dracunculus Medinensis.**—*Distribution.*—This worm is practically confined to certain regions of the tropics. These include Arabia Petræa, the shores of the Persian Gulf and Caspian Sea, the banks of the Ganges, Egypt, Abyssinia, the coast of

FIG. 40 A.

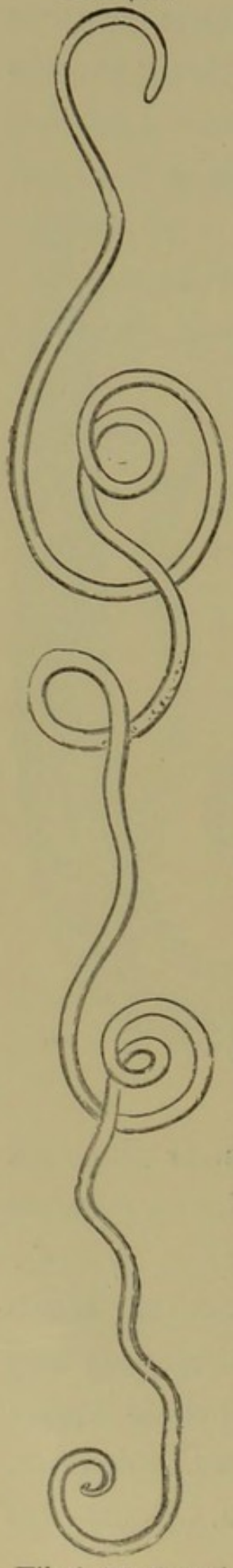
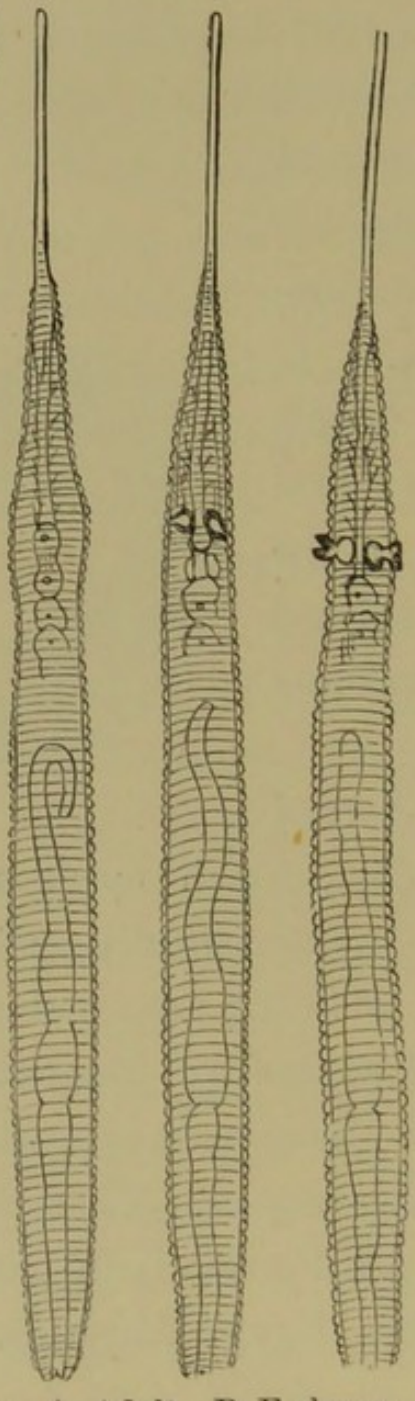


FIG. 40 B.



Filaria Medinensis. A. Adult. B. Embryos.

Guinea, and certain of the West Indian Islands. Cobbold considers that this parasite is identical with the fiery serpents mentioned by Moses.

Life-History.—The female worm is alone parasitic. It probably enters its human host through the skin, and takes about nine months to develop. When about to liberate its young, it causes abscesses to form, which, opening on the surface, allow the young filaria to escape. According to Cobbold, these filaria soon become sexually mature, and the male then dies. The female then penetrates the body of a minute crustacean cyclops, where it undergoes the earlier stages of its development.

Appearance. — Only the female

worm has been up to the present recognised. It measures from 60 to 100 cm. (6 to 12 feet) in length, and about 2 mm. in breadth (Fig. 40 A). The head is more or less flatly convex, and possesses a simple mouth, furnished with four crucially disposed papillæ. The body is uniformly cylindrical, and terminates in a curved filiform and retroverted tail.

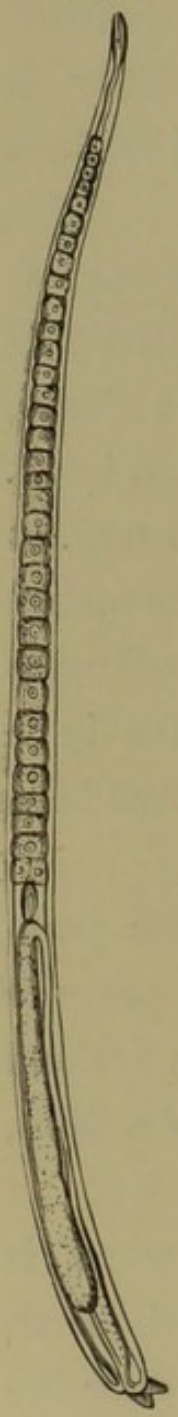
The uterus is large and sac-like, and as yet no vaginal orifice has been found. It appears probable therefore that the young filaria, which are crowded in the uterus, cannot escape without the rupture of the maternal body. The fully developed embryos measure about 0·8 mm. in length and 0·025 mm. in breadth (Fig. 40 B). The mouth is tri-lobed or tri-papillated. The anterior three-fourths of the body are cylindrical, and it terminates in a sharply pointed tail. No generative organs have been seen.

§ 104. **Trichina Spiralis.**—*Distribution.*—This parasite is somewhat rare in England, but in certain parts of Germany it is very frequent. It occurs also in Ireland and Southern Europe. It is found in dogs, cats, mice, rats, pigs, etc.

Life-History.—The immature form of this worm is found in the muscles of the pig. If the flesh of an animal so affected be eaten, the capsule surrounding the trichina becomes dissolved and the young worms are set free in the intestines. Here they soon arrive at maturity, and at about the sixth day young filiform embryos are liberated from the mother, which at once commence boring into the muscles, where they become

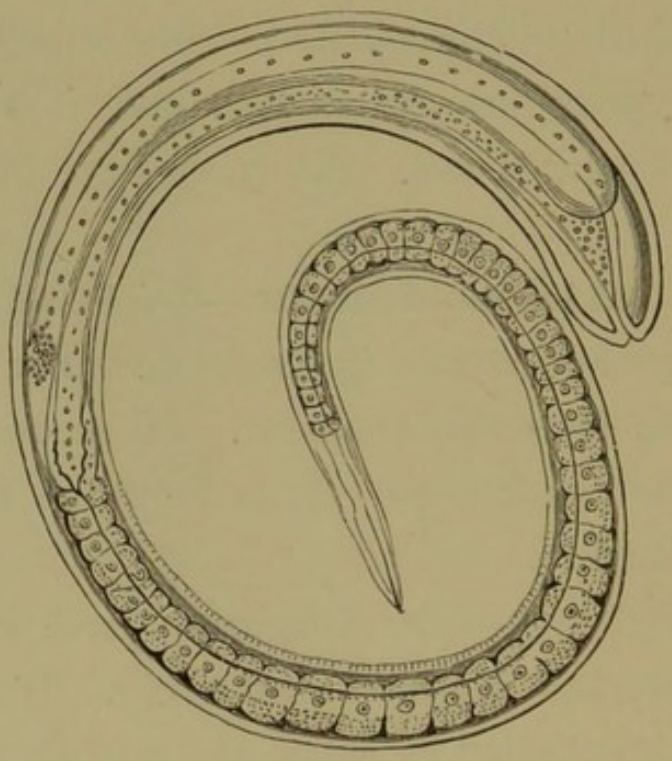
encapsuled. It is stated that one mature female can produce from 1000 to 1500 trichinæ.

FIG. 41 A.



Appearance.—The adult worm inhabits the intestinal canal, and only lives for a few weeks. The female measures about 3 mm. ($\frac{1}{8}$ inch) in length, whilst the male only attains a length of 1·4 mm. (Fig. 41 B). The head is narrow and pointed, and possesses a centrally situated mouth. The body is rounded and filiform, thicker behind than in front. In the male, the caudal extremity is furnished with two

FIG. 41 B.



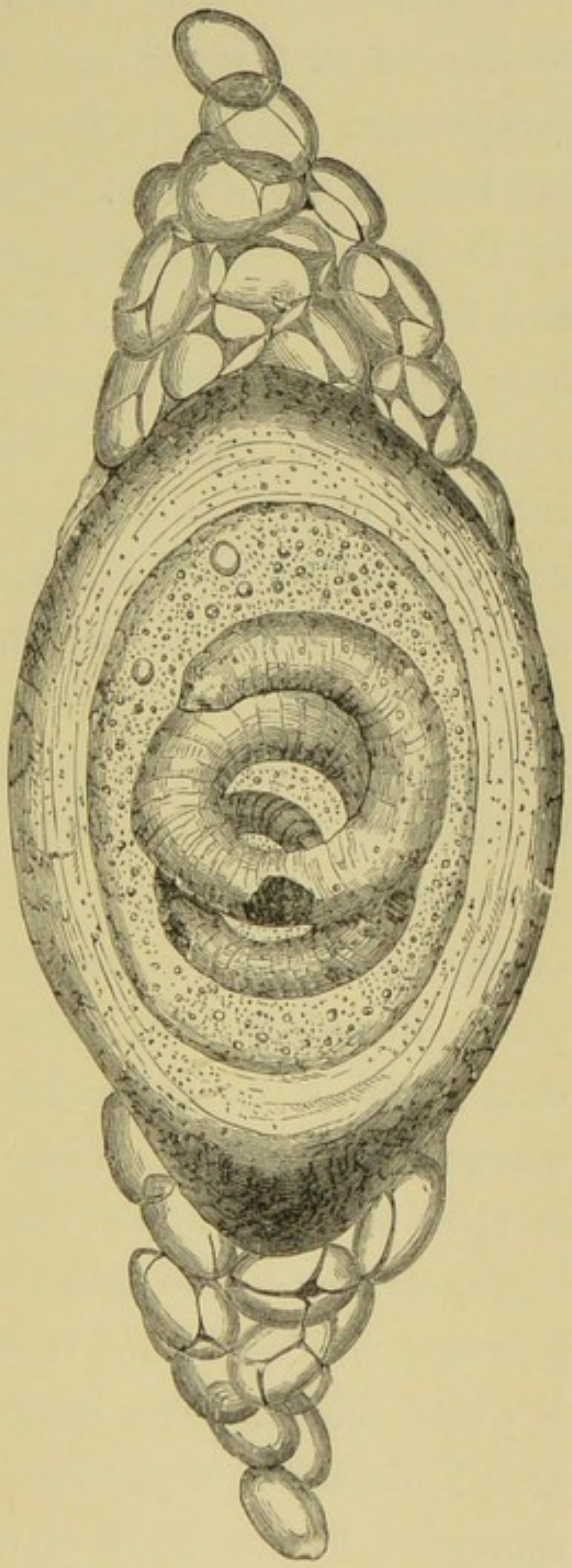
Trichina Spiralis. A. Male. B. Female.

mammillary protuberances, between which the cloacal aperture is situated (Fig. 41 A). The penis consists of a single spicule, and is somewhat V-shaped. In the female the caudal extremity is blunt, and the

vaginal opening is placed near the fore part of the body.

The young trichinæ, as before stated, are found encysted in the voluntary muscles, where they are seen as small spirally coiled worms in oval cysts. The cysts measure about 0·32 mm. in length, and 0·2 mm. in breadth (Fig. 42). The wall of the cyst often contains calcareous particles. The young trichinæ themselves measure about 1 mm. in length, and 0·04 mm. in diameter.

FIG. 42.



The embryos are born in a free state. Although the adult form dies within about six hours of the death of their hosts, the larval form will live for some considerable time, even after putrefaction has set in.

§ 105. **Filaria Sanguinis Hominis Nocturna.**—*Distribution.*—This parasite is found in almost all tropical and sub-

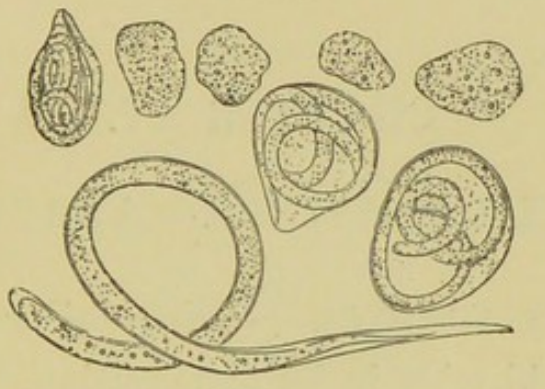
tropical countries, where a large number of the inhabitants harbour it. It is now proved to be the larval form of the *Filaria Bancrofti*, which is considered the cause of two diseases—chyluria and elephantiasis.

Life-History.—The *Filaria Bancrofti* is probably swallowed with water, and at once bores its way to the lymphatics. Here the female produces its young, which are born in a free state. These small filaria are wafted by the lymph stream into the blood. Their sojourn in this fluid is marked by a curious periodicity, as they are only present in the superficial parts during the night, whilst during the day they probably retire to the internal viscera. This periodicity is probably an accommodation to the habits of its intermediate host, a form of mosquito which bites most vigorously at night. On the mosquito biting the human host, the filaria curls round its proboscis and from thence is transferred to its stomach. Here the filaria sheds its chorional envelope, and bores its way into the muscles of the mosquito. Some remarkable changes now take place, including the development of an alimentary canal, and a circumcaudal circle of papillæ. The mosquito laying her eggs on the water, the filaria escapes, to be swallowed later by its human host.

Appearance.—The adult worm measures from 75 to 100 mm. in length, and 0·28 mm. in breadth, and is of a white colour. The head is furnished with a simple mouth, destitute of papillæ. The neck is narrow and is succeeded by the body of uniform thickness. The tail in the female is blunt and exhibits the anus just above

the tail. The uterus is tubular and usually packed with ova, and opens close to the head. The male is probably smaller in size. The *Filaria Sanguinis Hominis* (Fig. 43) averages 0·34 mm. in length, and 0·007 mm.

FIG. 43.



in breadth. It is enclosed in a transparent sac—the remains of one of the coverings of the egg. No reproductive organs are present.

These filariae are best seen by staining them with a weak solution of eosin for about two hours, then washing and mounting in balsam.

CHAPTER IV.

ANIMAL PARASITES.—ARACHNIDA.

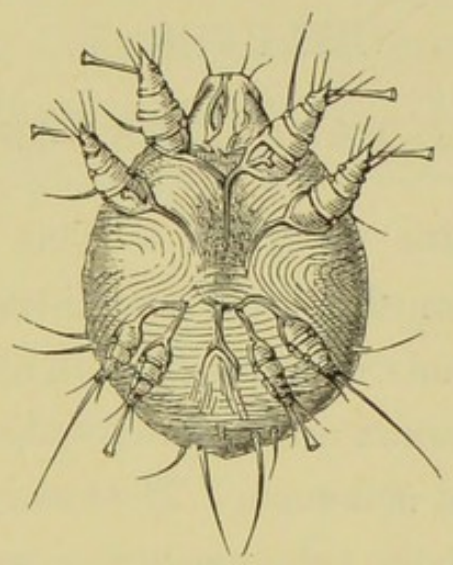
§ 106. **ACARUS SCABIEI.** — *Distribution.* — This small parasite is world-wide in its distribution. It attacks all parts of the body, but its favourite sites are the clefts between the fingers and the fronts of the wrists.

Life-History.—The eggs are hatched in the deep burrows bored by the female acarus. The young mite at first has only six legs, but about six days later it sheds its first skin and then appears with eight legs. A second shedding of the skin takes place about six days later, whilst eleven days after this the third and last shedding of the skin takes place, and the mites are now furnished with sexual organs. The males pass down the burrows, impregnate the females, and then die. The females burrow deeper and lay their eggs.

Appearance.—The *Acarus Scabiei* (Fig. 44) is a small oval animal, somewhat resembling a tortoise in shape. The belly is flat, whilst the back is convex. The back is covered with small transparent cones or teeth, which are doubtless used in boring into the tissues. The female is larger than the male, and measures 5 to 6 mm. in

length and 3 to 4 mm. in breadth. The four anterior legs are provided with terminal suckers, whilst the four posterior legs terminate in filamentous bristles. In the male, which is about half the size of the female, the last pair of legs are also provided with terminal suckers. As

FIG. 44.



stated above, the young acarus has only six legs, the last distinctive pair appearing after the first skin is shed. Each female lays about fifteen eggs, which take from eight to fourteen days to hatch.

The head is retractile, and consists of two movable upper lips and a massive immovable lower lip.

Another parasite, the *Acarus Folliculorum*, also infests man, but it is hardly of sufficient importance to be described here.

CHAPTER V.

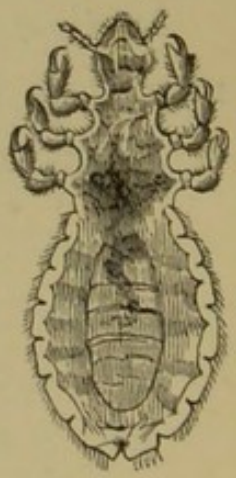
ANIMAL PARASITES.—INSECTA.

§ 107. **PEDICULUS CAPITIS.** — This small animal is about a line in length, and of a semi-transparent whitish colour. The female is larger than the male. The head and thorax are well marked off from the oval-shaped abdomen. The head is furnished with two short antennæ and a pair of large eyes. The animal possesses six legs, the last joints of which are developed into strong claws. On the back of the male a conical chitinous penis can be seen.

FIG. 45.

The eggs are oval and somewhat flat at their anterior extremity, where an operculum can be found. The opposite extremity is truncated and is attached to the hair.

FIG. 46.



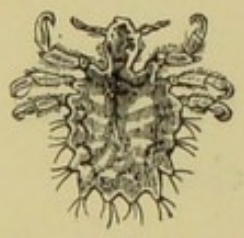
The young are hatched in about six days. This parasite is world-wide in its distribution.

§ 108. **PEDICULUS CORPORIS VEL VESTIMENTORUM.**—This species resem-

bles the above in almost every particular, but is somewhat larger and is possessed of larger claws. The colour is a dirty white. It infests the clothing, especially that made of wool. The ova are also deposited on the clothes, and are hatched in about five days.

§ 109. **PEDICULUS PUBIS.**—This louse is smaller and shorter than either of the preceding, the separation between the head and abdomen being less marked. The abdomen is rounded, and the louse has a slight general resemblance to a crab.

FIG. 47.



The anterior feet have not the claws well developed, but in the posterior ones the claws are very massive.

The eggs are smaller than those of the preceding species, but otherwise resemble them.

This louse infests the hair on the pubis, and is also sometimes found among the hairs in the axilla and the eye-lashes.

CHAPTER VI.

VEGETABLE PARASITES.

VEGETABLE parasites may be divided into three main classes :

1. Schizomycetes or fission fungi, which includes the whole of the bacteria, etc.
2. Saccharomycetes or yeast fungi.
3. Hypomycetes or moulds.

The first of these classes is dealt with separately in the section devoted to Bacteriology, and it is not necessary to do more than mention them in this place.

The more important members of the remaining two classes will now be briefly described.

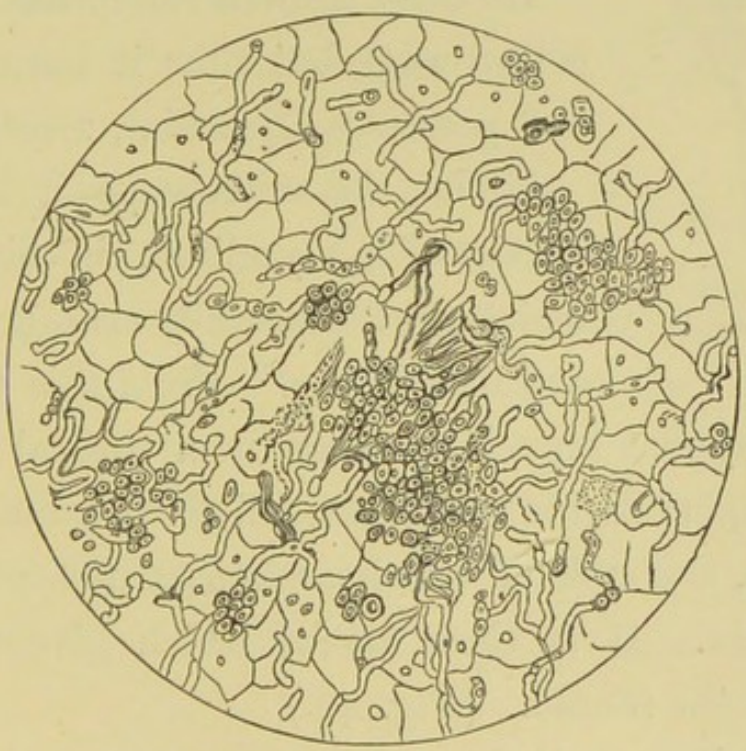
§ 110. **SACCHAROMYCETES.—Torula.**—This fungus consists of small ovoid cells about 0·0085 mm. in diameter. No nucleus has been observed, but the protoplasm usually presents one or two bright spots or vacuoles. A thin delicate cell envelope can be seen. Their method of propagation is by means of a process of germination. A small bud is seen on the side of the cell, which enlarges and becomes gradually cut off from the parent cell. Often, however, these cells remain attached by their

margins, and thus we usually see this fungus in chains of from three to five cells.

This fungus is the cause of vinous, acetous, and other fermentations, and is present normally in the intestines, in diabetic urine, &c.

§ 111. **HYPOMYCETES.**—To this group belong the fungi which are the cause respectively of tinea tonsurans, tinea favus, and pityriasis versicolor. These fungi greatly resemble each other, and will therefore be described together.

FIG. 48.



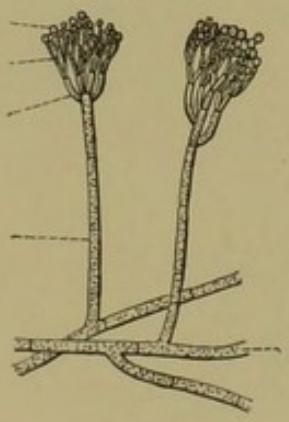
Trichophyton Tonsurans.

Tricophyton Tonsurans, Achorion Schönleinii, Microsporon Furfur.—These fungi consist of simple or branched tubes, which form the mycelium of the fungus, and which are sometimes jointed. The spore, from which the fungus is developed, elongates, thus forming

a mycelial filament. The protoplasm of the tube buds out laterally, and forms the spore-bearing tube. This then becomes segmented, the segmentation first appearing in the protoplasm, and finally spreading to the sheath. These spores remain for some time attached in threads, but finally become isolated, and repeat the process mentioned above.

§ 112. **PENICILLIUM GLAUCUM.**—This mould is very widely distributed, but though it is occasionally found on old ulcerating surfaces, it is rare as a human parasite.

FIG. 49.



Its early growth somewhat resembles cotton-wool, but later it becomes green and possesses a peculiar, musty odour.

Under the microscope it is seen to consist of a mycelium, the filaments of which are horizontally arranged, jointed, and undulating. From this the spore-bearing hyphæ branch upwards in a vertical direction. These hyphæ divide into forks, which terminate in fine filaments, the ends of which are segmented into rows of fine spores. It is these spores which give the green colour to the mould. (Fig. 49.)

Loeffler's methyl blue stains the mycelium and the hyphæ, but leaves the spores unstained.

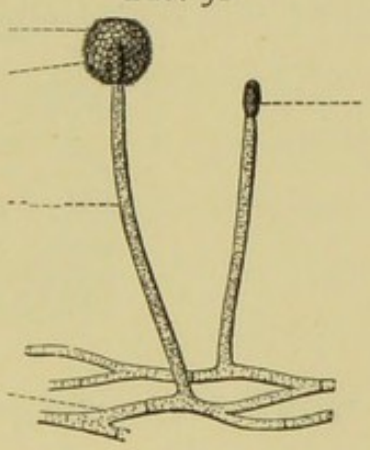
FIG. 50.

§ 113. **MUCOR MUCEDO.**—This mould is of very common occurrence, and is found in the excreta of many animals.

Under the microscope, the mycelium

is seen to consist of branching filaments, from which the hyphæ arise in a vertical direction. The heads of the hyphæ swell out into knobs, around which a capsule known as the sporangium forms. In this sporangium the spores are developed and escape only after the bursting of the capsule. (Fig. 50.) The spores are black, and about as large as poppy seeds. It is never found in man.

FIG. 51.



§ 114. **ASPERGILLUS NIGER.**—This mould is of a greyish-black colour. Like the varieties already described, the mycelium consists of a horizontally arranged system of filaments, from which the vertical hyphæ proceed. The distinctive feature of this mould is the swollen, club-shaped ends of the hyphæ from which the spore-carriers or sterigmata proceed, the upper ends of which become segmented off into spores. The bunches of spores appear as rounded, black swellings. (Fig. 51.)

§ 115. **ASPERGILLUS GLAUCUS.**—This mould resembles the above, only differing in fact as regards its colour.

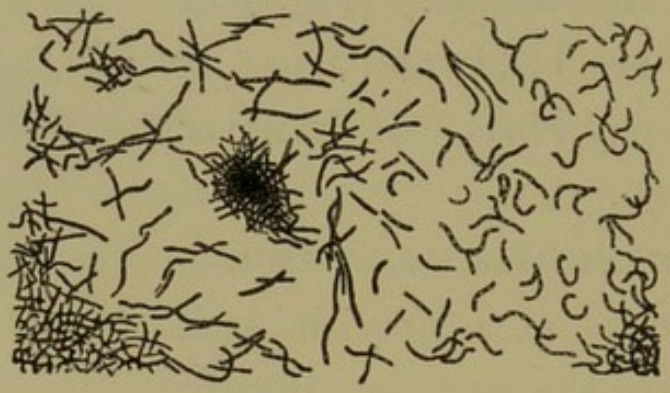
§ 116. **ACTINOMYCES OR RAY-FUNGUS.**—This organism is the cause of a disease, more or less common in cattle, known popularly as "wooden tongue." In man its principal seat is the liver, where it causes the formation of multiple abscesses.

The fungus forms small yellow tubercules, about the size of poppy-seeds. Under a high power these nodules

are seen to consist of a number of filaments, which radiate from the centre, each filament being thickened and clubbed at the peripheral end. (Fig. 52.)

At present there is much doubt as to the position

FIG. 52.



which this fungus should occupy in a systematic classification, but it seems probable that its true position will ultimately be found amongst the fission fungi.

§ 117. **OIDIUM ALBICANS.**—This fungus has for years been identified as the cause of the specific stomatitis, to which the name of Thrush is given. The white patches, so characteristic of this affection, consist of epithelial scales, mycelium fibres, leucocytes, and bacteria. The mycelium consists of branching fibres, from which the horizontal hyphæ proceed in a vertical direction. No special spore-bearing organs are developed, but the ends of the hyphæ become segmented, thus forming the spores.

§ 118. **BEGGIATOA ALBA, OR SEWAGE FUNGUS.**—This organism is widely distributed, and is found largely in sewage effluents, and the effluents of sugar and other factories. It belongs to the order *Lepto-*

trichea and is morphologically a bacterium. It consists of numerous filaments attached by their bases to some alga or other substance and measuring from 2 to 5 mm. in diameter. The maturer filaments always contain numerous grains of sulphur. On microscopic examination it is seen that the filaments are segmented into a number of thin discs, which later divide in the longitudinal axis of the filaments into quadrants. These quadrants after a time become detached and then appear as spherical cocci, containing two or three large grains of sulphur. The cocci move about for some time, and finally become attached as stated above, growing out into straight and spiral filaments. There is a tendency for the spiral filaments to break up into actively mobile fragments, resembling spirilla. These movements are due to a terminal flagellum.

CHAPTER VII.

EXAMINATION OF STARCHES.

§ 119. **METHOD OF EXAMINATION.**—The recognition of the various starch granules is of some importance, owing to the adulteration of flour, etc., with the commoner and cheaper varieties of starches, such as potato and rice.

In order to examine microscopically any starch, an exceedingly small quantity is dropped on a glass slide and one drop of water is then added. The starch should then be thoroughly mixed with the water and carefully covered with a cover-slip. Only a very thin film is required, as otherwise the granules become aggregated together and their outlines consequently blurred. Many of the starches to be presently described resemble each other very closely, and some considerable practice is necessary before the student will be able to differentiate them with any certainty. The student is recommended to first mount a series of preparations of all the principal starches and then make a drawing in his notebook of all the typical granules. This he should easily be able to do by comparing his preparations with the accompanying

plates. After he has gained a certain amount of proficiency, he should proceed to test himself by examining mixtures of two or more starches. It is not possible to make permanent preparations of starches. A quarter or sixth-inch power will be found the most suitable for this branch of work.

The characteristics of the various starches will be found below.

§ 120. **CANNA INDICA.**—The granules are large and irregular and somewhat oyster-shaped. The hilum is round and sometimes double. Well-marked concentric lines are to be seen. (Plate I. 1.)

§ 121. **CANNA EDULIS** or Tous le Mois resembles the former variety, but the concentric lines are much fainter. (Plate I. 2.)

§ 122. **BERMUDA.**—The granules are oval and irregular in shape and size and much smaller than in the preceding varieties. The concentric lines are almost indistinct, and the hilum is often slit-shaped. (Plate II. 1.)

St. Vincent and Natal arrowroots resemble this variety.

§ 123. **POTATO.**—The granules are very large and irregular and pass through all gradations in size down to very small ones. They are marked by indistinct, irregularly disposed concentric lines. There is usually to be found a circular hilum near the smaller extremity. Owing to its cheapness, this starch is often used to adulterate both arrowroot and wheat flour. (Plate II. 2.)

§ 124. **WHEAT.**—This starch consists almost entirely of two sizes of granules. The granules are all circular

and, as stated above, are either large or very small, very few granules intermediate in size being found. Faint concentric striæ may be seen in the larger granules, but the hilum is always indistinct. (Plate III. 1.)

§ 125. **BARLEY.**—This starch resembles wheat in consisting mainly of two sizes of granules, but the small size in this variety are even smaller than in the case of wheat. The large granules exhibit more distinct concentric granules, but the hilum is indistinctly marked. (Plate III. 2.)

§ 126. **RYE.**—The granules of this starch bear a general resemblance to the two preceding varieties. There are, however, three distinct sizes, the largest granules being larger than is the case in either wheat or barley, and there is also a well-marked intermediate size. The large granules often present a cruciform hilum, but the concentric rings are very indistinct. (Plate IV. 1.)

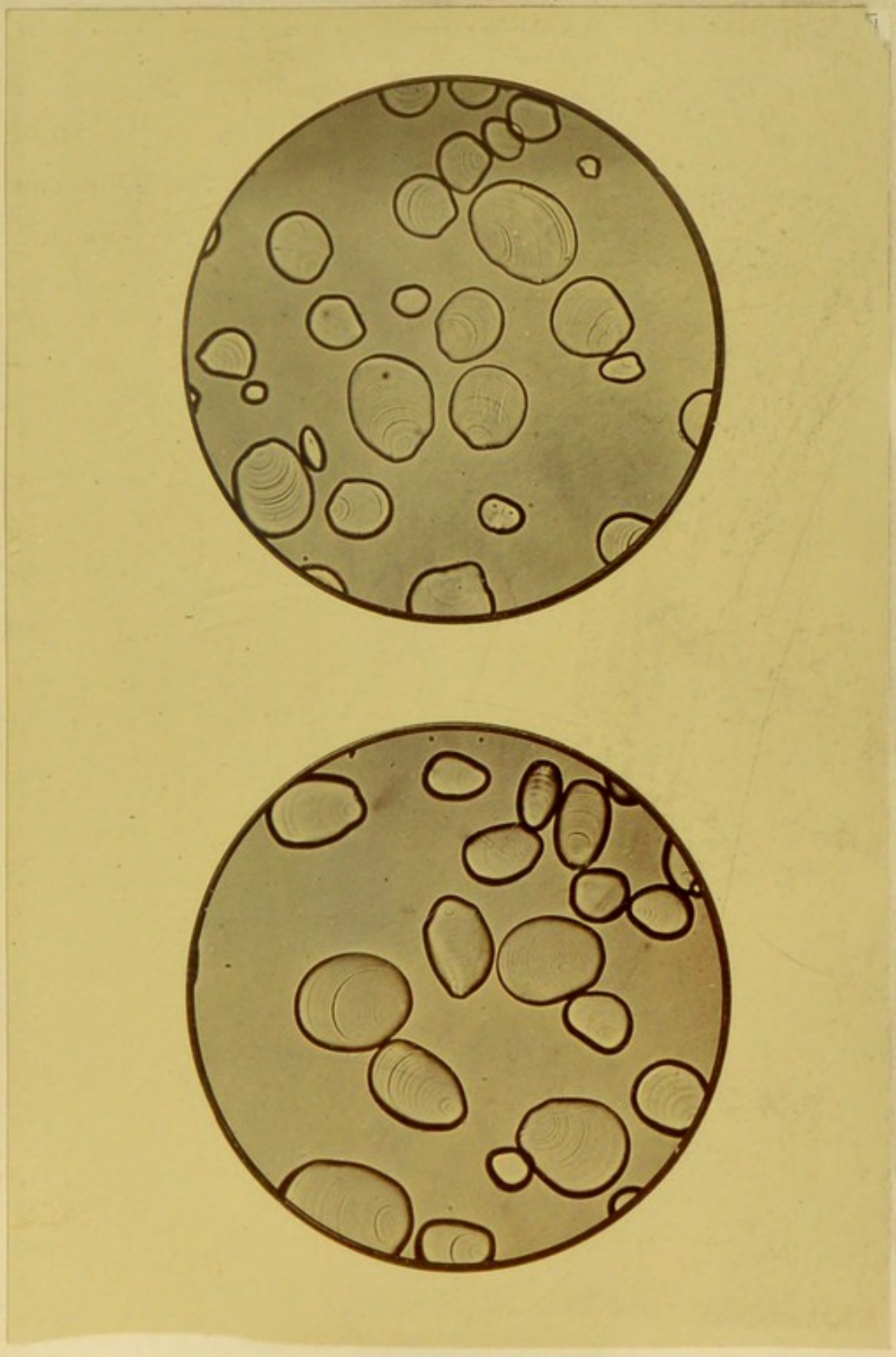
§ 127. **RICE.**—This starch consists of small angular and facetted granules of a uniform size. No hilum or concentric rings are visible. It is exceedingly often used as an adulterant, and it is perhaps fortunate therefore that its distinctive characteristics are so well marked. (Plate V. 1.)

§ 128. **MAIZE.**—This starch consists of large angular facetted granules, somewhat irregular in size and shape, and may be distinguished from rice and oats both by their larger size and by the fact that most of the granules contain a stellate or cruciform hilum. (Plate V. 2.)

§ 129. **OAT.**—This starch also consists of angular granules intermediate in size between rice and maize

STARCHES.

1. CANNA INDICA.



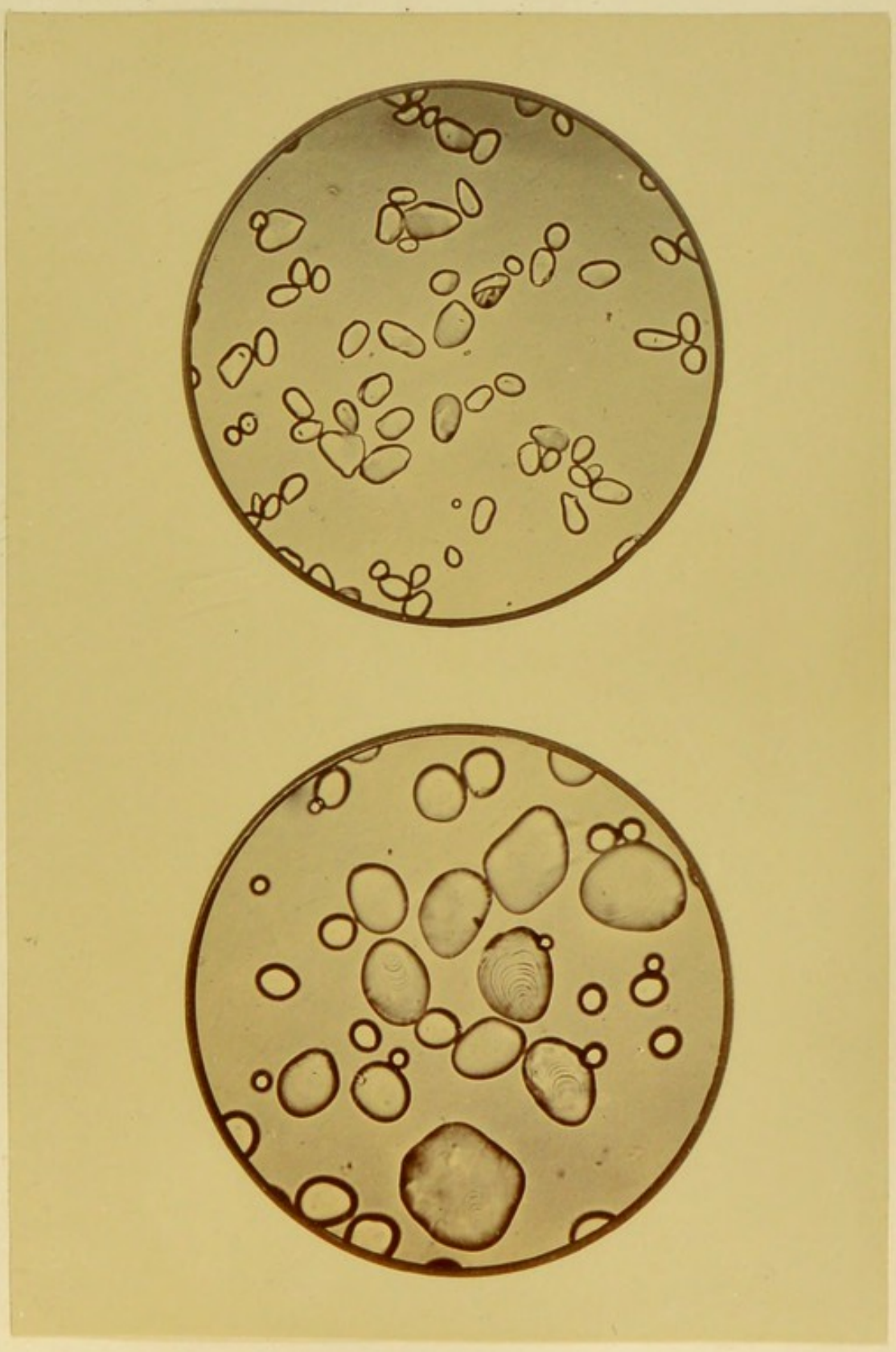
2. CANNA EDULIS.

× 160

STARCHES.

1. BERMUDA ARROWROOT.

Maranta arundinacea.



2. POTATO.

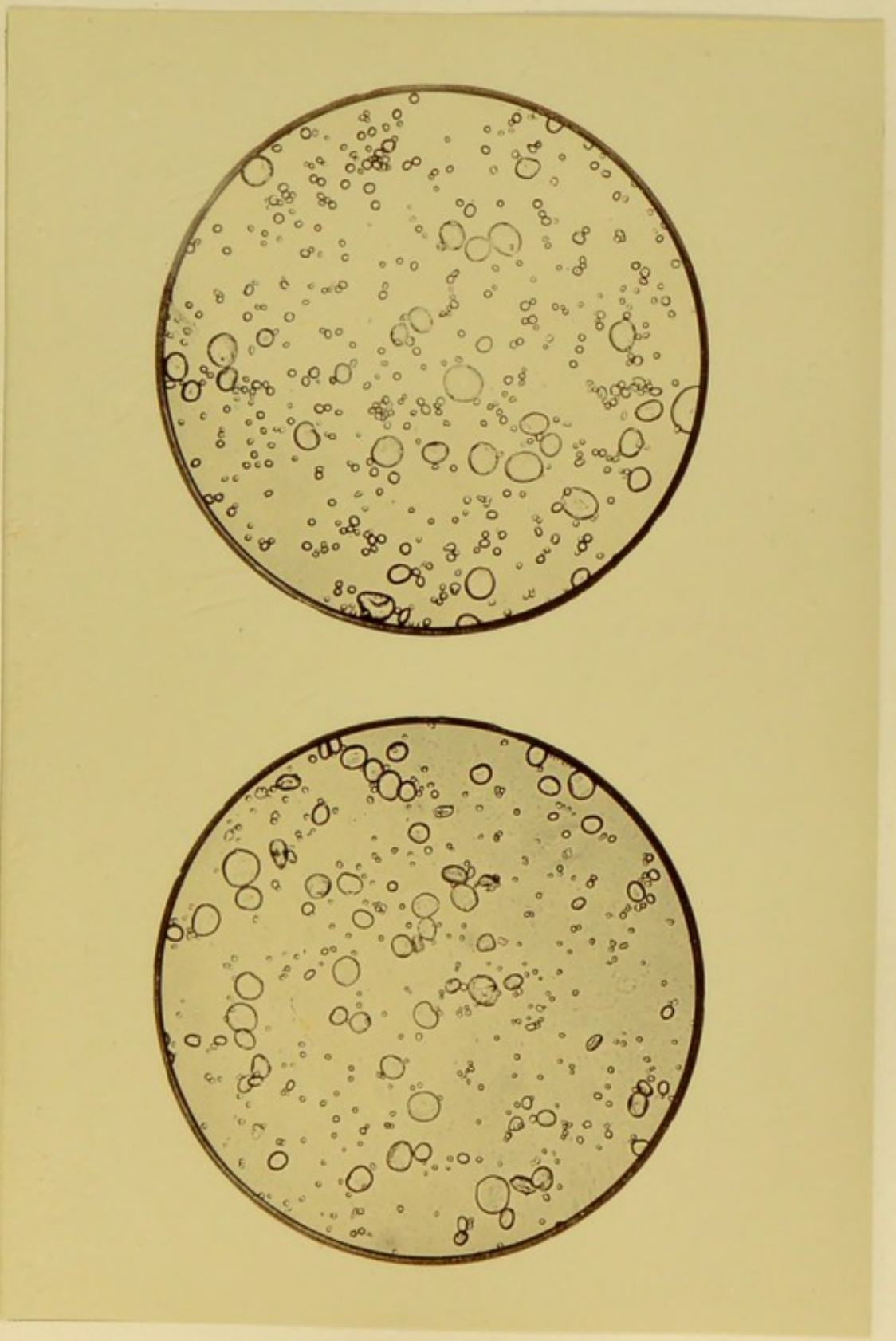
Solanum tuberosum.

× 160

STARCHES.

1. WHEAT.

Triticum vulgare.



2. BARLEY.

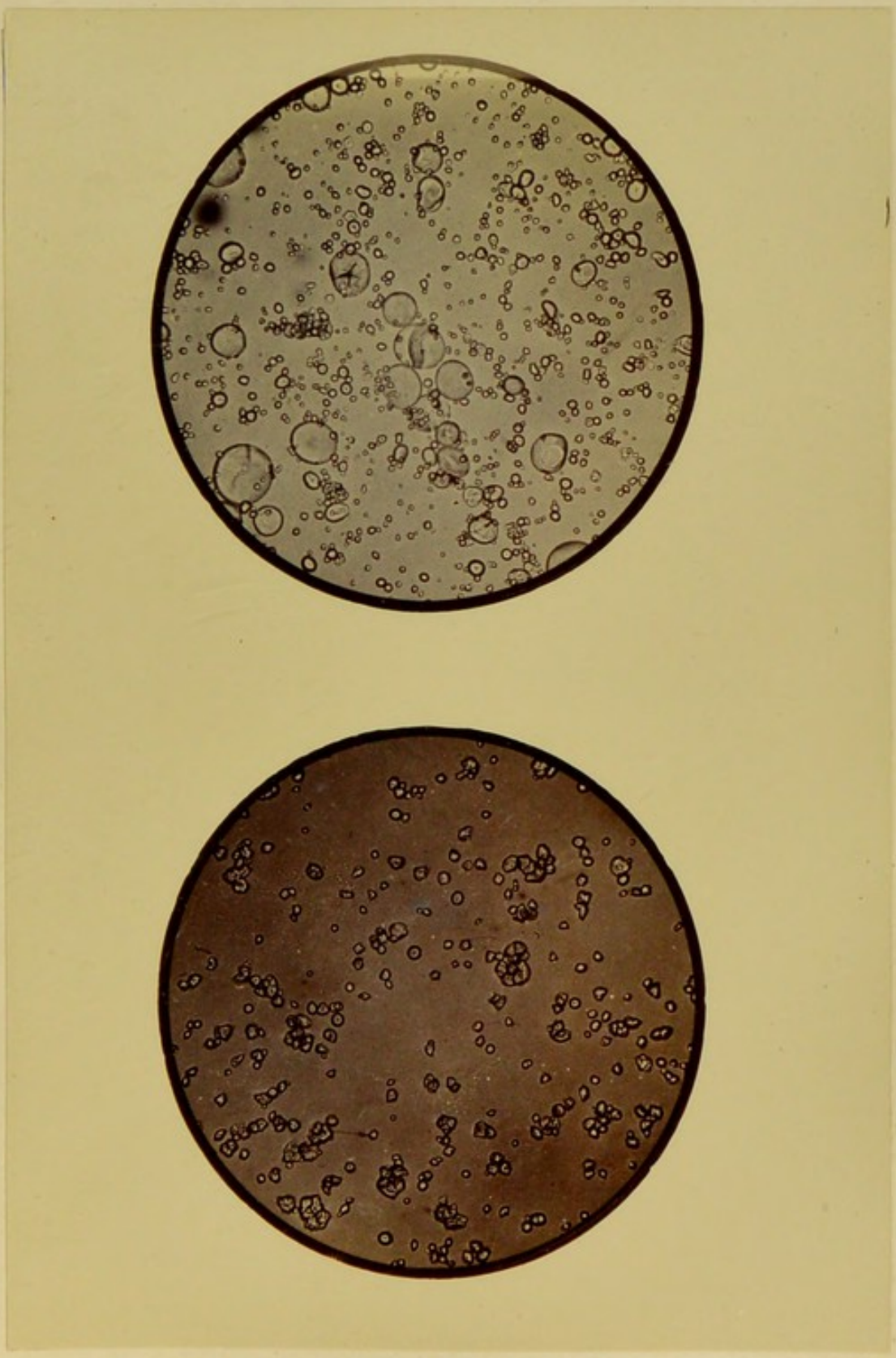
Hordeum vulgare.

× 160

STARCHES.

1. RYE

Secale cereale.



2. OAT.

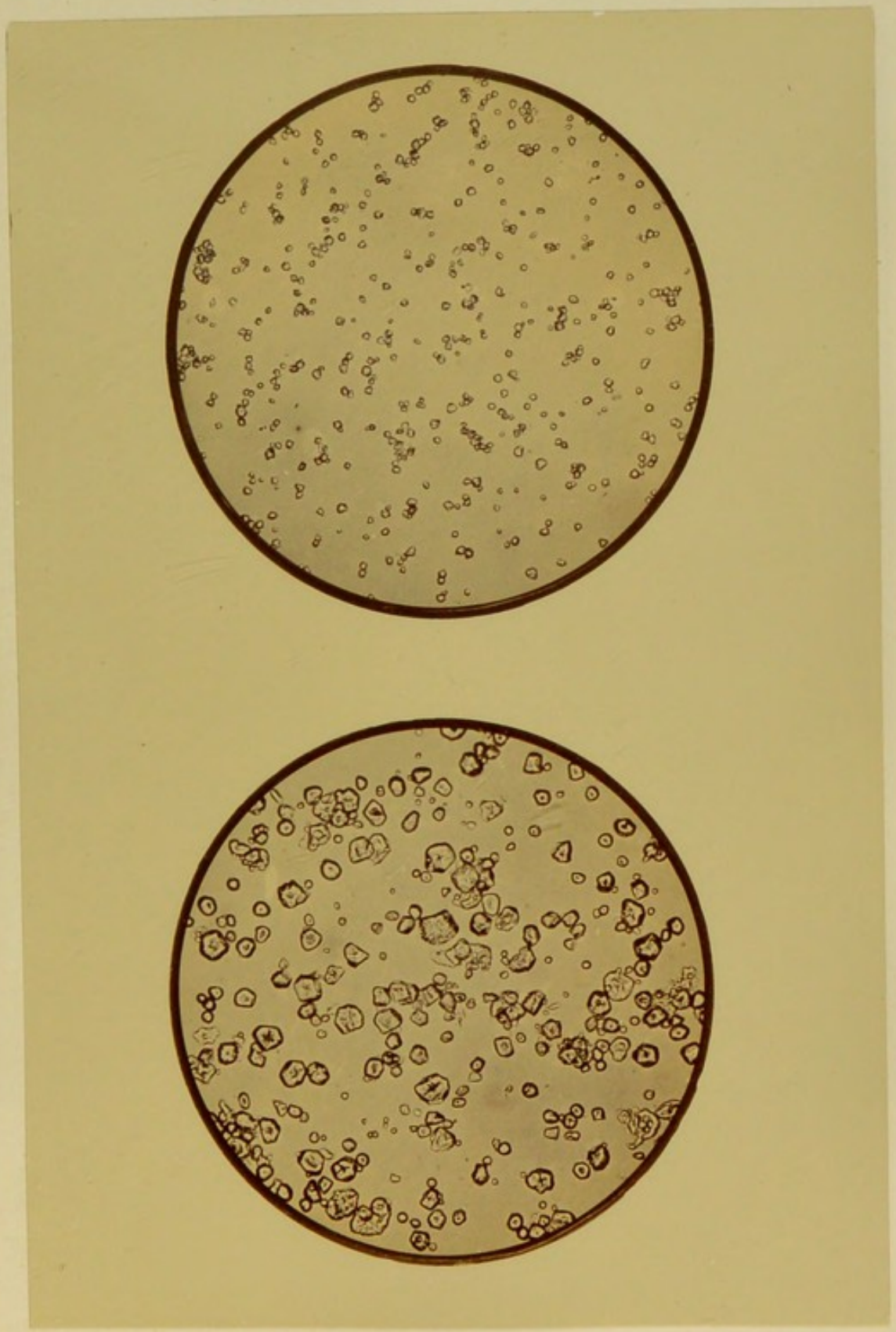
Avena sativa.

× 160

STARCHES.

1. RICE.

Oryza sativa.



2. MAIZE.

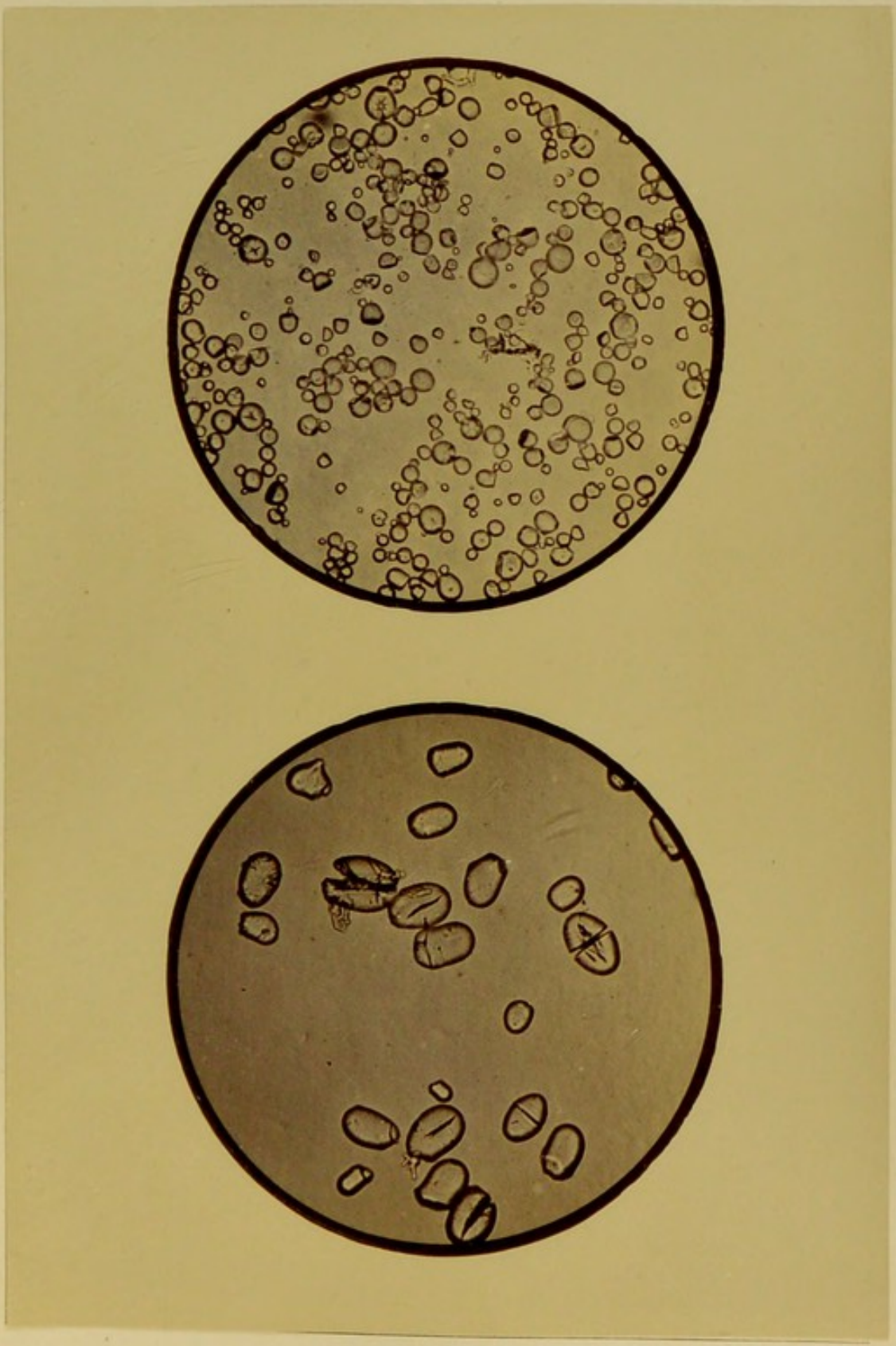
Zea Mays.

× 160

STARCHES.

1. TAPIOCA.

Manihot utilissima.



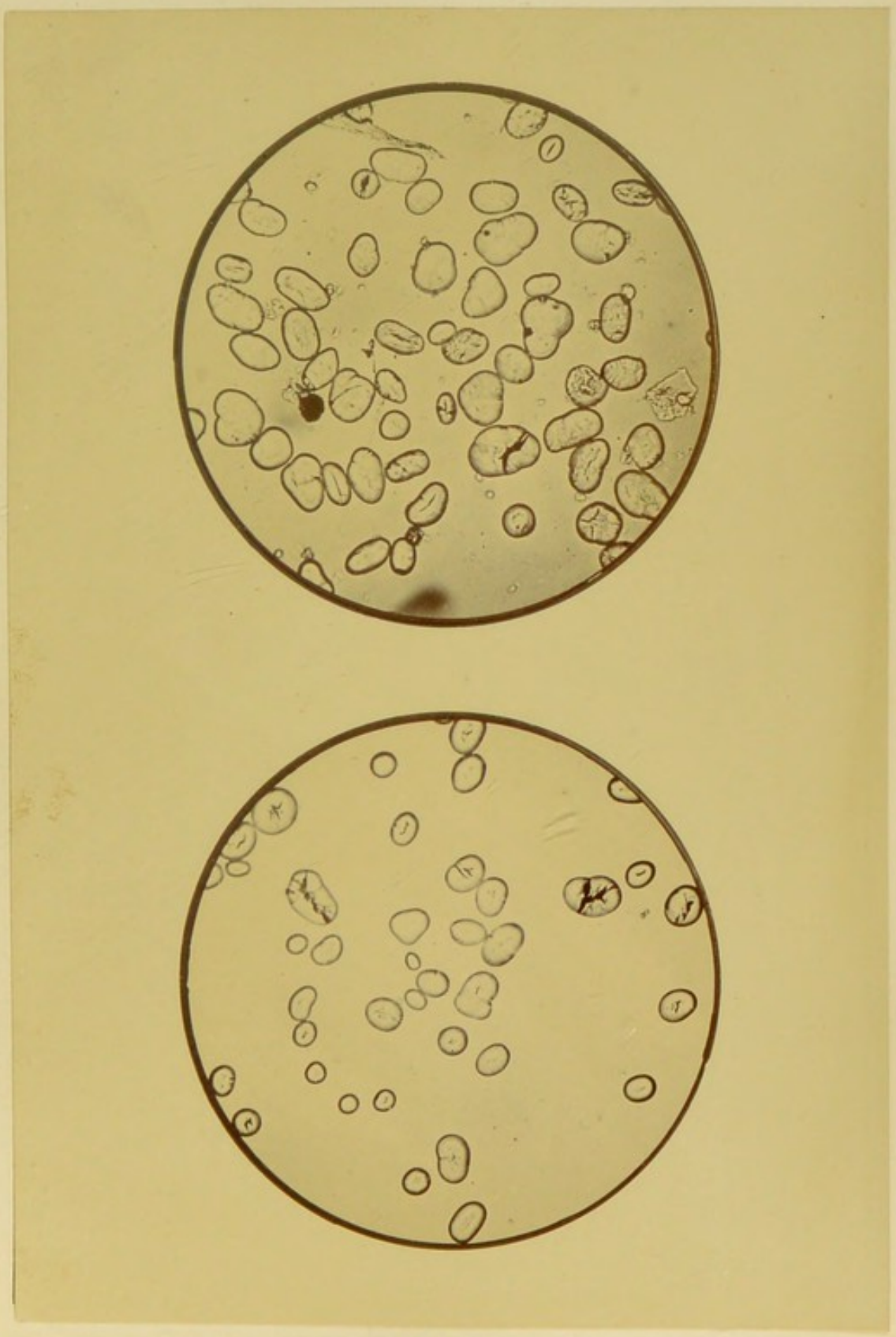
2. SAGO

Metroxylon sagu.

STARCHES.

1. PEA.

Pisum Sativum.



2. BEAN

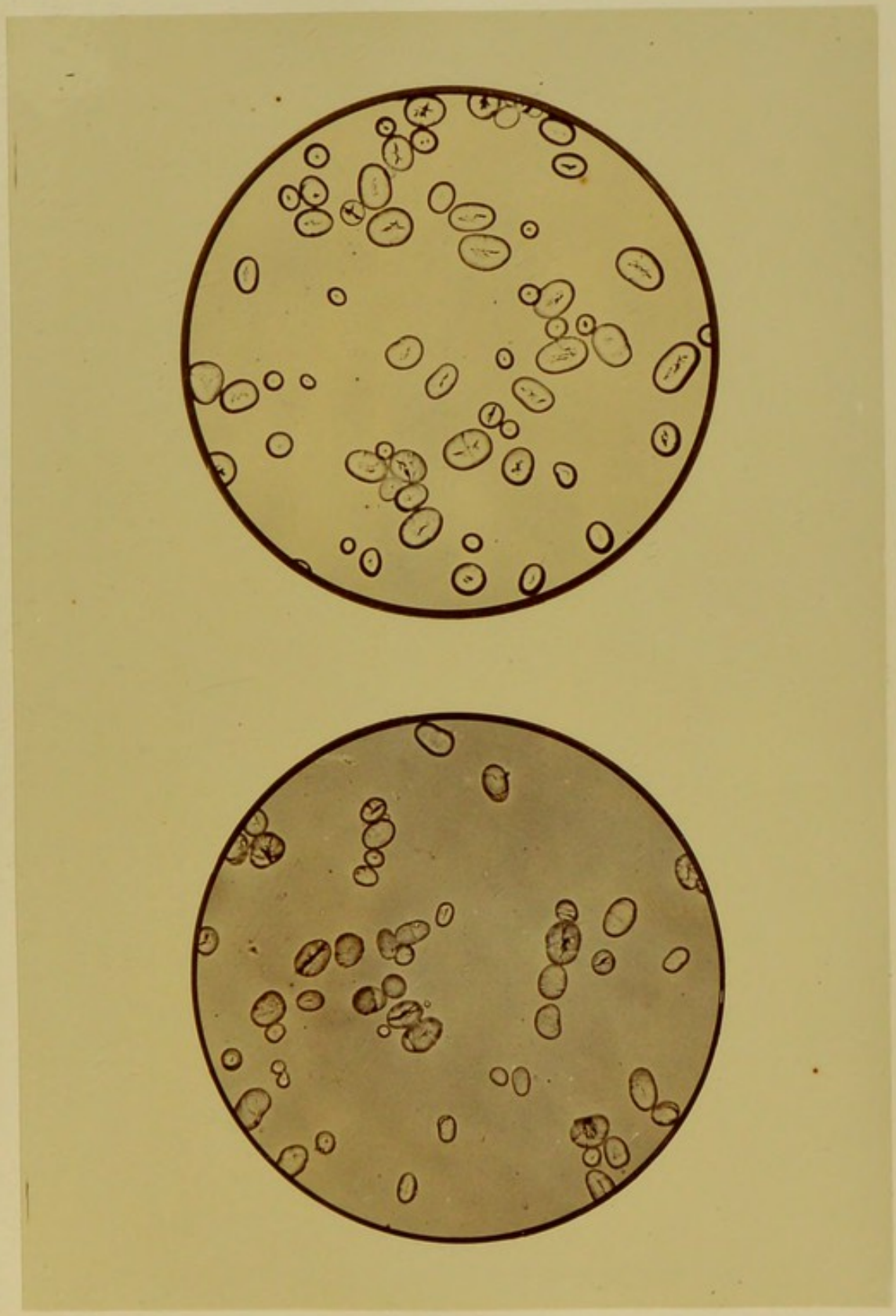
Faba vulgaris.

× 160

STARCHES.

1. HARICOT BEAN.

Phaseolus vulgaris.



2. LENTIL.

Lens esculenta.

× 160

starch. The granules exhibit a great tendency to adhere together and to form large circular masses. (Plate IV. 2.)

§ 130. **TAPIOCA.**—The granules which compose this starch are mostly of a medium size and circular shape. Some of the granules, however, are distinctly truncated and are thus bell-shaped. The hilum is usually distinct and is sometimes stellate in form. (Plate VI. 1.)

§ 131. **SAGO.**—The granules of this starch resemble the preceding, but are larger and usually irregularly oval. the smaller end often presenting a facet. The hilum is either linear or more often stellate. (Plate VI. 2.)

§ 132. **PEA.**—The granules are large and oval in shape, and are marked by very faint concentric lines. The hilum is usually slit-like and more or less puckered. (Plate VII. 1.)

§ 133. **BEAN.**—This starch is almost identical in its microscopical characters with the preceding. The linear hilum sometimes presents a branched appearance, but too much stress should not be laid on this point. (Plate VII. 2.)

§ 134. **HARICOT BEAN.**—This starch is also almost identical with the two preceding. (Plate VIII. 1.)

§ 135. **LENTIL.**—Although this starch resembles the three preceding varieties, it is possible to distinguish it, The granules are smaller and the concentric striæ are often more distinctly visible. The hilum is slit-like. (Plate VIII. 2.)

CHAPTER VIII.

EXAMINATION OF A WATER SEDIMENT.

§ 136. **METHOD OF PROCEDURE.** — The sediment of a water may consist either of mineral matter (clay, &c.) or of animal or vegetable matter (hairs, cotton, and linen fibres, starches, &c.), or else of living animal or vegetable organisms (torula, &c.).

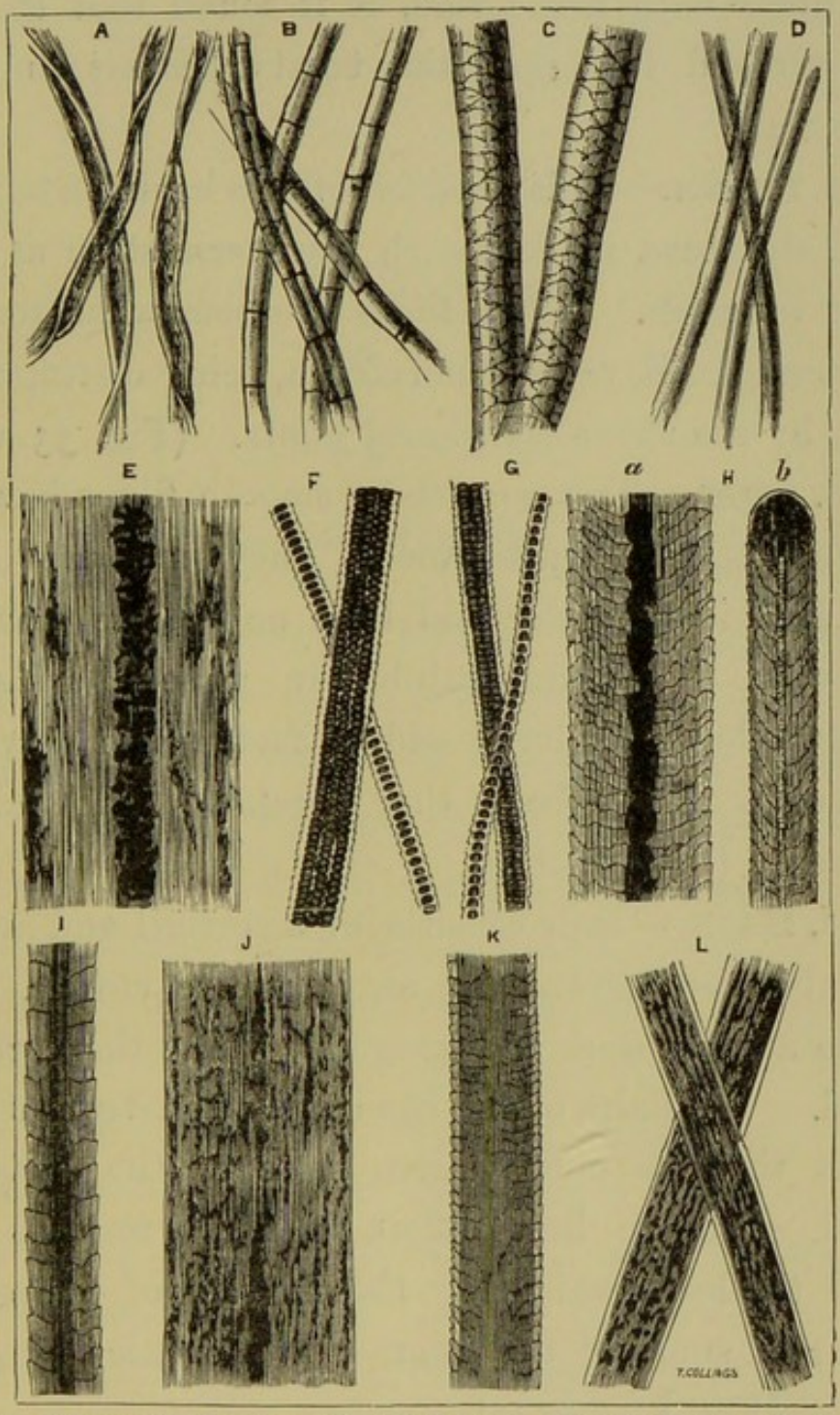
The water to be examined should be first poured into a tall glass and left for some time, in order that all the suspended matters may have time to sink to the bottom. A small portion of the sediment is then drawn off by means of a pipette, and a drop is placed on a slide and subjected to microscopic examination.

It is unnecessary here to deal in any detail with many of the matters found in a water sediment, as they have already been described in previous chapters, but no description has hitherto been given of the microscopic appearance of certain fabrics.

§ 137. **WOOL.** — Wool consists of rounded fibres covered with a peculiar spiral-like imbrication, which are usually colourless except when artificially dyed. When old, the fibres have a tendency to break up into

fibrillæ. The size of the fibres varies from 0·015 to 0·08 mm. (Fig. 53 c.)

FIG. 53.



A. Fibres of Cotton. B. Of Linen. C. Of Wool. D. Of Silk. E. Hair of Pig. F. Of Rabbit. G. Of Hare. I. Of Horse. J. Of Cow. K. Of Cat. L. Of Dog. H. Human Hair (*a*) from Head, (*b*) from Body.

§ 138. **COTTON.**—Cotton consists of riband-like twisted

fibres measuring about ·006 mm. in diameter, the edges of the fibres being somewhat thickened. On treating these fibres with nitric acid, it is found that they are not destroyed but that the twists become unrolled. (Fig. 53 A.)

§ 139. **LINEN.**—This consists of fibres having a tapering jointed structure, marked with transverse striæ at fairly regular intervals. These fibres are somewhat rounded and resemble silk rather than cotton, being distinguished from it by the above-mentioned joints. (Fig. 53 B.)

§ 140. **SILK.**—This consists of smooth fibres having a somewhat glassy appearance. They measure about 0·015 mm. in diameter and have no surface markings whatsoever. They are soluble in mineral acids and stained yellow by picric acid, a fact which serves to distinguish them from the vegetable fibres above described. (Fig. 53 D.)

§ 141. **HAIR.**—Hair consists of a cortical and medullary substance, covered by an imbricate cuticle. The medulla appears dark and irregular, whilst the cortex is striated. The hairs of the head are often truncated or split at the free end, whilst those of the body are pointed. Human hair differs in many respects from that of the lower animals. For the sake of comparison the hair of some of the commoner domestic animals is shown. (Fig. 53 E to L.)

The fact that any of these substances described above was found in a water sediment would furnish strong presumptive evidence of organic pollution.

PART VI.

BACTERIOLOGY.

CHAPTER I.

GENERAL CONSIDERATIONS.

§ 142. **OBJECT—DIFFICULTIES OF BACTERIOLOGICAL RESEARCH.**—It is not my intention to enter at length into the subject of bacteriological work, but merely in a general way to indicate the procedure which has to be adopted, and then more particularly to deal with the bacteriological examination of air and water.

The object of bacteriological research is to produce pure growths of various specific organisms, with the view of accurately determining their physiological and morphological characters; and to do this it is obvious that the method employed must be thoroughly trustworthy. Here, at once, the careful worker will recognise the difficulties which beset him, difficulties which arise from the extreme minuteness, the immense numbers, and the general distribution of micro-organisms. To leave instruments or vessels uncovered in the air for a short time is to afford facilities for the contamination by many varities of organisms, which either fall as spores upon

their surface with dust, or are brought in contact with it by currents of air. Contact with the hands, with clothes or with unsterilised vessels is attended with the same danger, and the worker must ever be on the alert to prevent this accidental and invisible contamination. To obviate this danger it is a matter of primary importance that all vessels, instruments, culture media, etc., should be bacteriologically clean or "sterile," by which I mean free from the presence of living micro-organisms.

§ 143. **METHODS OF STERILISATION.**—This sterilisation or cleansing may be effected either (1) by the application of heat, (2) by the use of chemicals, or (3) by filtration.

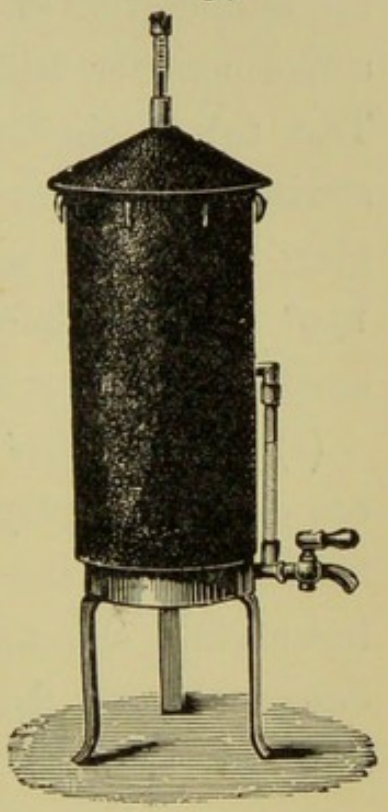
1. **Heat** is applied in three ways (a) as moist heat or steam, (b) as hot air, (c) by the direct application of a gas or other flame to the object to be sterilised.

(*a*) *Steam, or moist air sterilisation.* This is most usually effected by the use of a steam steriliser (Fig. 54), consisting of a metal cylinder covered with some such non-conductor as asbestos or felt, to prevent loss of heat, and fitted with a movable lid or cover, the centre of which allows of the insertion of a cork with a thermometer; the bottom is of copper, and heat is applied by one or more large Bunsen burners; water is placed in the cylinder below a movable perforated shelf or floor, upon which rest the vessels, etc., requiring sterilisation, and the height of the water is shown by a glass indicator placed outside.

The heat applied must be sufficient to keep the water

in a state of vigorous ebullition, and free vent must be given to the steam. In this way a uniform temperature of 100° C. is obtained, and exposure for one or two hours to such a temperature will usually effectually sterilise. But with some culture media (*e.g.* gelatine) in which the melting-point would be reduced, such prolonged exposure would be unadvisable, and such substances may be exposed for a short time, from twenty to thirty minutes, to this high temperature on two or three successive days in the manner suggested by Tyndall.

Fig. 54.



This method of sterilisation by intermittent applications of heat is based on the fact that the spores of the bacteria, perhaps by reason of the thicker capsule surrounding them, are much more resistant than the fully developed organisms. The bacilli being destroyed by the first heating, the spores left are given time to develop into bacilli, and are then destroyed in the second heating, the interval between the sterilisations not being long enough to allow of the development of spores in the newly formed bacilli.

In steam sterilisation the heat penetrates rapidly into the objects to be sterilised, and steam of the temperature of 100° C. sterilises much more rapidly than atmospheric air of the same or of a much higher tempera-

ture, which is probably to be explained by chemical action.

(*b*) *Hot air sterilisation* is effected in a hot-air oven, the temperature of which is governed by a gas regulator, a thermometer being fitted into the top of the oven. Test tubes, pipettes, plates, wool, etc., are all usually sterilised by means of dry heat. It is necessary to expose glass vessels for some two hours to a temperature of 150° C., and then it is advisable, in order to avoid cracking, to allow them to cool gradually in the oven.

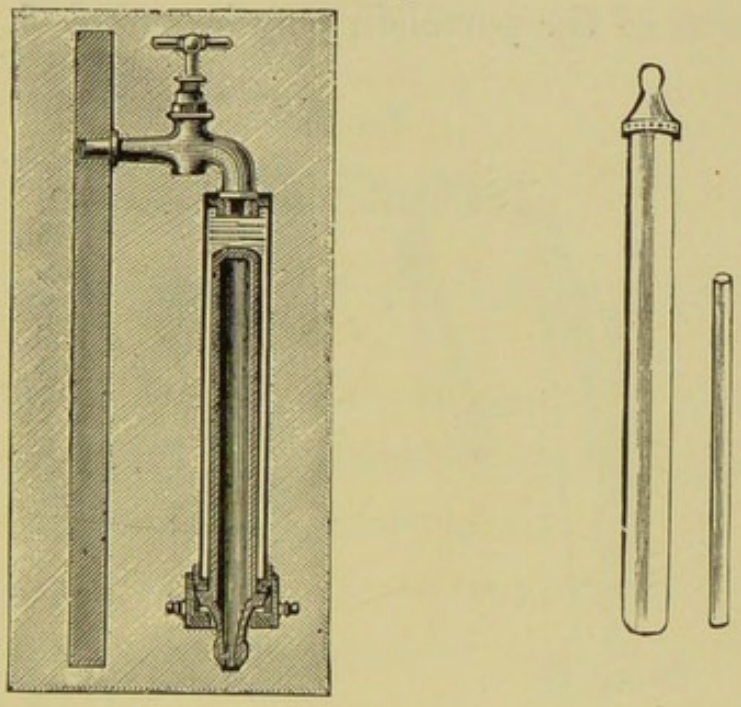
(*c*) *By the direct application of a flame.* This method can only be employed in certain cases, it is very useful for sterilising the platinum needle generally used for inoculating the media, as it can be heated to incandescence in the flame of a spirit lamp without injury, but knives, scissors, etc., cannot be so heated without completely destroying their temper.

2. **Sterilisation by Chemicals.**—Corrosive sublimate solution having a strength of 1 or 2 per 1000 may be used for cleansing the hands, instruments, morbid material, etc.; to prevent the precipitation of insoluble mercury salts, on the addition of some waters, such as well water, a little acetic acid may be added. Carbolic acid solutions of various strengths, may be substituted with advantage for many such purposes, as it does not injure steel instruments, which are quickly destroyed by the use of corrosive sublimate.

3. **Sterilisation by Filtration.**—As various chemical changes are induced by the action of heat in certain culture fluids which it is often necessary to avoid, their

sterilisation may be effected by means of filtration. There are various kinds of filters in use, but the most useful are the Pasteur-Chamberland filter, which is made

FIG. 55.



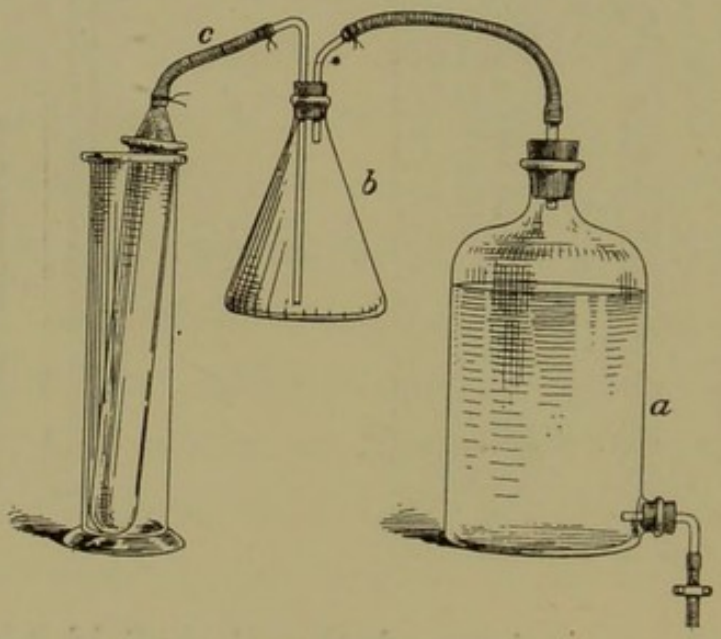
of unglazed porcelain, and the Berkefield filter, which consists of baked infusorial earth.

The Pasteur-Chamberland filter (Fig. 55) is the one in common use in the laboratory; it consists of a hollow porcelain cylinder, closed at one end and provided at the other with a funnel-shaped, glazed porcelain end piece, over which a flexible india-rubber tube can be pushed. The filter is immersed in the fluid to be sterilised, and the open end is connected with an aspirator. If the fluid to be sterilised is in too great quantity to be contained in the cavity of the filter, a flask for its reception is placed between the aspirator and filter.

The filter is usually employed for laboratory purposes either in the metal case sent out by the manufacturers,

or fitted up in a lamp glass (Fig. 56); and before being used a second time it must always be cleansed by being brought to a red heat by some such means as is afforded by a combustion furnace, in order that any organic matter in the pores of the porcelain may be removed. All the

FIG. 56.



apparatus used must be carefully adjusted and then the whole apparatus sterilised in the steamer.

In the case of water which, for experimental purposes, is required to be sterilised without such chemical changes as would be induced by boiling, filtration by means of a Chamberland or Berkefeld filter is all that is required. Milk also may be deprived of its fat in this way, a clear sterile fluid being obtained, and for many other purposes this method is most advantageous.

Gases also are readily deprived of any micro-organisms they may contain by filtering them through cotton wool, plugs of sterile cotton wool being commonly used for plugging the mouths of flasks, test-tubes, etc.

CHAPTER II.

THE ISOLATION OF MICRO-ORGANISMS.

This isolation is necessary in order to obtain pure cultures of particular organisms uncontaminated by the presence of other or foreign organisms.

§ 144. **BY THE METHOD OF DILUTION.**—This consists in largely diluting the liquid containing the micro-organisms, and then dividing the diluted fluid into a number of small fractions, so that each fraction contains not more than one micro-organism, the starting-point of a pure growth thus being obtained.

This method, although laborious, is under some circumstances most necessary, as with the case of nitrifying bacilli which do not grow on ordinary solid media. Miquel has also adapted this method to the examination of water.

§ 145. **THE METHOD OF PLATE CULTURES.**—**Gelatine Plate Cultures.**—These cultures are made either upon ordinary photographic glass plates or by using culture dishes (Fig. 57).

(*a*) If photographic glass plates are used they should be of quarter-plate size, and should be first soaked in

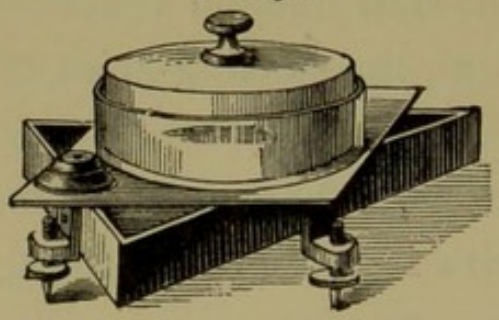
caustic soda, washed with water, dilute hydrochloric acid, then with water again, and finally rinsed with distilled water. They are then sterilised by placing them in a metal box, which is placed in a hot-air oven and exposed for some time to a temperature of 150° C. or 160° C.

FIG. 57.

They are retained in the oven, the gas of which has previously been turned out, until they are required for use.

It is desirable to have a cylindrical glass dish filled with ice and water. This is covered with a thick glass plate, care being taken that the dish is quite full, or otherwise air bubbles will be enclosed, which will prevent the uniform cooling of anything which may be placed on the plate-glass top. The dish is then put on a three-screw levelling stand (Fig. 58), and a spirit-level is placed on the plate-glass, so as to indicate when a level surface is arrived at; a glass bell jar is then placed on the glass plate.

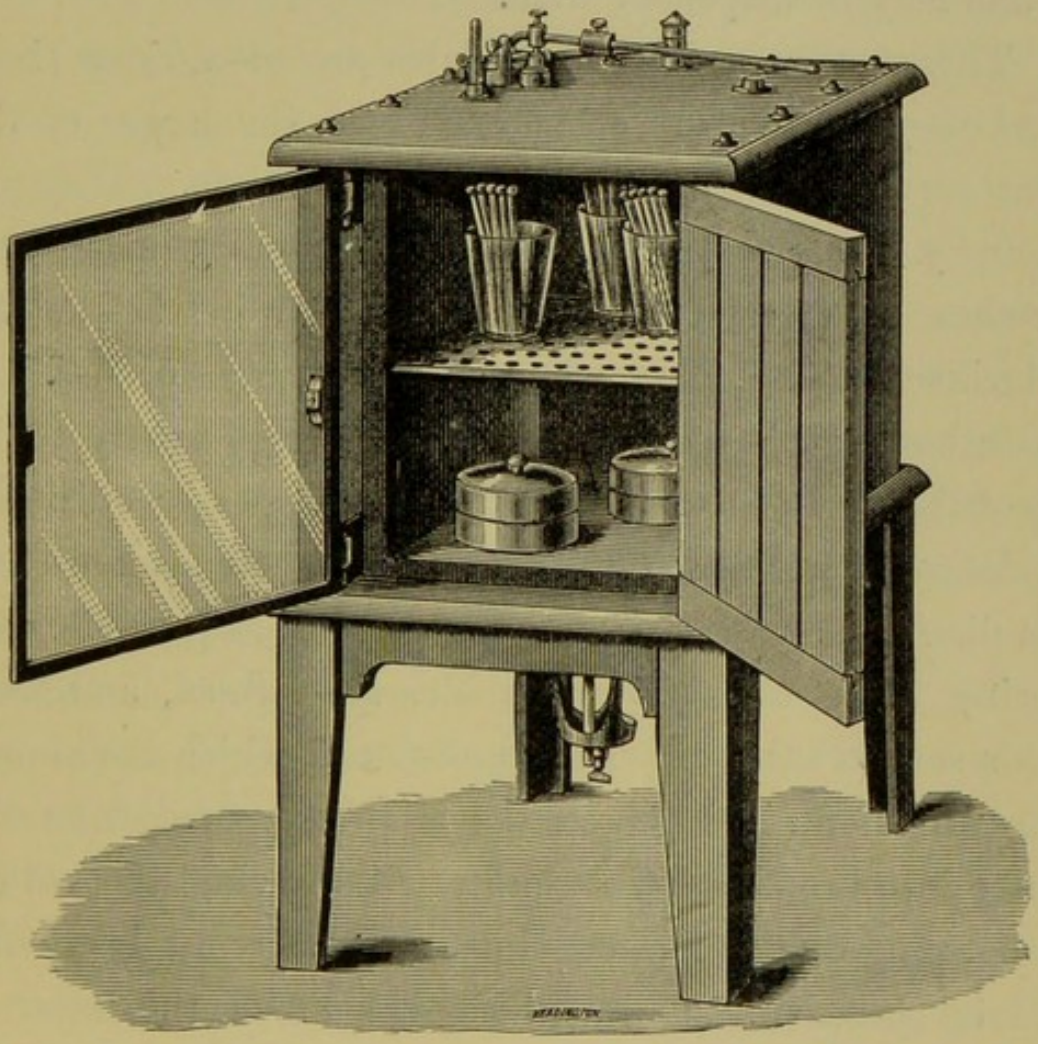
FIG. 58.



It being desired to make a cultivation upon one of the prepared plates, it is carefully removed from the steriliser, with the future upper surface held downwards, so as to prevent contamination, and it is placed with this face

upwards under the bell jar of the cooling apparatus with the view of receiving the liquefied gelatine, which has been purposely infected with the micro-organisms, and which is to be poured upon it from the test-tube, the bell jar being partly lifted for the purpose.

FIG. 59.



After congelation has taken place, the plate is placed in a damp chamber, which may conveniently be made by taking an ordinary dinner plate and adapting to it a bell jar, either placing a little sterilised distilled water in the hollow of the plate, or a little moist blotting paper in the upper part of the bell jar. The damp chamber

and plate are then placed in an incubator, in which a temperature of 18° C. to 22° C. is maintained.

(*b*) If culture dishes are used, the details of the procedure are much the same. This method is in many respects better than the one first described, for there is less risk of aerial contamination, and the levelling apparatus may be dispensed with.

These culture dishes or plates are of various sizes, but they go in pairs of unequal size, the larger of the two serving as a cover to the smaller plate. They are usually $\frac{1}{4}$ inch to $\frac{1}{2}$ inch in depth and from 3 inches to 4 inches in diameter, and are sterilised in the hot-air steriliser. The plate when cold is ready for use. A gelatine tube is inoculated in the way presently to be described. It is then placed in warm water until the gelatine is melted; the tube is then gently shaken so as to distribute the organisms as uniformly as possible, care being taken that no air bubbles are formed, and from this several other tubes are inoculated, which are known as "dilutions." The gelatine is then poured from the tube into the plate by carefully withdrawing the cotton wool plug of the former whilst in a more or less horizontal position, and by lifting the lid of the latter to such an extent only as is necessary to accomplish the object desired. The lid is then quickly replaced, and the dish gently moved about to equally distribute the gelatine over the surface. It is then placed aside to permit of the gelatine setting, and subsequently placed in a damp chamber and then in the incubator (Fig. 59).

§ 146. **THE METHOD OF TUBE CULTIVATIONS.**—

These are of two kinds: those in which solid media are used, and those in which fluid media are used, such as broth or milk.

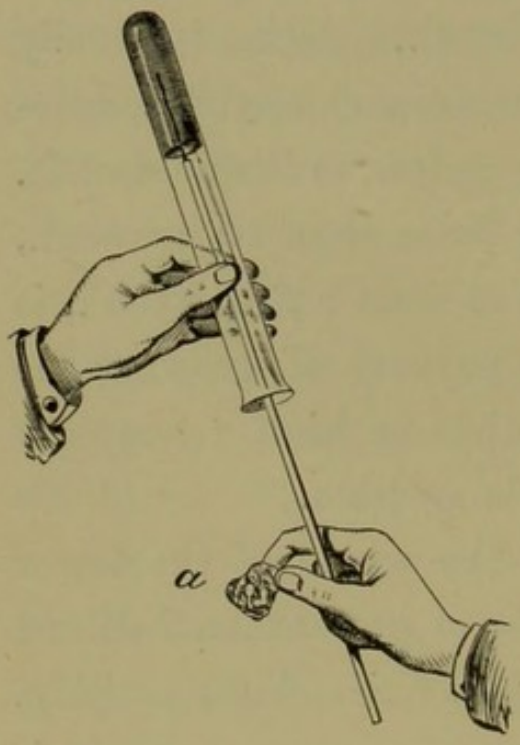
(*a*) Tubes in which solid media are used, such as gelatine, agar-agar, serum, etc. If the organism has to be grown at a low temperature, such as 20° C., gelatine is usually selected; if at a higher temperature, such as 30° C., agar-agar, serum, etc., gelatine being useless, as it would melt. A stronger gelatine, containing more than 10 per cent., which will stand a temperature of from 24° to 25° C., is not unfrequently used for the purpose of growing the diphtheria bacillus, which requires as high a temperature as can be obtained with this medium.

The medium either occupies the whole of the lower part of the tube, or it is made to present an inclined surface by allowing it to solidify in this oblique position. The former tubes are used for stab cultures when it is desired to study the mode of growth of the organism in the depth, the culture being made by a platinum needle armed with the organism stabbing the medium to some distance in its centre. The latter tubes are used for surface cultures, or growths, when the platinum needle is carried more or less over the whole surface, or for streak cultures, when the platinum needle is drawn once down the centre of the surface of the medium.

(*b*) Tubes containing fluid culture media may be inoculated in one of two ways: (1) With a platinum wire which has been sterilised in a gas flame, and, when cold, used for touching the colony it is desired to cultivate. The cotton-wool plug is withdrawn by steri-

lised forceps from the culture tube held in a slanting position, and the wire is dipped in the fluid and moved briskly about, with the object of detaching some of the organisms. The cotton-wool plug, previously held for a moment in the gas flame, is then re-inserted.

FIG. 60.



When the tube to be inoculated contains a solid medium, this is held in the left hand in an inverted position, whilst the tube from which the culture is to be taken is held in the same hand, either in an inverted or slanting position, according as the bacteria have or have not liquefied the gelatine. The cotton-wool plugs are then withdrawn, and a sterilised platinum needle is quickly plunged into the culture and removed. The tube is inoculated in the manner desired, and the cotton-wool plugs replaced. (Fig. 60).

Esmarch's Tube Cultures.—This is a modification of the method of plate culture. A gelatine tube is taken and inoculated in the usual way. The gelatine is then melted by placing the tube in warm water. The gelatine is then gently agitated to distribute the organisms. An india-rubber cap is fitted over the cotton-wool plug, and the tube is carefully rotated in a horizontal position in iced or cold water, so that the solidification of the gela-

tine may take place in an even layer over the whole internal surface of the tube.

In plate cultivations, or in Esmarch's tube culture, the colonies, as soon as they have sufficiently developed, are examined microscopically. Each colony, if it has sufficient space, is almost always a pure growth; so that if a portion be removed by means of a sterile platinum needle to a tube containing gelatine or other culture material, the organisms may be perpetuated in a pure state. When the colonies are few in number, it is easy enough to isolate the organisms in this way, and to facilitate this, certain ingenious instruments have been devised by Fodor[1] and Unna,[2] the former of which can be obtained from Calderons & Co., of Deakgasse, Budapest, the latter from Zeiss, of Zena.

[1] Fodor, "Central. f. Bakteriolojie," x. 721.

[2] Unna *Ibid.*, xi. 278.

CHAPTER III.

MICROSCOPIC EXAMINATION OF MICRO-ORGANISMS.

Such examination may be made by simply placing a little of the culture when obtained by a platinum wire from a gelatine or agar tube upon an object glass, rubbing it with a little salt solution, covering with a cover glass, and then examining it under the microscope. In the same way a droplet of a broth culture may be examined.

§ 147. **CLASSIFICATION OF STAINS.**—The identification of micro-organisms has been greatly facilitated by the use of staining agents. These may be roughly divided into two classes, the basic and the acid. Ehrlich has shown that the staining principle in the acid is ammonium picrate, and the basic of a staining base in combination with an acid that does not stain, acetate of rosaniline.

The basic coal tar dyes show a great affinity for the protoplasm of bacterial cells and for the nuclei of animal tissues.

The acid coal tar colours, on the other hand, when

applied to a section of animal tissue stain it throughout its entire extent.

The basic aniline dyes in most frequent use are fuchsine, magenta, gentian violet, methyl violet, methylene blue, Bismarck brown and vesuvin.

Ehrlich calls those dyes neutral which are formed by the union of an acid and a base, both of which stain, *e.g.*, picrate of rosaniline.

It is best to make a saturated alcoholic solution of each of these for stock purposes—*i.e.*, 25 grains of the dye to 100 grains of absolute alcohol—which for use should be diluted with ten times their volume of distilled water, but only small quantities of these diluted solutions should be made at a time, as they very soon lose their staining powers, with the exception of methylene blue. Vesuvian or Bismarck brown is best kept in powder, or if a solution be required this is best made by saturating it with equal parts of glycerine and water.

The colours in most common use are magenta, gentian violet, and methyl violet.

The principal acid coal tar colours are cosine acid, magenta safranine, picric acid, and aurantia.

§ 148. **PREPARATION OF STAINS.**—The staining powers of the aqueous alcoholic solution of the basic aniline dyes are sometimes greatly increased by the addition of certain substances, more particularly when it is desired to stain spores or flagella. These substances, such as potash, carbolic acid, aniline oil, or tannin act as mordants and show a tendency to unite both with the dye and the object to be stained, thus serving as a sort

of connecting link. The preparation of some of the principal of these may be given.

(1) Loeffler's alkaline methylene blue solution, 100 c.c. of a solution of caustic potash (1 : 10000) add 30 c.c. of a concentrated alcoholic solution of methylene blue.

(2) Ehrlich's solution, 4 to 5 c.c. of aniline (obtained from the benzene of coal tar) and shaken with 100 c.c. of distilled water, and allowed to stand for about twenty-four hours. It is then passed through a damp filter, so that the excess of undissolved oily aniline particles may be retained by the filter. To the clear filtrate or aniline water are added 11 c.c. of concentrated alcoholic solution of either fuchsine, gentian violet, or methyl violet; it is then frequently shaken during twenty-four hours, when the liquid becomes clear and ready for use. It only retains its staining powers for a limited period.

3. Loeffler has modified Ehrlich's solution by dissolving 5 grains of solid fuchsine or any other basic colour in 100 c.c. of aniline water prepared as described. This preserves its staining properties for from four to six weeks, and should be kept in the dark in a stoppered bottle.

4. Weigert and Koch solution—another modification of Ehrlich's solution—is to add to 100 c.c. of the aniline water 11 c.c. of a concentrated alcoholic solution of fuchsine or methyl violet and 10 c.c. of absolute alcohol. This solution will keep 10 or 12 days.

5. Ziehl's carbolic fuchsine—1 part of fuchsine, 10 parts of alcohol, and 100 of 5 per cent. carbolic acid. Kuhne has replaced the fuchsine by adding 1·5 grain of methylene blue to 10 c.c. of absolute alcohol, which is

mixed with 100 c.c. of a 5 per cent. aqueous solution of carbolic acid.

§ 149. **DECOLORISING AGENTS.** — Sometimes it is necessary, with the view of obtaining greater definition, to stain in two colours. For this purpose it is necessary to use certain agents which have the effect of removing the stain from some parts of the tissues whilst the other parts remain unaffected. A second stain is then used which does not affect in any way the already stained portion of the preparation.

The principal of these decolorising agents are :

(1) 5 per cent. aqueous solution of acetic acid.

(2) 20 per cent. aqueous solution of nitric acid.

(3) 3 per cent. alcoholic solution of hydrochloric acid (100 parts absolute alcohol and three parts hydrochloric acid).

(4) 25 per cent. of sulphuric acid solution.

(5) Iodine.

§ 150. **METHODS OF STAINING.**—Koch and Ehrlich's method. This is used for staining tubercle and leprosy bacilli. Aniline water is prepared as previously described, and alcoholic solution of fuchsine, gentian violet, or methyl violet is added to it until a fairly saturated solution in dilute alcohol is obtained. Small masses of sputum are smeared over a cover glass and then spread out by rubbing it with a second, so that both glasses become coated with a thin film. The glass after drying is then passed gently through the flame of a burner, so as to fix the film, the prepared side being held upwards; it is then placed with this face downwards in a watch glass which contains

the staining fluid, and left for some twenty-four hours at the temperature of an ordinary room or placed in an incubator at 20° or 30° for a shorter period. The cover glass is next lifted from the dye with a pair of forceps and placed in a solution of 33 per cent. of nitric acid for a few seconds, until the red colour of the preparation becomes a yellowish green; it is then washed in 70 per cent. alcohol. If fuchsine has been used for the staining, the contrast staining may be done with methyl blue, malachite green, or picric acid; but if the first staining has been done with gentian or methyl violet, then Bismarck brown may be used for the second. This secondary staining lasts from one to five minutes, until the colour used is plainly visible on the cover glass; it is then washed in water, dried, and mounted in Canada balsam. The cover glasses after washing in water may be brought into alcohol and subsequently treated with oil of cloves and Canada balsam. In this staining the nitric acid acts as a bleaching agent to all bacilli, with the exception of the tubercle and leprosy bacilli.

Ziehl and Neelsen's.—In this carbolic fuchsine is used instead of aniline water fuchsine. The decolorisation is effected in a 33 per cent. nitric or in a 5 per cent. sulphuric acid solution. In all other respects the process resembles the Koch-Ehrlich method, the advantage consisting in the fact that the fuchsine stains in five or ten minutes when warmed. Secondary staining is effected by means of malachite green, picric acid, or methyl blue. This method possesses the great advantage of quickness, and by it sputum can be stained in a few minutes.

Ehrlich's Method.—This is used for demonstrating the presence of tubercle bacilli in pus. The material should be spread in a thin layer on a cover glass and placed for one or two hours in cold aniline oil fuchsine solution and decolorised with sulphanil-nitric acid (1 part of nitric acid to 3 to 6 parts saturated solution of sulphanilic acid). The secondary staining is done with methyl blue.

Fraenkel's Method.—The cover glasses are stained with aniline water fuchsine and transferred to a fluid consisting of a saturated solution of methyl blue in 50 parts of water, 30 parts of alcohol, and 20 parts of nitric acid. When the preparation appears blue it is washed in alcohol and acetic acid, or in pure water, and is examined in water. In this method the process of decolorisation and second contrast stain are carried out in one solution.

Method of Pfuhl and Petri.—The preparations are stained in a mixture of 10 c.c. alcoholic solution of fuchsine to 100 c.c. of water and subsequently decolorised in glacial acetic acid. They are then washed in water and double stained with malachite green, again washed in water, dried, and mounted in Canada balsam. The glacial acetic acid is here the decolorising agent.

Gram's Method.—This depends upon the employment of iodine in aqueous solution combined with potassium iodide (1 part iodine, 2 parts iodide of potassium and 250 parts of water). After the preparation has been stained in aniline water solution of gentian violet, the iodine forms with the colouring matter a precipitate which adheres to the micro-organisms but can

be easily washed out of the tissues, and then the bacilli or micrococci appear isolated by the stain. The cover glass preparation or section is first placed in an aniline water solution of gentian violet for about five minutes, after which it is placed in the iodine solution for two minutes, and then washed with alcohol until no more colour is removed; it is then placed in clove oil, by means of which more colour is abstracted. By this method also it is found that certain micro-organisms lose the stain whilst others retain it, and thus a means of differentiation is provided between micro-organisms, *e.g.*, the bacilli of cholera, typhoid fever, and glanders, the spirilla of recurrent fever, etc., as well as the nuclei of cells. Its most important application is perhaps found in distinguishing between pus organisms and the gonococcus, the former only retaining the stain. It is also very useful in examining for diphtheria, since this organism and the pus microbes are both stained in the process.

Gunther has modified Gram's method by washing the preparation with a 3 per cent. alcoholic hydrochloric acid after the alcoholic washing of Gram and also by substituting xylol for clove oil.

Fuchsine, methylene blue and Bismarck brown cannot be used for Gram's method but only the pararosaniline colours, to which belong methyl violet, gentian violet, and Victoria blue, all of which have a strong affinity for iodine.

Weigert's Modification of Gram's Process.—The sections stained with gentian or methyl violet are not transferred to alcohol from the iodine solution but laid

upon slides and covered with aniline oil, which dehydrates and differentiates them. The aniline oil is then removed with blotting paper, xylol is poured upon the preparation and it is put up in Canada balsam in xylol.

Gram's method lends itself very readily to the use of a contrast stain for picking out the tissues in a section. It may be stained with picro-carmine before being subjected to the Gram method, or it may be stained with eosin or Bismarck brown after the decolorisation by alcohol before being cleared during the Gram process.

§ 151. **STAINING OF FLAGELLA.**—Loeffler stains the flagella of motile micro-organisms by taking 10 c.cm. of a 20 per cent. solution of tannin and a few drops of saturated ferrous sulphate solution with fuchsine, or 4 or 5 c.cm. of extract of logwood. Staining is effected with fuchsine in aniline water to which a 1 per 1000 solution of caustic potash has been added until it becomes turbid. For bacteria which form alkalies the mordant must be rendered correspondingly acid; for those which form acids, the mordant must be alkaline.

§ 152. **STAINING OF SPORES.**—For this purpose Ziehl's solution of carbolic acid fuchsine is used. The infected cover glass is left for an hour in the boiling dye, when the spores remain of a red colour after washing with water and decolorising with alcohol, or if double staining with methyl blue be carried out, the bacilli appear blue and the spores dark red.

Spores are also brought out distinctly as little granules by staining with dilute alkaline methyl blue, in which

case, after double staining with aqueous solution of Bismarck brown, they appear blue on a brown ground.

Moeller's method for staining spores is as follows. The cover glass preparation is brought for two minutes into absolute alcohol and for two more into chloroform washed in water; plunged for half a minute to two minutes into a 5 per cent. chromic acid solution and again rinsed with water, after which aqueous carbolic fuchsine solution is dropped on and the cover glass warmed in the flame until it has been once brought to the boil. The carbolic fuchsine is then poured off and the cover glass dipped into 5 per cent. sulphuric acid until decolorised, and once more thoroughly washed with water. An aqueous solution of methyl blue or malachite green is allowed to act on it for half a minute and then washed. The spores appear dark red in the interior of blue or green bacteria.

CHAPTER IV.

THE BACTERIOLOGICAL EXAMINATION OF AIR.

The micro-organisms which float in the atmosphere have been investigated in many ways.

There are two modes in use for collecting air and its organisms. In the one the dust is allowed to settle by its own weight, and in the other the air is drawn into a suitable apparatus by the use of an aspirator.

§ 153. **METHOD FOR QUALITATIVE ANALYSIS.**—The simplest method is to take several pairs of shallow glass trays, which should be sterilised at 150° C. Into the smaller of these should be carefully poured the media upon which the cultivations are to grow, this being most commonly peptonised meat infusion with agar or gelatine; the gelatine should be allowed to solidify and taken to the spot where the examination is to be made. The lids or larger of the trays are then taken off and the gelatine left exposed to the air for a longer or shorter time, varying with the abundance of germs; the lids are then replaced, and the plates set aside for the organisms to grow

at the temperature of the room, or they are placed in an incubator.

The gelatine, instead of being poured into trays, may be spread on glass plates which are then set within a pair of trays.

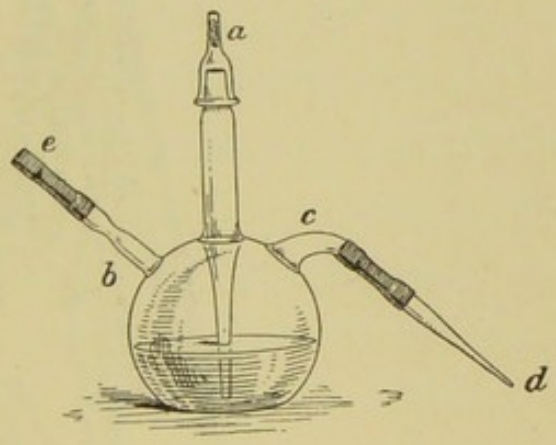
This method is to be recommended for the qualitative analysis of atmospheric air, but it of course affords no criterion of the number of micro-organisms in a given quantity of air.

§ 154. **POUCHET'S METHOD.**—The apparatus used by this observer for the examination of air consists of a glass cylinder capable of being made air-tight by means of a screw and clamps. It is placed vertically upon a stand, which is perforated above and below. In the upper aperture is a glass tube with a narrow exit; whilst the lower one is made to communicate with an aspirator by means of a piece of india-rubber tubing, and in the centre of the cylinder is a ridge supporting a small glass plate smeared with glycerine. The aspirator being put into action, air passes in through the upper opening, and in its passage deposits the greater part of its contents upon the glycerine plate, which, so soon as sufficient air has been drawn through, is removed from the cylinder. The deposit is distributed as evenly as possible through the glycerine by stirring it with a sterilised needle. The glass plate is then removed and covered with a second plate for the purpose of microscopical examination, or placed in an incubator under proper conditions. A calculation is made of the number of organisms in a litre of air by counting the colonies on several plates, so

as to ascertain the average number. In this way the number of organisms contained in a litre of air is ascertained.

§ 155. **MIQUEL'S METHOD.**—Miquel's apparatus consists of a flask with two natural tubes, and a third tube which fits by a ground glass joint into the aperture at the top, thus supporting a cap of glass which is closed with a cotton wool plug. (Fig. 61.) One of the lateral tubes is connected with an aspirator; the other is connected by a piece of india-rubber tubing with a narrow glass tube sealed at the end. The flask is then filled with 30 to 40 c.c. of water and sterilised in the steam steriliser. The glass cap is then removed and a given quantity of air admitted by means of the aspirator. The cap is then replaced, and by blowing air through the lateral tube which communicates with the aspirator, the water is driven up into the vertical tube, which thus washes it out. The point of the glass tube on the opposite side is then broken off and the fluid contained in the flask distributed into tubes of sterilised broth.

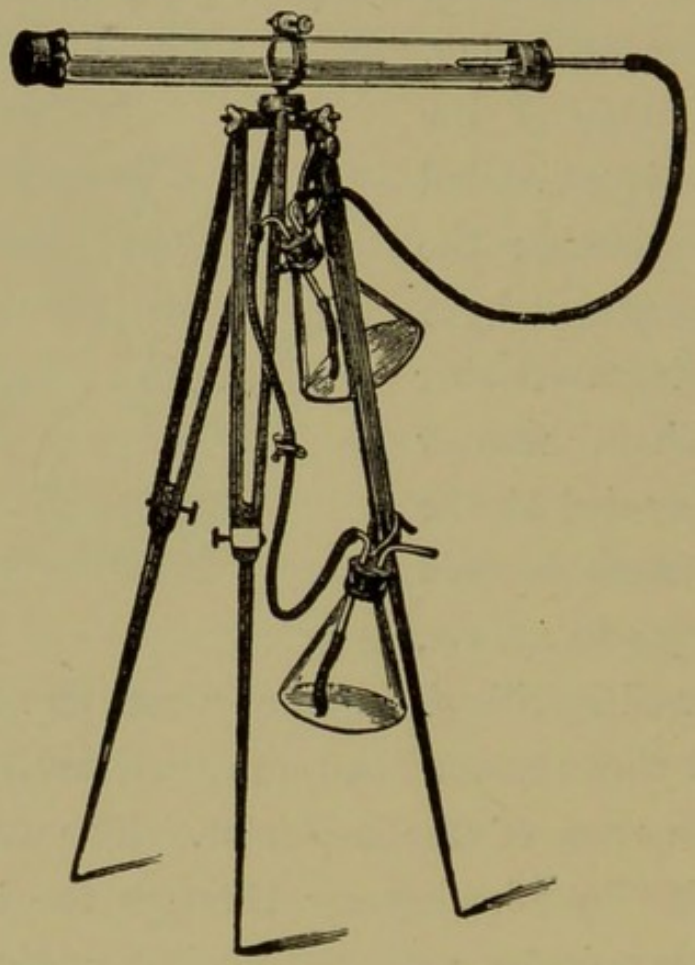
FIG. 61.



§ 156. **EMMERICH'S METHOD.**—By this method Emmerich draws the air slowly through an apparatus consisting of a coiled tube filled with nutrient broth, by which the organisms are retained.

§ 157. **HESSE'S METHOD.**—By this method the air is made to pass by means of a slowly acting aspirator through a sterilised tube, the walls of which are coated with gelatine. The tube, which is 70 cm. long, with a diameter of 3 to 4 cm., is placed horizontally and

FIG. 62.



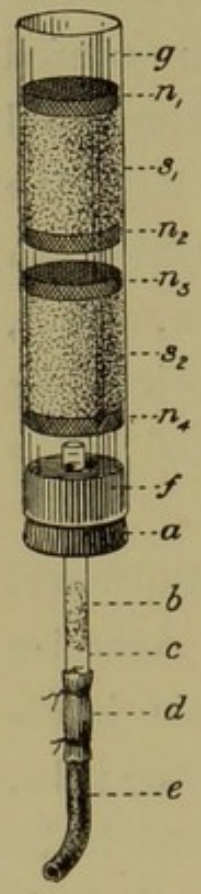
covered at one end with a tightly stretched india-rubber cap, which has a small hole in its centre, and over which a second non-perforated cap can be placed, whilst the other end, which is closed with a caoutchouc plug perforated in its centre, is connected with the aspirator by means of a small glass tube about 1 cm. wide and 10 cm. long. (Fig. 62.)

When the air is being aspirated the unperforated india-rubber cap must be removed. Two flasks are used for the purposes of aspiration, the one being filled with water and united by means of an india-rubber tube to the second flask, the other, which is empty, is placed at a lower level than that containing the water, so that this may be able to flow into it, and in this way a volume of air corresponding to the quantity of water used is aspirated into the receiving bottle. The heavier organisms, such as bacilli, are found chiefly in the fore part of the tube, whilst the spores of moulds are carried further into the interior. The experiment is concluded by replacing the unperforated cap. In a few days the gelatine is found to be covered with colonies which can be distinguished from each other by their form, colour, and action on the gelatine; they are then isolated by surface cultures in gelatine tubes and examined in the ordinary way.

Before using the Hesse's aeroscope, the stopper and caps are cleansed in a 1 per cent. solution of corrosive sublimate and then rinsed with boiled water. An india-rubber cap is then tied fast with thread to the tube, which is half filled with water. If it is proved to be water-tight by holding the end downwards a hole is clipped in the india-rubber cap and the second cap is tied over it. Its power to hold water is then again tested; the other end is now plugged and the whole apparatus, still containing water, is hung in the steam steriliser, when, after exposure for a quarter of an hour, it is removed, and after becoming somewhat cooled, the

water is poured out and replaced with all proper precaution by the liquid sterile gelatine. The stopper being again replaced, the apparatus is once more hung in the steam steriliser and exposed for ten to fifteen minutes to 100° C., after which it is removed and gently rotated in a horizontal position until the gelatine is nearly hard, when it is allowed to lie flat, so that one part of its circumference is more thickly coated than the rest. When required for use it is set upon a proper support, where the air is to be examined.

FIG. 63.



a, rubber stopper; n_1 to n_4, brass gauze caps; s_1 and s_2, sand filters; *c*, sterilised glass tube; *b*, *f*, & *g*, cotton wool plugs; *e*, lead tube; *d*, rubber tubing.

Hesse recommends that 1 to 5 litres should be passed through the apparatus for inhabited rooms at the rate of one litre in one to three minutes, and 10 to 20 litres out of doors, at the rate of one litre in 3 to 4 minutes. The air having been sucked through, the apparatus is closed by replacing the rubber cap, which has been washed in a solution of corrosive sublimate.

§ 158. **PETRI'S METHOD.**—This method depends upon the use of a sand filtering apparatus (Fig. 63), which consists of a tube 8 or 9 cm. long and 1·5 cm. in width, one end of which is closed with wire netting, upon which quartz sand (the grains of which vary from $\frac{1}{4}$ to $\frac{1}{2}$ mm. in size) is placed to a depth of 3 cm.; a second wire netting is now placed on it, and a second quantity of sand, and then a third piece of netting. 50 to 100 litres of air

are now drawn through the sand by means of a water aspirator, at the rate of 10 litres in a minute, the quantity being determined by a gas meter. When the aspirator is completed the sand filter is separated out into different glass capsules containing nutrient gelatine.

FIG. 64.

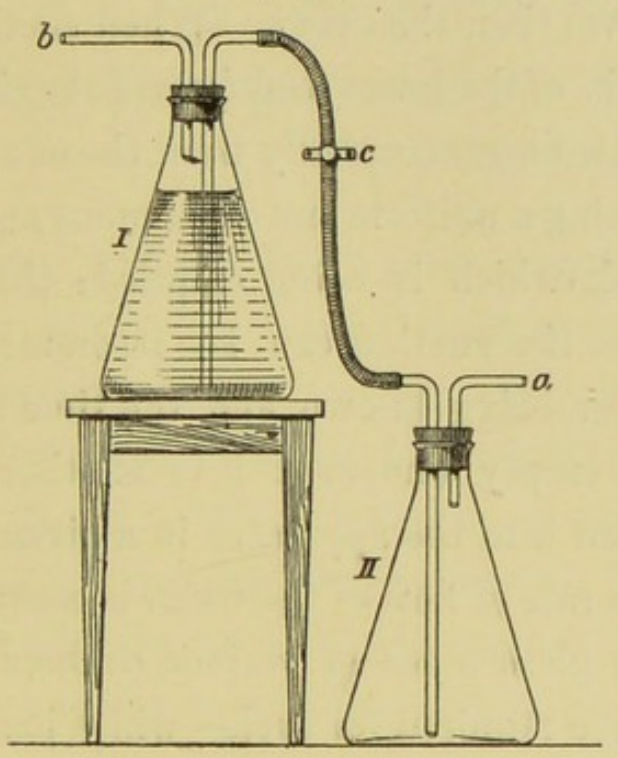
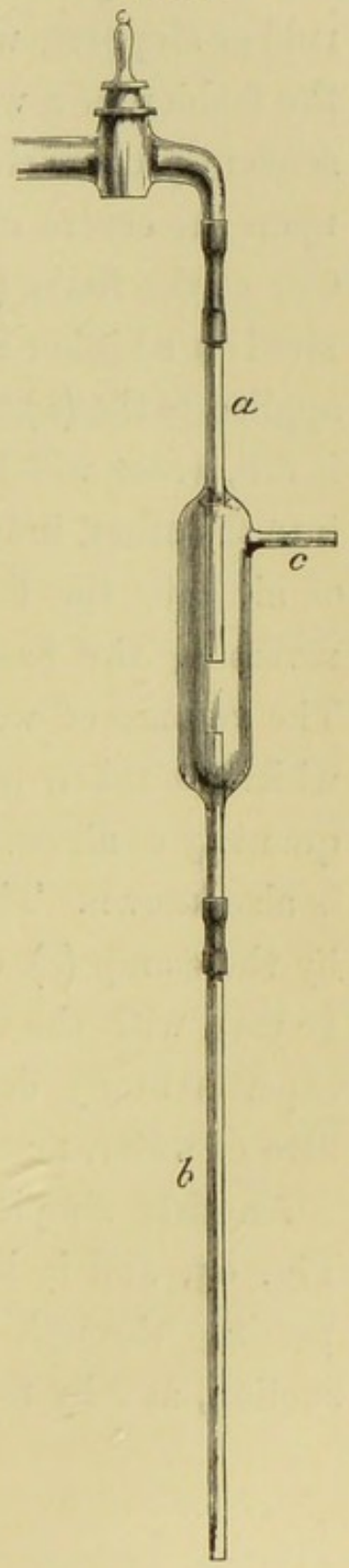


FIG. 65.



Miquel uses sulphate of soda instead of sand, and Percy Frankland glass wool, glass powder, and sugar. The advantage in the use of sugar is obvious, since it passes into solution in the gelatine medium, and thus does not interfere with the future examination of the colonies.

§ 159. **ASPIRATOR.**—A simple form of aspirator is that made by taking two conical flasks fitted with india-rubber stoppers, with glass tubes arranged in each after the fashion of a wash bottle, the two flasks being subsequently connected by means of an india-rubber tube, upon the centre of which is placed a clamp. (Fig. 64.) One of the flasks (I) is filled with water and placed on a stool on a higher level than the other. When suction is applied to the (*a*) tube of the lower flask (II) till the siphon is full, water will flow uninterruptedly from the one flask into the other, inducing a uniform and continuous suction of air into the flask which is being emptied; then by reversing the flasks the suction can be recommenced. The volume of water being known and the time noted which is taken to empty one flask into another, the quantity of air sucked into the apparatus in a given time is also known. The rate of flow of the water is controlled by the clamp (*c*), or glass tubes of various calibres may be used with the time required for each—which has been experimentally determined—to permit the passage of a litre of water, marked upon it.

Another simple form of aspirator is that shown in Fig. 65, and is known as the Drip Aspirator. Water passing through tubes *a* and *b* produces a powerful suction, at *c* by mean of which air is drawn out.

CHAPTER V.

THE BACTERIOLOGICAL EXAMINATION OF WATER.

§ 160. **COLLECTION OF SAMPLES.** — Samples of water for bacteriological examination may be collected in ordinary sterile flasks, the mouths of which are plugged with sterile cotton-wool, or in glass bottles with tightly fitting stoppers, or in sealed flasks, from which the air has been exhausted, the necks being subsequently drawn to a fine point, which can be easily broken under water, when the latter rushes in to fill the vacuum, the neck being subsequently sealed by the use of a spirit-lamp. Usually wide-mouthed glass-stoppered bottles are employed, from 60 to 100 cc. capacity. These, after careful cleansing with acid and distilled water, are placed in a steriliser to dry, the stopper being laid across the mouth of the bottle. When dry, the stopper is inserted and the bottle placed in a tin canister, which is then placed in the steriliser for some three hours at a temperature of 150° C., after which the gas is extinguished and the bottles allowed to cool.

These canisters with their contained bottles are then

taken to the place where it is intended to collect a sample of water, and all things being ready, the bottle is taken from its canister, and the stopper removed by a pair of sterile forceps, care being taken throughout that the hands do not touch the mouth of the bottle or stopper. The open mouth of the bottle is then placed under the tap or spout, the water being allowed to flow in until the bottle is nearly filled. The stopper, which throughout has been held by the forceps, is then replaced, and the bottle put away in an upright position in the tin canister.

If the sample is to be taken from a cistern, tank, stream, lake, or pond, the bottle should be immersed well below the surface before the stopper is removed, which, when the bottle is filled, should be replaced under water.

If the water is to be obtained from a tap it should be allowed to run for some minutes before the water is collected; if from a well, the water should be pumped for some considerable time, indeed for some hours, before a sample is collected.

For the collection of samples at great or specified depths, an apparatus has been devised by Miquel, consisting of a glass vessel capable of holding 50 cc. All air having been expelled from it, the neck is drawn out to a point and bent upon itself. This is supported by metal bands, to which is attached at the bottom a weight of some 3 kilogrammes, and at the other end a graduated cord, by which it is suspended. Running parallel with this latter is a copper wire attached to a ring, which

encircles the neck. The bottle is then lowered to the required depth in the water, and by means of the copper wire the neck is broken, when water rushes in to supply the vācuum. The bottle is then withdrawn, and the neck again sealed by the flame of a spirit lamp.

The examination should take place as soon as possible after the collection. If this cannot be done at once, the bottle should be kept in a cool place or packed in ice.

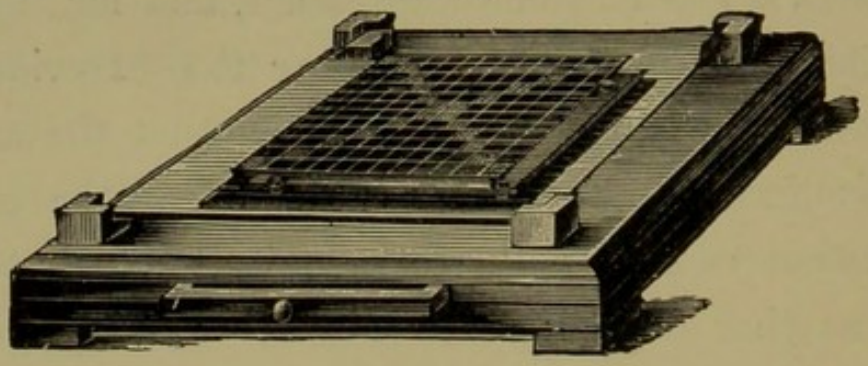
§ 161. **SELECTION OF MEDIA.**—The question of the nutrient media to employ for the cultivation of water organisms is one of some importance, and for this purpose it is necessary to remember the experiment of Beinsch on the subject. He showed that the addition of 0·01 grams of sodium carbonate to the gelatine peptone caused the number of colonies to be six times as great as that following the use of ordinary gelatine. The same observer added varying quantities of tartaric acid to ordinary gelatine, and he then found the number of colonies proportionately reduced. Others have confirmed and extended these observations.

§ 162. **METHODS OF EXAMINATION.**—To make a plate cultivation, the sample of water is taken and violently shaken for some minutes, to secure as far as possible even distribution of the organisms throughout the water. A definite quantity of the water is then removed by means of a sterilised pipette, and if the water is tolerably pure, say 1 cc. is taken for each plate, in all cases two plates being used for each sample of water; and if the water is expected to contain a large number of organisms, then 1 cc. of it must be diluted 50, 100, or more times,

as the case may be. Esmarch's tube-plates are exceedingly convenient for this purpose, as the inoculations may be made immediately the sample is taken.

The plates are incubated in the usual manner, and the counting of the colonies carried out with the assistance of a counting apparatus (Fig. 66), which consists of a wooden stand, on which is supported a glass plate, the centre part of which is etched out into squares, some of which are further divided up into smaller squares. The colonies

FIG. 66.



enclosed by each square are counted. If this is not practicable, the colonies in a few of the squares should be counted, and the average number of colonies then obtained by multiplying these by the number of squares on the plate. In this way, if the water originally was well shaken, a fairly accurate result may be obtained as to the number of colonies in 1 cc. of the original water.

Pfuhl's Method.—If the examination can be carried out immediately at the spring, the water to be used is poured into sterilised vessels, which are closed with plugs of sterilised cotton-wool. To obtain water without contamination with aerial organisms, flat-bottomed tubes are used, partially emptied of air, the ends of which are

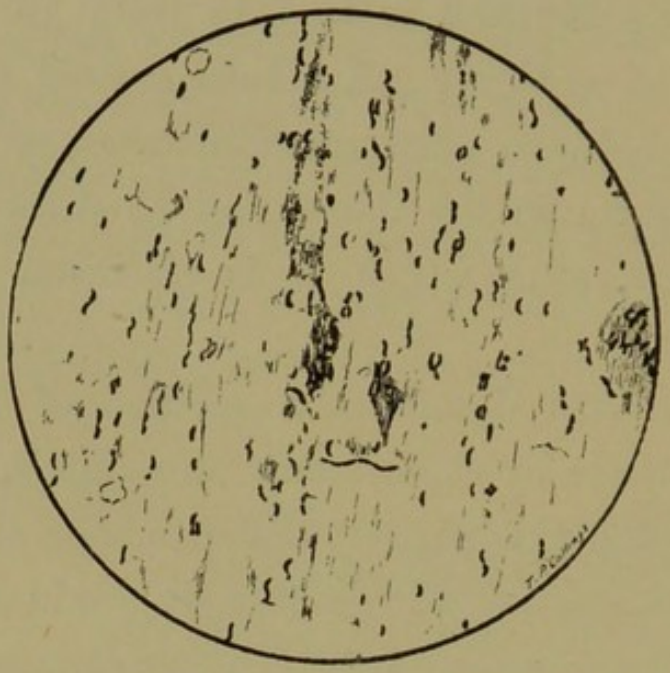
drawn out into capillaries, bent at a right angle and sealed. The points are broken off at the spring, and the tubes filled with water and sealed.

Kirchner's Method is to take from 36 to 44 cm. length of the ordinary glass tubing used for making connections between chemical appliances, to bend it to a V-shape form in the flame, and to draw both ends out to points, after which it is sterilised and sealed hermetically.

Blackstein has described a method of testing the purity of a water by experiments on animals, which may be mentioned at this place. It depends on the fact that true water organisms do not usually produce toxic products, whereas ordinary putrefactive or sewage organisms produce more or less toxic substances and are even in many cases infective. Tubes of sterilised broth, containing 10 c.c. each, receive 1 c.c. of the water to be tested and are incubated at a temperature of 30° C. In 48 hours one of the tubes is taken, and 2 c.c. of the culture injected intravenously into a rabbit, and a similar quantity subcutaneously or intraperitoneally into a guinea-pig. If the water is very impure the rabbit may die within a day or two, or at any rate the guinea-pig which received the injection subcutaneously will show marked local induration and inflammation. Where the 48 hours' culture has given a negative result, tubes which have been incubated for a week or a fortnight may be used in a similar manner: a positive result, however, in this case indicating less recent contamination. This method has not yet come into general use, but may frequently

be taken advantage of to confirm other methods of testing water.

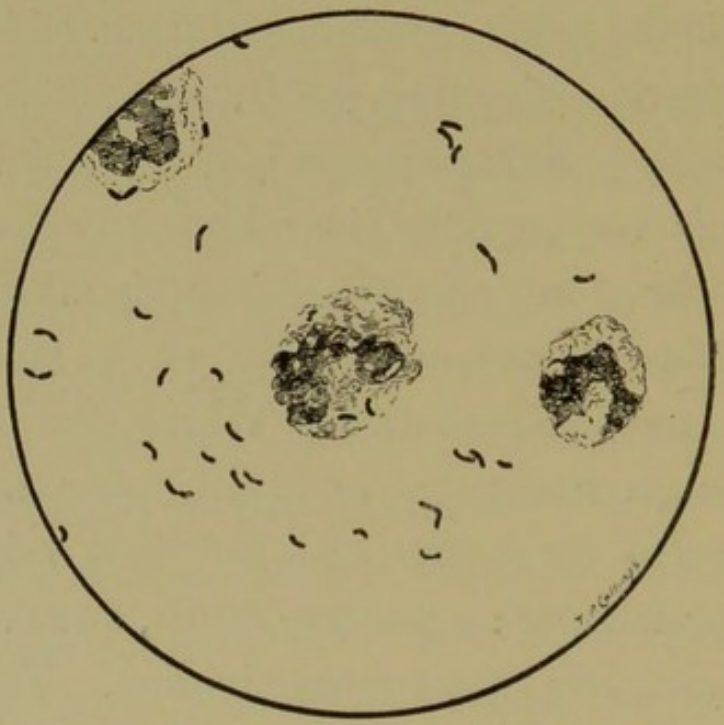
FIG. 67.



Cholera Stool. × 1000.

Of pathogenic organisms which occur in water there are only two species which need occupy attention—viz.,

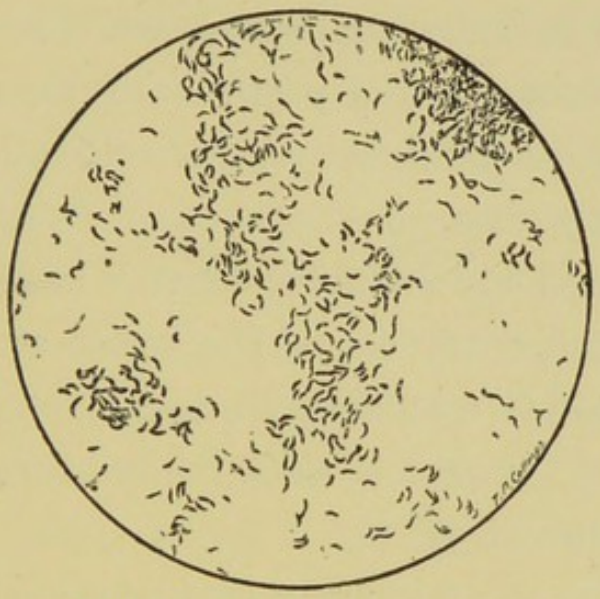
FIG. 68.



Pure culture. Koch's comma bacillus. × 1000.

the organism of cholera and the organism of typhoid fever.

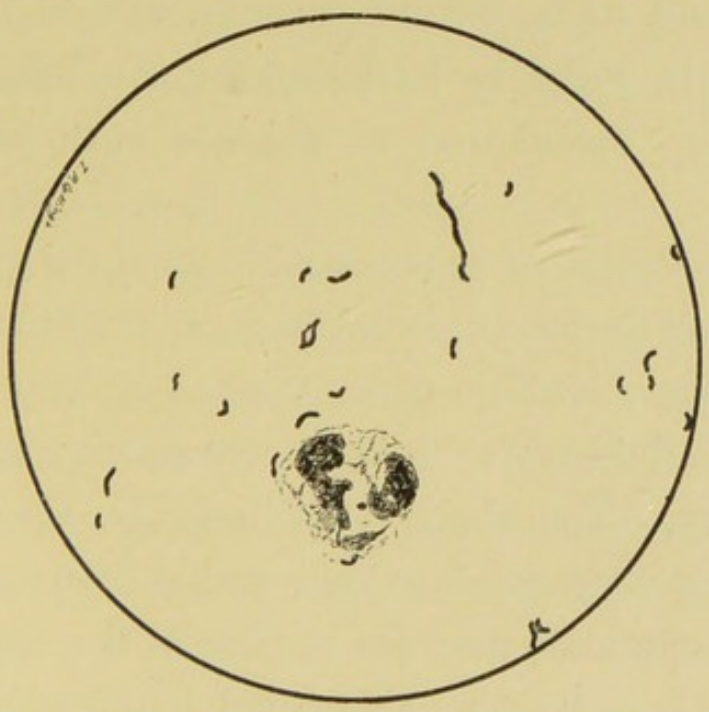
FIG. 69.



Cholera. Agar plate. × 1000.

§ 163. **DETECTION OF THE ORGANISM OF CHOLERA.**—The difficulties of demonstrating the pres-

FIG. 70.



Pure culture. Koch's comma bacillus. × 1000.

ence of the cholera bacillus in water are very great. Of the many examinations made during the epidemic in Hamburg in 1893 only Koch, Frankel, and Labarsch succeeded in demonstrating its presence. These difficulties are mainly owing to the presence of the many other species of bacteria which in artificial cultivation overgrow the cholera organism.

The best method to adopt is what is known as Koch's peptone method. 100 c.c. of the water are taken, and to them are added a sufficient quantity of peptone and common salt, 1 per cent. of each, the mixture is then kept at a temperature of 37° C.

After 10, 15, or 20 hours, agar plates should be made from the peptone cultivation, as by their use a speedy diagnosis may be arrived at owing to the higher temperature at which they may be incubated, and all colonies of suspicious appearance should be microscopically examined, and if found to be curved bacteria, should be further cultivated in order to be tested for the indol reaction and by the inoculation of animals such as guinea-pigs.

A pure solution of peptone with an equal quantity of common salt is an exceedingly good nutritive medium for the cholera bacteria when kept at a temperature of 37° C. The fluid must be distinctly alkaline or made so if necessary. The cholera bacilli (Figs. 67–70) need oxygen to a very great extent, and consequently they tend to accumulate near the surface of the fluid, where they multiply in large numbers; indeed, after the lapse of six hours pure cultivations of the comma bacillus may

be obtained from the surface if the organism has been present in large numbers. Examination of the surface growth should be made from this time up to twelve hours, so as to hit the best time for noting the maximum development of the organism; after this they may be overgrown by other bacteria, and if the examination be too long delayed they may no longer be found.

The peptone cultivation must be supplemented by the gelatine plate cultivation, for growth in this media is more characteristic though less rapid. A ten per cent. gelatine should be used, and the temperature should be 22° C., when in about forty-eight hours the colonies will present their peculiar appearance of cup-like depression, with indications of commencing liquefaction.

The agar plate cultivation of the cholera bacillus is not so striking as that of gelatine. They form moderately large colonies, of a clear grey-brown transparent appearance in eight to ten hours when kept at a temperature of 37° C.

Inoculations from the colonies regarded as cholera should also be made on potato and milk, as these media furnish us with a means of distinguishing cholera from many other comma-shaped bacilli. On potato it usually grows as a thin brown membrane. In milk frequently no change can be observed, but at other times a slow separation of the casein occurs after the elapse of several days.

The Cholera Red Reaction (Indol-Reaction).—This consists in the production of a red colour on the addition

of sulphuric acid to the broth cultivations of cholera which contain indol and nitrous acid. Several other bacteria of a curved form yield indol and nitrous acid simultaneously in their cultivations, and accordingly give the red reaction, such as the vibrio Meschnikovii and certain curved organisms occurring in water.

For this test certain precautions must be observed: the peptone must have the necessary amount of nitrate added to it if the peptone be poor in nitrates or free from it, and the sulphuric acid must be absolutely free from nitrous acid; and thirdly, the cultivation of cholera bacillus must be a pure one.

The reaction should always be sought with cultivations made in a peptone solution.

Experiments on Animals.—A certain quantity of the growth from the surface of an agar cultivation, say 1·5 milligramme, is placed in a cubic centimetre of sterile broth or salt solution and injected into the peritoneal cavity of a guinea-pig, the dose varying with the size of the animal.

This injection is speedily followed by a rapid fall of temperature, ending in the death of the animal.

In this connection it is important to note the rules laid down by Koch relative to the effects of sand filtration upon the presence of micro-organisms in a drinking water, the result of various bacteriological observations at the Berlin and Altona waterworks.

(1) The filtration rate must not be more than 100 millimetres an hour.

(2) Each filter basin should be examined bacteriologi-

FIG. 71.

Plate cultivation of the Typhoid Bacillus on Gelatine, 6–7 days at 20° C.

FIG. 72.

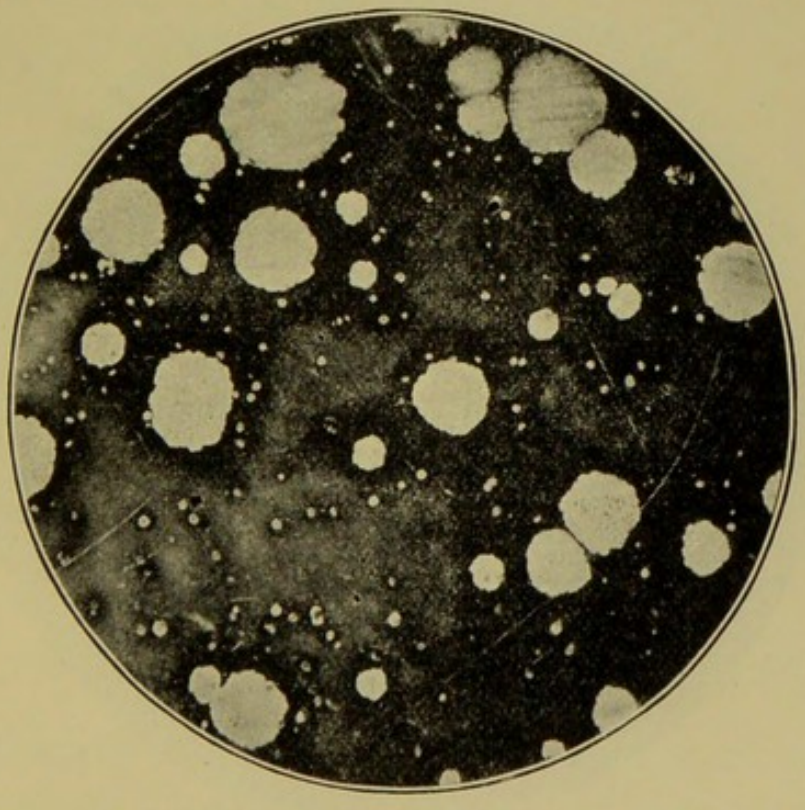
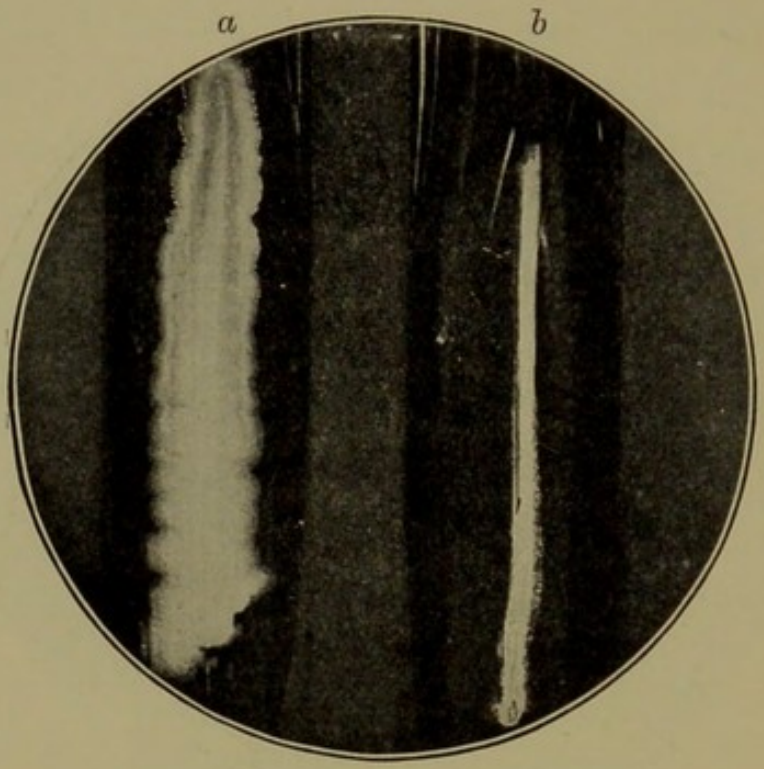


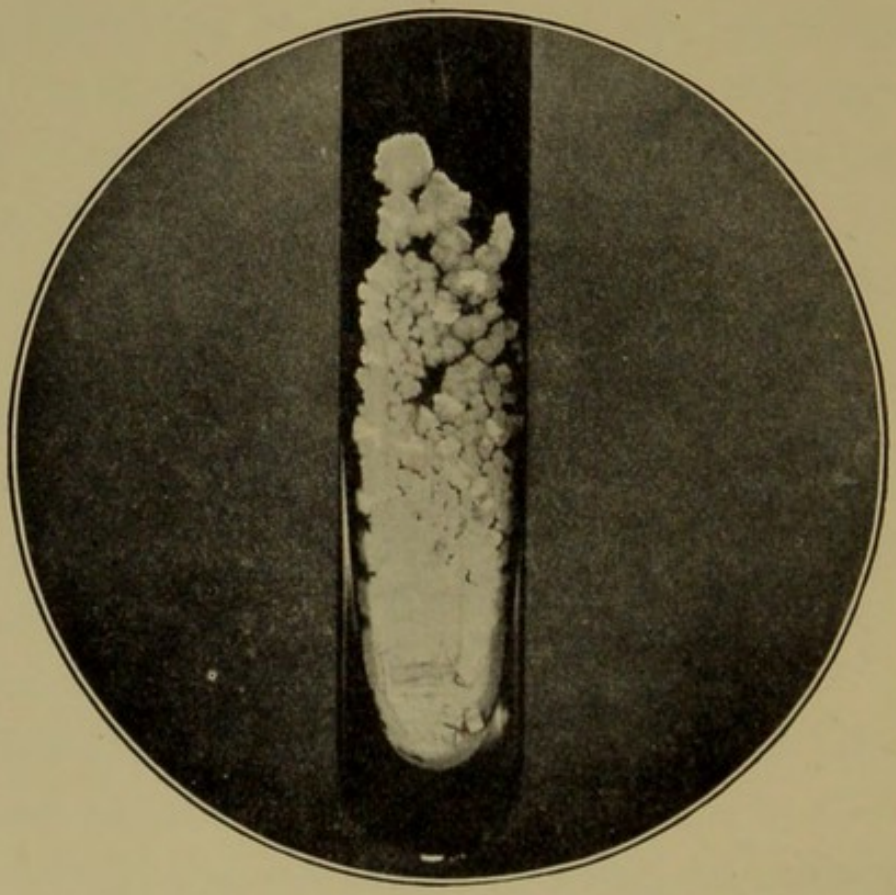
Plate cultivation of the Bacillus Coli Communis on Gelatine, 3–4 days at 20° C.

FIG. 73.



Streak Cultures on Gelatine of (*a*) the Bacillus Coli Communis and (*b*) the Typhoid Bacillus. 48 hours at 20° C.

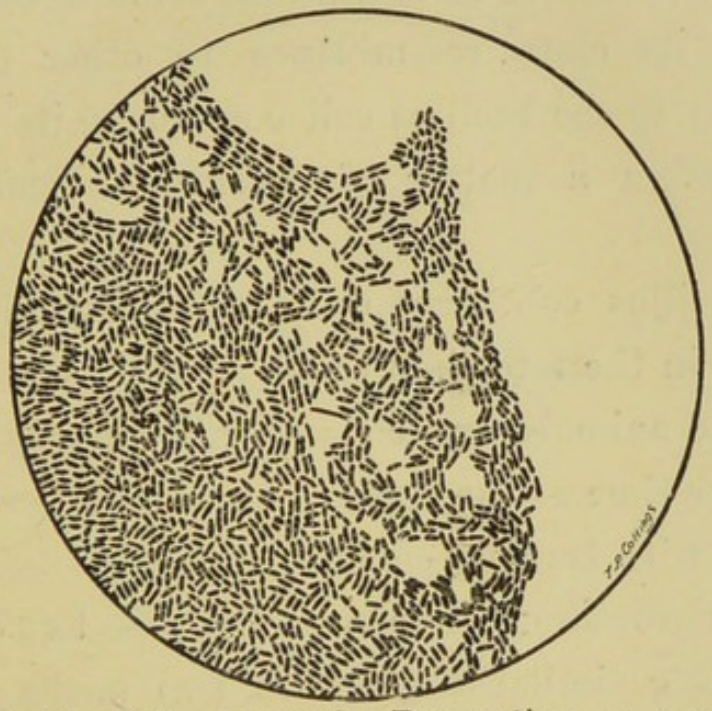
FIG. 74.



A pure culture of the Typhoid Bacillus.

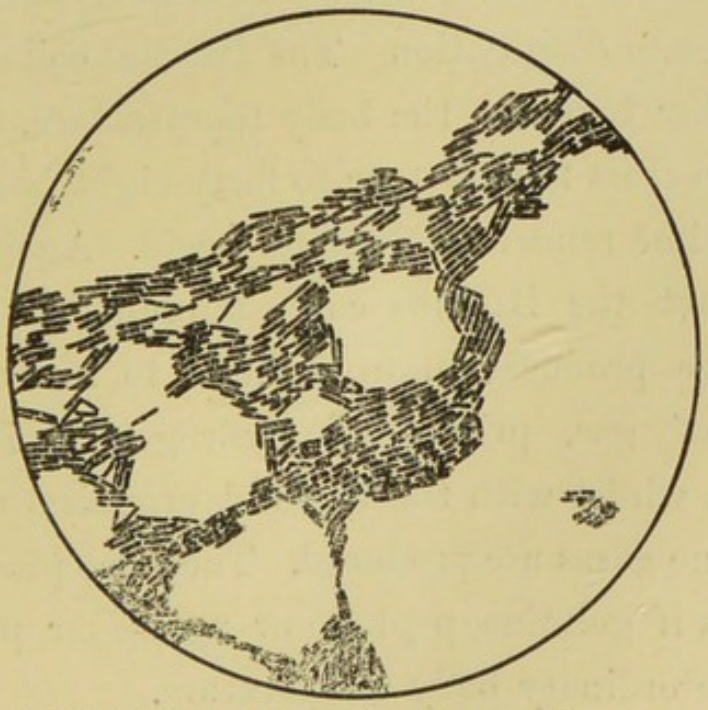
cally every day, the specimens being taken from water leaving the filter.

FIG. 75.



Bacillus Coli. Impression Preparation. × 1000.

FIG. 76.



Typhoid Bacillus. Impression Preparation. × 1000.

(3) Filtered water must not contain more than 100 germs capable of development per cubic centimetre.

§ 164. **DETECTION OF ORGANISM OF ENTERIC FEVER.**—The typhoid bacillus is the second pathogenic organism which may be found in a drinking water; but owing to its close resemblance to other organisms, particularly to the bacillus coli communis, its differentiation is often a matter of extreme difficulty (Figs. 71–76).

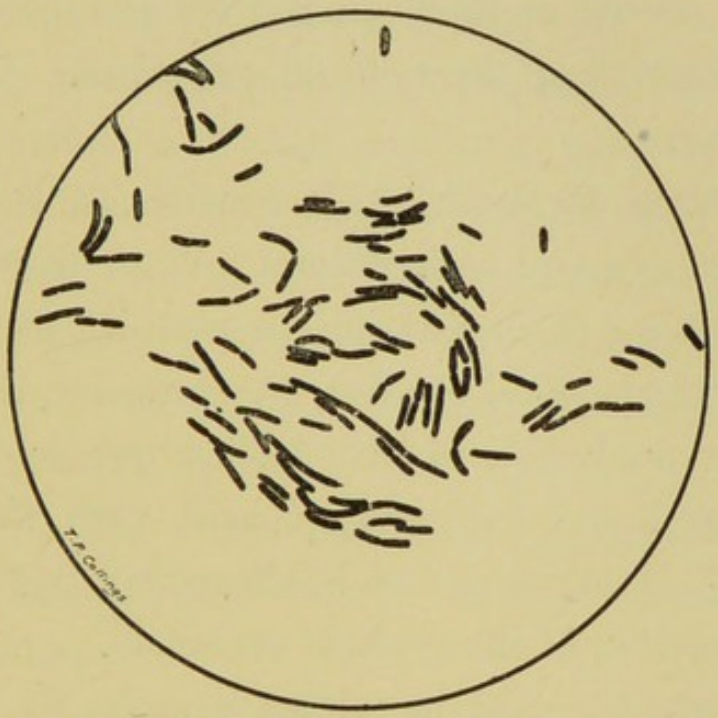
The Bacillus coli communis is found under normal conditions in the intestinal canal, as well as in the fæces of man and animals, and consequently it may be found at the same time as the typhoid bacillus in a water contaminated with typhoid emanation.

In their growth on various media these two organisms show striking similarities, but in two media there are marked differences. In sterile milk, for example, the typhoid bacillus renders the liquid slightly acid and does not give rise to coagulation. The Bacillus coli communis, on the other hand, at the body temperature, coagulates the milk in from twenty-four to forty-eight hours, and at the same time renders it distinctly acid. Again, in fluid meat extract the Bacillus coli communis at the body temperature produces in from three to twelve hours a quantity of gas, principally hydrogen and carbonic anhydride, whilst with the typhoid organism under like condition no gases are produced. The same production of gas follows if gelatine peptone or agar-agar peptone be used at the ordinary body temperature.

The bacilli of typhoid fever are short rods (Figs. 77 and 78), with rounded ends, the length of which is three times greater than their breadth, and they some-

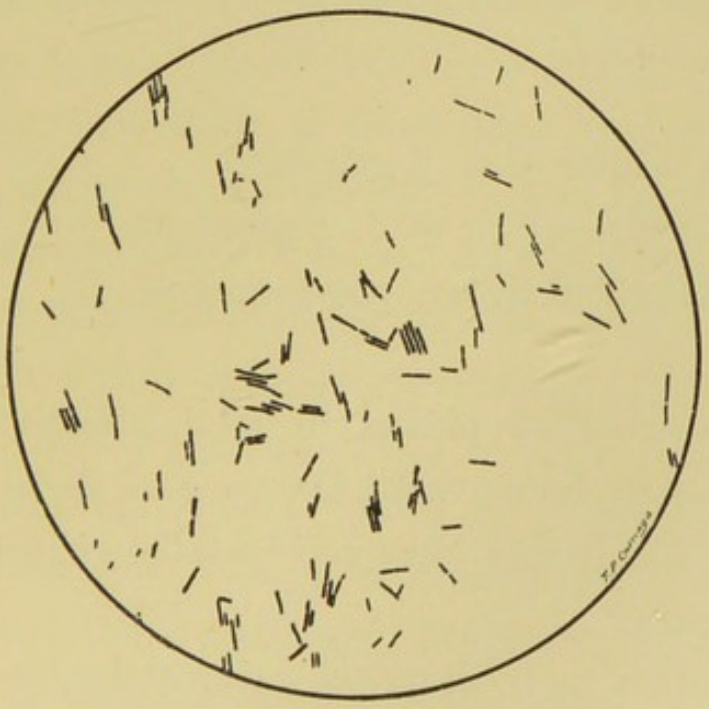
times unite to form threads. The bacilli are motile, the

FIG. 77.



Very young Colony Typhoid Bacillus. × 1000.

FIG. 78.



Cover Glass Typhoid Bacillus. × 1000.

motility depending upon the presence of flagella. The presence of oxygen is not essential to their growth, but

they appear to thrive more vigorously if it is provided. They stain well in watery solutions of the aniline dyes. There are several methods in use for the determination of the presence of the typhoid organism. It does not form spores.

Parrietti's Method.—This consists in the addition of carbolic acid and hydrochloric acid to neutral broth, with the object of eliminating all organisms with the exception of the typhoid bacillus. As, however, the Bacillus coli communis is able to withstand larger doses of both carbolic acid and hydrochloric acid than the typhoid bacillus, this method cannot be altogether depended upon for the isolation of the typhoid organism.

The method is used by taking a series of test tubes, each containing 10 c.c. of neutral broth and adding from 3 to 9 drops of hydrochloric acid phenol solution made up as follows:

Carbolic acid, 5 grammes.
Hydrochloric acid, 4 grammes.
Distilled water, 100 grammes.

The tubes are then placed in an incubator at 37° C. for twenty-four hours, with the view of destroying any organisms which may have accidentally gained access to the solution.

Then from 1 to 10 drops of water under investigation are added to each tube and the whole thoroughly shaken together, the tubes then being again placed in the incubator. If after another interval of twenty-four

hours turbidity is visible, it may be concluded with certainty that either the typhoid bacillus, or the Bacillus coli communis, or both, are present.

Ordinary plate cultivations of the turbid broth should then be made, and the resulting colonies carefully examined with a view to their identification.

Vincent's method is very similar to Parrietti's. It consists in adding the water under examination into a series of test tubes containing sterile peptone bouillon, to each of which is added 5 drops of a 5 per cent. solution of phenol. The tubes are then kept at a temperature of 42° C. This method is not effectual against the Bacillus coli communis.

Chantemesse and Widal have suggested another means for isolating the typhoid bacilli. They make plate cultivations of the water upon a gelatine peptone medium containing ·25 per. cent of phenol; but Holz and Dunbar have shown that such a percentage of carbolic acid altogether prevents the growth of the typhoid bacilli, and the latter observer has also shown that whilst the colonies of the Bacillus coli are impeded by small additions of phenol, yet they then present greater resemblance to the colonies of the typhoid bacillus; and, further, he confirms the observations of Vincent that the Bacillus coli communis can withstand a greater addition of phenol than the typhoid bacillus; and one other interesting observation of Dunbar's deserves notice, which is that the addition of ·1 c.c. of a 5 per cent. solution of phenol to 10 c.c. of gelatine has a distinct effect in preventing the liquefaction of the gelatine by foreign organisms, whilst

hardly any effect is produced upon the Bacillus coli communis.

The typhoid organism grown upon gelatine forms colonies of a greyish white iridescent character, with jagged, irregular edges. No liquefaction takes place.

Upon agar-agar a greyish-white moist expansion is formed. On potatoes it produces an almost invisible greyish transparent growth after forty-eight hours. On touching the surface with a needle a resistant pellicle is found. The Bacillus coli communis, on the other hand, when grown upon potato, produces a raised slimy yellow or greyish-white expansion.

Kitasato has suggested a chemical reaction by which a differentiation may be made between the Bacillus coli communis and the typhoid bacillus as follows:

To 10 c.c. of the ordinary alkaline peptone broth culture of the organism which has been growing for at least twenty-four hours in the incubator, 1 c.c. is added of a solution of potassium or sodium nitrite (·02 grammes in 100 c.c.) and then a few drops of strong sulphuric acid.

If the Bacillus coli communis is present an indol reaction is obtained, consisting of a rose to a deep red coloration, which depends on the action of nitrous acid with indol to form nitrosoindol nitrate. No such action is produced with the typhoid bacillus.

Intravenous injections of the typhoid bacillus will kill rabbits in about twenty-four hours, the bacilli

being subsequently found in the urine, blood, and secreta. A subcutaneous injection of the bacillus coli communis will kill rabbits in from one to three days, the animal dying with the symptoms of diarrhœa and collapse.

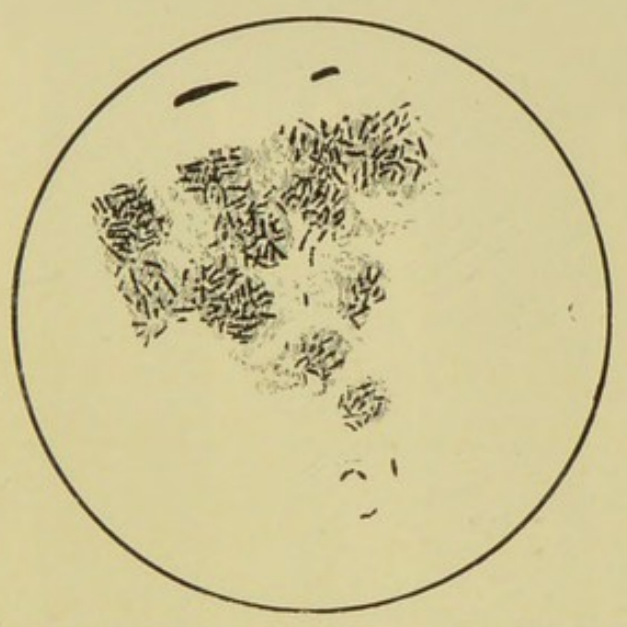
CHAPTER VI.

THE BACTERIOLOGICAL EXAMINATION IN DIPHTHERIA AND TUBERCULOSIS.

§ 165. **THE BACTERIOLOGICAL DIAGNOSIS OF DIPHTHERIA.**—A portion of the exudate or membrane is taken from the throat and examined microscopically at once, and later on by means of cultivations. The material for examination is obtained by means of a swab, which consists of a short rod of iron wire, to the extremity of which a pledget of cotton wool is attached. The swab is brought into contact with the affected throat or tonsils, and, when withdrawn, and brushed over the surface of a tube of Loeffler's blood serum, and afterwards can be used for the purpose of making one or more cover glass preparations for immediate examination. As the diphtheria bacilli can be stained by means of Gram's method, we are able by its use to at once eliminate other organisms which are decolorised by this process. The cover glasses, when dry, are first fixed by being passed three times through the flame of a Bunsen burner. They are then stained by being placed for a minute or two in Weigert's gentian violet solution,

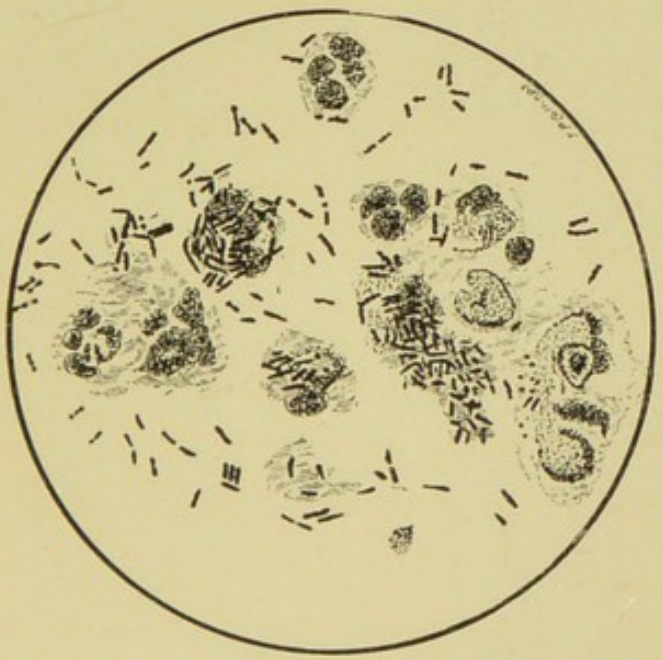
made by the addition of the alcoholic stain to a saturated solution of aniline oil and water. It is then passed into

FIG. 79.



Superficial portion of false membrane from larynx of a child dead of acute diphtheria, showing numerous bacilli.

FIG. 80.

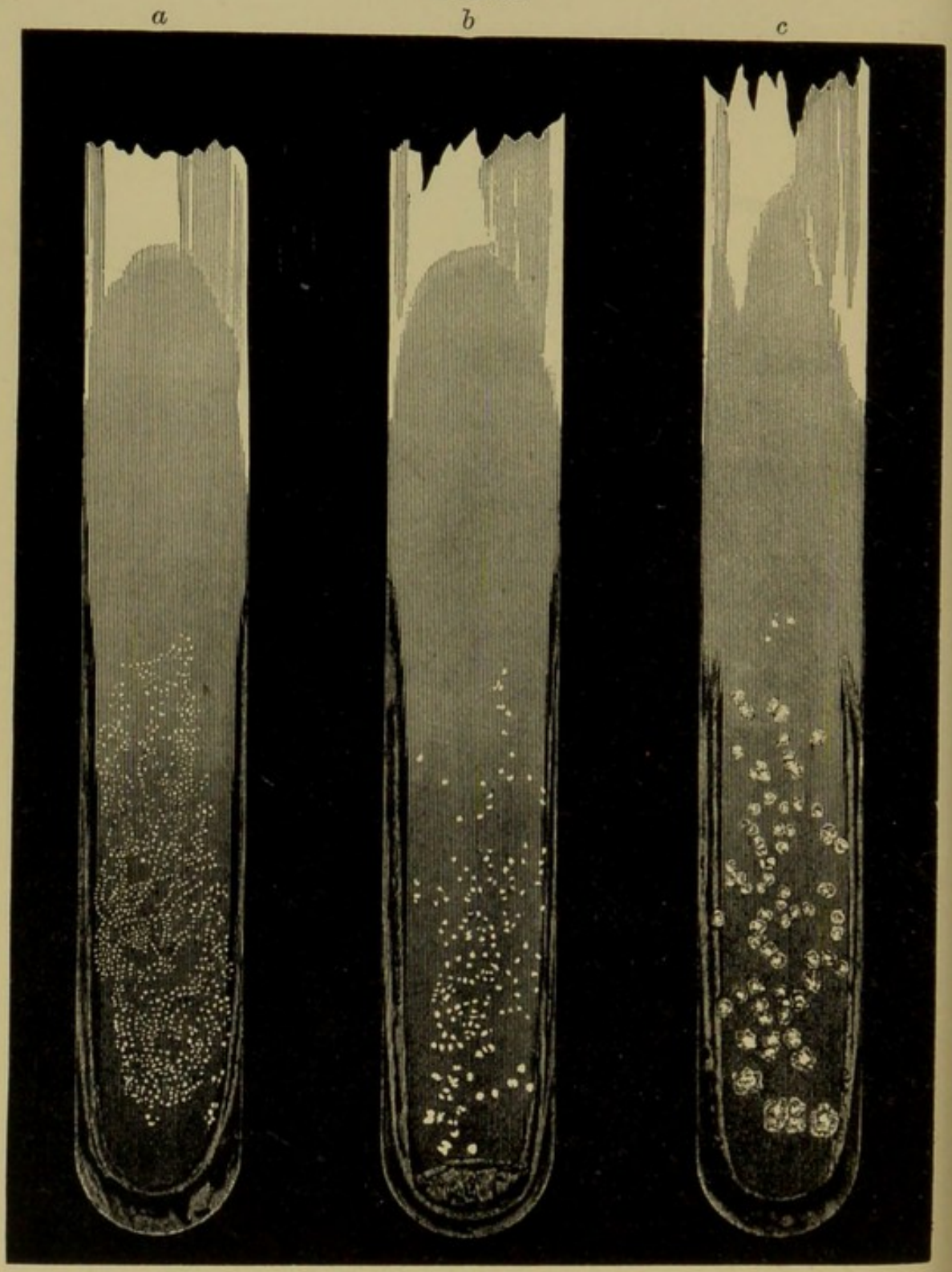


Coverglass specimen of Diphtheria Bacilli, taken from membrane in pharynx.

the iodine solution for two or three minutes till the violet colour has disappeared; it is now thoroughly

washed in alcohol, and can then be passed through

FIG. 81.



Colonies on surface (slanting) of gelatine of Diphtheria Bacillus
(*a*) after one week's incubation at 20·5° C.
(*b*) „ two weeks' „ „
(*c*) „ three „ „ „

water and dried, and mounted in Canada balsam. Very

frequently a shorter method of staining is made use of: a few drops of Loeffler's alkaline methylene blue solution being dropped on the cover glass, and allowed to remain for ten minutes, when the specimen may be washed with water, dried, and examined. When examined under the microscope, the diphtheria bacilli are seen as single rods or in pairs, varying in diameter from 0·5 to 8·75 mm., and in length from 1·5 to 6·5 mm. The rods are straight or, more usually, slightly curved, and are frequently swollen at the ends, or pointed at the ends and swollen in the middle portion. (Figs. 79 and 80.) The blood serum culture is incubated at 37° C. and may be examined after twelve or eighteen hours' incubation. On examination after twelve hours in a positive case, a number of very small colonies are easily recognisable by the naked eye. After eighteen hours the colonies are much larger, and can be distinguished with more or less ease from the other organisms which have now begun to manifest themselves. (Fig. 81.) A drop of water is now placed on a clean cover glass, and a platinum wire having been drawn over the surface of the blood serum, and the colonies thus separated distributed over the surface of the cover glass. The specimen is then dried and stained as already described. On examination the characteristic diphtheria bacilli are usually recognised without any difficulty, although they are usually accompanied by very numerous cocci, due to the pus organisms which are almost always present. In not more than 1 in 20 cases is there any difficulty in making a diagnosis from a culture.

§ 166. **THE BACTERIOLOGICAL DIAGNOSIS OF TUBERCULOSIS.—Examination of Sputum.**—The sputum should be spread out in a thin layer in a watch glass, which then should be placed on a dark background, by which means one of the characteristic yellow particles may be picked out with a pair of fine-pointed forceps.

The suspected matter should then be squeezed between two clean cover glasses, by which means thin films are prepared. This should be allowed to dry in the air, and then gently dried over a flame as previously described. The films are then floated on warm carbol fuchsine solution for three or five minutes.

One of the films should then be removed, thoroughly washed in water to remove the excess of the stain ; it should then be decolorised in a 25 per cent. solution of hydrochloric acid by holding the cover glass with a pair of forceps and dipping it into the acid until the red colour is discharged, then immediately washed in 60 to 70 per cent. spirit, which at first causes the red colour to reappear, and continue to wash until no more red comes off. It should then be again washed in water, and the film dried between filter paper, and passed three times through the flame.

It should now be stained in Loeffler's methylene blue for ten to twenty seconds, washed in water, dried between filter paper, and again passed through the flame to dry it. Now mount in zylol balsam, and examine with an oil immersion, the tubercle bacilli will be found stained red on a blue ground. (Fig. 82.)

If no tubercle bacilli are found, the second film, which was left in the stain, should be treated and examined as just described.

To facilitate the procedure, the water, acid, spirit, and methylene blue, should be kept in small, wide-necked, stoppered bottles. The cover glass, held in a pair of forceps, may then be passed successively from

FIG. 82.

B. Tuberculosis Sputa. × 1000.

one to the other. The process can be repeated by using carbol gentian violet instead of carbol fuchsine, and vesuvine or Bismarck brown instead of methylene blue.

The carbol gentian violet is prepared by taking ten volumes of 5 per cent. solution of carbolic acid, and one volume of a concentrated alcoholic solution of gentian violet.

The specimen should remain in this stain, which has been previously warmed, for at least five minutes; but

care must be taken that the film does not remain too long in the hydrochloric acid.

The vesuvine must be used in a concentrated solution.

Tubercle bacilli will be found stained blue on a brown ground.

Sputum kept in Carbolic Acid.—This affords the best method for detecting the tubercle bacilli. 10 to 20 c.c. of the sputum should be added to 100 c.c. of a 5 per cent. carbolic acid solution in a small flask, and the whole shaken vigorously for five or six minutes until the sputum is thoroughly disintegrated. The contents of the flask should then be poured in a conical glass and allowed to stand from twelve to twenty-four hours. The supernatant fluid should then be decanted, and by means of a fine pipette a little of the sediment from the bottom should be taken and rubbed between two cover glasses. The films thus formed should be allowed to dry in the air, and then passed three times through the flame, washed in a mixture of equal parts of alcohol and ether for two or three minutes, dried between filter paper, and again passed through the flame. The films are then stained with carbol fuchsine or carbol gentian violet, and the mounting completed as before described.

If the sputum be watery 100 c.c. of it may be placed in a flask to which 5 c.c. of liquefied carbolic acid may be added; shake for five to eight minutes and proceed as before.

Urine suspected of containing tubercle bacilli may be treated in a like manner as watery sputum.

APPENDIX I.

DIRECTIONS FOR OBTAINING SAMPLES OF WATER.

THE quantity of water ordinarily required for a sanitary analysis is half a gallon, while if a complete mineral analysis is desired, it will be necessary to obtain not less than two gallons.

Water samples should be collected and forwarded in "Winchester quarts," with *stoppers*, not corks. The stoppers should be tied down and sealed and then covered by a piece of oiled silk.

In taking a sample of drinking water care should be taken to obtain a sample under the same conditions under which it would be drawn for drinking. That is to say, the first portion running from either pump or tap should not be discarded unless this is also done in practice.

In taking samples from a stream or river, care should be exercised not to allow any floating scum to enter the sample bottle, and the place chosen to take the sample at should not be a point at which the current is so violent as to stir up mud, etc., not normally present in the water.

In addition, attention should be paid to the state of the weather previous to collection, as excessive rainfall may cause alterations in the normal composition of the water. This is of course most important in the case of streams and wells supplied with surface water.

The "Winchester quart" should in no case be one that has ever held ammonia, and should be washed out thoroughly with ammonia-free water when it leaves the laboratory, and when the sample is taken it should be well rinsed with some of the same water as the sample.

APPENDIX II.

THE PREPARATION OF THE STANDARD SOLUTIONS USED IN WATER ANALYSIS.

Estimation of Chlorine.

Solution of Nitrate of Silver—4·79 grams of pure dry silver nitrate is dissolved in water and made up to a litre. 1 c.c. = ·1 mgrm. (·001 gr.) Cl. The potassium chromate used must be purified as described in § 25.

Estimation of Ammonia.

Nessler Solution.—Two solutions are made separately, one of 35 grams of potassium iodide in 100 c.c. of water, and the other of 17 grams of mercuric chloride in 300 c.c. of water. The mercury solution is gradually dropped into the iodide with stirring until a precipitate is permanent. Now a solution of caustic potash (20 per cent.) is added until the whole measures a litre. The liquid becomes clear on standing and should then be siphoned off into small bottles for use. It improves on keeping. Two c.c. should be used for each test.

Standard Solution of Ammonia.—The weak solution in ordinary use is made up most conveniently by diluting down a solution of 100 times the strength. This concentrated ammonia solution is made by dissolving 3·15 grams of pure ammonium chloride in 1000 c.c. of water (1 c.c. = 1 mgm. NH_3). 10 c.c. of this made up to a litre with distilled water that has been previously ascertained to be free from ammonia, gives the ordinary working solution (1 c.c. = ·01 mgrm. NH_3).

Sodium Carbonate.—This reagent, which is only used in the case of waters that are not already alkaline, is best ignited on a platinum spatula, or crucible lid immediately before use.

Alkaline Permanganate Solution.—200 grams of caustic potash, 8 grams of potassium permanganate are dissolved in about 1100 c.c. of distilled water, and the solution rapidly boiled (preferably in a porcelain dish), until the volume is reduced to a litre. It must be kept closely corked up; and as glass stoppers usually become cemented in by the potash, an ordinary cork well paraffined and coated with tin foil, should be used.

Ammonia Free Water.—The water used for making up the standard solution of ammonia should be so free from ammonia that 100 c.c. of it with 2 c.c. of Nessler re-agent show no appreciable tint in five minutes. The Society of Public Analysts recommend that ammonia free water should be prepared by boiling ordinary distilled water with 1 in 1000 pure ignited sodium carbonate. Another method which gives equally good results is to fix all the ammonia present in a water before distillation by the addition of 1 c.c. of pure phosphoric acid per gallon of water to be distilled.

Re-agents required in the Tidy-Forchammer Process.

Standard Solution of Potassium Permanganate.—0·395 grams of potassium permanganate is dissolved in 1000 c.c. of organically pure distilled water. [1 c.c. = 0·1 mgm. of available oxygen.]

Potassium Iodide.—Must be quite free from iodate. A crystal dropped into dilute sulphuric acid must not liberate any iodine at once.

Dilute Sulphuric Acid.—250 c.c. of pure concentrated sulphuric acid is poured into 750 c.c. of distilled water. A weak solution of potassium permanganate is now dropped in with constant stirring until a faint pink colour is permanent. The whole is now warmed for some hours at 80° F.; a very faint coloration should be observable.

Sodium Thiosulphate.—One gram of the crystallised salt (sodium "hyposulphite") is dissolved in a litre of distilled water.

Starch Paste should be made each day as required. A little starch (about ·1 gr.) is ground up with a little cold water to an emulsion, and this slowly poured with stirring into about 50 c.c. of boiling distilled water, and the whole cooled in a beaker under the tap before use. May be filtered if thought necessary, but the operation is a tedious one.

Estimation of Oxidised Nitrogen.

The Zinc-couple Couple.—The preparation and use of this is fully described in the text (§ 35).

Colour Method of Estimating Nitrates.

Phenol-sulphonic Acid.—37 c.c. of strong sulphuric acid are added to 3 c.c. of water and 6 gr. of pure phenol [Leffman and Beam].

Standard Solution of KNO_3.—0·722 gr. of pure dry potassium nitrate, dissolved in water and made up to a litre. 1 c.c. = ·0001 gr. N as NO_3.

Estimation of Nitrites.

Meta-phenylene-diamine Solution.—One gram of the base is dissolved in 200 c.c. of water to which a drop or two of dilute sulphuric acid has been added.

Dilute Sulphuric Acid.—35 c.c. of concentrated acid are made up to 100 c.c. with distilled water.

Standard Nitrite of Sodium Solution.—Since silver nitrite is the only stable nitrite which can be made (or purchased) in a pure dry condition, it is usual to start with this: 1·1 grm. of pure silver nitrite ($AgNO_2$) is dissolved in boiling distilled water, and a solution of pure sodium chloride added until all the silver is

precipitated. This is made up to a litre, the silver chloride allowed to settle, 100 c.c. of this withdrawn, and this again made up to a litre with well-boiled out water. The solution must be kept in small stoppered bottles, completely filled, and in the dark.

1 c.c. = ·01 mgm. N as (NO_2).

Determination of the Hardness of Water.

The preparation of the solutions required is described in detail in the text (§ 39).

APPENDIX III.

THE INTERPRETATION OF ANALYTICAL DATA.

It is impossible to lay down hard-and-fast rules for the interpretation of the analytical data yielded by a water, nor must reliance be placed on any one or two figures, but judgment must be formed on the results taken as a whole.

It is rarely possible to say that a given water is certainly wholesome, but it is frequently possible to assert positively that it is dangerous; and in the latter case we may, by analysis, often be enabled to predict and so lead to the discovery of a hitherto unsuspected source of pollution.

It is of great importance to have as much knowledge as possible of the source from which the water is derived, *i.e.*, the geological strata, height above sea-level, nearness to possible sources of pollution; as, for example, graveyards, cesspools, or sewers.

Many persons who submit a sample of water to an analyst show a disposition to withhold all information on the points above-indicated, thereby defeat their own object, which is to obtain the most accurate opinion possible as to its fitness for domestic use. Where it is possible to get information supplied, the analyst should not fail, in sending instructions for the taking of samples, to request answers to questions framed by him in such a manner as to elicit clear replies to main points respecting the surroundings of the well and the character of the ground.

Bearing in mind, as stated above, that it is impossible to lay down hard-and-fast rules as to quantities of various constituents that should lead to the condemnation of a water, the student

may gain some idea as to how to form an opinion on the character of a water by careful examination of the figures given in the following table:

(*All figures are given in grains per gallon.*)

	A.	B.	C.	D.
Physical Characters:				
Colour in 2 in. tube	bluish	yellowish brown	faint yellow	bluish
Smell	normal	flat	stale	normal
Suspended matter	none	very considerable	slight	none
Chemical Characters:				
Total solids	22·0	50·0	45·0	56
Mineral solids	19·0	38·0	36·0	48
Loss on ignition	3·0	12·0	9·0	8·0
Chlorides (as Cl)	1·5	3·0	2·5	2·0
Chlorides (as NaCl	2·9	4·6	4·1	3·3
Nitrites	none	none	traces	none
Nitrates (as N)	·2	·6	1·0	·6
Saline ammonia	·002	·008	·01	·005
Albumenoid ammonia	·003	·02	·02	·006
Hardness	16·0	15·0	12·0	19.0
Oxygen absorbed in 15 mins.	·01	·03	·035	·015
Poisonous metals	none	none	none	none

A. Tap-water as supplied to King's College. Rather hard, but otherwise of excellent quality

B. Thames water, from the embankment, illustrating vegetable and other pollution.

C. Polluted well-water.

D. Chalk-water, hard, but wholesome.

APPENDIX IV.

NUTRIENT MEDIA AND THEIR PREPARATION.

THREE principal types of media are employed for the growth of bacteria.

The first of these is *alkaline beef broth* or meat infusion, which may be employed at any temperature, and is useful to obtain a large and rapid growth when we do not desire to obtain a characteristic appearance.

The second, *nutrient gelatine*, consists of beef broth thickened with gelatine, and is suitable for growing on a solid medium those organisms which flourish at 20° to 22° C. It cannot be employed for blood heat, as it melts at about 24° C.

The third, *agar-agar*, is prepared in the same way as the last mentioned, only that agar is substituted for the gelatine. This medium is suitable for organisms that require a blood-heat, as it remains solid at 39° C., or even higher. In preparing media, it is therefore customary to make a large stock of alkaline beef broth, some of which is kept for use without addition, while to one portion gelatine is added, and to another agar-agar.

Preparation of Beef Broth Media.

To prepare 1000 c.c. of beef broth take 1 pound of beef-steak, mince finely, and set to soak in a covered flask with 1000 c.c. of distilled water. After standing twelve hours, boil and strain. The liquid is then filtered, 1 per cent. of peptone is added, and 5 per cent. of common salt. It is then neutralised by cautious additions of a solution of carbonate of soda until

faintly alkaline to red litmus paper. After this it is again boiled, and then requires re-filtering. It must be placed in a flask plugged with cotton wool, and sterilised on three successive days.

It is most convenient to prepare three litres of broth, one each of which is finally made into gelatine and agar.

Preparation of Gelatine Media.

To prepare nutrient gelatine take one litre of alkaline beef broth and raise to the boiling point, then add 50 to 100 grams as you require, 5 per cent. or 10 per cent gelatine which has been previously soaked for half an hour, and bring the whole to the boil. After boiling a few minutes it is filtered.

Gelatine usually filters easily and clear; if it does not, recourse must be had to the addition of white of egg, which will be described under Agar-agar.

The gelatine medium must not be heated too long nor too often, for each successive heating diminishes its power of "setting."

Preparation of Agar-agar Media.

The preparation of agar is similar to that of gelatine, except that only 1 per cent. of agar is required instead of 10 per cent. of gelatine. Some workers suggest previous soaking of the agar, but it generally dissolves readily if it is added little by little to the boiling broth. The whole is then well boiled and stirred with a stick to prevent burning at the bottom of the saucepan. It sometimes happens that the agar can then be filtered clear, but as a general rule it is necessary to add white of egg.

The whites of two eggs are sufficient for 1 litre of medium, and the medium must be partially cooled before the white of egg is added or it will coagulate into large flocks, and very little clarification will be effected. After the white of egg has been added, the liquid should be well stirred and rapidly raised to the boiling point, and retained at this temperature for a few minutes.

We may then filter; or another plan is to pour the whole into a tall cylinder and allow it to cool and "set." It will then be found that nearly all the flocculent matter has subsided, and if the vessel is warmed the cylinder of agar can be slid out, and the end containing the precipitated matters cut off, while the clear portion is re-melted and filtered. Various special papers are made for the filtration of agar, none of which are very successful, and its filtration through ordinary filter paper is exceedingly tedious.

It may, however, be filtered through flannel or, still better, through an ordinary jelly-bag. In any case, during filtration the medium should be kept hot by being placed in a steam chamber.

Preparation of Potato Media.

For certain purposes we require potato media.

This is prepared by cleaning potatoes with a solution of mercuric chloride and then, with the aid of a cork-borer, cutting out cylinders about $2\frac{1}{2}$ inches long. These cylinders are then cut from end to end, so that each one forms two wedges, which are placed in sterilised test-tubes, with a small plug of wet cotton wool at the bottom to prevent them from becoming too dry.

Potato serves excellently for the growth of all the chromogenic organisms, and is also useful to bring out points of distinction between certain organisms, as, for example, between the Eberth-Gaffky bacillus and Bacterium coli commune, or between the Spirillum choleræ Asiaticæ, and the Finkler Prior spirillum.

Various other kinds of media have been proposed and used for special purposes, but those above described are the only ones of general application.

Preparation of Test Tubes for Media.

To prepare test tubes for gelatine or agar, it is best to wash them out with strong nitric acid, and then to rinse them twice

with water and once with methylated spirit. They are then drained and sterilised. When it is required to fill them they are placed in a rack, and about 10 c.c. of the melted media run in from a large pipette, taking care not to drop any on the edge or near the top of the tube. Any tube so smeared should be poured out, as, if it is used, the wool used for plugging will become glued to the tube, and it will be almost impossible to withdraw it. When the tubes are filled they are plugged tightly with cotton-wool and sterilised for one hour on three successive days. It is well to cover the tops of the tubes, so as to prevent condensed water from running down and wetting the cotton wool plugs. The tubes may eventually be "capped" with small rubber caps to prevent loss of moisture.

The only other medium of any importance is the blood-serum medium of Koch or Loeffler. This is useful to grow the tubercle bacillus, and in the examination of diphtheritic membrane. It may, however, be replaced for either of the purposes by glycerine agar, which is more easily prepared.

APPENDIX V.

ATOMIC WEIGHTS OF THE ELEMENTS.

Element.	Symbol and Atomicity.	Atomic Weight.
Aluminium	Al III. IV.	27·5
Antimony	Sb III. V.	120
Arsenic	As III. V.	75
Barium	Ba II.	137
Beryllium	Be II. III.	9·4
Bismuth	Bi III V.	208
Boron	Bo III. V.	11
Bromine	Br I. III. V. VII.	80
Cadmium	Cd II.	112
Cæsium	Cs I.	133
Calcium	Ca II.	40
Carbon	C IV. II.	12
Cerium	Ce III. IV.	138
Chlorine	Cl I. III. V. VII.	35·5
Chromium	Cr IV. VI.	52·5
Cobalt	Co II. IV.	59
Copper	Cu II.	63
Didymium	Di IV.	145
Erbium	E II.	169
Fluorine	F I.	19
Gallium	Ga IV.	69
Gold	Au I. III.	196·7
Hydrogen	H I.	1
Indium	In III.	113·4
Iodine	I I. III. V. VII.	127
Iridium	Ir II. IV. VI.	193
Iron	Fe II. IV. VI.	56
Lanthanum	La IV.	139
Lead	Pb II. IV.	207
Lithium	Li I.	7

Element.	Symbol and Atomicity.	Atomic Weight.
Magnesium	Mg II.	24
Manganese	Mn II. IV. VI. VII.	55
Mercury	Hg II.	200
Molybdenum	Mo VI.	96
Nickel	Ni II. IV.	58·8
Niobium	Nb V.	94
Nitrogen	N III. V.	14
Osmium	Os II. IV. VI. VIII.	199
Oxygen	O II.	16
Palladium	Pd II. IV. VI.	106·5
Phosphorus	P III. V.	31
Platinum	Pt II. IV. VI.	197·18
Potassium	K I.	39
Rhodium	Rh II. IV. VI.	104
Rubidium	Rb I.	85
Ruthenium	Ru II. IV. VI. VIII.	104
Selenium	Se II. IV. VI.	79
Silicon	Si IV.	28
Silver	Ag I.	108
Sodium	Na I.	23
Strontium	Sr II.	87·5
Sulphur	S II. IV. VI.	32
Tantalum	Ta V.	182
Tellurium	Te II. IV. VI.	128
Thallium	Tl I. III.	204
Thorium	Th IV.	231·5
Tin	Sn IV.	118
Titanium	Ti IV.	48
Tungsten	W IV. VI.	184
Uranium	U VI. IV.	240
Vanadium	V III. V.	51·2
Yttrium	Y IV.	89
Zinc	Zn II.	65
Zirconium	Zr IV.	90

APPENDIX VI.

ALCOHOL TABLES.

Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.	Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.
1·0000	0·00	0·00	0.00	·9749	17·33	21·29	37·30
·9999	0·05	0·07	1·12	·9739	18·15	22·27	39·03
·9989	0·58	0.73	1·28	·9729	18·92	23·19	40·64
·9979	1·12	1·42	2·48	·9719	19·75	24·18	42·38
·9969	1·75	2·20	3·85	·9709	20·58	25·17	44·12
·9959	2·33	2·93	5·13	·9699	21·38	26·13	45·79
·9949	2·89	3·62	6·34	·9689	22·15	27·04	47·39
·9939	3·47	4·34	7·61	·9679	22·92	27·95	48·98
·9929	4·06	5·08	8·00	·9669	23·69	28·86	50·57
·9919	4·69	5·86	10·26	·9659	24·46	29·76	52·16
·9909	5·31	6·63	11·62	·9649	25·21	30·65	53·71
·9899	5·94	7·40	12·97	·9639	25·93	31·48	55·18
·9889	6·64	8·27	14·50	·9629	26·60	32·27	56·55
·9879	7·33	9·13	15·99	·9619	27·29	33·06	57·94
·9869	8·00	9·95	17·43	·9609	28·00	33·89	59·40
·9859	8·71	10·82	18·96	·9599	28·62	34·61	60·66
·9849	9·43	11·70	20·50	·9589	29·27	35·35	61·95
·9839	10·15	12·58	22·06	·9579	29·93	36·12	63·30
·9829	10·92	13·52	23·70	·9569	30·50	36·76	64·43
·9819	11·69	14·46	25·34	·9559	31·06	37·41	65·55
·9809	12·46	15·40	26·99	·9549	31·69	38·11	66·80
·9799	13·23	16·33	28·62	·9539	32·31	38·82	68·04
·9789	14·00	17·24	30·26	·9529	32·94	39·54	69·29
·9779	14·91	18·36	33·19	·9519	33·53	40·20	70·46
·9769	15·75	19·39	33·96	·9509	34·10	40·84	71·58
·9759	15·54	20·33	35·63	·9499	34·57	41·37	72·50

Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.	Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.
·9489	35·05	41·90	73·43	·9109	53·17	61·02	106·93
·9479	35·55	42·45	74·39	·9099	53·61	61·45	107·69
·9469	36·06	43·01	75·37	·9089	54·05	61·88	108·45
·9459	36·61	43·63	76·45	·9079	54·52	62·36	109·28
·9449	37·17	44·24	77·53	·9069	55·00	62·84	110·12
·9439	37·72	44·86	78·61	·9059	55·45	63·28	110·92
·9429	38·28	45·47	79·68	·9049	55·91	63·73	111·71
·9419	38·83	46·08	80·75	·9039	56·36	64·18	112·49
·9409	39·35	46·54	81·74	·9029	56·82	64·63	113·26
·9399	39·85	47·18	82·69	·9019	57·25	65·05	113·99
·9389	40·35	47·72	83·64	·9009	57·67	65·45	114·69
·9379	40·85	48·26	84·58	·8999	58·09	65·85	115·41
·9369	41·35	48·80	85·53	·8989	58·55	66·29	116·18
·9359	41·85	49·34	86·47	·8979	59·00	66·74	116·96
·9349	42·33	49·86	87·37	·8969	59·43	67·15	117·68
·9339	42·81	50·37	88·26	·8959	59·87	67·57	118·41
·9329	43·29	50·87	89·15	·8949	60·29	67·97	119·12
·9319	43·76	51·38	90·03	·8939	60·71	68·36	119·80
·9309	44·23	51·87	90·89	·8929	61·13	68·76	120·49
·9299	44·68	52·34	91·73	·8919	61·54	69·15	121·18
·9289	45·14	52·82	92·56	·8909	61·96	69·54	121·86
·9279	45·59	53·29	93·39	·8899	62·41	69·96	122·61
·9269	46·05	53·77	94·22	·8889	62·86	70·40	123·36
·9259	46·50	54·24	95·05	·8879	63·30	70·81	124·09
·9249	46·96	54·71	95·88	·8869	63·74	71·22	124·80
·9239	47·41	55·18	96·70	·8859	64·17	71·62	125·51
·9229	47·86	55·65	97·52	·8849	64.61	72·02	126·22
·9219	48·32	56·11	98·34	·8839	65·04	72·42	126·92
·9209	48·77	56·58	99·16	·8829	65·46	72·80	127·59
·9199	49·20	57·02	99·93	·8819	65·88	73·19	128·25
				·8809	66·30	73·57	128·94
·9198	49·24	57·06	100·00 Ps.	·8799	66·74	73·97	129·64
				·8789	67·17	74·37	130·33
·9189	49·68	57·49	100·76	·8779	67·58	74·74	130·98
·9179	50·13	57·97	101·59	·8769	68·00	75·12	131·64
·9169	50·57	58·41	102·35	·8759	68·42	75·49	132·30
·9159	51·00	58·85	103·12	·8749	68·83	75·87	132·95
·9149	51·42	59·26	103·85	·8739	69·25	76·24	133·60
·1930	51·83	59·68	104·58	·8729	69·67	76·61	134·25
·9129	52·27	60·12	105·35	·8719	70·08	76·98	134·90
·9119	52·73	60.56	106·15	·8709	70·48	77·32	135·51

Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.
·8699	70·88	77·67	136·13
·8689	71.29	78.04	136·76
·8679	71·71	78·40	137·40
·8669	72·13	78·77	138·05
·8659	72·57	79·16	138·72
·8649	73.00	79·54	139·39
·8639	73·42	79·90	140·02
·8629	73·83	80·26	140·65
·8619	74·27	80·64	141·33
·8609	74·73	81·04	142·03
·8599	75·18	81·44	142·73
·8589	75·64	81·84	143·42
·8579	76·08	82·23	144·10
·8569	76·50	82·58	144·72
·8559	76·92	82·93	145·34
·8549	77·33	83·28	145·96
·8539	77·75	83·64	146·57
·8529	78·16	83·98	147·17
·8519	78·56	84·31	147·75
·8509	78·96	84·64	148·32
·8499	79·36	84·97	148·90
·8489	79·76	85·29	149·44
·8479	80·17	85·63	150·06
·8469	80·58	85·97	150·67
·8459	81·00	86·32	151·27
·8449	81·40	86·64	151·83
·8439	81·80	86·96	152·40
·8429	82·19	87·27	152·95
·8419	82·58	87·58	153·48
·8409	82·96	87·88	154·01
·8399	83·35	88·19	154·54
·8389	83·73	88·49	155·07
·8379	84·12	88·79	155·61
·8369	84·52	89·11	156·16
·8359	84·92	89·42	156·71
·8349	85·31	89·72	157·24
·8339	85·69	90·02	157·76
·8329	86·08	90·32	158·28
·8319	86·46	90·61	158·79
·8309	86·85	90·90	159·31
·8299	87·23	91·20	159·82

Specific gravity, 15·5°.	Absolute Alcohol by weight. Per cent.	Absolute Alcohol by volume. Per cent.	Proof Spirit. Per cent.
·8289	87·62	91·49	160·33
·8279	88·00	91·78	160·84
·8269	88·40	92·08	161·37
·8259	88·80	92·39	161·91
·8249	89·19	92·68	162·43
·8239	89·58	92·97	162·93
·8229	89·96	93·26	163·43
·8219	90·32	93·52	163·88
·8209	90·68	93·77	164·33
·8199	91·04	94·03	164·78
·8189	91·39	94·28	165·23
·8179	91·75	94·53	165·67
·8169	92·11	94·79	166·12
·8159	92·48	95·06	166·58
·8149	92·85	95·32	167·04
·8139	93·22	95·58	167·50
·8129	93·59	95·84	167·96
·8119	93·96	96·11	168·24
·8109	94·31	96·34	168·84
·8099	94·66	96·57	169·24
·8089	95·00	96·80	169·65
·8079	95·36	97·05	170·07
·8069	95·71	97·29	170·50
·8059	96·07	97·53	170·99
·8049	96·40	97·75	171·30
·8039	96·73	97·96	171·68
·8029	97·07	98·18	172·05
·8019	97·40	98·39	172·43
·8099	97·73	98·61	172·80
·7999	98·06	98·82	173·17
·7989	98·37	99·00	173·50
·7979	98·69	99·18	173·84
·7969	99·00	99·37	174·17
·7959	99·32	99·57	174·52
·7949	99·65	99·77	174·87
·7939	99·97	99·98	175·22

Absolute Alcohol.			
·7938	100·00	100·00	175·25

APPENDIX VII.

THE METRIC SYSTEM.

MEASURES OF LENGTH.

	Metres.	Inches.	Feet.	Yards.	Miles.
Kilometre .	1000				0·6214
Hectometre	100				
Decametre .	10				
Metre .	1	39·37	3·28	1·0936	
Decimetre .	0·1				
Centimetre.	0·01				
Millimetre .	0·001	0·03937			

MEASURES OF AREA.

	Square Metres.	British Measures of Area.
Square Kilometre .	1,000,000	0·3861 sq. mile.
,, Hectometre, or Hectare	10,000	2·4711 acres.
,, Decametre, or Are	100	119·6 sq. yds.
,, Metre . .	1	10·764 sq. ft.
,, Decimetre .	0·01	15·5 sq. ins.
,, Centimetre .	0·0001	0·155 ,,
,, Millimetre .	0·000001	0·00155 ,,

SOLID MEASURES.

1 cubic	Decametre, or Kilostere	equals	35,316·5	cubic ft.
1 ,,	Metre, or Stere	,,	35·316	,,
1 ,,	Decimetre, or Millistere	,,	61·025	cubic ins.
1 ,,	Centimetre	,,	0·061	,,
1 ,,	Millimetre	,,	0·000061	,,

MEASURES OF WEIGHT.

	Grammes.	Grains.	Avoir. ozs.	Avoir. lb.
Kilogramme .	1000	15,432	35·3	2·204
Hectogramme	100			
Decagramme .	10			
Gramme .	1	15·432	0·0353	0·0022
Decigramme .	0·1			
Centigramme	0·01			
Milligramme .	0·001	0·0154		

MEASURES OF CAPACITY.

	Cubic Centimetres.	Fluid ozs.	Pints.	Gallons.	Cubic ins.
Kilolitre .	1,000,000				
Hectolitre .	100,000				
Decalitre .	10,000				
Litre .	1,000	35·3	1·76	0·22	61·027
Decilitre .	100				
Centilitre .	10				
Millilitre .	1				

FACTORS REQUIRED FOR CALCULATING EQUIVALENTS OF WEIGHT, VOLUME, &c.

To convert	grammes	to	pounds	multiply by	0·0022
,,	,,	,,	grains	,,	15·432
,,	,,	,,	ounces	,,	0·0353
,,	grains	,,	grammes	,,	0·0648
,,	ounces	,,	,,	,,	28·349
,,	pounds	,,	,,	,,	453·592
,,	kilogrammes	,,	pounds	,,	2·204
,,	,,	,,	ounces	,,	35·3
,,	litres	,,	gallons	,,	0·22
,,	,,	,,	fluid ounces	,,	35·3
,,	,,	,,	pints	,,	1·76
,,	,,	,,	cubic feet	,,	0·354
,,	,,	,,	,, inches	,,	61·027
,,	gallons	,,	cubic feet	,,	0·1605
,,	,,	,,	litres	,,	4·5434
,,	pints	,,	,,	,,	0·5679
,,	,,	,,	cubic centimetres	,,	568·1818
,,	,,	,,	,, inches	,,	34·6592
,,	cubic metres	,,	gallons	,,	0·0036
,,	,,	,,	pints	,,	0·0288
,,	,,	,,	fluid ounces	,,	0·5813
,,	,,	,,	cubic centimetres	,,	16·4
,,	cubic feet	,,	,, metres	,,	0·0283
,,	,,	,,	litres	,,	28·2153
,,	,,	,,	gallons	,,	6·2322
,,	fluid ounces	,,	cubic inches	,,	1·72
,,	,,	,,	,, centimetres	,,	28·35
,,	square feet	,,	square metres	,,	0·0924
,,	,,	,,	,, yards	,,	0·111
,,	square metres	,,	,, feet	,,	10·7641
,,	inches	,,	metres	,,	0·0254
,,	,,	,,	millimetres	,,	25·4
,,	metres	,,	inches	,,	39·37
,,	,,	,,	feet	,,	0·30479
,,	feet	,,	miles	,,	0·000187
,,	yards	,,	,,	,,	0·00057
,,	,,	,,	centimetres	,,	2·54
,,	centimetres	,,	inches	,,	0·3937
,,	millimetres	,,	,,	,,	0·03937
,,	kilometres	,,	miles	,,	1·6
,,	sq. kilometres	,,	square miles	,,	2·5899
,,	hectares	,,	acres	,,	0·4046

INDEX

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Physician to and Lecturer on Clinical Medicine, Belfast Royal Hospital;
Consulting Physician to the Belfast Ophthalmic Hospital and Consulting Physician to the Ulster Hospital for Women and Children;
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