PUBLIC HEALTH

CHEMISTRY AND BACTERIOLOGY:

A HANDBOOK
FOR D.P.H. STUDENTS

BY

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PREFACE

THE present work is based on the notes prepared by the writer for his class while teaching Public Health Chemistry and Bacteriology. These were compiled from various standard works, and from articles appearing in the medical journals from time to time. No originality is therefore claimed for them, and grateful acknowledgment is made to the sources indicated.*

The book is intended to assist in, and supplement, actual laboratory teaching, and not in any way to supersede it. It has been the writer's endeavour therefore to make it as complete as possible, while leaving out matters which can be more satisfactorily taught and demonstrated than written about. No illustrations have been introduced, as the various instruments and apparatus are seen and used in the actual work.

I desire to express my hearty appreciation of, and my best thanks for, the assistance received from a number

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* I am especially indebted to the following works:—Pakes' "Hygiene," Notter & Firth's "Hygiene," Moor & Partridge's "Aids to the Analysis of Foods and Drugs," Richter's "Organic Chemistry," the "Harmsworth Self-Educator," the "Encyclopædia and Dictionary of Medicine and Surgery," Jordan's "Bacteriology," Muir & Ritchie's "Bacteriology," Hiss and Zinsser's "Bacteriology," Abel's "Laboratory Handbook," Stitt's "Practical Bacteriology," Sims Woodhead's "Bacteria and their Products," and the *Lancet*, *Public Health*, and the Bacteriological Supplement to the *Medical Officer*.

of friends, and very specially from Dr. R. M. Buchanan, Bacteriologist to the Corporation of Glasgow, and Dr. J. Hume Patterson, Bacteriologist to the County Council of Lanarkshire. To Dr. John A. Wilson, Assistant Bacteriologist, Glasgow, I owe a careful revision of the chapter on Special Bacteriological Examinations, including the Table on pp. 354–5, and much helpful criticism. To Dr. Archibald MacMillan I am indebted for criticism of some parts of the Chemical portion of the book, as a result of which certain changes have been made.

DAVID McKAIL.

GLASGOW, 1912.

CONTENTS

Addenda and Erratum

Page 38, line 7 :
after "present"
add "as chloride, sulphate, etc."

Page 79, *Preservatives in Milk* : See new Regulations (1912) on page 388.

Page 80, line 30 :
after "tubes"
add "plus a drop of phenolphthalein solution"

Page 84, *Preservatives in Cream* : See new Regulations (1912) on page 388.

Page 233, *Antityphoid vaccine* : Leishman sterilizes the typhoid culture at 53° C. for one hour.

Page 277, line 2 from foot :
after "growth"
add "on glycerin egg medium (see page 391)"

Page 257, line 5 from foot :
for "Limond," *read* "Simond"

PUBLIC HEALTH CHEMISTRY AND BACTERIOLOGY.

INTRODUCTORY.

PUBLIC HEALTH Chemistry and Bacteriology do not differ fundamentally from general chemistry and bacteriology, and in fact are based on these subjects, of which they are specialized departments. The same principles underlie the part as the whole, and the accumulation of scraps of knowledge derived from the parent sciences, under the heading of Public Health Chemistry and Bacteriology, is justifiable only on the score of convenience and the importance of economizing the student's time. It is therefore necessary to remember that the whole is greater than the part, and that to have wide and luminous views of the subjects so designated, the study of them should not be strictly utilitarian, but be extended in all necessary directions as much as possible.

A knowledge of Public Health Chemistry and Bacteriology has become more urgently called for, owing to the increase of Public Health work, to participate in which it is necessary to possess a Diploma in Public Health.

The General Medical Council at their meeting on 1st December, 1911, adopted in an amended form the resolutions and rules which form the Regulations for the Diploma in Public Health. These are printed in full in an Appendix to this volume. For our present purpose it is sufficient to quote here Rule 2.

Rule 2. Every Candidate for a Diploma in Sanitary Science, Public Health, or State Medicine shall have produced satisfactory evidence that, after obtaining a registrable qualification, which should be registered before admission to examination for the diploma, he has received practical instruction in a laboratory or

laboratories, British or foreign, approved by the licensing body granting the diploma, in which chemistry, bacteriology, and the pathology of the diseases of animals transmissible to man are taught.

Note.—The laboratory instruction shall cover a period of not less than four calendar months, and the candidate shall produce evidence that he has worked in the laboratory for at least 240 hours, of which not more than one-half shall be devoted to practical chemistry. The laboratory course should be so arranged as to lay special stress on work which bears most directly on the duties of a medical officer of health.

The amendments in this rule reduce the number of months of instruction from six to four, and define the hours worked as 240, of which at least 120 shall be devoted to Bacteriology. The net result is that students will thus require to do as a minimum 15 hours' practical laboratory work per week for four months. Many students will find it more convenient to do 12 hours per week for a so-called six months' term of 20 weeks.

The final clause of the rule, namely, "The laboratory course should be so arranged as to lay special stress on work which bears most directly on the duties of a medical officer of health" might usefully have been made more specific, and been extended to include the phrase, "and the Examiners for the Diploma shall have special regard to this recommendation."

The student will be well advised to exceed the minimum periods above laid down, as far as he can, and more especially if he is not already well informed in the subjects of chemistry and bacteriology.

The scope of the work and the methods may be thus summarized on the basis of the Syllabus of the Scottish Conjoint Board :—

DIPLOMA IN PUBLIC HEALTH.

Public Health Laboratory Work Course

comprises :—

Physical, Chemical, and Bacteriological Examination of water, sewage and sewage effluents, air and other gases, food stuffs, beverages, soils, and building materials.

Examination of disinfectants, antiseptics, deodorants.

Detection of Poisons in foods, dress, decoration.

Examination of parasites and other animal organisms found in the body and human food stuffs.

Bacteriology and Bacteriological Methods: apparatus, media, modes of culture; culture and recognition of the principal pathogenic organisms; bacteriological examination of water, air, and foods; antitoxins; principles of serumtherapy and immunization; cultivation and recognition of micro-organisms in relation to epidemic and other diseases.

Modes of Examination.

Physical.—Inspection by the unaided senses—colour, odour, taste, transparency, turbidity, etc.; specific gravity; microscopic examination; spectroscopic examination; polariscopic examination.

Chemical.—Examination for proximate principles, e.g., water, solids, fat, sugar, etc.; search for and identification of chemical impurities such as ammonia, lead, borax, etc.; quantitative estimations of above.

Bacteriological.—Enumeration of bacteria present; search for pathogenic forms; if found, isolation and identification.

PART I.

PUBLIC HEALTH CHEMISTRY.

IT is necessary, in the first place, to consider carefully certain points of chemical practice, which form the basis of much of the subsequent work, and which must be thoroughly understood to enable the latter to be readily followed and apprehended.

CHAPTER I.

CHEMICAL ANALYSIS.

THIS is of two kinds—*Qualitative* or *Quantitative.*

1. **Qualitative.**—Consists in proving the presence or absence of certain metals or salts, or generally of chemical elements or radicles or compounds in a substance or solution, by the use of a series of tests.

2. **Quantitative.**—Consists in separating out the constituents of any composite body and accurately estimating the amount of each of them. This may be done in three ways: Gravimetrically, Volumetrically, Colorimetrically.

Gravimetric method.—The desired constituent is separated out in a known form, and this is accurately *weighed.* As a method it is often very complicated, very lengthy, and requires elaborate apparatus and much skill.

Volumetric method.—This consists in submitting the substance to certain characteristic reactions, a measured quantity of a solution of known strength being added until a change looked for occurs. From the quantity of reagent used, the amount of the substance found can be calculated by known chemical laws. It is less elaborate, much more quickly accomplished, needs simpler apparatus as a rule, is susceptible of great accuracy, and the skill required is less specialized.

Colorimetric method.—This consists in using a reaction which produces a coloured tint, which is compared with the tint obtained from the same treatment of a known

quantity of the substance under investigation. Exact matching of the tints is arrived at, either by dilution of the stronger, or by putting up several standard tints.

Requirements for Volumetric Analysis.—

1. Solution of reagent or test, the chemical power of which is accurately known; this is called the Standard Solution.

2. A graduated vessel, from which portions of the standard solution may be accurately delivered: the Burette.

3. Some indication, unmistakeable to the eye, that the reaction is terminated or concluded: the Indicator.

The process is called, a *titration*, i.e., an estimation of the titre or strength of a solution, and the person is said to titrate the solution.

Volumetric methods may be classified thus:—

1. Neutralization of acids by alkalies, and vice versa—acidimetry and alkalimetry.

2. Reduction or oxidation of substances—for example, ferrous sulphate titrated with potassium permanganate illustrates both actions.

3. Precipitation of some insoluble and definite combination—for example, precipitation of AgCl in testing for chlorides.

STANDARD SOLUTIONS.

A standard solution of any substance is made when a known quantity of that substance is dissolved in a known quantity of water. Then the strength can be expressed definitely as—

So many pounds per gallon, or pint, or ounce; or
" grains " " " or
" grammes per litre or cubic centimetre (c.c.); or
" milligrammes " " "

Thus we might have a standard solution of NaCl of these strengths: (1 gallon = 10 lbs. = 70,000 grs.).

1 lb. per gallon; then 1 fluid grain = 0·1 grain NaCl.
1 drachm per fluid ounce; then 1 minim = 0·125 gr. NaCl.
1 gramme per litre; then 1 cubic centimetre = 0·001 gramme, or 1 milligramme of NaCl per c.c.

The metric system of weights and measures is found to be very convenient for such solutions, because—
1 gramme dissolved in 1 litre = 1 milligramme in 1 c.c. ; or
n grammes dissolved in 1 litre = *n* milligrammes in 1 c.c.

Standard solutions often have their strength expressed in terms of some other substance which they measure—usually an elementary substance. For example—

$$\underset{170}{AgNO_3} + NaCl = AgCl + NaNO_3 \quad (23 + 35{\cdot}5).$$

From this equation we see that 170 parts of silver nitrate precipitate completely 58·5 parts of sodium chloride containing 35·5 parts of chlorine. If we wish to estimate the amount of Cl present in a solution of unknown strength, we can titrate with a solution of silver nitrate of known strength—that is, a standard solution. What strength shall we make it ?

a. 170 grm. $AgNO_3$ in 1 litre of water will precipitate 35·5 grm. Cl; then 1 c.c. will precipitate 35·5 mgr. Cl.

b. 17·0 grm. $AgNO_3$ in 1 litre of water will precipitate 3·55 grm. Cl ; then 1 c.c, will precipitate 3·55 mgr. Cl.

c. $\frac{170}{35{\cdot}5} = 4{\cdot}78$ grm. in 1 litre of water will precipitate 1 grm. Cl; then 1 c.c. will precipitate 1 mgr. Cl.

The strength chosen depends on—(1) The simplicity desired ; (2) The strength of solution to be tested ; (3) Whether any one of these strengths will be more useful than the others for other estimations, and so save needless duplication of solutions.

NORMAL SOLUTIONS.

These are standard solutions made to a certain strength on the basis of chemical theory and practice. Thus, from the equation—

$$\underset{40}{NaOH} + \underset{36{\cdot}5}{HCl} = NaCl + H_2O$$

we see that 40 parts of sodium hydrate are exactly neutralized by 36·5 parts of hydrochloric acid. If therefore we

make a standard solution of NaOH 40 grammes to 1 litre, and one of HCl 36·5 grammes to 1 litre, then—

1 c.c. stand. sol. NaOH = 1 c.c. stand. sol. HCl,

and if we can build other solutions on the same plan, we shall have a whole range of solutions which are chemically equivalent c.c. for c.c. This will obviate having a strength of acid for titrating soda, another strength for potash, another for baryta, and so on, and reversely. This result is obtained, or rather attained, by using normal solutions, which are thus defined :—

A normal solution is one which contains in 1 litre of distilled water at 16° C. the hydrogen equivalent of the active reagent weighed in grammes, hydrogen being taken as one gramme. Such a solution is usually indicated by the mark N or $\frac{N}{1}$. If diluted, the degree of dilution is indicated by a denominator, thus—

$\frac{N}{2}$ $\frac{N}{10}$ $\frac{N}{100}$ $\frac{N}{1000}$

which are respectively—

seminormal *decinormal* *centinormal* *millinormal*

The hydrogen equivalent of a substance is found by taking its molecular weight, the number of atoms usually replaced by hydrogen, and the valency of these atoms. Divide the molecular weight by the product of the number of atoms and their valency. Thus—

NaCl m.w. =58·5 no. of replaceable atoms=1 valency=1 ; therefore—

N/1 NaCl=58·5/1×1=58·5 grm. to 1 litre of water.

Similarly—

HCl m.w.=36·5 n.r.a.=1 v.=1; hence N/1=36·5 grm. per litre.

H_2SO_4	m.w. = 98 grm.	N/1 = 49 grm.	per	litre.
HNO_3	m.w. = 63 ,,	N/1 = 63 ,,	,,	
NaOH	m.w. = 40 ,,	N/1 = 40 ,,	,,	
KOH	m.w. = 56 ,,	N/1 = 56 ,,	,,	
Na_2CO_3	m.w. = 106 ,,	N/1 = 53 ,,	,,	
K_2CO_3	m.w. = 138 ,,	N/1 = 69 ,,	,,	

Where the substance possesses molecules of water of crystallization, the weight of these must be added to the sum of the molecule proper, in order to arrive at the right figure for the normal solution. Thus—

$H_2C_2O_4+2H_2O$ m.w.=126 N/1=63 grm. per litre (oxalic acid).

$H_3C_6H_5O_7+H_2O$ m.w.=210 N/1=70 grm. per litre (citric acid).

$H_2C_4H_4O_6$ m.w.=150 N/1=75 grm. per litre (tartaric acid).

$HC_2H_3O_2$ m.w.=60 N/1=60 grm. per litre (acetic acid).

Some salts with enlarged molecules :—

Ferrous sulphate, $FeSO_4+7\ H_2O$ molecular weight =278
Copper sulphate, $CuSO_4+5\ H_2O$,, ,, =249
Lead acetate, $Pb(C_2H_3O_2)_2+3\ H_2O$,, ,, =379
Sodium thiosulphate, $Na_2S_2O_3+5.H_2O$,, ,, =248

The hydrogen equivalent of some reagents is not so easily arrived at ; for instance, potassium permanganate, variously written as

$$K_2Mn_2O_8 \quad \text{and} \quad KMnO_4.$$

The molecular weight of the first formula is 316, and of the second is 158. Nevertheless, the normal solution is not 158 grammes per litre, but is 31·6 grammes per litre. This is because potassium permanganate does not react—as its formula might suggest—as a manganate, but reacts as a double salt of potassium and manganese, as if it were written thus—

$$K_2O + Mn_2O_7,$$

and in reaction the latter oxide is reduced and oxygen liberated ; thus—

$$\underset{316}{K_2Mn_2O_8}+3\ H_2SO_4=K_2SO_4+2\ MnSO_4+3\ H_2O+5\ \underset{80}{O}$$

We here see that 316 grammes of permanganate of potash liberate 80 grammes of oxygen, which are chemically equivalent to 10 grammes of hydrogen, and so 31·6

grammes of the salt are equivalent to 1 gramme of hydrogen. Hence—

N/1	$KMnO_4$	= 31·6 grm. per litre.
N/10	do.	= 3·16 do. do.
N/100	do.	= ·316 grm. do.

Non-Standardized Solutions :—
Ammonia free water.
Organically pure ammonia free water.
Nessler's solution.
Methyl-orange solution (1 grm. per litre of water).
Phenolphthalein solution (1 % in 50 % alcohol).
Starch solution (5 grm. per litre of boiling water).

Baryta water (5 grm. of crystallized barium hydrate in 1 litre of freshly boiled distilled water. Stopper and set aside for three days, and decant off clear liquid. About 0·5 %.)

Metaphenylene-diamine (5 grm. per litre aq. dest. slightly acidulated with a few drops of sulphuric acid).

Naphthylamine acetate in sulphanilic acetate :—

a. 3 to 4 grm. of sulphanilic acid dissolved in 1 litre of dilute acetic acid.

b. ⅔rds grm. of naphthylamine are boiled with 150 c.c. of aq. dest., the colourless liquid poured off and diluted to 1 litre with dilute acetic acid.

c. Mix equal bulks as required for testing.

Phenol-sulphonic acid (32 c.c. of pure concentrated sulphuric acid are added to 4 c.c. of pure phenol. Heat to 100° C. for 2 to 3 hours. Cool, and add 110 c.c. of distilled water).

INDICATORS.

These may be classified under two heads :—

(1) *Neutrality indicators*, which give a special reaction with acid or alkaline liquids, or with both; (2) *All others*, such as starch, iodine, chromate of potash, permanganate of potash, and soap lather.

Neutrality indicators are divided thus by R. T. Thompson :—

(1) *Methyl-orange group*: are most susceptible to alkalies; methyl-orange, lacmoid, cochineal, congo-red; (2) *Phenolphthalein group*: are most susceptible to acids; phenolphthalein, turmeric; (3) *Litmus group*: are intermediate in susceptibility—litmus, rosolic acid, phenacetolin.

Sensitiveness.—Phenolphthalein, lacmoid, rosolic acid, and phenacetolin showed change of colour with one-fifth quantity of acid or alkali required by methyl-orange and litmus; that is to say, if the two latter required in 100 c.c. of acid or alkali 0·5 c.c. to show change of colour, the former required only 0·1 c.c.

Neutral point of one indicator does not coincide exactly with that of other indicators. Thus, saliva is generally neutral to litmus, alkaline to lacmoid or congo-red, and acid to turmeric. Fresh milk shows similar variations.

1. *Litmus solution* is violet coloured. Acids change it to red; alkalies to blue.

In cold solution it may be used for the titration of—

Hydrates of soda, potash, ammonia, lime, baryta, etc.
Nitric, sulphuric, hydrochloric, and oxalic acids.
Arsenites and silicates of soda and potash.

In boiling solution—

Carbonates and bicarbonates of K, Na, Ca, Mg, Ba.
Sulphides of sodium and potassium.

2. *Methyl-orange* is orange-brown in colour. Acids change it to pink; alkalies to faint yellow.

Only used in cold solution, and then for titration of—

Hydrates, carbonates, bicarbonates of K, Na, NH_3, Ca, Mg, Ba, etc.
Sulphides, arsenites, silicates, borates of K, Na, NH_3, Ca, Mg, Ba, etc.
All the mineral acids.
Sulphites.
Half the base in the alkaline and earthy alkaline phosphates and arseniates.
NOT for organic acids, nor in presence of nitrous acid or nitrites, which decompose it.

3. *Phenolphthalein* is colourless in solution. Acids cause no change in colour; alkalies change it to purple-red.

Used in the cold for titration of—
Alkaline hydrates, except ammonia.
Mineral acids.
Organic acids (oxalic, tartaric, acetic, citric, and others).
Carbonates to bicarbonates.

May be used in alcoholic solutions, and hence for organic acids insoluble in water. Also for acids combined with bases, like morphia, quinine, brucine, etc., the organic base having no effect on it.

4. *Rosolic Acid* is pale yellow in solution. With acids, unchanged; with alkalies, violet-red.
Good for mineral acids and oxalic.
Not reliable for organic acids.

5. *Turmeric*, yellow in colour. With acids, bright yellow; with alkalies, reddish-brown.

6 *Lacmoid*, blue and red papers are best form for use. These are an excellent substitute for methyl-orange when latter cannot be used. It is a derivative of resorcin, and is allied to litmus.

The other indicators will be alluded to as their use is required.

Rules as to use of indicators commonly employed:—
Methyl-orange for mineral acids.
Phenolphthalein for organic acids.
Litmus for organic acids in presence of free CO_2 (e.g. in beer).

ALKALIMETRY AND ACIDIMETRY.

Perform the following exercises:—

1. Titrate 1 c.c. 50 per cent NaOH diluted with a little water (distilled) with N/1 H_2SO_4, using a few drops of litmus as indicator.

Take the NaOH in a porcelain basin—add the water and the litmus solution. Then take a burette and fill it with the normal acid solution; be careful that the nozzle is

full, and that the lowest part of the meniscus is opposite the zero mark.

Now add the acid 1 c.c. at a time, stirring after each addition, with a glass rod. Continue the process until the colour of the solution changes to red. The result will be accurate to 1 c.c. With practice the end reaction can be judged more accurately; but, as already mentioned, a certain quantity of acid is required for change of colour, and this is greater with litmus than with some other indicators.

Say that 13 c.c. of N/1 H_2SO_4 were required. How much NaOH was present in the 1 c.c. of sample?

1 c.c. N/1 H_2SO_4 = 1 c.c. N/1 NaOH
But N/1 NaOH = 40 grm. per litre
Then 1 c.c. N/1 NaOH = 0·040 grm.

Hence 1 c.c. N/1 H_2SO_4 = 0·040 grm. NaOH
Then 13 c.c. ,, ,, = 0·52 grm. NaOH.

But 13 c.c. were required to neutralize 1 c.c. of sample.

Therefore 1 c.c. of sample contains 0·52 grm. NaOH,
or 100 c.c. ,, will contain 52 grm. NaOH.

The discrepancy between 50 grm. in 100 c.c. and 52 grm. may be due to:

(1) Inaccuracy in making the soda solution originally;
(2) Inaccuracy in measuring the 1 c.c. of soda solution;
(3) Inaccuracy in the strength of the normal acid solution;
(4) Inaccuracy in titration.

2. Repeat the experiment, using 1 drop methyl-orange as indicator.

3. Repeat the experiment, using 1 drop phenolphthalein as indicator.

4. Titrate 5 c.c. 25 per cent H_2SO_4 diluted with a little water, with N/1 NaOH, using a few drops of litmus as indicator.

5. Repeat the experiment, using 1 drop methyl-orange as indicator.

6. Repeat the experiment, using 1 drop phenolphthalein as indicator.

Calculate out strength of solution by method similar to above.

Thus, say that 25 c.c. of N/1 soda are required to neutralize 5 c.c. of sample, said to be 25 per cent sulphuric acid.

Then 1 c.c.	N/1 soda	= or is chemically equivalent to 1 c.c. N/1 sulphuric acid.
But	N/1 H_2SO_4	= 98/2 or 49 grm. per litre.
Hence 1 c.c.	,, ,,	= 0·049 grm. H_2SO_4
Hence 1 c.c.	N/1 NaOH	= 0·049 grm. H_2SO_4
And 25 c.c.	,, ,,	= 25 × 0·049 = 1·225 grm. H_2SO_4.

But 25 c.c. N/1 NaOH were required to neutralize 5 c.c. of sample; therefore 5 c.c. of sample contain 1·225 grm. of sulphuric acid, and 100 c.c. of sample contain 24·5 grm. of sulphuric acid; that is, if 100 c.c. are taken as 100 grm., 24·5 per cent. The sources of error are as before.

WEIGHING AND MEASURING.

Measuring of Solutions.—Small quantities, like 1 c.c., 2 c.c., etc., up to 25 c.c. or 50 c.c., are most accurately measured by pipette, or on some occasions by burette. For larger amounts, like 100 c.c., 250 c.c., 500 c.c., and 1000 c.c., flasks with a narrow neck and a mark thereon are the best. When extreme accuracy is not essential, the ordinary graduated measure is quite efficient. Be careful in pipetting certain liquids not to get any drawn into the mouth. Never pipette strong sulphuric acid or ammonia by mouth suction.

On Weighing with the Chemical Balance.—To weigh a certain quantity of a substance the necessary weights are placed in the right-hand pan of the balance. Some of the substance is then placed in the left-hand pan, or preferably in a watch-glass of known weight, or balanced by a similar one in the other pan. The handle or screw is now turned, and the balance put in action. If the pointer swings more on the side away from the weights, that is more to the left side, the amount of substance is too little. *The handle must now be turned down, and the balance thus placed*

at rest, before any further manipulations are tried. Thereafter more of the substance is added, and the same technique carefully observed.

To obtain the weight of a substance or dish, or both, these are placed in the left-hand pan, and a trial weight is put on the right pan. If this is too much, the next lower weight is tried, and so on, being careful to observe the technique outlined above. The weight is read from the vacant spaces in the case, and checked on removing the weights. Always use a watch-glass in weighing a salt or substance not contained in a vessel of some kind.

Atomic Weights.—The following table gives for some of the elements the older atomic weight, and that more recently assigned.

	OLDER ATOMIC WEIGHT.	NEWER.
Aluminium	27	26·9
Arsenic	75	74·4
Barium	137	136·4
Boron	11	10·9
Bromine	80	79·4
Calcium	40	39·7
Carbon	12	11·9
Chlorine	35·5	35·1
Copper	63	63·1
Hydrogen	1	—
Iodine	127	126·0
Iron	56	55·5
Lead	207	206·4
Magnesium	24	24·18
Manganese	55	54·52
Mercury	200	198·5
Nitrogen	14	13·9
Oxygen	16	15·9
Phosphorus	31	30·8
Potassium	39	38·8
Silver	108	107·1
Sodium	23	22·9
Sulphur	32	31·8
Tin	118	118·1
Zinc	65	64·9

Volume and Density of Gases.—All gaseous molecules, at the same temperature and pressure, occupy the same volume. This is another way of stating Avogadro's law, that "equal volumes of all gases (at a temperature sufficiently remote from their condensation-point—the so-called permanent gases) at the same temperature and pressure, contain the same number of molecules."

From this it follows, that if we know the volume occupied by one gaseous molecule at standard temperature and pressure, we thereby know the volume of all gaseous molecules at the same temperature and pressure. The volume occupied by the molecular weight of hydrogen, expressed as grammes, that is 2 grammes of hydrogen gas at 0° C. and 760 mm. pressure, is 22·32 litres. Hence the statement, "the molecular weight of any gas, expressed in grammes, measures 22·32 litres, at standard temperature and pressure, that is, 0° C. and 760 mm., or 32° F. and 29·9 inches of mercury."

The Crith.—One litre of hydrogen gas at standard temperature and pressure weighs 0·0896 gramme. This weight has been called a crith. It follows from the above statement of Avogadro's law, that one litre of oxygen gas will contain the same number of molecules, each one 16 times the weight of the hydrogen molecule (as 32 : 2), and hence the weight of one litre of oxygen gas will be 16 criths, or 16 × 0·0896 grm. Similarly, one litre of carbon dioxide gas weighs 22 criths (as 44 : 2). The weight, therefore, of one litre of an elementary gas (with exceptions) is equal to its atomic weight in criths; and of one litre of a compound gas, is equal to half its molecular weight in criths.

Metric System.

The weights and measures of the metric system are those used nowadays in most Public Health work, although statements like "grains per gallon" still persist. The chief units employed are the following:—

Length.—

1 *metre* = the length of a rod of platinum at the temperature of melting ice. This rod is kept at Paris, with official copies in the large towns. Equals 39·37 inches.

1 decimetre $= \frac{1}{10}$ of a metre.
1 centimetre $= \frac{1}{100}$ of a metre.
1 millimetre $= \frac{1}{1000}$ of a metre.
1 micron (μ) $= \frac{1}{1000}$ of a millimetre.
1 kilometre = 1000 metres.

Mass.—

1 *gramme* = the mass of 1 cubic centimetre of distilled water at the temperature of its maximum density, 4° C. or 39·2° F., 1 c.c. at 16° C. or 60·8° F. = 0·9989 gramme. 1 gramme equals 15·432 grains.
1 decigramme $= \frac{1}{10}$ of a gramme.
1 centigramme $= \frac{1}{100}$ of a gramme.
1 milligramme $= \frac{1}{1000}$ of a gramme.
1 kilogramme = 1000 grammes.

Volume.—

1 *litre* = the volume or capacity of 1 kilogramme of distilled water at 4° C. Equals 35·196 imperial fluid ounces.
1 decilitre $= \frac{1}{10}$ of a litre.
1 centilitre $= \frac{1}{100}$ of a litre.
1 millilitre $= \frac{1}{1000}$ of a litre.
1 *cubic centimetre* $= \frac{1}{1000}$ of a litre.

FACTORS FOR CONVERSION FROM ONE SCALE TO THE OTHER.

Grammes into grains	×	15·432
,, into ounces, avoirdupois ..	×	0·03527
Kilogrammes into pounds	×	2·2046
Grains into grammes	×	0·0648
Avoirdupois ounces into grammes ..	×	28·35
Troy ounces into grammes	×	31·104
Cubic centimetres into fluid ounces, impl.	×	0·0352
Litres into fluid ounces, imperial ..	×	35·2
Fluid ounces into cubic centimetres ..	×	28·42
Pints into litres	×	0·568
Metres into inches	×	39·37
Inches into metres	×	0·0254

The following tables give metric equivalents of imperial measures of mass and capacity :—

Length.—

1 mm. (millimetre) = $\frac{1}{25}$ of an inch.
1 cm. (centimetre) = $\frac{2}{5}$ of an inch.
1 inch = 25·4 millimetres or $2\frac{1}{2}$ centimetres.

Mass.—

1 mgr. (milligramme) = 0·01543 grain (or approx. $\frac{1}{64}$ gr.).
1 grm. (gramme) = 15·4323 grains.
1 kgr. (" kilo " or kilogramme) = 2 lb. $3\frac{1}{4}$ oz. avoirdupois.
1 pound avoirdupois = 453·592 grammes.
1 ounce avoirdupois = 28·35 grammes.
1 grain = 0·0648 gramme or 64·8 milligrammes.

Capacity.—

1 centimil = 0·17 minims (approx.) imperial measure.
1 decimil = 1·7 minims (approx.) imperial measure.
1 c.c. (cubic centimetre) (or 1 mil) = 16·9 minims, imperial measure.
1 L. (litre) = 35·196 fluid ounces (35 fl. oz., 1 fl. dr., 34 min.), imperial measure.
1 fl. ounce, imperial measure = 28·42 cubic centimetres.
1 pint, imperial measure = 568·34 cubic centimetres.
1 gallon, imperial measure = 4·546 litres, or 10 lb. avoirdupois of pure water at 62° F., and under an atmospheric pressure of 30 inches of mercury.

CHAPTER II.

WATER ANALYSIS.

THE examination of water samples is most commonly made to determine the presence or absence of evidence of sewage pollution. If the pollution is gross, the evidence of the unaided senses will cause its rejection. Few people would use for domestic purposes water which was turbid, or contained suspended matter, or had a peculiar colour, or smelt badly, or had a disagreeable taste. Yet a water may have none of these characteristics, and still be dangerous or unfit for domestic use. Chemical analysis is then often of service in distinguishing good from bad waters. Except in the case of a poisonous metal, the analysis does not aim at finding things deleterious in themselves, but the search is made for constituents which suggest the presence of deleterious or dangerous substances. In the case of sewage pollution, the latter are micro-organisms, and the former are those constituents of sewage which are readily detected, namely, chlorides from the urine, ammonia from the urea, and so-called albuminoid ammonia from any albuminous matter. As the average adult excretes 6 to 9 grammes of chlorine daily as chlorides, and 1 part of chlorine per 100,000 parts of water is easily estimated, the pollution produced by one day's excretion of urine into a water would thus be inferable from a rise of the chlorine content 1 per 100,000 even where the dilution was into 600–900 litres or 120–180 gallons of water. The ammonia estimation is much more sensitive, a rise of 1 part in 50,000,000 being detectable. The urea excretion of an adult averages 33 grammes per day, and by the influence of the micrococcus ureæ this is changed to ammonium carbonate, thus :—

$$\underset{60}{CO(NH_2)_2} + 2H_2O = \underset{96}{CO_3(NH_4)_2}$$

Hence, if 60 grammes of urea give rise to 96 grammes of ammonium carbonate, containing 34 grammes of ammonia, 33 grammes of urea will give rise to the formation of 18·7

grammes of ammonia, which diluted into 900,000 litres or 200,000 gallons of water, would still have caused an appreciable rise on the amount (if any) of ammonia already present. This illustrates the delicacy of some of these tests.

Various other estimations are carried out, such as, to determine the presence or absence of poisonous metals, the degree of hardness, etc. These are usefully summarized thus :—

Water Analysis.

This consists of three parts :—

1. Physical examination.
2. Chemical examination.
3. Bacteriological examination.

Physical Examination—

Transparency—clear and bright—turbid.

Suspended matter—stand for twenty-four hours in glass with conical bottom.

Colour—two-foot tube.

Taste—uncertain—iron detectable in $\frac{1}{5}$ gr. per gallon—NaCl in 75 grains per gallon.

Smell.

Microscopic characters of sediment—

Particles of animal, vegetable, and mineral origin.

Micro-organisms, bacterial and protozoal.

Chemical Examination.—

Reaction.

Dissolved solids—Total.
Fixed.
Volatile.
Charring on ignition—fumes—odour.

Chlorine.

Poisonous metals—Pb, Cu, Fe, Zn, As, Sn.

Lime and magnesia.

Phosphates and sulphates.

Free carbonic acid—bicarbonates—carbonates ; dissolved oxygen ; sulphuretted hydrogen.

Hardness—Total.
Permanent.
Temporary.

Free and saline ammonia.
Albuminoid ammonia.
Oxygen absorption.
Nitrates.
Nitrites.

Bacteriological Examination.—

(*a*) Absolute minimum.

1. Enumeration of bacteria growing in a medium at air temperature 18°–22° C.
2. Search for Bacillus coli. If found—identification and enumeration.

(*b*) Additional.

3. Enumeration of bacteria growing at blood heat 37° C.
4. Search for and enumeration of streptococci.
5. Search for Bacillus enteritidis sporogenes.

(*c*) Special procedures.

6. Isolation of Bacillus typhosus from water.
7. Isolation of Spirillum choleræ.

COLLECTION OF SAMPLE.

A fair average sample should be taken in a clean glass vessel with a glass stopper. In filling the vessel from a pond, lake, reservoir, or river, the mouth of it should be sunk two inches below the surface, and the vessel should be filled and emptied once or twice. If a surface specimen is wanted, then of course the sample will be so taken. When sampling water from a pipe or tap, unless the effect of the water on the pipes is under examination, the water should be allowed to run to waste for a few minutes before filling the vessel.

The stopper should be tied in but not sealed.

A convenient receiver is a Winchester quart bottle which holds half a gallon, and this is a suitable quantity for the usual analysis.

Along with the sample, a written statement should be sent, giving full particulars as to mode of collection, place, time, recent meteorological conditions, reason why analysis is desired, etc., etc.

The examination should be undertaken as soon as possible, since changes take place on keeping. If delay is unavoidable, changes should be kept at a minimum by packing in ice.

PHYSICAL EXAMINATION.

Transparency.

Suspended matter.

Colour.—Best, bluish or greyish; greenish, from algæ; yellow or brown suspicious, except peaty.

Taste.

Smell.—Place 250 c.c. in a glass-stoppered bottle. Put on water-oven at 30° C. for a few minutes. Remove stopper, and smell at once.

Sediment.—Let water stand for a few hours, pipette a few c.c. from bottom, centrifuge, mount a drop on a clean slide, and examine. The deposit may contain a very large number of things.

1. Mineral matter, such as sand, clay, etc.
2. Vegetable matter: (*a*) Living—such as bacteria, yeasts, moulds, diatoms, desmids, rotiferæ; (*b*) Dead—vegetable cells, husks of grain, cotton or linen fibres, starch granules.
3. Animal matter: (*a*) Living—such as ova, insects, worms, etc.; (*b*) Dead—such as hairs, scales, muscle fibre.

CHEMICAL EXAMINATION.

Reaction.—Most drinking waters are alkaline in reaction. Upland surface water is often acid from humic and ulmic acids; and this is important, as these acids dissolve lead. Sewage-contaminated waters usually retain their alkalinity. Waters polluted by refuse from chemical or dye works are sometimes acid in reaction.

Dissolved Solids.—The suspended matter is usually allowed to settle before testing for the solids in solution. The latter are estimated as total, fixed, and volatile. Also note, when igniting dried solids, presence or absence of fumes, odour, and charring.

Total Solids.—(1) Take a weighed platinum or porcelain dish of sufficient size; (2) Add 100, 200, 250, 500, or

1000 c.c. of water sample ; (3) Reduce bulk by moderate heat, avoiding boiling or spurting. Or, to a small dish successive quantities of the sample are added, a note being kept of the amount ; (4) Evaporate to complete dryness at 100° C. (212° F.) on water-bath ; (5) Now place in water-oven at 100° C. for half an hour to remove all traces of moisture. Some analysts advise this drying to be done at 105° C. in hot-air chamber ; (6) Cool in dessicator ; (7) Weigh. To make quite sure that residue is dry, items 5, 6, and 7 can be repeated until weight is constant ; (8) Subtract weight of dish ; difference is weight of total residue in amount of sample taken ; (9) Calculate as parts per 100,000, and as grains per gallon.

Fixed Solids.—(1) Incinerate the dried solids at as low a heat as possible ; (2) Watch the process for blackening or charring, fumes or odour. A piece of dry starch and KI paper held over crucible will detect any nitric oxide given off ; (3) Cool and weigh ; (4) Difference from weight of dish gives fixed solids in amount of sample taken. Calculate as before.

Volatile Solids.—Total solids less fixed solids, gives volatile. Consist of organic matter, nitrates, nitrites, ammoniacal salts, combined water, combined carbonic acid, and sometimes chlorides. Should not exceed 1·5 per 100,000 in a very good water.

EXAMPLE.—Evaporated 200 c.c. sample water to dryness, dried in air-oven, cooled in dessicator, and weighed :—

Weight	19·674	grammes.
Weight of platinum dish..	19·554	,,
Difference	0·120	gramme.

i.e., 0·120 grm. in 200 c.c. water, or 0·060 grm. in 100 c.c. or 100 grm., or 60 grm. in 100,000 grm., or 60 parts per 100,000 parts.

Incinerated total residue at low heat. No blackening, fumes, odour, nor change in starch and KI paper. Cooled and weighed :—

Weight	19·650	grammes.
Weight of platinum dish..	19·554	,,
Difference	0·096	gramme.

i.e. 0·096 grm. of fixed residue in 200 c.c. of sample water, or 0·048 grm. in 100 c.c. or in 100 grm., or 48 parts of fixed solids per 100,000 parts of sample. Then volatile solids = 60 — 48 = 12 parts per 100,000 parts.

Chlorine (present as Chlorides).—This is estimated by precipitation with silver nitrate—the end of the process being known by the use of a few drops of potassium chromate, which gives a red precipitate with silver nitrate. So long as there is any chlorine in solution, however, the red precipitate which forms when each drop of silver nitrate solution is added, is immediately dispelled. The first indication of the red colour persisting is taken as the end of the reaction.

The water sample must be neutral, and certainly not acid. It should also be colourless, or nearly so.

Solutions required : (1) 5 per cent solution of potassium monochromate, K_2CrO_4, free from chlorine ; (2) Silver nitrate solution, either decinormal or standard, say 1 c.c. = 1 mgr. Cl.

Process.—(1) Take 100 c.c. sample in a clean porcelain basin ; (2) Add a few drops of chromate solution, which gives the liquid a yellow tinge ; (3) Fill burette with silver nitrate solution, and level ; (4) Run in the solution drop by drop, stirring the while ; (5) Stop when the least permanent red tint is got ; (6) Calculate amount present in parts per 100,000, and grains per gallon.

After a preliminary trial, the end reaction can be more accurately watched, and a second estimation should always be done.

EXAMPLE.—100 c.c. sample took 6·5 c.c. standard silver nitrate solution, of which 1 c.c. = 1 mgr. Cl. Therefore, there are—

6·5 × 1 = 6·5 mgr. Cl in 100 c.c. sample
in 100 grm. ,,
in 100,000 mgr. of sample

that is, 6·5 parts per 100,000 parts.

For grains per gallon multiply result by 0·7, thus :—

6·5 × 0·7 = 4·55 grains per gallon.

The result is sometimes required in terms of NaCl.

Every molecule of NaCl = 58·5 parts, of which 35·5 parts are Cl. Therefore one part of Cl = 58·5 ÷ 35·5 = 1·65 part NaCl. Then 6·5 parts Cl per 100,000 parts of sample becomes 6·5 × 1·65 = 10·7 parts NaCl per 100,000 parts of sample.

With decinormal silver nitrate solution the process is similar, but as 1 c.c. = 3·55 mgr. of Cl, much less solution will be required. On this account a larger quantity of sample is frequently taken, say 250 c.c. When more than 10 c.c. of standard silver solution are required in the titration, it is advisable to repeat the process after diluting the sample water with distilled water. In this way a more accurate result is obtained.

The purest water as a rule contains less than 1·5 parts Cl per 100,000. Increase may be due to sea-water, salt-bearing strata, sewage, etc., and gives cause for suspicion until explained satisfactorily.

Poisonous Metals.—Under this heading are usually included Pb, Cu, Fe, and Zn.

The presence of lead, copper, or iron in appreciable amount can be determined by taking 100 c.c. in a Nessler glass, and adding one or two *drops* of ammonium sulphide solution, when some darkening of the sample will occur in proportion to the quantity present. If no change is noted, compared with a control, then the sample will require to be concentrated, and tests applied.

A delicate qualitative test is to take two 100 c.c. Nessler glasses, and fill one to the 100 c.c. mark with sample and the other with distilled water. To each then add a few drops of solution of permanganate of potash to give them a distinct pink tinge. Then add to each 1 c.c. of sulphuric acid and 1 c.c. of potassium ferrocyanide, and compare the tints.

Iron gives a blue tint; copper gives a brown; zinc gives a white; and lead gives no change. The control shows no change.

Qualitative Test Table.—Concentrate sample to one-fifth of its bulk, say 200 c.c. to 40 c.c., and test thus :—

To 5 c.c. in a test tube add a few drops of Am_2S solution.

Black precipitate may be lead, copper, or iron.
White ,, ,, zinc.

1. If precipitate is black, divide into two portions.

a. To one portion add dilute HCl; if precipitate dissolves, then iron is present. Confirm for iron. Take two tubes with 5 c.c. of concentrated sample in each, after heating for a few minutes with a pinch of potassium chlorate to oxidize the iron to the ferric state. To one, add solution of KCNS; blood-red colour produced. To other, add solution of K_4FeCy_6; prussian blue colour.

b. To other portion add KCN; if precipitate dissolves, copper present. Confirm for copper. As above, take two tubes with concentrated solution, but without treating in any way. To one, add AmOH; blue colour. To other, add K_4FeCy_6; bronze precipitate.

c. If precipitate does not dissolve; then lead. Confirm for lead. As above, take two tubes with concentrated solution. To one add KI solution; yellow precipitate, soluble on boiling. To other add K_2CrO_4 solution; yellow precipitate, soluble in KOH.

2. If precipitate is white, confirm for zinc. Take two tubes as before. To one, add AmCl, AmOH and Am_2S; white precipitate. To other, add K_4FeCy_6; white gelatinous precipitate.

Arsenic.—Take a litre of the water sample, add pure sodium carbonate until alkaline, and evaporate nearly to dryness. Test concentrated liquid by Reinsch's or Marsh's test. The former is described under Beer (page 129).

Tin.—Evaporate a litre of water sample to dryness, ash the residue; exhaust ash repeatedly with strong HCl, evaporate portions to dryness on water-bath, add some water, boil, and filter. Test filtrate with H_2S; a dingy, yellow precipitate, soluble in Am_2S, indicates the presence of tin. The precipitate is also soluble in the caustic alkalies.

Quantitative Estimation.—This depends on a colorimetric method. 100 c.c. of the sample water are taken in a Nessler glass, and a measured quantity of a suitable precipitating agent is added. According to the amount of metal present, a certain depth of coloration is obtained. 100 c.c. of distilled water are now taken in a Nessler glass, and the same quantity of precipitant added to them. From a burette, successive small quantities of a standard solution of the metal being tested for are added to the glass containing

the distilled water ; and after each addition the coloration produced is compared with that in the glass containing the sample water. This is done by putting both glasses together on a white tile, or on a Nessler stand, and looking down through the liquids. If the tints are alike in depth, then the glasses have been matched. If the sample is the darker, more standard will require to be added to the other glass, and a further comparison made. If the sample is the lighter, a fresh amount of distilled water will require to be put up, and less standard solution added at first. In any case the comparisons are continued until matching of the tints is obtained, and then the amount of standard solution which has been added to the distilled water is held to measure the amount of metal present in the 100 c.c. of sample water.

The process of comparing the tints or colorations to a match is called "Nesslerizing," and will frequently be used in subsequent work.

Determination of Lead.—A standard solution of lead acetate is required, of such a strength that 1 c.c. = 0·1 mgr. ($\frac{1}{10}$ mgr.) of lead. $Pb(C_2H_3O_2)_2 + 3H_2O$; the molecular weight = 379, and contains 207 parts of lead. Therefore 379/207 = 1·83 parts of lead acetate contain 1 part of lead. That is, 1·83 grm. of lead acetate contain 1 gram. of lead. If we dissolve 1·83 grm. of lead acetate in 1 litre of distilled water, 1 c.c. will contain 1 mgr. of lead. This solution diluted ten times gives a standard solution of lead acetate such that 1 c.c. = 0·1 mgr. Pb. A solution of Am_2S in water is also required.

Process.—Take two 100 c.c. Nessler glasses, with distinctive marks affixed. To one add 100 c.c. of sample water. To the other add 100 c.c. distilled water. To both add 2 c.c. of Am_2S solution, and stir. Now take a burette, and fill it with the standard solution of lead acetate. Add 1 c.c. of this to the distilled water and stir. Compare coloration produced with that in glass containing sample. If the sample is darker, add another c.c. of standard solution to the other glass, stir, and compare. Repeat procedure until a match is obtained. If the sample is lighter than the coloration produced by 1 c.c. of standard solution, begin again and add $\frac{1}{2}$ c.c. of standard solution.

EXAMPLE.—Suppose 3 c.c. of standard solution were required to match sample. 1 c.c. of standard solution lead acetate = 0·1 mgr. Pb. Therefore 3 c.c. of standard solution lead acetate = 0·3 mgr. Pb. Hence there is 0·3 mgr. Pb in 100 c.c. of sample water, or 0·3 mgr. Pb in 100 grm. of sample water, or 0·3 mgr. Pb in 100,000 mgr. of sample water; that is, 0·3 part of Pb in 100,000 parts of sample water. By this method 0·05 part per 100,000, or $\frac{1}{30}$ gr. per gallon, may be easily detected.

Many waters, especially soft and peaty waters, possess a coloration sufficient to equal that produced by 0·5 c.c., or even 1 c.c. of standard lead solution. In such a case, carefully match the natural coloration in terms of the standard solution, and deduct the amount obtained from the amount required in the regular test. Where the coloration is deeper still, and a poisonous metal is suspected, evaporate 100 c.c. to dryness, ignite to get rid of vegetable colouring matter, digest the ash with HCl, filter, collect filtrate, washing filter-paper with distilled water, and make up bulk of filtrate to 100 c.c. Now test as before.

As lead is a cumulative poison, its presence in a water should disqualify that water for domestic use.

Copper.—Copper is similarly estimated, using a standard solution of copper sulphate, $CuSO_4 + 5H_2O$. The molecular weight of this salt in crystals is 249, and as this amount contains 63 parts of copper, 249/63 or 3·95 parts will yield 1 part of copper. Hence 3·95 grm. of copper sulphate crystals dissolved in 1 litre of distilled water gives a solution such that 1 c.c. contains 1 mgr. of copper. This diluted ten times gives the desired standard solution, 1 c.c. = 0·1 mgr. Cu.

Copper can also be estimated by precipitation with HCl and potassium ferrocyanide, which gives a bronze coloration.

Iron.—Iron is best estimated by oxidizing it, if necessary, to the ferric state, and then adding potassium sulphocyanate, which produces a blood-red colour. Reagents required: (1) Standard solution of ferrous sulphate, $FeSO_4 + 7H_2O$, 0·496 grm. dissolved in 1 litre of distilled water (acidified with H_2SO_4), 1 c.c. of this solution = 0·1 mgr. Fe. Dilute ten times in use, then 1 c.c. =

0·01 mgr. Fe; (2) Dilute HNO_3 solution: make up 30 c.c. of pure concentrated acid to 100 c.c. with distilled water; (3) Potassium sulphocyanate solution: 15 grm. KCNS dissolved in 100 c.c. of distilled water.

PROCESS.—To each of two Nessler glasses add 5 c.c. of each solution (2) and (3). Add 1 c.c. of standard solution to one, and to the other add a measured quantity of sample, say 10 c.c., and note depth of tint produced, compared to standard. If the two are near each other, proceed as before to match. If the sample is much too dark, use less; if much too light, use more. Always make up bulk in each Nessler to be approximately equal to each other by adding distilled water, before finally matching. If more than 3 c.c. of standard solution are required, the tint gets too deep.

Iron is perceptible to taste when present to the extent of $\frac{1}{5}$ gr. per gallon, or 1 part in 350,000 parts of water.

Zinc is usually determined gravimetrically. It may be done volumetrically with standard solution of potassium ferrocyanide, using copper sulphate as an indicator, the brown copper precipitate not being formed until all the Zn is precipitated. The standard solution is made 0·324 grm. of $K_4Fe(CN)_6 . 3H_2O$ per litre. Then 1 c.c. = 0·1 mgr. Zn.

Lime and Magnesia.—These are often present together from strata.

Lime.—Ammonium oxalate gives a turbidity with 9 parts per 100,000, and a white precipitate with anything over 20 parts per 100,000.

Magnesia.—Precipitate any lime present with ammonium oxalate, filter, then add AmCl, AmOH, and sodium phosphate. Crystals of so-called triple phosphate will separate out in twenty-four hours. ($MgNH_4PO_4$.)

For this and the next test it is better to concentrate to $\frac{1}{50}$.

Phosphates.—Add some dilute nitric acid, stir, add excess of ammonium molybdate, and heat. If phosphates are present, a yellow colour will form.

Sulphates.—Add dilute HCl and barium chloride solution—a white precipitate of sulphate of barium, insoluble in all acids.

Quantitative Estimation of Lime, Magnesia, Phosphates, and Sulphates.

These are all estimated for gravimetrically by precipitating as above, collecting the precipitate, which settles after twelve hours, on a filter-paper of known ash; drying, and igniting; and weighing ash. The ash of lime is weighed as calcium carbonate, of magnesia as magnesium pyrophosphate ($Mg_2P_2O_7$), of sulphates as barium sulphate. The phosphates are precipitated as triple phosphates of magnesium and weighed similarly. In the case of magnesium, the lime salts must be removed before precipitating, hence the filtrate from the lime estimation is suitable for the purpose.

GASES IN WATER.

Carbon Dioxide in Water.—Exists as free CO_2; bicarbonate and carbonate; and free CO_2 and bicarbonate.

1. *Free* CO_2.—Determined by titration with N/20 sodium carbonate solution, using phenolphthalein as indicator. On adding the N/20 solution from a burette to the sample, a red colour appears which gradually fades as the carbonate absorbs the free carbon dioxide and changes to bicarbonate, which is neutral to phenolphthalein.

$$\underset{106}{Na_2CO_3} + \underset{44}{CO_2} + H_2O = 2NaHCO_3$$

N/1 sod. carb. = 53 grm. per litre and absorbs 22 grm. carbon dioxide per litre; hence N/20 sod. carb. = 2·65 grm. per litre and absorbs 1·1 grm. carbon dioxide per litre; hence 1 c.c. N/20 will absorb 0·0011 grm. free CO_2.

Process.—Take 100 c.c. of sample in an Erlenmeyer flask on a white slab, and add one drop of 1 per cent alcoholic solution of phenolphthalein.

Fill burette with N/20 solution of sodium carbonate and add to sample, c.c. by c.c., until a permanent red tint remains after waiting a minute or two.

Calculate out result.

Free carbon dioxide is almost constantly present in ground waters, and in inverse ratio to the amount of dissolved oxygen. It may be as high as 13 parts per

100,000, probably derived from ground air, increasing with the depth and decreasing with the porosity of the soil.

2. *Carbonate and Bicarbonate.*—The carbonate is first determined by titration of the sample with the standard solution of oxalic acid, in presence of phenolphthalein, which gives a pink colour with carbonates and alkalies, and is colourless with bicarbonates and acids.

$$H_2C_2O_4 2H_2O + 2Na_2CO_3 = 2NaHCO_3 + Na_2C_2O_4 + 2H_2O$$
$$90 + 36 \qquad 2 \times 106$$

From the equation we see that 126 grm. of crystallized oxalic acid react with 212 grm. of sodium carbonate, containing 88 grm. of CO_2, and change it to sodium bicarbonate. Thus 126 grm. of oxalic acid crystals measure the change of 88 grm. of CO_2 from the state of carbonate to that of bicarbonate, or 126/88 = 1·43 grm. measure the change in 1 grm. of CO_2. Hence a standard solution of oxalic acid crystals, 1·43 grm. per litre, is of such a strength that 1 litre = 1 grm. CO_2, or 1 c.c. = 1 mgr. CO_2. When all the carbonate is changed to bicarbonate, the solution becomes colourless, and the number of c.c. used measures the carbonate present. Boil the fluid in the flask briskly for ten minutes, when all the bicarbonate, whether originally present or derived from carbonate, is decomposed with formation of carbonate (signalized by the return of the pink colour) and evolution of CO_2, as shown thus :—

$$2NaHCO_3 = Na_2CO_3 + H_2O + CO_2$$

Cool and repeat the titration with the standard oxalic solution ; the number of c.c. required measures the amount of carbonate now in the sample. But this figure represents only half the CO_2 present before boiling, half of the CO_2 having gone into the atmosphere. Hence the figure has to be doubled, and then measures the total amount of CO_2 present originally in the sample. The amount present as carbonate is known from the first titration, and so the amount present as bicarbonate is measured by the difference between the number of c.c. used in the first titration, and double the number used in the second titration. The rationale may be shown thus:—

(1) $\underset{Na_2CO_3}{Na_2CO_3} + H_2C_2O_4 + Phth = \underset{NaHCO_3}{NaHCO_3} + Na_2C_2O_4$

(2). $NaHCO_3$ + solution boiled = $Na_2CO_3 + H_2O + CO_2$ (into air)
$NaHCO_3$

(3). $2Na_2CO_3 + H_2C_2O_4 + Phth = 2NaHCO_3 + Na_2C_2O_4$

EXAMPLE: 100 c.c. of sample, plus phth., required 8 c.c. of std. oxalic sol. to decolorize. Boiled; cooled; and titrated again, when 11 c.c. were required.

CO_2 as Carbonate, 8 parts per 100,000.
CO_2 as Bicarbonate, $22 - 8 = 14$,, ,,

3. *Free Carbon Dioxide and Bicarbonate.*—These are estimated by the addition of excess of alkali in the shape of a known quantity of baryta solution. The baryta fixes the free carbon dioxide and that half bound in the bicarbonates, precipitating both as barium carbonate. The excess of alkali which remains unused is measured by titration with standard oxalic acid solution.

$$CO_2 + BaH_2O_2 = BaCO_3 + H_2O$$
$$CaCO_3{\cdot}CO_2 + BaH_2O_2 = BaCO_3 + CaCO_3 + 2H_2O$$
$$H_2C_2O_4 + BaH_2O_2 = BaC_2O_4 + 2H_2O$$

From these equations we see that one molecule of oxalic acid and one molecule of CO_2 are each able to neutralize one molecule of baryta in solution. Therefore, 126 grm. of crystallized oxalic acid neutralizes 171 grm. of baryta, which fixes 44 grm. of CO_2; or $126/44 = 2{\cdot}86$ grm. of oxalic neutralizes $171/44$ grm. of baryta, which fixes $44/44 = 1$ grm. CO_2. Hence a standard solution of oxalic acid 2·86 grm. per litre is such that 1 c.c. measures the quantity of baryta which fixes 1 mgr. of CO_2.

PROCESS.—To 100 c.c. of sample in a flask or bottle, add a drop of phenolphthalein, and then 5 c.c. of $BaCl_2$ solution, 5 c.c. of AmCl solution, and 40 c.c. of baryta solution (0·5 per cent). If excess of baryta is present, the liquid should become red, and remain so. Stopper the vessel and set aside for twelve hours. Thereafter titrate the whole sample (or an aliquot part) with standard oxalic acid solution. Titrate 40 c.c. of fresh baryta solution. The difference between the number of c.c. required for the fresh baryta solution and that required for the baryta mixed with sample, measured in c.c. of standard oxalic solution the amount of baryta used up in fixing free CO_2 and changing bicarbonate

to carbonate. But as each c.c. of oxalic equals 1 mgr. of CO_2, then the number of them gives the total number of mgr. of CO_2 in the water sample. The free CO_2 is determined as before by N/20 sodium carbonate solution, and the difference gives the amount present as bicarbonate.

Dissolved Oxygen.—Winkler, Dibdin, Thresh, and Mohr have all devised methods for determining the amount of oxygen dissolved in water samples. Any such method must be simple, speedy, and accurate, and the water must not be operated on in an inert atmosphere, or there might be a rapid loss by diffusion. Winkler's is perhaps the most simple and readily applied, and needs no special apparatus. The following solutions are required: (*a*) Manganous chloride solution free from iron (80 grm. $MnCl_2 + 4H_2O$ in 100 c.c. of distilled water; (*b*) KI and NaOH solution (10 grm. KI in 100 c.c. of 33 per cent NaOH). This solution when diluted, and sulphuric and starch solution added, should not give any blue colour; (*c*) N/100 iodine (1·27 grm. I and 2 grm. KI dissolved in 1 litre). This is used to standardize thiosulphate; (*d*) N/100 thiosulphate of soda solution (2·48 grm. $Na_2S_2O_3 + 5H_2O$ per litre); (*e*) Starch solution.

Process.—Take a glass bottle provided with a well-fitting glass stopper and of about 300 c.c. capacity. Determine accurately the capacity when stoppered. Wash it out with some of the water to be examined, and then fill it to overflowing with sample water (avoid splashing). Introduce, by different pipettes, 1 c.c. of each of solutions (*a*) and (*b*), doing this carefully so that they are delivered close to the bottom of the bottle. Put in the stopper tightly, enclosing no air bubbles. Mix the contents by lightly swinging the bottle. A precipitate forms which is allowed to settle. This takes a variable time; usually fifteen minutes is sufficient. When it has settled and the upper part of the fluid is clear, introduce by pipette, so as to fall on to the precipitate, 5 c.c. of strong HCl, replace stopper and swing until precipitate dissolves, when the fluid becomes yellow-coloured from liberated iodine. The contents of the bottle are now poured into a clean beaker, the bottle washed out with distilled water, and the washings added. It is then titrated with the N/100 thiosulphate

solution, every c.c. of which used equals 0·00008 grm. O, or 0·055825 c.c. oxygen. Starch solution is used for the end reaction. The amount of oxygen found is present in the capacity of the bottle, less the 2 c.c. of solutions added. The result is returned in parts by weight per 100,000, or in cubic centimetres per litre.

1. $2MnCl_2 + 4NaOH = 4NaCl + 2Mn(OH)_2$.
2. $2Mn(OH)_2 + O + H_2O = 2Mn(OH)_3$.
3. $2Mn(OH)_3 + 6HCl = 2MnCl_3 + 6H_2O$.
4. $2MnCl_3 + 2KI = 2MnCl_2 + 2KCl + I_2$.

The process must be done rapidly. Nitrites liberate iodine and so vitiate the result, increasing it. Much organic matter interferes with the method, as it absorbs the liberated iodine, thus diminishing the result. Rapid working diminishes the latter interference.

The amount of dissolved oxygen in a water is influenced mainly by temperature, being less in summer and more in winter. Ordinary tap water in this country contains on an average 7 c.c. per litre, which is about 1 part by weight in 100,000. Water is saturated at 5° C, 10° C, 15° C, and 20° C. respectively, by 8·68 c.c., 7·77 c.c., 6·96 c.c., and 6·28 c.c. per litre.

Suphuretted Hydrogen.—This is estimated by titration with N/100 iodine, which is decolorized by the H_2S; thus $H_2S + I_2 = 2HI + S$.

PROCESS.—Take 10 c.c. N/100 iodine in a white porcelain basin. Fill a burette with sample, and add to basin until colour is gone, using starch for end reaction. The N/100 iodine is made as above, and is standardized against N/100 thiosulphate solution. Every c.c. of N/100 I equals 1 c.c. of N/100 H_2S. But N/1 H_2S is 17 grm. per litre, therefore 1 c.c. N/1 = 0·017 grm., and 1 c.c. N/100 = 0·00017 grm. H_2S, or 0·17 mgr. Hence the number of c.c. of N/100 I used × 0·17, gives the number of mgr. of H_2S in the amount of sample run in from burette to decolorize the iodine.

HARDNESS (TOTAL, TEMPORARY, OR PERMANENT).

Hardness is due to the presence in a water of metallic salts which form insoluble compounds with the fatty acids usually present in soap. A soap is the oleate, stearate,

or palmitate of sodium or potassium. Hard soap has soda for its base, and soft soap has potash for its base. These soaps are soluble in water and form a lather therewith on shaking. When soap is used with a water in which lime, magnesia, baryta, iron, alumina, or other such substances are present, oleates, etc., of these bases are formed, which being insoluble are precipitated, and no lather can be produced until an excess of soap is present. A certain amount of the hardness is removable by boiling, and this is called temporary hardness, and is chiefly due to the carbonates of lime and magnesia held in solution by carbonic acid gas, and by sulphates of these, with salts of silica, alumina, and iron when present. The permanent hardness, or what still remains in solution after boiling, consists mainly of some sulphates, chlorides, and nitrates of calcium and magnesium, with a little iron and alumina. Free carbonic acid gas in water also consumes soap, two equivalents uniting with one of soap as ordinarily estimated. Amount of hardness is expressed as grains per gallon (Clark's degrees), or parts per 100,000 (metrical degrees) in terms of calcium carbonate. In Germany the hardness is expressed as metrical degrees of CaO per 100,000.

The total hardness of a water should not exceed 30 parts per 100,000, if for domestic purposes. Hard waters vary from 20 to 30 degrees on the metrical scale ; a soft water from 8 to 15 ; and a very soft water from 8 downwards. The greater the permanent hardness, the more objectionable is the water ; and of a good water it should not exceed 5° metrical, or 3° to 4° Clark.

Determination of Hardness.—

1. By Standard Soap Solution Method.

Dissolve 10 grm. of castile or soft soap in 1 litre of a mixture of equal parts of distilled water and methylated spirit. Standardize the solution so that 1 c.c. completely precipitates 1 mgr. of calcium carbonate or an equivalent salt. The $CaCO_3$ may be dissolved in the least possible quantity of HCl, then evaporated to dryness twice, to get rid of the HCl, and then the resulting $CaCl_2$ dissolved in the proper amount of distilled water ; that is, 1 grm. of the carbonate is treated as above, and the resulting

chloride dissolved in 1 litre of aq. dest., then 1 c.c. = 1 mgr. $CaCO_3$. The soap solution is then tested with 50 c.c. of aq. dest. (recently boiled to get rid of CO_2), to determine how much of it is required to produce a permanent lather; that is, a lather which remains as a uniform film $\frac{1}{4}$ in. thick on the surface of the water five minutes after the bottle has been laid on its side. The soap solution is added from a burette, $\frac{1}{10}$ c.c. at a time. The 50 c.c. are contained in a stoppered bottle, 150 c.c. capacity, which is well shaken after each addition, then laid on its side, and the character of the lather noted. If it quickly disappears, then more soap solution is added, until the lather has the permanence described. The amount usually required by 50 c.c. of aq. dest. varies from 0·2 c.c. to 0·6 c.c., and should be determined not once for all, but at intervals, as it will vary with the strength of the soap solution, and this latter tends to deteriorate on keeping.

The soap solution is now standardized against the standard $CaCO_3$ solution, 5 c.c. of which are added to 45 c.c. of recently boiled aq. dest., contained in a glass-stoppered bottle of about 150 c.c. capacity. The soap solution is now added from a burette, 1 c.c. at a time. Shake briskly after each addition. When the proper lather is formed, the shaking of the bottle produces a soft sound which is different from the hard sound at first emitted, and heard when the bottle is held near the ear. Say that 4 c.c. of standard soap solution were required to produce the permanent lather, and that 0·5 c.c. were necessary for 50 c.c. of aq. dest., then $4 - 0{\cdot}5 = 3{\cdot}5$ c.c. have been used in precipitating the 5 mgr. of $CaCO_3$ contained in the 5 c.c. of standard calcium solution. But we wish the standard soap solution to be 5 c.c. = 5 mgr. or 1 c.c. = 1 mgr. calcium carbonate. Hence it is too strong, and we must dilute it (with a mixture of equal parts of methylated spirit and aq. dest.) so as to make every 3·5 c.c. up to 5 c.c., or every 35 c.c. up to 50 c.c. The solution is now of standard strength, but requires to be re-standardized at intervals, as it is somewhat unstable.

The standardizing can also be done against a standard solution of $Ba(NO_3)_2$ which has a molecular weight of 261 compared to 100 for $CaCO_3$. Therefore if 2·61 grm.

of barium nitrate be dissolved in 1 litre of aq. dest. 1 c.c. = 1 mgr. $Ba(NO_3)_2$ equal to 1 c.c. = 1 mgr. $CaCO_3$.

Total Hardness.—Take 50 c.c. of sample in bottle and test with standard soap solution as above until a permanent lather is obtained. Deduct 0·5 c.c. (say) necessary to produce lather, and double the answer gives the total hardness in metrical degrees. This multiplied by 0·7 gives it in grains per gallon, or Clark's degrees. If more than 8 to 9 c.c. be required, it is advisable to dilute 25 c.c. of the sample with 25 c.c. recently boiled aq. dest., and redetermine the hardness.

Permanent or Fixed Hardness.—Take 100 c.c. of sample water and make up to 200 c.c. with aq. dest. Boil down to one-half its bulk, and a little more. Allow to cool to 60° F. (15·5° C.), and make up to 100 c.c. with aq. dest. Remove 50 c.c. and determine hardness as before.

By boiling, all the free and half-bound CO_2 is driven off, and nearly all the $CaCO_3$ is precipitated. The $CaSO_4$ and the $CaCl_2$ are not affected if the evaporation is not carried too far. Some of the $MgCO_3$ at first thrown down is redissolved as the water cools.

Temporary or Removable Hardness.—This is the difference between the total and the fixed hardnesses.

Notes.—The lime salts precipitate at once with the soap solution; the magnesium salts precipitate slowly; hence it sometimes happens that all the Ca is precipitated and a lather formed which shortly disappears, and more soap solution is needed. The presence of magnesium salts is said to cause the lather to be brown in colour and to break very easily. More soap is required to produce a lather with a certain amount of magnesium, than with the equivalent amount of Ca, though this is ignored in practice.

2. By Hehner's Alkalimetry Method.

Temporary.—Titrate 50 or 100 c.c. of sample with N/50 H_2SO_4, using methyl-orange as indicator, until a permanent pink is got. The sulphuric acid decomposes the $CaCO_3$ with evolution of CO_2, and until all the carbonate is decomposed, no sulphuric is free to attack the methyl-orange. The number of c.c. of sulphuric gives the number

of mgr. of calcium carbonate in the amount of sample taken, because 1 c.c. N/50 H_2SO_4 = 1 c.c. N/50 $CaCO_3$. But the molecular weight of $CaCO_3$ is 100 and N/50 = 1 grm. per litre, hence 1 c.c. = 1 mgr. $CaCO_3$.

Permanent.—To a fresh lot of sample add sufficient N/50 Na_2CO_3 to precipitate as carbonate all the Ca and Mg present, noting carefully amount used.

Evaporate mixture to dryness on water-bath.

Dissolve soluble part of residue in 10 to 20 c.c. of aq. dest. and filter through small filter-paper. Wash out dish and filter-paper with a little more distilled water to ensure complete removal of all the Na_2CO_3.

Titrate filtrate with N/50 H_2SO_4, using methyl-orange as indicator, until a permanent pink colour is obtained.

The difference between the number of c.c. of N/50 Na_2CO_3 used and the number of c.c. of N/50 H_2SO_4 required to neutralize, is the number of c.c. of N/50 sodium carbonate used up in precipitating the Ca and Mg. Every c.c. of same equals 1 c.c. of N/50 $CaCO_3$ = 1 mgr. $CaCO_3$. Thus we arrive at the amount of permanent hardness in the quantity of sample taken.

Total.—The sum of the temporary and permanent hardnesses, as determined above, gives the total hardness.

ORGANIC MATTER IN WATER.

This is derived from vegetable and animal pollution, and is estimated in a variety of ways.

Frankland's Method.—The water is evaporated to a residue, which is ignited in a hard combustion tube with cupric oxide; the evolved gases are collected and measured, and the amount of carbon and nitrogen found in these returned as Organic C and Organic N. In a good water suitable for domestic use, the Organic C should not exceed 0·2 part per 100,000, and the Organic N should not exceed 0·02 part per 100,000. The ratio of Organic C to Organic N furnishes a valuable indication of the nature of the organic matter present in unoxidized waters. Thus, unoxidized peaty waters give a high ratio of from 8 to 12 to 20 or even more, the average being about 12, and such a ratio is held to indicate organic matter of vegetable rather than of animal origin. In unpolluted upland surface

water, the ratio varies from 6 to 12; in surface water from cultivated lands, from 4 to 10; in shallow wells, from 2 to 8; in deep wells, and springs, from 2 to 6; in sea water it averages about 1·7; and in sewage it varies from 1 to 3, averaging about 2.

TABLE.

	Organic C.	Organic N.
Unoxidized peaty waters	8 to 20; avge. 12	1
Unpolluted upland surface waters ..	6 to 12	1
Surface water from cultivated land	4 to 10	1
Shallow wells	2 to 8	1
Deep wells, and springs	2 to 6	1
Sea water	Avge. 1·7	1
Sewage	1 to 3; avge. 2	1

In waters subjected to oxidation, the ratio tends to be reduced when the organic matter is mainly vegetable, and the reverse when it is animal. Loch Katrine water (average of five years) gave Organic C, 0·148 part per 100,000, and Organic N, 0·016 part per 100,000, and the ratio as 9·2. This method is for trained chemists only.

Wanklyn, Chapman, and Hall's Method recognizes that organic matter tends to resolve itself into simpler substances, and chooses to estimate the amount of ammonia present, free in solution or as salts, as an index of the amount of organic matter so resolved. Further, the water is so treated subsequently that any organic matter remaining undecomposed has its nitrogen split off as ammonia; this is measured and furnishes an index of the amount of such organic matter. The absolute amounts of these two ammonias (the first called "the free and saline," the second "the albuminoid ammonia"), and their relative amounts, give valuable evidence of the state of a water with regard to organic pollution.

Forschammer Process, as modified by Tidy, is commonly called *Tidy's Process*, and consists in measuring the oxygen-consuming or absorbing power of a water, and inferring therefrom the amount of organic matter present. It has many limitations, but under proper conditions furnishes another item on which to found an estimate of a water.

Kjeldahl's Process, in which the organic nitrogen is converted into ammonia and estimated by distillation along with the free and saline ammonia. This method is very useful in highly polluted waters and sewage effluents, where the estimation of the albuminoid ammonia is tedious and difficult. It is much used to determine the total nitrogen in food-stuffs, from which the total proteins is got by multiplying by a factor which for meat foods is 6·25, and varies for other foods.

Free and Saline Ammonia.—

By Wanklyn's Method.—Solutions required: (1) Nessler's reagent. This is a saturated solution of mercuric-potassic-iodide in ammonia-free distilled water, the whole being rendered strongly alkaline with caustic soda or potash. It may be made thus: Dissolve 35 grm. of potassium iodide in 200 c.c. of ammonia-free distilled water and 12·5 grm. of corrosive sublimate in 300 c.c. of ammonia-free distilled water. Add the iodide solution to the sublimate one, when a yellow to scarlet precipitate is obtained, which re-dissolves in the excess of potassium iodide present. (Mercuric iodide is almost insoluble in water.)

$$HgCl_2 + 2KI = HgI_2 + 2KCl.$$
$$HgI_2 + 2KI = HgI_2.2KI.$$

Now add carefully a cold saturated solution of corrosive sublimate, stirring all the time, until a slight red precipitate remains permanent. In this way excess of potassium iodide, above that required to keep the mercuric iodide in solution, is used up. 120 grm. of caustic soda in stick are now added to the mixture and allowed to dissolve and cool. If the red precipitate has disappeared, add again a little of the saturated solution of corrosive sublimate, until a slight permanent red precipitate appears. Make up bulk to 1 litre with ammonia-free distilled water. The solution is now ready for use.

Nessler's solution gives a yellowish tinge with the faintest trace of ammonia, and if much ammonia is present a yellow-brown precipitate forms of di-mercuric-ammonium-iodide :—

$$NH_3 + 2HgI_2 = NHg_2I + 3HI.$$

(2) Standard solution of ammonium chloride, such that 1 c.c. = 0·01 mgr. NH_3. Ammonium chloride, NH_4Cl, has a molecular weight of 53·5, of which 17 parts are due to ammonia NH_3. Since the standard solution is 1 c.c. = 0·01 mgr. of ammonia, 1 litre will contain 0·01 grm. of ammonia.

17 : 0·01 : : 53·5 : x = 0·03147 grm. of NH_4Cl will yield 0·01 grm. of ammonia. Hence, dissolve 0·03147 grm. of ammonium chloride in 1 litre of ammonia-free distilled water, and 1 c.c. will contain 0·01 mgr. of ammonia.

Process.—Take a retort or boiling-flask of about 700 c.c. capacity, cleanse it well and rinse it out with ammonia-free distilled water. Now put into it 200 c.c. of ammonia-free distilled water, connect to a condenser, start the water flow in latter, and distil over 100 c.c. to rid the apparatus of any traces of ammonia. Test the distillate with Nessler's solution, and if ammonia is found in the last portions, repeat the distillation. If not, cool flask, wash out with ammonia-free water, and proceed.

Introduce into flask 500 c.c. of sample water and render this alkaline by the addition of some recently-heated sodium carbonate. Connect to a condenser, start the water supply for the latter, and place a clean 50 c.c. Nessler glass at the end of the condenser to catch the distillate. Make sure that all parts of the apparatus are properly connected and adjusted. Now apply the flame of a Bunsen burner to the flask, which may be protected by a piece of gauze. Heat gently at first, but once the parts have got heated, increase the flame. Try to distil over at the rate of 50 c.c. every fifteen minutes. When the first Nessler glass is filled to the 50 c.c. mark, remove it and put another clean one in its place, and so on. Have a stock of six ready for the purpose.

The first 50 c.c. of distillate is then tested by adding to it 2 c.c. of Nessler solution and mixing. Place the glass on a white slab, or on the glass shelf of a Nessler stand, and on looking down through the liquid, the amount of coloration produced, or its absence, is easily made out. With experience the depth of colour will suggest how much standard solution will be required to match it. The next step is to put up three trial glasses for comparison.

Take three 50 c.c. Nessler glasses, and from a burette add to the first 1 c.c., to the second 2 c.c., and to the third 3 c.c. of standard solution of ammonium chloride; 1 c.c. = 0·01 mgr. NH_3. Fill all three up to the 50 c.c. mark with ammonia-free distilled water, and then add to each 2 c.c. of Nessler solution, and mix. Put these glasses, distinctively marked, on the slab or shelf, and compare the first 50 c.c. of distillate with them as to depth of coloration. If the distillate matches any one of them, the result is attained. If it does not match any of them, it may be intermediate between any two of them, or be darker than the 3 c.c. or lighter than the 1 c.c. glass. In any case, fresh trial glasses should be put up for, as the case may be, ·5 c.c., 1·5 c.c., 2·5 c.c., 4 c.c., 5 c.c., 6 c.c., of standard solution. If the distillate is darker than the coloration given by 6 c.c. of standard solution, it is better to dilute it with ammonia-free distilled water, and then to proceed to match. The same procedure is carried out with the second 50 c.c. of distillate and succeeding lots. The distillation is stopped when no coloration is given with 2 c.c. of Nessler, or at least less than will match 0·5 c.c. of standard solution. This usually happens with the fourth lot of 50 c.c. The sum of the amounts of ammonia found in each lot of distillate, is the total free and saline ammonia present in 500 c.c. of sample water. This is reduced to the amount in 100 c.c., and thereafter expressed as parts of ammonia per 100,000. The distilling over in separate lots is the mode recommended by the Society of Public Analysts, but Wanklyn recommends that only 50 c.c. be distilled, and that the amount found in it be increased by one-third, on the ground that in his experience three-fourths of the ammonia comes over in the first lot of 50 c.c.

EXAMPLE.—

First	50 c.c.	of dist.	matched	5.0 c.c.	std. sol. of	NH_4Cl.
Second	50 c.c.	do.	do.	1·5 c.c.	do.	do.
Third	50 c.c.	do.	do.	0·5 c.c.	do.	do.
Fourth	50 c.c.	do.	do.	nil		
				7·0 c.c.	do.	do.

That is, the 500 c.c. of sample water yielded ammonia sufficient to match 7 c.c. of standard solution of ammonium chloride. (1 c.c. = 0·01 mgr. NH_3), hence 7 c.c. standard solution equals $7 \times 0{\cdot}01 = 0{\cdot}07$ mgr. NH_3, and there is—

0·07 mgr. of free and saline NH_3 in 500 c.c. of sample.
or 0·014 mgr. of do. do. do. in 100 c.c.
or do. in 100 grm.
or do. in 100,000 mgr.
or 0·014 part of do. do. do. in 100,000 parts.

NOTE.—In Nesslerizing, always add the AmCl solution first to the trial glasses, and the Nessler later. If the order is reversed, a turbidity is produced which prevents accurate comparisons.

Likewise always use distilled water, ammonia-free. Pure natural water, ammonia-free, will not do, as it appears muddy when compared with distilled water.

The residue in the flask is used to determine the "albuminoid ammonia," as now described.

Albuminoid Ammonia—is determined by breaking up the nitrogenous organic matter in the water sample by the action of an alkaline solution of potassium permanganate, the nitrogen being converted into ammonia, which is distilled off and estimated as described for free and saline ammonia. All the nitrogenous organic matter is not so decomposed, but the proportion of it which does so is sufficiently uniform to form a basis for deductions. The albuminoid ammonia is approximately one-tenth of the nitrogenous organic matter in the water. Solution required is "alkaline permanganate," made by dissolving 8 grm. of potassium permanganate and 200 grm. of caustic potash in 1100 c.c. of distilled water, and boiling down the bulk to 1000 c.c. (1 litre).

The amount of alkaline permanganate used should be about one-tenth of the bulk of sample taken. It should be mixed with three volumes of water, and boiled down to three volumes. That is, in present case, take 50 c.c. of alkaline permanganate, add to them 150 c.c. of water, and boil down to 150 c.c., which are added to the residue in

flask. This gets rid of any free or saline or albuminoid ammonia in this added fluid.

PROCESS.—Take the residue from the estimation of free and saline ammonia, and keeping it still in the flask, add to it 50 c.c. of freshly-boiled permanganate solution, and about 100 c.c. of ammonia-free distilled water, to increase the bulk. Some fragments of pumice stone, which have been heated to redness in a Bunsen flame and cooled, are also added. The apparatus is now fixed together and distillation resumed, the distillate being collected as before in 50 c.c. Nessler glasses. The determination of the amount of ammonia is made in precisely the same way as for free and saline ammonia. The number of lots of 50 c.c. to be collected cannot be approximately stated, as the splitting up of the organic matter occurs irregularly, and in this way more ammonia may be found in the second or third lot than in the first. The process should be continued until no reaction with Nessler is got. In some cases it may be necessary to stop the process and allow the apparatus to cool, then add more distilled water, and then resume the distillation. The amount of ammonia found is of course derived from the original 500 c.c., and must be calculated accordingly.

Free and saline ammonia represents the ammonia combined with carbonic, nitric, or other acids, and also what may be derived from urea, or other easily decomposable substances, if present. The limit in pure waters is 0·002 mgr. per 100 c.c., and in a usable water it should not exceed 0·005 mgr. per 100 c.c.

Albuminoid ammonia in drinking waters of good quality should not exceed 0·01 parts per 100,000. Much albuminoid ammonia with a small amount of free ammonia usually indicates vegetable contamination, particularly if the chlorides and nitrates are low. Peaty waters yield large quantities of albuminoid ammonia, which is slowly evolved; whereas badly polluted waters as a rule yield their high proportion more rapidly.

Oxygen Absorption or Consuming Power.—Tidy's process is based on the fact that much of the organic matter in a water is capable of oxidation, and especially by permanganate in acid solution. Unfortunately, different

substances reduce different proportions of permanganate, and slight variations in temperature and acidity influence the readiness of the permanganate to part with its oxygen to an appreciable extent. Nevertheless, the process yields results which, taken in conjunction with other analytical facts, aid materially in forming an opinion of a water sample.

Reagents required : (*a*) Standard solution of potassium permanganate :—

$$2K_2Mn_2O_8 + 6H_2SO_4 = 2K_2SO_4 + 4MnSO_4 + 6H_2O + 5O_2$$
$$2 \times 316 \qquad\qquad 5 \times 32 ;$$

that is, 632 parts of potassium permanganate liberate 160 parts of oxygen, or 1 part of O will be set free by 3·95 parts of permanganate. Hence, if 3·95 grm. of the latter be dissolved in 1 litre of aq. dest., then 1 c.c. = 1 mgr. O. The solution is usually diluted ten times in use, so that 10 c.c. = 1 mgr. O. (*b*) KI solution, 10 per cent in aq. dest. (*c*) Starch solution, 1 grm. per half litre, freshly boiled and filtered. (*d*) Sodium thiosulphate solution, 1 grm. to the litre of distilled water. (*e*) Sulphuric acid, 25 per cent in aq. dest.

Process.—Take two stoppered flasks or bottles of at least 300 c.c. capacity, and into one put 250 c.c. of sample water, and into the other put 250 c.c. of distilled water. To each add 10 c.c. of the 25 per cent sulphuric acid, and place them both on a water-bath at 80° F. or 26° C. When the required temperature is reached, 10 c.c. of the permanganate solution are added to each lot. A pink colour will result. Maintain the temperature, and observe carefully whether the pink colour is discharged. If so, then another 10 c.c. of the permanganate solution is added to the sample and the control, and more if necessary to keep them markedly pink. Further addition of sulphuric acid is not needed. At the end of a specified time, which may be fifteen minutes, half an hour, one hour, two hours, three hours, or four hours, or any combination of these (the commonest being fifteen minutes and four hours), the oxidizing process is stopped by the addition of 1 c.c. of the KI solution, when the unused permanganate reacts thus, through its loosely held oxygen ; $5O_2 + 20KI + 10H_2O =$

$20KOH + 10I_2$. The liquid turns a yellow colour from the iodine set free. The quantity of iodine liberated is strictly proportional to the amount of unused permanganate. It remains, therefore, to estimate the amount of iodine set free, which measures the amount of oxygen unused, and this deducted from the amount known to have been added, gives the amount absorbed. This is done by titrating the yellow solution with the thiosulphate solution until the yellow colour is nearly gone, and then adding 1 c.c. of starch solution to give a more distinct end reaction. The titration is finished when the blue is just gone :—

$$I_2 + 2Na_2S_2O_3 = 2NaI + Na_2S_4O_6.$$

Both the sample and the control are treated thus. In the control presumably no oxidation takes place, so that the number of c.c. of thiosulphate solution required for it, is a measure of the iodine liberated by all the oxygen free to cause oxidation. The amount of thiosulphate solution used for the sample measures the unused oxygen, and the difference between these two numbers gives the proportion of oxygen used up. For example, say that 10 c.c. only of permanganate were required to be used, and that after adding KI solution the control took 40 c.c. of thiosulphate solution to decolorize, and the sample took 30 c.c.; then 40 c.c. of thiosulphate measure 1 mgr. of oxygen, and 40 c.c. − 30 c.c. = 10 c.c. measure 10/40 = 0·25 mgr. O, and this is the quantity absorbed by the 250 c.c. of sample taken. This multiplied by 0·4 gives 0·1 mgr. O absorbed per 100 c.c., or 0·1 part per 100,000.

Waters of great organic purity will not consume more than 0·05 part of oxygen per 100,000 in fifteen minutes at 80° F., and if the amount absorbed exceeds 0·1 part in fifteen minutes, the sample may be considered of doubtful purity. After four hours' exposure, an absorption of more than 0·3 part must be regarded with suspicion.

Ferrous salts, nitrites, and sulphuretted hydrogen, if present, vitiate the test.

Kjeldahl's Process for the determination of organic nitrogen is performed only in very polluted waters. The process is described under Sewage and Sewage Effluents.

NITRITES AND NITRATES IN WATER.

Ammonia present in water, derived either from the decomposition of organic matter or by synthesis from urea, tends in its passage through the soil to become oxidized, first into nitrites, then into nitrates. Nitrates, however, may be present in water which has dissolved it out of strata through which it has passed. Sometimes these nitrates become reduced, first to nitrites, then to ammonia, and this has been specially observed as due to iron salts in the ferrous state. In the London basin, the deep-well waters from the "greensands" strata have been noted as yielding ammonia thus derived. Waters polluted with vegetable matter yield little nitrites and nitrates relatively, as plant life removes these, and vegetable matter contains little nitrogen.

Nitrites.—

Qualitative Tests.

1. *Starch Iodine Test.*—Take 50 c.c. of sample water in a Nessler glass and 50 c.c. distilled water in another. To each add a few drops of KI solution and a few drops of freshly-made starch solution. Now add a few drops of dilute sulphuric acid to each tube. The presence of nitrites is indicated by an immediate blue colour.

2. *Naphthylamine Test.*—Take two Nessler glasses as above and acidulate with acetic acid. Add to each a few drops of naphthylamine solution in sulphanilic acid. With nitrites a beautiful pink colour develops in two to three minutes.

Quantitative Tests.

Griess's Test.—Solutions required :—

a. Metaphenylene-diamine solution, 5 grm. per litre, slightly acidulated with sulphuric acid, decolorized by boiling with pure animal charcoal.

b. Sulphuric acid, one part of strong acid to two parts of aq. dest.

c. Standard nitrite solution. This is made from silver nitrite because it is the most stable salt. $AgNO_2 + KCl = AgCl + KNO_2$. 154 parts of silver nitrite, when treated with KCl, give rise to 85 parts of KNO_2, or 46 parts of nitrous acid as represented by NO_2, or 308 parts are equivalent to 76 parts of N_2O_3. Hence, if $308/76 = 4{\cdot}06$

grm. silver nitrite are dissolved in boiling distilled water, and precipitated by slight excess of KCl, 1 grm. of nitrous acid as N_2O_3 is left in solution in combination with potassium. The bulk is made up to 1 litre, the precipitate allowed to settle, and 10 c.c. are taken and diluted to 1 litre, then 1 c.c. = 0·01 mgr. N_2O_3 (equal to 1 per 100,000). Standard nitrite solution is also made so that 1 c.c. = 0·01 mgr. N, and also of millinormal strength, and then 1 c.c. N/1000 = 0·046 mgr. NO_2.

Process.—To 100 c.c. of sample in a Nessler glass, 1 c.c. of the dilute sulphuric acid and 1 c.c. of the metaphenylene-diamine solution are added as a preliminary test. If an orange colour is immediately produced, the tint will prove too deep for comparison. In such a case 50 c.c. should be tried, and if found suitable such an amount diluted to 100 c.c. with aq. dest. is to be used in the real test, which is done thus :—Having decided the amount of the sample to be used, it is taken in a 100 c.c. Nessler glass and made up to 100 c.c. if required. Three other Nessler glasses are taken, and 1 c.c., 2 c.c., and 3 c.c. of standard nitrite solution added to each respectively, and the bulk is made up to 100 c.c. in each case with aq. dest. To all the glasses is added 1 c.c. of each of the reagents, namely M–P–D and H_2SO_4, and this is done as quickly as possible, so that the colours in the glasses may develop from as nearly as possible the same time. The glasses are set aside for fifteen to twenty minutes and are then compared in the manner known as " Nesslerizing." If the sample matches one of the standards, then the amount in it is known. If not, fresh trial glasses are put up, the amount of standard being gauged from the preceding experiment.

The colour produced is Bismarck brown or triamido-azo-benzol. Griess's test is a very accurate one but requires that the water and the reagent should be colourless or be decolorized. The reagent may be bleached by pure animal charcoal.

Ilosvay's Naphthylamine Test.—

a. Solution of sulphanilic acid 0·5 grm. in 150 c.c. of diluted acetic acid (specific gravity 1·04).

b. Solution of naphthylamine made by dissolving 0·1 grm. in 20 c.c. of aq. dest., filtering, and adding 180 c.c. diluted acetic.

PROCESS.—Take 100 c.c. of sample in a Nessler glass, and in another the same quantity of aq. dest. and 1 c.c. standard nitrite solution. To each add 2 c.c. of each of the above solutions, *a* and *b*. Set aside for five minutes and then compare tints. If not equal in tint, abstract some fluid from the darker by pipette and make up the bulk with aq. dest. If the colours still do not match, more fluid is removed, and bulk made up as before. Suppose sample is darker, and that 40 c.c. are removed, and bulk made up; and that again, 30 c.c. are removed, when finally tints match. Then we get :—100 × 60/100 × 70/100 = 42 c.c. of the original 100 c.c. match 1 c.c. of standard nitrite solution, say 1 c.c. = 0·01 mgr. N : then 42 c.c. of sample contain 0·01 mgr. N, and therefore 100 c.c. will contain 0·01 × 100 ÷ 42 = 0·023 mgr. N, or 0·023 part of N as nitrite per 100,000 parts.

The nitrites first act on the sulphanilic acid and form a new compound which reacts with the naphthylamine and forms the substance which gives the pink colour to the liquid.

A water containing nitrites is not safe for domestic use, and should be rejected on that evidence alone, unless unexceptionable in all other respects.

Nitrates.—

QUALITATIVE TESTS.

1. *Brucine Test.*—Take 5 c.c. of sample and add 5 c.c. of brucine solution (1 in 1000), then mix, and pour carefully down the side of the test tube some pure, strong sulphuric acid, free from nitrates, when a positive result is denoted by the appearance of a pink ring at the junction of the liquids on gentle shaking. The test is also performed by evaporating 10 c.c. of sample to dryness in a clean porcelain basin, then adding a crystal of brucine, and then allowing one drop of pure sulphuric to run down the side of the dish, over the solids; when in the presence of nitrates a pink is obtained. Detects 0·7 part per 100,000. Unreliable in the presence of nitrites, which should be first destroyed by addition of urea and sulphuric acid to sample; allow to stand aside for an hour, when test can be applied as before.

2. *Diphenylamine Test* $(C_6H_5)_2NH$.—Take 5 c.c. of sample, add as much diphenylamine solution, mix, and run down pure strong sulphuric, when a blue colour forms at junction of liquids in presence of nitrates.

Quantitative Tests.

1. *Phenol-sulphonic Acid.*—This reagent is made by adding 6 grm. of pure carbolic acid to 3 c.c. of aq. dest. and then adding mixture to 37 c.c. of pure sulphuric acid.

A standard solution of potassium nitrate is required, 0·072 grm. to 1 litre, and then 1 c.c. = 0·01 mgr. N as nitrates. This contains 1 part N in 100,000 parts of standard.

Process.—To two porcelain dishes are added respectively 10 c.c. of the sample and 10 c.c. of the standard. These are placed on the water-bath until their contents are just evaporated to dryness. To each of the residues add 1 c.c. of phenol-sulphonic acid, and mix well with a glass rod (if a large amount of nitrates is present the liquid will turn red). Set aside for fifteen minutes, and then wash out each dish successively into two clean 100 c.c. Nessler glasses with 25 per cent ammonia solution in distilled water. Add more ammonia until effervescence ceases, and make up to mark with aq. dest. The nitrates present convert the phenol-sulphonic acid into picric acid, with which the ammonia forms a picrate having a yellow colour, and the amount of this is proportional to the amount of nitrates present. The two glasses are now compared as to tints, and the darker one is diluted as before described under Ilosvay's test for nitrites. If the water is very pure, a larger amount of sample should be evaporated down, say 20 c.c., 50 c.c., or 100 c.c., and a smaller quantity of standard, say 5 c.c. If rich in nitrates, then less should be taken of the sample, say 5 c.c. or 1 c.c.

Aluminium Process.—If aluminium foil be added to a strongly alkaline water, decomposition of the water ensues with the evolution of hydrogen, which in the presence of nitrites or nitrates reduces these, converting their contained nitrogen into ammonia. Thus :—

$4Al + 4NaOH + 4H_2O = 2Al_2Na_2O_4 + 6H_2$: and $3KNO_3 + 12H_2 = 3KOH + 6H_2O + 3NH_3$.

Required: (1) Thin aluminium foil; (2) 10 per cent solution NaOH.

PROCESS.—Take 100 c.c. of the water sample and 100 c.c. of the NaOH solution in a 300 c.c. boiling-flask. Add a piece of aluminium foil about 1·5 inch square. Cover but do not cork. Set aside for six hours at least. Then connect the flask to a condenser and distil over the ammonia into 50 c.c. Nessler glasses, collecting three lots. The amount of ammonia is determined by comparison of the coloration developed in these glasses by adding to each 2 c.c. Nessler's solution, and that developed in glasses containing 50 c.c. ammonia-free distilled water, plus 1 c.c., 2 c.c., and 3 c.c. respectively of standard NH_4Cl (1 c.c. = 0·01 mgr. NH_3), and similarly treated. The amount estimated by this method includes ammonia present in the sample, ammonia derived from nitrites, and ammonia derived from nitrates. The two former are separately estimated and deducted, and the remainder is the amount derived from nitrates, and is readily converted back into terms of NO_3 or of N.

EXAMPLE.—100 c.c. gave ammonia equal to 40 c.c. of standard AmCl = 40 × 0·01 = 0·4 mgr. NH_3. But the water contained 0·006 mgr. of free and saline ammonia per 100 c.c., and 0·042 mgr. N as nitrites per 100 c.c. = 0·042 × 17 ÷ 14 = 0·051NH_3 per 100 c.c. Hence, 0·4 − (0·006 + 0·051) = 0·343 mgr. NH_3 due to nitrates = 0·282 mgr. N as nitrates per 100 c.c. or 100,000 mgr.

Copper-Zinc Couple Method.—This method is similar in principle to the above. A bright piece of thin zinc foil, 3 in. × 2 in., is cleansed with dilute sulphuric acid. It is then rolled into a coil, so that it may fit into a 200 c.c. wide-mouthed bottle. Now immerse the coil for three minutes in a 3 per cent solution of copper sulphate. The zinc becomes coated with a black deposit of metallic copper. Remove the coil carefully, wash in ammonia-free distilled water, wash in sample water, and then immerse in 110 c.c. of sample water contained in a wide-mouthed bottle. Stopper tightly, place in a cool dark place for twenty-four hours. The "Copper-zinc couple" acts electrically on the sample, changing any nitrates present

to nitrites, and then to ammonia. It thus acts also on any nitrites originally present, so that the process estimates nitrates and nitrites. The reaction is finished when no free nitrites are found in the solution. This is determined by removing 10 c.c. and testing by Griess's test. If nitrites are found to be present, more time must be given. If they are absent, the remainder of the sample water is poured into a 700 c.c. boiling-flask, and the bottle washed out repeatedly with ammonia-free distilled water, the washings being added to the flask, and more water added to bring up the bulk to about 500 c.c. The water is then distilled as in the estimation of free and saline ammonia, and the amount of ammonia determined. This is restated as nitrogen by multiplying by 14/17 ($N : NH_3$). If the sample was found to contain any free ammonia, the amount of this would require to be deducted before assigning the amount found by this process to nitrates and nitrites.

Indigo Method.—Another method, which is a rapid and convenient one, but subject to great irregularity, is the indigo method. 20 c.c. of sample are taken in a beaker, and 20 c.c. of pure strong sulphuric acid are added. From a burette allow standard indigo solution to run into the hot mixture, until the colour of the indigo ceases to be discharged, and a faint greenish tinge becomes permanent. The estimation should be repeated, adding half a c.c. of indigo less to 20 c.c. of the sample, and then the sulphuric. When the colour is discharged, the indigo is run in drop by drop until colour is again permanent. The indigo solution is standardized against standard nitrate solution similarly treated. The strong sulphuric liberates free nitric acid, which in the hot liquid oxidizes the indigo to isatin, which is colourless. Owing to the heat evolved, the titration is best done with the beaker resting on an asbestos mat.

The method is unreliable in the presence of organic matter, the results being too small. It also requires that all the procedures should be carried out exactly alike for the titration of the sample and the standardizing of the indigo solution. Otherwise it is a very simple, rapid, and delicate method.

No water used for drinking purposes should contain

more than 0·35 part of nitrogen as nitrates per 100,000 parts, unless there is some satisfactory explanation. This amount equals about one grain per gallon when expressed as N_2O_5, or 1·5 parts per 100,000 when expressed as NO_3.

ICE.

Ice is frozen water, and it is not usually purer in content than the water from which it is derived. Whatever may be frozen out of the water is usually mineral matter, such as salt; suspended matter is likely to be enclosed. Microbic content, when composed of the common sewage organisms, is little affected by the temperature of freezing, for the most part only being rendered torpid. As far as possible, therefore, ice should only be used when made from pure water, and by a process in which it is not subject to risk of serious contamination. The analysis of ice proceeds on the same methods as for water, the ice being first melted.

MINERAL WATERS AND AERATED WATERS.

These are examined on the same principles. In the case of artificial waters, the spring or supply from which they are made should also be examined. In such also a search should be made for poisonous metals, such as lead and antimony, iron, copper, zinc, and even arsenic.

In natural mineral waters the same careful examination should be made. In these the mineral content is often considerable, and a thorough analysis of the different metals present is very important. The temperature and the amount of carbonic acid gas are also noted. Nowadays the presence of metals of the radium group has acquired a new significance, and their occurrence is specially noted.

INTERPRETATION OF THE RESULTS OF A WATER ANALYSIS.

This must not be based on any one item, but on a careful consideration of the following points:

1. Local inspection for any source of possible pollution.
2. Bacteriological examination made as soon after collection as possible.

3. Chemical analysis made as soon after collection as possible.

It is rare for a sample of water to yield results under the second and third headings, where careful *local inspection* has failed to suggest danger of pollution. It should, therefore, be thoroughly carried out.

Bacteriological examination absolutely condemns a water for domestic use when pathogenic organisms are found in it. Unfortunately the detection of these is not always an easy matter, and so their presence or absence is inferred from the abundance or scarcity of associated forms which are more readily found and identified. The result of this is that the bacteriological examination mostly furnishes evidence confirmatory to that derived from other sources.

Chemical analysis is more rapidly accomplished than the other procedures, and was formerly regarded as a sufficient basis for diagnosis of a water sample, in regard to its wholesomeness or otherwise for domestic purposes. This is so no longer, because it is recognized that the constituents sought for and actually found in a particular water sample, for the most part are of themselves non-deleterious, and by their excess or deficiency simply suggest the presence or absence of the actual *materies morbi*. Chemical evidence, therefore, must be used in conjunction with ALL the other evidence before a definite opinion is formed, and even then the judgment may be wholly based on negative findings, which here, as elsewhere, may at any time not bear the interpretation put upon them. Nevertheless, the following statements, when cautiously used, are helpful in interpreting results.

High chlorine and oxidized nitrogen, associated with marked free and albuminoid ammonia, suggest present or recent animal pollution.

High chlorine and oxidized nitrogen (not from strata), with little free and albuminoid ammonia, suggest past or remote animal pollution.

Low chlorine and oxidized nitrogen, and very low free and saline ammonia with high albuminoid ammonia, suggest pollution of vegetable origin.

Deep wells often show a large amount of chlorides and

free ammonia, without these necessarily indicating pollution. This is especially notable in wells sunk into strata like the London greensands.

The organic nitrogen in a water is mostly determined by the estimation of the albuminoid ammonia which only partially measures it. If the organic nitrogen by Frankland's process be 1 part per 100,000, then the albuminoid ammonia of the same water would be about 0·615 part per 100,000, containing 0·506 of organic nitrogen; and the organic nitrogen by the Kjeldahl process would be about double that in the albuminoid ammonia, or 1·012 part per 100,000.

SPECIMEN ANALYSES (from Notter and Firth).

	Chlorine	Free NH_3	Alb. NH_3	Oxygen absorbed	Nitrates	Nitrites
1. Upland surface ...	1·0	0·003	0·012	0·290	0·16	nil
2. Shallow well ...	2·2	0·011	0·009	0·200	0·002	nil
3. ,, ,, ...	1·0	nil	0·003	0·040	0·800	nil
4. ,, ,, ...	12·5	0·005	0·006	0·150	1·500	traces
5. Deep well ...	2·8	0·010	0·004	0·060	0·030	nil
6. ,, ,, ...	29·0	0·055	0·002	0·110	0·110	nil
7. ,, ,, ...	19·0	0·018	0·004	0·110	0·390	traces
8. ,, ,, ...	22·0	0·011	0·004	0·060	0·090	nil
9. Spring in a copse...	1·6	0·020	0·001	0·015	1·100	nil
10. ,, near ditch	4·0	nil	0·006	0·200	1·700	nil
11. ,, in meadow	3·9	0·008	0·030	0·180	0·200	nil
12. ,, protected...	3·0	0·009	0·006	0·122	0·600	nil

NOTES.

Local Inspection:

In numbers 1, 2, 5, 8, and 12, sources of pollution were absent or well guarded against.

Numbers 3 and 7 were in farmyards.

In numbers 4, 6, 9, 10, and 11, defects in construction or in protection from possible pollution were found.

Bacteriological Examination:

In numbers 4, 6, 7, 10, and 11, sewage organisms were found.

Opinion:

Numbers 1, 3, 5, 8, and 12 were returned as safe; number 2 as doubtful; and numbers 4, 6, 7, 9, 10, and 11, as unsafe.

SEWAGE AND SEWAGE EFFLUENTS.

The subjoined table will suggest ideas on this subject.

Contents of Sample in Parts per 100,000	Samples: Midden Towns Sewage	Water Closet Towns Sewage	Birmingham Sewage	Staffordshire Experiments: Sewage	Staffordshire Experiments: Septic Tank	Staffordshire Experiments: Effluent 3ft. filter	Standards: Effluents: Ship Canal Manchester	Standards: Effluents: Sewage Commission
Total solid residue ..	121·5	136·9	193·6	170·9	107·8	101·8	—	—
Dissolved solids	82·4	72·2	119·3	107·4	100·2	101·6	—	under 3
Suspended ,, total ..	39·1	44·7	74·3	63·5	7·6	0·14	—	under 3
,, ,, organic ..	21·3	20·5	44·9	28·5	3·8	0·06		
,, ,, mineral ..	17·8	24·2	29·4	35	3·8	0·08		
Organic carbon	4·2	4·7	—	—	—	—	—	—
,, nitrogen	2·0	2·2	—	—	—	—	—	—
Total combined nitrogen ..	6·5	7·7	—	—	—	—	—	—
Free and saline ammonia ..	5·4	6·7	4·05	2·154	1·716	0·009	under	—
Albuminoid ammonia ..	—	—	1·57	0·972	0·340	0·031	0·14	—
Nitrites and nitrates as N...	—	—	0·92	0·049	nil	1·755	—	—
Nitrites	—	—	nitrites	0·029	nil	0·005	—	—
Chlorine (chlorides) ..	11·5	10·7	20·2	11·0	9·9	9·4	—	—
Oxygen absorbed in 4 hours at 80° F.	—	—	*a* 27·56 *b* 15·79	5·019	2·184	0·244	under 1·4	0·5 in 24 hours
Oxygen absorbed in 3 minutes before incubation	—	—	—	1·862	0·836	0·060	—	1·0 in 48 hours
Oxygen absorbed after incubation for 3 days at 27° C.	—	—	—	2·176	1·571	0·052	—	or 1·5 in 5 days
Column necessary to obscure test lines (inches)	—	—	—	0·5	1·5	over 24	—	—
Percentage purification :—								
On free and saline NH_3	—	—	—	—	—	99·5%	—	—
On albuminoid NH_3 ..	—	—	—	—	—	96·8%	—	—
On oxygen absorption in 4 hours at 80° F. ..	—	—	—	—	—	95·1%	—	—

(a) Filtered. *(b)* Unfiltered.

Examination of a Sample.

Equal quantities are taken every hour and mixed, and a sample is then taken. This is examined for the following constituents and characteristics. The bottle containing the sample should be completely filled, and examined at once or kept in an ice chamber.

Chlorine.—Dilute with as much distilled water, and examine as in water analysis.

Ammonias.—In a crude sewage or unfiltered effluent, 5 c.c. should be diluted to 500 c.c., but in a good effluent a dilution of ten times will usually be sufficient. Test a little with Nessler, and judge from coloration produced.

Oxygen Absorption.—Dilute ten to one hundred times, and be careful to watch for decolorization of the permanganate, as several lots may be needed.

Nitrates and Nitrites are estimated as in water; but as seen in the table above, nitrates may be present in large amount, especially in a good effluent, and the coloration produced will be much stronger than that in the standard.

Suspended Solids can be estimated by filtering a known quantity of the sample through a weighed filter paper, and drying and weighing again. The filter paper is then ignited in a weighed platinum or other crucible, which is cooled and weighed. The increase in weight of the crucible, less the weight of filter-paper ash, gives the mineral-content in the suspended solids. The loss or difference between the total and the mineral part is the amount of organic matter in suspended solids.

Dissolved Solids are estimated in the filtrate.

Incubator Test is a test for putrescibility laid down by the Mersey and Irwell Joint Committee for effluents discharged into the Manchester Ship Canal. The effluent is tested in the fresh state for oxygen absorption in three minutes. A bottle is then completely filled with the sample, stoppered, and incubated at 80° F. (27° C.) for a week. The contents are thereafter tested for oxygen absorption in three minutes. If the amount absorbed is the same or less, the effluent is considered harmless as regards its power of absorbing oxygen from any stream or river into which it may be poured. If the oxygen

absorption has increased, putrefaction is inferred, and the effluent is considered unsuitable. Less absorption may be noted, due to oxidation having taken place at the expense of the nitrates and the dissolved oxygen.

Dissolved Oxygen.—Winkler's process, given under water, was stated to be unsuitable in the presence of much organic matter. It is, however, the method used by the Glasgow Corporation Chemist for the effluents submitted to him daily for analysis. The interference of the organic matter is got rid of by the addition of a few drops of weak permanganate solution, until a faint pink colour remains permanent. The process is then proceeded with as before. Another process described is that of Letts and Blake. In it a solution of ferrous sulphate is added to a measured quantity of the sample contained in a bulb having two openings, one closed with a stopper, and the other leading to a smaller bulb from which it is separated by a stopcock. Some ammonia is added, which precipitates ferrous hydrate. This absorbs any dissolved oxygen in the sample, becoming ferric hydrate. Sulphuric acid in excess is then added, which dissolves the two hydrates, forming the corresponding sulphates, which being more stable allow the end titration to be done in an open vessel without risk of further oxidation. The amount of ferrous sulphate taken is titrated with standard potassium permanganate solution, or standard potassium bichromate solution, each of which is of such a strength that 1 c.c. = 1 c.c. oxygen. This preliminary titration measures the amount of oxygen required to oxidize the ferrous sulphate, and the end titration with one of the same solutions measures the amount of ferrous salt still unoxidized, and the difference between the two titrations gives the number of c.c. of oxygen supplied by the quantity of sample taken, that is, the amount of dissolved oxygen. Nitrites have a very disturbing effect on the process, and to obviate this 2 c.c. of a mixture of 3 volumes permanganate solution and 1 volume 50 per cent sulphuric are added, and the sample is allowed to stand ten minutes before proceeding. The preliminary titration of the ferrous sulphate, mixed with the same amount of sample and sulphuric, is similarly treated.

Solutions required: (*a*) Ferrous sulphate solution (5 per cent in 1 per cent H_2SO_4); (*b*) Standard potassium permanganate solution (5·638 grm. per litre), 1 c.c. = 1 c.c. oxygen at normal temperature and pressure; (*c*) Ltd. pot. bichromate solution (8·79 grm. per litre), 1 c.c. = 1 c.c. oxygen at normal temperature and pressure; (*d*) Sulphuric acid, 50 per cent in distilled water.

PROCESS.—Measure the exact capacity of the large bulb. Fill it by siphonage to avoid absorption of atmospheric oxygen. Insert stopper and drain off excess of fluid. Remove stopper, withdraw 7 c.c. of fluid, add 5 c.c. of ferrous sulphate solution by pipette to bottom of bulb, fill up mouth with strong ammonia solution, and insert stopper. Mix by swinging. A precipitate of greenish ferrous hydrate forms, which absorbs dissolved oxygen, becoming brownish ferric hydrate. Allow to stand fifteen minutes. Then add 50 c.c. of the sulphuric acid solution to the small bulb, and opening the stopcock allow it to mix into the larger bulb. The hydrates are dissolved, and sulphates formed. Pour out into a beaker, and titrate with permanganate in case of water, and with bichromate for sewage. For latter, end reaction is got by removing a drop of liquid from the beaker, and touching a drop of solution of potassium ferricyanide, when if oxidation is incomplete, a blue colour (Turnbull's blue) is produced within two minutes. A control or preliminary titration is made by taking the same quantity of sample (less 7 c.c.), adding first 50 c.c. of the sulphuric acid, then 5 c.c. of the ferrous sulphate solution, and then titrating. The end of the titration with permanganate is a permanent faint pink tint.

The Sewage Commission recommend that the suspended solids should be removed before estimating the dissolved oxygen, because "small variations in the amounts of solids in suspension in effluents may seriously affect the rate at which effluents take up oxygen."

Kjeldahl's Method for Total Nitrogen.—Owing to the large amount of organic matter in sewage, the estimation of albuminoid ammonia is alleged to be unreliable, and the estimation of the total nitrogen is preferred.

PROCESS.—Take a Kjeldahl flask (a 200 c.c. flask with a round bottom, and made of fire-resisting glass), pipette into it 10 c.c. of sewage or 25 c.c. of the effluent. Add 1 c.c. of strong sulphuric acid, mix well, and evaporate slowly over a small flame guarded with wire gauze. When the fluid is reduced to very small bulk, add 5 grm. potassium sulphate, and 20 c.c. strong sulphuric acid. Heat over a small Bunsen flame, very slowly at first, until all frothing ceases. Continue heating for about two hours, or until the liquid is colourless and clear. Then cool the flask and contents, and carefully transfer the liquid to a 700 c.c. boiling-flask, washing the small flask out repeatedly with ammonia-free distilled water, and adding the washings to the large flask, making up the bulk to about 200 c.c. Now add sufficient strong KOH solution to neutralize the excess of acid, and some extra to make alkaline. Add a few pieces of granulated zinc to prevent bumping, connect with a Kjeldahl's safety bulb and condenser, and distil over the ammonia into N/1 sulphuric acid, the end of the tube from the special condenser dipping under the surface of 20 c.c. of the N/1 acid in an Erlenmeyer flask. About 100 c.c. are distilled over, slowly. Thereafter the acid is titrated with N/1 NaOH, using methyl-orange as indicator. The number of c.c. of soda required deducted from the number of c.c. of normal acid used (20) gives the number of c.c. of normal acid neutralized by the ammonia distilled over. Each c.c. of normal acid is chemically equivalent to 0·014 grm. of N. The organic N is got by deducting from the result obtained the amount previously found to be present as free and saline ammonia. (When this process is applied to food stuffs, this correction is not necessary.)

The *rationale* of the process is that the organic matter is broken up by the acid and sulphate, and the nitrogen converted into ammonia, which is fixed by the excess of acid as ammonium sulphate. On adding excess of KOH, the ammonia is liberated, and is then distilled over. It is received into N/1 sulphuric acid, forming again ammonium sulphate. The amount of acid left unneutralized is then estimated, and by difference the amount combined with the ammonia.

GLASGOW SEWAGE.—Average of the daily analyses of sewage treated at Dalmarnock and Dalmuir sewage works for the year ending May, 1910; and of the unfiltered effluents discharged after treatment by precipitation processes. In parts per 100,000.

	DALMARNOCK		DALMUIR	
	Crude Sewage	Effluent	Crude Sewage	Effluent
Suspended solids:				
Total	47·2	—	25·0	—
Organic	23·4	—	14·1	—
Mineral	23·8	—	10·9	—
Chlorine	21·4	20·0	7·0	7·2
Free and Saline Ammonia..	1·89	1·18	1·94	1·60
Albuminoid Ammonia ..	·522	·305	·407	·263
Oxygen absorbed in 4 hrs. at 80° F.	6·818	2·617	4·610	2·837
Per cent. purification—				
On Free and Saline NH_3 ..	—	37·5 %	—	17·5%
On Albuminoid NH_3 ..	—	41·5 %	—	35·3%
On Oxygen absorbed ..	—	61·6 %	—	38·4%

CHAPTER III.

EXAMINATION OF AIR.

THE chief points to determine are odour, temperature, pressure, humidity, carbonic acid, ozone, oxidizable and organic matter, noxious emanations, micro-organisms, suspended matter, carbon monoxide, oxygen.

EXAMINATION OF A SAMPLE.

Collection.—Large wide-mouthed jars with rubber caps, and holding about 4000 c.c. (4 litres), are most convenient. These are thoroughly cleansed with distilled water before use, run dry by inverting, and capped. Their actual capacity should be ascertained, and marked on them. To collect a sample of air, one of two methods may be employed, namely:

1. Place jar where sample is to be taken, and blow in the surrounding air by a pair of bellows having a long nozzle reaching down to the bottom of the jar. The contained air is thus displaced from the jar.

2. Fill the jar with distilled water, and empty it at the place named for sampling by inverting it, and allowing it to drain dry. It is then capped and labelled, and the label inscribed with the observed temperature and pressure and, if not already noted, the capacity of the jar.

Great care must be exercised not to contaminate any sample with the air breathed out by the observer.

Odour.—The sense of smell exceeds in acuteness any other means used at present to demonstrate the presence of minute particulate matter. It also has a special value in detecting the peculiar fœtid odour so noticeable on first entering an occupied room from the open air. De Chaumont was the first to emphasize this, and he further pointed out the importance of observing it immediately on entering, as the sense of smell is soon blunted. He further pointed out the marked influence of atmospheric humidity in rendering the smell of organic matter more perceptible, an increase of 1 in the humidity being as

powerful in this respect as a rise of 2·32° C. or 4·18° F. (see "Gordon Report on Ventilation of Houses of Parliament," 1905).

Temperature.—By the thermometer.

Pressure.—By the barometer fitted with a vernier (e.g. Fortin's standard barometer).

Humidity.—By the hygrometer (direct or indirect). Daniell's, Regnault's, Dines', Mason's (wet and dry bulb). Absolute humidity, relative humidity, dewpoint, Glaisher's factors, Apjohn's formula.

Carbonic Acid Gas.—The determination of CO_2 affords an important index as to the extent to which other impurities exist. It is usually estimated by Pettenkofer's method. Solutions required: (*a*) Baryta water 0·5 per cent; (*b*) Standard oxalic acid solution 1 c.c. = 0·5 c.c. CO_2 at normal temperature and pressure (2·822 grm. per litre).

Process.—This consists in exposing a measured quantity of baryta water to a known volume of air enclosed in a jar. The baryta water absorbs the carbonic acid gas, becoming barium carbonate, which is precipitated. The amount of baryta water unused or unchanged is estimated by titration of a measured portion of the baryta water removed from the jar, with standard solution of oxalic acid in the presence of phenolphthalein. Oxalic acid is used in preference to sulphuric acid, because the latter would attack particles of barium carbonate floating in the liquid, and thus give rise to some degree of error.

$$BaH_2O_2 + CO_2 = BaCO_3 + H_2O$$
$$BaH_2O_2 + H_2C_2O_4{\cdot}2H_2O = BaC_2O_4 + 4{\cdot}H_2O.$$

From these equations we see that one molecule of baryta water is neutralized by one molecule of CO_2 and one molecule of oxalic acid. Hence the two latter are chemically equivalent, and 1 molecule of oxalic acid measures indirectly 1 molecule CO_2; that is, 126 grm. (cryst.) oxalic measure indirectly 44 grm. CO_2, or 126 grm. (cryst.) oxalic measure 22·32 litres CO_2, or 2·822 grm. oxalic measure 0·5 litre CO_2. Hence, if we dissolve 2·822 grm. of crystallized oxalic acid in 1 litre of distilled water, 1 c.c. = 0·5 c.c. CO_2.

METHODS.—A large jar is filled with the air sample as directed above. Fifty c.c. of the baryta water are added, the jar is capped, and shaken now and then over a period of half an hour. Thereafter 25 c.c. are removed from the jar and titrated with the standard oxalic, using phenolphthalein as indicator until the red colour is just discharged. Twenty-five c.c. of fresh baryta are similarly titrated, and the number of c.c. required noted. The difference between this latter and the number of c.c. required for the 25 c.c. removed from the jar, measures in oxalic the amount of baryta which has had its alkalinity neutralized by absorption of CO_2 from the air in the jar. This amount doubled measures the quantity so neutralized in the 50 c.c. taken, and as the oxalic per c.c. = 0·5 c.c. CO_2, on multiplying the result by 0·5 we get the number of c.c. of CO_2 absorbed from the volume of air in the jar. The CO_2 is at normal temperature and pressure, and the air in the jar is at the temperature and pressure noted on collection of the sample, and so these two volumes are not quite comparable. The volume of sample in the jar must first be corrected to the volume it would occupy at standard temperature and pressure. This may be done after several fashions, but the one here recommended is to use the formula

$$\frac{V^\circ \times P^\circ}{T^\circ} = \frac{V' \times P'}{T'}$$

where V° P° and T° denote respectively volume at standard pressure (760 mm. or 29·92 inches of mercury) and at standard absolute temperature (0° C + 273 or 32° F. + 459), and V′, P′, and T′, the volume of the jar as measured (less 50 c.c. displaced by the baryta water added), and the pressure and temperature at the time and place of taking the sample, the temperature being changed to the absolute scale.

Thereafter the proportion of CO_2 present is calculated and the result expressed, which may be stated as a percentage, or parts per 1000 or per 10,000. The air of the open country averages almost exactly 3 parts per 10,000.

Haldane's Method requires a special apparatus, but once facility of manipulation of the apparatus has been acquired, an accurate result can be obtained in ten minutes and

without any calculation. The method consists in subjecting 25 c.c. of air to exposure to caustic potash, which absorbs the CO_2, and the diminution in volume is measured under the same conditions of temperature and pressure, and the divisions on the graduated portion of the burette are each $\frac{1}{10000}$th part of the whole capacity of the burette, so that the result is read off in parts per 10,000.—$2KOH + CO_2 = K_2CO_3 + H_2O$.

Hesse's Modification of Pettenkofer's Method is to collect the sample in a flask, from 250 c.c. to 1000 c.c. capacity, closed with a rubber stopper with two holes plugged with glass rods. One of the plugs is removed, and a pipette containing 10 c.c. of baryta is inserted in its place. The other plug is loosened, and the baryta allowed to run into the flask. The pipette is then removed and the plugs are reinserted, and the flask shaken from time to time. After half an hour a plug is withdrawn, a drop of phenolphthalein added, and the nozzle of a burette containing standard oxalic one-tenth the strength of that used in Pettenkofer's process is placed in the vacant hole, and the baryta in the flask titrated. The other titration to ascertain the oxalic equivalent of 10 c.c. of fresh baryta is done as before. The advantage of the method is that there is less exposure of the baryta to the air not in the flask. Equations—

$$Ba(OH)_2 + CO_2 = BaCO_3 + H_2O$$
$$Ba(OH)_2 + H_2C_2O_4 = BaC_2O_4 + 2H_2O.$$

Pettenkofer's name is attached to another method in which air is aspirated through a known bulk of baryta water spread out lengthwise in a tube so that the air bubbles through the baryta solution.

Lunge and Zeckendorf's Method is to take a known quantity of N/500 Na_2CO_3 in a glass bottle fitted with a two-holed stopper and two glass tubes as in a wash-bottle. An indiarubber bulb of a standard capacity with an inlet and an outlet tube, both fitted with valves, has its outlet tube attached to the glass tube leading to the bottom of the bottle and under the surface of the solution of sodium carbonate. The bulb is compressed slowly, and the air expressed bubbles through the fluid in the bottle, and

the latter is then shaken. The manœuvre is repeated until the fluid (which had been coloured pink by the addition of phenolphthalein) becomes colourless from the Na_2CO_3 absorbing CO_2 and forming $NaHCO_3$. The number of compressions of the bulb required are counted, and by reference to a table the proportion of carbonic acid gas present in the air tested is obtained. The method is very tedious if the air is pure, as forty-eight compressions are required to detect 3 parts per 10,000.

Scurfield's Apparatus.—Air is aspirated through weak baryta solution, 3 parts per 10,000, coloured red by addition of phenolphthalein.

Ozone.—

1. *Houzeau's paper.* A piece of neutral or faintly reddened litmus paper soaked for a quarter of its length in neutral KI solution and dried (keep in dark). When exposed to ozone the KI is decomposed and K_2O formed, which turns the litmus blue at soaked part only. Cl and Br would not do this. N_2O_3 would turn rest red. NH_3—use control.

2. *Schönbein's paper.* KI and starch paper turned blue. Cl, Br, N_2O_3, H_2O_2 all give same result.

3. KI and phenolphthalein papers turned red. H_2O_2 gives same result. Cl, Br, and N_2O_3 give a brown.

4. *Arnold-Mentzel paper.* Chromic acid paper is negative. Hydroxyl gives a blue.

5. *Engler and Wild's manganous chloride paper.* Ozone turns it brown. Hydroxyl has no action, but ammonia also gives a brown.

Oxidizable and Organic Matter. — This is best determined by washing the air (by shaking with distilled water or slowly aspirating through the same) and examining the washings, as in water analysis, for total nitrogen-free, saline and albuminoid ammonias, nitrous and nitric acids, and oxygen absorption.

Carnelly's process is sometimes used, but is not very reliable. A millinormal solution of permanganate is acidulated with sulphuric acid, and 50 c.c. are added to an air jar containing the sample, and exposed with occasional shakings to its influence for about half an hour. There-

after 25 c.c. are removed and compared with the same quantity of fresh solution, and the number of c.c. of same required to be added to bring its colour up to that of control is noted. From this number the amount of oxygen absorbed can be calculated and the result expressed in parts per million.

Noxious Emanations.—Under this heading is considered the search for foreign gases and vapours in the air, such as fumes of hydrochloric, nitric and nitrous, carbonic, and sulphurous acids, sulphuretted hydrogen, chlorine, ammonia, carbon monoxide, ammonium sulphide, carbon bisulphide, carburetted hydrogen, roburite, nitrobenzol.

Scheme for detection.—Take sample in jar.

1. Remove cap or stopper, smell, and replace stopper. Chlorine, HCl, SO_2, ammonia, ammonium sulphide, sulphuretted hydrogen and carbon bisulphide, all have characteristic odours. Carbonic, nitrous, and nitric acids have not.

2. Take a piece of red and a piece of blue litmus paper; moisten in some neutral distilled water, attach to a piece of stick, and hang down into jar free of sides. After waiting a minute, note change of colour.

3. If reaction acid or alkaline, pour rapidly into jar 10 c.c. of ammonia-free distilled water, replace stopper, and shake vigorously. Remove half of this water, and test for dissolved gas.

A. If the reaction was acid, then it is likely to be carbonic, hydrochloric, sulphurous, nitric, or nitrous acid. Add to water removed from jar a few drops of silver nitrate solution.

White precipitate denotes either :—

a. Carbonic acid : precipitate very slight, acidity also very faint, baryta water added to jar becomes turbid after shaking, and turbidity is increased by adding ammonia.

b. Hydrochloric acid : precipitate marked, acidity ditto, precipitate insoluble in nitric acid, soluble in ammonia, and also in KCN.

c. Sulphurous acid: precipitate marked, soluble in nitric, soluble also on heating, but the solution darkens from formation of sulphide of silver. The water will also decolorize iodide of starch solution, and warmed with Zn and HCl gives off H_2S, which darkens lead acetate paper. Odour characteristic.

No precipitate, infer nitric or nitrous acid. Test for these as described under water analysis.

B. If the reaction was alkaline, gas is either ammonia or Am_2S.

To water from jar add a little Nessler's solution. Yellow to amber colour—ammonia. Characteristic odour.

Black colour, Am_2S; nitroprusside of Na gives a violet; smell of H_2S.

4. If litmus is unaffected, may be either :—

H_2S, PbAc papers blackened, odour characteristic.

CS_2, colourless volatile liquid giving off an inflammable vapour with a garlicky odour. The liquid burns with a blue flame giving off sulphur dioxide fumes, and leaving a deposit of sulphur.

5. If the blue litmus is first slowly reddened and then bleached, the gas is—

Chlorine: filter paper moistened in KI solution is first darkened and then bleached. Odour characteristic. Red colour, with mixture of proto-salt of iron and KCNS.

Estimation of some Gases detected as above.—Hydrochloric, nitrous, and nitric acids are absorbed in freshly distilled water, and tested for as in water analysis. Chlorine by pure KI solution, from which it liberates iodine, which is titrated with thiosulphate of sodium. Bromine similarly. Sulphurous acid by absorption in a decinormal solution of iodine. Sulphuretted hydrogen similarly. Carbon bisulphide by absorption in a strong solution of potash in alcohol, and after titration with standard iodine solution.

In all these cases the air to be tested is slowly aspirated through the liquid named. Ammonia can be absorbed in pure water and Nesslerized.

Micro-organisms.—See BACTERIOLOGY.

Suspended Matter.—Aspirate large quantities of air through small amounts of water in a series of wash-bottles; evaporate down to aliquot part, mount a drop and count number of particles, or to dryness and weigh residue, which may then be ignited and cooled and re-weighed for non-volatile part. Pouchet's aeroscope. Hesse's apparatus. Sugar filter. Aitken's method. Is composed of animal, vegetable, and mineral matter. Varies in towns from 5 to 25 mgr. per c.m., or otherwise expressed from 10,000 to 2,000,000 particles per c.c. Shaw calculates that 400 tons of soot are thrown into the air of London per day. In London 40 cwt. of soot are deposited on each acre of ground per annum, and in Glasgow 22 cwt. in summer and 25 in winter, making 47 in all.

Carbon Monoxide.—From stoves, in water gas, 6 per cent in coal gas, in mines. Affinity for hæmoglobin 300 times that of oxygen. Kills when blood is saturated up to 60 to 80 per cent. Haldane advises the use of birds and mice as indicators in mines.

Haldane's Method.—Take 5 c.c. dilute blood solution in a clean dry bottle, aspirate in some suspected air, cork and shake for ten minutes, protecting from light. Pour out into a test tube and compare with some of original blood. If CO present, the treated blood will be pink. The test is made quantitative by adding carmine solution to the normal blood until tints equal, and repeating with normal blood saturated with coal gas.

Spectroscope: spectrum similar to OxyH, but not reduced by Am_2S. May also be absorbed by copper subchloride in a Hempel's gas burette.

Oxygen.—This may be estimated by combustion with hydrogen, or by absorption in an alkaline solution of pyrogallic acid, or by absorption by nitric oxide. The two latter are done in a Hempel's gas burette, and in the pyrogallic method the carbonic acid is also absorbed and has to be separately estimated and deducted. In these

methods care must be taken that the temperature and pressure do not vary while the experiment is in progress.

Gases in Mines.—The atmosphere in mines is liable to become dangerously contaminated by noxious gases issuing from the coal face, such as sudden discharges of marsh gas from accumulations under pressure in the coal measures (so-called " blowers "), by accumulation of dust which may fire, and by the gases resulting from fires and explosions. The following table gives the principal gases found in mines, and their popular and other names.

Miner's Name	Composition	Occurrence	Remarks
Black-damp or Stythe	A mixture of gases, containing 85 to 88% of nitrogen	In coal mines	Does not support life, nor combustion
After-damp or Choke-damp	CO_2 carbon dioxide carbonic acid	In coal mines In lead mines	do.
White-damp	CO carbon monoxide carbonic oxide	In coal mines	Very poisonous and explosive
Fire-damp	CH_4 marsh gas, methane, carburetted hydrogen	In coal mines	Highly explosive
—	H_2S sulphuretted hydrogen	In sulphur mines	Very poisonous Inflammable
—	Coal dust	In coal mines	Highly inflammable

CHAPTER IV.

SOILS.

Soil is the term used to denote that portion of the earth's crust which by its condition or properties can affect health. It is conveniently spoken of as composed of two layers: (1) an upper or surface soil, and (2) a deeper or subsoil layer. The upper layer contains the products of the decay of animal and vegetable matter, constituting mould or "humus." The subsoil layer is intermediate between the upper layer and the underlying formations or strata. Both layers are originally derived from these deeper layers by "weathering," a geological term which includes all those forces that make for denudation of surface.

Examination of a Sample.

Ground Air.—The amount of air in soil varies with its porosity, and its state in regard to moisture. Thus a dry porous soil may contain, if loamy, 70 per cent; if loose sand, 40 to 50 per cent. Such air is collected by aspiration through a tube leading to a perforated bulb, sunk into the soil, in which an opening has been made. The analysis of ground air is made for: CO_2 (increases with depth, most in summer and autumn, least in winter and spring, more in impure porous soils; varies from 4 to 8 per cent in January, to 8 to 24 per cent in August); Moisture: 85 per cent; Oxygen: 21 to 18 per cent. The amount, in a quantity of soil, may be estimated by filling a burette to the zero mark, with sample of soil, and connecting the nozzle with that of another burette containing 50 c.c. of water, by a piece of rubber tubing. On opening the stopcocks and raising the burette containing the water, the water flows into the other burette, wetting the soil. The process is stopped when the water reaches the zero mark, by closing the stopcocks. The loss of water from the one burette is a measure of the amount absorbed by the soil displacing the contained air.

Ground Water—Is divided into (1) Moisture, which is the water present along with ground air, simply moistening the particles ; and (2) Subsoil water, which is the condition when the particles and their interstices are full of water.

The amount of moisture in a soil is estimated by drying a weighed portion of it ; the loss of weight being calculated as moisture.

The level of the subsoil water is studied by digging a pit or well, and observing from time to time the varying levels.

Soil Temperature—Is taken at the depth of 4 feet by a specially sluggish thermometer, enclosed in a protecting case. The soil temperature attains its maximum in July and August.

Soil is also examined chemically for total nitrogen, phosphates, sulphates, nitrates, and peaty acids. An aqueous extract of a weighed quantity of it may be examined for chlorides, ammonias, etc. It is also separated mechanically into particles of different sizes, and into clay, sand, etc.

CHAPTER V.

FOODS.

EXAMINATION OF MILK.

AVERAGE composition of cow's milk:—Water, 87 to 88 %; proteid, 3 to 3·5 %; fat, 3·5 to 4·5 %; sugar, 4 to 5 %; mineral matters, 0·7 %.

Physical Characters.—Placed in a narrow glass it should be quite opaque, of full white colour, without deposit, without peculiar smell or taste, and when boiled it should not change in appearance. The temperature should be taken.

Reaction.—Should be slightly acid, or neutral, or very feebly alkaline. Fresh milk is sometimes both acid and alkaline to indicators, that is, amphoteric, turning red litmus blue and turmeric to brown. Strongly alkaline: cow ill, or much colostrum, or addition of sodium carb. Strong acidity: lactic or butyric acids, and indicative of retrograde change.

Cream.—Stand 100 c.c. in a measure for twenty-four hours in a still atmosphere. Read off proportion of cream. Should be $\frac{6}{100}$ to $\frac{11}{100}$; generally about $\frac{8}{100}$. Alderney cows give $\frac{30}{100}$ to $\frac{40}{100}$. Time of year and breed to be considered.

Specific Gravity.—At 15° C. or 60° F. Varies from 1027 to 1034, being less as fat is greater. The specific gravity is raised by skimming and can be reduced by adding water, so that this factor alone is not a reliable index to the character of a sample. The specific gravity falls 1° for each rise of 10° F. above 60°, and at 60° F. there is a loss of 3° of specific gravity for every 10 per cent of water added.

Three methods—Specific gravity bottle, lactometer, and Westphal balance.

Total Solids.—Ought not to be below 11·5 per cent; but are more usually 12 to 13 per cent. Take 2 c.c. of the milk in a flat shallow dish of known weight. Evaporate to dryness over the water-bath, and then in the water-oven

for half an hour, and weigh. The increase in weight is the amount of total solids in 2 c.c. If the specific gravity of the milk is known, the weight of 2 c.c. is readily calculated, and the percentage of total solids is then worked out. Some analysts weigh 5 grm. of milk into the dish, and thus avoid using the specific gravity.

Ash.—The dried solids are incinerated at as low a heat as possible, and, on cooling, the dish is reweighed and the percentage calculated. It averages in normal milks about 0·73, and should not fall below 0·7. Watering makes it less. Test ash for effervescence; if marked, suggests addition of a carbonate.

Fat.—This is a very important determination. Several methods.

Werner-Schmidt Method.—Ten c.c. of the milk are pipetted into a Stokes' tube, and strong HCl is added to the 20 c.c. mark. The mixture is now heated in the water-bath, or carefully over a flame, until it turns a brown colour. Now cool the tube and its contents in water, and then add ether to the 50 c.c. mark. Cork the tube firmly, and mix the contents well by inverting slowly ten to twelve times. Set the tube down in an upright position to allow the ether to separate and become clear. Pipette off 10 c.c. or 15 c.c. of the ether into a weighed platinum capsule, or other dish, evaporate the ether on water-bath at 60° C., dry at 100° C., cool and weigh. The increase of weight is weight of fat dissolved in the amount of ether taken by pipette. Now observe number of c.c. of ether still in the tube, plus $\frac{3}{4}$ of the fluffy layer, separating the clear ether from the dark liquor. Calculate by proportion amount of fat dissolved in this quantity of ether, and add the result to former; the sum gives the amount of fat derived from 10 c.c. of milk sample. Express result as weight of fat per 100 grm. of milk. To do this specific gravity of sample must be observed.

EXAMPLE.—Twenty c.c. of ether removed from tube gave a residue of 0·286 grm. Ether still in tube (plus $\frac{3}{4}$ fluffy layer) 2·8 c.c. Therefore as 20 : 22·8 : : 0·286 : x = 0·326 grm. of fat in 10 c.c. of milk sample. But specific gravity of sample was 1030·5, hence 10 c.c. weigh 10·305

grm. Then by proportion—10·305 : 100 : : 0·326 : x = 3·16 grm. of fat in 100 grm. of milk, or 3·16 per cent.

Adams' Process.—Using a Soxhlet apparatus is the official method of the Society of Public Analysts.

Take a strip of Adams' fat-free paper, and from a pipette spot over it 5 c.c. of milk sample. Dry the paper high over a Bunsen flame, and finally in the water-oven. Roll paper up into a coil, and put it into a Soxhlet apparatus attached below to a clean dry flask (wide-mouthed) of known weight and above to an invert condenser. Sufficient ether (specific gravity 0·720) should be used to fill the Soxhlet tube to the top of the siphon one and a half times. The flask is supported in an evaporating-basin containing water. The basin is heated by a small flame. The ether evaporates and is condensed, running back over the paper, soaking it, and filling the tube until, when the level reaches the top of the siphon, the latter acts and empties the whole amount back into the flask, carrying with it the dissolved fat. Twelve such siphonings at least should take place, and then (the flame being meanwhile withdrawn), the condenser is fixed in the usual position, and the ether distilled over. The flask is then detached and dried in the water-oven (laid on its side) to a constant weight. The weight obtained, less the weight of the flask, gives the weight of fat in 5 c.c. of milk sample, and this is calculated out as in above example to a percentage.

Never heat the flask containing ether over a naked flame, but in a vessel of water at about 60° C., keeping the ether in a gentle state of ebullition.

Gerber's Process is somewhat similar, using a Gerber apparatus.

Leffmann-Beam Process.—Using a special set of graduated bottles and a special centrifugal machine. Into one of the bottles 15 c.c. of sample are introduced by means of a pipette, and then 3 c.c. of a mixture of equal parts of amylic alcohol and strong HCl, and these are thoroughly mixed. Then 9 c.c. of pure concentrated sulphuric acid are added slowly, 1 c.c. at a time, shaking after each addition. The milk will gradually assume a chocolate colour passing on to a deep brown. Now fill up bottle to zero mark with a hot and freshly-made mixture of one part of sulphuric acid to

two parts of water. The bottle is placed in the machine, and balanced with a similar one filled with sulphuric (1 in 2), and the machine rotated for at least two minutes. On stopping, the fat will be seen to have separated out as a layer on the top, and the percentage is read off, each graduation representing 0·1 per cent of fat by weight. The reading is from the extreme top of the fat column to extreme bottom.

There is also a Gerber process similar in principle.

Maceration Process.—Is used at Somerset House in the Government Laboratory, and is specially suited to the analysis of sour milks. It consists in neutralizing the acidity with N/10 strontia, evaporating to the consistency of soft cheese, repeatedly washing with ether, which is run through a filter-paper of known weight, weighing the fat-free solids plus the filter-paper washed free of fat, and thus the solids-non-fat are obtained, an allowance being made for the strontia added. This figure deducted from the total solids obtained from another portion of the sample gives the amount of fat. (Several corrections are made in calculating the solids for alcohol, for volatile acid, and for ammonia.)

Calculation Method by Hehner and Richmond's Formula.—$F = 0{\cdot}859 \times T - 0{\cdot}2186 \times G$, where F represents the percentage of fat, T the percentage of total solids, and G the last two figures of the specific gravity (including any decimal). For skim milks, where $G \div T$ exceeds 2·5, the following modified formula is used: $F = 0{\cdot}859 \times T - 0{\cdot}2186 \times G - 0{\cdot}05 (G \div T - 2{\cdot}5)$. A third formula gives T where G and F are known. $T = 0{\cdot}25 \times G + 1{\cdot}2 \times F + 0{\cdot}14$.

Solids-not-Fat—Are obtained by subtraction of the fat from the percentage of total solids. Should not be less than 8·5 per cent.

Calculation of Amounts of Adulteration (by Skimming and Added Water).—A milk may be skimmed of fat, or have water added, or be treated in both ways. The Board of Agriculture has fixed the standard for fat in milk at not less than 3 per cent, for solids-not-fat not less than 8·5 per cent, and for total solids not less than 11·5 per cent (3 + 8·5). Most pure milk samples give total

solids = 12 per cent to 13 per cent. If a milk sample gives a lower figure for fat or solids-not-fat than these standards, adulteration is assumed to have been practised, and the burden of proof to the contrary rests with the vendor of the milk sampled.

1. If the solids-not-fat figure is less than 8·5 per cent, and the fat figure is 3 per cent or above, the milk has been watered, and the amount of water added is calculated from the formula :—$(8{\cdot}5 - \text{SnF}) \times \frac{100}{8{\cdot}5}$ = percentage of added water.

2. If the fat figure is less than 3 per cent, and the SnF figure is 8·5 per cent or above, then fat has been abstracted, usually by skimming, or already skimmed milk has been added. The percentage of fat present deducted from 3 per cent, when multiplied by 100 and divided by 3, gives the percentage of fat abstracted. $(3 - \text{F}) \times \frac{100}{3}$ = percentage fat abstracted.

3. If both the figures (SnF and F) are below the standard, the percentage of added water should first be calculated, and a further calculation made to see if the addition of this amount of water would account for the lowness of the fat figure. If it does not, the amount of fat removed is calculated thus :—The percentage of fat present deducted from that found by calculation after allowing for the water added is multiplied by 100 and divided by 3. Thus a milk having 7·65 per cent of SnF and 2·7 per cent of F would be returned as having 10 per cent of added water. If the SnF are still 7·65 per cent, and the F is now 2·4 per cent, it would be said to have 10 per cent of added water and 10 per cent of fat abstracted.

Lactose in Milk.—May be determined by the Saccharometer (certain proteins having been first removed), or by Fehling's test as now described. Take 10 c.c. of the milk sample in a test tube, add a few drops of acetic acid, and warm. The casein coagulates, carrying the fat with it. Pour into a Nessler glass, washing out all the curd, and make up the bulk to 100 c.c. Break up the curd and filter several times, until whey is as clear as possible. Fill a burette with the whey. Take 10 c.c. of standard Fehling's solution in a porcelain basin, add 50 to 80 c.c. of aq. dest.,

and bring to the boil, and just keep boiling. Now add the whey from the burette until all the blue colour is discharged. The end reaction is difficult. Allow to settle, place a drop of supernatant liquid on a white tile, add a drop of K_4FeCy_6, and then a drop of acetic acid. A brown precipitate shows that some copper is still unreduced.

Read the amount of whey added, divide by 10. This gives the amount of milk which exactly reduces 10 c.c. of Fehling's solution. But 10 c.c. of Fehling's solution are reduced by 0·0667 grm. of lactose, so that this quantity of milk contains 0·0667 grm. of lactose, and the amount in 100 c.c. and 100 grm. is readily calculated.

Fehling's Solution: synonyms, potassio-cupric tartrate solution, or alkaline cupric tartrate solution—consists of 34·65 grm. of crystallized copper sulphate, $CuSO_4 \cdot 5H_2O$, 176 grm. of Rochelle salt, $KNaC_4H_4O_6 \cdot 4H_2O$, 77 grm. sodium hydrate, NaOH, dissolved in water and bulk made up to 1000 c.c. Of this solution 10 c.c. are completely reduced by 0·05 grm. of glucose, lævulose, or invert sugar. Fehling's solution does not keep well, and so it is often made up in two parts, equal measures of which produce, when mixed, Fehling's solution. Solution No. 1: 34·64 grm. of copper sulphate crystals dissolved in water, 0·5 c.c. sulphuric acid added, and bulk made up with water to 500 c.c. Solution No. 2: 176 grm. Rochelle salt and 77 grm. caustic soda dissolved in water, and bulk made up to 500 c.c.

Cane Sugar in Milk.—(Present in preserved milks, but here described for completeness.) Take a portion of the whey from the above process, and boil it with 1 c.c. of strong HCl. Cool and neutralize with anhydrous sod. carb., and make up bulk originally taken to three times with distilled water. Estimate the invert sugar produced as for lactose. Subtract the percentage of lactose previously found from the percentage of invert sugar now obtained, and the remainder is the amount of invert sugar derived from cane sugar. This multiplied by 0·95 gives the percentage of cane sugar present.

Nitrogen in Milk—Is the most constant ingredient, and never falls below 0·5 per cent. Ten grams. of milk are weighed into a dry Kjeldahl flask. Evaporate to dryness on the water bath, and to the dried residue add 20 c.c.

strong sulphuric and 5 grm. pot. sulphate. Heat gently over a Bunsen flame until all frothing ceases, and then place on a stand over a small Bunsen flame until the liquid is colourless and quite clear. Cool the flask and contents, and add 50 c.c. of water, and neutralize with strong NaOH solution, adding slight excess to make alkaline. Distil over the ammonia into 20 c.c. of normal acid sulphuric, the distillation taking about an hour. Titrate with decinormal alkali, using methyl orange as indicator, and the difference in the titre found and what the titre should be represents the amount of nitrogen in the amount of milk taken, and this is converted into albuminoids by multiplying by 6·39.

Microscopic Examination.—This is always advisable. The strictly normal constituents are round oil globules of various sizes in an envelope and a little epithelium. The abnormal constituents are epithelium in large amount, pus, conglomerate masses, and casts of the lacteal tubules. The added matters may be starch grains, portions of seeds, and chalk.

Bacteriological Examination.—See BACTERIOLOGY.

Proteids in Milk.—*Ritthausen's Method.* Consists in precipitating with copper hydrate, which carries down the fat and proteids. The precipitate is collected, dried, and weighed on the filter paper. Deductions are made for the amounts of fat, salts, and copper hydrate precipitate, and the weight of filter paper, and the remainder is the weight of proteids in the quantity of milk taken.

Boiled Milk.—To 10 c.c. of milk sample add 1 c.c. of solution of ortol and a few drops of peroxide of hydrogen solution. In unheated milk a crushed-strawberry colour is produced. If the milk has been heated above 72° C. (181·6° F.) no colour will be produced, but on the addition of a little unheated milk the colour will appear. As milk is pasteurized at 159° F., such milk will be positive to this test. Paraphenylenediamine (replacing ortol) gives an indigo-violet in milk not heated above 78° C.

Preservatives in Milk.—*L.G.B. Circular*, 1906. "The presence in milk of formalin to an amount which is ascertained by examination within three days of collecting the sample to exceed 1 part in 40,000 (1 part per 100,000 of formic aldehyde) raises a strong presumption that the

article has been rendered injurious to health, and that the purchaser has been prejudiced in the above sense; also that similar presumption is raised where boron preservatives are present in milk to an amount exceeding 57 parts per 100,000 or 40 grains per gallon." A Departmental Committee of the Board of Agriculture (1901) recommended "that the use of any preservative or colouring matter whatever in milk offered for sale in the United Kingdom be constituted an offence under the Sale of Food and Drugs Acts," but this recommendation has not yet received the force of law.

The commonest preservatives in use are boric acid or borates and formic aldehyde (formaldehyde). Sodium carbonate is sometimes added to restrain the formation of lactic acid. Salicylic acid, benzoates, salt, sulphites, fluorides, boro-glycerin, and hydrogen peroxide have all been used at various times.

1. *Borax and Boric Acid.*—The ash of the milk is treated with a little dilute HCl, so that it is distinctly but not strongly acid, and a piece of turmeric paper is placed in the liquid, and the dish is slightly warmed for a few minutes. The turmeric paper is removed and dried at a low temperature. If even so small a quantity as 0·01 per cent (1 in 10,000) of boric acid be present it will produce on the paper a reddish colour when dry. On moistening with a drop of an alkaline solution the red turns a greenish black. Boric acid and borates are the only substances which will give this change of colour in an acid solution.

Richmond's Test. Take equal quantities of the milk sample in two test tubes, and add N/10 caustic soda to each, drop by drop, until a faint pink colour appears. To one tube add an equal quantity of water, and to the other the same quantity of neutral 50 per cent solution of glycerin. If boric acid be present, the latter tube will turn white, while in its absence both will remain the same colour.

Another test is to take part of ash and add strong sulphuric acid and some alcohol. Put in a dark place and light the alcohol, when in the presence of boric acid it burns with a green flame.

2. *Formaldehyde.*—(1) Hehner's test. Take 5 c.c. of milk in a test tube and add as much water. Some 90 per cent

commercial sulphuric acid is then run down the side of the test tube, so that it forms a distinct layer at the bottom of the tube. Pure milk, free of formaldehyde, gives a greenish ring, but when formaldehyde is present a violet ring is formed. Pure sulphuric will not give the test, but will do so after the addition of a few drops of ferric chloride. The test, therefore, can be varied thus. To the dilute milk add the ferric chloride, and then some strong sulphuric, when the milk becomes a reddish-purple colour; but carbolic and salicylic acids, if present, would give confusion colours. (Hehner's test reacts with 1 part in 200,000, but fails with milks containing overo ·5 per cent).

(2) Add a drop of carbolic acid to the dilute milk, and repeat Hehner's test, using pure H_2SO_4, when a red ring indicates the presence of formaldehyde.

(3) *Jorissen's Test.*—To 10 c.c. of milk in a tube are added several drops of a 10 per cent aqueous solution of phloroglucinol, the mixture shaken, and a few drops of NaOH or KOH added. Normal milk gives no reaction, but a milk containing as little as 1 part of formalin in 20,000 gives a fleshy-pink coloration.

(4) Distil 100 c.c. of the milk, and use the distillate for these tests.

(*a*). Repeat test number (2) above. Detects 1 in 200,000.

(*b*). *Schiff's Magenta Test.*—Take a very dilute solution of magenta (fuchsin or nitrate of rosanilin), and decolorize with sulphurous acid, adding drop by drop. Add a few c.c. of distillate, and watch for some minutes (or set aside). Traces of formaldehyde bring back the colour slowly. The test is also said to be got in the filtrate of curdled milk.

(*c*). *Tatten-Thomson Test.*—To 20 c.c. of distillate add 5 to 10 drops of a reagent made by adding ammonia to a 2 per cent solution of silver nitrate until the precipitate first formed just dissolves. Set aside in a dark place for twenty-four hours. Darkening almost to blackness is due to formaldehyde, but a slight browning may be disregarded (often seen in silver solutions).

Formalin is a 40 per cent solution of formic aldehyde (CH_2O) in water. Two to three drops keep a pint of milk fresh for 3 to 4 days, and 0·05 per cent preserves milk for

months. In the milk trade a much more dilute solution is used, namely 1 of formalin in 80 of water (2 oz. per gallon) equal to 0·5 per cent of CH_2O. Rideal states that one-fourth of a pint of such a solution in 17 to 18 gallons of milk (= 1 in 126 to 144) keeps the milk fresh for at least three days, and does not give it any taste or smell. This dilution is roughly 1 part of CH_2O in 100,000.

3. *Salicylic Acid.*—Now rarely used in this country, is still largely employed on the Continent. It is best detected by Pellet's method: 100 c.c. of milk sample are diluted with as much distilled water, and heated to 60° C.; 1 c.c. of acetic acid, and some mercuric nitrate are then added. The resulting curd is filtered off and rejected. The filtrate is repeatedly extracted with ether, the various extractions are mixed, a portion is evaporated, the residue dissolved in distilled water, and the solution tested with ferric chloride solution. A violet coloration not discharged by acetic acid, is positive. When present in considerable amount, the direct addition of ferric chloride gives a pale brown colour, and in the filtered whey a violet may be detected.

4. *Benzoates.*—Curdle the milk with acetic acid, extract the whey with chloroform, neutralize carefully after dilution. Add ferric chloride, when benzoates give a buff-coloured precipitate insoluble in acetic acid.

5. *Sulphites.*—

(1) Add dilute phosphoric acid and heat gently; observe odour (SO_2).

(2) Take 20 c.c. in a tube, add Zn and HCl. Place paper soaked in lead acetate solution over mouth of tube and heat latter gently. Darkening indicates presence of sulphites, but a negative result is more reliable.

Hydrogen Peroxide.—First suggested by Budde: milk said to be "Buddeized." To 10 c.c. of milk sample, add 1 c.c. of 1 per cent. ortol solution (freshly made), when in presence of hydroxyl a dull crimson colour is produced unless the milk has been heated above 72° C., when the addition of a little fresh milk is required. Paraphenylene-diamine similarly gives a blue coloration. Schardinger's reagent is decolorized by normal milk, but not by treated milk. In the presence of organic matter hydroxyl splits

up slowly into water and free oxygen, and so after six to eight hours will not be found.

Pasteurized Milk.—By this term is meant milk which has been heated to a temperature sufficient to kill the less resistant pathogenic bacteria without altering the flavour. The temperature used varies from 70° to 85° C. (158° to 185° F.). The higher temperature has been advised by Professor Bang. The one mostly used is 75° C. (167° F.) for half an hour, cooling quickly thereafter. Over 90 per cent of the organisms are killed, but the sporing forms are not destroyed. Souring of pasteurized milk does not take place owing to the destruction of the lactic acid bacilli. It does not keep for more than three days.

Buddeized Milk.—Fifteen c.c. of a 3 per cent solution of hydroxyl are added per litre of milk (1 of H_2O_2 in 2222), and the mixture heated for three hours at 51° C. (123·8° F.). Milk so treated is normal in taste, and keeps fresh for eight days, even in hot weather.

Homogenized Milk.—Under 200 to 400 atmospheres' pressure, milk is forced through very small openings, and the size of the fat globules reduced to 0·001 mm. in diameter. This prevents the fat from rising. Adams' process gives too low an estimate of fat in such milks.

Dried Milk.—By passing a thin layer of milk between two heated rollers, the milk is immediately desiccated and reduced to a fine powder, which merely requires the addition of water to bring it back to the condition of ordinary milk. The temperature of the rollers is 110° C. (230° F.). Such milk may be lacking in the antiscorbutic properties necessary for infants, but appears to possess all the solids of the original milk in a sterile form.

Humanized Cow's Milk.—This is prepared on the large scale by diluting the milk with an equal quantity of pure water, and subjecting the mixture to centrifugalization. This divides it into two equal parts, one of which contains practically all the fat of the original milk, but only half of the other ingredients. The only constituent, therefore, notably deficient, will be the sugar, which is added in the proper proportion. The amount of proteid tends to be

too low, and the mineral salts too high. Paget's Perfected Milk Food is a concentrated humanized milk of this class.

Dirt in Milk.—In Dresden, a standard of not more than 8 mgr. per litre exists. In this country Dr. Houston has suggested (1905) two standards for dirt in milk: (1) Amount of filth that settles on standing to be less than 100 mgr. per litre; (2) This apparent filth is diluted with water and put in centrifuge, when amount of deposit should be less than 50 mgr. per litre. Otherwise expressed, (1) is 10 parts per 100,000, and (2) is 5 parts.

CREAM: CONDENSED MILK: INFANTS' FOODS.

Cream.—The composition is similar to that of milk, but the fat is much higher in amount. No standard is laid down in this country; but in the state of New Hampshire, cream must contain 18 per cent of fat. Cream is at times thickened by the addition of such agents as gelatin, starch paste, saccharate of lime (called viscogen), and condensed milk.

Colouring with annatto and coal-tar dyes is known. Annatto may be detected by adding bicarbonate of sodium, and then immersing a strip of white filter-paper and standing overnight. A brown stain indicates the presence of annatto. Coal-tar dyes of the azo group give a pink colour with diluted mineral acids.

Fat is estimated by the Leffmann-Beam process: 2 grams. of cream are mixed with 12 c.c. of water, and the mixture is poured into the bottle, the remainder of the procedure being as before (page 75). The result is multiplied by 7·77. The Werner-Schmidt and Adams' processes may also be used.

The Departmental Committee on Preservatives advised that only borax and boracic acid be allowed in cream, and in an amount not exceeding 0·25 per cent, expressed as boric acid; and the amount to be notified by a label affixed.

Cream prepared by centrifugalizing methods contains 45 to 65 per cent of fat. Devonshire, Cornish, or clotted cream is prepared by warming milk in pans for several

hours. The cream rises in a more coherent layer (50 to 60 per cent of fat).

Condensed Milk.—Consists of unsweetened milk or sweetened milk or sweetened skimmed milk, concentrated by evaporation, usually to one-third of its volume. The addition of two volumes of water should, therefore, produce a strength equal to the original.

The unsweetened milks are well prepared, keep well, and contain the due proportion of fat.

The sweetened milks form the largest class, and for the most part are good. The dilutions recommended in nearly every case produce a milk very much below the standard of ordinary milk. Such milks usually contain rather more cane sugar than milk solids. For example (a good specimen): Fat 11 per cent, proteins 10 per cent, milk sugar 14 per cent, ash 2·2 per cent, and cane sugar 38 per cent. (Solids not cane sugar, 11 + 10 + 14 = 35 per cent.)

The sweetened skimmed milks, or separated milks, or machine-skimmed milks, are very inferior to the above, containing as little as 0·2 per cent of fat, and generally about only 1 per cent. Such milk is unfit for the sole nourishment of children; and it would be better if such a statement had to be put on the label.

ANALYSIS.—Mix the contents of the tin well, weigh out 10 grm., dissolve in water, and make up the bulk to 100 c.c. Analyse as in the case of ordinary milk, except that for fat the Werner-Schmidt method is inapplicable in sweetened milks, owing to the charring of the cane sugar. In the Adams' process use carbon tetrachloride instead of ether.

Humanized Condensed Milk.—This is a condensed milk with added milk sugar and cream, but no cane sugar. When diluted with water in the proper proportion it is practically identical in quantitative composition with human milk.

Infant Foods.—These are sold as a substitute for mother's milk. As they are in the dried state, their composition should approximate to that of dried mother's milk, which, according to Hutchison, is as follows:—

	Water	Proteid	Fat	Carbo-hydrate	Mineral matter
Dried mother's milk	nil	12·2	26·4	52·4	2·1
,, diluted 1 : 7·5	88·3	1·52	3·30	6·55	0·26
Fresh mother's milk	87·7	1·62	3·14	6·26	0·27
Fresh cow's milk	87·5	3·5	4·0	4·3	0·7

Most of these substitutes are deficient in fat, some are deficient in proteid, and many of them contain unaltered starch. They may be divided into three groups :—

1. Cow's milk desiccated, with additions or alterations. These only require the addition of water. Includes Allenbury (Nos. 1 and 2), Horlick's malted milk, Carnrick's soluble food, Milo food, Manhu infant food, and Maltico. They are all deficient in fat and too rich in carbohydrate. The first three and the last contain no unaltered starch.

2. Cereals, usually wheat, of which the starch has been partly or wholly transformed into dextrins or malt sugar. In Mellin's food, Cheltine maltose food, and Hovis babies' food (No. 1), all the carbohydrate is in a soluble form in the powder, and there is no starch present. In Savory & Moore's food and Allenburys' malted food, the powder consists of wheat flour mixed with malt. When prepared according to the directions, most but not all of the starch is converted into soluble forms. Benger's food is a mixture of wheat flour and pancreatic extract, and in it, too, most but not all of the starch is converted into soluble forms. The proteid is also partially digested. These are all made with milk, or milk and water.

3. Cereals, wheat flour, oats, and barley, with the starch unaltered. To some, sugar has been added. Made with milk, or milk and water. Such are Ridge's food, Neave's food, Frame food, Scott's oat flour, Robinson's patent barley and groats, etc.

Analysis.—Examine for starch (microscope). Total nitrogen, by Kjeldahl × 5·7 = proteid. Phosphates, sugar, starch, dextrin, and cold water extract : see under Cereals and Wheat Flour. Fat, estimate by Soxhlet, or with petroleum ether (see under Coffee).

TABLE OF INFANT FOODS (HUTCHISON).

	Water	Proteid	Fat	Carbo-hydrate	Mineral matter
Dried mother's milk ..	nil	12·2	26·4	52·4	2·1
Allenbury, No. 1	5·7	9·7	20·0	60·85	3·75
,, No. 2	3·9	9·2	15·0	69·1	3·50
Horlick's Malted Milk ..	3·7	13·8	9·0	70·8	2·70
Mellin's Food	6·3	7·9	trace	82·0	3·8
Hovis B. F., No. 1	3·7	7·7	0·20	86·6	1·82
Savory & Moore's ..	4·5	10·3	1·4	83·2	0·6
Benger's Food	8·3	10·2	1·2	79·5	0·8
Frame Food Diet	5·0	13·4	1·2	79·4	1·0
Scott's Oat Flour	5·8	9·7	5·0	78·2	1·3

BUTTER.

Butter is the fat of milk clotted together by shaking or beating at a low temperature. A thin bluish fluid separates, which is called buttermilk. Butter consists of neutral fats mixed with water, a small amount of casein, and traces of salts, and there may be added salt (NaCl). The average composition is :—Fat, 78 to 94 per cent ; water, 8 to 12 per cent ; curd, 1 to 3 per cent ; salt, 0 to 7 per cent. The fats are present as glycerides of certain fatty acids, namely : butyric, caproic, caprylic, capric, myristic, palmitic, stearic, and oleic acids. The first four are soluble in hot water, and are known as the " soluble fatty acids," the rest as the " insoluble fatty acids." Bell's analysis of butter *fat* is : butyric acid, 6·1 per cent ; caproic, caprylic, and capric acids, 2·1 per cent ; myristic, palmitic, and stearic acids, 49·4 per cent ; oleic acid, 36·1 per cent ; glycerol (calculated), 12·5 per cent. These are present chiefly as tributyrin, tripalmitin, and triolein (: $C_3H_5(O{\cdot}C_4H_7O)_3$: $C_3H_5(O.C_{16}H_{31}O)_3$: $C_3H_5(O.C_{18}H_{33}O)_3$:).

ADULTERATIONS.—The important ones are : Addition of water in excess, the substitution of foreign fats, salt in excess, starch, boric acid.

EXAMINATION OF BUTTER FOR WATER, SALT, CURD, FAT, AND BORIC ACID.

The fat is further examined by the Valenta test, for specific gravity, for volatile fatty acids, for fixed fatty

acids, refractive index, iodine absorption, and foreign oils. Butter may also be examined for starch, annatto, or other colourings, benzoic and salicylic acids, and with the polariscope.

Water. — Weigh 2 grm. of butter into a weighed capsule, and dry in the water-oven to constant weight. The drying may be expedited by adding 1·5 c.c. of absolute alcohol after melting the butter in the water-oven. The amount of water should not exceed 16 per cent (" Butter Regulations," 1902). The same standard now applies to margarine (" Butter and Margarine Act," 1907).

Salt.— The residue, after burning off the fat and curd from the dried butter, may for all practical purposes be taken as salt. Or, melt 2 grm. of butter in some hot water, make up the bulk to 200 c.c. with hot water, and put into a separating funnel. Allow fat to separate to top, take 20 c.c. of the clear liquid, titrate with standard silver nitrate solution, and calculate result in terms of NaCl. It rarely exceeds 10 per cent. Often high in Irish butter.

Curd or Casein.—Varies from 0·3 to 4 per cent. It is usually calculated by difference.

Fat.—The amount of fat may be estimated in a Soxhlet apparatus, using a prepared thimble made of filter-paper; or by washing the dried solids with ether. For the other processes the fat is separated by filling a Nessler glass with butter, and melting on the water-bath. The fat gradually rises to the top, and the water and curd sink, so that three layers are noted; fat, curd, and water. The fat is now filtered through a dry filter-paper placed in a funnel surrounded with hot water. Care is taken not to pour any of the wet curd or water on to the paper. The water-free fat is then used for the various processes.

1. *Reichert-Wollny Process* for the estimation of the volatile fatty acids.

Five grms. of the liquid fat are introduced into a 300 c.c. boiling-flask; 2 c.c. of 50 per cent NaOH solution (free from CO_2), and 10 c.c. of 92 per cent alcohol, are added. The mixture is heated for fifteen minutes (under a reflux condenser) on the water-bath kept at 100° C. The fats are thus saponified. Remove the condenser, and continue the

heating for half an hour, or until the soap is dry. This is to get rid of the alcohol. Freshly boiled aq. dest. is added cautiously, while still hot, to the amount of 100 c.c., and the flask carefully heated until the soap is dissolved. Thereafter, 40 c.c. of N/1 H_2SO_4 are added, and 3 to 4 pieces of pumice stone, and the flask is rapidly connected to a condenser by a tube having a bulb. Heat at first with a small flame to melt the insoluble fatty acids (liberated with the soluble fatty acids by the action of the sulphuric acid), but do not boil. When fusion is complete, increase the heat, and distil over 110 c.c. in thirty minutes. Shake the distillate, filter 100 c.c., add 0·5 c.c. of 1 per cent phenolphthalein solution, and titrate with N/10 soda or baryta. The number of c.c. used $\times$ 1·1 is called the Reichert-Wollny number, and for genuine butter varies from 24 to 32. A blank experiment, using the reagents alone, supplies a correction for any acidity due to these.

This is an official process, recognized by the Government Laboratory and the Society of Public Analysts. Sundry details are given in the official description, such as the length of neck of flask, size of tubes and condenser, etc., all to secure uniformity of method and apparatus, and so give comparable results. When the Reichert-Wollny figure has been got, if it is 24 or above, the sample is looked upon as genuine, and as 1 c.c. N/10 soda = 0·0088 grm., or 8·8 mgr. of butyric acid, the percentage of soluble volatile fatty acids returned as butyric acid is easily calculated. With the Reichert-Wollny varying from 24 to 32, the percentage of acid will vary from 4·2 per cent to 5·6 per cent.

Pure Margarine Fats give a Reichert-Wollny figure of 0·2 to 1 per cent. As a maximum of 10 per cent of butterfat is allowed to be added for flavouring purposes, the maximum Reichert-Wollny figure for a saleable margarine will be 1 + 10 per cent of 32 (= 3·2), or 4·2. The figure usually taken is 3. If a butter gives a Reichert-Wollny of say y, let x stand for the percentage of margarine, then $3 \times x + 24\ (100-x) = y \times 100$; or $x = (2400 - 100y) \div 21$.

Unfortunately for the value of this process, vegetable fats which give a moderate Reichert-Wollny figure are now being used for food. Thus coco-nut oil has a Reichert-

Wollny of 7, palm-nut fat of 5, and certain fish oils are said to give values above 40. In the case of coco-nut oil, the figure is due to the presence of the glyceride of caprylic acid, with a little caproic, but no butyric glyceride. To meet this difficulty, several tests have been devised. Here we describe that of Polenské, which is the most generally adopted one.

Polenské Method.—Is based on a determination of the insoluble volatile fatty acids, which are distilled over in the Reichert-Wollny process. Certain arbitrary conditions must be observed to get results which will be comparable with those of Polenské and others.

Process.—Five grm. of the fat, 20 grm. of glycerol, and 2 c.c. of 50 per cent NaOH (an alcoholic solution of soda *must not* be used) are taken in a 300 c.c. boiling-flask, and heated over a naked flame, with constant shaking, until a clear solution is obtained. The soap formed is dissolved carefully in 90 c.c. of hot water; 50 c.c. of N/1 sulphuric acid are added; and 0·1 grm. of pumice, which has been powdered and then sifted through muslin. The flask is quickly connected to the top of an upright Liebig condenser, by means of a glass tube with a bulb just above the cork, and the tube thereafter bent, first at an obtuse, and then at an acute, angle. Distillation is commenced, and the flame so regulated that 110 c.c. are distilled over in nineteen to twenty-one minutes. When 110 c.c. have been received into a small flask, the latter is replaced by a 25 c.c. cylinder, and the flame withdrawn. The cylinder receives the drainings. The flask containing the 110 c.c. of distillate is, without shaking the contents, put into a bath of water at 10° C. for ten minutes, the level of the water outside being just above that of the distillate inside the flask. (At this stage, it may be noted that the insoluble fatty acids are almost invariably opaque and white in the case of butter, while 10 per cent or more of coco-nut oil gives clear oily globules.) The distillate flask is now shaken, and the contents are filtered. The Reichert figure may be obtained on 100 c.c. of the filtrate. The filter-paper is kept; and the distillate flask, the condenser, and the cylinder are washed out with 18 c.c. of distilled water, which is then poured on to the filter-paper. This paper

now contains all the insoluble volatile fatty acids which have distilled over. These are dissolved in alcohol, and the solution is titrated with N/10 baryta + phth. The number of c.c. required is called the "new butter value" of the fat. This varies with the Reichert figure, being 1·3 where the Reichert is 20, and 3·0 where the Reichert is 30. An increase of 0·1 in the new butter value corresponds to an addition of 1 per cent of coco-nut oil. The mode of calculation is thus: The new butter value of a pure butter having the same Reichert figure is subtracted from the new butter value of the sample. The difference multiplied by 10 gives the percentage of coco-nut oil present. Ten per cent and under is not detectable with certainty. (See article on "The Estimation of Coco-nut Oil in Butter-fat," by F. W. Harris, F.I.C., in the *Analyst*, November, 1906.)

Insoluble or Fixed Fatty Acids.—About 5 grm. of the fat are taken in a flat porcelain dish or in a flask, melted on the water-bath, and 50 c.c. of methylated spirit or absolute alcohol added. A clear yellow solution is formed. Now add 2 grm. of NaOH or KOH, and continue the heating, stirring all the while. The fats are saponified. In five minutes add a few drops of water; if turbidity ensues, all the fat has not been saponified; continue the heating. Repeat until no turbidity ensues. (If too much water is added, some of the fat may be precipitated from solution. In that case add more alcohol.) Continue to heat until all the alcohol is evaporated and a jelly of soap remains. Then add water, almost filling the dish, and in this the soap dissolves. Dilute HCl is now added until strongly acid, and the fatty acids are thus liberated. Heat for half an hour on the water-bath, filter through a weighed filter-paper (5 inches diameter) placed in a hot-water jacket, washing all the fatty acids from the dish with repeated amounts of boiling water, until the filtrate is no longer acid. The insoluble fatty acids remain on the filter-paper. They are solidified by putting cold water in the jacket. The paper is then carefully removed, placed in a small weighed beaker, and dried for two hours, and then the beaker and contents are weighed. The percentage of insoluble fatty acids in the butter fat is then calculated,

and ranges from 86·3 to 88·5, on average about 87·3. All other animal fats give an average of 95·3.

Valenta Test.—Depends on the intermiscibility of butter fat and strong acetic acid at a low temperature, whereas animal and vegetable fats (except coco-nut oil) do not mix until a much higher temperature is reached. Glacial acetic acid (99 per cent) is used. Take a test tube and add 3 c.c. of the fat and 3 c.c. of the acid. Immerse it in hot water to heat the contents, which are stirred all the while by a thermometer. If the sample is pure butter fat, it will have cleared at 40° C., and it is then allowed to cool, still stirring, and the temperature noted on the first appearance of turbidity. For butter fat this should be from 32° to 36° C. Margarine fat will not clear under 75° C. An abnormally low Valenta figure suggests the presence of coco-nut oil.

Specific Gravity of Butter Fat.—Is now seldom taken at ordinary temperatures, it being more convenient to take it with the fat in a molten state, at 100° F. The fat at 110° F. is poured into an ordinary specific-gravity bottle, which is then placed in water at 100° F. The stopper is pushed home, and the bottle dried, cooled, and weighed. The weight of the same bulk of water at 100° F. is similarly ascertained, and the specific gravity calculated. Pure butter fat ranges from 910·7 to 913·5, but mostly between 911 and 913. Margarine fat ranges from 901·5 to 906, at 100° F. The specific gravity is also taken at 100° C. by the Sprengel tube or the Westphal balance, and ranges thus: Margarine, 856 to 860; butter, 865·3 to 866·8; coco-nut oil, 868 to 872—compared with water at 15·5° C.

Refractive Index.—Is determined on a special instrument. At 35° C. it varies for butter from 44° to 49°, generally 46°. Margarine gives about 54°, and coco-nut oil about 43°. Its use is less valuable now than formerly.

Boric Acid.—Is detected in the ash, as in the case of milk. In order to prevent loss when ashing, a few drops of milk-of-lime solution should first be added. To estimate, take 25 grm. of butter in a beaker, add 25 c.c. of a solution of lactose (6 per cent) and sulphuric acid (4 c.c. of N/1 per cent). Melt in water-oven, mix by stirring, allow to settle, and pipette off 20 c.c. of the aqueous portion. Titrate at 100° C. plus phth. with N/2 NaOH, until a faint

pink appears. Then add 12 c.c. of glycerol, when boric acid is liberated and pink disappears. Titrate again; note number of c.c. used, and the difference × 0·0368 gives amount of boric acid in 20 c.c. This × (100 + percentage of water in butter) ÷ 20 = percentage.

Saponification Equivalent of Koettstorfer for butter fat varies from 242 to 253, and for margarine fat the mean figure is about 284. The saponification of oils by alkalies is a definite reaction, and may be represented by the following general equation where F stands for the radicle of the fatty acid:—$C_3H_5(OF)_3 + 3KOH = C_3H_5(OH)_3 + 3KOF$. Therefore, if we know exactly the amount of alkali necessary to saponify the oil under examination, we can to some extent determine the nature of the glycerides present. The amount of alkali required varies with the composition of the fatty acids; the lower the molecular weight, the higher will be the amount of soda or potash needed to saponify. This amount may be stated in three ways: (1) As a percentage, that is, the number of grammes of alkali absorbed per 100 grm. of fat; (2) The number of milligrammes of alkali absorbed per 1 grm. of fat. This is called the saponification value, and the figure for it is ten times the percentage, since it is parts per thousand; and (3) As the number of grammes of fat which would be saponified by 1 litre of normal alkali; that is, in the case of potash, by 56 grm. of alkali. This is called the saponification equivalent, and may be got by dividing the percentage of alkali absorbed into 5600, or if the exact atomic weights are used, with oxygen = 16 as the basis, into 5610. The following table from Moor & Partridge summarizes these facts, in regard to the chief fats:—

Glyceride	Formula	Mol. Wt.	KOH abs. %	Sn Value	Sn Equiv.
Butyrin	$C_3H_5(O.C_4H_7O)_3$	302	55·7	557	100·67
Laurin ..	$C_3H_5(O.C_{12}H_{23}O)_3$	638	26·4	264	212·67
Palmitin	$C_3H_5(O.C_{16}H_{31}O)_3$	806	20·9	209	268·67
Stearin	$C_3H_5(O.C_{18}H_{35}O)_3$	890	18·9	189	296·67
Olein ..	$C_3H_5(O.C_{18}H_{33}O)_3$	884	19·0	190	294·67
Pure butter fat	composite	—	22·15 to 23·3	221 to 233	254 to 240·7

PROCESS. — Weigh 2 grm. of sample into a 200 c.c. flask, add 25 c.c., approximately, N/2 (seminormal) alcoholic solution of KOH. A like amount of KOH solution is run into an empty flask for a blank experiment. The flasks are fitted with corks carrying vertical tubes 4 ft. long to act as condensers. Both flasks are then heated on the water-bath for not less than thirty minutes, with frequent agitation. Thereafter a few drops of phth. are added to each flask, and both are titrated with exactly N/2 HCl solution, 1 c.c. of which = 0·02805 grm. KOH; therefore the difference between the two titrations, multiplied by this factor, gives the amount of KOH taken up by the oil, and from this the percentage is easily calculated, and the Sn value and the Sn equivalent. The Sn value shows the number of milligrammes of KOH required to saponify 1 grm. of the oil, since 55·7 per cent equals 557 per 1000, or 557 mgr. per 1 grm.

Iodine absorption of butter fat ranges from 23 to 38 per cent, and for margarine from 40 to 55 per cent, but is not of much value in determining the amount of foreign fat in butter. The fats of the oleic series readily unite with a definite quantity of I, Br, or Cl, the others being indifferent.

Sesame Oil.—To 10 c.c. of fat sample add 10 c.c. strong HCl containing 0·1 grm. of cane sugar. Shake thoroughly and allow to stand, when in the presence of even 2 per cent of sesame oil, the aqueous liquid becomes crimson coloured.

Cotton-seed Oil.—Mix equal volumes of the fat and a saturated solution of Pb acetate, add AmOH and stir quickly. On standing, the surface layer turns an orange-red colour.

Starch.—Melt sample in a small beaker or tube, pour off fat, and add KI solution to water and curd. Normal butter gives a reddish coloration only, while a very small trace of starch will give a blue. Examine curd microscopically.

Polariscope.—Pure butter (using gaslight) on rotation, so that the two Nicol prisms are at right angles, gives the whole field equally dark. If 20 per cent or over of margarine is present, it is impossible to darken the whole field, no matter how the prisms be placed, but a cloudy

appearance is got. No selenite plate should be used, and the butter should not have been melted.

Annatto and other Colouring Matters.—If the colouring matter of a butter can be extracted with alcohol, foreign colours are present, as the natural colouring matter is not soluble in alcohol. Annatto may be detected by extraction with methyl alcohol and carbon disulphide. The latter dissolves the fat and sinks, the alcohol dissolves the colour and floats. Separate alcohol and evaporate: yellow stain, which turns blue-green on adding a drop of strong sulphuric. Turmeric, saffron, saffronette, marigold, and the azo dyes are also used.

Butter v. Margarine.—

1. Digest fat of sample with alcoholic solution NaOH, when butter gives pleasant odour of butyric ether or pineapples, and pure margarine gives a tallow smell.

2. Burn a small piece of sample on platinum foil and extinguish flame: butter, rather pleasant smell; margarine, tallow smell.

3. Melting-point of fat. Take a platinum loopful, dip in water alongside thermometer, and heat until fat just translucent: butter fat, 30° to 35° C.; margarine fat, rarely above 28° C; coco-nut oil, 20°–26° C.

4. Valenta test.

CHEESE.

Cheese is made from milk by the action of rennet, and consists of coagulated casein with varying proportions of water, fat, and salts. It may be made from whole milk (Cheddar, Cheshire, Gloucester, and some American cheeses), from skimmed milk (Dutch, Parmesan, Suffolk, and Somerset cheeses), from whole milk and cream (Stilton). Parmesan is made from goat's milk (partly skimmed); Roquefort from ewe's milk (partly skimmed); and Gorgonzola by adding in the process of manufacture, powdered bread crusts on which moulds have been allowed to grow. Cream cheese consists of the fresh curd which has been moderately pressed, and is eaten before being allowed to ripen. "Ripening" of cheese is supposed to be a decomposition whereby the casein undergoes a fatty change with the

formation of lime salts of the fatty acids, and a soluble compound of phosphoric acid with the casein. Average composition of Cheddar cheese per cent: water 33·9, ash 4·2, fat 30, nitrogen 4·3, casein 27·3. Water is very high in cream cheeses, which are also very deficient in fat, in nitrogen, and in casein. The Reichert figure for the fat of cheese is, from its similar origin, the same as that for butter fat. There is no standard for cheese in this country, and a cream cheese can be purchased containing less fat than a milk one, unless double cream be asked for. A good standard would be not less than 30 per cent of true butter fat and no starch or other extraneous matter: for cream cheese, a 40 per cent standard. U.S.A. standard: 50 per cent milk fat in dried solids.

Analysis. — *Moisture*: dry 2 to 5 grm. of sample cut in thin slices, to constant weight in water-oven at 105° C. (absolute alcohol hurries drying).

Ash.—Ignite dried cheese at as low a temperature as possible.

Nitrogen.—Treat 1 grm. of sample by the Kjeldahl process. $N \times 6{\cdot}38 =$ proteins.

Fat.—Take dried cheese, put into filter-paper thimble, and extract in Soxhlet for two hours; or grind 50 grm. of sample in a mortar with sand, put mixture in tall stoppered cylinder and extract with four portions of ether, using in all about 500 c.c. Make to a definite volume, take aliquot part, evaporate to dryness, and weigh residue. Or by Leffman-Beam.

Reichert-Wollny Process. — Extract fat by melting in water-oven.

Poisonous Metals in Rind.—Lead, copper, and arsenic have been found in rind.

Salt 5 to 6 per cent; tyro-toxicon, acarus domesticus, moulds.

CEREALS: WHEAT-FLOUR: BREAD.

The cereals consist of the edible grains, such as wheat, oats, barley, rye, maize, rice, millet, and buckwheat. From these, various products are got which are largely used for food; as wheat flour, oatmeal, barley meal, pearl barley, rye flour, corn flour (from maize or Indian corn),

buckwheat flour, flaked rice, ground rice, rice flour, hominy (split maize). The proteid varies in the different cereals. Wheat flour has the largest proportion of gluten, and therefore makes the best bread. Cane sugar is found in all cereals.

The analysis of these consists in estimating the moisture, ash, fat, phosphoric acid, total nitrogen, and proteids; sugar, starch and dextrin, cellulose, extract and acidity, and true albuminoids.

Phosphoric Acid.—Dissolve ash in diluted HCl, boil and filter. Precipitate lime with $Am_2C_2O_4$, filter after heating on water-bath for one hour, and precipitate phosphoric acid from filtrate with AmCl, AmOH, and $MgCl_2$. Stand for twelve hours, filter, dry, ignite, and weigh as magnesium pyrophosphate $Mg_2P_2O_7$.

Sugar, Starch and Dextrin.—Take 5 grm., and boil with 250 c.c. aqua and 50 c.c. N/1 HCl under an invert condenser for six hours. If hydrolysis of starch is complete, a drop of the solution will give no blue colour with iodine solution. Neutralize with 50 c.c. N/1 NaOH, filter into a litre flask, and with washings make up to the mark. Estimate with Fehling's solution, either volumetrically or gravimetrically. The Pavy-Fehling method is more reliable than the ordinary way. Pavy's solution is made from Fehling's thus: 120 c.c. of Fehling are taken, and 300 c.c. strong AmOH (sp. gr. 0·880) and 400 c.c. NaOH solution (12 per cent) are added, and the volume is made up to 1 litre; 100 c.c. of this solution has the same oxidizing effect on glucose as 10 c.c. of ordinary Fehling solution, that is, 100 c.c = 0·05 grm. glucose. The cuprous oxide is not precipitated in the presence of ammonia, and the titration is continued until the solution is colourless. In working, take a 300 c.c. boiling-flask fitted with a two-holed stopper. Through one hole goes the nose of the burette containing the dilute sugar solution, and through the other a tube to lead away the ammonia; 50 c.c. of the Pavy solution are taken in the flask and brought to the boil. The sugar solution is then run in slowly from the burette till the blue colour is discharged. To convert glucose to starch × 0·9.

Adulterations.—Talc, gypsum, French chalk; substitution; blending; bleaching with NO_2 (also O_3, SO_2, SO_3,

Cl, etc.); addition of "improvers" (acid-phosphates of K, Mg and Ca; H_3PO_4; diastase, etc.).

Animal and Vegetable Parasites.—The corn weevil (*Calandra granaria*), the ear cockle (*Vibrio tritici*), ergot (*Claviceps purpurea*), smut (*Uredo segetum*), bunt (*Uredo fœtida* or *Tilletia caries*), rust (*Puccinia graminis*), *Mucor*, *Aspergillus* and *Penicillium*; *Acarus farinæ*.

Wheat Flour.—Examine microscopically, physically, and practically (by making bread). Should be white in colour, silky to touch, free from smell or odour, and not yellow or gritty. Moisture: dry 5 grm. in water-oven (should not exceed 18 per cent). Ash: ignite (not more than 1 per cent). Total proteid, from Kjeldahl N × 6·25 (not less than 8 per cent). Cold-water extract, (10 per cent) (should not exceed 4·7 per cent). Acidity, none.

Glutin.—The proteins consist chiefly of a globulin and an albumose which, when acted on by water, unite to form glutin (from gliadin and glutinin). Estimate thus: weigh 10 grm. of flour and put into a porcelain dish. Add pure water from a burette, stirring the while with a glass rod so as to make a well-mixed dough. Be careful to add the water slowly, using about 4 c.c. When made, let the dough stand for fifteen minutes, then pour a little water on and stir about with the rod to let the water wash out the starch. Repeat until washings are free from starch, as tested by iodine. (After a time the glutin becomes so coherent that it can be worked with the fingers.) Dry, and weigh. If time does not permit of drying, weigh moist glutin and divide weight by 2·9. Dry glutin ranges from 8 per cent to 12 per cent (reject if below 8 per cent).

Cold Water Extract.—Weigh 20 grm. into a dry flask, add 200 c.c. aq. dest., and shake for five minutes. Allow to stand twenty-five minutes. Decant off clear liquid and filter through a dry paper. Evaporate 50 c.c. in water-oven to constant weight, and this gives total soluble extract, consisting of sugar, gum, dextrin, soluble albumin, and phosphate of potash. Ignite, and weigh ash, which is phosphate of potash. The acidity (if any) can be determined by using 100 c.c. of the cold-water extract.

Mineral Matter.—Shake 100 grm. of the flour with 200 c.c. of chloroform in a separator. Allow to stand, when

the flour floats to the top and any mineral matter sinks to the bottom. Draw off, dry, weigh, and examine microscopically. Any soluble matter can be dissolved and tested for alum, while the insoluble can be tested for $CaSO_4$, $BaSO_4$, and $CaCO_3$. To test for alum: add NaOH, white precipitate solution in excess of NaOH, but re-precipitated on adding AmCl. The sulphuric acid present in alum is precipitated by barium chloride; precipitate insoluble in HCl.

Bleaching of Flour.—Chiefly with nitrogen peroxide (NO_2), produced by the action of HNO_3 on $FeSO_4$, or combustion of NH_3 in air, or by electric sparks. The NO_2 with the moisture in the flour forms nitric and nitrous acids, the latter of which is readily demonstrated in a 10 per cent watery extract by the Ilosvay method (page 48). ($\frac{1}{2}$ to 3 parts as N per million). (See Hamill and Monier-Williams, *Journal of Hygiene*, vol. xi, No. 2, July, 1911).

Alum.—Make 10 grm. of flour into a paste with 10 c.c. of water. Add 1 c.c. of each of two solutions, namely, (1) Fresh tincture of logwood, and (2) Saturated solution of ammonium carbonate. Mix well with a glass rod. If the flour is free from alum, the mixture is a pinkish colour fading to a dirty brown. If alum is present, the pink changes to a lavender tint or even to a blue. In such a case the sample should be put in the water-oven for two hours to make sure that the colour is permanent.

Adulterations.—Those mentioned under cereals; water; "improvers"; also foreign seeds. One of these, the purple cow-wheat (*Melampyrum arvense*), is not injurious, but gives bread from the flour a smoky-violet or bluish-violet tint. Two others, the corn-cockle (*Agrostemma githago*) and darnel seeds (*Lolium temulentum*) possess toxic powers. The corn-cockle is detected by its appearance as large black seeds, while the darnel seeds are detected by the repulsive taste they give to the flour and the greenish colour produced on the addition of alcohol (pure flour gives a straw-coloured solution with a more or less agreeable taste). Darnel seeds do not affect the colour of the bread, but produce in those eating it, vertigo, hallucinations, delirium, and narcosis.

Bread is made by kneading wheat flour with water, the coherence of the dough being due to the moist glutin

formed. The porosity of bread, which is essential to its easy digestion, is produced by enclosing in the dough minute bubbles of carbonic acid gas. This is accomplished in one of three ways, viz. :—

1. By the use of yeast which sets up fermentation of a small portion of the starch, forming alcohol and carbonic acid gas.

2. By the use of baking-powders containing an acid salt and a bicarbonate, which on being moistened give off carbonic acid gas.

3. By kneading the dough with water charged with carbonic acid gas under pressure (Dauglish's system), so-called " aerated bread."

The " germ," an important constituent of the grain, and rich in fat and proteid, is removed in modern milling processes, and its absence makes the flour whiter.

Analysis.—In sampling, take crumb and crust.

Moisture.—Take 10 to 50 grm. and dry to constant weight in water-oven (should not exceed 40 per cent).

Ash. — Take 10 grm. of bread, moisten with strong solution of ammonium nitrate, dry, and carefully ignite. If ash is still very carbonaceous, repeat, and a clean ash will usually be obtained (should not exceed 2 per cent, and part insoluble in HCl should not exceed 0·2 per cent).

Alum.—Take 10 grm. of bread free from crust, in a dish. Pour over them 100 c.c. of water containing 5 c.c. each of the logwood and ammonium carbonate solutions described above. Stand for five minutes, drain off excess of liquid, and dry in dish at 100°C. Violet or blue tint if alum is present ; if not, a brown or buff. Detects 7 grains in a 4-lb. loaf. Test unreliable when bread is sour.

Acidity.—Soak 10 grm. of bread in 100 c.c. of aq. dest. for one hour, macerate, and then titrate with N/10 NaOH and return result in terms of acetic acid : 1 c.c. N/10 NaOH = 0·006 grm. glacial acetic (part of the acidity is due to lactic acid). Some extract with hot water, or digest on water-bath and filter a portion. Use phenolphthalein as indicator. Should be less than 0·115 per cent or 8 gr. per lb. Alum test is unreliable when the bread is sour.

Copper Sulphate has been detected in bread, probably due to the corn having been steeped in a copper solution to

prevent a growth of fungus. It is detected by soaking in a solution of K_4FeCy_6, acidulated with acetic, when a purplish or reddish-brown coloration indicates the presence of copper.

Ergot in Flour. — (1) Microscope; (2) Place some flour in a test tube, and a few c.c. 10 per cent KOH, and warm gently: smell of propylamine (like warm urinous napkin); (3) Shake up 2 grm. of flour with 10 c.c. of alcohol (70 per cent) containing 1/2 c.c. HCl. On standing, a red colour slowly forms.

Baking-Powders.—Definition: "A baking-powder may be defined as a salt or mixture of salts with or without a diluent such as starch, which evolves carbon dioxide when moistened, and on heating" (Moor). The Sale of Food and Drugs Act, 1899, enables authorities to take cognizance of the constitution of a baking-powder as an article which "ordinarily enters into or is used in the composition or preparation of human food."

The best mixture is one containing the proper chemical proportions of good cream of tartar ($KHC_4H_4O_6$) and baking soda ($NaHCO_3$), with a filling of about 20 per cent of pure starch. The chemical action forms rochelle salt ($KNaC_4H_4O_6$). This keeps well, reacts slowly and in a definite way; and the rochelle salt formed has a very weak retarding action on ferments, and so does not disturb the digestive processes.

Alum is objectionable from inhibiting the digestive ferments, precipitating the phosphoric acid and phosphates, and liberating sulphate of soda, which is purgative. Bisulphate of potash also liberates the last-named salt.

Tartaric acid and acid phosphate of lime liberate the CO_2 too quickly.

Ammonium carbonate and superphosphate of lime (free from sulphate) are not objectionable.

Available carbon dioxide should be at least 8 per cent by weight.

$$KHC_4H_4O_6 + NaHCO_3 = KNaC_4H_4O_6 + H_2O + CO_2.$$

Numerous prosecutions for the presence of alum have been made.

STARCHES.

Take a clean slide, and put on it a small drop of water. With a platinum loop, transfer a portion of the starch

powder to the water-drop, and mix. Put on a cover-glass, remove excess of water, and examine with a microscope, using first a $\frac{2}{3}$ inch objective and then a $\frac{1}{6}$ inch. Observe carefully the following points: (1) *Shape*; (2) *Size*; (3) *Hilum* (presence or absence of); (4) *Striations* (presence or absence of). Iodine solution shows up the striæ. (1–1000).

Starches fall into five groups:—

1. Contour *round*—the wheat group—wheat, barley, and rye. These all have grains large and minute (rye has also intermediary sizes, as also has barley to a less extent), and have no very apparent hilum or striæ.

2. Contour *oval*—pea, bean, and lentil. These all have grains of large size with a longitudinal hilum, with very faint striæ.

3. Contour *ovoid*—potato and arrowroot. Large granules, with a distinct hilum and well-marked concentric rings or striæ. The hilum is at the smaller end (oyster shape) in potato, and at the other end in arrowroot, the granules of which are smaller (except tous-les-mois variety).

4. Contour *semi-faceted*—sago and tapioca. These have a hilum and ill-defined rings. The sago grains are the larger.

5. Contour *faceted*—maize, oats, and rice. The rice granules are the smallest, the maize the largest, and they have a stellate hilum.

The polariscope gives valuable aid in differentiation.

CARBOHYDRATES.

Carbohydrates are compounds of carbon, hydrogen, and oxygen, the two latter being present in the same proportion in which they combine to form water. The class is a large one, and its components are widely distributed in Nature. The carbohydrates may be arranged into three groups, viz.:

1. **Monosaccharids** or **Glucoses,** including dextrose (or grape sugar), lævulose (or fruit sugar), and galactose.

2. **Disaccharids** or **Sugars,** including saccharose (sucrose or cane sugar), lactose (or milk sugar), and maltose (or malt sugar).

3. **Polysaccharids** or **Starches,** including starch, dextrin, gum and cellulose, inulin, and glycogen.

From allied groups certain substances used in bacteriology are derived, and are enumerated here for the

sake of completeness. These are raffinose or melitose (tri-saccharid), arabinose (pentose), and mannite, dulcite, and sorbite (hexahydric alcohols, hence better called mannitol, dulcitol, and sorbitol).

The carbohydrates are distinguished from one another not only by their chemical constitution but by their relative sweetness, their source, their power of crystallization, their oxidation products, their power of reducing Fehling's solution, their action on polarized light, their reaction to the yeast and other ferments, and other tests.

Commercially, sugar is obtained from the sugar cane (West Indies), beetroot (Western Europe), the maple tree (Canada and U.S.A.), various palm trees, such as date palm (India), sago palm (Ceylon), Palmyra palm (South America and Australia), a grass plant called sorghum (U.S.A.), and maize or Indian corn (Mexico). These plants all yield the **Sugar** known under the various names of *sucrose, saccharose, cane sugar, beet sugar*, etc. Sugar is soluble in $\frac{1}{3}$ part of its weight of water at 15° C., in $\frac{1}{2}$ part in cold water, and in all proportions in boiling water. It dissolves with difficulty in alcohol. Its sp. gr. is 1·606. Its aqueous solution is dextro-rotatory, its specific rotatory power at 20° C. for sodium light being $A_d = +66{\cdot}5$. It melts at 160° C., and on cooling forms an amorphous glassy mass known as "barley-sugar," which in time loses its transparency and becomes crystalline. At 190° to 200° C. it changes to a brown non-crystallizable mass called caramel, which is used for colouring liquids. It does not reduce Fehling's solution nor ferment with yeast. When boiled with dilute acids it is decomposed into dextrose and lævulose, both of which reduce Fehling and ferment with yeast, but rotate the plane of polarized light in opposite directions. The lævulose has the greater rotating power, with the result that the solution has now a lævo-rotatory action, and is hence called "**Invert Sugar.**" Cane sugar forms compounds with metals, metallic oxides, and salts, and these are named saccharates or sucrates, such as sodium sucrate, chloride of sodium sucrate, and lime sucrate. Cane sugar crystallizes in large monoclinic prisms. Boiled with nitric acid it oxidizes to oxalic and saccharic acids and inactive tartaric acid.

Glucose and Lævulose are found in equal proportions in all sweet fruits. It is likely that cane sugar first forms in the plants, and that a ferment at once breaks it up into grape sugar and fruit sugar, the mixture forming invert sugar. The saccharine substance which bees collect and form into honey is a mixture of these two sugars, forming "invert sugar," and hence pure honey is lævo-rotatory.

Glucose, or *dextrose*, or *grape sugar*, or *blood sugar*, reduces Fehling's solution, ferments with yeast, is dextro-rotatory (+ 52·7), and forms six-sided crystals.

Lævulose, or *fructose*, or *fruit sugar*, closely resembles glucose, but rotates polarized light to the left (— 95·5).

Lactose, or *sugar of milk*, occurs in the milk of mammals. It crystallizes with one molecule of water in rhombic prisms, has a faintly sweet taste, is sparingly soluble in water (1 in 6 of cold and 1 in 2·5 of hot) and is insoluble in alcohol. It reduces Fehling's solution, ferments readily with lactic ferment, but not with yeast (or very slowly). With dilute acids it yields galactose and dextrose. Lactose is dextro-rotatory (+ 52·7) for A_d.

Galactose is got from lactose by heating with dilute sulphuric acid. It reduces Fehling, ferments with yeast, and is dextro-rotatory (+ 83·3 for A_j).

Maltose is a variety of sugar formed together with dextrin by the action of malt diastase upon starch. It can also be produced by the action of dilute sulphuric acid on starch. It ferments with yeast, reduces Fehling, is soluble in alcohol, is dextro-rotatory, and crystallizes in white needles + 1 molecule H_2O. In the sugaring of starch by diastase at 60° C, two-thirds maltose and one-third of dextrin are produced thus :—

$$\underset{\text{Starch}}{3C_6H_{10}O_5} + H_2O = \underset{\text{Maltose}}{C_{12}H_{22}O_{11}} + \underset{\text{Dextrin}}{C_6H_{10}O_5}$$

Starch, or amylum, is found in the cells of many plants in the form of granules of varying size (0·002 m.m. to 0·185 mm.). These are insoluble in cold water and in alcohol. When heated with water the granules swell up at 50° C., burst, partially dissolve, and form starch paste, which turns the plane of polarization to the right. The soluble portion is called granulose; the insoluble, starch

cellulose. Alcohol precipitates a white powder (soluble starch) from the aqueous solution. The blue coloration produced by iodine is characteristic of starch. Heat discharges the coloration, but it reappears on cooling. By dilute acids, starch is converted into dextrin, maltose, and dextrose; by diastase, into maltose and dextrin, as shown above; and by the saliva, into dextrin and maltose.

Dextrin is really a name which denotes several isomeric substances usually found in mixture and resembling each other very closely. They form gummy amorphous masses whose aqueous solutions are dextro-rotatory, hence the name dextrin. They do not reduce Fehling's solution and are not directly fermented by yeast, but in the presence of diastase, the dextrin is changed to dextrose and then fermented. They give a red colour with iodine, and are much used as substitutes for natural gums.

Inulin is a polysaccharid found in the roots of dahlia, in chicory, and in many compositæ. It is a white powder, soluble in boiling water to a clear solution. It gives a yellow colour with iodine. When boiled with water it is completely changed to fruit sugar (lævulose).

Glycogen occurs in the liver of mammals. It is a mealy powder, soluble in hot H_2O, gives a reddish-brown colour with iodine; ferments change it to maltose, and dilute acids to dextrose. It is precipitated from solution by alcohol.

Raffinose, or *Melitose*, is a trisaccharid found in Australian manna, in the flour of cotton seeds, in small amounts in sugar beets, and is crystallized from the molasses. It is very soluble, is dextro-rotatory, is easily fermented with yeast, but does not reduce Fehling's solution.

Arabinose was formerly thought to be a glucose, but is really a pentose; that is, contains but five carbon atoms. It is made from gum arabic by boiling with dilute sulphuric acid. It crystallizes in prisms, is slightly soluble, is dextro-rotatory, reduces Fehling's solution, but does not ferment with yeast. Boiling mineral acids convert it into furfurol.

Mannite, or *Mannitol*, exists in three states, namely, dextro, lævo, and inactive. It has a sweet taste and is found in "manna," the dried sap of the manna ash (*Fraxinus ornus*). It is also made (with difficulty) by the action of nascent hydrogen on glucose. It oxidizes to saccharic acid.

Dulcite is another of the hexahydric alcohols, and is found in a manna from Madagascar. It is made artificially from lactose or galactose by treating them with nascent hydrogen. It oxidizes to mucic acid.

Sorbite occurs in mountain-ash berries. With one molecule of water it forms small crystals which dissolve readily in water.

Asparagine is the monamide of aspartic or amidosuccinic acid. It occurs in many plants (asparagus, beetroot, peas, beans, etc.). It may be crystallized from the pressed juice in rhombic prisms + $1H_2O$. It ferments in the presence of albuminoids to ammonium succinate. There are lævo and dextro varieties.

Inosite (muscle-sugar) is a crystallizable substance, with the same empirical formula as glucose, but it is not a carbohydrate but a hexahydric phenol of the benzene series. It is also found in unripe peas and beans.

TABLE OF CHIEF CHARACTERISTICS OF AFOREMENTIONED SUBSTANCES.

NAME	Formula	Source	Rotatory	Fehling's	Yeast
Glucose ..	$C_6H_{12}O_6$	Sweet fruits	Dextro	+	×
Lævulose	,,	,,	Lævo	+	×
Galactose	,,	Lactose ..	Dextro	+	×
Saccharose	$C_{12}H_{22}O_{11}$	Cane, beet ..	,,	−	−
Lactose ..	,,	Milk ..	,,	+	−
Maltose ..	,,	Starch ..	,,	+	×
Raffinose..	$C_{18}H_{32}O_{16}$	Molasses ..	,,	−	×
Arabinose	$C_5H_{10}O_5$	Gum arabic	,,	+	−
Dextrin ..	$(C_6H_{10}O_5)n$	Starch ..	,,	−	−
Starch ..	,,	Plant cells ..	,,	−	−
Inulin ..	,,	Chicory, etc.	Lævo	−	−
Mannite ..	$C_6H_8(OH)_6$	Manna ..	Dextro	−	−
Dulcite ..	,,	,, ..	,,	−	−
Sorbite ..	,,	Mountain ash	,,	−	−
Asparagine	$C_4H_8N_2O_3$	Asparagus ..	D & L		
Inosite ..	$C_6H_{12}O_6{\cdot}2H_2O$	Muscle ..			

+ Reduces. − Not. × Ferments.

Adonite, $C_5H_7(OH)_5$ is a pentite (from *Adonis vernalis*). *Salicin* ($C_{13}H_{18}O_7$) and *Coniferin* ($C_{16}H_{22}O_8{\cdot}2H_2O$) are glucosides.

Analysis of Cane Sugar.—Moisture, 0·5 per cent in refined sugar, to 6 per cent in raw sugar. Ash, from a

trace to 2 per cent. Glucose, not more than 0·1 per cent in refined, or 2 per cent in raw. Saccharose or sucrose, 93 per cent upwards. Other organic matter. Mineral matter. Colouring matter. Sugar mite (*Acarus sacchari*).

Moisture.—Dry 5 grm. in air-oven at 105° C. to a constant weight (about two hours).

Ash.—Treat residue with pure sulphuric acid and ignite to whiteness (takes twenty minutes). Nine-tenths of the sulphated ash are taken as the true ash. The ash of sugar consists of salts of potash and soda, lime, alumina, and silica.

Glucose.—Estimate by Fehling's solution, volumetrically or gravimetrically.

Saccharose, or *Sucrose*, is estimated either (1) directly, by the polarimeter (saccharimeter), or (2) after "inversion," with Fehling's solution.

1. *Polarization* is employed in all commercial transactions, and for the assessment of duty by the Customs authorities. It depends on the optical property of some bodies to rotate the plane of polarized light. The specific rotatory power of an optically active substance is the amount of angular rotation (in degrees) of the ray of polarized light which is produced when a solution of the substance containing 1 grm. per c.c. is examined in a column 1 decimetre (100 m.m.) long. If a be the observed angle of rotation in the sample, p the weight of the substance per 100 c.c. of solution, l the length of the tube in decimetres, and d the sodium light, then the specific rotatory power $(A)_d$ is determined from the formula—

$$(A)_d = \frac{a}{l \times p/100} = \frac{100 \times a}{l \times p} \text{ and } p = \frac{100 \times a}{l \times (A)_d}$$

To determine $(A)_d$ for pure cane sugar, dissolve 10 grm. in water and make up to 100 c.c. Put some of the solution in a polarimeter tube, examine, and substitute in formula. Now examine sample similarly and compare results. The ratio of these, multiplied by 100, gives the percentage of pure sugar. Or, in the formula, simply substitute already ascertained values for $(A)_d$,—

+ 66·5	being	taken for	saccharose,
+ 52·7	,,	,,	dextrose, and
− 95·5	,,	,,	lævulose

at a temperature of 20° C. Clarify dark-coloured solutions with basic lead acetate. If cloudy only, add 3 c.c. cream of alumina, mix well, add one drop of PbAc, shake, and filter. If yellow, repeat, adding more PbAc. If brown or black, add 2 c.c. of 10 per cent Na_2SO_3, then PbAc solution till no further precipitate.

2. *Estimation by Fehling's Solution.*—The sugar must first be inverted by boiling with a dilute acid and then estimated either volumetrically or gravimetrically. (*a*) Volumetrically. Take 1 grm. of sample, dissolve in 50 c.c. aq. dest., add 5 c.c. concentrated HCl, and heat to 70° C. for 10 to 20 minutes to invert—

$$\underset{\text{Saccharose}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Dextrose}}{C_6H_{12}O_6} + \underset{\text{Lævulose}}{C_6H_{12}O_6}$$

Cool, and neutralize with NaOH, make up to 100 c.c. (*i.e.*, 1 per cent), and put into a burette. Take 10 c.c. of Fehling's solution in a flask or porcelain dish, add 40 c.c. of water, and bring to the boil. Run in the dilute sugar solution carefully until all the copper is reduced, keeping the liquid at the boiling-point all the time. The end point is the chief difficulty in this method. Filtration is helpful, but an indicator is commonly used. Allow to settle, remove a drop of the supernatant fluid, and test on a white tile with potassium ferrocyanide until a brown precipitate is no longer given on acidifying with acetic acid. Or, using Harrison's indicator, take several drops of a freshly-made mixture (of 0·05 grm. of starch boiled with water, 10 grm. of KI added, and the bulk made up to 100 c.c.) on a white tile, and from time to time a drop of the hot liquid (precipitate and all) is removed and placed on a spot and a drop of acetic acid is superimposed. A blue colour develops until all the copper is reduced. 10 c.c. of Fehling's solution = 0·0475 grm. inverted cane sugar and 0·0500 grm. dextrose, lævulose, or invert sugar, and 0·0678 grm. lactose and 0·0807 grm. maltose. (*b*) Gravimetrically. Proceed as above until the 1 per cent inverted saccharine solution is obtained. Thereafter take 20 c.c. to 50 c.c. of Fehling and boil. Add a measured quantity of the 1 per cent solution (but always less than will completely precipitate the Fehling solution) and continue the

boiling for two to six minutes, when there should be a red precipitate and the liquid should still be blue. Now filter rapidly, washing the precipitate at first by decantation and then on the filter-paper, until the washings contain no copper. Dry, ignite, and weigh as CuO (black copper oxide). Weight $\times$ 0·4308 gives cane sugar, and $\times$ 0·4535 gives glucose. The CuO is hygroscopic; therefore, for accuracy, at least two ignitions and two weighings are necessary. Also prolonged ignition is needed to oxidize all the Cu_2O (the filter-paper having a reducing action).

Insoluble Mineral Matter.—Dissolve a considerable amount in water, filter on to weighed paper, dry, and weigh.

Colouring Matter.—Extract with alcohol and steep a piece of wool mordanted with aluminium acetate. Aniline dyes are dissolved and stain the wool. The natural colour is not so removed. Ultramarine is used to "whiten" sugar, as also is methyl-violet, both correcting the slight yellow tint natural to sugar. To fix the natural yellow tint, chloride of tin was formerly used, but not now. Demerara sugar is made by adding sulphuric acid to the massecuite (the finished mass of sugar crystals and syrup formed by concentrating syrup in a vacuum pan) so as to slightly char the sugar grains. About 3 gallons of pure sulphuric acid, diluted with 1·5 gallons of water, are used for 5 tons of sugar. This gives the bright yellow colour so much admired. Animal charcoal, sulphurous acid, and peroxide of hydrogen are also used in various processes.

The sample is dyed if, when treated with a few drops of concentrated HCl, a pink colour forms.

Sugar Mite, or *Acarus sacchari,* by microscope.

Sugar of Milk or *Lactose* is made on the large scale from whey or skimmed milk.

Glucose, or *Starch Sugar,* is made on the large scale from the starch of maize, potato, rice, and sago, by treating with weak (pure) mineral acid, aided by boiling under increased pressure until some of the liquid added to alcohol gives no precipitate of dextrin or no iodine reaction. The acid is then carefully neutralized and the resulting glucose solution refined. Poisoning has arisen from the use of impure sulphuric acid in the process (arsenic in beer).

GOLDEN SYRUP: HONEY.

Golden Syrup.—When the syrups no longer yield sugar they are made into treacle. Golden syrup or invert-sugar syrup is also largely made from the sap of the maple in Canada and U.S.A., thus saving the time and fuel required to extract the sugar. The various drainings from the crystallized sugar are called molasses. This passed through a charcoal filter becomes bright and clear, and, when concentrated to the required viscosity, forms golden syrup. In the case of maple sap, it is first boiled, and skimmed and strained while still hot, and the evaporation is continued to a density of 1325, or equal to 11 lb. of sugar to the gallon. It is then poured while hot into perfectly clean pans or tins, which are then sealed to keep out the air. Syrups of this strength will not granulate under ordinary conditions. The steps of the process can be gauged by a thermometer, the thin sap boiling at about 213° F., and a syrup of the desired strength at 235° to 240° F., giving a polarimeter figure of over 80°, for which a bounty was at one time paid by the state of Vermont, U.S.A.

Molasses of inferior grades is used, when inverted, for the manufacture of alcohol, for feeding cattle, or for fuel. Sorghum juice is also a source of syrup.

The chief adulterant of golden syrup is glucose, which is added to thicken it to improve the appearance, and where the syrup contains some crystallizable sugar, to prevent its deposition. The presence of glucose is determined by the polarimeter, the specific rotatory power of genuine golden syrup being taken as + 16°, and of glucose syrup as + 110°, then if a_d be the observed rotation, the percentage of glucose will be

$$100(a_d - 16) \div (110 - 16)$$

The reducing power of golden syrup is due to glucose and invert sugars present. A second estimation, after inverting any cane sugar present, shows how much, if any, is present. A third, after inverting for three hours, shows, if giving a higher result than the second, the presence of maltose and dextrin, which would confirm the presence of glucose or starch-derived sugar.

Honey consists of the saccharine substance collected by bees from the nectaries of flowers, and deposited in the cells of the honeycomb. Honeydew is a secretion of the leaves of various trees and plants, and is also gathered by bees.

Honey contains dextrose and lævulose, and hence, like invert sugar, is lævo-rotatory from -4 to -15, and the reading is not altered to any extent by inversion, showing the absence of cane sugar. If the reading is $+$, it indicates the presence of glucose or cane sugar, and if after inversion it is still $+$, then the substance is glucose. If the reading is $+$ and very high (above 100), the presence of glucose is almost certain, and in this case if no cane sugar is present at the same time, inversion will not change the reading. Cane sugar is stated to be present in natural honey at times, up to 8 to 10 per cent, and so a reading of $+2$ may be passed.

Honey is largely adulterated with glucose, starch paste, malt extract, and cane sugar, and imitated by adding a piece of genuine honeycomb to a jar of glucose syrup. On microscopical examination, genuine honey will always show the presence of pollen grains, which would generally be absent from a filtered honey. (*Mel depuratum* B.P. is the honey of commerce melted in a water-bath, and strained while hot through flannel previously moistened with warm water.)

Beeswax melts at about 64° C. (147° F.) and is carbonized by strong sulphuric acid on boiling; paraffin wax melts at 54° to 57° C. (130° to 135° F.) and is not carbonized on boiling with strong sulphuric acid.

ANALYSIS.—*Water* varies from 15 per cent to 25 per cent, and is estimated by dissolving 5 grm. in water, making up to 100 c.c., and drying 10 c.c. mixed with 10 grm. of sand at 96° C. until weight constant. *Ash.*—Ignite 2 to 5 grm. at a low heat. Varies from 0·1 to 0·3 per cent, and is alkaline. If more, it suggests glucose; test for $CaSO_4$, and neutrality.

Polariscope Reading.—Dissolve the "normal weight" of honey in water and make up to 100 c.c., filter through a small quantity of bone charcoal to clarify, and examine in saccharimeter.

Glucose.—Syrup; sticky precipitate with one part of water and ten of methyl alcohol.

MUSTARD.

Mustard is the seed of *Sinapis alba* and *S. nigra*. It is commonly sold as a powder. Pure mustard contains 14 per cent of carbohydrates, 0·66 per cent of volatile oil, 35 per cent of fixed oil. The chief adulterations are: the addition of starch, bringing up the percentage of carbohydrates to 67 per cent; the abstraction of oil, reducing it to as low as 7 per cent; the addition of turmeric to colour, and cayenne pepper to make the taste sharper. Examine with microscope for hexagonal cells in white mustard.

PEPPER.

Black pepper is derived from *Piper nigrum*. White pepper is made from the inside of the berry.

Moisture, 9 to 11 per cent; ash, 2 to 5 per cent; ash insoluble in HCl, under 1 per cent for white, under 2 per cent for black pepper; fibre, 4 to 6 per cent in white, 8 to 11 per cent in black; carbohydrate, 65 per cent in white, 50 per cent in black; piperin and fixed oil, 8·2 per cent in white, 7·8 per cent in black. Adulterations: linseed, mustard husks, wheat and pea flour, rape cake, ground rice, ground olive stones (poivrette), sweepings.

GINGER.

Ash, under 3·9 per cent; soluble ash, over 1·7 per cent; cold water extract, over 8·7 per cent.

PEAS.

Test peas, especially when tinned or bottled, for copper, used in "greening" them.

Qualitative Test.—Acidify with HCl and put a piece of bright steel in the liquid in which the peas are immersed. Stand all night. Deposit of copper, of a coppery colour.

Quantitative Test.—Ash 20 grm. of peas, boil ash with dilute sulphuric acid. Filter, and make up filtrate to 50 c.c. Test amount of copper colorimetrically, or gravimetrically.

MEAT EXTRACTS AND ESSENCES.

Total nitrogen, by Kjeldahl; protein, N × 6·25; moisture; ash; reaction; carbohydrate; fat; antiseptics; poisonous metals.

CHAPTER VI.

BEVERAGES.

COFFEE.

THE seed or berry of the plant *Coffea arabica*. Each coffee bean contains two seeds. These are removed, roasted, and ground, producing coffee. The chief constituent is caffeine, identical with theine, or trimethylxanthin $C_5H(CH_3)_3N_4O_2$, and on the average 1·2 per cent is present.

The chief adulterant is chicory, or the wild endive (*Cichorium intybus*), but this can be legally used as a diluent for coffee if the article is sold as a mixture. Chicory is the root of the plant, dried, and powdered. It contains no caffeine, much less fat than coffee, and much more sugar.

Other adulterants which have been used are: dandelion root, mangel-wurzel, turnips, bean, pea, rye, and wheat flours, caramelized condemned sea-biscuit. The berries themselves are sometimes spurious, being moulded from a composition of chicory and other substances. Chicory itself is sometimes adulterated with some of these substances and occasionally with roasted beetroot.

ANALYSIS.—

Moisture.—Dry 5 grm. to a constant weight at 100° C. Should not exceed 6 per cent (chicory 10 per cent).

Total Ash.—Ignite 5 grams. until a nearly white ash is obtained. From 3·5 to 5 per cent. Chicory ash is reddish from the presence of iron, and is about 5 per cent. Four-fifths of coffee ash is soluble in water; one-third of chicory ash.

Caffeine is extracted by successive boilings with water, the albuminous matter precipitated by acetate of lead, filter, concentrate the filtrate to small bulk, and extract four or five times with chloroform. On evaporating the chloroform, pure caffeine is left. Should be 1·1 per cent to 1·3 per cent (chicory none).

Fat.—Soak in petroleum ether for several hours, pipette off 20 c.c. of ether and evaporate off ether in a tarred vessel. Averages 11 to 14 per cent (chicory 1 to 2 per cent).

Sugar.—Extract with hot water, invert, and estimate. Under 1 per cent (chicory 10 to 18 per cent).

Starch.—Make a 10 per cent decoction, boil with animal charcoal to decolorize (or acid permanganate), cool, and test with iodine solution. Pure coffee contains almost no starch, and the presence would indicate adulteration with breadcrumbs, etc. Mounting in iodine (1 in 1000) brings out the concentric rings.

Detection of Chicory.—

Qualitative.—Throw a pinch on to the surface of a glass of water. Pure coffee remains floating on the top for some minutes at least, whilst chicory sinks almost instantly, and colours the liquid. The fragments which fall to the bottom are taken out separately and examined by the fingers (coffee is hard, chicory is soft). They are then examined microscopically for the long testa cells of coffee berry and the ladder-shaped structures, and in chicory for the cells and dotted ducts.

Quantitative.—Take 10 grm. of sample in a boiling-flask, add 100 c.c. of water, bring to the boil, and continue for one half minute. Strain through muslin or a fine sieve, and then through a filter-paper. Cool to 15·5° C. (60° F.) and take specific gravity by bottle. Under these conditions pure coffee gives a decoction having a sp. gr. rarely exceeding 1009·5, and chicory about 1024 to 1025, or say 1024·5. Thus a difference of 15 per 1000 represents the difference between 100 per cent coffee and 100 per cent chicory, and from these data the amount of adulteration can be calculated. Say that the sp. gr. of sample is 1014·5, that is a rise of 5 ; therefore, as 15 : 5 : : 100 : 33·3 per cent of chicory, and a fall of 10 from the sp. gr. of chicory, and therefore as 15 : 10 : : 100 : 66·6 per cent of coffee.

A second method is to take 10 grm., add 100 c.c., bring to the boil, filter, boil dregs with another 150 c.c. for five minutes, allow to settle, decant off clear liquid, mix with previous filtrate, make up to 250 c.c. with aq. dest., mix thoroughly, pipette off 50 c.c., evaporate to dryness in weighed capsule over water-bath, weigh, and calculate

as a percentage. Coffee gives a remarkably constant percentage of 24, and chicory a mean percentage of 70, or a standard difference of 46, so that percentage of coffee = 100 (70—percentage of extract found) ÷ 46. A third method is to calculate the percentage of soluble ash and then calculate adulteration on this basis.

TEA.

Tea is the prepared leaf of the shrub, *Camellia thea*, which grows in China, India, Ceylon, and Japan. It is nearly always blended to a standard quality and flavour by mixing two or more kinds of leaves. When the leaves are baked (to dry them) immediately after picking, green tea results; but if first allowed to ferment and then baked, black tea is obtained. The chief constituents of tea are moisture, caffeine, tannin, albuminous matters, ethereal oil, gum, dextrin, fat, wax, chlorophyll, woody fibre, etc. Tea is now rarely adulterated, since it is examined by the Customs authorities, and samples found to be adulterated are not allowed to be imported. The usual adulterants were foreign leaves (sloe, willow, elder, hawthorn, beech, etc.), sweepings, tea dust, clay, mineral matter, ground olive stones, catechu, gum, starch, and exhausted tea-leaves.

Moisture.—Dry 5 grm. of sample to a constant weight. Varies from 4 to 11 per cent; average 6 per cent.

Total Ash.—Ignite dried tea at lowest possible temperature to get a grey, not a green ash. Varies from about 5 per cent to 7 per cent, averaging about 6 per cent.

Insoluble Ash.—The total ash after weighing is washed on to a filter-paper, and thoroughly extracted with about 400 c.c. of very hot water, the washings being carefully preserved. The filter-paper is then dried, ignited, and weighed, and the weight of filter-paper ash being deducted, the amount of insoluble ash is known. Varies from 2 per cent to 4 per cent, of which less than 1 per cent is insoluble in HCl.

Soluble Ash.—Deduct amount of insoluble ash from total ash, and result is soluble ash. Varies from 2·8 to 4 per cent, but should be at least half the total ash. If the

soluble is low, the inference is an admixture with exhausted leaves.

Alkalinity of the Soluble Ash is determined by titrating the washings of the total ash (taking 50 c.c.) with N/10, HCl or H_2SO_4, using methyl-orange as indicator and returning the answer in terms of K_2O. Varies from 1·3 to 2 per cent. 1 c.c. N/10 acid = 0·0047 grm. K_2O.

Extract.—Dry some of the leaves at 100° C.; then weigh out 2 grm. and exhaust thoroughly by boiling under a reflux condenser for one hour. Filter off water, and repeat with more water until no more colour is imparted to the water. Collect the exhausted leaves, dry, and weigh. The difference from original weight gives the amount of extract, which should be from 35 to 40 per cent of the dried tea.

Caffeine.—Extracted as indicated under "Coffee." Varies from 1·8 to 3·5 per cent; average 2·6 per cent.

Tannin.—Treat 2 grm. as described under "Extract," but keep the filtrates and make up to a known bulk (1 litre). Take an aliquot part (say 100 c.c.) in a beaker, add excess of 5 per cent copper-acetate solution, and boil. The precipitate contains all the tannin. Filter and wash precipitate until the washings are free of copper. Dry, ignite, weigh, and deduct filter ash. The weight of ash × 1·305 = tannin. The ash is CuO. The amount of tannin varies from 13 to 18 per cent.

Caffeine - Tannate. — Experiments in the "Lancet" laboratory (*Lancet*, 1911, vol. i, page 46; and vol. ii, page 1573) led to the conclusion "that an infusion of tea is a solution of caffeine-tannate in an alkaline medium." On neutralization (with HCl) the caffeine-tannate is gradually thrown out of solution, it being only slightly soluble in cold water but readily soluble in hot water. Caffeine-tannate is a compound of one part of caffeine with three parts of tannic acid. When these substances are present in these proportions in a tea infusion, they neutralize each other's effects by combination, and are precipitated by the gastric juice. Excess of either is detected by the palate as bitterness or astringency in the infusion.

Aroma is mainly due to a volatile oil, which is present to the extent of 0·5 per cent.

Microscopic Characters are important. Soak leaves in hot water, when they are easily laid out flat on a slide, and examined. The tea leaf has a serrated margin (but not quite to the stalk), the primary veins run out from the midrib nearly to the margin and then loop: possesses stomata and long hairs on the under surface, and internally shows long branched cells, called "idioblasts." The apex of the leaf is described as notched or emarginate. Average size, 1 in. to 2 in. by ½ in. to 1 in.

Fresh v. Exhausted Leaves.—

	Total Ash	Soluble Ash	Alkalinity—K_2O
Fresh	4·8 to 7 %	2·8 to 4 %	1·3 to 2 %
Exhausted	4·4 %	0·7 %	0·2 %

COCOA.

Cocoa is the prepared seed of *Theobroma cacao* and allied trees, growing in the West Indies, Mexico, Brazil, etc. The seeds are contained in a pod packed in a pulpy substance. Each pod holds 25 to 30 seeds. The ripe pods are allowed to ferment, when the seeds are easily separated. These are then dried in the sun, or in ovens, roasted in iron cylinders, cracked by machinery, and the husks separated from the cocoa beans or "nibs." The nibs are then ground, but either some of the fat has to be removed or some diluent (as starch or sugar) added before they can be made into a powder. A further treatment is to add alkali, which may be potassium carbonate or the sodium or ammonium salt. The added alkali emulsifies the fat and saponifies any free fatty acid, so that on addition of hot water there is less tendency for the fatty globules to separate the so-called "soluble cocoa." Cocoa contains the following: moisture, fat, starch, theobromine, proteins, cellulose, and mineral matter (including phosphates).

Moisture.—Dry 2 grm. in water-oven. In raw cocoa, 4·5 per cent; commercial, 8 to 13 per cent.

Fat is estimated by the Soxhlet process; 51 per cent in raw cocoa and about 30 per cent in prepared. It is also known as cacao butter, or oil of theobroma of the B.P., and is used for suppositories. It is yellowish in colour, and contains stearin, olein, and theobromine. Melting point

31° to 34° C. (Distinguish from coco-nut oil, from *Cocos nucifera*, a tropical palm. Melting point 20° to 26° C.)

Ash.—Ignite 5 grm. in a platinum dish at a low red heat. If red, examine for iron. Is about 3 per cent in raw cocoa (rock cocoa), and 5 per cent in commercial varieties. Contains about 1 per cent of phosphoric anhydride, but this varies.

Soluble Ash is not determined from the ash but from the soluble extract. It should not fall below 2 per cent.

Soluble Extract (cold water extract).—Five grm. of the sample are rubbed up in a mortar with 250 c.c. of cold water until a smooth mixture results. Shake at intervals, and allow to stand over night. Filter 50 c.c. (= 1 gram. of sample) and evaporate to a constant weight (dryness). Should not exceed 18 per cent. Any material excess is probably due to added sugar. This residue is now ignited at a gentle heat, cooled, and weighed, and the result called the "soluble ash" (better, the ext. ash).

Theobromine is allied to caffeine, being a dimethylxanthin, and like it is not precipitated by potassic mercuric iodide, or by I in KI. Estimate by treating with petroleum ether; remove latter on water-bath; extract with alcohol; evaporate alcohol; add water, and clarify with PbAc; remove lead with H_2S, and extract with chloroform. Amount varies from 1·3 to 1·7 per cent. Theobromine contains 31·1 per cent of nitrogen.

Proteins.—Estimated approximately by Kjeldahl process. Deduct N due to theobromine and × by 6·25. Averages 2·2 per cent except where proteins added (plasmon cocoa).

Starch.—Cocoa contains about 10 per cent of natural starch. The granules are small and round and are easily distinguished from any likely to be added as an adulterant.

Sugar is determined by extracting 10 grm. in a filter-paper cylinder in a Soxhlet to remove the fat. Dry and extract with alcohol, which dissolves the sugar and other matters. Remove latter as before, boil for ten minutes with 2 per cent HCl, neutralize, and estimate the invert sugar by Pavy's process. The residue contains the starch. Boil for six hours with 200 c.c. of 2 per cent

H_2SO_4. The new residue, treated with 2 per cent NaOH and then weak HCl, and filtered, gives "insoluble fibre."

Adulterants.—Potassium carbonate, ground cocoa-shells, red sanders wood, iron oxide, sugar, and starch. Microscopic examination detects most of these.

Paraguay Tea, known as "maté," contains 1 per cent of theine. Used in South America.

Guarana contains 5 per cent of theine or caffeine. Used for migraine. From seeds.

Kola.—From the seeds of a tree growing wild on the West Coast of Africa. It contains 2·42 per cent of caffeine, little fat (0·68 per cent), starch and sugar 36·5 per cent, and proteins 6·7 per cent. Spurious kola nuts containing no caffeine are much sold.

Coca.—From the leaves of *Erythroxylon coca*; contains the alkaloid cocaine. The reputed sustaining powers of coca leaves are not marked in the case of Europeans, probably from their ordinary dietary being rich in stimulant extractives of the xanthin group. On the natives the effects are notable.

LEMON JUICE AND LIME JUICE.

Lemon Juice—Is the expressed juice of the *Citrus limonum*, and is a slightly turbid, yellowish liquid, with a sharply acid taste. The British Pharmacopœia standard is: specific gravity, 1030 to 1040; citric acid, 30 to 40 grains per fluid ounce; and ash, not more than 3 per cent. The Board of Trade standard for lemon and lime juices is: sp. gr. (when de-alcoholized) 1030, and 30 grains per ounce of citric acid. As found in the Merchant Service, or in the Royal Navy, these juices have sugar added to them, and have 1 ounce of brandy added per 10 ounces of juice, or are pasteurized at 145° F., or are boiled. The alcoholic juice keeps better, and freezes at a lower temperature. The latter point is of importance in Arctic and Antarctic expeditions. Good juice keeps about three years; bad juice becomes turbid, stringy, and mucilaginous; glucose and CO_2 being formed from the decomposition of the citric and malic acids present. One ounce

of lemon juice per head per day must be issued when a vessel has been ten days at sea, except when in harbour if fresh vegetables can be had.

Lime Juice—Is the expressed juice of the *Citrus limetta*. Like lemon juice, it contains free citric acid, traces of other organic acids, citrates, sugar, and albuminous and mucilaginous bodies. The average composition of these juices is :—

	Total Solids	Sugar	Citric Acid	Mineral Matter	Potash	P_2O_5 (sol.)
Lemon Juice ...	8·80 %	2·30 %	4·57 %	0·35 %	0·15 %	0·010 %
Lime Juice ...	8·64 %	0·70 %	5·60 %	0·35 %	0·12 %	0·065 %

According to the above standards, the percentage of citric acid should be 6·6 to 8·8 per cent. Few juices reach the higher figure. The important points in the analysis are : specific gravity, total solids, free citric acid, combined acidity, ash, free mineral acid, tartaric acid, alcohol, sulphites, and salicylic acid.

Specific Gravity.—Lime juice is usually about 1035 to 1037 (= 32 grains per oz.).

Total Solids.—Evaporate 10 c.c. over water-bath, and dry in oven to constant weight. Vary from 5 to 9 per cent.

Ash.—Ignite the residue. For lemon juice, should not exceed 3 per cent, and be neutral.

Free Citric Acid.—Titrate 20 c.c. with N/1 or N/2 NaOH, using phenolphthalein as indicator. 1 c.c. N/1 NaOH = 0·07 grm. crystallized citric acid, $H_3C_6H_5O_7.H_7O$. The percentage × 4·375 = grains per fl. oz. Varies from 5 to 9 per cent.

Combined Acids.—The neutralized juice from the above process is evaporated to dryness on the water-bath, and the residue ignited at a low temperature. The citrates are changed to carbonates. Cool, and extract mass with hot water, add sufficient N/1 sulphuric acid to make acid (noting the quantity), boil to get rid of CO_2 and then filter. Now titrate with N/1 NaOH, using methyl-orange as indicator, to estimate amount of N/1 sulphuric, added in excess. This amount deducted from the total amount

of sulphuric used, gives the amount of N/1 sulphuric required to decompose the carbonates formed from the citrates by ignition. These citrates are partly present as such, and partly from neutralization of the free acid by soda, some of which is not citric. The result, therefore, gives the total organic acid, free and combined, in the sample, calculated as citric acid. The combined acid expressed as citric acid, is got by subtracting from the total acid the amount of free acid in sample. 1 c.c. N/1 sulphuric acid = 0·07 grm. crystallized citric acid.

Free Mineral Acid.—Place several drops of methylene-violet solution on a white slab. Add a drop of juice; if free mineral acid is present, a greenish coloration is got. With logwood solution dried on a slab, a drop of juice, if it contains free mineral acid, gives a red coloration.

Sulphites.—Add Zn and HCl, and heat; if sulphites are present, H_2S is given off, and will blacken lead acetate paper (moist). Quantitatively: take 50 c.c. of sample and add 25 c.c. N/1 KOH, and shake at intervals for fifteen minutes. Add 10 c.c. 25 per cent sulphuric and starch solution, and titrate rapidly with N/10 iodine until a permanent blue colour, lasting two minutes, is produced. 1 c.c. N/10 iodine = 0·0032 grm. SO_2. When the sulphurous acid is present in the free state, a known excess of N/10 iodine may be added to a measured or weighed quantity of the sample, the whole allowed to stand, with occasional shaking, for one hour, and then titrated with standard thiosulphate solution to find excess of iodine. Another process for sulphites, is to take a quantity of the sample in a flask, add a large excess of water, and then HCl. Distil over the liberated SO_2 into a known quantity of N/10 iodine, and find excess of iodine solution used by titration with standard thiosulphate. The difference is the amount of N/10 iodine used in oxidizing sulphurous acid to sulphuric.

$$H_2SO_3 + I_2 + H_2O = H_2SO_4 + 2HI.$$

Alcohol.—By distillation (see under "Beer," page 128).

Salicylic Acid.—Precipitate albuminous matters with lead acetate solution; filter, extract filtrate several times with ether, evaporate ether, and dissolve residue in distilled

water. Test with ferric chloride ; a violet coloration, not discharged by acetic acid, is positive.

Tartaric Acid.—Neutralize with NaOH, add calcium chloride, and shake well : if tartaric is present, a white crystalline precipitate of calcium tartrate falls. To estimate, take 25 c.c., add potassium acetate and alcohol, stand for some hours, stirring occasionally, filter, washing with saturated solution of potassium acid tartrate, dry, and weigh as cream of tartar, $KHC_4H_4O_6$, or KHT.

Poisonous Metals Test for as on page 124.

VINEGAR.

Vinegar may be defined as the " product of the alcoholic and acetous fermentation of a vegetable infusion." This definition includes all brewed vinegars, but excludes wood vinegar, made by diluting acetic acid derived from the destructive distillation of wood. The vinegars in common use are : malt, cider, wine, white or distilled, and wood vinegars, and vinegar from starch, glucose, and molasses. The essential constituent is acetic acid, which should not be under 3 per cent in a good vinegar. The other constituents, and their amount, vary with the mode of production : malt vinegar yielding the most, and distilled vinegar the least, extract or total solids.

Malt Vinegar is made from malted barley, which is first fermented to produce alcohol (by yeast), and the resulting liquid poured over piles of birch twigs, and exposed to the air. The vinegar plant (*Mycoderma aceti*) grows on the twigs, and converts the alcohol to vinegar, with formation of small quantities of acetic ether, aldehyde, and other bodies, which give such vinegar its pleasant odour. Genuine malt vinegar has the following composition : specific gravity at 15·5° F. 1019 ; acetic acid, 5·50 per cent ; extract, 2·50 per cent ; ash, 0·50 per cent ; N, 0·08 per cent ; P_2O_5, 0·08 per cent. It is dextro-rotatory.

Wine Vinegars are made from grape juice and inferior new wine, and vary in colour from straw to red (malt vinegar is brown). They usually contain 1 per cent of alcohol, 1 per cent of extract, 5 to 6 per cent of acetic acid, not less than 0·25 per cent of ash, and small quantities of

tartaric acid and KHT. The specific gravity varies from 1015 to 1022.

Cider Vinegar, from apple juice, is much used in America. It contains 5 per cent of acetic acid, some malic acid, is lævo-rotatory, and contains no aldehyde.

White Vinegar is made by distilling malt vinegar.

Wood Vinegar, by destructive distillation of wood.

Vinegars from starch and glucose run the same danger of arsenical contamination as beer made from these substances. They are dextro-rotatory.

Vinegar was formerly excisable, and to preserve it, sulphuric acid was allowed to be added, not exceeding 1/1000th part by weight (duty abolished in 1844).

Sodium carbonate or ammonia gives a purplish precipitate in wine vinegar, but not in malt vinegar.

ADULTERATIONS AND CONTAMINATIONS.—Water, mineral acids, pyro-ligneous acid, metals, colouring agents, preservatives, gypsum, capsicum, vinegar eels. Vinegar is at times pasteurized at 145° F.

ANALYSIS.—Specific gravity, total solids, ash, acetic acid, free mineral acid, poisonous metals, nitrogen, and phosphoric acid. Microscope for eels (*Anguillula oxyphila*).

Specific Gravity.—By sp.-gr. bottle or Westphal balance, 1015 to 1022. The sp. gr. of an artificial vinegar will vary with the amount of acetic acid, caramel, and malt vinegar it contains. Thus the acidum aceticum dilutum of the B.P., which contains 4·27 per cent (by weight) of acetic acid, and should yield no residue on evaporation, has a sp. gr. of 1006. The sp. gr. of these vinegars will therefore average above this figure.

Total Solids.—Evaporate 25 c.c. to constant weight in a tared dish. Varies with the vinegar: malt, 2·50 per cent; cider, 2 per cent; wine, 1 per cent; white, 0·2 or less.

Ash.—Ignite at a low temperature. Should be alkaline if free from mineral acid. Varies from 0·5 to 0·2 per cent in malt and cider, to 0·03 per cent or less in distilled.

Alkalinity of Ash.—Extract ash with hot water, titrate with N/10 acid, using methyl-orange as an indicator. 1 c.c. N/10 acid = 0·0047 grm. K_2O. About 0·026 per cent in malt, and 0·14 per cent in cider vinegar.

Acetic Acid.—Take 10 c.c. of sample in a porcelain dish. If dark coloured, dilute well. Titrate with N/2 NaOH, using phenolphthalein as indicator: 1 c.c. N/1 NaOH = 0·06 grm. glacial acetic acid. Should not be less than 3 per cent.

Malic Acid—in genuine cider vinegar. Gives a fairly copious precipitate with lead subacetate solution.

Free Sulphuric Acid.—A few drops of sample are taken on a white slab. Place near a drop of methyl-violet and bring into contact with a glass rod. If only a trace of free acid is present, the violet changes to blue; but if more than 1 part per 1000 be present, a green colour develops. Or test with logwood, as described under "Lime Juice." Or add five drops of methyl-violet solution to 5 c.c. of sample, and compare with control. Dilute sample if dark coloured.

Nitrogen.—Evaporate 25 c.c. to dryness, and proceed as usual (none in wood or distilled vinegars). Less than 0·1 per cent as a rule.

Phosphoric Acid is estimated by Stock's method. This consists in treating the ash with nitric acid, adding ammonia, then more nitric, till the precipitate formed dissolves; then more ammonia, until it returns slightly. Some fuming nitric is then added, the solution warmed to 70° C., and ammonium molybdate solution added, when a yellow precipitate forms and is collected and weighed. Weight × 0·0373 = P_2O_5. (None in distilled vinegars.)

Poisonous Metals.—Dissolve ash in boiling distilled water, or this failing, in boiling dilute HCl, or if necessary, in HCl (2 parts) + HNO_3 (1 part), and then test by usual analytical table. HCl precipitates Ag, Hg′, Pb; H_2S precipitates Pb, Hg″, Cu, Bi, Cd, As, Sb, Sn, Au, Pt; AmCl and AmOH precipitate Al, Cr, Fe; Am_2S precipitates Zn Mn, Ni, Co; Am_2CO_3 precipitates Ba, Sr, Ca; $AmHPO_4$ precipitates Mg.

Copper, if present in the vinegar of pickles, may be detected by inserting the bright blade of a steel knife.

Arsenic may be derived from arsenical malt, caramel, or glucose. Use Reinsch's test (see under "Beer," page 129).

Copper, lead, and tin may all be derived in the process of manufacture (cider vinegar) or from storage in metallic or metallic-covered vessels.

Potassium ferrocyanide is sometimes used to clarify vinegars, and is said to be decomposed, leaving in solution an unstable compound of prussic acid with the organic matter.

Estimation of the Free Sulphuric Acid.—Take 50 c.c. of sample, add 25 c.c. N/10 NaOH. Evaporate whole to dryness, and ignite. To ash add 25 c.c. N/10, HCl or H_2SO_4, 25 c.c. aq. dest., and boil to expel CO_2. Filter, wash paper with hot water, and titrate whole filtrate with N/10, NaOH, and phenolphthalein. The number of c.c. of soda used × 0·0049 = free sulphuric acid in 50 c.c. of sample.

Barium chloride cannot be used to test for the free acid, as vinegar is often made from hard waters containing sulphates, which would also precipitate the barium.

Microscope for vinegar eels. (1 to 2·5 mm. in length.)

Preservatives.—Test as elsewhere.

Vinegar, in doses of from a half to one ounce daily, is an anti-scorbutic, but is inferior to lime and lemon-juices.

BEER.

Beer is usually defined as "a fermented infusion of malt, flavoured with hops," but this definition must be extended to include beers brewed from saccharine fluids other than malt infusion, and flavoured with other bitters than hops. The malt substitutes are such as malted Indian corn, and starches chemically converted into sugars (rice, potato, and other starches) by the use of sulphuric acid. The latter may contain arsenic derived from the acid used, and so contaminate the beer. This happened in South Lancashire in 1900. The hop substitutes are used only in a few breweries, of which a list is published by the Inland Revenue authorities. They are such bitters as quassia, gentian, calumba, chiretta. Noxious bitters have at times been used, as nux vomica, picrotoxin, and picric acid.

Malt Beer is thus made:—Barley grain is shot into a cistern and covered with water to the depth of 5 inches and soaked for 40 to 60 hours, until it has swollen up and the process of germination has started. The wet grain is then removed from the steeping-tanks, piled up in

heaps, called "couches," and allowed so to remain for 20 to 48 hours, to enable it to develop sufficient heat to ensure equable and good germination. Thereafter it is spread out on floors, where rapid germination proceeds, the plumule growing up the back of the grain and the tiny rootlets being put forth. In 10 to 14 days the plumule is halfway to three-quarters up the back of the grain. Further growth is then stopped by running the malt on to the floors of the "kilns," where it is gradually heated until dry, when the heat is increased. If pale malt is desired, the temperature is not raised above 185° F., but for black or brown malts for black beers, the malt is caramelized. The heated malt is then sifted or screened, to get rid of the sproutlings; the siftings are ground into "grist," and the grist is run into the "mash-tun," a large covered vessel, containing a proper proportion of the brewing-water, at a temperature of 140° to 152° F. for pale ales, and 144° to 150° F. for black beers. In the mash-tun it is well mixed for 15 to 30 minutes, allowed to stand for two hours more, and then the liquor, or "wort," run off into a receiving vessel. More water is run into the mash-tun, and the solids are washed or "sparged," the water not being used hotter than 160° F. to prevent the solution of starch. After four hours' sparging the liquor is run off, the speed being increased as the solids settle, and is mixed with the first wort. The mixture is then boiled in the "copper," the larger portion of the hops added, and after one to two hours' boiling, run out and the hops allowed to settle. The bright, clear, supernatant liquid is then run into large flat trays or coolers, and over coils of pipes in which cold water is circulating (refrigerators), its temperature being reduced to a suitable one for fermentation (60° F. or under). At this stage the specific gravity of the wort is taken by the Excise officers with a Bates' saccharometer, which is a modified hydrometer. The Customs' standard is a sp. gr. of 1055 at 60° F. After allowing 6 per cent off for wastage, they charge 7s. 9d. per 36 gallons, as duty. The wort is now run into tuns made of wood, slate, or stone, about 1 lb. of yeast per barrel being mixed with it as it is run in. Fermentation now takes place, and is continued until the beer reaches the specific gravity desired by the brewer.

The yeast is removed by skimming or otherwise, and the beer run into barrels. When about to be sent out to the "trade," it is subjected to "fining." Finings are made of isinglass, and are white or brown. They are added in the proportion of 1 to 2 pints per barrel; 4 lb. of isinglass dissolved in a hogshead of weak acid or sour beer constitutes the usual finings (hogshead = 63 wine, or 52·5 imperial gallons).

Beers from starches, glucose, invert sugar, etc., are similarly treated, beginning with the mash-tun.

Brewing-Waters.—A hard water free from nitrates and organic matter is preferred for brewing. It should also contain 10 to 15 grains per gallon of NaCl. The composition of Burton water is taken as a standard in this country, and it contains 11 to 15 grains of carbonates of lime and magnesia, 40 to 60 of sulphate of lime, 12 to 30 of sulphate of magnesia, and 5 of chloride of Na and K. In the Dublin water the carbonates are about 11 grains per gallon, the sulphates only 0·8, and the chlorides 1·8. The sulphates and carbonates of Na and K are injurious, as are also any iron salts. The hard water does not take up so much albuminous matter from the malt. Nitrates restrain the growth of the yeast. Organic matter in the water spoils the keeping quality of the beer.

Yeast.—In England the "high" or surface fermentation is used (top yeast); in Germany the "low" or sedimentary process (bottom yeast). The latter works at a lower temperature, and gives a more gaseous but less alcoholic beer.

Composition.—Beer consists of water, alcohol, maltose, dextrin, albuminoids, bitters, salts, carbonic acid gas, and acidity (acetic, lactic, succinic, etc.). Preservatives (salicylic acid, boric acid), saccharin (prohibited), and arsenic may be present. All the constituents vary greatly in the different makes.

Analysis.—The beer is shaken, and poured from one vessel to another to get rid of the CO_2 gas, and the froth is got rid of by filtering through cotton-wool. *Specific gravity*: by bottle or Westphal, varies from 1006 to 1030 (Bass 1013). *Extract*: evaporate 5 c.c. to dryness. See

also under Addenda, page 130. (Bass 7 per cent). *Ash*: ignite extract at a low temperature. Under 0·5 per cent. *Sodium chloride*: extract ash with water and titrate with standard $AgNO_3$.

Acidity.—Total. Titrate 20 c.c. of sample (well diluted) with N/10 NaOH and litmus, and note number of c.c. required. Calculate as glacial acetic acid (1 c.c. N/10 soda = 0·006 grm. Ac). Is commonly 0·182 per cent, or 16 grains per pint.

Fixed:—Dilute 20 c.c. to 100 c.c. and evaporate down to 50 c.c. Dilute again, and titrate with N/10 soda. 1 c.c. = 0·009 grm. lactic acid. Calculate to a percentage.

Volatile:—Deduct number of c.c. required for fixed acid from number required for total, and calculate as acetic.

Alcohol.—Two methods:—

1. *By direct distillation.*—100 c.c. of sample are taken in a distilling-flask, a small piece of pumice stone is added, and about 90 c.c. are distilled over. The distillate is cooled to 15·5° C., and made up with aq. dest. to 100 c.c. at the same temperature. The specific gravity is now taken by the bottle or Westphal balance. The amount of alcohol is then found from tables.

2. *Indirect or Tabarie's method.*—Take sp. gr. of sample carefully (at 15·5° C.). Take 100 c.c. and evaporate down to one-third of the bulk to get rid of alcohol and other volatile substances. Cool, make up to original bulk at 15·5° C., and take sp. gr. again. Then the original sp. gr. ÷ new sp. gr. = sp. gr. of an alcoholic water of same strength as beer. Find percentage from tables. Or, deduct difference between the two specific gravities from 1000, and the result is sp. gr. of an alcoholic water of same alcoholic strength as the sample of beer (average 5 per cent).

Original Gravity of Beer Wort.—This is sometimes needed to calculate the rebate or drawback of duty allowed when beer is exported. It is obtained by deducting the sp. gr. of the alcoholic distillate in (1) above from 1000. The number got is the "spirit indication," and from a table we get with its help the number of degrees of "gravity lost." This number, added to the sp. gr. obtained by Tabarie's method for the de-alcoholized beer, gives the original gravity of the beer wort. A further addition for

excess of acidity (over 0·1 per cent acetic) is also made by consulting another table. (Bass, 1056).

Saccharin.—Treat dried extract with anhydrous ether; evaporate to dryness; if residue sweet, infer presence of saccharin.

Preservatives.—*Boric acid:* test ash (as on page 80). *Salicylic acid:* kiln-dried malt contains a principle which gives an identical reaction with ferric chloride. Hence use Spica's test: Take 100 c.c. of beer, acidify with H_2SO_4; extract with ether; separate ether; evaporate spontaneously; and warm residue carefully with a drop of strong HNO_3. If salicylic acid is present, picric acid is formed. Add AmOH or NaOH, and a bright-yellow picrate is formed, which will stain a woollen thread immersed in it.

Arsenic.—By Reinsch's test. Take 200 c.c. of sample, add 30 c.c. strong HCl and a piece of clean bright copper foil $\frac{1}{2}$ in. $\times$ $\frac{1}{4}$ in. Boil for forty-five minutes, adding aq. dest. to keep up bulk as required. Cool, and examine copper foil. If clean and bright copper-coloured, *As*, *Sb*, and *Hg* are absent. If changed, there may be *As* or *Sb* if deposit is *black;* but if *silvery*, it is *Hg*. Wash gently with water, alcohol, and ether in succession. Dry at 100° C. Put in a perfectly dry 2-in. glass reduction tube, the upper part of which has been previously warmed. Heat gently. *As*, *Sb*, and *Hg* all sublime and condense in the upper part of the tube as, respectively, As_2O_3 (crystals); Sb_2O_3 (amorphous); and Hg (globules). Examine sublimate magnified 200 times. *Arsenic* gives octahedral crystals. *Antimony* gives an amorphous mass. *Mercury* gives globules. If *arsenic* found, wash out sublimate with weak KOH solution and put two portions on a white slab: (1) Touch one with ammonia-cupric solution, when Scheele's green is formed; (2) Touch other with ammonia-silver-nitrate solution — canary-yellow precipitate. If *antimony* found, wash out with weak tartaric acid, which forms tartrated antimony. Add HCl and H_2S—orange precipitate. If *mercury* found, expose to iodine vapour—yellow iodide formed. A control should be done with the distilled water and the HCl and copper foil.

Marsh's and Gutzeit's tests are also used.

ADDENDA.—A pint of good beer contains roughly 1 fl. oz. of alcohol, 1·5 oz. of sugary extract, 20 grains of free acid, 14 grains of salts, and 1 pint of CO_2 gas in solution. The extract may be got from the sp. gr. of the de-alcoholized beer by dividing the excess over 1000 by 3·86. The difference between the sp. gr. of the beer and the sp. gr. of the de-alcoholized beer may be taken as the approximate spirit indication in calculating the original gravity of the beer wort, and deducted from 1000, gives sp. gr. of alcoholic water of same alcoholic strength as beer.

WINE.

Wine is the fermented juice of the grape, but much of it is made otherwise. The grapes are pressed or trodden (mechanical pressure extracts an excess of tannin and colouring matter from red grapes), and the juice or "must" ferments spontaneously from the yeast existing naturally on the skins, the glucose or grape sugar being converted into alcohol and CO_2. When fermentation has ceased, the wine is run off from the residue or "lees," composed mainly of yeast cells and cream of tartar (KHT). The wine is kept in casks in which a further fermentation takes place, resulting in the deposition of more KHT. It is then put into casks to mature or "age." In making white wine the must is separated from the skins and stalks, while for red wine the skins of purple grapes are fermented with the must. In grapes the relative amount of sugar and albuminous matter varies. The yeast fungus lives on the latter, and if it is used up before all the sugar is fermented a sweet wine results; if the contrary happen, a non-sweet or "dry" wine is obtained. The grape juice also contains tartaric acid and its salts, and the proportion of acid to sugar best adapted for wine production is 1 to 40. In some parts, if the acid is in excess, the must is diluted, and sugar (glucose or cane sugar) added in the necessary amount. To make dry wines, white of egg, gelatin, or isinglass is added to feed the fungus until it ferments all the sugar.

ANALYSIS.—

Specific gravity: varies. *Extract.*—2·4 per cent.
Alcohol.—By distillation: 6 to 17 per cent.

Ash.—0·1 to 0·3 per cent. About one-sixth is P_2O_5.

Acidity.—(1) Fixed as tartaric. Dilute 20 c.c., boil down, repeat, add water, and titrate with N/10 NaOH, using phenolphthalein as indicator (0·5 per cent and under); (2) Volatile acid: 20 c.c. of the wine are well diluted, and then titrated with N/10 soda, using phenolphthalein as before. Deduct number of c.c. required for fixed acid from number now obtained, and multiply difference by 0·006 = volatile acid as acetic. The fixed acidity is calculated as tartaric: 1 c.c. N/10 NaOH = 0·0075 grm. tartaric acid.

Sugar.—Take 50 to 100 c.c., boil off the alcohol, remove colouring matter and other bodies with slight excess of basic lead acetate, filter, remove lead, and treat with Fehling's solution after dilution to 200 c.c. Varies from 0 to 5 per cent; in champagne, 4 to 10 per cent.

Preservatives.—Salicylic acid, formaldehyde, sulphites. Boric acid is said to be naturally present in some wines.

Colouring Matter.—Soak gelatin (10 per cent) cubes in sample for twenty-four hours, then cut them diagonally. Natural wine colour penetrates less than one-eighth of an inch, but artificial colours through and through. Or, the Paris Municipal Laboratory test: Take a piece of recently calcined lime and wet it with a few drops of the wine. Natural red wine gives a yellowish-brown coloration; wine coloured with fuchsin or Brazil wood gives a rose colour, and wine coloured with logwood gives a reddish violet. Or, baryta water is added until solution is green, and then acetic ether, and shake. Allow to stand until acetic ether separates, when if coloured, basic dyes present, if not, probably only natural colour. Or, add diluted KOH until alkaline, then mercuric acetate, filter; filtrate is red or yellow if acid aniline dyes present, colourless if pure wine colour.

Adulteration.—Addition of tannin, alum, catechu; plastering (addition of gypsum); blending; pasteurization; saccharin.

Piquette.—An artificial substitute for wine, manufactured in France (50 million gallons were made and consumed in 1898). One pound of raisins and one pound of dried apples are added to one gallon of water; expose mixture in an open vessel to the air for three days; then

bottle, adding one-half teaspoonful of sugar and a small piece of cinnamon to each bottle.

Cider.—Is the fermented juice of the apple. Contains about 3 to 5 per cent of alcohol.

Perry.—The fermented juice of the pear; alcoholic strength tends to be higher than in cider.

SPIRITS.

Spirits are all made by the distillation of alcohol produced by the fermentation of various saccharine or starchy materials, such as fruit juices, grain, and molasses. The essential constituent is ethyl alcohol, but they also contain varying proportions of other alcohols, ethers, and other fragrant bodies, and in some cases added substances.

Ethyl Alcohol ($C_2H_5 \cdot OH$) is commonly denoted simply alcohol. It is a liquid which boils at 78° C., and at 20° C. has a sp. gr. of 789. It oxidizes to acetic acid ($CH_3 \cdot COOH$.)

Brandy is made by the distillation of fermented grape juice. It is at first colourless, but is stored in casks which give it an amber colour. Sp. gr. is usually about 930, alcohol is 48 to 56 per cent, and total solids are about 0·1 per cent. Besides alcohol and water, it contains traces of various ethers, aldehydes, and acids (chiefly acetic). Imitation brandy is made from grain spirit flavoured with various esters and oils (cloves, cassia), and coloured with caramel.

Whiskey or **Whisky** (Gaelic, uisgebeatha—water of life) is made by distillation from malted barley, oats, or rye which has been fermented. It is stored in sherry casks which flavour and colour it. Numerous imitations exist, especially from potato spirit. Alcohol, 44 to 50 per cent; total solids, 0·15 per cent; acidity as acetic, 0·1 per cent. Specific gravity, 935 to 945.

Rum is made by the distillation of the fermented juice of the sugar cane or from molasses (the drained syrup from which sugar does not crystallize on boiling) and coloured with caramel. Its characteristic odour is due to ethyl butyrate. Alcohol, 40 to 50 per cent; total solids, 0·7 to 1·5 per cent.

Gin is made by distillation from fermented grain flavoured with juniper berries, oil of turpentine, oil of

juniper, coriander seeds, capsicum, etc., with or without the subsequent addition of cane sugar (to sweeten it). It acts on the kidneys. Alcohol, 30 to 40 per cent. Hollands and Schnapps are varieties from rye.

Proof Spirit is a term in use for excise purposes, denoting a dilute spirit of definite strength. It contains of absolute alcohol if expressed as (1) Volume in volume, 57·05 per cent ; (1 in 1·753) ; (2) Weight in volume, 42·46 per cent (1 in 2·355) ; (3) Weight in weight, 49·25 per cent (1 in 2·03). Spirits weaker than proof spirit are said to be under proof, and when stronger to be over proof. Sp. gr., 919·8 at 15° C. Whisky containing 60 per cent of alcohol volume in volume, is equal per 100 measures to 60 $\times$ 1·753 = 105·18 measures of proof spirit, and is said to be 5·18° over proof ; but if it contains only 30 per cent of alcohol volume in volume, then 100 volumes less 52·59 = 47·41 under proof.

ANALYSIS.—

Alcohol.—By direct distillation after diluting sample with an equal bulk of water to prevent loss of alcohol from too rapid evolution and imperfect condensation. By the Sale of Food and Drugs Amendment Act, 1879, brandy, whisky, and rum must not be weaker than 25° under proof (= 75 per cent proof spirit), and gin not weaker than 35° under proof (= 65 per cent proof spirit).

Acidity.—The fixed may be determined on the residue from the distillation by titration with N/10 baryta or soda, and phenolphthalein. The volatile is got by titrating the sample, deducting the number of c.c. required for fixed acid, and calculating as acetic acid. The fixed is returned as tartaric acid. Spirits should not contain any fixed acid.

Total Solids.—Evaporate on the water-bath and dry to constant weight in water-oven.

Ash.—Ignite residue.

Ethers (compound).—These are calculated as so many parts per 100,000 of alcohol, and the usual amount in brandy is about 100, in whisky from 10 to 90, and in rum the extremes are as great as 30 to 400.

Furfurol is an aldehyde, and is present in pot-still, but not in patent-still spirit. It is tested for by adding 10 drops of colourless aniline oil to 10 c.c. of distillate (made down to 50 per cent of alcohol), and 1 c.c. of acetic

acid (free from alcohol). A rose tint appears, which is compared with that from 10 c.c. of standard solution of furfurol (0·005 grm. per litre) similarly treated. From 1 to 3 parts per 100,000. Formula : $C_4H_3O{\cdot}CHO$.

Fusel Oil (*Amyl Alcohols*, $C_5H_{11}{\cdot}OH$).—Spirits should never contain more than 0·1 per cent amyl alcohol, as such. Tests :—Distil off ethyl alcohol from 100 c.c. on water-bath and take residue. Add an equal bulk of water, cool, and extract with ether. Let ether evaporate at room temperature and divide residue into three parts.

1. Heat one portion with sulphuric acid and a little potassium bichromate ; a smell of valerianic acid is obtained.

2. Heat with sulphuric acid and sodium acetate, when the odour of jargonelle pears (acetate of amyl) is got.

3. Warm with double its volume of strong sulphuric acid ; violet-red colour of amyl sulphuric acid is formed.

Aldehydes.—Vary from 10 to 40 parts per 100 litres of alcohol (100,000 parts).

Higher Alcohols.—From 100 to 250 parts per 100,000 parts of absolute alcohol.

Total Secondary Products, or " the co-efficient of impurities," is the sum total of the free acid, aldehyde, furfurol, ethers, and higher alcohols. According to the *Lancet* Commission this co-efficient varies for a specially fine brandy from 300 to 646, but may fall to 250 per 100,000 of absolute alcohol in inferior but genuine brandy. Grain and beet spirits are comparatively free of secondary products, furfurol especially being absent. Gin is also low in total secondary products. Jamaica rum is very high in ethers (400 grm. of ethyl acetate per 100 litres of absolute alcohol present) and contains more acids and furfurol than brandy. Whisky closely resembles brandy, but the furfurol is high.

Specific gravity is frequently ascertained by Sykes' hydrometer, the temperature of the liquid being noted, and the result obtained from special tables, which give the amount that the sample is over or under proof. If there is much solids the spirit must be distilled as for beer and wine.

Methyl Alcohol, $CH_3{\cdot}OH$ (wood spirit).—Is a liquid which boils at 66° C., and at 20° C. has a sp. gr. of 796. From it are derived formaldehyde ($H{\cdot}CHO$) and formic acid ($H{\cdot}COOH$).

CHAPTER VII.

DISINFECTANTS, ANTISEPTICS, AND DEODORANTS.

A DISINFECTANT is an agent which destroys the causes of disease and their products, such as fire, steam (saturated), boiling water, hot air, and chemicals in a proper strength (5 per cent carbolic acid, 5 per cent permanganate of potash, 0·1 per cent of perchloride of mercury, formalin, cyllin, lysol, etc., and Cl, Br, and I and ozone).

An antiseptic is an agent which arrests or impedes the growth of micro-organisms without destroying their vitality. Most of the disinfectants act thus in the weaker strengths, as do also borax and boracic acid, sulphites and sulphurous acid, salicylates and salicylic acid, essential oils, quinine and other alkaloids, and common salt.

A deodorant is an agent which masks or destroys the effluvia produced by certain micro-organisms. Deodorants include nitrous acid fumes, chlorine fumes (from chloride of lime), sulphurous acid fumes, fumes of wood, tar, and burnt paper, and some of the disinfectants in virtue of their oxidizing power or their strong odour, such as permanganate of potash and carbolic acid.

Direct sunlight and fresh air are powerful factors in, and aids to, disinfection, antisepsis, and deodorization.

Bleaching Powder is prepared by passing Cl gas over slaked lime, when a compound is formed having the formula Ca (OCl) Cl, which liberates Cl gas on treatment with acids. Theoretically it should contain 56 per cent of available Cl; a good bleaching powder commercially is one which gives 35 per cent of available Cl.

$$Ca(OCl)Cl + 2\,HCl = CaCl_2 + H_2O + Cl_2.$$

Process. Pinot's Method.—

Solutions required: (1) Standard arsenious solution. Dissolve 4·95 grm. of pure arsenious oxide in about

250 c.c. of water, along with 25 grm. of sodic carbonate. Boil for some time until all the oxide is dissolved, cool, and make up to 1 litre. This is decinormal strength and can be standardized against N/10 I.; (2) N/10 iodine solution; (3) Starch and KI paper as indicator. Take 2·5 grm. of sample and rub up with successive quantities of water until all the powder is transferred to a 250 c.c. flask to which the washings are added. Make up to the mark, shake well, and taking 20 c.c. of the milky fluid, titrate with the standard arsenious solution until starch and KI paper is no longer blued on being touched with a drop of the mixture removed on a glass rod. As excess of the arsenious solution is easily added, it is usual to now add starch to the titrated liquid and titrate with N/10 iodine until a permanent blue is got. The amount of the N/10 I used is deducted from the amount of arsenious solution required, and as 1 c.c. As_2O_3 solution = 0·00355 grm. Cl, or 3·55 mgr. Cl, the amount of Cl is easily calculated and is returned as a percentage.

$$2\,Ca(OCl)Cl + 2\,H_2O + As_2O_3 = As_2O_5 + 2\,CaCl_2 + 2\,H_2O.$$

Formalin is a 40 per cent solution in water of formaldehyde (CH_2O), the qualitative tests for which have been given on page 80. Quantitatively it is tested as follows: Weigh out 2·075 grm. of formaldehyde sample, and dilute with water to 500 c.c. Take 10 c.c. of this dilution and mix with 25 c.c. of N/10 iodine solution in a flask. Add NaOH solution (15 per cent) drop by drop until the liquid becomes clear yellow, and allow to stand for ten minutes. Then add sufficient dilute HCl to liberate the iodine not acted on by the formalin, and titrate the amount of I with N/10 thiosulphate; the number of c.c. of thiosulphate required subtracted from 25, gives the number of c.c. of N/10 I absorbed. 1 c.c. N/10 I = 0·0015 grm. CH_2O.

$$H{\cdot}CHO + H_2O + I_2 = H{\cdot}COOH + 2HI.$$

Permanganate of Potash.—The solution is titrated with standard ferrous sulphate solution in presence of sulphuric acid. Take 5 c.c. or 10 c.c. of sample (the smaller the amount the stronger the sample), add 10 c.c. of 25 per cent H_2SO_4, and from a burette add standard $FeSO_4$ solution until colourless. Calculate to a percentage.

Ferrous Sulphate.—Reverse the above process, using a standard solution of permanganate: $K_2Mn_2O_8 + 10\,FeSO_4 . 7\,H_2O + 8\,H_2SO_4 = K_2SO_4 + 2\,MnSO_4 + 5\,Fe_2(SO_4)_3 + 15\,H_2O$. The standard solutions can be made any suitable strengths, but as we have already in use a solution of permanganate, 3·95 grm. to 1 litre, it can be used for the ferrous sulphate: 1 c.c. = 0·03475 grm. $FeSO_4\,7H_2O$. Similarly, standard ferrous sulphate solution 34·75 grm. per litre, 1 c.c. = 3·95 mgr. permanganate.

Carbolic Acid, or phenol, C_6H_5OH, is detected *qualitatively:* (1) By its odour; (2) Add a few drops of ferric chloride—a violet coloration discharged by acetic acid (distinction from salicylic acid); (3) Bromine water gives a yellowish-white precipitate of tribromophenol, even in very dilute solutions; (4) Add one-fourth volume of ammonia and then a few drops of dilute bleaching-powder solution—a blue colour develops.

Quantitatively, carbolic acid is estimated by the process of Koppeschaar. The phenol is precipitated as tri-bromophenol by the addition of excess of bromine solution. The overplus of bromine is determined by adding potassium iodide from which the bromine displaces iodine, and the amount of the latter is found by titration with N/10 sodium thiosulphate solution.

$$C_6H_5\cdot OH + 3Br_2 = C_6H_2Br_3\cdot OH + 3HBr.$$

$$Br_2 + 2KI = 2KBr + I_2.$$

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI.$$

The bromine solution used may be N/10, or can have its strength estimated at the time in terms of iodine and sodium thiosulphate.

Process.—Weigh out 1·556 grm. of the sample, and dissolve in sufficient water to make 1000 c.c. Take 25 c.c. of dilution (= 0·0389 grm. of specimen) in a glass-stoppered bottle; add 30 c.c. N/10 bromine solution; 5 c.c. of strong hydrochloric acid; and 5 c.c. of potassium iodide solution (20 per cent weight in volume). Stopper bottle quickly and shake well. Remove stopper and wash neck and stopper, allowing washings to run into bottle. Add 1 c.c. of chloroform and shake mixture. Titrate with N/10

sodium thiosulphate solution, and number of c.c. of same required subtracted from 30 gives number of c.c. of N/10 bromine solution absorbed. From the quantities taken, this number, multiplied by four, gives percentage of phenol in sample.

The chief impurities in carbolic acid are the light and heavy coal-tar oils, which are largely composed of hydrocarbons of the benzene series. The light oils float on water, and the heavy sink; hence the terminology. In " carbolic powders " there is sometimes very little phenol, it being replaced by cresols or sulphites. Even when present the phenol may be rendered inert by having lime as a basis instead of silica. Pure phenol crystallizes in long colourless prisms, melts at 42° C., boils at 183°C., is soluble in water (1 part in 15 at 20° C.), and is very soluble in alcohol, ether, benzene, chloroform, carbon disulphide, and glacial acetic acid. Commercial phenol is a colourless crystalline mass, which gradually acquires a reddish colour, and deliquesces on exposure to the air.

Cresols are found in coal tar, give a blue colour with ferric chloride, and are otherwise similar to phenol. They are hydroxy derivatives of toluene or methyl-benzene, and are known as ortho-, meta-, and para-cresol, all of them being present in coal tar and in the tar from pine and beech woods. Ortho-cresol melts at 31° C. and boils at 188° C.; meta-cresol at 4° to 5° C. and 201° C.; and para-cresol at 36° C. and 198° C., respectively. Creosote is obtained by distillation of wood tar, and contains among other things cresols and guaiacol.

Carbolic Powders.—A good powder should contain not less than 15 per cent of tar acids (crude carbolic acid), of which 62·5 per cent is crystallizable at 15° to 20° C. when examined by C. Low's test. The base should contain no lime or chalk. Test reaction of powder with litmus. If alkaline, it contains free lime. If otherwise, the base is probably siliceous.

EXAMINATION.—

1. If the base is silica. Mix the powder well and take 50 grm. Extract with 150 c.c. of methylated spirit, which dissolves all the tar acids not in combination with lime. Now separate, and mix extract with 50 c.c. of 10 per

cent KOH or NaOH. Distil off spirit and evaporate to about 30 c.c., and cool. If any tar oils separate out, filter them off, run the filtrate into a burette, and add cautiously and a little at a time, 50 per cent sulphuric acid, until the alkali present is completely neutralized. The tar acids are liberated, and will rise to the surface and form a separate layer, the volume of which can be read off. As the sp. gr. of crude carbolic acid is about 1050, the volume $\times$ 1·05 will give the weight, and the result $\times$ 2 gives the percentage of available carbolic acid present in the powder.

2. This method extracts all the carbolic present, and is suitable for samples which contain free lime. 50 grm. of the previously well-mixed powder are placed in a large mortar, and a cold mixture of equal parts of sulphuric acid and water (i.e., 50 per cent) is added drop by drop, with constant stirring, until all the lime has been converted into sulphate, and the mixture is just acid. This is determined by repeated testing with litmus, removing and moistening a small fragment of the powder. The process must not be hurried, or heat sufficient to volatilize the free phenol present will be generated (takes one hour). The result is a dry powder, free from lumps. If it is moist, add some calcium sulphate. Now extract with four lots of ether, filtering each time the supernatant ether through a small filter into 50 c.c. of 10 per cent NaOH. Agitate well and distil off the ether (almost). Transfer to a separator, washing out flask with small quantities of water and ether, which are added to the same. Separate, wash ethereal layer with water, separate again, and mix with first lot. Reduce bulk by evaporation to 30 c.c., transfer to a burette, and treat as in (1).

Sodium Sulphite and Sulphurous Acid.—*See* page 121.

Zinc Chloride.—Estimate gravimetrically by precipitation with Am_2S, filter, dry, ignite, and weigh as ZnO.

Copper Sulphate.—Precipitate as CuO with NaOH. Collect, dry, ignite, and weigh as CuO.

* * * *

The "Lancet" Commission appointed to inquire into the Standardization of Disinfectants, issued a report, which

is published in the "Lancet" for 1909, *Vol.* ii., *pages* 1454 (*Chemical*), 1516 (*Bacteriological*), *and* 1612 (*Summary and Conclusions*).

The chemical part is here dealt with. Disinfectant is taken to mean a substance capable of destroying disease germs, or a "germicide," which is a preferable word.

Chemical Analysis.

Arguing that disinfection is a chemical process, the standards laid down are these :—

1. A standardized disinfectant should contain a certain proportion of an accredited germicidal substance ; and

2. If it is presented as a soapy mixture, which makes an emulsion with water, such dilution in water should show active Brownian movements of the particles distributed in the mixture.

The coal-tar disinfectants form the majority of those sold to the public. They consist of varying quantities of phenolic bodies, with inert tar oils, and in many cases soap and resins or other emulsifying agents, such as gelatin or dextrin, etc. The analysis of these mixtures necessitates the separation of the soap, resin, or oily hydrocarbons present, before the presence of phenol, cresol, or other phenoloid body can be properly tested for. The existing methods were found to be inconvenient, tedious, and troublesome, and the following method was devised and worked out by testing a large series of these mixtures. Distillation methods were avoided, because of the relatively large quantity of disinfectant required, and the inconvenience of the operations in any but a technical laboratory, while solvent processes were objected to, on account of the difficulty in separating the solvent, and also because no differentiation of the kind of phenol present was attempted.

The Lancet-Acetone-Baryta (L.A.B.) Method.—1. For fluids containing soaps and resins as emulsifiers. The process consists in making an emulsion with a known weight of the disinfectant mixed with water ; precipitating the soaps and (or) resins by adding a strong solution of

baryta ; filtering, when all the phenols are in the filtrate, and are estimated as later described. The residue on the filter contains the soaps and resins as barium compounds, and the neutral oils. On shaking it up with acetone, the neutral oils are dissolved out, and on filtration again, the residue left, if treated with HCl, yields free fatty acids and resins, which can be dissolved out with ether, the ether evaporated, and the residue weighed. The acetone filtrate containing the neutral oils can be examined for these.

Process.—Take 10 grm. of disinfecting fluid, in a 300 c.c. Erlenmeyer flask. Add 100 c.c. of distilled water, and shake well. Now add 15 grm. of barium hydrate crystals, attach flask to a reflux condenser, and digest at 100° C. for half an hour by immersion in a water-bath, shaking at intervals. Cool, and while cooling spread out the soaps and resins with a glass rod. Decant off clear liquid (baryta solution), or filter through an asbestos plug by aid of water suction. In either case, wash out flask with warm baryta solution, and decant after settling, or filter into previous filtrate, making up bulk to 250 or 300 c.c. exactly. Of the filtrate, 50 c.c. are taken in a separator, HCl is added till acid, and then some $CaCl_2$. The liberated phenols are extracted with ether. On separation the ether is evaporated by putting the fluid in a tall glass flask, which is put on a hot plate at 37° C., and evaporation encouraged by blowing a current of air into the flask. The residue is in this way also made water-free, and on cooling is weighed in the flask. The weight of the flask is deducted, and the remainder is the weight of phenol bodies present. This is tested, as if it were pure phenol, by the *bromine absorption process*. The residue is dissolved in NaOH, excess of bromine solution in NaOH added, and then HCl. The phenol present absorbs bromine. The amount of unused Br is measured by adding KI, and titrating the iodine liberated in place of bromine, with N/10 sodium thiosulphate solution. The difference from the amount of bromine added (determined by a blank experiment) is the amount of bromine absorbed. This multiplied by the figure 0·195, gives the equivalent amount of carbolic acid present. Any notable difference of this amount from the weight of

the residue, shows that the phenoloid present is not phenol (C_6H_5OH).

$$\underset{94}{C_6H_5.OH} + 3.\ \underset{480}{Br_2} = C_6H_2Br_3.OH + 3.HBr.$$

The water present may be obtained by adding to 25 grm. of the fluid, exactly 10 c.c. of 10 per cent sulphuric acid, and then 25 c.c. of petroleum (white spirit). Shake well, and allow to settle. The clear under liquid is accurately measured in a narrow graduated cylinder, and the increase above 10 c.c. is taken as amount of water in 25 grm. of disinfecting fluid.

The analyses undertaken showed a range of 5 to 66 per cent in phenolic bodies, and 39 to 94 per cent in inert bodies such as soap, resin, neutral oils, alkalies, and water.

2. For fluids containing neither soap nor resin as emulsifiers. There are preparations in which gelatin (in izal) and dextrin (in okol) are used as emulsifiers. In these the use of baryta is omitted, but the phenoloids are dissolved right away in excess of absolute alcohol or acetone, which also precipitates the gelatin or dextrin. Any neutral oils present are in this case along with the phenoloids, so that the residue left is the dextrin or gelatin. The neutral oils are got by adding to the solution in acetone, 10 per cent NaOH, and diluting freely. They may be dissolved out by addition of 20 c.c. of petroleum spirit. From the filtrate, the phenoloids are estimated as before.

Summary and Conclusions.

On tabulating the results along with the carbolic acid co-efficients found in the bacteriological investigation it was seen that the wider the difference between the percentage of phenoloids present and the equivalent of these in carbolic acid (calculated from the bromine absorption of the phenoloids), the higher was the carbolic acid co-efficient of the disinfectant for B. Coli. In fact, the differences between these two figures, that is the percentage of phenoloids and the calculated equivalent of this in carbolic acid, formed a series running parallel with that of the co-efficients, with few

exceptions. When these differences were all divided by 3, the series of numbers were in many cases identical with, or very close to, the carbolic acid co-efficient independently arrived at for each substance. In the cases where there was a divergence, the disinfectant did not form an emulsion with water, and the solution did not exhibit Brownian movements. Thus, it was deduced that the formula $(P - B) \div 3$ gives the carbolic acid co-efficient for B. coli of the disinfectant tested, where P is the percentage of phenoloids present, B the carbolic acid equivalent of these (calculated), and 3 an arbitrary constant.

From a chemico-physical point of view, it is concluded that for tar disinfectants, at any rate, they should contain a reasonable quantity of active bodies, phenols or phenoloids, and that the dilution of them in water should show active Brownian movements, that is, should form a satisfactory emulsion. The formula $(P - B) \div 3$ gives a ready means of estimating the carbolic co-efficient of a disinfectant, and its value should be at least over unity, that is, be equal to pure carbolic acid. A number of the disinfectants at present in common use give values of 5 to 9 by this method.

CHAPTER VIII.

CHEMICAL APPENDIX.

Table of Glaisher's Factors.

Reading of the dry-bulb therm. F.	Factor	Reading of the dry-bulb therm. F.	Factor	Reading of the dry-bulb therm. F,	Factor	Reading of the dry-bulb therm. F.	Factor
10°	8·78	33°	3·01	56°	1·94	79°	1·69
11°	8·78	34°	2·77	57°	1·92	80°	1·68
12°	8·78	35°	2·60	58°	1·90	81°	1·68
13°	8·77	36°	2·50	59°	1·89	82°	1·67
14°	8·76	37°	2·42	60°	1·88	83°	1·67
15°	8·75	38°	2·36	61°	1·87	84°	1·66
16°	8·70	39°	2·32	62°	1·86	85°	1·65
17°	8·62	40°	2·29	63°	1·85	86°	1·65
18°	8·50	41°	2·26	64°	1·83	87°	1·64
19°	8·34	42°	2·23	65°	1·82	88°	1·64
20°	8·14	43°	2·20	66°	1·81	89°	1·63
21°	7·88	44°	2·18	67°	1·80	90°	1·63
22°	7·60	45°	2·16	68°	1·79	91°	1·62
23°	7·28	46°	2·14	69°	1·78	92°	1·62
24°	6·92	47°	2·12	70°	1·77	93°	1·61
25°	6·53	48°	2·10	71°	1·76	94°	1·60
26°	6·08	49°	2·08	72°	1·75	95°	1·60
27°	5·61	50°	2·06	73°	1·74	96°	1·59
28°	5·12	51°	2·04	74°	1·73	97°	1·59
29°	4·63	52°	2·02	75°	1·72	98°	1·58
30°	4·15	53°	2·00	76°	1·71	99°	1·58
31°	3·60	54°	1·98	77°	1·70	100°	1·57
32°	3·32	55°	1·96	78°	1·69		

Short Alcohol Table.

Specific gravity at 60° F.	Volumes per cent of alcohol	Specific gravity at 60° F.	Volumes per cent of alcohol	Specific gravity at 60° F.	Volumes per cent of alcohol
1000·0	0·00	990·2	7·00	979·0	17·00
999·9	0·05	989·0	8·00	978·0	18·00
999·8	0·15	987·8	9·00	977·0	19·00
999·1	0·55	986·6	10·00	976·0	20·00
998·5	1·00	985·4	11·00	970·9	25·00
997·0	2·00	984·3	12·00	965·4	30·00
995·6	3·00	983·2	13·00	959·2	35·00
994·2	4·00	982·1	14·00	951·9	40·00
992·9	5·00	981·1	15·00		
991·5	6·00	980·0	16·00		

TABLE SHOWING THE WEIGHT OF 1 CUBIC FOOT OF WATER VAPOUR.

Temp. F.	Weight in grains of a cubic foot of vapour	Temp. F.	Weight in grains of a cubic foot of vapour	Temp. F.	Weight in grains of a cubic foot of vapour	Temp. F.	Weight in grains of a cubic foot of vapour
0°	0·55	26°	1·68	51°	4·24	76°	9·69
1°	0·57	27°	1·75	52°	4·39	77°	9·99
2°	0·59	28°	1·82	53°	4·55	78°	10·31
3°	0·62	29°	1·89	54°	4·71	79°	10·64
4°	0·65	30°	1·97	55°	4·87	80°	10·98
5°	0·68	31°	2·05	56°	5·04	81°	11·32
6°	0·71	32°	2·13	57°	5·21	82°	11·67
7°	0·74	33°	2·21	58°	5·39	83°	12·03
8°	0·77	34°	2·30	59°	5·58	84°	12·40
9°	0·80	35°	2·39	60°	5·77	85°	12·78
10°	0·84	36°	2·48	61°	5·97	86°	13·17
11°	0·88	37°	2·57	62°	6·17	87°	13·57
12°	0·92	38°	2·66	63°	6·38	88°	13·98
13°	0·96	39°	2·76	64°	6·59	89°	14·41
14°	1·00	40°	2·86	65°	6·81	90°	14·85
15°	1·04	41°	2·97	66°	7·04	91°	15·29
16°	1·09	42°	3·08	67°	7·27	92°	15·74
17°	1·14	43°	3·20	68°	7·51	93°	16·21
18°	1·19	44°	3·32	69°	7·76	94°	16·69
19°	1·24	45°	3·44	70°	8·01	95°	17·18
20°	1·30	46°	3·56	71°	8·27	96°	17·68
21°	1·36	47°	3·69	72°	8·54	97°	18·20
22°	1·42	48°	3·82	73°	8·82	98°	18·73
23°	1·48	49°	3·96	74°	9·10	99°	19·28
24°	1·54	50°	4·10	75°	9·39	100°	19·84
25°	1·61						

TABLE FOR ASCERTAINING THE SPIRIT VALUE OF ACETIC ACID IN BEER.

Percentage of acetic acid	Corresponding degrees of spirit indication.									
	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
0·0	—	0·02	0·04	0·06	0·07	0·08	0·09	0·11	0·12	0·13
0·1	0·14	0·15	0·17	0·18	0·19	0·21	0·22	0·23	0·24	0·26
0·2	0·27	0·28	0·29	0·31	0·32	0·33	0·34	0·35	0·37	0·38
0·3	0·39	0·40	0·42	0·43	0·44	0·46	0·47	0·48	0·49	0·51
0·4	0·52	0·53	0·55	0·56	0·57	0·59	0·60	0·61	0·62	0·64
0·5	0·65	0·66	0·67	0·69	0·70	0·71	0·72	0·73	0·75	0·76
0·6	0·77	0·78	0·80	0·81	0·82	0·84	0·85	0·86	0·87	0·89

TABLE SHOWING DEGREES OF SPIRIT INDICATION WITH CORRESPONDING DEGREES OF GRAVITY LOST.

Spirit Indication	Hundredths of a degree.									
Degrees and tenths	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
4·0	15·10	15·14	15·18	15·22	15·26	15·30	15·34	15·38	15·42	15·46
·1	15·50	15·55	15·60	15·65	15·70	15·75	15·80	15·85	15·90	15·95
·2	16·00	16·04	16·08	16·12	16·16	16·20	16·24	16·28	16·32	16·36
·3	16·40	16·44	16·48	16·52	16·56	16·60	16·64	16·68	16·72	16·76
·4	16·80	16·85	16·90	16·95	17·00	17·05	17·10	17·15	17·20	17·25
·5	17·30	17·34	17·38	17·42	17·46	17·50	17·54	17·58	17·62	17·66
·6	17·70	17·75	17·80	17·85	17·90	17·95	18·00	18·05	18·10	18·15
·7	18·20	18·24	18·28	18·32	18·36	18·40	18·44	18·48	18·52	18·56
·8	18·60	18·65	18·70	18·75	18·80	18·85	18·90	18·95	19·00	19·05
·9	19·10	19·14	19·18	19·22	19·26	19·30	19·34	19·38	19·42	19·46
5·0	19·50	19·54	19·58	19·62	19·66	19·70	19·74	19·78	19·82	19·86
·1	19·90	19·95	20·00	20·05	20·10	20·15	20·20	20·25	20·30	20·35
·2	20·40	20·45	20·50	20·55	20·60	20·65	20·70	20·75	20·80	20·85
·3	20·90	20·94	20·98	21·02	21·06	21·10	21·14	21·18	21·22	21·26
·4	21·30	21·35	21·40	21·45	21·50	21·55	21·60	21·65	21·70	21·75
·5	21·80	21·84	21·88	21·92	21·96	22·00	22·04	22·08	22·12	22·16
·6	22·20	22·25	22·30	22·35	22·40	22·45	22·50	22·55	22·60	22·65
·7	22·70	22·74	22·78	22·82	22·86	22·90	22·94	22·98	23·02	23·06
·8	23·10	23·15	23·20	23·25	23·30	23·35	23·40	23·45	23·50	23·55
·9	23·60	23·65	23·70	23·75	23·80	23·85	23·90	23·95	24·00	24·05
6·0	24·10	24·15	24·20	24·25	24·30	24·35	24·40	24·45	24·50	24·55
·1	24·60	24·65	24·68	24·72	24·76	24·80	24·84	24·88	24·92	24·96
·2	25·00	25·05	25·10	25·15	25·20	25·25	25·30	25·35	25·40	25·45
·3	25·50	25·55	25·60	25·65	25·70	25·75	25·80	25·85	25·90	25·95
·4	26·00	26·04	26·08	26·12	26·16	26·20	26·24	26·28	26·32	26·36
·5	26·40	26·45	26·50	26·55	26·60	26·65	26·70	26·75	26·80	26·85
·6	26·90	26·95	27·00	27·05	27·10	27·15	27·20	27·25	27·30	27·35
·7	27·40	27·44	27·48	27·52	27·56	27·60	27·64	27·68	27·72	27·76
·8	27·80	27·85	27·90	27·95	28·00	28·05	28·10	28·15	28·20	28·25
·9	28·30	28·35	28·40	28·45	28·50	28·55	28·60	28·65	28·70	28·75

TABLE SHOWING DEGREES OF SPIRIT INDICATION WITH CORRESPONDING DEGREES OF GRAVITY LOST (*continued*).

Spirit indication	Hundredths of a degree.									
Degrees and tenths	0·00	0·01	0·02	0·03	0·04	0·05	0·06	0·07	0·08	0·09
7·0	28·80	28·84	28·88	28·92	28·96	29·00	29·04	29·08	29·12	29·16
·1	29·20	29·25	29·30	29·35	29·40	29·45	29·50	29·55	29·60	29·65
·2	29·70	29·75	29·80	29·85	29·90	29·95	30·00	30·05	30·10	30·15
·3	30·20	30·25	30·30	30·35	30·40	30·45	30·50	30·55	30·60	30·65
·4	30·70	30·75	30·80	30·85	30·90	30·95	31·00	31·05	31·10	31·15
·5	31·20	31·25	31·30	31·35	31·40	31·45	31·50	31·55	31·60	31·65
·6	31·70	31·75	31·80	31·85	31·90	31·95	32·00	32·05	32·10	32·15
·7	32·20	32·25	32·30	32·35	32·40	32·45	32·50	32·55	32·60	32·65
·8	32·70	32·75	32·80	32·85	32·90	32·95	33·00	33·05	33·10	33·15
·9	33·20	33·25	33·30	33·35	33·40	33·45	33·50	33·55	33·60	33·65
8·0	33·70	33·76	33·82	33·88	33·94	34·00	34·06	34·12	34·18	34·24
·1	34·30	34·35	34·40	34·45	34·50	34·55	34·60	34·65	34·70	34·75
·2	34·80	34·86	34·92	34·98	35·05	35·10	35·16	35·22	33·28	35·34
·3	35·40	35·45	35·50	35·55	35·60	35·65	35·70	35·75	35·80	35·85
·4	35·90	35·96	36·02	36·08	36·14	36·20	36·26	36·32	36·38	36·44
·5	36·50	36·55	36·60	36·65	36·70	36·75	36·80	36·85	36·90	36·95
·6	37·00	37·05	37·10	37·15	37·20	37·25	37·30	37·35	37·40	37·45
·7	37·50	37·55	37·60	37·65	37·70	37·75	37·80	37·85	37·90	37·95
·8	38·00	38·06	38·12	38·18	38·24	38·30	38·36	38·42	38·48	38·54
·9	38·60	38·65	38·70	38·75	38·80	38·85	38·90	38·95	39·00	39·05
9·0	39·10	39·16	39·22	39·28	39·34	39·40	39·46	39·52	39·58	39·64
·1	39·70	39·75	39·80	39·85	39·90	39·95	40·00	40·05	40·10	40·15
·2	40·20	40·25	40·30	40·35	40·40	40·45	40·50	40·55	40·60	40·65
·3	40·70	40·75	40·80	40·85	40·90	40·95	41·00	41·05	41·10	41·15
·4	41·20	41·25	41·30	41·35	41·40	41·45	41·50	41·55	41·60	41·65
·5	41·70	41·75	41·80	41·85	41·90	41·95	42·00	42·05	42·10	42·15
·6	42·20	42·25	42·30	42·35	42·40	42·45	42·50	42·55	42·60	42·65
·7	42·70	42·75	42·80	42·85	42·90	42·95	43·00	43·05	43·10	43·15
·8	43·20	43·25	43·30	43·35	43·40	43·45	43·50	43·55	43·60	43·65
·9	43·70	43·75	43·80	43·85	43·90	43·95	44·00	44·05	44·10	44·15

Part II.

PUBLIC HEALTH BACTERIOLOGY.

BACTERIOLOGY is one of the more recent sciences, and is becoming larger and more complex every year. It is now of extreme importance to the Hygienist, as it is also to the Agriculturalist, and in various Arts and Industries. A short glance at its evolution will serve to make the methods and processes to be hereinafter studied more intelligible.

Leeuwenhoek, a native of Delft, in Holland, produced the first really good microscope and, on examining with it the contents of the intestinal canal in horses, frogs, pigeons, and fowls, and his own diarrhœa stools, he saw small moving and living forms. This was in 1675, and eight years later he examined tartar scraped from teeth, and described and depicted minute organisms such as we recognize at the present day. These discoveries accorded with various theories which were prevalent at the time, and a micro-organismal basis for disease became widely accepted, which, though much the same as prevails now, was built on very slender foundations. Plenciz thereafter insisted on the specific character of the contagious diseases, and explained the incubation period as dependent on the growth of a germ in the body, which had not yet made its presence manifest. Müller systematized the morphology. Then the theory of spontaneous generation or abiogenesis arose. Dr. Needham boiled a beef infusion, kept it in a well-stoppered bottle, and found that, on keeping, it putrefied. He argued that the boiling killed all the organisms in the infusion, and that therefore the putrefaction of the infusion pointed to the existence of a special vegetative force which produced fresh organisms. Spallanzani worked at the subject, and found that on boiling for one hour and then hermetically sealing, no putrefaction ensued. It was objected that the air was shut from the vessel, and that this might be necessary. This objection was met by Schulze in 1836 by fitting a flask with right-angled tubes,

and filtering all the air through sulphuric acid in the one and potash in the other. Schroeder and von Dusch found (in 1854) that filtration through cotton-wool sufficed, and then Hoffmann, Chevreul, and Pasteur showed that it was quite sufficient to draw out the neck of the bottle and bend it downwards; and they argued that germs obeyed the laws of gravitation in the absence of wind. Pasteur demonstrated that there was a causal relation between certain lowly organized parasitic organisms and certain diseases of animals and insects. His conclusions were attacked on two grounds: (1) that these organisms were not the cause of disease at all, and (2) that the germs were not specific, i.e., one for each fermentation or disease. The second objection was a forcible one because, so far, he had not been working with pure cultures. The first one was met by the researches of Lemaire, who proved that carbolic acid was hurtful to the life of the higher animals and plants, and that the addition of a small quantity of it to fluids prevented the incidence of putrefaction and fermentation but did not retard the action of diastase or synaptase. He applied the same reasoning to the treatment of wounds, and reduced pus to a minimum and got rapid healing, which results he attributed to the destruction of the microzoa and infusoria by the carbolic acid lotions. Lister saw the great importance of Pasteur's work, and on it and independent research he built up against considerable opposition the theory and practice of antiseptic surgery. Pathogenic bacteria were also being studied, beginning with Bacillus anthracis, which was first observed by Pollender in 1849, and described by Davaine and Rayer in 1850 as motionless, thread-like organisms and rods, found in the blood taken from animals affected with splenic fever. In 1863 Davaine suggested that these rods were the actual and specific cause of the disease, and in 1864 he demonstrated (not rigorously) that malignant pustule and splenic fever were forms of one infection. Confusion was introduced by the revival of the theory of polymorphism in the form that all contagia and miasmata are the products of fungi or algæ, and on account of their small size are able to pass through the fine capillary vessels, and that when a micrococcus was found it was only

necessary to trace it back to some parent fungus or mould. This not improbable theory was, even in its crude form, not easily met, as at that time no one was working with pure cultures. Klebs in 1872 described in pus, rod-like bodies and "microspores," grouped in short chains or in longer threads; and he filtered the pus through baked clay cylinders, and found that the filtrate on injection into the blood or under the skin gave constitutional symptoms, but did not induce suppuration nor cause death; though if to it were added a small quantity of the micro-organisms, a true pyæmia was produced.

Koch in 1876 demonstrated the specificity of the anthrax bacillus by making it satisfy the following four conditions, commonly called Koch's postulates, namely:

1. The anthrax bacillus was invariably found in the blood or tissues of animals affected with the disease.

2. The bacillus was cultivated in artificial media for an indefinite number of successive generations.

3. The same disease was produced by inoculation of a susceptible animal with the last cultivation.

4. In every such inoculated animal the specific microbe was found, and similarly distributed as in animals infected in the ordinary way.

To these Martin adds:—

5. The secondary infective agent or toxin separable from the tissues in the natural disease, should have similar chemical and physiological actions to the products obtained from a pure cultivation of the organism.

Koch succeeded in doing this by being able to make pure cultures of the anthrax bacillus on the aqueous humour of the eye of the ox, and in this way was able to carry out the further procedures, and to accept the results as due to the single substance inserted.

Numerous similar investigations were now made, and served to corroborate the soundness of the germ theory of disease.

The introduction of solid media by Koch in 1882 paved the way for an enormous advance in bacteriological technique, and numerous discoveries of specific organisms followed, or specificity was established:—Staphylococci in 1880–83, Streptococci 1881–84, Micrococcus tetragenus

1881, Gonococcus 1879–85, Pneumobacillus 1883, Pneumococcus 1884, Meningococcus 1885, Tubercle bacillus 1882, Bacillus mallei 1882, B. typhosus 1880–84, B. coli, 1886, Klebs-Loeffler 1883–84, Micrococcus melitensis 1887, B. enteritidis 1888, B. tetanus 1884–89, B. pestis 1894, B. enteritidis sporogenes 1895, Cholera v. 1883, B. botulinus, B. paracolon and paratyphosus, and B. Morax-Axenfeld, all in 1896.

CHAPTER IX.

GENERAL PRINCIPLES.

BACTERIOLOGICAL MEDIA

may be thus classified :—

Nutrient Broth, standardized.
Derivatives : Glucose broth, lactose broth, nutrient gelatin, nutrient agar-agar, glycerin agar, glucose agar, lactose agar.

Peptone Water.
Derivatives : Glucose peptone water, lactose peptone water, sucrose and mannite peptone water.

MacConkey's Media.— Bile-salt litmus glucose peptone water ; bile-salt neutral-red lactose agar.

Other Media.—Milk, potato, blood serum, ascitic fluid, urine, whey, gelatin agar, beer wort, bread, eggs ; nitrate media, synthetic media ; animal tissues, etc.

Nutrient Broth.—500 grm. of lean beef finely minced are steeped in one litre of ordinary water for twenty-four hours in a cool place. The fat particles are then skimmed off, the fluid strained off, and the juice well pressed out. This is then boiled for half an hour to coagulate the albumins, filtered, and the bulk made up to 1 litre. One per cent of Witte's peptones and ½ per cent of common salt are then added and dissolved by the aid of heat. The broth is now tested as to its reaction, and is usually acid. Its acidity is determined by taking 5 c.c., diluting to 50 c.c.

with water, adding 1 c.c. of phenolphthalein, and titrating with N/10 NaOH. Boil one minute before titration. Calculate the amount of N/1 NaOH required for the litre of broth made. The convention at present in use is to leave the broth acid to phenolphthalein to the extent that 1 c.c. of N/1 NaOH is required to neutralize 100 c.c. of broth, or 10 c.c. per litre, and the medium is said to be "acid + 10," or + 1 per cent. Therefore add the calculated amount of N/1 NaOH less 10. (Some add the full amount of soda and then make acid to the desired extent with N/1 HCl.) Heat to boiling and test again; if it needs a further correction, boil again. Allow to cool to bring down the precipitate of MgAm phosphate caused by the change of reaction. Filter, place in flasks or tubes (about 5 c.c.), and sterilize in autoclave at 120° C. for 15 minutes, or at 130° C. for 1 minute, or for 15 to 60 minutes on three successive days in a steam sterilizer at 100° C.

Broth can also be made from Liebig's Extract of Meat, using 0·5 per cent of it instead of mince-meat. The other procedure is similar. Neutralization can also be effected by adding saturated solution of NaOH until red litmus is just turned blue.

Meat Extract or Fleischwasser can be used as a basis for broth and the media derived from it. It is made by warming 500 grm. of minced beef or horseflesh with 1 litre of water at 50° C. for half an hour, and then boiling for half to three-quarters of an hour. Filter, strain, make up to 1 litre, and then pour into a flask; if not to be used at once, sterilize.

Broth contains some muscle sugar or inosite, and to get rid of this is at times inoculated with a young culture of B. coli and incubated at 37° C. for 18 hours, and then boiled to kill the organisms.

Glucose and *Lactose* are added to sugar-free broth (usually 1 per cent). Such media are not sterilized in the autoclave but in a steamer, because the sugars are not stable at high temperatures.

Nutrient Gelatin is made from broth by adding 10 per cent in winter and 15 per cent in summer of "gold label" gelatin. Heat on water-bath (as little as possible) to dissolve; readjust reaction (gelatin makes the medium

acid), filter, and sterilize in the steamer. If filtrate is not clear, cool to 60° C., add whites of two eggs per litre, re-heat for half-an-hour in steamer, and filter through Chardin paper in warm filter-jacket.

Nutrient Agar.—Add 1·5 per cent of powdered agar to broth. Melt in the steamer at 100° C. for 1·5 hours. Standardize, and replace in steamer for 20 minutes to precipitate phosphates. Cool to 60° C., add two whites of egg per litre, reheat for half an hour, filter through Chardin paper by aid of a hot-water funnel or in a steamer, or filter through glass-wool. Tube, and sterilize. Agar melts between 90° and 100° C. and remains fluid down to 40° C.

Glycerin Agar. — Add 6 per cent of glycerin after filtration ; tube, and sterilize.

Glucose and Lactose Agar.—To agar made with sugar-free broth, add 2 per cent of glucose or lactose. If to be tinted with neutral red, add before filtration 2 per cent of a solution of neutral red ($\frac{1}{2}$ per cent).

Litmus Lactose Agar.—Add to nutrient agar prepared from sugar-free broth 1 to 2 per cent of lactose, and sufficient litmus to give a good colour (about 5 to 10 c.c. of a 1 per cent litmus solution per 100 c.c. of total medium). Conradi and Drigalski's medium is similar, plus nutrose and crystal-violet.

Peptone Water.—Dissolve by the aid of heat 1 per cent of peptones and $\frac{1}{2}$ per cent of NaCl in distilled water. Tube, and sterilize. For water investigations it is usual to keep a stock solution ten times this strength.

Glucose, lactose, sucrose, and mannite are used with peptone water plus Durham's fermentation tubes. Mostly in 1 per cent strength.

MacConkey's Media are much used in water examinations in this country.

Bile-salt Litmus Glucose Peptone Water is made in single strength, and in triple strength thus :—Peptone, 20 or 60 grm. ; glucose, 5 or 15 grm. ; taurocholate of sodium, 5 or 15 grm. ; litmus solution (10 per cent sterile) 100 c.c., and water to 1 litre. Put peptone, glucose, bile-salt, and water in a flask and heat in steamer for 45 minutes. Filter through Chardin's paper, add the filtered litmus

solution, place in tubes, and put in Durham's fermentation tubes. Steam for 45 minutes on two successive days. Double strength is also used. In tubing put 10 c.c. of single strength, 10 c.c. of double, and 50 c.c. of triple, into suitable tubes. To these are added respectively 1 c.c., 10 c.c., and 100 c.c. of the water sample.

Bile-salt Neutral-red Lactose Agar is composed of agar 20 grm., peptones 20 grm., lactose 10 grm., bile-salt 5 grm., neutral-red aqueous sterile solution (1 per cent) 4 c.c., and water to 1 litre. Dissolve the agar, peptones and bile-salt in 500 c.c. of water by heating in the steamer for 90 minutes. Add rest of water, cool to 60° C., add the white of one egg, heat in steamer for 45 minutes, filter through a moistened Chardin filter-paper in a warm filter-jacket, heat filtrate in steamer for 15 minutes, add lactose and neutral red, put in tubes, and steam for 30 minutes on two successive days. The medium requires no alkali.

Other Media.—

Milk.—Fresh milk free from preservatives, with the cream removed, and giving an amphoteric reaction to litmus, is poured into tubes and heated in the steamer for three successive days at 100° C. (Above 110° C. browns it.) To prove sterility, incubate for at least three days at 37° C. before using.

Litmus Milk.—Add sufficient litmus to colour.

Potato. — Scrub and wash a potato; bore a cylinder-shaped piece; split it diagonally and put each half into a sterile tube, with a pad of wool at the bottom, and half an inch of aq. dest. Plug, and sterilize at 100° C. on three days.

Blood Serum. — Collect blood in a sterile cylinder and allow to clot. Set aside for twenty-four hours in an ice chest. Pipette serum into tubes and place these in a sloping position in inspissator at 75° C. for one hour. Repeat on two successive days. When cool, incubate for twenty-four hours at 37° C., and if no growth, they may be considered sterile.

Litmus Whey (Petruschky's). — Mix milk with equal quantity of water, heat to 40° to 50° C., and add dilute HCl to precipitate casein. Filter, neutralize with NaOH, and heat for one or two hours in steamer, filter till

clear, and if necessary neutralize again. Add sterile litmus until a violet hue is produced. Tube, and sterilize. A good medium for observing change of reaction.

STERILIZATION AND DISINFECTION.

1. Dry heat:
   - (*a*) Bright red heat of flame: for platinum needles.
   - (*b*) Dull red heat of flame: for knives, glass rods, etc.
   - (*c*) Hot air: 170° C. for 1 hour: for glass-ware and cotton-wool.
2. Moist heat:
   - (*a*) Boiling in water at 100° C.: for 5 minutes kills all non-sporing forms: for 1½ hours kills spores also.
   - (*b*) Steam at 100° C.: Koch's steam sterilizer: 1½ hours' full steaming, or 15 minutes' full steaming on three successive days: used for all media.
   - (*c*) High-pressure steam: in autoclave: at 115° C., 2 minutes for germs, 15 minutes for spores. Never used for gelatin media, or will not re-solidify. Never used for carbohydrate media, as decomposed into other sugars.
3. Chemicals: 5 per cent carbolic, 0·1 per cent perchloride of mercury, etc. Allow to remain in contact for half-an-hour.

Discontinuous sterilization at 57°–75° C. is used for media, like blood serum, that are changed at higher temperatures. The object is submitted to 60 minutes' heating, and kept at 20° to 37° C. until next day, when the heating is repeated, and the same procedure repeated for 3 to 8 days. This is to cause spores present to assume the vegetative form and then to kill the same on re-heating.

CULTURAL METHODS.

1. Inoculation of liquid media, solid media, living media.
2. Isolation of pure cultures by (*a*) serial inoculation; (*b*) plate cultivation (serial dilution); (*c*) differential

sterilization; (*d*) aerobic and anaerobic cultivation; (*e*) deterrent media; (*f*) favouring media; (*g*) inoculation into susceptible animals.

3. Preparation of toxins, vaccines, and sera.

4. Post-mortem examination of bodies and tissues.

5. Examination of blood, pus, sputum, urine, cerebro-spinal fluid, exudates, and dust, air, water, milk, sewage, soil, shell-fish, water-cress, etc.

MODES OF STUDY.

Cultures.—Growth on or in various media; liquefaction of media; gas production; acid or alkali production; indol formation; colour formation (pigment); colour reduction; proteinchrome formation; sulphuretted hydrogen production; phosphorescence; nitrate reduction; toxin formation; ferment production and effects.

Morphology.—Form, motility, flagella, sporing, pleomorphism, colour, staining reactions, capsulated.

Resistance to desiccation, dry heat, moist heat, chemical agents, sunlight, ultra-violet rays.

Optimum Temperature for growth, and toxin and ferment formation.

Pathogenicity for (*a*) man, (*b*) animals, (*c*) plants.

Products of Growth in Host and Culture—toxins soluble and insoluble, ferments.

Habitat.

Immunity.—Mode of production; antitoxins, alexins, complement, phagocytosis, opsonins, amboceptors, antibodies, agglutinins, precipitins, aggressins.

Anaphylaxis (from Gr. "against protection")—*opposite of Immunity.*—A state of excessive susceptibility induced in animals by the injection of certain substances (blood serum, white-of-egg, milk, etc.)

CULTURAL REACTIONS.

Inoculate various media and observe results from day to day on incubation at 20° or 37° C. (as directed). Label tubes with name of organism and date of inoculation, or mark with pencil.

Broth.—Inoculate from agar cultures three broth tubes, one with each of the following bacilli: B. fluorescens liquefaciens, B. subtilis, and B. mycoides. Incubate at 20° C. and examine in twenty-four or forty-eight hours. Culture of B. fluorescens liquefaciens, quite turbid, or "universal turbidity"; of B. subtilis, quite clear but scum is formed; of B. mycoides, quite clear but deposit is formed.

Gelatin.—Three forms of culture: (1) slant or streak, (2) stab, (3) shake.

1. *Slant* or *streak* — for non-liquefying organisms. Inoculate sloped tubes with B. coli communis and Torula alba. Incubate at 20° C. and examine after forty-eight hours.

B. coli communis, spread over surface; Torula alba, growth limited to line of inoculation.

2. *Stab culture*—to observe presence or absence of liquefaction. Inoculate gelatin tubes by stabbing with B. mycoides, B. megatherium, and Vibrio Finkler-Prior. Incubate at 20° C. and examine after twenty-four, forty-eight, and seventy-two hours.

B. mycoides, horizontal liquefaction; B. megatherium, funnel of liquefaction medium width; V. Finkler-Prior, funnel of liquefaction wide.

3. *Shake culture*—to observe gas formation. Melt gelatin at 40° C. on water-bath and inoculate as in the case of broth. Place in rack, and allow to solidify. Incubate for forty-eight hours. Use B. coli communis and B. subtilis.

B. coli communis, gelatin full of gas bubbles; B. subtilis, no gas formed.

Agar.—Inoculate sloped agar tubes with the following germs, incubate at 37° C., and examine after twenty-four hours.

Results should be as follows:—B. subtilis, dry mycoderma; B. mycoides, fine filaments; B. megatherium, confluent moist raised growth; B. proteus, thin transparent growth over whole surface.

Potato.—Inoculate, incubate at 37° C., and examine in twenty-four hours.

B. subtilis, flesh-coloured mycoderma ; Streptococcus, invisible growth ; B. megatherium, a yellow, raised, moist growth.

Blood Serum.—Inoculate, and incubate at 37° C. for two to three days, and examine.

B. coli communis, serum solid ; B. pyocyaneus, serum liquefied.

Milk.—Inoculate, incubate at 37° C. for forty-eight hours, and examine.

B. coli communis, milk clotted and acid ; B. denitrificans, no clot, alkaline ; B. pyocyaneus, casein precipitated and partly dissolved ; Streptococcus, no clot, slightly acid.

MacConkey's Broth with Durham's tubes. Inoculate, incubate at 37° C., and examine daily.

B. coli, acid and gas ; B. typhosus, acid, no gas.

Peptone Water.—Inoculate, incubate at 37° C., and examine in four or five days.

B. coli communis, indol formed ; B. typhosus, none ; Sp. choleræ, nitroso-indol.

Aerobic and Anaerobic.—Make gelatin stabs and incubate at 20° C. for two days.

B. zopfii, growth only on surface (strict aerobe). Torula alba, on surface and in depth (aerobe and facultative anaerobe). B. butyricus, growth only in depth (strict anaerobe).

Colour Formation.—

1. Inoculate two agar tubes with B. prodigiosus, and incubate ; (1) at 37° C., (2) at 20° C. Examine in forty-eight hours. (1) is white or grey, (2) is pink.

2. Presence of oxygen is necessary in most cases for pigment to be developed. Make two gelatin stab cultures of Bacillus fluorescens liquefaciens. Incubate one aerobically and the other anaerobically. Examine in forty-eight hours. The aerobic culture is pigmented, the other is not.

3. A few require absence of oxygen. Make gelatin stab of Spirillum rubrum and incubate at 20° C. for 3 to 4 days, when growth is found in depth to be red and on surface to be white.

Colour Reduction is measured by the addition of some easily discoloured substance to the medium. Litmus, methylene-blue, and sodium sulphindigotate are used. As the bacteria grow, the colour is discharged in the anaerobic parts of the culture. In a fluid medium, shaking restores the colour.

Proteinochrome formation is observed in 5 per cent peptone broth or 3 per cent peptone water. Add a few drops of acetic acid and then fresh chlorine water, when a red-violet colour indicates proteinochrome formation.

Test for Indol Formation.—To a pure culture in broth or peptone water add 1 c.c. of 0·01 per cent sodium nitrite solution and 1 c.c. of purest sulphuric (25 per cent), or HCl. A red coloration within five minutes indicates that indol is present. A second test which gives a positive result with B. coli within forty-eight hours at 37° C. is to add 1 c.c. of an acid solution of paradimethylamido-benzaldehyde, when a rose or cherry-red colour develops in 2 or 3 minutes if indol is present. (Solution A.: para. 8 grm., HCl 160 c.c., absolute alcohol 760 c.c.; Solution B.: cold saturated solution of potassium sulphate. Use 1 c.c. of A, and shake, and add 1 c.c. of B. and allow to stand.) If there is any suspicion of the micro-organism having the power of reducing nitrates to nitrites, add the sulphuric acid first and wait; if no coloration develops, then add the nitrite solution.

Spirillum choleræ has this power, and hence the addition of the sulphuric acid is alone required. This is called the *nitroso-indol* reaction.

LIQUEFACTION OF GELATIN.

This is due to the development of enzymes from the growth of bacteria in proteid media. These proteolytic enzymes are not always secretions of the bacterial cell, but are in some cases closely bound to the cell-body, and are separable from it only after its death. When they are true secretory products, they can be separated from the micro-organisms by filtration through a Berkefeld filter candle, and from such filtrates they can be obtained in

the dry state by precipitation with alcohol. Such enzymes are usually more thermostabile than when in solution. Thus, most enzymes are readily destroyed in solution at 70° C., but dry enzymes may withstand 140° C. for 10 minutes. (As usual, moist heat is more effective than dry heat.)

This proteolytic (protein-splitting) or peptonizing power varies for the different proteids, and is usually tried on gelatin, blood fibrin, and casein of milk. Thus, Staphylococcus pyogenes liquefies gelatin and blood-serum, and clots milk, but does not dissolve the casein ; Streptococcus pyogenes does not liquefy gelatin, nor blood serum, nor casein ; B. coli communis is likewise negative to all three tests ; some varieties of B. proteus are positive to all three ; B. pyocyaneus is positive to the three ; Spirillum choleræ liquefies gelatin and blood serum, but not casein ; and so on. These tests are still very useful in dividing the bacteria into groups, and so narrowing the field in the difficult task of concluding, with moderate certainty, the race of a particular germ.

Gelatin—liquefying	Gelatin—non-liquefying
Staphylococci	Streptococci
B. anthracis	Pneumococci
B. tetani and botulinus	M. tetragenus and melitensis
B. enteritidis sporogenes	Colon-typhoid group
B. œdematis maligni	B. diphtheriæ
Sp. choleræ and most Sp.	B. mallei
B. cloacæ	B. pestis
B. proteus	Friedlaender's pneumobacillus
B. subtilis	Yeasts (most)
B. pyocyaneus	
Actinomyces	
Moulds (most)	

Note.—Organisms which do not grow on gelatin or at air temperature cannot be thus classified.

HÆMOLYSIS.

Hæmolysis will be treated of under immunity, but the present reference is to the hæmolytic action of certain organisms when grown on blood-agar plates. (Blood agar is made from defibrinated blood 1 part, and agar 2 parts.)

In such a medium, hæmolysis (destruction of the red blood-cells) is shown by a yellow transparent halo around the colonies. Organisms producing hæmolysins, are:—*Staphylococci*, *Streptococci*, *some Spirilla* (but not *Sp. choleræ*).

STAINING REACTIONS AND METHODS.

Saturated alcoholic solutions are kept as stock, and diluted 1 in 10 with water as required, and filtered. Rather use dilute stains and for a longer time, than have precipitate of stain on preparation. Stains can be reduced in intensity if necessary by using dilute acids, commonly acetic 1 per cent. Acid stains, like eosin, stain the protoplasm of cells, whereas basic stains, like gentian-violet, methylene-blue, and fuchsin, stain the cell-nuclei and bacteria.

Blood, pus, and smears from agar plates stain most sharply with methylene-blue, but stain fades rapidly on keeping. Certain bacteria need special stains.

Loeffler's Methylene-blue.—Saturated alcoholic solution methylene-blue 30 c.c.; solution KOH (0·01 per cent) 100 c.c. Keeps well.

Aniline Oil-Water Stains.—Made with saturated alcoholic solutions of gentian-violet and fuchsin, which are mixed 1 in 10 of aniline oil-water. The latter is a mixture, made by shaking 5 c.c. of aniline oil in 100 c.c. distilled water; filter, and keep in dark. These keep badly.

Carbol-fuchsin (Ziehl-Neelsen).—Ac. carbolic (5 per cent) 100 c.c.; saturated alcoholic solution fuchsin 10 c.c. Diluted 3 to 4 times it stains more slowly but better. Keeps well.

Carbol-glycerin-fuchsin.—Fuchsin 1 grm., ac. carbolic liq. 5 c.c., glycerin 50 c.c., and aq. dest. 100 c.c. Dilute in use 4 to 10 times. Keeps well.

Carbol-methylene-blue.—Methylene-blue 1·5 grm., absolute alcohol 10 c.c., ac. carbolic (5 per cent) 100 c.c. Keeps well.

To Make a Film.—Take a cover-slip (in Cornet's forceps), and put on it a drop of distilled water (small for fear of plasmolysis). Take two strokes of culture with platinum needle and rub into drop, and spread out. Dry in air

(should dry at once). Fix by passing three times through the flame. Stain for two or three minutes. Wash with water and examine on clean slide in water-drop (using oil immersion). In water-drop bacteria look larger, can be restained, and can be kept longer than when mounted direct in Canada balsam. To preserve: allow to dry, remove from slide, roll up, and label.

Counterstain with eosin or other stain in dilute solution for 1 to 2 minutes. Eosin stains cell protoplasm red.

Films are also made on slides, which are more easily handled than coverslips.

Gram's Method of Staining—Depends on the fact that some bacteria when well stained retain the stain after treatment with a solution of iodine and subsequent washing with alcohol (strong or absolute). This is believed to be due to the stain and the iodine forming a combination which resists decoloration.

TABLE.

Gram-positive	Gram-negative
(*Retain the gentian-violet*)	(*Take the counterstain*)
Staphylococcus pyogenes	Meningococcus
Streptococcus ,,	Gonococcus
Pneumococcus	Micrococcus melitensis
Micrococcus tetragenus	,, catarrhalis
Bacillus anthracis	Colon-typhoid group of bacilli
,, subtilis	Cholera group of spirilla
,, diphtheriæ	Bacillus pestis and group
,, tetani	,, mallei
,, botulinus	,, influenzæ
,, tuberculosis and other acid-fasts	,, pyocyaneus
,, aerogenes capsulatus	,, proteus
,, enteritidis sporogenes	,, Koch-Weeks
,, of swine erysipelas	,, Morax-Axenfeld
,, of mouse septicæmia	,, maligni œdematis
,, of potato	,, anthracis symptomatici
Yeasts and many moulds	,, of fowl cholera
Streptothrix actinomyces	,, of rabbit septicæmia
	Spirillum Obermeieri (spirochæte)
	Friedlaender's diplobacillus

METHOD.—Stain for 5 minutes with aniline-oil-gentian-violet, or carbol-gentian-violet. Pour off excess of stain

and cover with Lugol's (or Gram's) solution of iodine (iodine 1, KI 2, aq. dest. 300) for 30 seconds to 2 minutes. Wash with 97 per cent alcohol (or methylated spirit) until washings are no longer coloured (takes about 30 sec. to 2 min.). Examine in water, or dry and mount in balsam. To counterstain: remove alcohol with water and cover with dilute carbol-fuchsin for a few seconds (or saturated watery solution of Bismarck brown for longer). Wash in water, dry, and mount. Result: Bacteria blue-black, or colourless, or red; tissues red. Those bacteria which are blue-black are said to be Gram-staining or Gram-positive. The others are said to be Gram-negative. (See Table on previous page.)

Acid-fast Bacteria.—Some bacilli stain with difficulty with ordinary dyes, requiring the aid of heat or a mordant (as carbolic). Such bacilli usually retain the stain even when treated with dilute acids and alcohol, and hence are called "acid-proof" or "acid-fast." This resistance is believed to be due to the presence in the cell-body of a waxy substance (an alcohol). The members of this group are: Bacilli of human, bovine, avian, and fish tuberculosis; Moeller's Timothy-grass bacilli (1) and (2); Mist-bacillus; Rabinowitch's butter bacillus; Korn's butter bacilli (2) and others; Johne's bacillus (of chronic bovine pseudo-tuberculous enteritis); Bacillus smegmatis (smegma bacillus); Bacillus lepræ (leprosy bacillus).

METHOD.—Flood slide or cover-glass with carbol-fuchsin and heat for 3 minutes. Wash and decolorize by dipping into 5 per cent sulphuric acid and 60 per cent alcohol alternately until film looks colourless. Wash in water. Counterstain with aqueous methylene-blue for a half to one minute. Wash and examine. The acid-fast bacteria are stained red, while the others and the matrix are stained blue.

Alcohol-fast Bacteria.—In specimens of urine being examined for tubercle bacilli, acid-fast smegma bacilli may also be present. To distinguish: counterstain film in a saturated solution of methylene-blue in *absolute* alcohol for 5 minutes. Tubercle bacilli remain red, while smegma bacilli become blue.

Capsule Staining.—Many bacteria possess a mucoid or gelatinous envelope, though it is only in a few species

that it is easily demonstrable. It is known as the "capsule" and varies in thickness from being only just visible to 4 or 5 times the size of the bacterium itself. It is mostly seen in preparations taken directly from animal tissues or fluids or exudates, or from cultures in media containing animal serum or milk. It is best seen in the Diplococcus pneumoniæ, Micrococcus tetragenus, B. aerogenes capsulatus, and the bacilli of the Friedlaender group. *Hiss's method*: Make a cover-slip film, and preferably by using a drop of animal serum instead of water. Dry in air and fix by heat. Stain for a few seconds with dilute fuchsin or gentian-violet (1 of saturated alcoholic solution in 19 of aq. dest.), meanwhile heating the preparation over a flame until steam arises. Wash off dye with 20 per cent watery solution of copper sulphate. Blot dry (do not wash with water), and mount direct in Canada balsam. The capsule appears as a faint blue halo around a dark purple cell-body.

Spore Staining.—Prepare film as usual and fix in the flame. Place in $CHCl_3$ for two minutes. Wash in water. Place in 5 per cent chromic acid for half a minute to two minutes. Wash in water. Float cover-slip, film side down, on carbol-fuchsin solution in a small porcelain basin and heat stain gently until it steams; continue in stain for 3 to 5 minutes. Decolorize in 5 per cent sulphuric acid for 5 to 10 seconds. Wash in water. Stain with saturated watery methylene-blue for 30 to 60 seconds. Wash and examine; or dry, and mount in balsam. The spores are stained red and the cell bodies blue.

Spores are believed to be an encysted or resting stage of bacteria, and not a method of reproduction, or rather multiplication. In most cases only one spore is produced by one bacillus, and the latter becomes extinct when the spore is fully developed. Spore formation is not very common among bacteria, and is found almost exclusively among the bacilli, less commonly in the spirilla, and rarely, if at all, in micrococci. The anaerobic bacilli are almost all spore-forming, but amongst aerobes the only sporing bacterium pathogenic to man is the anthrax bacillus. This materially facilitates and simplifies the disinfection and treatment of infectious diseases, as spores are extremely

resistant to injury by heat, light, drying, and chemicals. True spores or endospores are to be distinguished from arthrospores, the existence of which is now seriously questioned. An arthrospore is a bacterium which enters into a resting stage without any new formation within the protoplasm. It stains well with ordinary stains, and has no distinct capsule, but is stated to have increased resistance to external agents. A true spore (1) resists the ordinary staining method; and (2) shows very great resistance to destruction to the usual agents.

Flagella Staining. — Flagella are hair-like organs used for locomotion, and have been described as occurring on bacilli, spirilla, and a few species of cocci. They are best seen in young cultures, 10 to 18 hours old, at 37° C. McCrorie's method gives admirable results when the technique is carefully followed.

McCrorie's Flagella Stain.—Measure out and mix:— Night-blue, 1 grm. in 20 c.c. of absolute alcohol; potash alum, 1 grm. in 20 c.c. of distilled water; tannin, 1 grm. in 20 c.c. of distilled water. Allow mixture to stand for twenty-four hours, and filter supernatant fluid. Keep stain in incubator and filter again when using.

METHOD.—

(1) Take some distilled water at 37° C. in a watch-glass; place therein a loopful of young agar culture, and allow to swim off and diffuse without stirring.
(2) Take several loopfuls of this solution, and deposit them singly, without smearing, on a clean cover-slip.
(3) Dry in the incubator at 37° C.
(4) Apply stain (also at 37° C.) and replace in incubator for 10 minutes.
(5) Wash off stain by dipping cover-slip edgeways several times into water at 37° C.
(6) Dry in the incubator.
(7) Mount: or counterstain bodies with strong fuchsin solution for 2 minutes; wash, and dry as before; mount.

Flagella are blue, bacillary bodies are red.

Granules.—Diphtheria bacilli when stained show oval bodies, which stain more deeply than the rest of the cell. Loeffler's methylene-blue (page 161) shows them well, but a contrast stain is often used, such as that of Neisser.

Neisser's Method.—Two solutions are used: Solution 1: methylene-blue 1 grm. + 20 c.c. alcohol (96 per cent) + 50 c.c. glacial acetic acid + 950 c.c. aq. Solution 2: Bismarck-brown 2 grm. dissolved in 1 litre of boiling distilled water. Make a film, fix, stain with Solution 1 for 30 to 60 seconds. Wash, and pour on Solution 2, and after 30 seconds wash off with water. Dry, and mount. Bodies of the bacilli are brown, and the granules are blue.

Paraffin-section Staining. — Sections must first be fixed on slides by one of two modes :—

1. Float section on warm water (under 40° C.), insert slide underneath, with a needle fix one corner, and withdraw slide. Dry for 24 hours in incubator at 37° C.

2. Place a drop of solution of egg-white (10 per cent in aq.) on a slide, draw on section as before, and incubate at 37° C. for 30 minutes; or, remove excess of moisture, heat over small flame until paraffin melts, and then until vapour arises.

Staining. General Method.—

1. Preparation: Remove paraffin with xylol, and xylol with absolute alcohol. Wash in water (unless alcoholic solution of stain is used).
2. Staining: Use methylene-blue for 15 minutes; carbol-thionin-blue (5 minutes), or aniline-oil-gentian violet, carbol-fuchsin, etc. For over-staining reduce with very weak acid for 5 to 30 seconds. This also decolorizes the tissues.
3. Counter-staining: Wash in water, stain with $\frac{1}{4}$ per cent eosin for 30 seconds, and wash in water.
4. Dehydration and Clearing: Remove water with absolute alcohol (some organisms are decolorized very easily at this stage, and hence treatment must be rapid). Remove alcohol with xylol, and mount in Canada balsam.

Weigert advises aniline oil, aniline-xylol, xylol, and balsam.

Gram's Method.—(1) Prepare ; (2) Stain for 5 minutes with aniline-oil-gentian-violet ; (3) Pour off excess, do not wash, flood with Gram's iodine solution repeatedly, until purplish black, and allow to act for 1 minute ; (4) Do not wash, but decolorize with absolute alcohol or methylated spirit until faint violet tint ; (5) Wash in water, counter-stain with ¼ per cent eosin for 1 minute ; (6) Dehydrate, clear, and mount. Bacteria are blue-black, and tissues are pink.

In Weigert's modification of the Gram method, the section is first stained for 30 minutes in lithia-carmine. Wash in water and proceed as in Gram's method, except that the dehydration is done with aniline oil.

Acid-fast Bacilli in sections.—Tubercle bacilli, etc.

(1) Prepare ; (2) Stain in carbol-fuchsin for 5 minutes in hot solution, or 24 hours in cold ; (3) Wash in water ; (4) Decolorize in 12 per cent sulphuric acid ; (5) Wash well in water (colour should just be a faint pink) ; (6) Contrast stain with saturated watery solution of methylene-blue for 30 seconds ; (7) Wash in water, dehydrate, clear, and mount. Bacilli are red, tissues are blue.

Note.—If a section has been hardened in corrosive sublimate, the latter must be removed after the paraffin. This is done by using equal parts of Gram's solution and water for a few minutes, and then removing the iodine with methylated spirit.

POLYCHROME STAINS.

These are of value for the staining of micro-organisms in pus and exudates, and for blood films, in all of which the relation of the bacteria or protozoa to the cellular elements is to be determined. In all these stains the basis is a mixture of solutions of methylene-blue and eosin, which stain the various elements separately and in combination, thus bringing out in a marvellous way the details of the structural and foreign bodies. This mixture is called the Romanowsky stain, and various modifications of it are now in use.

Jenner's Stain.—A simple stain, excellent for blood work, but not so good for parasites as others given below. No

alkali is used in its preparation. Equal parts of watery solutions of (*a*) Gruebler's water-soluble eosin (1·2 per cent), and (*b*) Gruebler's medicinal methylene-blue (1 per cent), are mixed, and the mixture allowed to stand for twenty-four hours. A coarse granular precipitate forms, is filtered off, dried at 55° C., washed with distilled water, filtered, washed again, filtered, and dried. Of the dried powder, 0·5 grm. is dissolved in 100 c.c. of Merck's methyl alcohol. In use, a few drops are placed on the dried unfixed film for one to three minutes, then poured off, and the slide is washed with distilled water until pink in colour. Dry with filter-paper, and mount in xylol balsam.

Leishman's Stain.—The methylene-blue is alkalinized with 0·5 per cent of sodium carbonate, weaker eosin solution is used, and the technique of preparation is varied. The stain will keep for a long period. In use, a few drops are placed on the unfixed preparation for fifteen to thirty seconds, the film being tilted from side to side to prevent drying at any part. In this way the film is both fixed (by the methyl alcohol) and stained by one operation. About twice as much distilled water is now added, and the diluted stain allowed to act for five minutes longer. Now wash in distilled water, mount, and examine.

Giemsa's Stain and Method.—This is a modified Romanowsky, of great value in staining Spirochæta pallida, Vincent's spirilla, protozoa, and Negri bodies. The stain used is methyl-azure, which Giemsa believes to be the essential constituent of the Romanowsky stain. In use, the film is first fixed with alcohol, dried, covered with the stain, diluted, well washed, drained, dried, and mounted. For ordinary staining, fifteen minutes are enough; for the spirochæte and Negri bodies, one to twelve hours may be necessary.

Staining.—By Romanowsky, red cells are stained orange to pink; eosinophile granules, red; neutrophile granules, yellow to lilac; nuclei, shades of violet; blood platelets, purplish; malarial parasites, blue; chromatin, red to rose-pink.

Blood Films.—May be made on cover-slips or on slides. With cover-slips, touch one to the exuding blood, drop it on another, and then draw the cover-slips apart. For slides, touch a drop of blood near one end of slide, and smear

out with another by drawing it slowly along the first, sloped to it at an angle of about 45°. The slides used must be clean, and are usually stored in absolute alcohol, which is burnt off just when using. When the film is stained, it can be examined at once, with a drop of cedar oil, and afterwards mounted, if desired.

Fixation is accomplished by methyl alcohol after air-drying, when using the Romanowsky method. For other processes it is attained by one of the following methods:—

(*a*). Half an hour in a hot-air chamber at 120° C.

(*b*). Half an hour in a mixture of alcohol and ether (equal parts). Wash, and dry.

(*c*). Five minutes in formol-alcohol (1—9). Wash, and dry (Gulland).

(*d*). Two to three minutes in saturated solution of corrosive sublimate. Wash well, and dry.

For wet films, which give the histology better, the methods are varied. The films, while still wet, are placed film downwards in the fixative. Gulland's combination of (*b*) and (*d*) is said to be an excellent one.

INOCULATION OF ANIMALS.

Inoculation of animals, or the animal experiment, as it is called, is used for a variety of purposes, is made in a variety of ways, and the number of different animals used is now considerable. It is an important way of getting a pure culture in difficult cases. It also determines the pathogenicity of a pure culture injected. By the occurrence of special symptoms following injection of suspected material, it serves to establish the presence of a particular micro-organism in the material. If the injection of known products of certain organisms is followed by certain reactions, the presence in the animal's body of the organism from which the product has been derived is inferred (tuberculin and mallein tests). By passing a particular organism through a series of susceptible animals in succession, its virulence may be exalted, and the same experiment through resistant animals may depress its vitality; this is Pasteur's "method of passage." Animal inoculation is also used for the production of antitoxins and antibacterial bodies.

The inoculation may be: (1) Cutaneous, that is, rubbing into the unbroken skin; (2) Subcutaneous, with a syringe, or by cutting the skin and putting the material in a pocket in the subcutaneous tissue and stitching up the skin wound; (3) Intraperitoneal; (4) Intramuscular; (5) Intrapleural; (6) Intravenous; (7) Into the stomach by a tube, or by ordinary feeding; and (8) By inhalation.

The various processes are described as required under the particular microbes concerned. The average temperatures of the more commonly used animals and a few others may be here conveniently tabulated. The table is compiled from Abel's "Laboratory Handbook of Bacteriology," and from various other sources.

TABLE OF ANIMAL TEMPERATURES.

	Rectal Temperature		Pulse	
	Centigrade	Fahrenheit	Rate	Where usually observed
Guinea-pig	37·3° to 39·5°	99° to 103°	—	—
Horse ..	37·7° to 38·3°	100° to 101°	35 to 45	jaw
Cow ..	37·7° to 38·9°	100° to 102°	45 to 55	jaw
Calf ..	38·4° to 39·9°	101° to 103·8°	—	—
Sheep ..	38·8° to 40°	102° to 104°	70 to 80	heart
Pig ..	ditto	ditto	ditto	ditto
Goat ..	38·6° to 39·7°	101·4° to 103·4°	—	—
Dog ..	38·6° to 39·5°	101·4° to 103°	80 to 90	—
Cat ..	37·7° to 38·3°	100° to 101°	—	—
Rabbit ..	38·3° to 39·9°	101° to 104°	—	—
Chicken ..	41° to 42·5°	105° to 108·5°	—	—
Pigeon ..	ditto	ditto	—	—
Linnet ..	44°	111°	—	—
Rhesus Monkey..	38·1° to 39·5°	100·5° to 103°	—	—
Chimpanzee	37° to 38°	98·4° to 100·4°	—	—
Bat ..	41	106°	—	—
Narwhal ..	35·5°	96°	—	—
Reptiles ..	28°	82·5°	—	—

UNICELLULAR MICRO-ORGANISMS.

Fungi.—Fungi are members of the class of plants called Thallophyta, which show no division into root and stem. They are distinguished from the algæ of the same class by not possessing chlorophyll.

Schizomycetes, or fission-fungi—multiply by fission: coccus, bacillus, spirillum, streptothrix.

Blastomycetes, or budding-fungi—multiply by budding: the yeasts or torulæ.

Hyphomycetes, or branching-fungi—multiply by branching. The branches are called hyphæ, and the network of interlacing threads is called the mycelium: the moulds.

Protozoa.—Unicellular members of the animal kingdom. They are divided into groups like the following:—

Sarcodina.—Naked or cased organisms which throw out pseudopodia: amœba.

Flagellata.—Endowed with organs of locomotion—flagella: trypanosoma, spirochæta.

Infusoria.—Locomotion by means of short flagella, called cilia, of which many are present: balantidium.

Sporozoa.—Non-motile in adult state. Reproduce by spores. Feed by osmosis. Exclusively endoparasites: plasmodium malariæ or hæmosporidia, piroplasma, coccidium.

Schizomycetes may be classed in various ways under the following heads:—

Parasites	or	Saprophytes
Aerobes	or	Anaerobes

The parasites, saprophytes, aerobes, and anaerobes may be either obligatory or facultative.

Pathogenic	or	Non-pathogenic
Sporing	or	Non-sporing
Motile	or	Non-motile
Flagellated	or	Non-flagellated
Gelatin-liquefying	or	Non-liquefying
Gram-staining	or	Non-Gram-staining
Chromogenic	or	Non-chromogenic.

Other characters used to distinguish bacteria into groups are: their action on the various sugars, the production of indol in certain media, reduction of nitrates, their behaviour to the dyes, e.g., acid-fast or not, polar staining, etc.

CHAPTER X.

RESULTS OF BACTERIAL ACTIVITY.

PRODUCTS.

THESE result mainly from the cleavage of proteids and fats, and the fermentation of carbohydrates. The basis of our knowledge on this subject was laid by Pasteur, who also was the first to prove the part played by micro-organisms in these processes. The actual work of cleavage is carried out by ferments or enzymes. A ferment or enzyme is a substance produced by a living cell, which substance is able to bring about enormous chemical change (in proportion to its bulk) without itself suffering decomposition. The accumulation of its products often causes its action to cease, but if these are removed, the action is indefinitely prolonged. We shall see that the toxins of bacteria have been compared to enzymes, and while to some extent there is a resemblance in their action, the toxins in a certain amount are able to produce only a definite result, which is less than that produced by a larger dose.

The various enzymes are grouped as proteolytic (in culture, gelatin-, fibrin-, serum-liquefying), fat-splitting, and carbohydrate-splitting (produce alcohol, simpler sugars, lactic acid, butyric acid, acetic acid).

Other activities are: denitrification, nitrification, light-production, colour-production, sulphur-utilization (sulphur bacteria), etc.

Ptomaines.—The action of bacteria on dead animal matter by their proteolytic enzymes, produces substances called ptomaines, or "animal alkaloids." These bodies are toxic to the human species (and others), and are organic chemical compounds, basic in nature, which combine with acids to form salts. They have to be distinguished from leucomaines, similar substances formed in the *living* body during proteid metabolism, and not by bacterial action. They have also to be distinguished from the bacterial toxins,

which are developed by bacterial growth, independent of the medium in which grown, and have even been obtained in cultures in proteid-free media. Of the ptomaines, putrescin and cadaverin are extremely poisonous, and most cases of meat-poisoning, cheese-poisoning, and vegetable-poisoning are due to one or another of these ptomaines.

INFECTION.

The invasion of the animal body by bacteria is spoken of as infection if it gives rise to disease. The definition requires extension to cover the case of diphtheria, where the invasion by the micro-organism is often very slight, but where the disease is due to the invasion of the body by the toxins or bacterial products. In most cases the infection is due to both the bacteria and their products, in varying degrees.

In the first place it is useful to note that the skin and the mucous membranes of the alimentary tract, the mouth, the nasal passages, the upper respiratory tract, the conjunctivæ, and the genital passages, are normally inhabited by various species of bacteria. Some of these are facultative parasites, and seize the opportunity of a break in the surface, or other injury, to grow and multiply, and so produce disease. Others are pure saprophytes, non-pathogenic in any circumstances to the body on which they harbour.

The definition of the infective diseases will be useful here.

An infective disease, or rather a specific infective disease, is one which results from the introduction into the body, (1) by wounds, (2) by the air-passages, or (3) by the alimentary tract, of a definite ferment, or poison, or micro-organism, which grows and multiplies in the body.

In some of these diseases the poison is given off again, and they are then spoken of as infectious, or transmissible from person to person. Where contact is necessary for transmission, they are called contagious. The tendency is to give up the use of these terms, infectious and contagious, and simply to speak of infective diseases, which are transmissible in various ways, as by the air, by food, by contact, by fomites, by insects.

They are called specific, because they have a perfectly definite course, characterized by the stages of incubation, invasion, advance and death, or decline and convalescence. Some of them have also a skin eruption or rash.

The infection is given off again by the breath, exhalations from the skin and wounds, by desquamated portions of the epidermis, by the secretions and excretions (mucus of mouth and throat, saliva, sputa, fæces, urine, seminal fluid, milk).

Micro-organisms, then, are only relatively pathogenic or non-pathogenic, and in any particular instance of pathogenicity, the amount and kind of attack vary with a large number of factors. This is not a matter for wonder now, with our knowledge of the varied needs and differences in vitality of many micro-organisms, but was a stumbling-block in the early days. Following Muir and Ritchie, we may summarize the matter thus :—

Infection is conditioned by (1) the infecting agent, and (2) the subject.

1. The infecting agent produces its effect dependent on (*a*) its virulence, (*b*) its numbers, (*c*) its path of entrance.
2. The subject varies in its susceptibility or the reverse (resistance), according to (*a*) its species, (*b*) race, (*c*) age, (*d*) individual peculiarities, (*e*) vitality, (*f*) other disease.

Mode of Action.—Multiplication ; invasion of lymphatics ; invasion of blood-stream ; settlement in certain tissues ; chemical products (toxins), locally or diffused.

Effects of Bacterial Action.

1. Tissue changes :—
   (*a*) Local — tissue reactions or degeneration and necrosis, acute or chronic.
   (*b*) Distant—damage to special tissues, reaction of blood-forming organs.
   (*c*). General—malnutrition or increased waste, or both.
2. Metabolic changes : fever, etc.

BACTERIAL POISONS.

The knowledge of these is by no means complete, so that sharp distinctions between various kinds cannot be, at present, depended on. The first to study their production was Brieger, and it was while so engaged that he was led to the discovery of the ptomaine poisons. These bodies, however, did not, on injection, reproduce the symptoms of diseases associated with the bacteria concerned in their production, and so the ptomaines are not nowadays classed as true bacterial poisons. Roux and Yersin, in 1889, filtered broth-cultures of B. diphtheriæ through unglazed porcelain (Chamberland filter), and showed that the filtrate was bacteria free, and yet on injection the filtrate produced practically the same effects as the injection of the living bacilli. From this it was inferred that the filtrate contained the toxin of the diphtheria bacillus. The same method applied to other bacteria yielded no such result in most, and so the conception was reached that some bacteria secrete or excrete poisons which are soluble in the media in which they are grown, and some do not. Of the former class, diphtheria and tetanus are the types; of the latter, the tubercle bacillus may be taken as a type, but the class is a very large one, including all the bacteria except diphtheria, tetanus, botulinus, and the anaerobes generally (some to only a small extent). Other bacteria, such as dysentery and cholera, are said to produce soluble poisons, but the results are still discordant. As a consequence of these findings, and of the further observation that, in the bacteria not secreting soluble poisons, the injection of dead bacteria could reproduce many of the characteristic lesions of the disease associated with them in the living state, the division of bacterial poisons into two groups has arisen, viz :—

1. Extracellular toxins, true toxins, or soluble toxins.
2. Intracellular toxins, endotoxins.

After the removal of these bodies from the bacteria, a certain proteid residue remains, which, on injection, gives rise to localized reactions. Buchner calls this residue "bacterial protein," and believes it to be the same in all

bacteria, to be without specific toxic action, but having a positive chemiotactic action on the white cells of the blood, and so preparing the way for the formation of pus. It is still doubtful how much reliance can be placed on the total separation of the soluble and endotoxins from the bacterial protein, and until this doubt is resolved, these conclusions must be accepted with reserve.

Extracellular Toxins. — Of the extracellular or true or soluble toxins, that of B. diphtheriæ may be taken as the type. As a class they may be defined as the secretory products of the bacterial cells, passing out into the medium, and soluble therein. That the soluble toxins are only so produced has yet to be proved, and in the meantime they may be ascribed to the following sources, which may occur singly, or in any combination :—

1. Secretion or excretion from the bacterium.
2. Action of the bacterium on the medium.
3. Death of the bacterium and liberation of toxins from its disintegrated body.

The soluble toxins are easily obtainable in large quantities, nevertheless they have not yet been isolated in a pure form, and so our knowledge of them is derived from the study of the complex filtrates in which they are found. Their action is characterized by being selective or specific for certain tissues; for example, diphtheria, tetanus, and botulismus toxins all attack the nervous system. In the case of many of them, a definite time elapses before symptoms appear after injection. This has been called a period of incubation, though it is susceptible of other explanations. The extracellular toxins are apparently uncrystallizable, are soluble in water, are dialysable, are precipitated with proteids by absolute alcohol and by ammonium sulphate, are allied to albumoses, and are relatively unstable to heat, light, and chemicals.

Intracellular Toxins. — The endotoxins are either not excreted from the bodies of the bacilli, or are closely bound thereto, or are insoluble in the medium, and remain on filtration on the same side as the bacteria. Any of these theories would account for the known facts in regard to the endotoxins. The greater number of the pathogenic bacteria seem to act by poisons of this class. The poisons

are liberated only after the death of the bacteria, by the breaking up of their bodies, and even then they cannot be obtained apart from the bacterial protoplasm. Their action has therefore been mostly studied by injection of the dead bodies of bacteria. The effects produced are not specific, but are more those of general disturbances of metabolism; nor does much time elapse before the appearance of the symptoms, that is, there is no so-called incubation time.

The intracellular toxins are less sensitive to heat than the soluble ones, but are mostly destroyed by heating to 70° C. The notable exceptions to this are those of the tubercle bacillus, which are still toxic after digestion at 100° C., and those of the B. enteritidis (Gaertner) which remain toxic after the infected flesh has been cooked.

Some organisms, such as B. anthracis, possess no soluble toxin, nor does the injection of the dead bodies induce toxic effects. Yet in the disease produced by the living bacterium, symptoms which suggest toxin action are present. To meet such cases, the hypothesis has been made that these organisms only produce toxins in the animal tissues, or may produce complementary substances which assist the action of endotoxins. Such substances have been studied under the name of "*aggressins*." An animal is given a lethal dose of an organism, injected into a serous cavity, into which a serous exudation results. On the death of the animal, some of the exudate is taken, most of the bacteria are removed by centrifugalizing, and the few that remain by shaking up with toluol, and allowing to stand for some days. This fluid, on injection, is non-pathogenic, but has the power of increasing the effect of the particular bacterium which has caused its production, so that a non-lethal dose of the bacterium becomes a lethal one; and not only so, but the fatal effect is more quickly produced. These results are ascribed to a paralysing action of the "aggressins" on the phagocytic functions of the leucocytes. *Leucocidin*, a true soluble toxin produced by some strains of staphylococcus, causes the death and partial solution of the leucocytes, and this would suggest that the aggressins might after all be toxins, either of the extra- or intra-cellular variety.

Some bacteria give rise to both varieties, and it is now claimed that this is the case with cholera and dysentery organisms.

Nature of Toxins.—The nature of toxins is likewise ill understood. Sidney Martin found that the action of anthrax, diphtheria, tetanus, and ulcerative endocarditis organisms on albuminous bodies was to produce albumoses and peptones, thus resembling the gastric and pancreatic ferments. C. J. Martin, working at the same subject, found that the toxins could pass through a Chamberland filter, the pores of which had been filled with gelatin. From the fact that albumoses can also pass through, it is inferred that the toxins have a molecule of about the same size as the albumoses. Are the toxins of the nature of ferments? Sidney Martin suggests that the primary toxic agents are of this nature, and by digesting the tissues produce albumoses, which cause the symptoms. The labile nature of the toxins is also urged as a point of resemblance between them and the ferments, as also the so-called period of incubation which follows their injection. If it is a fact that the action of a toxin is strictly proportional to its dose, comparison between toxins and the ferments is rendered unnecessary, as this is a fundamental difference; the so-called resemblances are then mainly fortuitous.

Allied Animal and Vegetable Poisons. — Ricin, abrin, robin, and venins. Major Lamb calculates that 0·015 grm. (roughly, $\frac{1}{4}$ grain) of cobra venom is a fatal dose for a man, which is large in comparison with the minimum lethal dose of tetanus toxin for man of 0·00023 grm. (about $\frac{1}{280}$ grain). All these poisons resemble the soluble bacterial toxins, but are less easily dialysable, and hence have been called toxalbumins. The snake poisons are very complex bodies, containing one or more of several toxins, such as neurotoxins, cell toxins, hæmolytic toxins, etc.

Flexner and Noguchi discovered that the hæmolytic toxin of the cobra venom has no action by itself on the red cells, but requires the presence of normal serum. The latter is then said to contain a "complement" which "activates" the venom. Kyes and Sachs further showed

that lecithin (a highly complex fat found in the nervous system, and to a less extent in bile) has the property of activating the hæmolytic substance in cobra venom. This is very important, since it points to a definite chemical combination, leading to the formation of a toxin from two non-toxic bodies, and is in line with the observed formation in diphtheria of an antitoxin, which has many of the characters of a chemical antidote.

CHAPTER XI.

IMMUNITY AND ANAPHYLAXIS.

Immunity, or resistance, may be defined as that power or function of the living organism, natural or acquired, which enables it to repel or prevent infection of itself by micro-organisms or their products.

Anaphylaxis, or excessive susceptibility (hypersusceptibility, supersensitiveness), is defined as a state of extreme sensitiveness to the injection of certain substances, such as bacterial proteins, animal and vegetable albumins (blood serum, egg white, milk), brought about by one injection of the same substances or present from hereditary transmission.

Both these terms are relative, in most instances. Thus, birds, while immune from tetanus toxin in any doses likely to result from natural infection, may be killed by enormous doses given experimentally. Similarly, in man, the immunity conferred by one attack of a disease like small-pox, may be overcome in special circumstances of dosage and environment.

Absolute immunity does exist. Thus, so far, no animal has been infected with leprosy ; also, cold-blooded animals, under their normal conditions, are absolutely immune to the pathogenic bacteria of the warm-blooded animals. The wild carnivora have a very high degree of resistance to bacteria.

Absolute anaphylaxis of a kind also occurs. Thus, the injection (subcutaneously) of 0·25 c.c. of the serum of an eel into a rabbit causes the death of the rabbit in a few minutes. Also the offspring of animals which have been themselves sensitized by injection, show a high degree of anaphylaxis from birth. (The strict use of the term " absolute " would require that the rabbit should die no matter how small the dose of serum used. Thus its use here is relative.)

IMMUNITY.

Immunity may be natural or acquired, and may be considered under the following heads:—

Natural Immunity.—Depends on (*a*) individual, (*b*) race, and (*c*) species.

Acquired Immunity.—(A) By an attack of the specific disease; (B) By active immunization with living bacteria, dead bacteria, or with toxins or filtrates; (C) By passive immunization with antitoxic serum, or antibacterial serum.

NATURAL IMMUNITY, or the resistance to bacteria conferred by nature, is a characteristic of the living organism, which varies with the individual, race, and species. Thus, individuals vary greatly in their resistance to infection from slight wounds, from polluted water and milk, and from micro-organisms generally. The young animal is usually less resistant than the mature of the same kind. As regards race, negroes are noted for their high degree of resistance to yellow fever, and in a less degree to malaria, yet they quickly sicken and succumb to small-pox and measles. Among animals, the Algerian sheep are more highly resistant to anthrax than the European races. The influence of species is seen in the non-liability of the human to certain animal diseases, such as cattle plague, fowl cholera, and swine erysipelas, whilst animals are equally resistant to such human infections as cholera, influenza, measles, etc.

The causes of natural immunity are usually given as:—

(1) The action of certain leucocytes and other cells in engulfing and destroying the bacterial invaders, called *phagocytosis*, and

(2) The action of the blood serum.

1. **Phagocytosis.**—Metchnikoff in 1884 advanced the theory of phagocytosis, based on a careful study of the subject, and since confirmed by many observers. The phagocytes are in part wandering cells, and in part fixed tissue cells. The chief wandering cells are the polymorphonuclear and large mononuclear leucocytes and wandering tissue cells. Of the fixed phagocytes, possessing the power of amœboid movement, the cells lining the serous and lymph spaces, the cells of the spleen pulp, and bone

marrow, are the chief examples. Metchnikoff calls the polymorphonuclear leucocytes, **Microphages,** and all the other phagocytes, **Macrophages.** He observed the phagocytosis in a fungus disease of a water-flea (Daphnia), and in frogs infected with anthrax, where the death and dissolution of the bacilli could be seen going on inside the phagocytes. Later researches proved that the same phenomena could be observed in all infective processes, more especially if the animal were resistant to the infection. When the micro-organisms get into a part where few phagocytes are, a migration towards the affected spot occurs. This is part of the inflammatory reaction which follows infection. The cause of this migration is the presence of substances in the part, which attract the phagocytes, and is known as positive chemiotaxis. Negative chemiotaxis, or the repulsion of the phagocytes, also occurs. Buchner showed that dead bacteria, bacterial proteins, and closely allied substances, such as vegetable casein (legumin), have a positive **Chemiotaxis,** whilst the toxins of many virulent bacteria have a negative chemiotactic power. In natural immunity, phagocytosis is developed to a high degree, and it is of such constant and regular occurrence that we may often foretell from the degree of phagocytosis whether, in a particular infection, the animal being experimented with will gain the victory or not. A clinical application of these results is seen in the observation of the increase of the number of leucocytes in the blood during the progress of a disease like pneumonia. The increase is called "**Leucocytosis,**" and is almost wholly of the polymorphonuclear variety. In pneumonia an early and marked leucocytosis is a sign of favourable import, and may be from 12,000 to 40,000 per c.mm. The absence of leucocytosis, except in very slight infections, is highly unfavourable. (It is interesting to note here that whooping-cough gives a **Lymphocytosis,** as also do enlarged tonsils, rickets, scurvy, and a few other diseases.)

2. **The Action of the Blood Serum.**—Besides the direct action of the phagocytes, as described by Metchnikoff (the cellular theory), the blood serum was found to have bactericidal power. Von Fodor showed that freshly drawn rabbit's blood could destroy anthrax bacilli, as

also could defibrinated blood, the pericardial fluid and the aqueous humour, and that this power was lost by heating to 55° C. Buchner (with others) found that completely cell-free blood was bactericidal, and lost this power on heating to 55° C., but not on freezing and thawing. According to Buchner, fresh blood frozen and thawed loses its power because the red cells are destroyed by the process, and make the blood so suitable for bacteria that the bactericidal power is compensated for. These protective substances in the serum are called "**Cytases**" by Metchnikoff, and "**Alexines**" by Buchner. These are now believed to be derived from the leucocytes, Metchnikoff holding that they are only formed on the death of the leucocytes, or rather phagocytes ("phagolysis"), and that they do not exist in the body except under abnormal conditions. In any case when present they will probably prepare the bacteria for ingestion by the phagocytes, and thus are related to, if not identical with the "**Opsonins**" (feast-preparers). The cytases or alexines are of proteid nature and are very unstable. The withdrawal of the salts from the serum by dialysis suspends their activity, which is restored on again adding them. This fact is evidently related to the lessened amount of chlorides excreted in the urine in all acute febrile processes, and especially in lobar pneumonia. The cytases or alexines may be regarded as an appliance common to every animal organism, for the dissolution of organized substances, whether bacteria, foreign red corpuscles, or other foreign bodies. They are the "**Complements**" of Ehrlich's classification.

Acquired Immunity.—The immunity that is called natural is of a general kind, being a natural resistance to disease or bacteria of all kinds. That type of immunity called acquired, is, on the other hand, "specific" in kind, that is, an immunity from a definite or specific disease or infection. For this reason it is by some called *specific* immunity.

(a). *Acquired by an attack of a Specific Disease.*—The fact that an attack of small-pox followed by recovery protected the individual from further attack, was a notable one during the epidemic prevalence of that disease. Similar

protection was seen in regard to other eruptive and non-eruptive fevers such as scarlatina, measles, typhus, typhoid, and whooping-cough. On the contrary, some specific diseases do not protect, but one attack seems to render the individual more liable to another. Such are diphtheria, pneumonia, influenza, gonorrhœa, erysipelas, relapsing fever, and rheumatic fever. In all of these it is probable that some degree of immunity results, but is of very short duration, as has been definitely observed in cholera and some other diseases. In all cases, however short the immunity, it is absolutely specific against a certain infective agent or its poison, and is not due to a general increase of resistance. In fact the reduction of the general resistance following the specific infection is such as in some cases to predispose to other infections. Thus, tuberculosis not infrequently follows a severe attack of measles, whooping-cough, or typhoid. Recovery from an acute infective disease is due to a process of immunization going on during the progress of the disease, which at a certain point or stage is able to prevent the further action of the infecting agent. The substances formed do not always exterminate the virus from the mucous surfaces; and this is seen specially in typhoid fever, where the recovered patient may continue to excrete the living virus by the bowel or urinary discharges, and in diphtheria, where the virus may persist in the throat.

Artificial Immunity.—Under this head may be classed together forms (B) and (C) of acquired immunity.

(B). *By* **Active** *Immunization, or Protective Inoculation*—where the specific protective substances have to be formed in the body itself, as opposed to immunization by transference of protective substances formed by active immunization in another animal, and called passive immunization. In *active immunization*, the individual or animal must undergo an infection followed by a reaction. By this means the protective substances are formed, and so the immunity is obtained only after the lapse of a period of time, when the immunizing apparatus of the organism is able to produce the protective substances in sufficient amount. The immunity thus evoked is of a more persistent type than that obtained by simply transferring

the protective substances from an immunized animal, because, in the first case, an immunizing apparatus has been set up which is able to produce an (apparently) indefinite amount of protective substances and over a long period, whereas in the second case, a definite amount of the protective substances is injected, and when this amount is used up the protection is at an end. In active immunization, we must distinguish a "**specific immunity to bacteria,**" and a "**specific immunity to their toxins,**" just as we have a natural resistance or immunity to bacteria, which is different from the natural resistance or immunity to certain poisons. Thus the immunity after diphtheria is mainly to the toxins (antitoxic). On the other hand, the immunity purchased by the injection of cholera vibrios, is merely to the bacteria and not to their endotoxins. Hence the injection of cholera spirilla into an animal previously immunized to the same, is followed by the death and dissolution of the spirilla, but if the dose is large enough, also by a fatal intoxication of the animal by the cell poisons thus suddenly set free. This is the chief cause of failure of immunization to those bacteria which do not act (as diphtheria and tetanus do) through soluble toxins, diffused into the blood stream. The immunization would require, in such cases, to be of a double nature, namely, antibacterial and antitoxic. Apparently, as usually induced, the former mainly, if not entirely, results.

The methods of active immunization are based on the work and discoveries of Pasteur and his associates, and may be summarized thus:—

(1) With living bacteria, virulent or attenuated.
(2) With dead bacteria.
(3) With the bacterial cell substances.
(4) With soluble toxins or filtrates.
(5) By feeding with toxic substances.

1. *With Living Bacteria or Virus, Virulent or Attenuated.* —(*a*). With virulent virus. Though the virus of small-pox is still unknown with certainty, inoculation of the small-pox, as introduced into England in 1718 by Lady Mary Wortley Montagu (see her "*Letters*"), may be given as an example. The inoculated disease was usually mild in type,

but at times was severe and even fatal. In contagious pleuro-pneumonia of cattle, subcutaneous injection of the lymph of a newly killed animal, into the tail, has proved protective. The animal sometimes loses its tail in part, the brunt of the infection apparently remaining localized.

(*b*). With attenuated virus. This is accomplished in one or more of the following ways :—

By cultivation of the organism in oxygen or a current of air, as first discovered by Pasteur in the case of the bacilli of chicken cholera. The attenuated bacilli, on injection, produced a non-fatal disease, which immunized the fowl, especially on repetition.

By cultivation of the bacteria at high temperatures, e.g., anthrax bacilli at 42° to 43° C.

By passage through a less susceptible species. This is the presently accepted explanation of vaccination for small-pox. Used by Pasteur for swine erysipelas, the bacillus of which is lessened in virulence by repeated passage through rabbits, but increased by passage through pigeons. Two inoculations were given, the first of the attenuated bacilli from rabbits, the second of the exalted bacilli from pigeons.

By drying the virus, as in hydrophobia, where Pasteur found that the virus was exalted in virulence by successive subdural passage through rabbits, but diminished by passage through apes. He tried the immunization of dogs by the use of the diminished and increased viruses, but the results were too variable, so he tried drying the spinal cord of a rabbit dead of the disease. A cord thus dried at 22° C. over KOH (to absorb CO_2) for 1 to 4 days, still causes rabies in 7 days (the incubation period shortened from 14 days by the exaltation of the virus); but if kept longer, the incubation period is prolonged, until one kept 12 to 14 days has become inactive. The treatment consists in beginning with injection of an emulsion of this cord, and daily repeating with an emulsion of a less dried cord until within 15 days in mild cases and 21 days in severe, the strength arrived at is a 3-day-dried cord. Complete immunity thus takes 3 to 4 weeks, and so in cases coming under treatment at a late period, the method is condensed. Hoegyes uses fresh cord emulsified

in salt solution, of which dilutions are made, and injections made in reverse order of dilutions. Fewer accompanying symptoms (as erythema at the point of injection, backache, muscular pains, and occasionally temporary paralysis) are noted, and this result is attributed to the less amount of nerve tissue injected.

By cultivation in a medium containing antiseptics in a dilute state; e.g., in presence of carbolic acid 1–600, or potassium bichromate 1–5000, or sulphuric acid 1–200.

By addition of weak antiseptic solutions to virulent broth-cultures preparatory to their injection, as advised by Behring for immunization of horses to diphtheria and tetanus. The antiseptic advised is iodine terchloride, ICl_3, in strengths varying from 0·05 per cent to 0·4 per cent. Lugol's solution of iodine is also used.

2. *With Dead Bacteria.*—This method is simpler and safer, and in many cases confers the same degree of immunity, which is chiefly antibacterial. It is also used preliminarily to injection of living cultures, in the active immunization of animals. In the human being, it is used for cholera, plague, typhoid, and in the treatment by "vaccines" generally, as for staphylococcus infection, etc.

3. *With Bacterial Cell Substances.*—This method is a modification of that with dead bacteria. Instead of injecting the culture heated to 65° C., or some such temperature, to kill the bacteria, the culture is subjected to various processes as in the preparation of Koch's original tuberculin. This is not purely bacterial cell substances, but is intermediate between the simply heated culture and tuberculin-R, which is an emulsion of the bodies of bacilli from which all the soluble substances have been extracted by grinding and treatment with distilled water (tuberculin-O). Hahn, following Buchner, has used mechanical pulverization of bacilli mixed with infusorial earth and quartz sand, and subjected to 300 to 500 atmospheres' pressure by hydraulic means, and has so obtained what he calls the cell-juices or bacterial plasmins, which he has used for immunization. "Cholera plasmin" and "typhoid plasmin" have both proved effective in immunizing guinea-pigs against intraperitoneal infection with ten times the fatal dose of virulent

bacteria. "Tuberculo-plasmin" after filtration is a clear pale yellow fluid, containing nucleo-albumin, which keeps indefinitely on addition of 20 per cent of glycerin and 5 per cent of NaCl. This preparation has been used with favourable results in guinea-pig tuberculosis.

The reaction which follows the injection of a dead culture (local pain and swelling, rigor, depression, and anorexia) is not peculiar to any one bacterium, but follows upon the subcutaneous injection of all bacterial emulsions, and even of innocuous and living bacteria (Buchner, 1890).

4. *With Soluble Toxins or Filtrates.*—This method was first successfully used by Salmon and Smith, who showed that pigeons could be rendered immune to hog cholera by treatment with filtrates of hog-cholera bacilli (1886). It is now used for the immunization of horses to diphtheria and tetanus toxins, the immunity being afterwards heightened by the injection of virulent cultures, if Behring's advice is adopted. This use followed on the observations of Roux and Yersin, who showed in 1886, as a "result of splendid research" (Buchner) that the poison of diphtheria is extremely susceptible to heat (being destroyed at 65° C.), and is carried down mechanically by chemical precipitates such as calcium phosphates, properties which until then had been recognized mainly in the digestive ferments or enzymes. Brieger and Fraenkel confirmed these results, and showed that the poisons or toxins of diphtheria and tetanus can be obtained in a moderately pure form by precipitation with absolute alcohol. These poisons gave the reactions of albuminous substances, and were hence at first called "toxalbumins." As they are now believed to be non-proteid, the name "specific toxins" is to be preferred. A specific toxin is one which, on injection, causes all the symptoms of the infection in question.

5. *Active Immunization by Feeding* has been successfully used by Ehrlich for the poisons ricin and abrin, and with less success by Fraser against snake venom. In bacterial infections it has proved, so far, tedious, and the immunization slight in amount.

(c). *By* **Passive** *Immunization.*—Behring in 1890

discovered that the blood serum of an animal actively immunized against diphtheria, when injected into another animal, is capable of rendering the latter insusceptible to what would otherwise be a fatal dose of diphtheria toxin. That is, the serum was able to destroy the diphtheria poison. In conjunction with Kitasato he afterwards proved the same for the tetanus toxin. The transference of the immune serum, in both cases, protects against the specific poison, and so allows the natural resistance of the body to overcome the bacilli. As the new animal body has thus supplied to it an antidote to the microbic toxins which have been shown to produce the symptoms of the disease, no reaction to these toxins occurs (where they have been completely and early destroyed), and so no active immunization occurs. The immunity conferred is, therefore, of a transient nature, and lasts only as long as some of the immune body in excess persists in the blood. Any such excess is destroyed or excreted within eight to fourteen days, and so the immunity may be expected to be absent thereafter. These remarks apply to "antitoxic sera," like those obtained in diphtheria and tetanus. The other form of passive immunization by "antibacterial" or "antimicrobic" sera, has proved unsatisfactory in use, for the reasons given on page 185. The action of the latter sera is not so simple as that of antitoxic sera, but is due to one or more of the following factors: (1) Bactericidal or lysogenic action, that is, death or solution of the micro-organisms; (2) Opsonic action, or the rendering the bacteria more susceptible to the phagocytic action of the leucocytes; (3) Agglutinative and precipitative actions, that is, clumping of the bacteria, or precipitation of their soluble products.

Antitoxic Sera.—The actual mode of manufacture may be conveniently given here. Diphtheria antitoxin may be taken as the type. A culture of B. diphtheriæ in meat-infusion broth (containing 1 to 2 per cent of added peptones, and after being made neutral to litmus, having 7 c.c. of N/1 NaOH added per litre) is incubated for three weeks at 37° C. A strongly toxic fluid is thus produced, which is filtered through a Chamberland candle into a sterile flask, care being taken to avoid exposure to bright light.

At the first attempts at immunization, the animals died of chronic poisoning. To avoid this, it is now usual to begin with very small doses of the toxin weakened by the addition of iodine terchloride or Lugol's solution (used in Gram's method of staining, I in KI in water). A young vigorous healthy horse (4 to 6 years old) is chosen, and 0·5 c.c. of toxin mixed with an equal quantity of Lugol's solution is injected subcutaneously in front of the shoulder-blade, using a large needle connected with a syringe by a piece of rubber tubing. (The skin is previously shaved and sterilized.) After the reaction has subsided, usually in 5 to 8 days, a few days' interval is given, and the next dose is administered, either 1 c.c. + 1 c.c. Lugol's solution or 0·5 c.c. pure toxin. The amount given is thus gradually increased by ½ c.c. until finally in three to four months the antitoxic value of the animal's serum is such that a dose of 300 c.c. of active toxin may be borne. The immunizing process must not be pushed too rapidly, otherwise the health of the animal will suffer. Serum is then obtained by inserting a sterile needle into the jugular vein of the horse, and collecting the quantity of blood desired (up to 6 litres at a time) through a rubber tube into sterile Erlenmeyer flasks containing solution of citrate of soda. These are allowed to stand until the corpuscles settle, and the plasma is poured off into another flask, and allowed to clot. Separate the serum, and filter. If filtered at once, it is found to precipitate again on standing, hence it is better to allow it to stand a few days before filtration. Bottle or tube, after adding 0·5 per cent carbolic, but before this it should be standardized.

Standardization of antitoxic serum is very important, so that accurate dosage may be determined, and also so that one strength may be aimed at, since in the making it is liable to variation. The making of such a standard is not an easy task, because no two samples of toxin are of exactly the same strength, nor are even two samples of the same toxin tested at different times. This is another way of saying that toxin is a very variable substance, but fortunately antitoxin is not so variable. Hence Ehrlich chose as his "*immunity unit*," the amount of antitoxic serum which will neutralize 100 times the minimum lethal

dose (M.L.D.) of toxin for one guinea-pig of 250 grm. weight and which kills it within 5 days; the serum and toxin being mixed together and made up to 4 c.c. bulk, and injected subcutaneously, the animal survives the time-limit. A serum containing one such unit in 1 c.c. is called "*normal serum*" or "*normal diphtheria antitoxin*" (D.A.N.), while a serum 1 c.c. = 1,000 M.L.D. is spoken of as ten times normal (D.A.N.)^{10}, etc. One c.c. of the normal serum is said to contain one "*immunization unit.*" Working back from quantities of serum of known strength (antitoxic) and preserved in a dried state in a vacuum and in a dark cool place, the potency of any toxin at hand can be determined, and against this latter, any newly prepared serum can be standardized. In this way a fairly uniform standard can be maintained. The usual antitoxic serum on the market contains 2000 "immunity units" or shortly, units in 4 to 5 c.c. of serum and equal to 200,000 M.L.D. High-potency sera are prepared so that 5 c.c. contain 5000 units, and correspondingly. The serum keeps very well for at least one year in a cool dark place. The durability of the serum is tested by keeping back some of the bottles, and from time to time examining their activity, and if it is found to rapidly diminish, all the bottles bearing the same number are recalled.

OTHER IMMUNITY PHENOMENA.

Pfeiffer's Phenomenon. — In 1894, Pfeiffer showed that when cholera spirilla are injected into the peritoneal cavity of cholera-immune guinea-pigs, the spirilla rapidly swell up, become granular, and often undergo complete solution. The same result could be observed in a normal animal, if a protective amount of cholera-immune serum were injected at the same time. The constituents of the blood serum which cause this result are spoken of as "**Bacteriolysins.**" It was soon shown that the same result followed the mixing of the spirilla and the serum in a test tube under suitable conditions. The same phenomenon was thereafter observed for other micro-organisms.

Agglutination. — In 1896, Grueber and Durham, investigating Pfeiffer's phenomenon, found that when a

quantity of immune serum is added to a broth culture of the respective bacterium, flake-like clumps sink to the bottom of the tube, and the supernatant liquid becomes clear. Grueber also showed that the immune serum would affect in the same way, though less powerfully, closely allied bacteria. The substances causing this are called "**Agglutinins,**" and were thought to be the same as the "immune-body" concerned in Pfeiffer's reaction. Both are comparatively thermostabile, but the agglutinins cannot be reactivated by the subsequent addition of normal serum. They do not act if NaCl is absent.

Precipitation.—In 1897, Kraus showed that precipitates were formed when *filtrates* of cultures were mixed with the corresponding immune serum. The "**Precipitins,**" like the agglutinins, are inactivated by heat (60° to 70° C.) and cannot be reactivated (see SERUM PRECIPITATION, below).

Hæmolysis.—Bordet, in 1898, showed that the serum of an animal which has been repeatedly injected with the red corpuscles of another, acquires the power of dissolving the red cells of that other, and that this power is lost on heating to 55° C. but is regained by the addition of serum of a non-treated animal. Other "cytotoxins" (cell-destroying antibodies) similarly produced are: leucotoxin, nephrotoxin, spermatotoxin, hepatotoxin, pancreatoxin, suprarenal toxin, etc.

Serum Precipitation.—Like hæmolysis, this subject is closely allied to the reactions induced by bacteria. When the serum of one animal is injected repeatedly into another animal of a different species, a substance forms in the first animal's serum, which causes, in a mixture of the two sera, a cloudiness or precipitate to form. This substance is called "precipitin," and is specific for each species, in high dilutions, as in the case of the other reactions. The precipitins, whether formed from bacterial or serum stimulation (or casein of milk, etc.), are all inactivated by heating to 60° to 70° C., but can *not* be reactivated by the addition of normal serum or any known method. Such inactivated serum, however, if mixed with a certain amount of active serum, is able to prevent

the latter giving a precipitate. The precipitins are therefore conceived to be built up of two atom groups, one thermolabile and the other thermostabile. Unlike agglutinins, they have not been, so far, shown to exist in normal serum. This reaction is used in forensic work, to determine the character of blood-stains, whether human or not.

Opsonic Action.—In 1903–4, Sir Almroth Wright undertook a systematic study of the phenomenon of phagocytosis, and showed that phagocytosis depended on a substance present in the serum, which acts on the bacteria (and not on the leucocytes), becomes fixed to them, and makes them a prey to the leucocytes. To this substance he gave the name "opsonin" (feast preparer). This substance is present in normal serum, but can be increased by immunization. It is destroyed by heating to 55° C. Leucocytes washed with salt solution have no phagocytic action. On the other hand, if the bacteria are exposed to the action of serum, and then washed free of it, they can be phagocytozed by the washed leucocytes. It is hence inferred that the opsonin becomes bound to the bacterium. A similar substance has been described in immune sera after heating (bacteriotropin), but it is specific for the corresponding bacterium, while the opsonin in normal serum is non-specific. There are thus two opsonic substances; that present in normal serum, which is thermolabile, and that present in immune sera, which is thermostabile. In opsonic estimation, both factors are at work where the person is being gradually immunized to a particular bacterium. The whole question is still complex and full of difficulties, and requires further elucidation.

Technique of Opsonic Estimation.—The method of Leishman is very simple. Take a capillary pipette, fitted with a rubber nipple. Make a mark on the stem; draw up fresh blood from the finger to the mark. Then draw up a little air to make an air-bubble, which separates the blood from the bacterial emulsion now drawn up to the same mark. The two fluids are then mixed by being blown out on a glass slide, and drawn back repeatedly. Finally, the drop is placed on the slide, covered with a

cover-slip, and incubated at 37° C. for 15 minutes. Thereafter a film is made and stained by Leishman's method, and the number of bacteria present in 50 polymorphonuclear leucocytes is observed. This number divided by 50 gives the "opsonic index." Wright's method is more elaborate and specific. He uses (1) Leucocytes from the observer's blood repeatedly washed with saline solution (0·85 per cent); (2) Bacterial emulsion in salt solution; (3) Serum from the blood to be tested, free of clot and cells. These are now mixed, as above, in equal amounts, in a capillary pipette, and the mixing is made thorough by blowing out and sucking in, for ten times. The mixture is then drawn into the tube of the pipette, the end sealed, the rubber nipple removed, and the tube put into the incubator for 15 minutes. The tube is then removed, the end broken off, the contents are again mixed, and films made, dried or fixed, and stained as desired (Jenner, Leishman, or Giemsa), and bacteria counted in 50 to 100 cells. A control is done with normal serum, and the "**Opsonic Index**" taken is the quotient of that got with the patient's serum divided by the index got with the normal serum. The latter, to minimize variation, may be made by mixing the serum of several healthy individuals. The number of bacteria per leucocyte (polymorphonuclear), in any one estimation, is also spoken of as the "**Phagocytic Index,**" and the opsonic index is thus the proportion between the phagocytic index for the patient's serum and that for the normal serum. A modification of the method is to take a number of dilutions of the patient's serum and of the normal serum and to estimate in all these not the phagocytic index, but the percentage of leucocytes which act as phagocytes, i.e., the "**Percentage Index,**" and the indices of the corresponding dilutions can be compared. The bacterial emulsion used is likewise thinner than in Wright's method. By the same process, the dilution of the serum at which phagocytosis is absent or very slight, can be determined. This is called the "**Opsonic Coefficient of Extinction.**"

Wright's Vaccine Therapy consists in injecting killed bacterial cells into the infected individual, in order to raise the phagocytic index to that particular cell. He

began with chronic staphylococcal infections, and in such cases good results were obtained. The method has been applied to infections by tubercle bacilli, streptococci, gonococci, pneumococci, and other bacteria. If possible, a culture is made from the actual bacteria causing the infection. From the culture so made, an emulsion in sterile salt solution is obtained, and the emulsion sterilized at the lowest possible temperature (usually 65° C. for one hour), and an agar inoculation made from the presumably sterile emulsion, and incubated for twenty-four hours to see if really sterile. The bacterial content of the emulsion must be estimated, so as to be able to know how many are being injected, and to inject a definite quantity. This is done by mixing equal quantities of the emulsion (whole or diluted) and fresh blood, and making films, and staining. The number of bacteria to red cells is noted over a field made by drawing a circle with a blue pencil on the lens of the eye-piece of the microscope. The number of red cells in the blood being known (say 5 million per c.mm.), and the relative proportion of bacteria in undiluted emulsion to the red cells being, say, 700 to 400, then as $400 : 700 : : 5{,}000{,}000 : x = 8{,}750{,}000$ bacteria per c.mm. If the emulsion had been diluted, then the result would have to be multiplied by the number of the dilutions. It is preferred that the content be estimated before sterilization, as some of the bacteria may undergo disintegration during that process. Where the blood serum has an agglutinating effect, the red cells are separated and mixed with salt solution. It is better to render motile bacilli still by having a little formol in the saline solution. From the stock emulsion thus standardized and sterilized, appropriate doses are made by dilution with 0·5 per cent phenol or lysol solution, and put in glass bulbs, with capillary ends which are sealed. The ordinary dosage is to begin with 100 million and to repeat, if necessary using a larger dose, after estimating the opsonic index, and only if this is rising. Numerous observations after the injection of such vaccines have shown that the opsonic index falls for some time thereafter (**"Negative Phase"**) and then begins to rise, and usually ascends to a higher level than before. Another injection during the negative

phase tends to accentuate it, while after the increasing or "**Positive Phase**" has begun, an injection causes it to reach a still higher level. The negative phase is usually completed in twenty-four hours, and the positive in three to four days. Wright recommends that the succeeding injection should be given when the positive phase has just reached its summit. In tuberculosis, Koch's bacillary emulsion is used, and the dose is minute, $\frac{1}{4000}$ mgr., gradually increased to $\frac{1}{1000}$ mgr. In vaccination against, and in, enteric fever, a standardized strain of Bacillus typhosus is used.

Leucocyte Extract.—The action of the leucocytes in phagocytosis, and of the alexines, which some believe to be derived from them, led Hiss to experiment with leucocytic extracts. These were obtained by the intrapleural injection of aleuronat, which produced a copious cellular exudate in 24 hours. The animal being used (a rabbit) is killed, and the exudate removed, with every precaution to ensure avoidance of contamination, and the cells are obtained by centrifugalization. The deposited cells are treated with sterile distilled water, and thoroughly beaten with a platinum spatula. Smears are made, stained by Jenner's method, and examined for bacterial contamination; cultures are made to detect the same; more sterile water is added, and the steps are repeated after incubating for 8 hours. If no bacteria are found, the resulting fluid is put into the refrigerator until used. Such extracts of exudate cells, on intraperitoneal or subcutaneous injection, have markedly modified the course of infections in the rabbit and guinea-pig, prolonging life, and in some cases preventing a fatal issue from an otherwise lethal dose. Beneficial effects have been observed in man in lobar pneumonia, erysipelas, and in staphylococcal infections. The action of the extract on the bacterial products or toxins seems to be a neutralizing or destroying one. The substances present in these extracts have been called "endolysins," and are different from the serum bacteriolysins, (1) in not being inactivated under 80° C.; (2) when heated above 80° C. they are destroyed, and cannot be reactivated by the addition either of fresh serum or of unheated leucocyte extract. They are not increased by immuniza-

tion, each leucocyte probably having a definite quantity within its substance.

Aggressins.—The great susceptibility of some species of animals to infection by certain bacteria, while their serum nevertheless possessed marked bactericidal power against these bacteria, suggested to Bail the theory that these bacteria secrete definite substances, which protect them against phagocytosis. Such substances he called "aggressins," and they are therefore antagonistic to the opsonins. They are probably not produced in test tube, or only to a slight degree. He based this theory on two observations, namely, that sub-lethal doses of bacteria, injected along with a small quantity of "aggressins" were rapidly fatal; and that animals could be immunized against the corresponding bacteria by the injection of the aggressins. The aggressins were got, as detailed on page 177, in the exudate into a serous cavity of an animal killed by the injection of a dose of a particular bacterium into the serous cavity, and from which exudate the bacteria are carefully removed. This theory has been attacked on the ground that the aggressins are merely the bacterial toxins, probably endotoxins, liberated in the living body. The fact that such exudates are usually cellular, along with what has been said above of the action of leucocyte extract in some infections, tends to confirm this criticism. In fact, it may be put this way: When there is a high natural resistance to a bacterium, the alexines and opsonins are able to overpower it in all average infections and prepare it for phagocytosis and subsequent destruction. On the other hand, where the natural resistance is low, these agents do not succeed in preventing the growth of the bacterium, which in its growth elaborates various substances, some of which reduce the resistance still further, and so progressive infection results. If this infection is not too severe, the immunizing apparatus throughout the body, stimulated by the diluted toxins (extra- or intra-cellular) present in the blood stream, produces an excess of antibodies and anticells (phagocytes), and in this way may attain the objective of active immunization. If the infection is too acute, paralysis of the immunizing apparatus is the result.

THEORIES OF IMMUNITY.

The rational explanation of all the phenomena of immunity which are known, is a task yet to be accomplished. It is perhaps better to have a working hypothesis only, as our ideas are being continually enlarged and modified. The many elaborate and complex experiments which have been performed and repeated by many observers are attended by so many consenting circumstances, of some of which we are totally ignorant, that it is not surprising that the inferences from the same experiment are so varied and even at times so conflicting. The words of Pasteur, used in another regard, seem quite appropriate here: "In experimental science, it is always a mistake not to doubt, when facts do not compel affirmation. . . . In my opinion the question is whole and untouched by decisive proofs."

Any theory must take account of phagocytosis, the bactericidal power of normal serum, the results of immunization as seen in the formation of antitoxins, bacteriolysins, agglutinins, precipitins, and opsonins, and any other phenomena which emerge in the further consideration of these. When the theory is built around the phagocyte, it is called a "cellular" theory; if the body fluids are taken as the key, a "humoral" theory. The final explanation will probably lie in a judicious blending of these two theories.

Metchnikoff's Phagocytic Theory. — In this theory immunization leads to a more rapid and greater leucocytosis in response to subsequent infection by the same agent. At the seat of invasion there is also emigration of the microphages from the blood-vessels into the tissues, or if in a serous cavity, the exodus is into the same, giving a cellular exudate. On examination of these cells, many of them are found (in bacterial infections) to contain bacteria in their substance. These are not simply dead bacteria, in process of removal, but living and virulent ones. At a later stage, the bacteria may be seen swollen, granular, and vacuolated, and finally disintegrated. On the other hand, the phagocyte may not be able to digest the engulfed bacteria, and may itself be killed; and in that

case, the leucocyte itself will disintegrate. The substance present in normal blood, which is bactericidal, Metchnikoff calls "**Cytase,**" and he holds that it is secreted by the phagocytes. The substance formed in immunization which, like the cytase, is bactericidal, he calls the "**Fixateur,**" and also looks upon as a derivative of the leucocytes. He believes that these substances (at least, the cytase) are only set free in the blood-stream by the destruction of the phagocytes. The action of opsonins and leucocyte extract all tend to confirm the importance of phagocytosis, and the probability that these cells, retaining the characters of the amœba, retain also its marvellous adaptability, which is not usually seen or expected of the fixed tissue cells, which are so very highly specialized as to function. Metchnikoff therefore believes that for every infection the leucocytes develop a power of resistance, which may be revived on any subsequent infection, and so protect to a greater or less degree.

Ehrlich's Theory is more complex. The discovery of antitoxins led to explanations of their action. At first they were thought to destroy the toxin, but this simple explanation was set aside by the experiments of Calmette on snake poison, which is thermostabile up to 100° C. He noted that non-toxic mixtures of the toxin and antitoxin became toxic again on heating, the inference being that the toxin was bound or inactivated by the antitoxin, which is destroyed on heating above 60° C., and so the more stabile toxin is again left free. Further, C. J. Martin and Cherry demonstrated the close resemblance of the union to that of definite organic compounds, by an experiment in which they tried to pass toxin-antitoxin mixtures through a Chamberland bougie, the pores of which were filled with gelatin. In previous experiments they found that under 50 atmospheres of pressure, toxin passed through but antitoxin did not. In toxin-antitoxin mixtures, if filtered at once, all the toxin came through; but after standing for variable periods, less came through the longer the time, until two hours after mixing, no toxin passed through the filter (or dialyser). Then Ehrlich showed, using ricin and antiricin, that definite quantitative proportions of the toxin and antitoxin entered into the

reaction. The standardization of diphtheria toxin and antitoxin was the next step. Von Behring called a toxin containing 100 minimum lethal doses (for a 250 grm. guinea-pig) in 1 c.c., a "normal toxin solution" ($D.T.N^{1}.M^{250}$), and a serum capable of neutralizing it c.c. for c.c., a "normal antitoxin" or an "antitoxin unit."

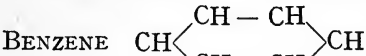
Ehrlich, in working at the subject, more exactly measured the toxin unit by introducing a time-limit, namely, that one unit must kill the guinea-pig in 4 to 5 days. He also varied von Behring's method of testing the antitoxin, by first mixing the toxin and antitoxin outside the body, and thereafter injecting; whereas von Behring injected them separately and at different parts. He prepared in this way an antitoxin, which he kept in a stable condition by drying in a vacuum and preserving in the dark in a dry atmosphere and at a low temperature. With this antitoxin he is able to standardize new toxins, and from them new antitoxins. In the course of this work he made some discoveries. In the first place, while the death of a guinea-pig in 4 to 5 days gave a fair measure of 1 toxin unit, when 100 such units were mixed with the amount of antitoxin necessary to neutralize, namely, 1 antitoxin unit (which was determined from previous measurement), and injected into a guinea-pig, it was not easy to estimate whether there was exact neutralization, or less, or more. If the antitoxin were markedly insufficient to neutralize the toxin, then symptoms such as paralysis, etc., would arise which would proclaim this. But the conditions of the experiment were such that no marked signs could be expected. The further test, therefore, was devised, namely to find the amount of toxin which, plus 1 unit of antitoxin would still be able to kill a guinea-pig, on injection, in 4 to 5 days. (When a new serum is to be standardized, the amount of serum which, mixed with this last-mentioned amount of toxin, just suffices to prevent the death of the guinea-pig before 4 days, is taken as one unit of antitoxin.) Theoretically one might have expected that, if 1 toxin unit killed a 250 grm. guinea-pig in 4 to 5 days, and 1 antitoxin unit exactly neutralized 100 toxin units, the injection of 1 antitoxin unit mixed with 101 toxin units would have left 1 toxin unit free to have killed

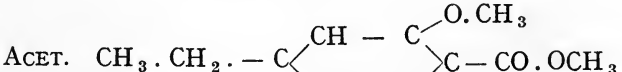
the guinea-pig in 4 to 5 days. Practically this was not found to be so, but that a considerable excess of toxin units is required to kill the guinea-pig, (stated by Ehrlich to be 100 toxin units, and by others as intermediate amounts). On this basis Ehrlich has built a whole structure of epitoxoids, toxoids, prototoxoids, syntoxoids, and toxons, designed to explain on the laws of chemical equivalence and differences in chemical affinity, the apparent contradiction of these results. It is right to point out, however, that the basis is not a chemical one. The death of a guinea-pig in 4 to 5 days cannot be compared, though it follows on an injection of toxin, to the appearance, more or less immediately, of a precipitate in a liquid to which some other chemical body has been added. Also, the relationship between the mixture of toxin and antitoxin which just causes no symptoms, and that mixture which kills a guinea-pig in 4 to 5 days, is an arbitrary one, and any apparent numerical relationship should be regarded as fortuitous until other evidence shows that it is more than that. Again, the admitted instability of the toxins, and probably of antitoxins, even under every precaution, renders the results equivocal. In brief, Ehrlich's theory is that antitoxin has a valency of 200 for toxin, and that some of the bonds of antitoxin can be satisfied by degeneration products of toxin, prototoxoids, deuterotoxoids, tritotoxins, of alpha and beta varieties, and by toxons, which are not derived from toxin but are present in the toxin fluid at first. [" Valency = 200 " cannot be accepted in the chemical sense. It only means that, starting with an amount of antitoxin which neutralized 100 toxin units elsewise defined, it was found that when the conditions were changed, the antitoxin, in some way or other, neutralized 200 of these same toxin units, or in the experiment appeared to do so. If the amount of antitoxin used at first had been the amount required to neutralize 1 toxin unit (and von Behring advised the larger quantity only for safety), the amount found in the second experiment would have been 2 by inference. The mixture of the toxin and antitoxin before injection may be a factor of moment in regard to this change of valency.] Ehrlich calls the quantity of toxin which just neutralizes

1 antitoxin unit, "limes zero," expressed as L_o; and the quantity required to neutralize 1 unit of antitoxin and yet, on injection, kill the guinea-pig in 4 to 5 days, "limes death, expressed as L +. Then, according to his theory, if T represent 1 toxin unit,

$$\mathbf{L_o} = 100 \times T \text{ and } \mathbf{L+} = \mathbf{L_o} + 101 \times T = 201 \times T.$$

Ehrlich's side-chain theory is based on his previous researches on the oxygen requirements of the organism, linked up with those on diphtheria antitoxin. Borrowing the language of organic chemistry, he likens the highly complex albuminous and other molecules of animal nutrition, to those complex compounds of the aromatic series which chemists have dissected into a central group, in which the elements may be represented as a hexagon or ring of the benzene type, and the various other parts as side-processes or side-chains. Thus benzene has derivatives like the following, the added groups of which may be spoken of as side-chains.





Applying the same ideas to living cells, Ehrlich believes that these cells have side-chains which have certain special affinities. In this way the diphtheria toxin may be supposed to be bound to certain nerve cells; and likewise tetanus toxin. The side-chains he calls receptors. When thus bound by a toxin molecule, they are supposed to be useless to the cell and are cast off into the blood-stream, and the cell is supposed to be stimulated to produce more; not only so, but stimulated to produce an overplus which is alleged to be then cast into the blood-stream, as the cell would become overstocked. Thus he accounts for the presence of antitoxin free in the blood, the free receptors acting as antitoxin to the toxin circulating. The toxin, which thus unites with the antitoxin, he conceives as

having two affinities: one which unites with the antitoxin, the haptophore; the other, the toxophore, by which the harmful effects are produced. These two affinities are the affinities of two different atom-groups, of which the toxin is supposed to be composed. In the toxoid bodies, the toxophore group is altered or wanting, but they can still bind antitoxin, in virtue of their haptophore group.

For other forms of immunity, which are more complex than the toxin-antitoxin one, some elaboration is required. In natural immunity, the blood contains a thermolabile substance which is bactericidal. To this substance Buchner gave the name "**Alexine**"; Metchnikoff spoke of it as "**Cytase**"; and Ehrlich renamed it "**Complement.**" In active immunization, a more thermostabile substance, bactericidal in nature, appears in the serum, and has been variously called "**Fixateur**" (Metchnikoff), "**Substance Sensibilisatrice**" (Bordet), "**Immune Body** or **Amboceptor**" (Ehrlich). This substance is found to act only in the presence of complement, and hence Ehrlich's conception that it has two combining affinities which must be satisfied to produce bacteriolysis. The one affinity binds it to complement, the other to the immunizing substance (bacterium or red blood cell, leucocytes and other body cells, toxins, ferments); called the **Antibody-producer,** or **Antigen.** The immune body he therefore called an amboceptor, or receptor with two hands, which he described as the cytophile haptophore and the complementophile haptophore. He also believes that the complement is composed of two parts, a haptophore group and a zymophore group. According to Ehrlich, the complement is unable to act directly on the antigen or antibody-producer, but only when connected by the immune body or amboceptor. Bordet, however, believes that neither antigen nor immune body has any affinity for complement, until when they are united they can absorb the complement, but not through the immune body. The hypotheses of Ehrlich have been used to explain agglutination, precipitation, and other phenomena, with sundry modifications. It is at present unnecessary to follow the theory further, because in these fields its explanations have been most called in question. This is not to be

wondered at considering the enormously increased and increasing complexity of the subject-matter.

Under either theory of immunity, the immune-body produced in active immunization is specific, that is, there is a special immune-body produced for each antigen. On the other hand, the complement, or alexine, or cytase, is believed to be one and the same, though Ehrlich and his school have argued in favour of specific complements for specific amboceptors.

FURTHER IMMUNITY PHENOMENA.

These can be more easily followed after the terms used in the theories have been acquired.

1. **Filtration of Serum.**—Muir and Browning found that on filtering serum through a Chamberland bougie, the immune-body passed through, and the complement did not.

2. **Fixation of the Complement.**—Bordet and Gengou planned an experiment, called the "Bordet and Gengou Reaction," to demonstrate the presence in a given serum of a specific immune-body, even in very small quantities. To this reaction the term "**Fixation of the Complement**" is now commonly applied, and has its best known practical use in the "**Wassermann Reaction.**" They performed two parallel experiments, (1) and (2), in which they used the following mixtures:—

(1). Heated immune plague serum + plague bacilli emulsion + fresh normal serum.

(2). Heated normal serum + plague bacilli emulsion + fresh normal serum.

Set aside for five hours at blood heat. Then added to each mixture:

(*a*) Heated hæmolytic serum + washed red blood cells.

Observe results: Mixture (1) shows no hæmolysis; mixture (2) shows hæmolysis.

The explanation of this phenomenon is after this manner: Looking at (*a*) we see that the mixture there requires the addition of complement to produce hæmolysis, since the complement in the serum has been destroyed by heat. Therefore, when mixture (2) produces hæmolysis, we infer that it must have supplied complement; and

similarly that mixture (1), since it did not produce hæmolysis, could not have supplied any complement. We have therefore to examine mixtures (1) and (2) carefully to determine the cause of their different actions. To do this, they may be rewritten thus :—

(1). Specific immune-body + specific antibody-producer + complement.

(2). Non-specific immune-body + specific antibody-producer + complement.

They differ only in their immune-bodies (normal serum is believed to contain non-specific immune-bodies). It is therefore inferred that in (1), since no complement is left free, it has become bound by the joint action of the specific immune-body and the corresponding antibody-producer. In (2), since the immune-body and the antibody-producer do not correspond, no binding or fixation of the complement occurs, and so hæmolysis takes place on adding (*a*). It should be noted here, that the quantity of complement used is determined by previous experiment, as the presence of an excess over the quantity which can be bound by the amounts of immune-body and antibody-producer would lead to hæmolysis, even in (1).

This reaction is capable of many applications in the determination of specific immune-bodies in a serum and of specific antibody-producers (antigens) in a serum. We shall here describe briefly the so-called "Wassermann test" for the diagnosis of syphilis, by determining the presence in the patient's blood of an immune body, capable when mixed with syphilitic antibody-producer (antigen) of causing fixation of complement.

Wassermann Test.—Requisites :

(1) Specific antibody-producer, referred to usually as the antigen.

(2) Red blood cells of a sheep, washed free of complement.

(3) Serum of a rabbit's blood, hæmolytic to sheep's red cells, heated before use, to destroy its complement.

(4) Fresh guinea-pig's serum, to supply complement.

(5) Serum from the patient, heated to 56° C., which serum is to be tested for the presence of specific immune-body or antibody.

1. The *antigen* at first used was a salt solution extract of the liver of a syphilitic fœtus. Alcoholic extracts have also been used, and it has been discovered that the extracts of normal liver and spleen, and even a 1 per cent emulsion of lecithin, will act as antigen. (This shows how little we must know of possible fallacies, in tests like this, with highly complex bodies like liver extract. It does not impugn its specificity for pure antigens, though it suggests the possibility of two different substances, with similar affinities for the immune-body produced by one of them. Of course the probability of this other substance being present, under the conditions of the experiment, is small, but must never be lost sight of.) The quantity of antigen to be used has to be carefully predetermined, as large excess of antigen has been found to cause binding of the complement, even in the absence of the immune-body. A series of trials of varying quantities of antigen with the same amount of complement in each case, shows the largest quantity of antigen which can be used without exerting this action. This amount of antigen is arranged, by proper dilution, to be present in 1 c.c.

2. *Washed Red Blood Cells.*—Some sheep's blood is gathered under aseptic precautions into a small sterile flask, containing sterile solution of sodium citrate (0·5 per cent) and sodium chloride (0·85 per cent). The corpuscles are separated by centrifugalizing, and washed repeatedly in the same way with sterile salt solution, to get rid of serum-complement and serum-precipitins. They are then brought down to a 5 per cent emulsion in salt solution, by mixing them with 19 times their bulk of the same.

3. *Hæmolytic Serum for Sheep's Cells.*—This is obtained by injecting a rabbit with washed red blood cells of a sheep, obtained as above. Of the 5 per cent emulsion, three or four injections are given at intervals of 5 to 6 days; the first injection of 5 c.c., the second of 10 c.c., the third of 15 c.c., and the fourth of 20 c.c.; intravenously or intraperitoneally. Ten days later than the final injection the serum is obtained by drawing blood from the carotid artery, allowing it to clot, and pipetting off the serum. A high-potency serum is desirable so that a small quantity only may be required. This obviates or reduces the risk

of precipitates, due to precipitins in the rabbit's serum for sheep's serum (from insufficient washing of the injected corpuscles), acting on any sheep's serum present, from insufficient washing of the corpuscles used in the test. The serum is inactivated by heating to 56° C. The quantity of hæmolytic serum to be used must be accurately determined, in relation to a definite amount of complement. This is the more necessary since it has been shown that, (contrary to Ehrlich's commonly accepted conception that immune-body and complement react in definite combining proportions), the hæmolytic immune-body and a complement react in inverse proportions; that is, the more immune-body, the less complement is required. The bearing of this on the test is that a very small quantity of complement left over by the combination of syphilitic-immune-body and antigen might suffice, in presence of large excess of hæmolytic immune-body, to cause hæmolysis; thus giving a negative result in a positive case. The quantity is determined by putting up a number of mixtures, each containing 0·1 c.c. of fresh guinea-pig serum to give complement, and 1 c.c. of the 5 per cent emulsion of sheep's corpuscles. To each mixture inactivated hæmolytic serum is added, in smaller and smaller quantities. The smallest quantity which gives complete hæmolysis is taken as the unit amount to be used in the test. The hæmolytic unit may therefore be defined as "the smallest quantity of inactivated hæmolytic serum which, in the presence of a stated amount of complement, is able to cause complete hæmolysis in 1 c.c. of a 5 per cent emulsion of washed red blood cells."

4. *The Complement.*—This is obtained by drawing blood from a guinea-pig, allowing it to clot, and pipetting off the serum, or separating by the centrifuge. Such serum, kept at a low temperature, preserves its complement in a fairly constant amount for three days.

5. *The Serum to be tested* is got from the patient under aseptic precautions, from the median basilic vein, from the finger, or from the ear. Three to four c.c. are withdrawn, as 1 c.c. of clear serum is required to go over the tests and controls. It is inactivated by heating to 56° C. in a water-bath for 20 minutes. Noguchi advises

54° C. Serum can also be obtained from cerebrospinal fluid, etc.

PROCESS.—

(*a*). In a test tube, put the following :—
0·1 c.c. of complement (fresh guinea-pig serum).
0·2 c.c. of inactivated test serum (from patient).
1·0 c.c. of standardized antigen (liver extract, etc.).
Add salt solution to make up bulk to 3 c.c. ; shake thoroughly, and place for 1 hour in incubator at 37·5° C.

(*b*). Now add,
1·0 c.c. of 5 per cent emulsion of sheep's red cells.
2·0 units of hæmolytic serum (determined as above).
Shake thoroughly, and incubate again at 37·5° C. for 1 to 2 hours.

(*c*). Observe result.
(1). *No hæmolysis.* Immune-body or antibody is present ; test *positive.*
(2). *Complete hæmolysis.* Immune-body or antibody is absent ; test *negative.*

Several controls should be put up at the same time to preclude error. Such are : a test with known normal serum ; tests with antigen and complement alone to see that antigen is not fixing complement ; test of hæmolytic serum and cells and complement with and without antigen to see that hæmolysis is actually possible, and tests with known syphilitic serum with and without antigen. In all these controls, except that with known syphilitic serum with antigen, complete hæmolysis should occur.

Noguchi has modified the test by using human red cells and an anti-human hæmolytic serum. This simplifies the test in that the patient's red cells may be used in testing his own serum, and in that no antibody for his red cells exists in his own serum (human serum at times contains an antibody to sheep's cells). Anti-human hæmolytic serum is prepared and standardized in a similar way to that described as used in preparing anti-sheep hæmolytic serum. Human serum, when used to provide complement, is said to vary more in its content than guinea-pig serum, to absorb 10 times as much immune-body, and to be less sensitizing.

Fleming's modification is to use the hæmolytic immune-body in human serum for sheep's corpuscles, and thus he can use the complement in the patient's blood. This simplifies the process.

D'Este Emery uses human red cells, sensitized with heated immune serum from a rabbit which has been injected with human cells. He advises only 5 minutes' incubation in a water-bath at 38° C., which very appreciably shortens the time taken for the test.

Browning, Cruickshank, and McKenzie describe "a method of carrying out the reaction which is very reliable in practice, and depends on the fact that the amount of complement absorbed by a mixture of serum and lecithin is increased on the addition of cholesterin, if the serum is syphilitic; but not, if the serum is normal. Accordingly, two series of tests are carried out simultaneously—in the one, complement is added to the mixture of serum and lecithin; in the other, to the mixture of serum, lecithin, and cholesterin. If more complement is absorbed in the second series than in the first, then the reaction is positive."

Value of the Test.—Like the agglutination or Widal test for enteric fever, the fixation of complement or Wassermann test for syphilis has its limitations.

It is almost always negative in healthy subjects, but quite often gives a positive reaction in those suffering from leprosy, scarlet fever, jaundice, yaws, sleeping-sickness, and the acute stage of malaria.

In the primary stage of syphilis, a negative result is usually got in the first fortnight. Thereafter one may expect a positive result in 50 per cent of the cases, and its absence has some weight.

In the secondary stage, in the presence of a suspected rash, a positive reaction is got in 50 to 70 per cent of the cases.

In the tertiary stage, with progressive lesions present, the absence of the reaction is almost conclusive proof that another diagnosis than syphilis must be sought.

In later stages, a positive reaction shows that the patient is not cured. Does a negative reaction show that he is cured? If a positive reaction was got previously, then it

is likely, always excluding treatment with mercury, which inhibits the reaction.

If, at any stage, the patient is under treatment with mercury, a negative reaction has no value for diagnosis. Stop treatment for one to two months, and test again. Salvarsan or "606" also inhibits the test.

In a few cases of syphilis, no reaction is given at any time.

In congenital syphilis, the reaction may persist throughout life, and be present even where there are no evidences of active pathological processes. The examination of the blood of the mother will usually give corroboration, and that of the father may, but not necessarily.

In tabes dorsalis, general paralysis, and aneurysm, a large number of positive results have been obtained; about 60 per cent in tabes, and 99 per cent in general paralysis. In the latter it is got in the cerebrospinal fluid.

Porges-Meier Reaction.—Equal parts of clear blood serum and a 1 per cent emulsion of lecithin or other lipoid substance in carbolized salt solution, are mixed and allowed to stand at room temperature for 5 hours. Normal serum causes no precipitate, but syphilitic serum does. This test is not nearly so delicate macroscopically as Wassermann's, and hence, while much more simple, is not so readily interpreted by the naked eye. Jacobsthal has studied it microscopically, by the aid of the ultra-microscope. He found that with normal serum, the particles of the lecithin emulsion appeared as isolated brilliant points, showing active Brownian movements. With a syphilitic serum, these brilliant points were seen to run together to form a large and brilliant mass. Intermediate reactions were noted, in which partial agglomeration occurred into small brilliant masses or brilliant chains. These phenomena were tested against the Wassermann reaction, and were found to run parallel to it.

Determination of an Antigen by Complement-Fixation.—The same principles apply, but in this case the immune-body present in the serum is known, and a serum is tested which is supposed to contain the corresponding antigen It has been applied to test blood for

the antigen of B. typhosus, using highly potent anti-typhoid serum obtained from an immunized rabbit.

Deviation of the Complement.—Neisser and Wechsberg, experimenting with mixtures of specific inactivated immune sera, specific antigen (bacteria) and complement, found that, beginning with the complement in excess, more and more bacteria were destroyed as the amount of immune-body was increased, up to a maximum. Beyond this, increase of the amount of immune-body lessened bacteriolysis, and finally in great excess, seemed to stop it entirely. To this phenomenon they gave the name of "deviation of the complement," in accordance with their theory that free immune-body has a greater affinity for antigen than immune-body joined to complement; and so in great excess of immune-body, the immune-body appropriates all the bacterial receptors, to the exclusion of the immune-body joined to complement; hence the cessation of bacteriolysis. This explanation cannot now be accepted, and so the term "deviation of the complement" is unfortunate and should be dropped. It is possible that the explanation of the phenomenon should be sought on physical lines, the excessive dilution of the mixture with serum containing immune-body reducing the chances of bacteriolysis by the complement in the same time-limit; and it is possible the complement may be inhibited by other constituents of the serum added.

Heterolysins, Isolysins, Autolysins.—A hæmolysin produced in the blood of one animal by the injection of the red cells of another species, is called a "heterolysin." When the hæmolysin is produced by injection of red cells of a member of its own species, it is called an "isolysin." Both of these have been produced. The production of a hæmolysin in an animal by the injection of its own red cells, has not been accomplished. If such a hæmolysin were produced, it would be called an "autolysin." The injection of isolysins produces "anti-isolysins," which like the heterolysins and isolysins are specific. The search for autolysins is clinically significant, as a possible theory for paroxysmal hæmoglobinuria.

ANAPHYLAXIS.

I. General Principles.—

As already defined, anaphylaxis is a supersensitiveness induced in man and animals by the injection of certain substances, mostly, so far as is known, of an albuminous nature. The ideas on this subject have grown around anomalous phenomena following on the injection of diphtheria toxin and antitoxin. Thus v. Behring and others noted that occasionally animals highly immune to the toxin showed excessive susceptibility to *small* doses of the toxin. Very soon after the introduction of the serum treatment of diphtheria in 1894, certain symptoms were observed to follow the injection of the serum, which, at first ascribed to the antitoxin contained in it, were finally found to be due to the horse serum which carried the antitoxin. These symptoms were hence called "**Serum Sickness**" or "**Serum Disease**" and were mostly a rash of an erythematous nature, and fever. These come on in most cases about the ninth day, but vary from the third to the nineteenth. Fever is not always present, and the rash is quite often urticarial, and occasionally scarlatiniform or morbilliform, is local to the point of injection or becomes general, is fugitive or persistent. Articular pains may accompany the rash, and may be severe. The frequency of these symptoms varies; from 30 to 55 per cent of the cases treated with serum are stated to show them in some degree or other. Some œdema is also noted by Currie, as accompanying the rash. The similarity of these symptoms to those of the specific infective diseases is striking, and led to the name "serum disease," which has therefore an incubation period (after subcutaneous injection) averaging nine days, and a duration of two days on the average. That these symptoms were due to the horse serum, and not to the antitoxin, has been proved by such symptoms following the injection of the normal serum of the horse. Another group of symptoms which on rare occasions followed the use of serum, was not at once recognized as due to it, but by the accumulation of cases and certain special features connected with them, the causal action of the serum was

obviously suggested, and experiments on animals proved the truth of the inference. These symptoms differed from those of serum disease in that they came on immediately or within an hour after the injection, were very severe, and in some cases ended fatally. The earliest recorded case is that of the son of Professor Langerhans, who was given a prophylactic dose of serum, took ill at once, and died shortly afterwards. The next recorded cases were three communicated by Goodall to the Antitoxin Committee of the Clinical Society (of which Committee he was a member). The first of these was that of a girl, aged 4 years, admitted to hospital suffering from diphtheria. She was given 4000 units on October 24th, 1897, and the same amount on October 25th and 26th. On Nov. 3rd there was a slight urticarial rash. On Nov. 30th she had a well-marked relapse of diphtheria, and was injected with 4000 units of antitoxin. "Within twenty minutes of the injection, she was seized with shiverings, quickly followed by two convulsions. Seen a few minutes later by the assistant medical officer, the convulsions had ceased, but the child was in a drowsy state, and the temperature had risen to 105° F. . . . There was no rash. . . . During the night the child vomited several times; about 6 a.m. on Dec. 1st a rash was noticed, and was a multiform erythema. It persisted till Dec. 5th, and while it was present the temperature remained up and the pulse was very rapid. On Dec. 5th and 10th there were twitchings of the mouth, and throughout the child was drowsy and apathetic, and had a bad colour. She slowly recovered and left the hospital well on Feb. 3rd." Similar cases could be multiplied, and some given in which the immediate reaction is limited to a local or general rash.

Such cases giving an immediate reaction fall into two groups: (1) Where the serum has not previously been injected; and (2) Where a dose of horse serum has been administered on a previous occasion (excluding doses given within the incubation period of the serum disease).

1. In the first group numerous fatalities have been recorded. In most of these, a history of asthma, or some similar condition, has been noted, and this is very important, since the subcutaneous injection of diphtheria

antitoxin has been recommended as a cure for asthma. A record of a fatal result in a man of 52 years, who died in tonic spasm ten minutes after receiving the serum, elicited particulars of 16 similar cases, and in the "*Therapeutic Gazette*" for March 15th, 1909, a short account is given of these, with 14 others, making 30 in all, in which alarming symptoms followed shortly after the injection, and ended fatally in 16 cases. In 22 of the 30 cases there was a history of asthma or some similar affection. (Goodall in *Public Health*, January, 1911).

2. Where there is a record of a previous administration of serum, while the immediate symptoms may be very alarming and dangerous, so far no fatality has been reported. The use of serum day after day in a severe case of diphtheria is not followed by these manifestations, unless a period of at least ten days separates the first dose from that causing the symptoms. It is this feature which suggested that the symptoms were due to supersensitiveness to serum, following on an attack of serum disease. The condition, beginning ten days after the first injection of serum, has been present in persons after five years, and may yet be found at a longer period. Experimental work on the subject has shown that rabbits injected with horse serum are very sensitive to a subsequent dose, show severe symptoms, and often die. This has been called the "**Phenomenon of Arthus.**" The "**Phenomenon of Theobald Smith**" is that guinea-pigs used to standardize antitoxin, when injected with a toxin-antitoxin mixture were always killed on the subsequent injection, after ten days, of normal horse serum. Otto, Rosenau, and Anderson, working independently, showed conclusively that the action of the serum was without relation to its antitoxin content; that sensitation of the guinea-pig was most marked after 10 days; that very small doses were efficient (0·001 c.c. or less); that the condition was transmissible from mother to offspring; that it was specific for the particular serum used; that it was not a hæmolytic nor precipitin action; that the condition could be conferred on another animal by injecting it with the serum of a sensitized animal; that a considerable dose of serum (5 c.c.) is required for the second injection; and that the symptoms are prompter in

appearing if the second injection is made intraperitoneally, cardially, or cerebrally, than if given subcutaneously. Sensitized animals which recover from the second injection are thereafter immune **(Antianaphylaxis).** This immunity may also be purchased by the injection of large quantities of the sensitizing serum towards the middle or end of the incubation period, but the duration of this immunity is believed by Otto to be short.

In a number of cases where a second injection is given after an interval, no immediate reaction (within 24 hours) follows, but an "**Accelerated Reaction,**" that is, the local and general symptoms of serum disease are noted earlier than in the previous attack, or than is the average where no record exists.

The simplest explanation of the phenomena of anaphylaxis is that of Wolff-Eisner, who holds that all proteid substances contain a toxic part, which does not produce an antibody when injected into animals. On the first injection a lysin is formed which breaks up the proteid, liberating the toxic part. A second injection results in the rapid liberation of the toxic part by the action of the already-present lysin, and hence the toxæmia. The profound affection of the nervous system, the general vaso-dilatation, and the more rapid action on intracranial injection, all suggest some substance which acts as a toxin on the nerve tissues. In this connection the use of serum for the cure of asthma is interesting, because it could be explained (if the patient survive) as diminished nerve-irritability to proteid.

II. Anaphylaxis to White-of-Egg.—

Besredka and Bronfenbrenner in their most recent mémoire (*Ann. de l'Institut Pasteur*, Mai, 1911), have studied very carefully the anaphylaxis produced by the injection subcutaneously of white-of-egg, both raw and heated to 100° C. They produced *active* anaphylaxis by the injection of 0·5 c.c. of white-of-egg, diluted with an equal quantity of normal saline solution. The state of anaphylaxis appeared in 16 to 20 days; with a smaller dose ($\frac{1}{100}$ c.c.), it appeared in 12 days. The injection of white-of-egg heated to 100° C. into other guinea-pigs,

produced a state of milder anaphylaxis than the raw white-of-egg. They hence conclude that the constituent of the white-of-egg which causes sensitation, while attenuated by heat, is thermostabile. This active state of anaphylaxis lasts for several months.

Passive anaphylaxis was produced within 24 hours after the injection into a guinea-pig of the serum of a guinea-pig which had been previously treated with white-of-egg. The injection thereafter into the jugular vein of the sensitized guinea-pig of $\frac{1}{500}$ to $\frac{1}{100}$ c.c. of white-of-egg, produced the classical symptoms of anaphylaxis, with death in 1 to 3 minutes. On the other hand, when the serum and the white-of-egg were injected at the same time, one guinea-pig showed a marked dyspnœa and some malaise immediately after the injection, but quickly recovered ; another showed slight respiratory distress ; and a third showed no reaction. In all three the injection was into the jugular vein ; in the first two it consisted of 2 c.c. of anaphylactic serum mixed with $\frac{1}{100}$ c.c. of white-of-egg ; in the third guinea-pig, it consisted of 1·5 c.c. of serum mixed with 1 c.c. of 10 per cent solution of white-of-egg, equal to $\frac{1}{10}$ c.c. of white-of-egg. Passive anaphylaxis, thus quickly induced, disappears more quickly than the active form, namely in about a fortnight. The mixture of the anaphylactic serum of the rabbit sensitized to white-of-egg, with white-of-egg, produced a precipitate. This was collected and diluted in normal saline, and injected into the jugular vein of two fresh (non-sensitized) guinea-pigs, and produced no reaction.

Whether active or passive anaphylaxis was conferred, the injection of a minute dose intravenously or intracerebrally produced the classical symptoms and shock resulting in death, described in the anaphylaxis due to serum and milk. Injected intraperitoneally, it is rare to get grave symptoms ; subcutaneously, no anaphylactic symptoms have been noted. This is markedly different from serum anaphylaxis, where the subcutaneous route is as fatal as the others. From this Besredka and Bronfenbrenner deduce that the most important thing in the production of the anaphylactic shock is the rapidity with which the antibody in the blood comes into contact

with the antibody-producer or antigen. White-of-egg being a viscous liquid is slowly absorbed from the peritoneum or subcutaneous spaces, and so the combination of the antibody and antigen has not the suddenness or instantaneousness which follows intravenous injection. (This deduction suggests that the antigen may act in a catalytic manner, causing an amount of chemical action out of proportion to its own mass injected. In such a case, the symptoms may be partly due to the accompaniments of rapid chemical action, such as the evolution of heat, etc.)

Antianaphylaxis can be produced to white-of-egg, as for serum, by the method of injecting small doses during the " incubation " time of anaphylaxis ; or by the injection of a minute dose, followed by a larger dose in 10 minutes, and so on for four doses. A guinea-pig, previously passively sensibilized, had $\frac{1}{2000}$ c.c. injected intravenously ; in 10 min. another injection of $\frac{1}{500}$ c.c. ; in another 10 minutes, a third injection, this time of $\frac{1}{50}$ c.c. ; and finally one of $\frac{1}{5}$ c.c. Thereafter the injection of 2 c.c. of white-of-egg, non-diluted, caused uneasiness but nothing further. Antianaphylaxis can also be established by oral and by rectal feeding, in two to four days. The state of anti-anaphylaxis is not so lasting as in the case of serum ; it lasts about three weeks, and two weeks where obtained by the oral or rectal method.

Besredka and Bronfenbrenner also found that the reactions were strictly specific, and that the anaphylaxis produced by white-of-egg was in the main specific. Feeble reactions were given by the white-of-egg of pigeon and turtle-dove. The anti-anaphylactic is strictly specific. Similar experiments were made with heated white-of-egg, with like results. The one protects little against the other, so that they conclude that their chemical constitution is different.

III. Serum-Globulin.—

Turro and Gonzales have investigated the subject of anaphylaxis to determine the nature of the substance which causes anaphylaxis with blood serum. The globulins were precipitated from horse's serum, and the

residual serum was kept. Guinea-pigs were injected with 1 c.c. of a 0·33 per cent solution of the globulins; and 12 days after, it was found that the minimal test dose was 1 c.c. of a 0·66 per cent solution (= 2 c.c. of the former solution). Anaphylactic shock developed rapidly, and there was a rapidly ascending paralysis, beginning in the hind limbs and causing death through asphyxia when the respiratory centres became affected, in 2 to 4 minutes. There were no convulsions, but in the male animals there was an abundant emission of serum.

Another set of fresh guinea-pigs were given 1 c.c. of a 1 per cent dilution of the globulin-free serum; and in twelve days thereafter, a test dose of the globulin solution produced no symptoms of anaphylaxis. (No mention is made of a test with the globulin-free serum itself.) An animal injected with normal serum, and later with the globulin solution test dose, showed intense muscular tremors, (the animal jumping about), but all ended in recovery.

When the blood of a sensitized guinea-pig is mixed with globulin solution, and 1 c.c. injected into the jugular vein of a normal guinea-pig, the animal dies in 2 to 3 minutes with symptoms identical with those described in animals sensitized with pure globulins. They proceeded to study the anaphylactic poison, which they found is readily destroyed by oxidation and light, is dialysable, is thermostabile, and is soluble in alcohol and ether. They conclude that it is alkaloidal in nature, and should be considered a leucomaine, that is, a toxic substance produced in the living body by proteid metabolism, and not by bacterial action.

IV. Classification.—

The foregoing *resumé* of the subject of anaphylaxis shows that it can be classified in the same manner as immunity into:—

1. *Natural Anaphylaxis.*
2. *Acquired Anaphylaxis.*

And each of these into sub-groups, thus:—

Natural anaphylaxis depends on—

a. Species of animal, e.g., cholera in man; anthrax in cattle; glanders in horses.

b. Age, e.g., diphtheria in children; erysipelas in elderly individuals.

c. Individual, e.g., to white of egg, blood serum even by ingestion. ("One man's food is another man's poison.")

Acquired anaphylaxis depends on—

a. An attack of the disease, e.g., erysipelas and diphtheria.

b. The injection of dead cells, e.g., tuberculin.

c. The injection of nitrogenous matter, e.g., blood serum and white of egg.

The subject of anaphylaxis has been dealt with here because of its intrinsic importance, and because it would seem to demand a restatement, in the near future, of the philosophy not only of infection but of medicine generally. The old idea of "diathesis" acquires anew its importance (having received direct experimental proof), and many apparently worn-out theories and old-fashioned explanations of disease may have new life put into them.

CHAPTER XII.

MICROCOCCI.

THESE organisms consist of cells more or less globular in form and varying in size from 0·5 micron to 2 micra in diameter, but most measure about 1 micron ($\frac{1}{1000}$ mm., or $\frac{1}{25000}$ of an inch). They are usually classified according to the mode of division or the resultant shape. Thus we have streptococci, staphylococci, diplococci, tetracocci (tetragenus), and sarcinæ. None show endogenous spore formation, but of some it is alleged that they form arthrospores. Most are non-motile, but a few motile species possessing flagella have been described (none pathogenic).

STAPHYLOCOCCI.

These were first demonstrated in pus by Pasteur in 1880 and Ogston in 1881, and in pure culture by Becker in 1883. Rosenbach in 1884 established specificity as cause of some forms of wound-suppuration and of osteomyelitis. They are so named from their growth in grape-like clusters. Several hundred species have been described, but the chief varieties are: Staphylococcus pyogenes (aureus, albus, and citreus); Staphylococcus epidermidis albus; Staphylococcus cereus (albus and flavus).

The common characteristics are: Grape-like clusters of cocci, 0·9 micron in diameter; non-motile; non-sporing; grow readily on most media; stain readily; Gram-positive; gelatin-liquefying; produce acid and clot in milk; form indol; reduce nitrates to nitrites; show colour reduction with litmus, methylene-blue, and rosanilin (fuchsin); aerobes, but facultative anaerobes; optimum temperature for growth, 28° to 30° C. Range from 8° to 42° C. Thermal death-point 30 min. at 80° C.; freezing useless.

Cultures.—Put up (1) broth, (2) agar slope, (3) agar

plate, (4) gelatin stab plate and slope, (5) milk, (6) potato, (7) peptone water, (8) nitrate, and (9) sugar media.

In broth, uniform turbidity with thin surface, pellicle ultimately settling as a heavy mucoid deposit, with a sour odour like weak butyric acid.

On agar slope, abundant growth in 24 hours at 37° C.; with smooth shining surface and resembling a streak of oil paint. Single colonies are circular.

On agar plate, numerous small, shining, pin-head shaped colonies; round, finely granular, with smooth edges, remaining discrete, and varying greatly in size.

On gelatin plate, growth occurs readily at 20° C., and shows much the same as in agar. The colonies are not flat, but rise from the surface as the segment of a sphere. Liquefaction of the gelatin; and gradually (after 48 hours or more), shallow saucer-shaped depressions are formed, which grow larger and finally become confluent. Liquefaction of gelatin by staphylococci is due to a ferment-like body elaborated by them and spoken of as "gelatinase" and which can be obtained apart from the cocci by filtration of cultures. It is an extremely thermolabile substance.

In gelatin stab, a streak of growth is visible in 24 hours, and liquefaction begins at the top in 2 to 3 days, forming a funnel with flocculent deposit of the bacteria. Ultimately fluidification extends to the wall of the tube.

In milk, coagulation takes place in 3 to 4 days with formation of lactic and butyric acids.

On potato, growth is abundant, rather dry, and usually deeply pigmented.

In peptone water, indol is formed.

Pigment formation is best seen in serum or starchy media and aerobically only. It is insoluble in water but soluble in alcohol, chloroform, ether, and benzol. It is a C, H, and O compound, a "lipochrome", or fatty pigment. Strong H_2SO_4 changes it to green or green-blue.

Toxic Products.—Endotoxins, hæmolysins, leucocidins.

Pathogenicity.—For man: abscesses, boils, carbuncles, endocarditis, osteomyelitis; for animals: rabbits most, mice medium, guinea-pigs least.

Immunization.—Take three-weeks-old culture, heat at

60° C. for one hour; inject 100 to 250 million of the dead staphylococci, repeating in 3 to 4 days later; opsonins increased.

Habitat.—Skin and mucous surfaces.

Differentiation: (Gordon).—Staphylococcus pyogenes aureus clots milk, liquefies gelatin, at times produces green fluorescence in neutral red broth, reduces nitrates to nitrites, produces acid in Lemco media containing maltose, lactose, glycerin, and mannite. Staphylococcus epidermidis albus gives the same reactions, except that it does not produce acid with mannite.

Houston's Lemco Medium: consists of distilled water containing 1 per cent of Lemco, 1 per cent of peptone, 0·1 per cent of sodium bicarbonate, and litmus solution to colour. Add 1 per cent of test substance (carbohydrate, etc.).

STREPTOCOCCI.

Ogston in 1881 first differentiated between the irregularly grouped staphylococci and the chain cocci or streptococci. Pure cultures were first obtained by Fehleisen in 1883 and Rosenbach in 1884. Named from tendency to form chains, and so the group includes micro-organisms which differ considerably from each other in cultural and pathogenic characters. The streptococci pathogenic to man mostly form chains of eight or more individual cocci, while the saprophytic varieties are apt to be united in shorter groups. On this basis streptococci have been divided into S. longi and S. breves, but the distinction is not a reliable one. Similarly, the streptococcus of ordinary pus formation was thought to be different from that of erysipelas. This is now proved not to be the case, and the two are regarded as one and the same. These statements enable one to attach the proper significance to the various names used; to wit: S. pyogenes, S. erysipelatis, S. longus, S. brevis. S. conglomeratus is a variety, and is so called from its forming in broth culture minute granules composed of very long chains (identical with S. anginosus or scarlatinæ). Streptococci grow well on all the richer media, and better in broth made from meat or veal than from meat extract. Animal serum and

glucose render media more favourable for streptococci cultivation, and alkalinity 0·5 per cent.

Streptococci are easily stained with the usual aniline dyes and are Gram-positive. They are non-sporing, non-motile, and non-flagellar. They (at least the pyogenic species) do not liquefy gelatin. Optimum temperature 37·5° C. Growth takes place at 15° to 20° C. (distinction from pneumococci). Aerobiosis is the suitable environment for most races, but strict anaerobiosis does not prevent development in suitable media. Size 0·5 to 1 micron.

Cultures.—In broth (bouillon) minute granules, which fall to the bottom and form a sparse powdery or sandy deposit. Diffuse clouding is rare. The long chain forms produce the coarser granules.

In glucose broth, the rapid formation of lactic acid arrests development (1 per cent of sterile $CaCO_3$ obviates).

In gelatin stab, in 48 hours a thin line forms which later is seen to be made up of minute rounded colonies of whitish colour reaching the size of a pin's head in 5 to 6 days. No growth on surface nor liquefaction.

On agar plates, the colonies are small, greyish, and delicately opalescent, are round, and tend to remain separate in stroke cultures.

In milk, ready growth with acid formation but no clot (in pyogenic varieties).

On potato, no growth.

On blood agar plates, most cause hæmolysis and decolorization (difference from pneumococci). Ferment lactose, saccharose, and salicin; not inulin in Hiss's medium.

Thermal Death-point: 10 minutes at 54° C.

Virulence varies; greatest for white mice and rabbits.

Habitat: skin and mucous membranes.

Diseases: cellulitis, erysipelas, osteomyelitis, bronchitis, pneumonia, empyema, pericarditis, otitis, pharyngitis, tonsillitis, septic endocarditis, septicæmia, puerperal fever.

Toxins: streptolysin (hæmolytic).

Immunization: variable results.

Differentiation: (Andrews and Horder).—Streptococcus *pyogenes* does not clot milk, nor give green fluorescence with neutral red broth, nor produce acid in Lemco media with raffinose, inulin, coniferin, and mannite. It produces

acid with lactose, saccharose, and at times with salicin; it grows on gelatin at 20° C.; it forms long chains, and is pathogenic to mice.

Streptococcus *salivarius* clots milk, produces acid with saccharose and lactose, and at times with raffinose; at times grows on gelatin at 20° C., and at times produces fluorescence. It is negative to the other tests, and grows in short chains.

Streptococcus *anginosus* gives the same reactions as S. salivarius, except that it never produces acid with raffinose; it forms long chains, and it is pathogenic to mice.

Streptococcus *fæcalis* (human) is positive to all the tests except production of acid with raffinose and inulin. It is non-pathogenic to mice, and it forms short chains.

Streptococcus *equinus* (in horse dung) produces acid with saccharose, salicin, and coniferin, and grows on gelatin. It is otherwise negative; forms short chains, and is non-pathogenic to mice.

The *pneumococcus* forms short chains, is pathogenic to mice, clots milk, forms acid in lactose, saccharose, and raffinose, and at times in inulin. It does not grow on gelatin at 20° C.

Examination of Pus.—By using solid media and method of 3 dilutions.

(1) Gelatin plates: Three tubes are melted at 30° to 35° C. in a water-bath. Inoculate one with a loopful of pus, replug, and mix by rotating tube. This is tube of 1st dilution. Inoculate second tube with 3 loopfuls from 1st tube: tube of 2nd dilution. Inoculate third tube with 3 loopfuls from 2nd tube: tube of 3rd dilution. Now pour out all three tubes into dry sterile Petri dishes. Allow to set, and incubate at 20° C.

(2) Agar plates: Same procedure, only melt at 100° C. and cool to 40° C. before inoculating, and work quickly. Warm the plates. Incubate at 37° C.

PNEUMOCOCCUS.

The pneumococcus has been at various times called Streptococcus pneumoniæ, Diplococcus pneumoniæ, Micrococcus lanceolatus, and Fraenkel's pneumococcus.

The sputum of cases of acute lobar pneumonia, injected into mice or rabbits, produced more constantly a septicæmia with fatal results than did the sputum of healthy individuals. In this " sputum septicæmia," lance-shaped cocci in pairs were most frequently found. Weichselbaum in 1886 examined 129 cases of all forms of pneumonia, and described four organisms which he found, the most frequently present being the one now known as the pneumococcus. It was present in all forms. The Streptococcus pneumoniæ (now believed to have been a more vigorous pneumococcus) was next in frequency, then the pneumobacillus, and lastly the Staphylococcus pyogenes aureus.

Description.—A small coccus, generally occurring in pairs, and surrounded by a definite capsule. The cocci are lance-shaped, like the flame of a candle; and in the pair have the pointed ends opposed. A close resemblance to a bacillus is thus formed. The capsule is characteristic in preparations from the sputum and tissues, but is only got in serum cultures, and is absent at times even in the sputum and in scrapings from the lung. The coccus is non-motile, non-flagellar, non-sporing. It is readily stained by the usual aqueous aniline dyes, and it is Gram-positive. The capsule is well shown in specimens stained by Gram and counter-stained: it takes the latter.

Cultures.—Growth on the ordinary media is variable. From sputum it is best isolated by animal injection and cultures from the heart's blood. The best temperature is 37·5° C. Growth does not take place as a rule below 25° C. The colonies are like drops of dew. Blood serum and blood agar are the best media. In gelatin stab (when growth takes place), a row of minute dots appears, but no liquefaction. In broth, a slight turbidity results, which settles to the bottom of the tube as a dust-like deposit. Cultures rapidly die out, and virulence is quickly lost. In milk, growth is rapid, with acid and clot, and capsules are usually formed. Prolonged life is said to have been got in cultures on ascitic agar and blood-smeared agar. The pneumococcus ferments saccharose, lactose, raffinose, and inulin.

Habitat.—The mucous membranes of the mouth, nose, throat, and conjunctiva.

Products.—No soluble toxin yet isolated; endotoxin by freezing and grinding.

Pathogenicity.—For mice and rabbits, very great; for guinea-pigs, less; for man, medium. Susceptibility is characterized by general septicæmic infection; resistance by occurrence of localized processes, e.g., in man: acute lobar pneumonia, pleurisy, empyema, pericarditis, meningitis, otitis media, etc.

Isolation.—Inoculate a mouse at the root of the tail with a little of the suspected material. The animal dies in 24 hours, and its blood is swarming with typical, encapsuled lance-shaped diplococci, if pneumococci present.

Immunity.—Agglutinins are formed, and agglutination is observed in 1–40 to 1–50 dilution with serum from pneumonic patient, and most marked at time of crisis. Passive immunization with sera has been unreliable so far. A leucocyte extract with water has given encouraging results.

Resistance.—Has been found alive in dried sputum after 55 days. Ten minutes at 52° C. is fatal. Very sensitive to weak solutions of disinfectants, but not in sputum.

STREPTOCOCCUS MUCOSUS.

This organism has been isolated from cases of meningitis, peritonitis, phlebitis, and parametritis, and from certain cases of pneumonia. It shows a marked tendency to form chains, but often appears in diplococcus forms. It is also capsulated, but never lance-shaped. It reacts to sugars as does the pneumococcus. It is pathogenic to white mice, but not so markedly to the rabbit as the pneumococcus. Growths are similar. It excites the formation of weak agglutinins, which can also in some cases agglutinate pneumococci. Also anti-pneumococcic serum frequently agglutinates it. These facts suggest a group relationship.

MENINGOCOCCUS.

First described by two Italian observers in 1884, but first cultivated by Weichselbaum from cases of cerebrospinal meningitis in 1887. It is a small coccus, very like the gonococcus, and like it occurring in pairs,

the adjacent sides being flattened like a coffee bean or two D's opposed to each other by the flat sides. In most cases it is present inside the protoplasm of the leucocytes in the exudation, the leucocytes being of the polymorphonuclear variety. Hence it has been called the Micrococcus intracellularis meningitidis. The variety of meningitis in which it is chiefly found is epidemic cerebrospinal meningitis, which is now a notifiable disease in a large number of districts. It is non-motile, non-sporing, non-flagellar, non-capsulated, and Gram-negative. It is found in the exudates, and specially in the spinal fluid obtained by lumbar puncture, and in such fluid it is well demonstrated in the cells by using Jenner's stain. It is easily stained by the usual dyes, and with methylene-blue stains irregularly. It is not readily cultivated on the ordinary media, and grows best on blood serum and ascitic agar. On blood serum, white shining viscid colonies appear in 24 hours. Cultures readily die out. It ferments maltose and dextrose with acid production (distinction from M. catarrhalis). Involution forms occur. In the patient, agglutinins and opsonins are formed. Distinguish from M. catarrhalis, which is found in the nose, and grows at room temperature, whereas M. meningitidis is not easily grown below 25° C. The sugar test is also helpful. M. pharyngis also grows at room temperature and ferments maltose, dextrose, saccharose, and lævulose. M. mucosus gives slimy growths. Other Gram-negative cocci are chromogenic.

GONOCOCCUS.

First found in urethral pus and pus from ophthalmia neonatorum by Neisser in 1879. Cultivated by Bumm in 1885 on human blood serum. Diploforms very similar to the meningococcus, and like it may be found in pus, intra- but also extra-cellularly. The gonococci are non-motile, etc., and Gram-negative. They are mostly extra-cellular in chronic discharges, and may be rather scarce. They do not grow on gelatin or agar, but on serum or ascitic agar, and best at blood heat. Growth ceases below 30° C. Colonies appear within 48 hours, but may not until four days. They are readily killed at 42° C. They

ferment dextrose but not maltose. A vaccine treatment for gonococcus has been used with success in chronic cases. An emulsion of gonococci in sterile salt solution (0·85 per cent) is heated to 65° C. for 1 hour. Inject 300 million, and give dose every 7 to 10 days, increasing to 1000 to 1200 million.

MICROCOCCUS TETRAGENUS.

Found in 1881 by Gaffky, in pulmonary cavities. Usually non-pathogenic in man. Grows well on ordinary media, clouds broth evenly, does not liquefy gelatin, clots milk with acid formation, is Gram-positive, and is capsulated in the body. It is pathogenic to white mice, causing septicæmia; slightly so to guinea-pigs and rabbits, and non-pathogenic to house mice and rats. It has been described as the cause of abscesses, on one occasion of meningitis, and on another of septicæmia.

MICROCOCCUS CATARRHALIS.

Found in patients suffering from catarrh of the upper respiratory tract. Its chief claim to attention is its similarity in staining and morphology to the meningococcus and gonococcus. From the latter it is distinguished by its rapid growth on the ordinary culture media. From the meningococcus, with which it may be found in the nasal passages, it is similarly distinguished, but here the difference is one of degree only. The sugar tests are very helpful.

	Dextrose	Maltose	Lævulose	Saccharose	Lactose	Galactose
Meningococcus ..	+	+	o	o	o	o
Gonococcus ..	+	o	o	o	o	o
M. catarrhalis ..	o	o	o	o	o	o
M. pharyngis sicc.	+	+	+	+.	o	o

MICROCOCCUS MELITENSIS.

This organism was first described by Bruce in 1887, as present in the spleen of patients dead of Malta (or undulant, rock, Mediterranean, or Neapolitan) fever.

This is an endemic pyrexial disease, occasionally prevailing as an epidemic, having a long and indefinite duration and an irregular course, with, almost invariably, pyrexial relapses of an undulatory type. The illness may last from 20 to 300 or more days, averaging 60 to 70 days, and having a mortality of about 2 per cent. The chief mode of spread formerly was the ingestion of goat's milk, in which the micrococcus was being excreted. The urine of patients is also infectious. The Mediterranean Fever Commission conclude that Malta fever is a septicæmia, in which the specific organism can be recovered from the peripheral blood, the urine, and the fæces. Infection is not conveyed by the sputum, sweat, breath, or skin-scrapings of patients. It does not take place if contact is limited to skin surfaces only, and if urinary and fæcal contamination are excluded. It is probably occasionally conveyed by sexual intercourse. About 10 per cent of the Maltese goats excrete the micrococcus, and 50 per cent give a positive agglutination reaction. Goat's milk is probably the prime source of the disease in most cases if not in all.

Characteristics.—Micrococcus melitensis is a very small coccus, 0·3 micron in diameter, occurs singly or in pairs, and at times in short chains. It is non-motile and Gram-negative; does not ferment glucose (unlike ordinary streptococcus), and renders milk slowly alkaline. It is easily stained. Some observers describe it as a minute bacillus.

Cultures.—On agar, it forms minute transparent colonies likened to dew-drops, but only after 2 to 3 days' culture at 37·5° C. On gelatin, liquefaction is not produced. In broth, no indol is formed, nor odour; but a slight turbidity. On potato, a moist transparent growth is formed.

Agglutination of the organism by the patient's serum is shown after the fifth day. In some cases it persists for years.

Resistance.—Is a vigorous organism and resists desiccation for weeks.

Malta Fever.—Other symptoms of the disease are: shifting rheumatic-like pains, profuse sweatings, constipation, local neuritis, emaciation, and almost always enlarged spleen. The micrococcus may be isolated by splenic puncture or from the blood.

CHAPTER XIII.

NON-SPORING BACILLI.

THE COLON-TYPHOID-DYSENTERY GROUP.

THIS is a large group, which includes the colon bacillus and its allies; the typhoid bacillus; paratyphoid bacilli; dysentery bacilli and allies; and Bacillus fæcalis alcaligenes.

Closely related to the group (but not properly within it) are the B. lactis aerogenes, B. mucosus capsulatus (Friedlaender's bacillus), and B. proteus.

All the members of the group are bacilli and are very similar morphologically, but exhibit minor differences insufficient to permit of accurate diagnosis from morphology alone. All are non-sporing, non-liquefying of gelatin, Gram-negative, and grow well at room and body temperatures on artificial media.

They are distinguished from one another by a careful cultural and biological study, to wit: reactions in special media (e.g. the FL-AG-IN-AC group of reactions:—fluorescence with neutral red, acid and gas with lactose, indol with peptone water, acid and clot in litmus milk); motility and flagella; and reactions with specific immune sera (chiefly agglutination).

Bacillus Coli Communis is a name which stands for a group of organisms, one member of which was first described by Buchner in 1885. The one taken as a type of the group was obtained from the stools of a breast-fed infant, and was described by Escherich in 1886 as the Bacterium coli commune. It is now usually designated B. coli (Escherich).

It is widely distributed in nature and has been isolated from air, water, and soil, but is found most abundantly and constantly in the intestinal tract of man and of many of the higher animals, from which habitat it probably finds its way into soil, water, and air. Its chief characteristics are: short plump rod, 2 to 4 micra long and 0·4 to 0·7 micron broad (very short oval and coccus-like forms are found,

especially in the animal tissues); grows well on usual media; curdles milk with acid production in forty-eight hours; forms acid and gas with dextrose and lactose (gas = hydrogen and carbonic acid gas in proportion of 2 to 1); some ferment saccharose; some are weak and aberrant; not usually pathogenic (agonal or post-mortem invasion excluded); sometimes causes peritonitis, cholecystitis, pyelitis, and cystitis; can precipitate cholesterin from solution (hence may cause gall-stones); cannot peptonize native proteids (casein and egg albumen, etc.).

Cultures.—In broth: uniform turbidity. In gelatin stab: growth along whole line of stab and film-like abundant growth on surface, but no liquefaction. On gelatin plate: surface colonies are apt to show the typical grape-leaf formation. (In gelatin stab, a few gas bubbles may form, see later.) On agar slope: a dense glistening white or greyish growth. Same on blood serum. On agar plate: surface colonies show grape-leaf structure. On potato: abundant growth, at first greyish-white, turning later to yellowish-brown. Cultures are characterized by a peculiar fœtid odour, not unlike that of diluted fæces. Grows well on media containing urine and bile. In peptone water: forms indol. In milk: acidity and clot. In lactose litmus agar: the medium becomes red along stab, and gas bubbles appear. In carbohydrate media: acid and gas are formed in presence of glucose, lactose, lævulose, galactose, maltose, raffinose, mannite, dulcite, and sorbite; and occasionally in saccharose (cane sugar) and in the glucosides, salicin and arbutin. Some varieties change neutral-red, first to a rosy-red and then to a green fluorescence (in glucose broth); and most reduce nitrates to nitrites. Aerobe, but facultative anaerobe. Motile, having from 4–12 peritrichal flagella.

B. Typhosus was discovered by Eberth in 1880 in the spleen and mesenteric glands of persons dying of typhoid or enteric fever. In such sections the bacilli occur in groups, scattered individuals being rare. Gaffky in 1884 first grew it in pure culture and studied its characters.

Characteristics.—Short plump rod with rounded ends, 1 to 3 micra long and 0·5 to 0·8 broad; actively motile; numerous peritrichal flagella (10 to 14); growth less

luxuriant than B. coli ; Gram-negative ; produces acid but no gas in dextrose broth and agar, no change with lactose or saccharose, no clot in milk, but in litmus milk slight acid at first, from small quantity of monosaccharid present ; later, deep blue from formation of alkali ; no indol in peptone water ; on potato slight moist glistening growth, becoming dull and velvety.

Cultures.—In broth : uniform turbidity. In gelatin stab : growth to bottom of stab, and on surface as a thin leaf-like film or pellicle with irregular wavy margin. In gelatin plate, the colonies are smaller, more delicate, and more transparent than those of the colon bacillus of the same age. In agar plates, in twenty-four hours, small greyish colonies are formed, at first transparent but later opaque. On acid potato : slight moist glistening growth.

Forms acid but no gas with dextrose, lævulose, galactose, maltose, mannite, and dextrin ; but no change with lactose and saccharose.

In Hiss's tube medium (agar, gelatin, dextrose, Liebig's extract, salt, water), it gives uniform clouding owing to its motility, but no gas ; whereas dysentery bacillus grows only along stab, and colon bacilli form sky-rocket-like figures, and the medium is broken with gas bubbles.

Optimum temperature, 37° C. ; range 15° to 41° C. Resistance like most non-sporing organisms, 30 min. at 60° C., or 2 to 3 min. at 100° C. In ordinary tap or distilled water it is usually found dead in three weeks (Frankland). The researches of Dr. A. C. Houston have shown that in the crude river water derived from the Thames, *raw* typhoid bacilli die out in one to two weeks, whereas *cultivated* typhoid bacilli may persist for five weeks. On this basis, the storage of water for thirty days, preliminary to filtration, is looked upon as of prime importance in all waters derived from sources polluted by sewage.

Does not multiply in water, even when impure. Is by preference a parasite, and when found outside the body can be usually traced to sewage of typhoid patient or convalescent (carrier). In natural bodies of water it retains its vitality for at least four to five days, and in sterile water it may be found surviving for three months. History of typhoid epidemics from sewage-polluted water

shows that danger is chiefly to be feared when pollution is recent. In soil and fæcal matter, however, duration of vitality is more prolonged (five months in privy refuse, and fourteen days after being spread on ground), but no genuine multiplication proved. This suggests one mode of pollution of surface water and surface wells after rains, by the washing of dormant bacilli into the same, and to the danger of using human excrement for manuring vegetable gardens and fields near water sources (Lincoln, 1905). Air-borne infection is rare. Sewer air is not regarded as an actual cause so much as a predisposing one.

Pathogenicity.—For man: enteric fever, typhoid fever, abdominal typhus (German), la fièvre typhoïde (French). For animals: do not multiply, and same effects by injection of dead bacilli, as that due to endotoxins. Chimpanzees have been infected by food, and have shown characteristic lesions. Intraperitoneal injections produce a short acute illness with pyrexia, etc., but non-specific.

Specificity has not been absolutely proved according to Koch's postulates. In enteric fever the bacilli are present in the blood, the bowel, the urine, the sputum and the rose spots. Toxins are intracellular.

Immunity usually follows one attack, and is due to bactericidal and bacteriolytic bodies.

Active Immunization is accomplished in animals by a first injection of 1 c.c. of a broth culture heated for ten minutes at 60° C., followed in five or six days by a larger dose, and so on, until finally living cultures are injected in considerable doses without serious consequences.

Wright's vaccination against typhoid has been used extensively in the British army. He uses a strain of bacillus standardized by passage through guinea-pigs, and sterilizes the culture by heating to 60° C. for five minutes. The first injection is of an amount of bacilli fatal to 100 grm. of guinea-pig, or alternatively, 750 to 1000 million of dead bacilli. The second injection given eleven days later should be double the first. The first dose is followed by tenderness and swelling locally and at the adjacent lymphatic glands, and some pyrexia, all of which usually subside in twenty-four to forty-eight hours. The method

is believed to reduce the case incidence and the mortality among vaccinated persons attacked.

Vaccine treatment of typhoid fever on similar lines has been tried by Leishman and Smallman, using one-fifth of the dose used for protective inoculation. If the temperature fall, the injection may be repeated every four days. Antityphoid serum has been used, but results are equivocal.

Agglutination.—The blood or blood serum of an animal previously inoculated with typhoid bacilli, when added to a suspension of living and motile typhoid bacilli, causes the latter to become motionless and aggregated (clumped). This reaction is one given in many diseases after the attack—a reaction of immunity; but in enteric fever it is given during the attack—a reaction of infection. The reaction is specific in high dilutions, but not absolutely so. Group bacilli are clumped, but in lower dilutions: for example, serum clumping typhoid bacilli in a dilution 1–40,000 will clump paratyphoid bacilli in a dilution 1–1000, and coli bacilli in 1-500. The reaction is used for : (1) *Diagnosis of disease* (*Widal's reaction*); and (2) *Diagnosis of bacteria* (*Grueber's reaction*).

1. **Widal's Reaction.**

a. Microscopic method.—Requirements: An eighteen to twenty-four hours' culture in broth of undoubted B. typhosus. Blood or serum. Platinum loop, a hollow ground slide, an ordinary slide or a watch-glass, cover-slips, a one-sixth-inch lens, and vaseline.

PROCEDURE.—1. Test motility of bacilli and absence of clumping by putting up a hanging drop of culture. If clumps, filter. If movements sluggish, warm.

2. Take a clean dry slide and put on it nine loopfuls of sterile broth arranged in a small circle. Put one loopful of serum in centre of circle and mix. Dilution 1–10. Now put one loopful of 1–10 dilution on a clean slide or hollow slide, mix with three loopfuls of sterile broth, and finally with one loopful of culture. Dilution is now 1–50.

3. Mount one loopful on a cover-slip, invert over hollow slide, and examine. Examine again in fifteen minutes and after one hour. If reaction is positive, the bacilli will be arranged in groups and be non-motile, and between the clumps will be clear spaces.

Or the serum may be diluted by means of a graduated pipette, such as a leucocytometer pipette, or by using a capillary pipette which is filled to a certain mark and emptied into a watch-glass, and the desired number of the full of the pipette of bouillon added and mixed. The mixture of a pipette-full of the diluted serum and of the bacillary emulsion is then examined, the final dilution being double that of the diluted serum.

b. Macroscopic method or Sedimentation.—Take a range of test tubes, 5 c.m. × 0·5 c.m., and put into each 1 c.c. of various serum dilutions and 1 c.c. of bacillary emulsion. Also put up a control with 1 in 20 normal serum. Plug, and keep vertical, and either incubate for three hours at 37° C., or keep at room temperature for twelve to twenty-four hours; or first incubate and then keep for twenty-four hours, reading the results at both stages. The result can be controlled by microscopic examination of the supernatant fluid.

The statement of the result should comprise all the conditions of the experiment, namely: kind of test (hanging-drop or tube), dilution of serum, times of observation, and intensity of reaction (complete, medium, or nil agglutination).

The test is also given by dead bacilli. Young cultures are used to prevent spontaneous agglutination. It is not always convenient to have young cultures, and so the following suspension of dead bacilli (which keeps well but agglutinates tardily though easily), may be used: To a twenty-hours' broth culture, add 1 per cent formalin, incubate for two days at 37° C., pour off the fluid from the precipitate and store in an ice chest.

Interpretation of Results.—A **positive** Widal reaction may be due to: (1) An attack of typhoid fever (especially if increasing); (2) A previous attack; (3) An attack of paratyphoid fever; (4) Some other disease (jaundice and tuberculosis).

A **negative** Widal may be due to: (1) Too great or too little infection; (2) Disease not typhoid; (3) Test applied too early (before eighth day); (4) Inhibition phenomena (some sera agglutinate in a dilution 1–100 and not in 1–20).

2. **Grueber's Reaction.**—Consists in determining the race of a bacterium by testing it against the blood serum of an animal immunized to an organism of known race. The reaction is the converse of the Widal one, but high dilutions (1–1000) must be used to avoid error due to group agglutinins. It is open to the fallacies that some bacilli are inagglutinable, though belonging undoubtedly to an agglutinable race (some typhoid bacilli), and bacteria giving a negative result may nevertheless have very similar pathogenic characters to those giving a positive result.

Principal Channels of Infection.—Water, milk, ice-cream foods, oysters, mussels, water-cress, lettuces, radishes, flies, dust, contact, chronic germ-carriers.

Paratyphoid Bacilli (also called paracolon bacilli)—are the probable cause of a disease clinically resembling mild typhoid (about 3 per cent of cases treated as typhoid are probably paratyphoid). Onset is usually sudden, with chills; mortality is low (2 per cent), and on post-mortem examination no characteristic ulceration of Peyer's patches is observed. Two chief varieties are described, namely, paratyphoid "A" and paratyphoid "B." Of these B is the more widely distributed in nature, and is more pathogenic to animals, and is believed by some to be the same as B. enteritidis (Aertryck) and B. typhi murium (mouse typhoid). In cultures A resembles the typhosus; and B the colon bacillus; and fermentative reactions likewise, except in milk, where A gives slight permanent acidity, and B slight acidity which after the third day gives place to alkalinity. Cases of illness due to A, resemble mild typhoid; and to B, are allied to food-poisoning with severe gastro-intestinal symptoms. Organisms of this group form endotoxins which are heat resisting, and therefore the ingestion of cooked food containing the bacilli may produce severe disturbance, thus relating them to Gaertner's bacillus (B. enteritidis). None form indol. All give fluorescence with neutral-red. These bacilli are motile, flagellar, show agglutination phenomena, and ferment dextrose, dulcite and mannite, but not lactose nor saccharose. They are present at times with the B. typhosus, and may thus produce a mixed infection.

B. Enteritidis (Gaertner).—In 1888 the flesh of a diseased cow was sold for food in a Saxony village. Gastro-enteritis followed the ingestion of the meat in the case of fifty-seven people. One young man ate 800 grm. (nearly 2 lb.) of the raw meat, and died in thirty-five hours. From his spleen and blood Gaertner isolated an actively motile bacillus, closely resembling the typhoid germ; and he obtained the same organism from the flesh of the cow. Similar bacilli have since been found in other outbreaks of meat-poisoning. Gaertner's bacillus is very pathogenic to laboratory animals, causing an intense hæmorrhagic enteritis. The symptoms are due to endo-toxins which are heat resisting, so that boiling does not readily destroy the toxicity. It grows more rapidly on gelatin than B. typhosus; forms no indol, but ferments dextrose, with formation of acid and gas. Closely related to the bacillus of Gaertner are the hog cholera bacillus and the B. psittacosis. The latter was first isolated in Paris in 1892 in a highly fatal pneumonia-like illness (49 cases, 16 deaths), which was traced to sick parrots from South America. B. icteroides and B. typhi murium are also of this group. Danysz's virus is supposed to consist of B. enteritidis (Ærtryck and Gaertner).

B. Dysenteriæ, or Shiga's bacillus. First isolated from the stools of patients (in Japan) suffering from acute dysentery, in which no amœba could be found. The bacillus was found by examining the stools for an organism which would agglutinate with the serum of the patients. In 36 cases one and the same organism was found to meet the test, and it was not found in the dejections of healthy persons or persons suffering from other diseases, nor did it agglutinate with their blood serum. It is now recognized as the specific cause of acute epidemic dysentery of temperate climates. Since then, several bacilli have been isolated in different parts of the world, all related to Shiga's bacillus but giving a variety of reactions to carbohydrates and to immune serum. Kruse isolated his organism from "pseudo-dysentery of the insane," and Flexner from dysentery in the Philippines. They all ferment dextrose but without gas formation. In milk, first slight acidity and

then increased alkalinity is produced. The Shiga-Kruse group produce no indol, but the Flexner group do. Short rod, non-motile, non-liquefying, non-Gram-staining, aerobe and facultative anaerobe.

Bacillary dysentery is an ulcerative colitis with little tendency to form liver abscess. It is spread like enteric fever, and is a scourge of armies as it formerly was of asylums. Animals are easily killed by injection, but show no characteristic lesions in the intestine, though the latter have been obtained by feeding experiments. The Shiga-Kruse bacilli form a soluble toxin which is very fatal to rabbits, and is resistant up to 70° C. This toxin causes profuse diarrhœa, and later paralysis, when injected intravenously into rabbits, being apparently excreted by the colon and cæcum.

Similar bacilli have been isolated in the summer diarrhœa of infants.

An anti-toxic serum has been prepared from horses.

B. Fæcalis Alcaligenes resembles B. typhosus morphologically and culturally, but is non-pathogenic.

Cultures.—Gives a luxuriant growth on potato; forms no acid with glucose, but alkali with milk whey and mannite. It is an occasional inhabitant of the ileum and colon.

Other organisms which have been described in connection with the colon-typhoid-dysentery group are: B. neapolitanus (in a choleraic disease); B. acidi lactici, of Hueppe; B. lactis aerogenes (resembles Friedlaender's diplobacillus), and other bacilli present in milk, which appear in the fæces of milk-fed persons.

Voges-Proskauer Reaction is not given by B. coli nor by any of the above, but by B. lactis aerogenes, B. cloacæ, and B. oxytocus perniciosus. Inoculate glucose-peptone solution and grow for 3 days; add KOH; stand for twenty-four hours. Red colour.

Differentiation of B. Typhosus from B. Coli.

The problem is to find a medium which will favour the development of B. typhosus and B. coli and yet will differentiate them. The usual method is to use coloured media + inhibitory agents or favouring agents.

1. *Drigalski and Conradi's Medium.*—This is a meat broth (1·5 lb. per litre) to which, besides the peptone and salt, 1 per cent of nutrose and 3 per cent of agar are added and dissolved. Thereafter litmus solution is used to dissolve pure lactose (quantity used = half quantity of agar), and the whole is added to the hot agar fluid. Render alkaline with sodium carbonate solution, and add a solution of crystal-violet. The medium must not be overheated or the lactose may be changed. It is a solid medium and is, shortly, a lactose-nutrose-agar.

The crystal-violet restrains the saprophytes. In twenty-four hours B. coli colonies are red; 2 to 6 mm. in diameter, and non-transparent. B. typhosus colonies are blue; 2 mm. in diameter, and glassy and dew-like.

The plates are inoculated by smearing the surface with a glass rod, dipped in, say, diluted fæces.

2. *Endo's Medium.*—This is a 3 per cent agar neutralized and then alkalinized with NaOH, and lactose and fuchsin (basic) solution added, and then Na_2SO_3 solution until decolorized. Put into test tubes (15 c.c.), sterilize, and keep in dark. When using, pour plates, and inoculate by surface smears, when in twenty-four hours B. coli colonies are red, and B. typhosus colonies are colourless.

3. *Loeffler's Medium.*—This is a 3 per cent agar to which malachite-green is added, and this retards growth of B. coli. B. typhosus colonies are minute glistening points; later, they colour agar yellow.

4. *Hoffman and Ficker's Medium.*—Convert water sample into medium by adding: caffein 2·5 per cent (restrains B. coli); nutrose 1 per cent; and crystal-violet 0·001 per cent (restrains saprophytes). Incubate at 37° C. for not more than 12 hours. The B. typhosus can then be isolated on plate media.

5. *MacConkey's Media*—Have been already described on page 153. The bile-salt assists growth of B. coli and B. typhosus, and hinders others. Where neutral-red is used, acid formation changes it to a rose-red.

6. *Bile Medium* (for blood).

7. *Hiss's Agar-Gelatin Media,* see p. 232.

TABLE OF CHARACTERISTICS.

	B. Coli	B. Typhosus	Paratyphosus A	Paratyphosus B	Dysenteriæ A	Dysenteriæ B	Fæcalis Alc.
Motility ..	+	+	+	+	—	—	+
Gram	negative	negative	negative		negative		neg.
Peptone water	indol	—	—	—	— indol		—
Glucose	A + G	A	A+G	A+G	A	A	nil
Lactose	A + G	—	—	—	—	—	—
Saccharose	—	—	—	—	—	—	—
Milk (litmus)	A + Clot	A (alk. in 4 to 10 days)	A (alk. in 3 days)		A (alk. in 3 days)		Alk.
Gelatin ..	non-liquefying	non-liquefying	non-liquefying		non-liquefying		non-liquefying
Pathogenic:							
For man	slight	distinct	distinct		distinct		nil
,, animals	do.	less	more		more		nil

SHORT TABLE OF CARBOHYDRATE REACTIONS.

	Glucose	Lactose	Saccharose	Dulcite	Mannite
B. typhosus ..	A	—	—	—	A
B. coli	+	+	—	+	+
B. acidi lactici	+	+	—	—	+
B. paratyphosus	+	—	—	+	+

A signifies acid; + acid and gas; - nil.

CAPSULATED BACILLI.

In this group are classed bacilli which are non-motile, and capsulated in certain cultures, but otherwise resemble the Bacillus coli.

Bacillus Pneumoniæ. — Otherwise called pneumobacillus, Friedlaender's bacillus. Was first described by Friedlaender in 1882 as the cause of acute lobar pneumonia. It was first called a micrococcus, and was confused with Fraenkel's pneumococcus, but was later recognized as a short bacillus. It is the cause of pneumonia in about 7 per cent of the cases. It is taken as the type of a group, "the mucosus capsulatus" group, and is also called B. mucosus capsulatus.

Characteristics.—A short plump bacillus with rounded ends, but showing some very long forms (0·6 to 5 micra long by 0·5 to 1·5 micron). The short thick forms are mostly found in animal tissues, and at times are almost coccoid. In sputum it shows a capsule, and in other preparations from the body. It occurs in pairs, and hence has been called a diplobacillus. It may also form short chains. It is non-motile, non-flagellar, non-sporing, non-gelatin-liquefying, and non-Gram (i.e., Gram-negative).

Cultures.—It grows readily on ordinary media and in gelatin at room temperature. It grows well on acid or alkaline media, is aerobic, and facultatively anaerobic.

In broth: rapid abundant growth with a pellicle, general clouding, and later a stringy sediment.

On agar: sticky mucus-like colonies of a greyish-white colour.

In gelatin stab: a white line of growth at first, but with increasing growth at surface a "nail-head" appearance is produced. This was at one time thought to be peculiar to Friedlaender's bacillus.

On potato: abundant, somewhat brownish growth.

In peptone water: no indol formation.

In milk: abundant growth with capsule formation. Acid and clot are slowly formed.

Pathogenicity.—For man: pneumonia of a severe and fatal type; ulcerative stomatitis and nasal catarrh; acute tonsillitis; in antral suppurations and in fœtid coryza; and, on rare occasions, in septicæmia.

For animals: a mouse injected at the root of the tail dies in two days of septicæmia. It is also pathogenic for guinea-pigs; less so for rabbits.

DISTINCTION FROM THE PNEUMOCOCCUS.

	Pneumococcus	Pneumobacillus
Growths on ordinary media	Sparse	Good
,, on gelatin	Almost none	Nail-head growth
,, in milk	Acid + clot	Acid + clot (late)
Staining	Gram-positive	Gram-negative

Allied bacilli are: B. ozænæ, found in fœtid nasal catarrh, which is scarcely separable from B. mucosus

capsulatus; bacillus of rhinoscleroma. Both these bacilli differ only in not fermenting dextrose.

B. Lactis Aerogenes—Is a widely distributed organism, and was isolated by Escherich in 1885 from the fæces of infants. It is almost constantly present in milk, fæces, sewage, and water. It differs from B. coli, which is found in like circumstances, in being non-motile and non-flagellar, in possessing a capsule in milk cultures, in fermenting saccharose and starch but not dulcite; and in not forming indol.

Cultures.—It grows readily on all media.

In broth, it forms a pellicle, and causes general clouding.

On agar and gelatin, it forms a heavy white growth.

In gelatin stab, it gives a nail-head growth.

On potato, it grows well and forms gas from the starch.

In Milk, acid formation and coagulation are rapid. The clot is not digested by the bacillus, and in ordinary souring of milk the germs present which produce proteolytic ferments have their growth restrained by the large amount of lactic acid formed by the more rapid action of B. lactis aerogenes. It is scarcely pathogenic, though flatulence in infants has been attributed to its action, and a cystitis in which gas was formed in the bladder, associated with an acid urine. For animals, its pathogenicity is not properly established, the reports being contradictory. It is an aerobe, but a facultative anaerobe. Optimum temperature, 25° to 30° C. B. lactis aerogenes is distinguished from Friedlaender's bacillus by its invariable and rapid curdling of milk; but some authorities consider it to be identical with that organism.

BACILLUS ACIDI LACTICI (HUEPPE).

This bacillus is present in milk, which it curdles and acidifies. It does not ferment saccharose or dulcite.

B. acidi lactici (Leichmann) is believed to be really a streptococcus, the Streptococcus lacticus (Kruse), which Heinemann states is a variety of the Streptococcus pyogenes. It is present on the cow's hide, in cow dung, and in milk from the first stage of milking.

MINUTE BACILLI.

Under this heading may be conveniently grouped the bacilli, of influenza, of acute epidemic conjunctivitis, and of whooping-cough. All are Gram-negative.

Bacillus Influenzæ—Was first described in 1892 by several observers, and has been called after one of them the Pfeiffer bacillus. It is very small even among micro-organisms, being only from 0·5 to 1·2 micron long by 0·2 to 0·4 micron thick. A tubercle bacillus, 3 micra by 0·3 micron is thus equal in length to several (3 to 6) influenza bacilli placed end to end.

It is then a small bacillus, of irregular length, having rounded ends, rarely forming chains, non-motile, non-sporing, Gram-negative, and not growing on gelatin or at room temperatures. It is not easily stained with the usual dyes; best with 10 per cent aqueous fuchsin, or Loeffler's methylene-blue, 5 minutes of either. The bacilli form irregular clusters; occasionally polar staining is noticed.

Cultures.—It is not easily cultivated, growing only in the presence of hæmoglobin. This is obtained on the ordinary media by smearing them with some blood drawn from the finger, or by mixing melted agar with fresh blood. The blood of the pigeon may be used. The medium is inoculated with the sputum coughed up from the bronchi, avoiding mucus from the mouth. Colonies appear in 18 to 24 hours, as minute transparent drops, colourless, and likened to drops of dew. Growth ceases in 2 to 3 days. Frequent subculturing and storage at room temperature are needed to keep the cultures alive.

It is aerobic, and shows no growth under strict anaerobiosis. It is readily killed at 60° C.; and by drying, in a few hours. Dies in culture media within a week. Its usual habitat during an epidemic is the nasal passages and bronchial tubes. It is said to remain in these places after recovery from the attack, and to persist for years. The immunity produced by an attack of influenza is very short. A pseudo-bacillus, differing only in its slightly larger size and its growth in threads, and showing involution forms, has been described, but its differentiation is doubtful.

Koch-Weeks Bacillus.—A bacillus similar to the above, but longer and more slender, was described by Koch in 1883, and by Weeks in 1887, in connection with an epidemic form of acute conjunctivitis.

Cultures.—It grows best on serum agar, and at 37° C; the colonies appear in 36 hours as dew drops.

The disease is characterized by a muco-purulent discharge, hyperæmia of the whole of the conjunctiva, and swelling of the lymph follicles of the lids, which show through the palpebral conjunctiva as slightly raised pinkish-grey bodies, a half to one millimetre in diameter. A film made from the discharge and stained with Loeffler's methylene-blue, shows the bacilli. The affection is very contagious, and to prevent epidemics in schools, common face-towels should be rigorously prohibited.

Bordet-Gengou Bacillus.—These observers found a small ovoid bacillus in the sputum of a child suffering from whooping-cough.

Culture.—In 1906, six years later, they succeeded in cultivating it on a special medium, after failing with ascitic agar and blood agar. This medium is a glycerin extract of potato with 4 per cent salt and 2·5 per cent agar, to which is added an equal quantity of defibrinated human or rabbit's blood. On this, inoculated from sputum, the colonies appear within 48 hours, and are small, greyish, and rather thick. In subcultures, they give a more luxuriant growth, and can then be grown on blood agar and in ascitic broth, in which it causes a viscid sediment but no pellicle. It is strictly aerobic, and grows moderately below blood-heat. It remains alive in culture for as long as two months. Specific agglutinins are developed in immunized animals, which serve to distinguish it from B. influenzæ. The washed and dried bacilli ground in a mortar and injected into a rabbit intravenously, usually kill it in 24 hours. Specific complement fixation has been used by Bordet and Gengou to prove the identity of the bacillus, using the serum of an infant suffering from whooping-cough.

The organism is present in the sputum in the early stages in predominating numbers, but later it is swamped by others. It is scattered among the pus cells,

and at times is intracellular. It is extremely small and ovoid, so that it is readily mistaken for a micrococcus. It is slightly larger than the bacillus of influenza, and more ovoid. It is Gram-negative, stains with Loeffler's methylene-blue, dilute carbol-fuchsin, or aqueous fuchsin. Toluidin blue in a special medium is advised. (Toluidin blue 5 grm., alcohol 100 c.c., water 500 c.c.; dissolve; add of 5 per cent aqueous carbolic acid solution, 50 c.c.; stand two days; filter.)

Bacillus of Ducrey—Is a small bacillus (1 to 2 micra × 0·5 micron), regularly found in soft chancre or chancroid. It is non-motile, non-flagellar, non-sporing, Gram-negative, and is found at times inside the leucocytes.

Culture.—It only grows on whole blood agar, and dies off at room temperatures. Colonies show in about 48 hours. Inoculation of pure cultures on the skin produces typical chancres in 4 to 6 days.

Zur Nedden's Bacillus.—A small slightly curved bacillus (1 micron long) which has been isolated in ulcerative conditions of the cornea. It grows well on the ordinary media. It is non-motile, Gram-negative, and does not liquefy gelatin. It clots milk, forms acid with glucose, but no indol in peptone water.

Culture.—It grows well on potato.

MORAX-AXENFELD DIPLO-BACILLUS.

This is a short, thick bacillus with rounded ends, found in a chronic eye condition called "angular conjunctivitis," in which there is slight redness of the edges of the lids, especially at the angles. The ocular conjunctiva is seldom affected. It mainly affects adults, and chiefly women. There is rarely any corneal trouble. It was described by Morax in 1896, and by Axenfeld a year later. The diagnosis is easily made, by taking a smear of the small bead of pus which gathers through the night at the angles, and staining with the usual dyes. The bacillus is easily stained, and is Gram-negative. In stained films, short and long chains of polar stained bacilli are seen.

Culture.—It grows best on Loeffler's blood serum at 37° C., causing small pits of liquefaction in 48 hours.

DIPHTHERIA BACILLUS.

Klebs first described the bacillus in the throat membrane in 1883, and Loeffler first cultivated it in 1884; hence called the Klebs-Loeffler bacillus. The toxins produced by it were investigated by Roux and Yersin in 1888–89, and the antitoxins by Behring and Kitasato in 1890.

Description.—A slender bacillus, 1 to 6 micra long and 0·3 to 1·1 micron broad. From the throat they are mostly 4 to 5 micra long. They are rarely of uniform thickness throughout, showing club-shaped thickening at one or both ends. They are straight or slightly curved, stain deeply with methylene-blue, often showing granules more darkly stained, so that a dotted, beaded, or striped or barred appearance results. The longer individuals often have a strong resemblance to short chains of streptococci. In 18-hour-old cultures, many of the bacilli show on staining deeply stained oval bodies situated most frequently at the ends, the so-called "polar" or "Babés-Ernst" bodies. These were first regarded as spores, but are now considered to be chromatic granules. B. diphtheriæ is an aerobe. Is non-motile, non-sporing, non-flagellar, and non-liquefying of gelatin. It is Gram-positive.

Cultures.—Grows on all media, but quickest and most characteristically on **Loeffler's blood serum**: [beef-blood serum, 3 parts, 1 per cent glucose broth (meat infusion), 1 part. Put in tubes, slant, coagulate at 70° C., and sterilize at 57° C. for 1 hour on eight days.] On this medium colonies form in from 12 to 24 hours at 37° C., as small circular discs of opaque whitish colour, like candle-grease spots, and enlarging rapidly, outstrip any accompanying colonies of streptococci.

On agar, a similar growth occurs, but less quickly, and is closely resembled by that of Streptococcus pyogenes.

In broth, a pellicle may form, and a turbidity which however soon settles to the bottom, leaving the fluid clear and producing a powdery deposit.

It grows well in milk but does not clot it.

Does not form indol. Ferments glucose, galactose, lævulose, maltose, and glycerin, but not mannite or saccharose. It reduces nitrate to nitrite.

Grows best at 37° C.; growth at 22° C., but not at 20° C.

Isolation.—Take sterile swab, rub on throat, then rub over serum in tube, and incubate for 18 to 24 hours at 37° C. Take platinum loop and rake all over surface of serum, and then make a film and stain with Loeffler's methylene-blue or Neisser. Distinguish from Hoffmann's bacillus, which is shorter and plumper, does not produce acid in glucose, is non-pathogenic to guinea-pigs, and does not show granules with Neisser. (B. diphtheriæ at times does not give Neisser, and occasionally pseudo-B. diphtheriæ does stain with Neisser.) The only sure method of differentiation is toxicity of culture; guinea-pig killed in 24 to 48 to 72 hours by injection of toxin of true B. diphtheriæ.

In the serum-water medium of Hiss, plus 1 per cent of glucose, lævulose, galactose, maltose, lactose, saccharose, mannite and dextrin, B. diphtheriæ produces acid in all except with mannite, lactose, and saccharose; B. xerosis produces acid with all except mannite, lactose, and dextrin; B. Hoffmanni does not produce acid with any.

The saccharose and dextrin media therefore serve to differentiate: B. *diphtheriæ*, acid with dextrin, not with saccharose; B. *xerosis*, acid with saccharose, not with dextrin; B. *Hoffmanni*, acid with neither.

In Vincent's angina, B. fusiformis is found: an anaerobe, Gram-negative, longish, and swollen in the middle.

B. xerosis is found in the conjunctiva, and is practically identical with the B. diphtheriæ: it is non-pathogenic to animals; produces no toxin; does not form acid with glucose, but ferments saccharose.

Habitat.—Mucous surfaces, mouth, throat, nose, conjunctiva, larynx, middle ear, vagina.

Thermal Death-Point.—Forty-five minutes at 55° C., (moist heat); dry membrane, 1 hour at 98° C.

Resistance.—Lives six to eight weeks on agar, five to six months on serum, twelve to fifteen months on dextrose serum. In dried membrane retains vitality for months.

Pathogenicity.—Guinea-pigs die in from two to three days after subcutaneous injection of young broth culture. Nephritis and paralysis are observed, but the characteristic feature is enlarged and hæmorrhagic condition of the

adrenals. Cats, dogs, and pigeons are very susceptible to mucus infection ; rats and mice are refractory.

A guinea-pig killed by the injection of a virulent culture of diphtheria bacillus shows congestion of all the organs, especially severe in the suprarenals.

Toxins.—Diphtheria toxin is an extracellular one, that is, it is soluble in the liquid media, and can be obtained separate from the bacillus by filtration through a Chamberland tube. Toxin formation is best got in meat-infusion broth with added peptones, and rendered alkaline, after two to three weeks' growth at 37·5° C. A free supply of oxygen is important to secure the greatest toxin formation.

The toxin is readily destroyed by bright light, by exposure in a liquid solution to 60° C., or if in dry state to a temperature over 70° C. Sealed and kept in the dark and in the cold, it may be kept for long periods. It is believed to be closely allied to the albumoses.

Antitoxin.—The mode of preparation and standardization is described under ANTITOXIC SERA (page 189). The value of antitoxin in the treatment of diphtheria is now well attested. The results are better the earlier the injection, so that few deaths occur in those injected within twenty-four hours of the onset, the rate gradually rising until by the fifth day the effect is slight. The dose given is not proportionate to the age, but to the severity of the attack and the time that has elapsed before coming under treatment. 2000 to 8000 units should be given to children on these principles, and up to 50,000 units have been given in one case. In large doses, it is better to give the higher-potency sera, in which 10,000 units can be had in 10 c.c. of serum. In about one-third of the cases injected, serum sickness or serum disease is noted. The symptoms are : an erythematous rash and fever coming on in a week to ten days after the injection. At times general pains and even arthritis may be present. These symptoms are more likely to be produced by large doses of serum. But more alarming symptoms than these occur soon after the injection, as has been referred to under ANAPHYLAXIS (page 212). On analysis these cases are found to occur in people who have not been previously injected, and in people who have had a previous injection

more than the incubation period of the serum disease (that is, ten to twelve days) before. On account of these facts, Goodall advises that prophylactic doses of diphtheria antitoxin should not be given to anyone without discrimination, and never to a person the subject of asthma (see page 213). In an actual attack of diphtheria in such a person the risk would have to be definitely considered, and a judgment come to on the relative dangers of the attack and the use of the antitoxin. In those who have had a previous dose, either for an attack or for prophylaxis, some time antecedent, one should be on the outlook for alarming symptoms if a second dose is being given. It is stated that so far no death has been recorded in these circumstances, which is to some extent reassuring. This class of case suggests the avoidance of prophylactic doses altogether, as if the person takes an attack more than ten days later, he is sensibilized to the now required injection. (See for further details, articles by Goodall in *Public Health*, January, 1911, and in *Encyclopædia Medica* ; and by Currie, *Journal of Hygiene*, January, 1907. Also annotation in *Lancet*, 1911, vol. i, page 1654.)

Antitoxic serum keeps well in a cool, dark place. Anderson has found that at 20° C. the average yearly loss of potency is 20 per cent ; at 15° C., it is about 10 per cent ; and at 5° C., it is only about 6 per cent. Dried, and kept at 5° C., its potency was practically unimpaired after 5·5 years. The addition of chloroform, tricresol, etc., to preserve, had no influence apparently on the rate of deterioration.

BACILLUS MALLEI.

The B. mallei is the bacillus of glanders, a disease of horses, mules, and asses. Horned cattle are quite immune, whilst goats and sheep are intermediate in susceptibility. Guinea-pigs and rabbits can be infected by inoculation, but rats are immune.

It was first obtained in pure culture and accurately studied by Loeffler and Schuetz in 1882, and from the human subject by Weichselbaum in 1885.

Description.—B. mallei is a medium-sized rod (3 to 4 micra × 0·5 to 0·75 micron), straight or slightly curved, usually with rounded ends. It is about the same length as the tubercle bacillus, but distinctly thicker. These bacilli show considerable variations in size, even in the same culture; and this is characteristic. They are non-motile, non-flagellar, non-sporing, and Gram-negative. They usually appear as single bacilli; on rare occasions short filamentous forms occur.

Staining.—The bacilli stain rather easily, but are as easily decolorized. The best results are got by staining with a mordant present, and simply washing in water, and drying; in the case of tissues, dehydrating by the aniline-oil method. Carbol-fuchsin and carbol-thionin-blue make good stains. Loeffler's methylene-blue, followed by slight decolorization in weak acetic acid, and then fifteen minutes in saturated solution of tannic acid, wash, dry, and mount, gives good results. As a counter-stain, 1 per cent acid fuchsin for half a minute may be used. With methylene-blue, the bacilli stain irregularly; granular, deeply staining areas alternating with unstained or faintly-stained portions. This has been ascribed to degeneration, and to preparation towards spore formation. It is probably a peculiarity of the cell protoplasm.

Cultures.—Grows well on all media at 35° to 37° C., indifferent to moderate degrees of acid or alkali. Glycerin or glucose render the media even more favourable.

On agar and on glycerin-agar: the growth is greyish white to yellow.

On gelatin: growth is slow, and greyish-white; no liquefaction takes place.

In broth: diffuse clouding; later, a heavy, tough, slimy sediment forms, and the broth becomes brown.

In milk: coagulation takes place slowly, with slight acid formation.

On **potato**: the growth at 37° C. is characteristic. Growth is rapid and abundant, and in forty-eight hours forms a transparent layer over the whole surface, and of a yellowish tint, like clear honey. The growth gets darker and more opaque, and on the eighth day it is reddish-brown or chocolate in colour, and at the edge the potato is stained a greenish-yellow colour. Spirilla choleræ and Metchnikovi, and B. pyocyaneus give similar cultures.

Resistance.—Killed by one hour at 75° C. or two hours at 60° C. In the dark, in sealed tubes and on artificial media, it may remain alive for years. In watering-troughs, it has been found after seventy days. Complete drying kills it in a short time; carbolic 1 per cent, in 30 min.; corrosive sublimate 0·1 per cent, in 15 min.

Pathogenicity.—Notable for horses, mules, asses, cats, dogs, guinea-pigs, rabbits, and field mice. Non-susceptible animals are: cattle, pigs, birds, rats, house mice, and white mice. In the horse, the disease takes two forms. When affecting the superficial lymphatic glands, it is called "farcy;" when affecting the nasal mucous membrane it is called "glanders," and is a much more serious disease. In glanders the course may be acute or chronic. In the acute form, chill is followed by general high temperature, and in a few days the nasal mucous membrane is studded with nodules, there is profuse nasal discharge, and later, ulceration of the nodules and swelling of the corresponding glands, and these also tend to break down. Finally the lungs are involved, and death takes place in from one to four weeks. In the chronic form, the onset is more gradual, the nasal swelling being accompanied by subcutaneous swellings all over the body, some of which tend to break down and ulcerate. These swellings are the so-called "farcy buds," and may persist for years.

In man, the onset is usually violent, with fever and general symptoms; and most cases terminate fatally within two to three weeks, sometimes within a few days. The infection is usually by a wound, which is followed by

lymphangitis, and then a general infection resembling a pyæmia. As in the horse, the nasal mucosa tends to become affected. At times the course is more chronic.

In the horse, the infection is usually by the mucous membrane of the nose or mouth, by wounds, or at times by the alimentary canal.

Toxin.—No soluble toxin is described, but a concentrated three-weeks' culture in glycerin broth, sterilized by heat and filtered, is used as a diagnostic agent under the name of mallein (fluid). Dry mallein has been prepared by filtering a broth culture, concentrating filtrate on a water-bath to one-tenth of its bulk, and precipitating with thirty times its bulk of alcohol. Mallein differs from many other bacterial extracts in being extremely resistant to heat and storage without loss of strength; 120° C. has no destructive effect on it.

Diagnosis of Glanders.—Three tests are used, namely: (1) Guinea-pig inoculation; (2) Mallein test; (3) Agglutination test.

1. *Inoculation of a Guinea-pig.*—A male guinea-pig is injected intraperitoneally with fragments of the diseased tissue, scrapings from ulcers, or nasal discharge of the suspected animal.

A positive reaction is shown by the testicles becoming red and swollen usually on the second or third day, due to inflammation of the tunica vaginalis. Severe general symptoms follow, and death occurs in twelve to fifteen days. Greyish nodules are found in the spleen and other organs. The test is not absolutely specific, but is useful when other tests are inapplicable. A culture on potato of the pus from the tunica vaginalis should be made.

2. *Mallein Test.*—A proper dose of mallein is injected subcutaneously into the breast or neck of the suspected animal. It is advised to inject a dose into a control animal. The temperature of the animal should be taken at least three times a day for one or two days before injection. The injection is made at 6.0 to 7.0 a.m., and the reaction will be at its height at or before 10 p.m. of the same day. The temperature is taken every two hours after the injection for at least eighteen hours. On the

succeeding day take the temperature at least three times. In a healthy animal free from glanders, a local swelling, not exceeding 3 inches in diameter, is produced at the seat of inoculation, and a rise of temperature not exceeding 1° C. (1·8° F.); and both swelling and temperature have much subsided in twenty-four hours. In a horse suffering from glanders, there appears within a few hours a firm, hot, diffuse swelling, which reaches a maximum size in twenty-four hours, is intensely tender during that time, and lasts from three to nine days. The size of the swelling reaches at least 5 inches in diameter. The temperature rises in six to eight hours 1·5° to 2° C. (2·7° to 3·6° F.), reaching 104° to 106° F. The high temperature continues for eight to ten hours (maximum about ten to sixteen hours after injection), and then gradually falls, but is distinctly above normal on the following day. This reaction is specific.

3. *Agglutination Test.*—The macroscopic or sedimentation method in high dilutions (1–1000) is preferred. Normal horse serum may react in 1–500.

Immunity.—An attack of glanders does not confer immunity. Artificial active immunization has been attempted but has so far failed.

Nodules.—The nodules found in glanders show more leucocytic infiltration and less proliferative change towards formation of epitheloid cells than does tubercle.

Prevention.—The Glanders or Farcy Order of 1907, issued by the Board of Agriculture, (1) Lays down compulsory notification of actual or suspected disease; (2) Empowers local authority to slaughter at once any diseased horse, ass, or mule; (3) Enables local authority to test suspected animals with mallein, and deal with contacts.

BACILLUS PESTIS.

This plague bacillus belongs to a group of bacilli which are all highly pathogenic to the animal world, and produce in them a hæmorrhagic septicæmia. The bacilli now usually classed in this group are: the bacillus of human plague, of swine plague, of chicken cholera, of septic pleuro-pneumonia in cattle, and of rabbit septicæmia. The group characteristics are: short, plump, non-motile bacilli; non-flagellar; non-sporing; Gram-negative; non-gelatin-liquefying; strongly aerobic, growing readily on simple media, easily stained but showing a marked tendency to stain more deeply at the ends than at the centre (bipolar staining). They are believed by some to be varieties of one organism.

Plague is a specific, infective disease, caused by the B. pestis, and characterized by inflammation of the lymphatic glands (buboes), carbuncles, pneumonia, and often hæmorrhages (Osler). In the past the plague has occurred in tremendous epidemics, and even to-day in India it proves a terrible scourge. The large and extremely fatal epidemic of pneumonic plague in China in 1910–11 shows that it still has very pathogenic powers for mankind.

In the sixth century, in the reign of Justinian, Emperor of Rome, half the population of the Roman Empire perished of the disease. In the fourteenth century the "black death" overran Europe and destroyed 25,000,000, or about one-fourth of the population. In the seventeenth century it raged virulently, and in London alone, in 1665, about 70,000 people died. During the eighteenth and nineteenth centuries its ravages lessened.

In 1893 an outbreak appeared at Hong-Kong, and since then the disease has occurred in many parts of the world, notably in India since 1896, in Egypt, in South Africa, and in several Mediterranean ports, and, after an absence from Great Britain of over two hundred years, it obtained a foothold in Glasgow in 1900. It reached New York quarantine station in 1899, and in San Francisco broke out in 1900, continuing until 1904. In Australia, cases appeared at Sydney and other ports. In the county of Suffolk, in England, an epidemic of rat plague, associated

with a limited outbreak of pneumonic plague in man, was (September, 1910) the occasion of considerable anxiety and of increased vigilance and action. In California the last case of plague was noted in 1909, but an epizootic of plague among squirrels, causing thousands of deaths among these animals, was only reported as quiescing in 1911.

In 1894, Kitasato and Yersin independently discovered the bacillus in large numbers in the buboes, cultivated it in pure growth, and reproduced the disease in susceptible animals by inoculation, and from them recovered the bacillus. The proof in the human subject was given later, when by an accidental infection a physician and a nurse died of plague.

Description.—B. pestis is a short thick bacillus with well-rounded ends, thus appearing as a small oval rod, two to three times in length what it is in breadth (1·5 to 1·75 × 0·5 to 0·7). The bacilli appear singly, though at times in pairs, and in fluid cultures in chains. In young cultures they show marked variations in size, and less polar staining. In old cultures, involution forms appear, as swollen coccoid forms, or as longer club-shaped diphtheroid forms. In the tissues they are sometimes found to possess a capsule. They stain readily with all the usual aniline dyes, dilute aqueous fuchsin and methylene-blue having been mostly used, and these show the polar staining well. Special polar stains have been devised. Involution forms are developed more rapidly when NaCl is added to the medium, and "salt agar" containing 2 to 5 per cent of NaCl is used for diagnostic purposes, the bacilli showing the usual shape on plain agar, on salt agar exhibiting coccoid, root-shaped, large, globular, and sausage-shaped forms, when the higher percentage is used; with the lower percentage, the most striking feature is a general enlargement of all the bacilli.

Dr. R. M. Buchanan, city bacteriologist of Glasgow, in a Report to the Local Government Board for Scotland ("Thirteenth Annual Report of the L.G.B., Scotland, 1907," page 81), describes a new culture medium for B. pestis as follows: "Rat agar as a culture medium for B. pestis. The susceptibility of the rat to plague suggested the use of rat tissues

instead of ox flesh in the preparation of a nutrient medium for the growth of Bacillus pestis. An extract is made from the carcases of rats deprived of skin, head, stomach, and intestines, and the further preparation of the medium is essentially the same as in that of ordinary agar, except that the extract is boiled for half an hour before straining. Rat agar has been used in the (city) laboratory for a number of years as a culture medium for Bacillus pestis, with most satisfactory results, growth taking place on this medium with much greater certainty, rapidity, and profusion than on glycerin agar. It is also noteworthy that on this medium the bacillus closely approximates to the form which it assumes in the body, and is sometimes much elongated. In this elongated form it may be difficult to recognize, so different is it from the familiar cocco-bacillary growth of glycerin agar."

B. pestis is Gram-negative, non-gelatin-liquefying, non-motile, non-sporing, and non-indol forming.

Cultures.—Grows readily and luxuriantly on all the meat-infusion media. The optimum temperature is 30° C., while below 20° C. and above 38° C. the growth is sparse and delayed. The best reaction is neutrality or slight alkalinity; but acidity does not prevent growth.

On agar: growth appears in twenty-four hours as minute colonies, whitish and compact in centre, and showing to hand lens a broad, irregular, indented, granular margin. Kept for a few days at room temperature, some colonies grow faster than others and become more opaque, almost suggesting a mixed growth (Muir and Ritchie).

On gelatin: a similar growth occurs in two to three days. In stab, a white line of growth takes place along the needle track, and little or no surface growth.

In broth: growth is slow, and usually forms a slightly granular or powdery deposit at the foot or sides of the tube or flask. If the surface is covered with "ghee" (in India, butter clarified by boiling, and thus converted into a kind of oil), delicate threads of growth extend from the surface downwards, the so-called "stalactite" growth, which however is not specific to the plague bacillus, nor is it shown by all races of the organism. To observe it, the culture must be kept absolutely at rest.

Milk is not coagulated. Litmus milk shows slight acid formation. Growth on potato and blood serum shows nothing of differential value.

In peptone water, no indol is formed.

Resistance.—Readily killed by heat, like all non-sporing forms ; ten minutes at 65° C., or one hour at 58° C. Drying kills them in six to eight days ; artificial drying in four to five hours. May live in pus or sputum for eight to fourteen days. In a moist dark place, may retain viability for months or even years. Freezing has little effect, bacilli surviving a temperature below 0° C. for forty days. Direct sunlight kills them in four to five hours ; carbolic 1 per cent in two hours, 5 per cent in ten minutes ; perchloride of mercury 1–1000 in ten minutes.

Pathogenicity.—For man, very pathogenic. For animals, very marked for rats, mice, guinea-pigs, rabbits, and monkeys. In rats and guinea-pigs, the mere rubbing of plague bacilli into the skin will often produce the disease, and this fact is made use of in isolating the bacillus from material contaminated with other bacteria. Animal passage increases virulence ; growth on artificial media diminishes it, when prolonged. After subcutaneous injection, a local inflammatory swelling follows ; then a swelling of the corresponding lymphatic glands ; thereafter a general infection. Mice usually die in one to three days, rats and guinea-pigs in two to five days, and rabbits in four to seven days. Post mortem, the chief changes are : the enlarged glands, congestion of the organs (sometimes with hæmorrhages), and enlargement of the spleen. The bacilli are numerous in the lymphatic glands, usually in the spleen, and throughout the blood. The blood of a plague-stricken rat may contain as many as 100 million bacilli per c.c.

Transmission.—Actual contact plays a very minor part in the transmission of the disease, as the virus is not given off by the skin. The chief modes of transmission are two in number, by : (1) *Inoculation by biting insects* (Limond, 1899), the usual insect being the rat flea (Pulex cheopis) ; (2) *Inhalation* : this is the mode of spread of the pneumonic form of plague.

1. By inoculation by biting we get the bubonic plague,

the course of which corresponds exactly to that induced in animals by subcutaneous injection, as described above. An outbreak of bubonic plague is always preceded by an increased death-rate among rats, and that from a disease now known to be due to the B. pestis. The rat flea becomes infected by sucking the blood of the rat, and infects other rats, or it may be human beings. It is now believed that the flea does not inject the bacillus when biting, as no bacilli have been found in its biting apparatus. It is therefore surmised that the mode of infection is by the inoculation of the biting wound by the rubbing in of the excreta and vomit of the flea, both of which are highly charged with B. pestis. The proof that B. pestis multiplies in the stomach of the flea is held to follow from the fact that abundant bacilli may be found in it up to twelve days or longer. It has also been observed that in India plague does not maintain itself in epidemic form after the mean temperature has risen above 80° to 85° F. (26·6° to 29·4° C.). This has been found to be associated with a rapid disappearance of the bacilli from the alimentary canal of infected fleas during the prevalence of this higher temperature. Transmission by inoculation must be extended to the infection of attendants on the sick and others, by the rubbing in of soiled linen, etc., and also from earth soiled by the excreta, vomit, and sputum of rats dead or dying of bubonic or pneumonic plague. Such transmission is urged by some as a likely one in the case of barefoot peoples, living in dwellings with earth floors and infested with rats. The transmission from rat to rat is believed to be by the flea, in which case the buboes are mainly cervical; and by ingestion of rats dead of plague by other rats, when the buboes are mesenteric.

The subject of transmission in this manner has been mainly studied in India. In Bombay there are two kinds of rats: (1) Mus decumanus, and (2) Mus rattus. Both kinds are infested by the same flea, the Pulex cheopis. The Mus decumanus is the large brown rat, and is the same species as that present in Suffolk, England, to-day. The Mus rattus is the black rat, and is identical with the English black rat. These two species of rats differ fundamentally in their habits. The Mus decumanus is a timid rat, which

avoids man as far as possible, and finds its food in sewers, ditches, fields, etc., rather than in inhabited houses. Mus rattus, on the other hand, is a domestic animal in India, and lives in close and intimate association with the home-life of the people. The Mus rattus, therefore, is chiefly responsible for the transmission of the disease to man; while the Mus decumanus is of special importance in maintaining the disease from season to season. The Indian Plague Commission conclude that plague is a rat disease, having a regular periodicity, namely (*a*) an epizootic season, from December to May inclusive, and (*b*) a non-epizootic season, from June to November inclusive. During the latter period there are few cases of plague in rats, fleas are scanty (this is given as a cause of the decrease in cases of plague in rats), and in some villages where the Mus rattus alone prevails, plague may actually die out each season. The Mus decumanus is more infested with fleas, and is thought to keep the infection going from season to season. A fresh epizootic first chiefly affects the Mus decumanus, then spreads to Mus rattus, and then to human beings. In this way are explained the outbreaks of plague in India, year after year since 1896, causing nearly a million deaths in 1904 among the natives, while the attendants and Europeans enjoy almost complete immunity, although both hospitals and camps abound with Pulex irritans. This is ascribed to the habits of this common flea of man, in that it rarely bites other creatures than man, and that in man with plague the blood is not so alive with bacilli as in the rat. The chances of infection by Pulex irritans are from these causes enormously reduced.

2. By inhalation of the virus, causing the pneumonic form of the plague, which is very rapid and fatal. It is not understood what factors determine the appearance of the disease in this form, but once started it is extremely infectious. The symptoms are very similar to those of acute lobar pneumonia (although the pneumonia is mainly lobular in its distribution), with high fever, rapid respiration, and hæmorrhagic sputa. The sputum contains the bacilli in enormous numbers, and almost in pure culture. In Egypt, the summer type is bubonic, and the winter type pneumonic.

Cimex lectularius, or the common bed-bug, has lately been investigated as a carrier of plague bacilli. Enormous numbers can be found in the stomach of the bug after infection, and for four to five days. Two bugs were still alive eighty-three days after the feeding, and broth and agar cultures were obtained from their bodies. Inoculation of mice produced typical results, and the B. pestis was recovered.

Other types of plague are described, which do not come under the above headings. These are : the *ambulant form*, in which the patient has a few days of fever, with swelling of the glands of the groin, and possibly suppuration. These cases are often found at the beginning of an epidemic, and are a source of great danger to the community, as the urine and fæces contain bacilli ; and the *septicæmic type*, in which the patient succumbs to a virulent infection before the buboes appear. There are also *cutaneous* and *intestinal types*, the former showing petechiæ and subcutaneous hæmorrhages ; the latter, diarrhœa and hæmorrhages from the mucous membranes, and sometimes the features of enteric.

In India, an analysis of 11,600 cases gave 77·65 per cent of bubonic type, 14·25 per cent of the septicæmic type, and 4·4 per cent of the pneumonic type. The mortality was highest in the pneumonic type (96·69 per cent), and almost as high in the septicæmic. In the bubonic form, out of 9,500 cases, 5,130 (54 per cent) showed the glands of the groin first affected, and usually on the third to the fifth day.* Resolution may occur, or suppuration, or in rare cases, gangrene. Suppuration is noted as a favourable feature.

The appearance of petechiæ, or " plague spots," or " tokens of the disease," gave to it in the middle ages the name of the " black death."

Toxins.—The filtrate of a plague culture has a very slight toxic effect, but not capable of inducing immunization. Hence it is believed that little or no soluble toxin exists. Injection of dead bacilli produces distinctly toxic

* The areas of skin surface which drain respectively into the glands of the groin, axilla, and neck, are as 5 : 1·8 : 1, and the number of primary buboes observed in hospital in these glands were as 5·8 : 1·3 : 1 respectively.

effects. These endotoxins are comparatively resistant to heat, being unaffected by exposure to 65° C. for one hour. By the injection of these endotoxins in suitable doses, a degree of immunity against living virulent bacilli is obtained. The serum of such immunized animals is found to confer a degree of protection on small animals.

Immunization.—

1. *Preventive inoculation.*—Haffkine's method. Cultures are made in flasks, with oil drops on the surface. Stalactite growths form, and the flasks are shaken every few days to break those formed, and so induce fresh crops. The incubation temperature is 25° C., and six weeks' growth is allowed. Thereafter the culture is sterilized by heating for one hour at 65° C., and carbolic acid is added to make the bulk contain 0·5 per cent. The contents are well shaken to distribute the sediment, and then bottled for use, the fluid thus containing the dead bodies of bacilli as well as any toxins that may be in solution. It is administered subcutaneously, and usually in one dose of 5 c.c. The susceptibility is said to be reduced to one-fourth, and the mortality among those inoculated who take the disease is about one-half of that among the non-inoculated. Protection begins a few days after inoculation and lasts for several months.

2. *Anti-plague Serum.*—Yersin has prepared a serum from horses, by injecting dead bacilli into the subcutaneous tissues, then into the veins, and finally, living bacilli intravenously. After a time, blood is drawn off, and the serum preserved in the usual way; 10 to 20 c.c. are injected daily. Some curative power has been observed.

Serum Diagnosis.—Specific agglutinins appear in the blood of some patients, but the potency of the serum is not strong, and the test is not easily carried out, owing to the tendency of the bacilli to adhere in clumps preventing a satisfactory emulsion being got. Hence the macroscopic or sedimentation method is preferable. Cairns, in the Glasgow cases, found that the reaction appeared about a week after onset of the illness, increased until the sixth week, and then faded away; being most marked in severe cases taking an early and favourable crisis, less in severe cases tending to a fatal issue, and feeble or absent in the mild cases. The best dilutions were 1–10 to 1–50.

Methods of Diagnosis.—

Bubonic.—(1) Prepare the skin over a bubo, and remove some juice by aspiration with a sterile hypodermic syringe, the needle of which is plunged into the bubo; (2) Make smears, and cultures on agar and salt agar; (3) Inoculate a guinea-pig with some of the material, by rubbing it in on the shaven skin with a glass spatula, or by subcutaneous injection.

In bubonic plague, a diagnosis in many cases can be made by microscopic examination alone, as in no known condition other than plague do bacilli with the same morphological characters occur in such numbers in the lymphatic glands.

Pneumonic.—(1) Microscopic examination of the sputum; (2) Make cultures, inoculate a guinea-pig; also a rat by smearing the sputum on its nasal mucous-membrane.

In pneumonic plague, a positive diagnosis should not be given from microscopic examination alone, especially in a plague-free district, as bacilli morphologically resembling the plague organism may occur in the sputum in conditions other than plague.

Post Mortem of Rat Dead of Plague.—Subcutaneous injection of the flaps of the abdominal wall is noted. Fluid in the pleural cavities, hæmorrhagic œdema of the neck glands, a creamy mottled appearance of the liver, and a somewhat similar appearance of the spleen. The Indian investigators laid stress on an abundant, clear, pleural effusion. The neck glands are chiefly involved in the rat because the flea prefers the skin of the neck; in California and Glasgow, however, the cervical bubo was not so commonly found as in India.

In chronic rat plague, enlargement of the spleen, with the formation of nodules in it containing plague bacilli, was the usual finding.

Prophylaxis.—

Destruction of rats, by poisoning, trapping, ferreting, and virus.

Separation of rats from mankind by better drainage, paving of yards, concrete floors, and general repairs.

Removal of rat food, by frequent scavenging and attention to the feeding of fowls, pigs, etc.
Slaughter-houses require special attention.
General campaign of cleanliness, avoidance of fatigue of body or mind, and temperance in all things.
Haffkine's prophylactic inoculation, at least to all those on the staff or otherwise specially exposed to infection.

Nursing.—Bubonic plague requires no special precautions, as it is not infectious. The liberal use of iodoform as a dusting powder, on the person and clothing, is strongly recommended to prevent the attacks of fleas.

Pneumonic plague is acutely infectious, hence both doctor and nurse should wear a cotton-wool respirator, as should also the attendants.

SUMMARY.—Plague is a rat disease. It is conveyed from rat to rat, and from rat to man, by the rat flea. The human flea is not involved to any extent in the matter. Bubonic plague is not an infectious disease, as this phrase is commonly understood. Pneumonic plague is most infectious, apart altogether from the question of fleas. Plague pneumonias breed true, i.e., give rise to other cases of pneumonic plague. The B. pestis blood-count is low in man, high in the rat. This affords an explanation of the high degree of infectivity of the rat flea as compared with the human flea. Insanitary conditions apart from the presence of rats play a secondary part. In Suffolk, Mus rattus is rare, Mus decumanus is common; therefore close contact with plague-stricken rats is unlikely, and hence the small epidemic. (Pringle, "The Outbreak of Rat Plague in Suffolk," *Public Health*, January, 1911.)

For an important article on the "Spread of Plague," by C. J. Martin, and the subsequent discussion, see *Brit. Med. Jour.*, 1911, vol. ii, p. 1249.

SUMMARY OF THE "LANCET" REPORTS ON THE PLAGUE IN CHINA, 1910–1911.

The outbreak of plague in China, beginning in Manchuria on October 12th, 1910, and extending rapidly until it had

invaded widely separated districts, was notable in several important particulars. The epidemic was almost without exception one of primary pneumonic plague. The fatality was extremely high, few cases of recovery having been reported. The infectious nature of the malady was very great, and the virus was apparently carried by the sputum. The origin of the plague was not ascribed to rats, but to marmots, a species of squirrel living in burrows. The question of infection by fleas is here of minor importance, once the epidemic is started. Its relation to the origin of the epidemic has not been worked out. Once begun, the propagation was apparently by direct inhalation of the virus.

The Chinese Government invited the other Governments to send representatives to an International Plague Conference, which began its sittings at Mukden on April 3rd, 1911. The following statements are taken from the reports of the proceedings published in the *Lancet* from April 29th, 1911, onwards, which include an exhaustive report by Dr. G. Douglas Gray, physician to H.B.M. Legation, Peking, the questions for discussion, and the Chairman's inaugural address.

Origin.—It had been known for many years that in Eastern Siberia and Mongolia the marmot, or tarabagan (Russian), or han ta (Chinese), a variety of the squirrel tribe, of the rodent genus, frequently suffers from a fatal disease which may be transmitted to man and produce symptoms indistinguishable from bubonic and pneumonic plague. This animal is hunted for its fur, which is used to imitate sable and other furs. One of its favourite haunts is a mountain range in the north-west of Manchuria, and here large numbers of Chinese are employed trapping it during the summer months. It was among these trappers that the present outbreak is held to have originated. The proof that the "tarabagan disease" in man, and plague, were one and the same disease, has not been given bacteriologically, and in 1905, 1906, and 1907, Russian scientific expeditions were sent to investigate and report. Dr. M. T. Schreiber concluded that: (1) Epizootics (a term applied to those animal diseases which behave as epidemics do in the human species)

undoubtedly do occur without human beings becoming infected; (2) Field mice do not contract the disease from the tarabagan, though they have every chance of doing so, and are known to be susceptible to plague; (3) Domestic animals also escape it, although dogs eat the flesh of the dead marmot.

Dr. B. A. Barykin reported in 1909 the following facts: In 1906 the marmots around a settlement called Abogaitui showed a high mortality from spring to autumn, but the inhabitants were aware of the danger and avoided all contact with the sick animals, except a Cossack, who was in indifferent health and had a craving for the flesh of a tarabagan. He got some, fell ill with the symptoms of plague, and died in four days. Others became ill, and in all eight died with symptoms of pneumonic plague. Post mortems were held in two cases, and bacilli indistinguishable from the plague bacilli were found in the organs; and mice injected with the splenic juice died in twenty-six hours with the typical appearances. This was in September, 1906. In the autumn of 1907, marmots were caught or killed by the party and examined for the presence of disease. In one of these animals showing no external signs of being ill save some degree of malnutrition, the spleen was found to be swollen and congested, and contained large numbers of bacilli identical in morphology and culture with the plague bacilli. A fortnight later, twelve miles from where this animal was discovered, the men of an isolated Cossack family, hunting the marmots around them, killed one showing clear signs of illness. In spite of the counsel of the elder men the animal was skinned and the body was given to a girl of thirteen years to take to the fields. She dragged its body (said to be 15 lb. weight) after her through the grass, and returned barefoot over the same path. On the next day she fell ill, a bubo appeared in the left groin, and she died some days later with all the symptoms of plague. From the bubo, from a pustule on a finger, and from the spleen, bacilli indistinguishable from plague bacilli were isolated, and being injected into mice caused their death in eighteen hours, of septicæmia. Neither the body nor the skin of the animal was recovered.

The rest of the family escaped. The same autumn a railway guard who had caught marmots, and a woman who had skinned them, both died of a disease resembling bubonic plague. In the guard, typical bacilli were found. A history of ten outbreaks in nine years in the district around one railway station was elicited. Various other localized outbreaks were reported, all tending to show that plague was not new to the districts where Mongolia, Manchuria, and Siberia adjoin.

Spread.—Beginning then in the north-west borders of Manchuria among the marmot hunters, it was carried by these in travelling back to their homes, many of them having come from the Shantung province, south of Peking. In the third week of October, 1910, about 10,000 of these men had gathered in Manchourie and Khailar, stations on the Vladivostock railway, waiting to sell the skins they had gathered and to return to the south for the winter and the new year festival. Cases of illness occurred here, with symptoms of headache, fever, spitting of blood-coloured sputum, and followed by rapid death. Apparently in spite of the risks not many hunters die on the plains; but when crowded into the poor hovels or inns of the market-towns,—where, in small badly ventilated rooms, twenty to forty may be found living, sleeping, and eating beside piles of raw pelts—the conditions for the encouragement of any epidemic disease are ideal. From these foci the infection spread by railway trains in which the hunters were carried to Harbin, then south to Chang-chun, thence to Mukden, on to Tientsin, and from there to their home villages in the Shantung province; and also by foot travellers who struck across country through Kirin city (eighty miles from a railway) to Dalny, and thence by boat to Chefoo, a port in the Shantung province. All the way men were falling ill and dying, and the sick and dead were thus deposited at all the places passed *en route*. The infection reached Harbin on November 7th, and in three months there were 5,000 deaths out of a population of 30,000. Two factors seem to have contributed largely to the virulence of the epidemic in the Chinese city. First, the severe climatic conditions, the thermometer registering at times $-30°$ C. ($-22°$ F., or 54

degrees of frost), which prevented the people going out of doors. Secondly, the low, dark, dirty, and overcrowded houses which form the majority of the dwellings. Nevertheless in Shuangcheng Fu, a finely planned city with wide streets, spacious compounds, and well-constructed houses, and with but little poverty, there were 1,500 deaths in seven weeks out of 60,000 inhabitants.

Symptoms.—The incubation period in the majority of cases was five days. There were no marked prodromal symptoms. Often a man had a normal pulse and temperature on one day and was dead the next. The invasion was without rigor, with feelings of illness, weakness, and giddiness. A sudden onset with headache, then bloated face and suffused conjunctivæ (septicæmic cyanosis), with temperature over 103° F., and fast fluttering pulse, was usual. The respirations averaged thirty-five per minute. Coarse crepitant râles were noted all over the chest, but little or no impairment of resonance. These râles are due to marked œdema of the lungs in the late stages of the disease. In the earlier stages râles are rarely present even in serious cases, and then they are usually fine. Blood-stained sputum is often the first sign of illness in pneumonic cases. The signs of cardiac involvement are always marked in advanced cases: very rapid feeble running pulse, agonizing dyspnœa, galloping rhythm of the heart sounds, and sudden heart failure. Death occurs from the intoxication, with paralysis of the heart. Death resulted in attempts to move patients and where the patients sat up in bed for a few minutes to take nourishment. Labial herpes was not observed in any of the patients seen in hospital, which is a point noted before and interesting in comparison with acute lobar pneumonia, in which it frequently occurs. In the septicæmic form there may be a flow of blood from the nose or mouth shortly before death. No glandular enlargements were noted except once at Harbin, where there was a submaxillary bubo followed by secondary plague pneumonia and death. The bacteriological diagnosis was the only certain one, as the symptoms were so variable. Many were able to walk about until within a few hours of death, and up to that time declaring that they were quite well.

Etiology and Pathology.—Dr. R. P. Strong and Dr. Teague performed twenty-five post-mortem examinations, and came to the following conclusions: That epidemic plague pneumonia results from inhalation, the primary point of infection being the bronchi. Through the bronchi the bacilli reach the lung tissue, and rapidly multiplying there they produce pneumonic changes of the lobular type and later more general lobar involvement. The blood becomes quickly infected and a true bacteriæmia results in every case. Secondary pathological changes occur, especially in the spleen, bronchial glands, heart, blood-vessels, kidneys, and liver. The fact that the bronchial glands at the bifurcation of the trachea are always much more affected than any of the other lymphatics, argues against the theory that epidemic plague pneumonia is primarily a septicæmia with secondary involvement of the lungs. Moreover, in the earliest stages of the disease, the blood may be free of plague bacilli. The condition observed in the trachea and bronchi in epidemic plague pneumonia is pathognomic of this condition alone. The throat and larynx may show characteristic appearances at times. The tonsils may become secondarily infected like other lymph follicles, but the duration of the disease is too short to allow of this as a rule. Primary infection by tonsil can occur, with enlargement of the glands of the neck early in the disease. The œsophagus was found normal in every case, which argues against primary intestinal plague infection, since plague bacilli must have been repeatedly swallowed in sputum and bronchial secretion in many of the cases.

Dr. Koulecha, who had dissected twenty-eight plague corpses, read a communication in which he differed *in toto* from the above in regard to the mode of infection. From the necropsies and microscopical examination of the tissues he concludes that pneumonic plague is a septicæmic disease, in which an overflooding of the blood and the lymphatic system with bacilli could be observed. Infectious matter entered the mouth, affecting *en route* the tonsils, mucous membrane of the trachea, bronchi, and neighbouring lymphatic glands; and from these the bacilli passed into the blood. The lungs were apparently affected secondarily from the blood, which he inferred from

the great accumulation of the plague bacilli in the perivascular spaces. On this view, pneumonic plague is a lobar pleuro-pneumonia of hæmatogenous origin, and should be classed with croupous pneumonia. Dr. Fujinami confirmed these findings. Professor Zabolotny observed that it had not been sufficiently proved how many were of direct pulmonary origin and how many were of hæmatogenous origin.

Rats and Fleas.—Professor Kitasato reported that of 30,000 rats examined in South Manchuria, 6 per cent were Mus rattus, but none of the 30,000 showed plague infection. In North China, of 3,000 rats examined living by Dr Andrew, all were Mus decumanus; he had never found Mus rattus. He had noted a seasonal flea prevalence, highest in September, October, and November. The only species noted was Pulex cheopis. Dr. Petrie found both Pulex cheopis and Ceratophyllus unisus among fleas examined at Mukden. Dr. Petrie also examined twelve tarabagans sent direct from Manchuria to Mukden. On these he found thirty-five fleas, twelve being on one alone. (Ticks were also observed on them.) The fleas found were unusually large, and on superficial examination appeared to belong to the genus Histricopsylla (eyeless, truncated head, comb on the inferior border of head, thorax, and part of abdomen, numerous long hairs over the body).

In an epidemic of plague in Tongshan, in 1908, bubonic cases predominated at the first, but the proportion of septicæmic and pneumonic gradually increased. Of the rats examined, 1 per cent were found to be affected. Some rats which remained well for days without any sign of glandular enlargement during life, were found post mortem to have Bacillus pestis in the splenic blood, and were evidently immune to infection, at least for the time being. Rats trapped in the mines in the neighbourhood were found to be less readily infected experimentally than those found above ground. The death-rate in the Tongshan epidemic was 800 out of 1000 cases.

Diagnosis.—Sputum is scanty at first, and the bacilli are difficult to find in it. Later, it is almost a pure culture. Professor Zabolotny advised Gram's method for staining

the sputum. He had often noted mixed infections, and a Gram-negative bi-polar-staining bacillus was seen at times, which was not the plague bacillus. It required further study. Involution forms bore no relation to virulence. Blood cultures could usually be got forty-eight hours before death. At least 1 c.c. of blood must be used for the test. In this way an earlier diagnosis could be made than by the sputum. In recovered bubonic cases, agglutination could be got in the second and third weeks with dilutions of 1–25 and 1–50. Agglutination and fixation of the complement experiments were of little value in pneumonic plague. Dr. Broquet advised the use of the following solution for the conservation of suspicious plague organs for future study or transmission to a laboratory at a distance: neutral glycerin, 20 c.c., calcium carbonate, 2 grm., distilled water, 80 c.c. Mix at 30° C., and immerse tissues.

Vaccination.—Protective vaccination with attenuated plague bacilli was advocated by Dr. Strong. Professor Galeotti advised the vaccine of Lustig and Galeotti for these reasons: (1) The toxin (Galeotti holds that it is an endotoxin and non-soluble; Zabolotny holds that it is a soluble toxin), and no other substance, is used; (2) It can be dried and standardized; (3) The plague nucleo-proteid can be stored in a sterile condition. The general experience was that no form of vaccination gave much protection against pneumonic plague.

Serum Therapy.—Dr. Martini advised passive immunization for all those exposed to infection, such as doctors and nurses, etc. Small doses were useless; 100 c.c. at least must be used, and repeated soon. As regarded the present epidemic, he thought the protective value was small. Professor Zabolotny reported that he had used up to 1 litre of serum without success, only prolonging the illness. Dr. Paul Haffkine had seen protective effect after large doses, but this did not last more than five days.

Quarantine.—The use of railway cars for this purpose was a new feature. This gives a segregation camp divided into small units, each completely isolated from the other, easy of disinfection, easy to supervise and for the detection of onset of sickness. There were 3000 suspects thus

isolated at Harbin, the railway cars being drawn into specially constructed sidings.

Disposal of the Dead.—With the ground frozen at —40° C., ordinary burial of such large numbers of bodies was not easy. In spite of Chinese traditions, the Government gave orders for the cremation of the bodies of those dead of plague. This was carried out thus at Harbin: A pit 20 feet square by 10 feet deep was made by blasting with dynamite. When bodies were in coffins, these sufficed to aid the burning; but if they were not, four pieces of round wood 4 inches in diameter by 2 feet long, were added per body. The pit held about 500 bodies, and into it kerosene was pumped from a fire-engine; about 10 gallons per 100 bodies. On being set alight the mass burned fiercely and rapidly, and little but ashes remained.

End of Epidemic.—The epidemic seemed to die out (April, 1911) when the temperature rose to — 20° C. (— 4° F.). The total mortality was over 40,000.

Summary of Conclusions.—The disease spread by direct infection from man to man. Whatever may have been its primary origin, there is no evidence that a concurrent epizootic in rodents played any part in its wide dissemination. From Russian sources reports of an epizootic disease among tarabagans have been received, and it is not unlikely that this is plague, but the bacteriological proof is not yet complete. The infection was introduced into towns and villages by persons actually suffering from, or by those in the incubation stage of, the disease. There has been no positive epidemiological evidence to show that the disease has been spread by clothing, merchandise, or other inanimate objects. So far as can be ascertained the only infective agent in the epidemic has been the sputum of the plague patient. In the majority of the cases the disease has been contracted by the inhalation of plague bacilli in droplets of sputum, causing infection of the lower portion of the trachea and of the bronchi. In infection by inhalation the risk to the person exposed bears a direct relation to his proximity to the patient and to the duration of the exposure. In view of this, masks and goggles should be worn by all who come in

contact with cases of the disease or suspected cases. The best form of mask is a three-tailed gauze bandage with a pad of cotton-wool. It should be destroyed or disinfected after each exposure to infection. The epidemic was, almost without exception, one of primary pneumonic plague, with an incubation period of from two to five days. An increased temperature and pulse-rate are usually the earliest signs observable, but a diagnosis cannot be made until the organisms are recognized in the characteristic blood-stained sputum.

An accurate diagnosis can only be made by a bacteriological examination of the sputum to exclude pneumonic infection due to other micro-organisms. Since the evidence points to the conclusion that in the epidemic all the cases became septicæmic, an examination of the blood microscopically or culturally may be a valuable aid in diagnosis. The physical signs of lung involvement are too indefinite and appear too late in the course of the disease to be of diagnostic value, and even in cases in which the condition of the patient is grave they may be very slight.

The fatality has been extremely high, scarcely any recovering. The general experience has been that no method of treatment has been of avail in saving life, but that the serum treatment seems in a few instances to have prolonged it. The decline of the epidemic has not been due to any loss of virulence of the bacillus, but probably to the preventive measures which were enforced either in accordance with scientific methods or by the efforts of the people to protect themselves.

The statistics of prophylactic inoculation collected during the past epidemic do not allow of any definite conclusion being formed about their value in plague pneumonia; but in bubonic plague it was argued that some degree of protection is conferred by the use of vaccines. Further experiments in animals are recommended, in reference to securing immunity against pneumonic plague infection. (*Lancet*, 1911, vol. 1, pp. 1614–16.)

THE TUBERCLE BACILLUS.

This bacillus is the cause of tuberculosis, an infective disease, characterized by lesions which are nodular bodies called " tubercles," or by diffuse infiltrations of tuberculous tissue, which may undergo caseation and finally ulcerate, or may become sclerosed, and in some cases calcified. The infectious nature of tuberculous material was for long suspected, was proved by Villemin in 1865, and by Armanni and Cohnheim and Salomonsen from 1870 to 1880. Baumgarten first described the bacillus in sections, but Koch first established its causal nature on a solid basis by: (1) Demonstrating the presence of the tubercle bacillus in a great variety of tissues and organs; (2) Preparing pure cultures of the organism from these; (3) Producing the disease by the inoculation of the bacillus derived from pure culture; and (4) Recovering the same organism from the diseased parts of inoculated animals. These four articles are known as Koch's postulates, and all of them have to be satisfied before an organism can be absolutely proved as the cause of a specific disease. At present, for quite a number of bacteria, some of these postulates have yet to be fulfilled as regards mankind.

Description.—Bacillus tuberculosis is a slender rod, often slightly curved, measuring 2·5 to 3·5 micra long by 0·3 micron in width. They are of uniform thickness, or may show slight swelling at the ends or even in their length. They may lie singly in the tissues or sputum, but often in small heaps or masses. In cultures, a remarkable filamentous growth has been repeatedly observed. In the sputum, long branching hypha-like filaments, sometimes with swollen ends, have been found. These are looked upon by some bacteriologists as involution forms, but many regard them as evidence of the relationship of the tubercle bacillus to the hyphomycetes. A capsular or enveloping substance is produced by the bacillus, more by the human form than by the bovine, and more the longer the growth on blood serum. When stained, the bacillus often appears beaded, the unstained spaces being regarded as vacuoles by some and as spores by others. Inasmuch as the bacilli

showing these have no increased resistance against heat and disinfectants, the spore interpretation is probably incorrect. The beads or highly stained portions have likewise been called spores, but the whole matter is at present unsettled.

The tubercle bacillus stains imperfectly or not at all with ordinary watery aniline dyes, and only after long exposure or heating, or more quickly if a mordant is used. Once stained, the bacilli retain the dye tenaciously, in spite of treatment with alcohol or strong acids, and for the latter reason they are spoken of as " acid-fast " bacilli (acid-proof would be a better term). This feature seems to be due to the presence of fatty substances in the cell, and has furnished the basis for differential staining methods. The fatty substances are really wax-like in nature, and are soluble in alcohol + ether.

B. tuberculosis is non-motile, non-flagellar, non-sporing, does not grow on gelatin, and is Gram-positive. Recently varieties have been described which are not acid-fast, but are stained by Gram's method prolonged, and these forms are stated to be present in old tuberculous lesions, where the ordinary form is not found and yet the material is virulent. These are, (1) A fine bacillary form, often showing granules, and (2) Free granules. *Much* also found that when acid-fast forms were added to milk (sterilized) and incubated, the acid-fast forms disappeared, and yet when the milk was injected into an animal, tuberculosis was produced and in the lesions acid-fast bacilli were demonstrable. If these statements are conclusively proved, they are of the first importance.

Chemical Analysis of Tubercle Bacilli.—Water 85·9 per cent, solids 14·1 per cent. The ash shows 55 per cent of P_2O_5, 12·6 per cent of CaO, 11·5 per cent of Mg, 13·6 per cent of Na_2O, 6·3 per cent of K_2O.

Cultures.—The bacillus is not easily cultivated ; growth fails, or is slow or scanty ; fails on agar and gelatin entirely, but on glycerin agar (3 to 6 per cent) and in glycerin broth (6 per cent) growth takes place in ten to fifteen days, becoming visible first as dry white spots, the extension and fusion of which form a dull, whitish, wrinkled pellicle or layer. These media are suitable for subcultures ; for

growth direct from the tissues, blood serum and Dorset's egg media are commonly used.

On blood serum, at 37·5° C., growth appears in ten to fourteen days as minute spots, rather irregular, raised above the surface, and comparable to small dry scales. The culture is got by inoculating with a sterile platinum loop from foci in a tuberculous gland; or by planting an actual piece of tissue on to the surface of the serum; or by inoculating with tubercle bacilli, derived by the anti-formin method (see later) from sputum or digested tissue.

On Dorset's egg media, vigorous growth takes place resembling that on blood serum. To make the medium: take the whole contents of four eggs, beat well, add 25 c.c. of water, and mix thoroughly; filter through muslin to remove air bells; fill into tubes, and heat these in sloped position for four hours at 70° C. They are now ready; but before inoculation, two drops of sterilized water are placed on the surface of the medium. When inoculating, the material is well rubbed over the surface, the plug is replaced, and is sealed over with a few drops of paraffin, and the tube incubated in the sloped position.

On glycerin potato, growth takes place, and on other purely vegetable media.

Optimum Conditions.—B. tuberculosis is markedly aerobic. It grows at human blood-heat, 37° to 38° C. The usual range is 28° to 42° C. but it can be acclimatized to grow at 22° to 23° C. In fluid media it is killed in fifteen to twenty minutes at 60° C., in five minutes at 80° C., and in one to two minutes at 90° C. It can resist dry heat at 100° C. for one hour. Simple drying is not efficient, as still virulent forms have been found in dried tuberculous sputum after two months. Similarly, putrefaction of sputa or tissue does not destroy the bacilli readily, for they have been found alive under such conditions after three weeks. Gastric juice failed to kill them in six hours, and several three-hour spells of freezing at — 3° C. had little effect. Direct sunlight rapidly kills them; 5 per cent carbolic kills them in a few minutes, but if, as in sputum, they are protected by mucus, complete disinfection takes five to six hours.

Perchloride of mercury is not very efficient, from the albuminate formed. The comparatively high powers of resistance (for an organism not admitted to have spores) are attributed to the protective qualities of the waxy cell membrane. The conclusion then is, that moist heat at 100° C. will kill the bacilli in fluids and tissues, provided time is given for penetration of bulky materials. In Germany, tuberculous ox flesh is thus sterilized (four hours boiling) and then allowed to be sold.

Pathogenicity.—For man is great: 10 per cent of all the deaths in Great Britain and in the United States of America are due to tuberculosis of one kind or another. This of course represents only a portion of the incidence of tuberculosis, as many people are attacked and succeed in throwing off the infection. In fact it may be said that the fatal attack of tuberculosis is in many cases only the last of a long series of attacks more or less successfully repulsed. The commonest type in man is phthisis pulmonalis, then affections of lymphatic glands, bones and joints, and serous membranes.

In America, 22 per cent of the deaths among the North American Indians, and 16 per cent among the negroes, are due to tuberculosis.

For animals, the pathogenicity varies, but most are vulnerable. The question is at present clouded by the statement that various types of tubercle bacilli exist, such as the human type, the bovine type, and the avian type, and that these types vary in their power over the different animal species.

Ignoring for the moment the different types, tuberculosis is mostly found in cattle and pigs. Dogs and cats occasionally suffer, and monkeys and apes (immune when in the wild state) are very subject to it and mostly die of it in captivity. Horses are rarely attacked, and sheep are practically immune. Of all the home cattle slaughtered in the Glasgow market in 1910 (67,849), 12·98 per cent showed lesions of tuberculosis, and of 39,724 swine, 6·12 per cent; while out of 307,784 sheep not one showed tuberculous lesions. The rate among cattle is excessive from the large proportion of milch cows included, some of which are imported into the city and are kept in byres

until sent to be slaughtered. Apart from that, however, the figures suggest the influence of open-air life in lowering the susceptibility of the sheep and the chances of infection.

B. tuberculosis of the **human** type is pathogenic for guinea-pigs, less so for rabbits, and still less so for dogs.

B. tuberculosis of the **bovine** type is very pathogenic for guinea-pigs, killing them more quickly and producing more extensive lesions than the human type; while intravenous injection in rabbits causes an acute tuberculosis with death in from two to five weeks; the human type causing a mild, slow disease, usually lasting for six months, and at times failing to kill the rabbit. This is the readiest method of distinguishing these two types.

The human type is found in the majority of human infections (46 out of 60 investigated), never in bovine tuberculosis.

The bovine type is found always in bovine tuberculosis, and in a considerable number of cases of human tuberculosis (14 out of 60 = 23 per cent), and in all the latter but one, the lesions were of the cervical lymphatic glands, or were lesions of primary abdominal tuberculosis, that is, were lesions which might fairly be attributed to feeding or alimentation. Moreover they were mostly in children, so that the deduction is made that bovine tuberculosis is transmissible to man, the milk of tuberculous cows being the usual vehicle.

Bovine tubercle bacilli, in cultures, are shorter, thicker, and more regular in size than the human type, and their growth on the various media is scantier. They are more pathogenic to cattle and swine, and less so to man, which may explain the chronicity of abdominal and cervical glandular tuberculosis.

A recent research by Park and Krumwiede confirms the results of previous investigations. They state that comparative luxuriance or sparseness of growth is in most cases absolutely to be relied on for differentiation.

Their results may be summarized thus :—

Form of Tuberculosis	Pulmonary		Cervical		Abdominal	
Type of bacillus found	Human	Bovine	Human	Bovine	Human	Bovine
Ages of persons examined :—						
16 years and upwards	290	1	13	1	14	3
5 years and up to 16	3	0	14	12	6	6
Under 5 years	7	0	9	8	6	10
Totals	300	1	36	21	26	19

The results in 606 cases of various kinds of tuberculosis, investigated by different workers, are tabulated thus (three cases showed both types) :—

	Total	Human Type	Bovine Type
Adults (16 years and upwards)	389	381 = 98 %	8 = 2 %
Children (5 to 16 yrs. inclusive)	78	54 = 69 %	24 = 30 %
Children (under 5 years) ..	136	99 = 72 %	37 = 27 %
Totals ..	603	534 = 88 %	69 = 11 %

The bacillus of avian tuberculosis shows a more luxuriant and moister growth than the human type, and grows at 43·5° C., is very pathogenic to rabbits, scarcely pathogenic to guinea-pigs, and not at all to dogs, even intravenously, whereas the human type produces an acute infection. Morphologically it is almost identical with the human type, and stains similarly. It is found in lesions in fowls and pigeons and some other bird species. Fowls fed on human tubercle bacilli are not found to become tuberculous. The identity of the two types is said to be established by the experiments of Nocard, who rendered mammalian tubercle bacilli pathogenic for fowls by keeping them in the peritoneal cavities of hens in collodion sacs for six months. Conversely, prolonged cultivation and passage of the avian type through the mammalian body are said to cause them to approach closely to the mammalian type.

The bacillus of a tubercle-like disease in a carp has

been called the bacillus of **fish** tuberculosis. It grows luxuriantly at room temperature (20° C.), and does not grow at 37° C., its range being 15° to 30° C. It is non-pathogenic for mammals, but kills frogs in a month. It shows some degree of acid-fastness.

Portals of Entry.—Inheritance, ingestion, inhalation, and inoculation. Cobbett recently traverses the theories of Behring, Calmette, and Guérin, that the portal of entry of the tubercle bacilli, even in the pulmonary form, is via the alimentary tract and thence via the lymphatics to the lungs. He concludes that the intestine is not a common port of entry for the tubercle bacilli which cause phthisis. (Cobbett, "Portals of Entry of the Tubercle Bacillus," *Jour. of Path. and Bact.*, vol. xiv., No. 4, 1910, p. 563). Leonard Findlay reaches the same conclusion from experiments on the production of pulmonary anthracosis in rabbits and guinea-pigs (Findlay, "The Origin of Pulmonary Anthracosis, an Experimental Study." *Zeitschrift für Kinderheilkunde*, 1911, vol. ii., part 2 (June), p. 293. *B.M.J.*, 1911, vol. ii., p. 1278.) Feeding experiments undertaken for the Royal Commission on Tuberculosis gave results more in consonance with Calmette's theories (see *resumé* on page 297; details in Appendix to Final Report, vol. i., pp. 48 and 52).

Toxins.—The tubercle bacillus secretes no soluble toxin or only in very small amount. The chief toxic principles are endotoxins or bacterial proteins. Dead bacilli will, if inoculated in sufficient numbers, produce tubercle-like nodules, in which giant cells and occasionally caseation are present. These results are obtained with intravenous and intraperitoneal injections, whereas subcutaneous injection produces a sterile abscess (cold abscess).

The hope of producing an active immunity led Koch to employ various means to extract from dead and living bacilli the complex bodies bound in them.

1. *Original Tuberculin, T.A.* (Koch 1890–91).—Tubercle bacilli are grown in 5 per cent glycerin broth for six to eight weeks. The entire culture is then heated on the water-bath at 80° C. until reduced to one-tenth of its original bulk. It is then filtered through sterile filter-paper or through porcelain filters. The filtrate is a thick,

brown, syrupy liquid, containing the products of growth in the culture medium and a 50 per cent extract of the bodies of the bacilli by glycerin; all in so far as these are indestructible by heat. Stored in a cool dark place it keeps indefinitely, the glycerin acting as a preservative. The dose of this preparation used in cattle is 0·25 c.c., but the stock is usually diluted with 0·5 per cent carbolic water four times, making the dose 2 c.c. Such a dose in a healthy man causes in three to four hours malaise, tendency to cough, laboured breathing, and moderate pyrexia, all passing off in twenty-four hours. In a man suffering from tuberculosis, such a dose would give rise to such an excessive reaction as probably to cause death. Even 0·01 c.c. causes all the above symptoms in an aggravated degree, together with marked inflammatory reaction around any tuberculous focus, resulting in necrosis but not killing the bacilli. This is now known as the "tuberculin reaction," and is much used in veterinary practice. It is also used in clinical work for help in diagnosis of obscure affections suspected to be tuberculous.

i. Subcutaneously: Use diluted tuberculin, 1–1000, so that 1 c.c. = 1 mgr. of tuberculin. Koch in 1890 used 1 mgr., but now most clinicians begin with 0·1 mgr. or 0·2 mgr. If no reaction occurs, after three to four days give 1 mgr. If again no reaction follows, wait three to four days and try 5 mgr., and finally, if still negative, 10 mgr., but no more. If still no reaction, the person may be considered tubercle-free. Take temperature regularly before and after test.

ii. Cutaneous test (von Pirquet, 1907). For this test von Pirquet first suggested a 25 per cent solution of "old tuberculin," but the latter is now used undiluted. The patient's skin on the flexor surface of the forearm is sterilized. Two separate drops of the tuberculin are placed on the skin, 2 to 4 in. apart. With a small metal bore the skin is scarified at a point midway between the two drops, and then that is covered by the drops. Within twenty-four to forty-eight hours in tuberculous patients redness round the points, a papule over the scarified surface, and minute vesicles all appear. The control area shows a slight traumatic redness which soon passes off. In a

negative result all three areas show this slight reaction. Intermediate reactions, in all degrees, are observed. 70 per cent of adults give a positive result, probably from healed tuberculosis.

iii. Inunction of lanolin, containing 50 per cent of tuberculin (Moro's test).

iv. Ophthalmo-tuberculin reaction (1907, Wolff-Eisner; Calmette). For this test, "old tuberculin" is treated with 95 per cent alcohol, and the precipitate dissolved in water, and re-precipitated and dissolved several times, being finally made up to a 1 per cent watery solution. One drop of this is instilled into the conjunctival sac and allowed to spread all over it. A sharp congestion of both the ocular and palpebral conjunctivæ results in six to ten hours in the case of a positive reaction, and passes off in twenty-four to thirty-six hours. In children, half the dose is used and the acme is reached sooner. In several cases the results of this test have been disastrous, the excessive reaction leading to destruction of tissue and blindness in the eye used for the test.

2. *New Tuberculin* (Koch, 1897)—Was introduced to produce anti-bacterial immunity. It was prepared thus: bacilli from young virulent cultures were ground in an agate mill, washed with distilled water, and centrifugalized. The clear fluid was decanted and called tuberculin–O. The deposit was dried, ground, and treated as before; and this was repeated several times until all the residue went into emulsion; the resulting fluids mixed together are tuberculin–R. Tuberculin–O gives no precipitate with glycerin added to make a 50 per cent solution. Tuberculin–R does precipitate in 50 per cent glycerin. Tuberculin–O gives a reaction on injection more readily than tuberculin–R.

3. *Bacillary Emulsion* (Koch, 1901).—This is a 1 per cent emulsion of pulverized bacilli in distilled water, allowed to sediment for several days, and then the supernatant liquid is mixed with an equal bulk of glycerin, making the whole 50 per cent. 1 c.c. = 5 mgr. solids. It resembles a mixture of tuberculin–O and tuberculin–R.

4. *Bouillon Filtré* (Denys, 1905).—A culture of tubercle bacilli in 5 per cent glycerin broth, filtered through

Chamberland filters and not heated, but 0·25 per cent phenol added to preserve. Corresponds to "old tuberculin," unconcentrated and unheated, and therefore supposed by Denys to contain important soluble but thermolabile substances.

Therapeutic uses of the Tuberculins. — (So-called Vaccine Therapy.)—For these purposes, tuberculin–R and bacillary emulsion are mostly used in doses beginning at 0·001 mgr. ($\frac{1}{1000}$), and gradually increased so long as the dose causes no greater disturbance of temperature than 0·5° F. In Wright's method of treatment the initial dose is usually $\frac{1}{4000}$ mgr., and is rarely increased beyond $\frac{1}{1000}$ mgr. The dose is administered after determination of the opsonic index of the patient, and subsequent doses are only administered during the positive phase of the reaction to the previous dose. To determine this, repeated observations of the opsonic index have to be made. As a result of experience gained by a large number of observations, some authorities advise the use of smaller doses, $\frac{1}{50000}$ mgr. every ten days, gradually increased to $\frac{1}{4000}$ mgr. in six months' time, without opsonic index estimation, but simply watching the clinical symptoms.

In the serum of patients so treated there is evidence of the formation of bodies antagonistic to tuberculin, of the nature of immune-bodies, precipitins, and opsonins.

Antituberculous Sera.— *Maragliano's Serum.*— Made by him by immunizing dogs, asses, and horses, by injecting a mixture of the filtrate of an unheated broth culture (1 part) and an aqueous bacillary extract at 100° C. (3 parts). The animal is bled after four to six months. Of the serum, 2 c.c. are injected subcutaneously every two days. Improvement has been noted in non-febrile cases. The serum is capable of protecting an otherwise healthy animal against a fatal dose of tuberculin.

Marmorek's Serum.—Marmorek believes the tubercle bacillus does not produce in ordinary media the same toxins that it does in the body, where it has to resist the antagonism of the body cells. To combat this he first grows the bacilli on a leucotoxic serum (produced by inoculating calves with guinea-pig leucocytes), and then on a medium containing liver extract, the liver being

regarded as the most antituberculous tissue in the body. He thereafter uses these bacilli (which he states yield no tuberculin) to immunize animals, and for the serum produced he claims high curative powers.

Tuberculin Tests applied to Cattle. — In cattle, tuberculosis may be present without any very apparent symptoms until an advanced stage is reached. Routine examination of herds by the tuberculin test has therefore become one of the necessary measures in public sanitation, in order that the milk of tuberculous cows may be excluded from consumption, and that such cows may be eliminated from herds (Bang's System). Mohler states that an accurate diagnosis is established in 97 per cent of the cases. Stall the animal and take the temperature in the rectum every two hours from 6 a.m. until midnight. Make the injection then (subcutaneously). Begin to take the temperature at 6 a.m. and continue as on the preceding day. The dose is usually 0·25 c.c. of old tuberculin. A positive reaction consists in febrile and constitutional signs, with marked congestion around any focus of tuberculosis. The febrile reaction begins six to ten hours after injection, reaches its height in nine to fifteen hours, and declines to normal in eighteen to twenty-six hours. A rise of 2° F. or more above the maximum of the previous day should be regarded as a positive reaction. In a doubtful case, repeat in four to six weeks. (Normal rectal temperature, 100° to 102° F.; normal pulse, 45 to 55 beats per minute.

Methods of Detection.—1. *Microscopic.*—In **sputum**: select a yellowish piece, make a film, and stain by acid-fast method. If not found, make solution of sputum by gradual addition of NaOH solution, boiling the while; then sediment or centrifuge. Or mix in 5 per cent carbolic or 2 per cent lysol, and stand; gradual solution, and tubercle bacilli precipitated. Or add antiformin (or a mixture of equal parts of solutions of NaClO and NaOH, each 7·5 per cent); dissolves in a few minutes. If a 20 per cent dilution of antiformin is used, the tubercle bacilli are not damaged, and all the other bacteria are killed. Centrifuge or sediment; wash twice with normal salt solution, and sediment can then be used to make culture,

or inoculate guinea-pig. In **urine**: sediment or centrifuge, make films, and examine. To avoid smegma bacilli, take specimen after cleansing meatus, or by catheter, and in staining decolorize with absolute alcohol after the acid (see pp. 163 and 285. In **pus**, fæces, and tissues: dissolve in antiformin as above, and examine deposit. In **milk**: centrifuge, take sediment and fat, and mix, make films, fix, remove fat with ether (not absolutely necessary), stain, and examine. Also inject 0·1 c.c., 1 c.c. and 3 c.c. of mixed sediment and fat into three guinea-pigs respectively; kill in three weeks and examine peritoneum, post-sternal glands, pancreas, spleen, and liver.

2. *Inoculation.*—Select a guinea-pig and inject, subcutaneously or intraperitoneally, the fluid to be tested, or the washed sediment from an antiformin solution of tissues, fæces, or sputum. The animal usually dies in six weeks, if tubercle bacilli are in the injected substance, with local and glandular changes, the spleen showing numerous tuberculous nodules and being swollen as a whole.

3. *Cultivation*—Is made from the inoculated animal, or from antiformin solution sediments, or from sputum treated with 2 per cent ericolin.

4. *Tuberculin Reactions.*

OTHER ACID-FAST BACILLI.

Besides the bacilli of human, bovine, avian, and fish tuberculosis, there are other bacteria which are acid-fast, as already enumerated on page 163. Such are: Moeller's Timothy-grass bacillus I (from infusions of Timothy grass), Moeller's Timothy-grass bacillus II (from the dust of a hay-loft), butter bacilli (isolated from butter by Petri, Rabinowitch, Korn, Tobler, Coggi, and others), mistbacillus (from dung by Moeller). All these are morphologically very similar to the tubercle bacilli, are extremely acid-fast, and produce lesions in guinea-pigs on injection, which closely resemble tubercles. They are, however, easily distinguished by their rapid growth on ordinary media, colonies being visible in twenty-four hours at 37° C. (in the case of the tubercle bacillus, the earliest is eight days), and by their growth in most instances at

room temperature. The cultures themselves are, however, similar to those of tubercle bacilli, and of one another.

Johne's Bacillus is the bacillus of "chronic bovine pseudo-tuberculous enteritis," a disease characterized by corrugated thickenings of the mucous membrane of the small intestine (especially), the bacilli occurring in large numbers in the lesions and in scrapings from the surface. The bacilli are like the tubercle bacilli, but slightly shorter; they are equally acid-fast. They have not yet been cultivated on artificial media.

Bacillus Smegmatis (the smegma bacillus) occurs in large numbers in the preputial secretions of the male, the external genitals of the female, and within the folds of the thighs and buttocks. The bacilli are usually found in clumps on the mucous membrane, and occasionally in the superficial layers of the epithelium, both inside and outside the cells. They were first described by Lustgarten in 1884, who found them in a number of syphilitic lesions, and who thereupon believed them to be the cause of that disease. Further work by Alvarez and Tavel, Klemperer, and others, showed that they were harmless saprophytes. They are very similar to the tubercle bacillus, but are more varied in size (usually distinctly shorter), at times slightly curved and short. They are not easily stained, and once stained resist decolorization by acids, but not so strongly as tubercle bacilli. They are said to give up the stain to absolute alcohol, but contradictory statements are made. On this basis is founded Pappenheim's method of staining, where a film stained with hot carbol-fuchsin is treated with absolute alcohol containing 1 per cent rosolic acid (corallin), methylene-blue to saturation, and 16 per cent of glycerin. The tubercle bacilli are red, the bacilli smegmatis blue. They are cultivated with great difficulty, and first on serum or ascitic media. They are non-pathogenic, so far as tested. Their growth on media is slow (five to six days); the colonies are yellowish-white, and corrugated like tubercle bacillus colonies. Bacilli of the smegma group have occasionally been demonstrated in sputum and in secretions from the throat and tonsillar crypts.

Bacillus Lepræ. — A bacillus closely resembling the

tubercle bacillus in size and in acid-fastness. These bacilli usually stain uniformly, not showing beading. They are non-motile, non-flagellar, and non-sporing. They have not been successfully cultivated, and attempts to inoculate animals have failed. They are readily stained by Gram's method. They are found in large numbers in the cutaneous lesions of tubercular leprosy, and occur for the most part within the protoplasm of the round granulation tissue cells. They are also found in the lymphatic glands, and in smaller numbers in the liver and spleen. The spread of the disease is by the lymphatics. The earliest lesion is usually a nasal ulcer at the junction of the bony and cartilaginous septum. In the anæsthetic form, or nerve leprosy, the bacilli are found in the diffuse infiltrations in the nerves, rarely in the trophic lesions resulting. Lepers react to tuberculin, and 50 per cent are said to give the Wassermann reaction. The nasal mucus and saliva (in a less degree) are the vehicles by which the disease is spread.

Diagnosis.—Animal inoculation is negative.

ACTINOMYCOSIS, OR THE RAY FUNGUS DISEASE.

Actinomycosis is a disease of cattle and man. It occasionally affects sheep, dogs, cats, and horses. Its usual sites are the regions of the face, mouth, and pharynx. In cattle, the lower jaw is most frequently affected, the disease taking the form of tumour formation, the so-called "lumpy jaw." The tumours are often nodulated and consist of fibrous tissue with irregular abscess cavities throughout. When an abscess discharges, the pus is of a yellowish-green colour, slimy in character, and contains small granular bodies, visible to the eye and distinctly palpable, and of a pale sulphur colour. These granules are found on examination to be composed of rosette-like masses of the fungus actinomyces, or ray fungus, first described by Boellinger in 1877. It is now classed among the trichomycetes or higher bacteria, and by some as a true mould; that is, forms composed of threads which show true branching and multiply by spore-shaped bodies, which usually appear in chains—the gonidia or spores. (Madura foot, or mycetoma, is similar in nature to actinomycosis.)

Description.—An anaerobe of slow growth, growing best at 37° C.; and in a shake culture in glucose agar, the colonies are most numerous 5 to 10 mm. below the surface of the medium, the inference being that a trace of oxygen is an advantage. The colonies are round, dense, and greyish-white in colour (chalky); sometimes they are rosette-shaped. Another variety has been described which is an aerobe, growing in three to four days to little transparent drops, becoming later amber, and then reddish-yellow in colour. This variety has been grown on gelatin, which it liquefies. In cultures, club-shaped forms have not been found in the aerobic variety, but have been noted by J. H. Wright in the anaerobic variety when grown in the presence of serum or other animal fluids. It is believed that several kinds have been described under the one name, and that further research is needed to differentiate these. The anaerobic form grows in broth, forming heavy flocculent masses (solid white mulberry granules) at the bottom of the tube; no clouding nor surface growth.

The fungus is described as having three forms: (1) Filaments, more or less radially arranged, 0·5 micron thick, and closely interlaced. These form the central core of the colony. (2) At the periphery, refringent club-shaped bodies, structureless and homogeneous; whereas the filaments show a sheath, enclosing a granular protoplasm. (3) Spores or gonidia are coccus-like bodies, found between the filaments of the central mass; are variously regarded as real gonidia, or as degeneration products, or contaminating cocci. In cultures, gonidia are developed at the ends of the filaments, and such gonidia have a higher resistance to heat than the simple filaments, half an hour at 75° C. being required to kill spore-bearing cultures, and the same time at 65° C. for spore-free cultures. The filaments are Gram-positive and acid-fast.

Pathogenicity.—In man, the disease tends to generalize; in the ox, to remain local. The point of entrance in man is usually by a carious tooth, by the tonsil, or by some abrasion. The corresponding glands are next affected, and later metastatic abscesses are formed in the skin and elsewhere. The symptoms resemble those of chronic tuberculosis, for which the patient is usually treated. The

disease is acquired probably from hay, straw, and grain, and possibly by milk of infected cattle. An actinomyces has been isolated from hay and straw, and in cattle, grains have been found embedded in the centre of growths. Inoculation of the ox has produced the disease; in the smaller animals, characteristic colonies and lesions may follow, but little growth.

Isolation.—May be easy or very difficult. The pus is washed in salt solution and sown in melted glucose agar. If much contamination is found, keep washed granules for several weeks in a dry state, and try again.

Summary of the Final Report of the British Royal Commission on Tuberculosis Issued in June, 1911.

The British Royal Commission appointed in 1901 to inquire into the relations of human and animal tuberculosis, issued its final report in June, 1911. The Commission was appointed on account of the diversity of opinion which was manifested at the International Congress on Tuberculosis, held in London in 1901, when the statement was made by Koch that human tuberculosis cannot be transmitted to cattle, and that bovine tuberculosis is not dangerous to man. The results of the work of the Commission, and of much other parallel work, are to traverse directly both statements. The Final Report extends to about fifty pages (there are 7 volumes of an Appendix), and may be usefully summarized thus:—

The report is unanimous. It is based on the isolation of the bacilli from the lesions of the natural disease; the investigation of the cultural characters of the bacilli isolated, and the study of their effects when introduced in varying doses and by several methods into different animals. The species of animals used have been cattle, rabbits, guinea-pigs, pigs, goats, chimpanzees, monkeys, horses, rats, mice, dogs, cats, and birds.

The experimental methods of infection used were: subcutaneous, intravenous, intraperitoneal, and by feeding (oral). Inhalation was not tried. The findings are based on the researches of their own staff.

Three types of tubercle bacilli are described :—

(1) *Bovine tubercle bacilli* — the only kind found in natural tuberculosis of cattle.

(2) *Human tubercle bacilli*—the kind most commonly found in man, but not the only kind so found.

(3) *Avian tubercle bacilli* — the only kind found in natural tuberculosis of birds.

1. **Bovine tubercle bacillus** is taken as the standard for comparison.

SUMMARY OF ITS CHARACTERS.—(*a*). Cultural.—Grows slowly on serum, and at the end of two to three weeks shows on surface as a thin, greyish, uniform growth, not wrinkled nor pigmented. According to the rate and luxuriance of growth on glycerin media, bovine tubercle bacilli may be divided into three grades. Nevertheless it is insisted that rate and kind of growth should not be the sole basis of identification as bovine type, but only when considered along with results of inoculation experiments.

(*b*). Effects on animals.—Produces characteristic effects when inoculated into calves and rabbits in certain doses.

Calves: subcutaneous injection in neck of 50 mgr. of culture under three weeks old, causes severe general tuberculosis, starting at point of inoculation; and death usually within eight weeks.

Rabbits: intravenous injection of 0·01 mgr. or 0·1 mgr. of culture causes generalized miliary tuberculosis, ending in death within 5 weeks; intraperitoneal injection of 0·1 mgr.—death in 13 to 48 days; intraperitoneal injection of 1·0 mgr.—death in 10 to 38 days; subcutaneous injection of 1 mgr.—death in 29 to 165 days; subcutaneous injection of 10 mgr.—death in 28 to 101 days.

These results are very striking and definite, and along with cultural tests afford a trustworthy means of recognizing bovine tubercle bacilli. Later it was considered sufficient to inoculate rabbits, as results are very reliable.

(*c*). Other properties.—Subcutaneous inoculation in very small doses invariably produces acute tuberculosis in the chimpanzee, monkey, and guinea-pig.

In the goat, the pig, and the cat, general tuberculosis is readily induced.

The rat and the mouse are highly resistant to sub-

cutaneous inoculation; intraperitoneally, the bacilli multiply in the body but do not produce tubercles.

Dogs are highly resistant to subcutaneous inoculation, but succumb to general tuberculosis when large doses are given intravenously or intraperitoneally.

In the fowl, intravenous injection of bovine tubercle bacilli caused death in 50 per cent, with wasting, œdema of lung, and pallor of liver. In a few, definite tubercles were found in the lungs and minute necrotic areas in the liver. Death is apparently due to toxæmia, as dead bacilli have the same effects. Intraperitoneally and intramuscularly, even in large doses, only local lesions are produced, and there is no dissemination.

Horses: subcutaneously or orally, no progressive tuberculosis is produced. Intravenously, 10 mgr. cause death from acute tuberculosis in twenty days.

(*d*). Stability in culture.—Subcultured for long periods, (one case, 1487 days = 4 years), no great loss of virulence was found.

2. **Human tubercle bacillus.**—The human type is taken as that bacillus which has been found in the majority of cases of human tuberculosis. Its chief characters are: on serum, it grows more rapidly than the bovine type, hence it is called "eugonic," as opposed to "dysgonic," the term applied to the bovine bacillus. On glycerin media, the growth tends to become wrinkled; and on all media becomes pigmented to a greater or less extent. Its effects on animals place it in still greater contrast to the bovine type.

Calves: subcutaneous injection in neck of 50 mgr. of culture under three weeks old, does not produce progressive tuberculosis, nor does it kill. Only a local lesion results, which later becomes fibrous. In about half the cases, the infection did not extend beyond the nearest glands.

Rabbits: intravenous injection of 0·1 mgr. to 1 mgr. of culture causes slowly progressive tuberculosis with limited lesions, and death (for the majority) after three months (thirteen weeks). Intraperitoneally, 1 mgr.; animal alive after three months. Subcutaneous injection of 1 to 100 mgr.; animals survived or were killed in 94 to 725 days. In certain cases 1 mgr. or 0·1 mgr. intra-

venously acted like the bovine type, killing with acute and rapid tuberculosis; in these cases, however, 0·01 mgr. never killed within three months, thus easily distinguishing the bacilli from those taken as the standard. Hence this dose is the best to use intravenously.

Effects on chimpanzee and monkey in producing acute tuberculosis, are similar to those produced by like doses of the bovine tubercle bacillus.

In guinea-pigs, it produces acute tuberculosis, but duration of life is longer than with the same dose of bovine tubercle bacilli.

In the goat, pig, and cat, great resistance is found, only slight retrogressive lesions being produced.

In the dog, the effects are similar to the bovine tubercle bacillus, that is, there is great resistance to subcutaneous injection, but large doses given intravenously or intraperitoneally cause generalized tuberculosis and death.

In the fowl, the effects are the same as those produced by the bovine tubercle bacillus.

In a horse, subcutaneous injection of 50 mgr. produced only local disease.

The human tubercle bacillus has not shown any alteration in cultural characters on prolonged cultivation.

Resumé.—The human tubercle bacillus is distinguished from the bovine tubercle bacillus by (1) Its more ready growth on artificial media; and (2) The results of inoculation into rabbits, calves, cats, pigs, and goats. They are alike in that they readily produce tuberculosis in chimpanzees, monkeys, and guinea-pigs, and in that the lesions produced in these animals are the same in distribution and structure.

3. **Avian tubercle bacillus.**—The avian tubercle bacillus forms a slimy, whitish growth, easily emulsified (difference from human and bovine). It grows badly on serum, but especially well on glycerinated media. Inoculation into animals produces effects markedly contrasting with those given by bovine and human tubercle bacilli.

Fowls are very susceptible to intravenous, intramuscular, and subcutaneous inoculation of the avian tubercle bacilli, and also to feeding. In the former modes, the lesions are in the spleen and liver, and frequently in the lungs, cervical

glands, muscles, and bones. After the feeding method, there are similar lesions, with, in addition, characteristic tuberculous lesions in the mucous membrane of the intestines.

Parrots show similar results, but by feeding method do not so regularly show intestinal lesions. Parrots are susceptible to both the bovine and human tubercle bacilli, by inoculation and feeding ; the effects are similar to those produced by the avian type, except that the bovine type is apparently the most virulent for parrots.

The rabbit and the mouse are the only two mammals in which the avian tubercle bacillus causes progressive tuberculosis.

Rabbits : moderately large doses, by inoculation, produce a fatal issue ; the bacillus is less virulent than bovine tubercle bacillus, but more virulent than human tubercle bacillus. The distribution of the lesions differs most markedly from that set up by the bovine and human types. Intravenously, 1 to 10 mgr. lead to speedy death, with great multiplication of the bacilli in the organs, which show general pallor ; slight œdema of the lungs, slight enlargement (with tubercles) of the spleen. If the animal lives four to five weeks, the spleen is greatly enlarged owing to formation of tubercles, and tubercles are found in the liver and to a less extent in the lungs. In 0·001 mgr. dose, the disease is very chronic, and resembles that produced by subcutaneous injection, the joints being affected. Subcutaneous injection of doses of 50 mgr. down to a fraction of 1 mgr. causes very chronic disease, and the lesions have the same distribution, irrespective of size of dose : local lesion and nearest lymphatic glands ; liver and spleen rarely affected ; kidneys vary ; but most commonly and characteristically there is a tuberculosis of the joints of the limbs, which runs a chronic course. Joint tuberculosis occasionally follows intravenous injection of human tubercle bacillus, but has not been observed after subcutaneous injection of the rabbit with the human virus. It has however been noted after subcutaneous injection of the bovine type, when the animal survives for a long period, i.e., the disease is chronic. By feeding, similar lesions are produced, with local intestinal ones.

Mouse: General tuberculosis by subcutaneous or intraperitoneal injection, and by feeding.

Calf, pig, goat, monkey, guinea-pig, horse, cat, and rat: all behave alike to the avian type. It never produces a progressive tuberculosis, but may kill them in a large dose given intravenously.

Dogs: are immune intravenously.

Chimpanzee: injected subcutaneously with 50 mgr. showed no lesion on death three years after.

Vitality in Culture.—The bacillus was found alive in culture after 1067 days (nearly 3 years).

Chemical Properties.—The investigators were unable to detect any definite and constant bio-chemical character by which tubercle bacilli of one type can be differentiated from those of nother.

BOVINE TUBERCULOSIS.—No further special investigation has been taken beyond that recorded in the second Interim Report, where in 30 cases of the natural disease in bovines, only one form or type of Bacillus tuberculosis was found.

HUMAN TUBERCULOSIS.—In all, 128 cases of all forms of tuberculosis in man were investigated. Twenty of these were of lupus, and are treated separately because the recognition of the type of bacillus was surrounded with special difficulties. The 108 cases remaining are tabulated in full in the Report, and briefly in Table I., page 294.

Of the cases of primary abdominal tuberculosis, the ages at death were as shown in Table II., p. 294. Twenty-four of the deaths were from some form of tuberculosis, the others from non-tuberculous affections. Of 15 of these cases, in which a plurality of lesions were examined on exactly parallel lines, 9 yielded none but human tubercle bacilli. In 12 cases in which a single lesion in each instance was examined (mesenteric glands in 11, cervical gland in 1), 4 yielded human and 8 bovine tubercle bacilli. Two cases yielded mixed human and bovine types, in one from the mesenteric glands alone, and in the other, also from the retroperitoneal glands.

The cervical gland cases show 3 bovine infections out of

TABLE I.—CASES OF HUMAN TUBERCULOSIS OTHER THAN LUPUS

NATURE OF CASES	NO. OF CASES	TYPE OF BACILLUS FOUND		
		Bovine	Human	Mixed
1. Primary pulmonary tuberculosis: (phthisis pulmonalis)				
A. Tissues examined post mortem (lung in 13 and bronchial gland alone in 1)	14	—	14	—
B. Sputum from other cases ..	28	2	26	—
2. General tuberculosis: various tissues	3	—	3	—
3. Tuberculous meningitis: cerebro-spinal fluid in 2	3	—	3	—
4. Bronchial gland tuberculosis ..	5	—	3	2
5. Cervical gland tuberculosis: .. (removed by operation) ..	9	3	6	—
6. Primary abdominal tuberculosis: mesenteric glands and other tissues	29	14	13	2
7. Joint and bone tuberculosis: scrapings and abscesses	14	—	13	1
8. Tuberculosis of testicle (1), kidney (1), and suprarenal (1)	3	—	3	—
Totals	108	19	84	5

NOTES.—The cases in group 1 *A* were all clinically cases of consumption, in which death resulted from the pulmonary disease.

The two patients who had bovine tubercle bacilli in their sputum died subsequently, but no post-mortem examination was obtained. The ages in the sputum cases were: 16 to 25 years, 19 cases; 26 to 33 years, 8 cases; and 50 years, 1 case.

TABLE II.—PRIMARY ABDOMINAL TUBERCULOSIS.

AGE AT DEATH	TYPE OF VIRUS FOUND PRESENT			TOTALS
	Bovine	Human	Mixed	
1 to 3 years ..	10	8	—	18
3 to 5 ,, ...	—	3	—	3
4 to 5 ,, ...	3	—	—	3
7 ,, ...	—	1	—	1
8 ,, ...	1	—	—	1
15 ,, ...	—	1	—	1
18 ,, ...	—	—	1	1
70 ,, ...	—	—	1	1
Totals	14	13	2	29

9 investigated, and these too are ascribed to pharyngeal or buccal infection, that is, alimentary infection. With the primary abdominal cases, this gives 38 cases of tuberculosis of presumably alimentary infection, in which the bovine bacillus alone was found in 17 instances, the human in 19, and a mixed infection in 2.

SUMMARY.

	Cases Examined.	Type of Virus Found.		
		Bovine.	Human.	Mixed.
Avenue of infection :				
(*a*) Respiratory tract (presumably)	47	2	43	2
(*b*) Alimentary tract (presumably)	38	17	19	2
Age :				
(*a*) Adolescents and adults ..	55	3	50	2
(*b*) Children	53	16	34	3

Lupus.—Out of 20 cases investigated, the virus was decided to be bovine in 9 cases, and to be the human type in 11. All but three of the cases presented difficulty in the detection of the type of bacilli. One was undoubtedly bovine by the already decided-on tests, two were likewise human ; but the remaining 17 furnished bacilli which conformed to the one type in some particulars and to the other in other particulars. Thus the other 8 finally called bovine showed the cultural characters of that type, but a lowered virulence for the calf and also for the rabbit, monkey, and guinea-pig. Two had their virulence raised by passage through the calf and rabbit, bringing it up to that of the bovine type. Those ascribed to the human type (11), but not typically so, had lowered virulence for all the test animals or some of them. The general result therefore seems to point to the existence of bacilli outside the types chosen ; these may be new types, or members of the other types with degraded virulence.

Tuberculosis in Swine.—In 59 cases investigated of

tuberculosis of all kinds in swine, the bovine virus was found in 50 cases, the human in 3, the avian in 5, and a mixed avian and bovine virus in 1 case.

In 33 of these cases the tuberculosis was generalized, and in 32 of these the infection was bovine, the other being the one of mixed bovine and avian infection. The other 26 cases showed local tuberculosis.

CONCLUSIONS: All three types of bacilli are capable of infecting the pig, but the bovine bacillus is the one much most frequently found, and in many instances it produces a severe and generalized disease.

Tuberculosis in Horses.—In most cases of tuberculosis in horses it is primarily an affection of the glands and organs in connection with the alimentary tract; the abdominal organs chiefly. This was so in the five cases investigated. In all, bacilli were recovered which were of the bovine type culturally; and three of them also had the bovine virulence. The other two had diminished virulence for the test animals in the test doses; passage experiments raised this to that of the bovine virus.

Tuberculosis in other Animals.—In a gnu which died from generalized tuberculosis in the London Zoological Gardens, the human virus only was found.

In an antelope, killed when ill, in the same Gardens, where it had been many years in captivity, extensive tuberculosis, with cavities in the lungs, was found. The human virus alone was isolated.

In a rhesus monkey, killed when ill at the quarantine station at Isleworth, tuberculosis of the lungs was found, and some elsewhere. Here too only the human virus could be got.

A chimpanzee died of acute miliary tuberculosis at the quarantine station. The tuberculosis started from the alimentary tract, and from a mesenteric gland the human virus was obtained.

A cat, suffering from naturally acquired tuberculosis, was investigated. A mesenteric gland gave a bacillus of the bovine type in growth and virulence.

Tuberculosis in Birds.—In 9 cases investigated (3 fowls, 3 pheasants, 1 pigeon, 1 demoiselle crane, 1 Senegal touracou) of tuberculosis occurring naturally in

birds, in every one the virus found was of the avian type.

In tuberculosis in pigs, the avian virus was found on 6 occasions in the submaxillary lymphatic glands; in 5 alone, and in 1 case in association with the bovine virus.

(*Query.*—Is this due to special exposure of the pig to the farmyard dust, and to its high body temperature?)

Behaviour of the Bacilli and their Fate in the Tissues of Inoculated Animals.—From a number of experiments detailed, the Reporters conclude that after subcutaneous inoculation of human and bovine viruses, rapid and abundant distribution of bacilli over the body takes place, provided the dose is large and the tissue conditions of the animal such as to allow the inoculation to take full effect. This is essentially a mechanical dispersion by the blood and lymph channels of a considerable proportion of injected bacilli, and occurs speedily after their insertion. The resulting acute tuberculosis is at first in strong contrast to the similar but more slowly developing generalized disease induced by smaller experimental doses, or such as occurs most commonly in nature. This more slowly developing form is due to a dispersion of bacilli, but not necessarily those primarily invading the animal; more probably their progeny, which have been able to get through the barriers and into the blood-stream.

Feeding Experiments.—In two pigs fed with large doses of **bovine** virus, the bacilli were demonstrated (by inoculation of guinea-pigs) in the submaxillary and mesenteric glands and lungs in seven and thirteen days after ingestion, but not in the other organs. In seven pigs fed with **human** virus, in from two to twelve days after ingestion the same distribution was proved in two; in the other five the virus was found in the glands alone. In a goat, eight days after ingestion of human bacilli, they were found in the submaxillary glands, mesenteric glands, and lung. In a cat fed with the same bacilli, in nine days they were found in the submaxillary and mesenteric glands, lung, liver, and spleen. Three rhesus monkeys, each fed with 50 mgr. of human tubercle bacilli, were killed; 1 in two days, showed no bacilli in the glands or internal organs; 1 in four days, showed bacilli in the mesenteric glands, liver, spleen, and lungs

and the third in six days, showed bacilli in the submaxillary and mesenteric glands and spleen, the lung condition, owing to the premature death of the guinea-pigs used for the test, not being determined.

Excretion of tubercle bacilli in milk was tested by injection of cultures into healthy cows and goats.

Subcutaneous injection of 100 mgr. of *bovine* culture into a healthy milch cow caused its death thirty days later of general tuberculosis. The udder was normal both to the naked eye and on microscopical examination, yet guinea-pigs fed with the animal's milk, by the end of the first week after injection and subsequently, developed tuberculosis.

Two other cows were injected with *human* tubercle bacilli. One received 100 mgr. subcutaneously; tubercle bacilli were recovered from her milk in twenty-four hours, and the milk in small doses caused tuberculosis in guinea-pigs at every time of testing right up to 155 days later, when the cow was killed and the udder was found normal. The other received 10 mgr. intravenously, and the milk contained the bacilli in twenty-four hours and up to fourteen days later, but not subsequently. The animal was killed in 182 days, and showed no tuberculous lesions.

Six milch goats were similarly tested with bovine and human bacilli, with confirmatory results.

In 12 experiments on heifers, subcutaneous injection of human virus (2), lupus virus (8), and bovine virus (2), in 50 to 100 mgr. doses, the heifers were killed in 62 to 127 days afterwards, and in eight cases tubercle bacilli were found in the sinuses of the undeveloped udder of the animals; in four, in such numbers as to suggest multiplication in these sinuses.

Modification of Bacilli.—Tests by cultural processes and by long-continued passage through animals have failed to effect any change of type from bovine to human, bovine to avian, human to avian, and *vice versa*. Certain difficulties were encountered with bacilli in lupus and in the horse, but while the Reporters think that further investigations may possibly disclose additional variations in the types of bacilli, they do not as a result of their investigations feel disposed to add a plurality of new types to the three already described by them. They are

not prepared to deny that the transmutation of one type into another may occur in nature, in view of the instances in which one and the same human body yielded both types.

Replies to the Terms of Reference.—The questions referred to them for investigation and report were as follows :—

(1) " Whether the disease in animals and in man is one and the same. (2) Whether animals and man can be reciprocally infected with it. (3) Under what conditions, if at all, the transmission of the disease from animals to man takes place, and what are the circumstances favourable or unfavourable to such transmission."

1. Morphologically, as grown on *serum*, the human and bovine types described are indistinguishable, but they are appreciably different in respect of their cultural characters and their capacity for causing disease in various species of animals. The question of the identity or non-identity of these two types clearly depends therefore upon the importance which it is permissible to attach to their cultural and pathogenic differences, and this depends on the fixity or variability of the differences in question. Though in the investigations no case was observed in which the mode of growth of one type was so modified that it was indistinguishable from the mode of growth of the other type, yet the bacilli referred to as the bovine type show so much variety among themselves as to luxuriance of growth, that the gap which separates those of that group which grow most luxuriantly from the human type, is not a wide one.

Again, as regards pathogenicity, it is more a matter of degree than of difference. The bovine tubercle bacillus produces a fatal tuberculosis in cattle, rabbits, guinea-pigs, chimpanzees, monkeys, goats, and pigs. The human tubercle bacillus readily produces a fatal tuberculosis in guinea-pigs, chimpanzees, and monkeys, and in large doses, only slight and non-progressive lesions in cattle, goats, and pigs. Its effects on rabbits are not uniform, for while in the majority of cases these animals are only slightly affected, in some cases extensive and fatal tuberculosis results.

In other words, guinea-pigs, chimpanzees, and monkeys are all highly susceptible to the effects of human or bovine tubercle bacillus; and the diseases produced in these animals by both types are histologically and anatomically identical.

In man, experiment is not permissible, and no opportunity has offered of generalized disease set up by accidental infection with the bovine tubercle bacillus. Nevertheless many cases of fatal tuberculosis caused by the bovine tubercle bacillus and nothing else have been investigated. Compared with parallel cases caused by the human tubercle bacillus, the two groups of cases were alike in their clinical histories and their fatal termination, and were indistinguishable anatomically when the lesions were examined after death. Man must therefore be added to the list of animals notably susceptible to bovine tubercle bacilli.

Are these two types, then, varieties of the same organism? This is the conclusion, in spite of the failure to transmute the one into the other. And, as a corollary, the lesions they produce, whether in man or in other mammals, are manifestations of the same disease. Whatever difference of opinion may be held on this conclusion, in a considerable proportion of cases of human tuberculosis the disease is one and the same as bovine tuberculosis, being caused by bacilli which are in every respect indistinguishable from the bacilli which are the cause of tuberculosis in cattle. In all such cases therefore the disease must unquestionably be pronounced as one and the same.

As regards avian tuberculosis, there does not appear to be sufficient evidence at present to answer the question in the affirmative.

2. The conclusion reached is that, excluding the fowl and other birds, mammals and man can be reciprocally infected with tuberculosis. The transmission to man has been conclusively shown by the study of fatal cases of tuberculosis, mostly in children; and from man to mammals, by feeding experiments.

3. Conclusions:—

(i.) Unmodified avian tubercle bacillus is a negligible factor in the production of human tuberculosis.

(ii.) It cannot be affirmed with confidence that man is

wholly free from risk of infection, through animal food, with that type of bacillus to which he is most prone, namely the **human** type; though the degree of danger to him in this sense must for the present remain undetermined. The pig, though experimentally it fostered human bacilli in a minor degree only, may have to be regarded as **a** possible source of this kind of infection, since particular glands of the pig's body, in which human tubercle bacilli have occasionally been found, are likely to enter into certain prepared foods.

(iii.) (*a*) The pig is a potential source of infection of man with **bovine** tubercle bacilli. This bacillus was present in 50 + 1 cases of tuberculosis in pigs, out of 59 cases of tuberculosis investigated. In 32 + 1 of these cases, it caused generalized tuberculosis, and in 18 cases, local tuberculosis. (The + 1 case was one of mixed bovine and avian infection.) There is no reason to suppose that the bovine tubercle bacilli are rendered less infective to human beings by previous residence in the tissues of the pig.

(*b*) The actual number of cases representing the various clinical manifestations of tuberculosis commonly found in man, on which the conclusions are based, is 128. So far as these have been examples of tuberculosis in adults, and especially when they have been cases of pulmonary tuberculosis, the lesions of the disease, when fatal, have been referable to the human tubercle bacillus, with but few exceptions.

In human abdominal tuberculosis, the experience has been very different, especially as regards children. Of young children, dying of primary abdominal tuberculosis, the fatal lesions could be referred to the bovine tubercle bacillus, and it alone, in nearly one-half of the cases.

In cervical-gland tuberculosis, in children, and often also in adolescents, a large proportion of the cases examined could be referred to the bovine tubercle bacillus.

In lupus, too, in the cases examined occurring in adolescents and children, the amount of infection with the bovine type was marked.

Whatever therefore may be the animal source of infection with the **bovine** type of bacillus in adult and adolescent mankind, there can be no doubt that a consider-

able proportion of the tuberculosis affecting children is of bovine origin, more particularly that which affects primarily the abdominal organs and the cervical glands, and further that primary abdominal tuberculosis, as well as tuberculosis of the cervical glands, is commonly due to ingestion of tuberculous infective material.

In what way are children most likely to obtain a large and fatally infective dose of tubercle bacilli? To this question there can be but one answer, namely, the evidence accumulated goes to demonstrate that a considerable amount of tuberculosis in children is to be ascribed to infection with bacilli of the bovine type, transmitted in meals consisting largely of the milk of the cow.

The child may be subjected to this feeding with infective material, and not develop a fatal tuberculosis; but still be injured, although it recover. Many cases of abdominal tuberculosis in children recover, and the proportion of bovine to human bacilli in these has not been estimated; and of cervical-gland tuberculosis, nearly all make some kind of recovery, with varying degrees of disfigurement; and a similar statement may be applied to lupus.

In adult and adolescent mankind (excluding lupus, in which, out of 10 cases, three yielded bacilli culturally bovine but with less virulence for the calf and rabbit than the bovine tubercle bacillus), fatal lesions due to bovine bacilli have been found, rarely in adolescents, and extremely rarely in adults. Yet, although of 55 cases scrutinized of tuberculosis in adults and adolescents, only 5 yielded bovine bacilli, it cannot be said that this figure adequately represents the proportion of like cases among the tuberculous population generally.

In view of the evidence adduced, the following pronouncements on administrative measures required at present to obtain security against transmission of bovine tubercle bacilli by means of food, are called for:—

In the interests of infants and children, and for the reasonable safeguarding of the public health generally, it is urged that existing regulations and supervision of milk production and meat preparation be not relaxed.

On the contrary, Government should cause to be enforced

throughout the kingdom food regulations, planned to afford better security against the infection of human beings through the medium of articles of diet derived from tuberculous animals.

More particularly it is urged that action in this sense should be taken, in order to avert or minimize the present danger arising from the consumption of infected milk.

Certain facts observed in reference to the elimination of bovine tubercle bacilli by the cow in her milk, are of such importance that they formed the subject of the third Interim Report, and deserve repetition here.

1. Bovine tubercle bacilli are apt to be abundantly present in milk, as sold to the public, when there is tuberculous disease of the udder of the cow from which it has been obtained. This fact is generally recognized, though not adequately guarded against.

2. Bovine tubercle bacilli may also be present in the milk of tuberculous cows presenting no evidence whatever of disease of the udder, even when examined post mortem.

3. In tuberculous cows, the milk leaving the udder may not contain tubercle bacilli, and yet it may and frequently does become infective by contamination with the fæces or uterine discharges of such diseased animal.

Convinced that measures for securing the prevention of the ingestion of living bovine tubercle bacilli with milk would greatly reduce the number of cases of abdominal and cervical gland tuberculosis in children, the Reporters advise that such measures should include the exclusion from the food supply of the milk of the recognizably tuberculous cow, irrespective of the site of the disease, whether in the udder or in the internal organs.

(A memorandum is appended in which reference is made to immunity experiments, on which no opinion is expressed, but which are fully reported in Vol. iii. of Appendix to this Report; and to several other subsidiary experiments.)

CHAPTER XIV.

SPORING BACILLI.

SPORING bacilli are comprised in two groups :—

1. *Aerobic* (facultative anaerobes). Non-motile : anthrax, anthracoides and radicosus. Sluggishly motile : mycoides, ramosus, vulgatus, mesentericus. Actively motile : subtilis, megatherium.

All the above are Gram-positive ; gelatin-liquefying ; non-indol-forming, and non-gas-forming in glucose or lactose ; coagulate milk slowly with little acid and then digest the clot ; digest blood serum.

2. *Anaerobic* (strictly).—Subcutaneous injection into animals causes :—

(i). No particular symptoms at site of inoculation, but absorption of the soluble toxin causing—(*a*) general symptoms of tetanus, B. tetani ; (*b*) botulism, pupillary symptoms, paralysis of tongue and pharynx, cardiac and respiratory failure, B. botulinus.

(ii). Local symptoms marked at the site of inoculation, causing hæmorrhagic emphysematous œdema ; (*a*) motile ; spores oval and central, B. œdematis maligni ; spores oval and excentric, B. anthracis symptomatici ; spore near one end, B. enteritidis sporogenes ; (*b*) non-motile ; B. aerogenes capsulatus of Welch and Nuttall.

SPORE-BEARING AEROBIC BACILLI.

Bacillus Anthracis is the cause of anthrax, a disease primarily of the herbivora, cattle and sheep, but occurring also in horses, pigs, and goats. Man is susceptible, and contracts it either directly from the living or dead animal, or from hides, wool, horse-hair, or dust arising from these. It assumes two forms, external anthrax or malignant pustule, and internal anthrax which in man takes the form of wool-sorter's disease and the form of intestinal anthrax, in which the symptoms are more like those of acute poisoning.

In human anthrax, bacterial invasion of the blood only

occurs late in the disease ; in animals, on the contrary, the blood-invasion is early.

The Algerian sheep and the white rat have a high degree of immunity. Pollender first described the anthrax bacillus as occurring in the blood of animals succumbing to splenic fever. Rayer and Davaine repeated the observation in 1850 (a year later) ; Brauell, in 1857, found the bacilli in the blood of a man affected with anthrax, and Davaine gave everything but absolute proof that they were the exciting cause of anthrax. Koch, by succeeding in getting a pure culture on the aqueous humour of an ox's eye, was able to prove its specificity. He also added largely to the knowledge of its life-history, and particularly to the mode of formation of spores.

Description.—B. anthracis is a straight rod, non-motile, with square or concave ends, 4·5 to 10 micra long by 1 to 1·5 micron thick ; forming chains in cultures, and sporing by oval spores one to each rod ; placed about the centre of the bacillus, and of about the same diameter, and highly refractile. Gram-positive ; gelatin-liquefying, and said at times to possess a capsule when recovered from tissues or blood, or grown on latter.

Cultures.—Grows well on all media, best at 37·5° C., but also from 12° to 45° C.

In broth : a heavy flocculent sediment, slight pellicle, remainder clear.

In gelatin stab : an invreted fir-tree growth, with gradual fluidification.

In gelatin plate : colonies develop within 24 to 48 hours as opaque white pin-head discs, later becoming larger and less regular, and under the microscope showing a hair-like tangle of threads—the so-called Medusa head.

In agar plate : the colonies magnified thirty times show wavy wreaths like locks of hair, the whole colony being probably one long thread. Such colonies are very suitable for making impression preparations, and in such the wreaths are seen to be made up of bundles of long filaments lying parallel with one another, each filament consisting of a chain of bacilli.

On potato : a thick white felted mass, useful for studying porulation.

Spores.—Only produced in the presence of oxygen (free), and hence not formed in blood of infected animals while in the unopened vessels or tissues. For this reason it is advised to cut into an animal dead of anthrax as little as possible, and to be specially careful not to spill the blood. The spores are very resistant, keeping for twenty years. They are killed by dry heat at 140° C. (248° F.) in 3 hours, and live steam at 100° C. in 5 to 10 minutes, or boiling water for 1½ hours. Their behaviour to chemical disinfectants is variable, some strains resisting 1–20 carbolic acid for forty days, while others are destroyed by the same solution in two days. Corrosive sublimate, 1–2000, kills most strains in 40 minutes. Direct sunlight destroys anthrax spores within 6 to 12 hours. Creolin (10 per cent) kills anthrax bacilli in 10 to 20 minutes, but anthrax spores can survive in a 60 per cent solution of creolin. Freezing has little effect on their vitality. Spores are formed best at 30° C., and by keeping the bacilli at 42° C. for eight days, the power of sporulation is lost, and is only regained by passing the bacilli through a series of animals.

Anthrax spores are often used for testing the value of "germicides." To do this, sterile silk threads are steeped in an emulsion of an anthrax culture and are dried over strong sulphuric acid in a desiccator. They are then placed in a solution of the "germicide" for a certain time, well washed with water, and laid on the surface of agar medium or dropped into broth, and incubated to see if any growth occurs. The culture used is first tested for spore formation.

Pathogenicity.—For man: great. For animals: mainly for cattle and sheep. In the German Empire in 1899, the following cases were reported: 3678 cattle, 307 sheep, 282 horses, 61 swine, and 6 goats.

In Great Britain, in the ten years 1896 to 1905, the total reported "outbreaks" in animals were 6203, and the number is increasing. In man, 512 cases were reported in 1901 to 1910, and of these 120 were fatal. Internal anthrax is usually fatal. In the external form, head and neck cases show a mortality of 85 per cent; and hand and arm cases 12 per cent.

Rabbits, guinea-pigs, and white mice are all very susceptible, the mice most so. Rats resistant, especially the white rat; dogs more so. Birds are highly immune, also amphibians (but toads are said to be very susceptible).

Toxins.—No toxins have yet been isolated, though it is highly probable that both extra- and intra-cellular toxins exist.

Vaccination.—In France, a death-rate from anthrax of 10 per cent among sheep and 5 per cent among cattle compelled attention to the problem of providing protection. Pasteur, in 1881, introduced his method by the use of two vaccines: (1) A broth culture of bacilli, whose virulence was reduced by being incubated at 42° C. for twenty-four days, and so made non-fatal to guinea-pigs but still fatal to white mice—premier vaccin; (2) A broth culture, incubated as above for twelve days, which would kill guinea-pigs but not rabbits—deuxième vaccin.

A sheep was inoculated in the subcutaneous tissues on the inner side of the thigh with 5 drops of the premier vaccin. Twelve days later a similar inoculation of the deuxième vaccin was given, and fourteen days later still an injection of an ordinary virulent culture produced no ill result. The method has given excellent results, and the immunity lasts about a year.

Passive Immunization.—Sclavo produced a serum from highly immunized asses, which has strongly protective and curative properties, and is used in the treatment of anthrax in man. In malignant pustule, four doses of 10 c.c. are injected into the abdominal wall, and if necessary repeated on the following day. Sclavo does not advise excision of the pustule. Sobernheim uses serum from sheep.

Isolation of B. anthracis from hairs, etc.: Add 5 grm. to broth and shake. Incubate: not a pure culture. Heat to 80° C. for 30 minutes; all non-sporing organisms killed. Take twenty samples of 1 c.c. on agar and grow. Infect animals and see if pathogenic. Plate again on second day.

Diagnosis. 1. In a case suspected to be malignant pustule, diagnose by (1) Making films from the fluid in the vesicles or from scrapings, and staining with watery methylene-blue, and also by Gram (be careful in scraping a pustule

before excision, not to manipulate it roughly, or bacteria may enter the circulation); (2) Making cultures from similar material, by successive strokes on agar tubes or plates; (3) Inoculation of the cultures into a guinea-pig or mouse, subcutaneously. If anthrax bacilli are present the animal usually dies within two days, and post mortem the tissues around the site of inoculation show intense inflammatory œdema, swelling, and gelatinous change, with small hæmorrhages. On microscopic examination, numerous bacilli are seen. The internal organs show congestion and cloudy swelling, and sometimes small hæmorrhages, and their capillaries contain enormous numbers of bacilli, so that they appear as if injected with them. The spleen is notably enlarged (especially in the ox dead of anthrax, being two to three times its natural size, hence the name "splenic fever"), is of a dark-red colour, and on section is soft and friable, at times almost diffluent. Films from the pulp contain enormous numbers of bacilli mixed with red cells and leucocytes of the lymphocyte and large mononuclear varieties. The lymphatic system is generally much affected, the glands and vessels being swollen and containing bacilli in very great numbers. The intestines are enormously congested, the epithelium is more or less desquamated, and the lumen filled with a bloody fluid. (Muir and Ritchie.)

2. *Methylene-blue Reaction.*—Depends on the disintegration of the capsules of the bacilli, which occurs when these are imperfectly fixed. It serves for the easy recognition of anthrax bacilli in blood and other bodily fluids, where putrefactive and other bacilli are present. Dry a loopful of blood on a slide; hold it for one second in the flame; repeat three times. Stain for a few seconds in old solution of methylene-blue, wash in water, and dry. Examine dry and without a cover-glass, when between and near the bacteria, violet or reddish-purple tinted granular or amorphous matter is seen. (M'Fadyean's test.)

Capsules can be demonstrated in smear preparations from organs, by staining in 2 per cent watery solution of methylene-violet (heating). Wash in water for 2 seconds. Wash in 1 per cent acetic for 6 to 10 seconds. Wash in

water and *examine in water-drop*. Another method is to stain (without fixing film) in a cold saturated solution of gentian-violet in formalin. Examine in water-drop.

Prevention of Anthrax.—The Home Office Order No. 1293, dated Dec. 12th, 1905, on this subject, is made under Section 79 of the Factory and Workshop Act, 1901. It provides for the prevention of dust from wool or hair by ordering the opening and sorting to be done only (1) after steeping in water; or (2) over an efficient opening screen, with mechanical exhaust draught, in a room set apart for the purpose and in which no other work than opening is carried on. Mohair, other than Van mohair, only needs to be sorted over a down draught. Van mohair, Persian locks, and Persian, must all be steeped before being opened. Alpaca, pelitan, East Indian cashmere, Russian camelhair, Pekin camelhair and Persian [or so-called Persian, if to be sorted or willowed] must be steeped before opening, or opened over efficient opening screen. It also provides for the use of overalls and respirators, cubic space per person (1000 c. ft.), temperature of room (not lower than 50° F.). Treatment of cuts and sores, washing of hands, burning of dust and other refuse, rules for treatment of damaged hair, proper kind of screen and board, lime-washing of rooms yearly, daily disinfection of floors and sweeping thereafter, etc., etc.

Anthrax orders are issued by the Board of Agriculture under the Diseases of Animals Act, 1894, and provide for the precautions to be used in treatment of a sick animal, and (1) the burial at a depth of not less than 6 ft., with 1 ft. of lime beneath and above the carcase of an animal dead of anthrax, or (2) the destruction of a dead animal by heat or chemical agents. The local authority must carry out the provisions of the order in these and other particulars.

Bacteria closely resembling B. Anthracis:—

1. **B. Anthracoides.** A Gram-positive bacillus; ends more rounded than those of B. anthracis; growth more rapid; gelatin liquefaction more rapid; non-pathogenic. Otherwise indistinguishable from B. anthracis.

2. **B. Radicosus.** Cultivated from water-supplies.

Larger, and more variation in size of individuals; grows best at room temperature; non-pathogenic.

3. **B. Subtilis.** The common bacillus of hay infusion, and found as a saprophyte in old wounds and infected sinuses; gives a heavy tenacious pellicle in broth; spores germinate equatorially; gelatin and casein are liquefied more rapidly than by B. anthracis; is actively motile in young cultures; non-pathogenic. Pathogenicity for man is now alleged, it having been isolated from a case of panophthalmitis in pure culture.

4. **B. Mycoides** (earth bacillus). Is obtained from the surface of the earth of cultivated fields or gardens. Grows best at 18° C., and on gelatin shows a mould-like growth. About the size of B. anthracis, which it resembles greatly. Grows in threads; motile; non-pathogenic.

5. **B. Megatherium.** 10 micra × 2·5 micra. First found on boiled cabbage leaves.

6. **B. Vulgatus** (potato bacillus). Grows rapidly on potatoes, showing marked wrinkling. Small thick rods with rounded ends, in pairs or fours.

SPORE-BEARING ANAEROBIC BACILLI.

Methods of Anaerobic Culture.

1. Liquid media: Boil vigorously for 15 minutes, to drive out dissolved oxygen; cool, and put layer of sterile oil on surface. (Pasteur.)

2. Piece of sterile mica on surface of agar or gelatin plates. (Koch.)

3. Deep inoculation in solid media, recently boiled for 15 minutes and cooled rapidly in ice, to prevent absorption of oxygen. It is advantageous to have 1 per cent of glucose in medium. The tubes are inoculated by deep stabs, and the top of the medium is covered with a thin layer of agar, gelatin, or oil, and the tube capped with rubber or sealed with sealing-wax. (Liborius.)

4. The solid medium may be inoculated before it solidifies,—a "shake culture"—as in the original method of Liborius. The colonies which develop are fished out after breaking the tube.

5. Tube fitted with two-holed cork and two tubes.

Pass in H or N until all the air is displaced, and then seal ends of tubes in flame.

6. *Buchner's Tube.*—A wide tube with a constriction near the closed end, so that an ordinary culture tube can be inserted and is held up by the constriction. In use, 1 grm. of solid pyrogallic acid and 20 c.c. of 10 per cent KOH are put into the bulb, the previously inoculated tube is inserted loosely plugged, and the Buchner tube is plugged and sealed with melted paraffin or closed with a rubber cap. The solution in bulb absorbs oxygen. The method can be used without the special tube, with a tall glass jar or a desiccator. It is better to add the alkali by pipette after the inoculated tube has been inserted.

7. *Bulloch's Method.*—A bell jar with an inlet and an exit tube, both having stopcocks. The bell jar is firmly bound down to a glass plate by ung. resinæ. Before fixing, a glass dish is set on the plate, and 3 to 4 grm. of pyrogallic acid are heaped to one side of it. Culture plates or tubes in a beaker are rested on a tripod stand placed in the dish. The bell jar is now fixed on so that the inlet tube will end in the dish at the side away from the pyrogallol. Hydrogen is passed in, and then a solution of KOH (109 grm. in 145 c.c.).

8. *Vacuum Method.*—Desiccator with stopcock. Exhaust air by burning alcohol on soaked filter-paper put into jar. Stopcock is needed to release pressure when opening.

B. Tetani.

The cause of tetanus or lockjaw, a disease characterized by the gradual onset of general stiffness and spasms of the voluntary muscles, beginning in the jaw muscles and those of the back of the neck. The disease is usually associated with a wound received four to fourteen days previously and infected with earth or dung. The majority of cases are fatal.

Kitasato first isolated the bacillus (in 1889) and in this fashion :—Pus from the local suppuration of mice inoculated from a human case, was smeared upon the surface of agar slants. These were permitted to develop at 37° C. for 24 to 48 hours. At the end of this time the cultures were subjected to a temperature of 80° C. for 1 hour. This destroyed all non-sporulating bacteria as

well as aerobic spore-bearers which had developed into the vegetative form. Agar plates were then inoculated from the slants and incubated in a hydrogen atmosphere, and on these tetanus bacilli grew. Rosenbach had previously pointed out the terminal spore formation of B. tetani, and Nicolaier had described the bacillus, but could not grow it in pure culture.

Description.—A slender bacillus, 2 to 5 micra long × 0·3 to 0·8 micron thick, with somewhat rounded ends. In young cultures it is slightly motile, and by special staining numerous peritrichal flagella are seen. In 24 to 48 hours the bacilli develop spores which are at full size three to four times the thickness of the bacillus in diameter, and are formed at one end of the bacillus. The bacillus and spore thus give the characteristic drumstick appearance. It is easily stained with the usual dyes, and is positive to Gram's method. Detached flagella often become massed together in the form of spirals, not unlike spirochætes. In specimens stained with watery solutions of gentian-violet or methylene-blue, the bacillary protoplasm stains uniformly, but any spores are unstained except at the periphery, and so look like rings. With carbol-fuchsin and time, the spores become uniformly coloured. Spores may be found free from the bacilli in which they were formed. The bacillus liquefies gelatin slowly, also blood serum, but does not coagulate milk. It is a strict anaerobe (obligatory), but can be habituated to aerobic life, though with loss of pathogenicity and toxin-forming power. Grows moderately in aerobic conditions where other organisms which use up the oxygen supply are present (symbiosis). Its growth is aided in all media by slight alkalinity, presence of glucose, maltose, or sodium formate (1 to 2 per cent), which act as reducing agents. With carbohydrates it produces acid. In gelatin and agar, moderate amounts of gas are produced, chiefly CO_2; but other substances which are volatile and cause a characteristically unpleasant odour are formed. This is described as a peculiar burnt odour, and as that of putrefying organic matter, and is said to be largely due to H_2S and $CH_3.SH$ (methyl mercaptan).

Cultures.—In deep glucose gelatin stab: growth begins

one inch or so below the surface, in fine straight threads, radiating from the needle track. Slow liquefaction, with slight gas formation, takes place.

In agar stab (glucose agar): the growth is somewhat similar.

In glucose broth: slight clouding, with later a thin powdery deposit on the walls of the tube. Ordinary broth is preferred for toxin production.

On blood serum: growth with liquefaction takes place.

In milk: acid is formed but no clot.

On potato: growth is delicate.

On agar plates: colonies show a compact centre, with loose feathery outline not unlike B. subtilis or anthrax.

Spores—Resist dry heat at 80° C. for 1 hour; live steam for 5 minutes; 5 per cent acid carbolic for 12 to 15 hours; 1 per cent corrosive sublimate for 2 to 3 hours. Direct sunlight diminishes their virulence and ultimately destroys them, otherwise they may remain virulent for years (in one case, 11 years). Are best formed at 37° C., but also form at 20° C. in 8 to 10 days.

Habitat.—Soil, street dust, horse-dung.

Pathogenesis.—In man: mostly from punctured wounds. The bacillus remains at the local site, but the toxins are carried to the nerve cells of the motor horns of the spinal cord, and of the motor ganglia of the brain. The manner of transmission is believed to be by absorption through the end-plates of the motor nerves in the muscles, and thence via the axis-cylinder processes to the respective nerve cell. The toxins have been shown to have no effect on the motor or sensory endings of the nerves, but solely as an exciter of the nerve cells concerned in reflex action in the cord, pons, and medulla. The affinity of the toxins for the nervous system varies in different animals; in the guinea-pig it is its chief affinity, whereas in the alligator it shows no affinity, and intermediate degrees exist. Section of a nerve, e.g., the sciatic nerve, followed by injection of the toxins into the muscles supplied by that nerve, prevents the toxins reaching the spinal cord; but if the nerve below the section be cut out and introduced into a mouse, the animal will die of tetanus. Similarly, infection of one side of the cord passes, when the dose is

large, to the other side via the commissure, and up the cord to the higher centres. The latter extension can be prevented by section of the cord. Lately, in India, the relation between subcutaneous or intramuscular injections of quinine (given for malaria) and the production of tetanus has been worked out. Such quinine injections are apt to have a destructive action on the tissues, and the foci of dead tissue produced serve as suitable anaerobic media for the growth of tetanus spores. The latter are believed to reach these foci by absorption from the bowel. This explanation will also probably serve for the Mulkowal outbreak (1902), when 19 persons developed tetanus (out of 107 injected) after inoculations of Haffkine's plague prophylactic. In India, tetanus spores seem to be present in the bowel in a considerable proportion of the natives.

Toxins.—Broth cultures grown anaerobically are usually highly toxic to animals, 0·000005 c.c. ($\frac{1}{200000}$) or less being fatal to a mouse of 10 grm. weight. Fatal dose for a man is given as 0·23 mgr., equal to 0·003 of a grain, or $\frac{1}{280}$. The maximum yield is given in 10- to 14-day-old cultures. After this it rapidly deteriorates. This also happens after separation from the bacilli by filtration, and in a few days it may have only $\frac{1}{100}$ of its original power. Von Behring, who first noted this change, attributed it to the action of light, temperature, and especially oxygen, on the toxins; and so such filtrates should be kept covered with a layer of toluol and in a dark cool place. Exposure for a few minutes to 65° C. destroys it, as do 20 min. at 60° C. and 1·5 hour at 55° C. Drying has no effect apart from temperature. It can be precipitated by over-saturation of the solution with ammonium sulphate, and thoroughly dried and stocked in vacuum tubes, together with anhydrous phosphoric acid, it may be preserved indefinitely without deterioration. The ordinary effects of the toxin are attributed to " tetanospasmin." Besides this, a substance named " tetanolysin " which has the power of destroying the red corpuscles of various animals, was discovered by Ehrlich. Tetanus toxin can be fully neutralized by mixing it with brain substance.

Tetanus toxin is peculiar in that, after introduction into an animal's body, a definite incubation period occurs

before symptoms arise. In the guinea-pig this is thirteen to eighteen hours, and in the horse five days. It is shorter after intravenous injection, probably through getting more quickly to the nerve centres. Crocodiles are resistant to tetanus toxin.

Immunity. — Produced by injection of filtered toxin in increasing doses. At Elstree, the serum-producing department of the Lister Institute, London, the horse is immunized by the injection of 0·5 c.c. filtered toxin + 0·5 c.c. of Lugol's solution of iodine (1–300), repeated at intervals of ten days, gradually increasing the dose until 10 c.c. of unreduced toxin are given. The iodine solution neutralizes the toxin to some extent. The serum of such an immunized animal is antitoxic; but the effect of its injection into an infected animal is not so good as is the case with diphtheria antitoxin, because the tetanus is mainly bound to the nervous tissue and is thus less susceptible to the action of the antitoxin. Von Behring believes that there is no hope of its being useful after symptoms have existed for 30 hours, but MacConkey and Green say that if much larger doses were used better results would be got in the human subject, comparable with those reported in horses. The serum is standardized so that 1 grm. will protect 100,000,000 grm. weight of mouse (v. Behring), or 1,000,000,000 grm. weight (Pasteur Institute). Of this 100 c.c. are advised to be injected subcutaneously, and in the case of the first, repeated. The largest dose that can be comfortably given at one spot is 20 c.c. Intravenous injection is said to give better results than subcutaneous injection. The serum is warmed to the body temperature and slowly introduced into an arm vein, 10 to 20 c.c. every few hours. Intracerebral injection has also been practised, but with no better results. Prophylactic doses (10 c.c.) are advised as a routine practice in ragged, bruised, and punctured wounds, especially if soiled with material likely to contain tetanus spores. The dose is given without unnecessary delay. In U.S.A., in 1903, out of 4449 Fourth of July accidents, 406 were followed by death from tetanus, while in 1907, only 62 tetanus deaths arose from 4413 accidents, and much of the decrease is attributed to the early use of a

prophylactic dose of antitoxic serum. In veterinary practice, prophylaxis has been used with great success.

Search for B. Tetani in a Suspicious Wound.—

(*a*) Microscopic examination of films for drumstick shapes.

(*b*) Cultivation in deep stabs in glucose media for forty-eight hours.

(*c*) Inoculation into mice and guinea-pigs.

A loopful of the discharge into the root of the tail of a mouse will soon give rise to characteristic symptoms if B. tetani be present. From cultures Kitasato uses splinters of wood dipped in same, then heated to 80° C. for 1 hour, which kills all non-sporing organisms and destroys any toxin developed. A splinter is introduced subcutaneously, and if death results it is from the spores which it carries.

Bacillus Botulinus.—Found in "meat-poisoning" by raw ham by van Ermengem in 1896. The symptoms of the illness resembled those following sausage (botulus) poisoning, frequently met with in Germany from the ingestion of raw sausage. The symptoms follow at the earliest in twelve to twenty-four hours after the eating of the food. They are due to the action of a soluble toxin on the medullary centres, causing dysphagia, salivation, dilated pupils, and respiratory and cardiac distress. Fever is usually absent and consciousness is retained.

B. botulinus is a large bacillus 4 to 9 micra long × 0·9 to 1·2 micron thick, with rounded ends. It is slightly motile, and has four to eight peritrichal flagella. It forms oval spores at one end, rather thicker than the bacilli, and these show slight resistance (1 hour at 80° C.). Strict anaerobe; liquefies gelatin; Gram-positive.

Cultures.—Characteristic growth on glucose-gelatin plates: round, yellowish, transparent colonies, composed of coarse granules which (under a low power) show a streaming movement, especially at the periphery. Forms gas in glucose, but not in lactose nor sucrose. Milk is not coagulated. All cultures have a sour odour. The toxin is closely related in its action to the toxins of diphtheria and tetanus. The bacilli do not seem to multiply in the body, but the toxin is absorbed from the alimentary canal and produces the symptoms. The infected ham or sausage

shows the bacilli in large numbers between the fibres. Thorough cooking destroys the toxin (not so in Gaertner meat-poisoning). The meat may be without any signs of ordinary decomposition. An antitoxin has been produced by Kempner, who also cultivated the bacillus from the intestine of the pig. Botulism is a dangerous affection, ending fatally in 25 per cent of those attacked.

Bacillus of Malignant Œdema.—Discovered by Pasteur in 1877, in guinea-pigs inoculated with putrefying animal tissues. Gaffky found it in the upper layers of the soil of gardens and in dust. It is widely distributed in nature, and has been found in the intestine of animals and man. Its spores are very resistant, and are placed in the centre or near it. They are oval-shaped and slightly bulge the bacterial body. Spore formation occurs above 20° C. and is usually well seen in forty-eight hours at 37° C. The guinea-pig, rabbit, sheep, and goat are susceptible to inoculation; the ox is immune to experimental infection, but has contracted the disease by natural channels. The bacillus is long (4 to 9 micra) and rather thinner than the anthrax bacillus, being 0·9 to 1·2 micron thick. The bacilli have somewhat rounded ends, and at times form threads. They are motile, have numerous peritrichal flagella, and are strict anaerobes. They stain readily by the usual aniline dyes; they are Gram-negative.

Cultures.—They grow best in the presence of glucose, and produce a heavy, putrid odour. They liquefy gelatin, and in deep stab show bubbles of gas around the colonies. They grow rapidly in deep stab in glucose agar, and here also gas forms and usually splits the medium. In broth, there is general clouding but no pellicle; a granular sediment forms. In milk, slow coagulation is produced. On blood serum, growth is luxuriant. On potato, growth readily occurs.

Inoculation of a guinea-pig (subcutaneously) produces death in twenty-four to forty-eight hours. There is an intense inflammatory œdema around the site of puncture and injection, which gradually extends to the surrounding tissues. The skin and subcutaneous tissues are infiltrated with a reddish-brown fluid, are softened, contain bubbles of gas, and are in places gangrenous. The superficial

muscles are also involved, and have a putrid odour. The internal organs are congested and show parenchymatous degeneration. The spleen is soft, but not much enlarged. Immediately after death bacilli are not found in the blood or internal organs; but thereafter the bacilli rapidly spread into the blood and organs. This account applies to the mixed infections (garden soil); in pure infection, little gas and odour are formed.

Toxins.—A small amount of soluble toxin is formed, and filtrates of cultures in fluid media produce the same symptoms (if used in sufficient quantity) as the bacilli themselves. Chamberland and Roux, in 1887, produced immunity in guinea-pigs by the injection of the toxin, obtained by filtration or by sterilization of cultures by heat, or by filtration from the serum of animals dead of the disease.

Pasteur called the disease "septicémie," but it is not a true septicæmia like anthrax, in which the bacilli invade the blood and organs. It is a rare disease, occurring in man after traumatism.

Quarter-Evil.—A disease of cattle, sheep, and goats, called by the Germans, "Rauschbrand," and by the French, "charbon symptomatique." It has never been observed in man. Infection takes place by some wound of the surface, and occasions inflammatory swelling with bloody œdema and emphysema of the tissues; the affected part becomes greatly swollen, and of a dark, almost black colour. The bacillus is found in the inflamed tissues and in small numbers in the blood of internal organs. It closely resembles the B. œdematis maligni, but is somewhat thicker and does not form such long threads (filaments). The spores also are more bulging and nearer the end of the bacilli. An acute disease of sheep in Northern Europe, called "braxy," is associated with the presence of a very similar, if not identical anaerobe. Active and passive immunization of sheep and goats and cattle are practised.

B. Enteritidis Sporogenes was first isolated by Klein in 1895 from diarrhœal stools. It was afterwards found in infantile diarrhœa and summer diarrhœa, and as a constant inhabitant of sewage. It is slightly motile, with a tuft of flagella at one pole (lophotrichal), 1·6 to 4·8 micra long by 0·8 micron thick; easily stained; Gram-positive; gelatin-

liquefying, and produces acid and gas in bile-salt glucose media and in peptone water + glucose or mannite. It forms a spore nearer one end. Its growth in milk is highly characteristic, and this medium is commonly used for its isolation.

METHOD.—A small quantity of the suspected material is inoculated into sterile milk (" whole milk "), using at least 15 c.c. of the medium. Heat for 10 minutes at 80° C. to destroy all non-sporing forms, cool the tube, and incubate anaerobically for twenty-four to thirty-six hours. If the casein is precipitated and torn into irregular masses, with a moderately clear whey and abundant gas formation, the result is positive, but it is desirable to verify by animal inoculation. (In the examination of water and milk, the result is observed after two days' incubation.) The culture has a smell of butyric acid, and numerous bacilli are found in the whey. If 1 c.c. of the whey be injected into a guinea-pig, the animal becomes ill in a few hours, and dies in twenty-four hours. At the point of inoculation the skin, subcutaneous tissues, and sometimes the adjacent muscles, are green, gangrenous, ill-smelling, and œdematous ; there may be gas formation. This pathogenic test serves to distinguish the B. enteritidis sporogenes from the B. butyricus of Botkin, which otherwise closely resembles it.

B. Aerogenes Capsulatus.—First observed by Welch in 1891, and obtained from the intravascular blood in a case of ruptured aortic aneurysm. The post-mortem took place six hours after death, and attention was called to the blood by the presence of gas-bubbles throughout the vessels. It was fully described by Welch and Nuttall in 1892, and in 1893 Fraenkel independently described (under the name of B. phlegmonis emphysematosæ) a bacillus, now considered to be identical with the B. aerogenes capsulatus. Klein's B. enteritidis sporogenes is believed by some to be the same organism, or a closely related one. B. aerogenes capsulatus is widely distributed in nature, being found in soil, dust, brackish water, and in the normal intestinal tract of man and animals. In size it is not unlike anthrax bacillus, but is more variable in length and somewhat thicker. The bacilli are generally

single or in short chains, and are shorter and thicker in cultures. Chain formation seems to occur in the blood chiefly, and never in artificial culture. Welch regards this as an important distinction from anthrax bacilli. When recovered from the body fluids it possesses a capsule. Each bacillus forms one spore, which may be central or excentric. Anaerobic; non-motile; non-flagellar; Gram-positive; gelatin-liquefying (in most), and forming acid and gas in glucose, lactose, and saccharose, but not in mannite. In milk the reaction is similar to that described under B. enteritidis sporogenes. It is highly pathogenic to guinea-pigs but not to rabbits.

Isolation.—Make a suspension of the suspected material (fæces, etc.) in sterile salt solution (1 c.c. in 5 c.c.). Thoroughly emulsify and filter through a sterile paper, and inject 1 to 2 c.c. of the filtered suspension into the ear vein of a rabbit. After 5 minutes, kill the rabbit and place its dead body in the incubator (37° C.) for 5 or 6 hours. At the end of this time the animal is usually found tensely distended with gas, and post mortem gas bubbles will be found throughout the body, most characteristically in the liver, where isolated bubbles are found on the surface. From the bubbles, smears and cultures may be taken. Identification is made from its morphology, capsule, non-motility, and gas formation. In man, infection usually follows traumatism. Distinction from B. enteritidis sporogenes: non-motility, non-flagellar, not fermenting mannite. Muir and Ritchie state that it is non-gelatin-liquefying and non-pathogenic to guinea-pigs, but American authors describe it as above.

SUMMARY.

Bacillus	Motility	Flagella	Gram.	Gelatin-liquefying	Spore
Tetani	+	peri-trichal	+	+	end (drumstick)
Botulinus	+	,,	+	+	near end
Malignant œdema	+	,,	—	+	central
Quarter-evil ..	+	,,	—	+	near end—racket shape
Enteritidis sporogenes	+	lopho-trichal	+	+	central or near end
Aerogenes capsulatus	—	—	+	+	,, ,,

CHAPTER XV.

SPIRILLA.

SPIRILLUM CHOLERÆ ASIATICÆ.

THE cholera spirillum was discovered by Koch in 1883 in the defæcations of sufferers from cholera. It is also called the " comma bacillus " and the " Vibrio choleræ."

Description.—Short, slightly curved rods, 1·5 to 2 micra long by 0·5 micron thick. *Ex.* (In pairs, may form an S-shape, thus ʃ. Actively motile, and swim like fish in lines, thus ≡ ≡ ≡ ≡. Possess one flagellum, situated at one end (monotrichal). Non-sporing; not phosphorescent; Gram-negative. Markedly aerobic, but can grow anaerobically. Optimum temperature 37° C.; growth usually ceases at 16° C. Gelatin-liquefying. Give nitroso-indol reaction with sulphuric acid within twenty-four hours.

Culture.—Grows readily on all usual media, but better if alkaline, and except on potato even at room temperature; characteristic on gelatin plates and in broth. On gelatin plate: minute whitish points in twenty-four to forty-eight hours; surface when magnified is coarsely granular and furrowed. Liquefaction follows and the colony sinks, showing a ring around. In gelatin stab: liquefaction begins at the surface, with gradual formation of a funnel of liquefaction. In broth: rapid clouding occurs with wrinkled pellicle on top, composed of spirilla in a very actively motile condition. In milk: growth but no visible change. In peptone water: rapid growth with production of indol, and reduction of nitrate to nitrite, hence a few drops of pure sulphuric acid will give a red colour—the so-called *cholera-red reaction.* Also given in broth culture, and in both in twenty-four hours, owing to rapid growth. Not absolutely specific, as it is given also by Sp. Metchnikovi. Blood serum is rapidly liquefied. In sugar media: no gas is formed, but acid with glucose. Does not produce hæmolysis, though very similar species do. Does not multiply in water.

Staining.—Readily with usual stains, best with Loeffler's methylene-blue and weak carbol-fuchsin. Loses stain by Gram's method.

Resistance.—Not great. Killed in ten minutes at 60° C.; on drying, in two hours. Mineral acids, 1 in 5000 to 1 in 10,000, destroy it in a few minutes. The gastric juice contains 2 parts of HCl per 1000; hence it is killed by gastric juice, but can flourish in intestine. Freezing kills it in three to four days.

Agglutination—Is shown by serum of cholera convalescents in dilutions of 1–15 to 1–120. Present eight to ten days after attack, most marked twenty-eight days after, and gradually diminishes. Has been noted as early as first day of disease.

Pathogenesis.—For man: has been established by laboratory accidents. Does not invade blood; immense numbers in stools; in rice-water stools, loosened epithelial cells loaded with vibrios. Not in urine. Infection by mouth. Disappears from stools in two to three days. Cholera carriers: healthy persons whose fæces contain virulent cholera spirilla. Mostly spread by water, fomites, fingers, flies. For animals: not established, though vibrios on teats of suckling mother have infected young, with choleraic symptoms. But as this result or similar ones have been given by other vibrios, specificity cannot be founded on this test. Intraperitoneal injection in guinea-pigs is followed by general symptoms, with abdominal distension, subnormal temperature, and profound collapse. There is peritoneal effusion which may be almost clear, or with flakes of lymph. There is little tendency to invade the blood-stream, and the symptoms are mainly due to an intoxication. It is not pathogenic to pigeons.

Toxins.—Filtered cholera cultures have as a rule little toxic action; hence it is inferred that little soluble toxin is formed, but mostly endotoxin. Results at present are conflicting.

Pfeiffer's Reaction.—If cholera spirilla are injected into the peritoneum of an immunized guinea-pig, they first lose their motility, then swell up and crumble into fragments, which finally melt away and disappear. This lysis is also manifested in a test tube in a mixture of serum

plus vibrios. Or, inject a mixture of one loopful (2 mgr. of recent agar culture and 1 c.c. of broth containing 0·001 c.c. of anti-cholera serum into the peritoneum of a guinea-pig. Remove some fluid at regular intervals and examine. If above reaction is got, then said to be positive, and in case described, organism is proved to be true cholera spirillum. If the latter is used against an unknown serum, then the anti-power of the serum can be determined and, if positive, by using various dilutions, the bacteriolytic power.

Immunization.—A guinea-pig is easily immunized by repeated injections of non-fatal doses of dead spirilla; later small doses of living organisms may be used. A high degree of immunity is thus developed, and the blood serum of such an animal (anti-cholera serum) when injected into another guinea-pig has marked protective power. This is not due to any antitoxic substances, but to antibacterial power. Cholera-immune serum is thus bacteriolytic, not antitoxic. This power is specific, and does not apply to other closely related organisms.

Haffkine's Vaccine.—First vaccine: Attenuated virus by long cultivation at 39° C. or by other methods. Second vaccine: Given five days later, of virulent virus (by passage through guinea-pigs). Both given subcutaneously. Lately has given only one, and that the "virus exalté." General conclusions as to efficacy: (1) Protective effect of anti-cholera vaccine commences soon after operation and increases rapidly for first four days, and lasts fourteen months, after which it diminishes and completely disappears. Larger doses cause longer effects. (2) During period of its activity, the number of cases among vaccinated is one-tenth of number among others. (3) Mortality among those attacked differs but little, and the course of the disease is not affected by the previous inoculation.

Isolation.—

1. *From Fæces.*—Make pre-culture in peptone water. (*a*) Inoculate peptone water in Erlenmeyer flasks (1 loopful in each). (*b*) In eight hours, if a film appears or not, make hanging drop from surface, and if you get fish trains, dry and stain to see if form is typical. (*c*) Subculture from film on gelatin plates, and smear over agar plates.

(*d*) After eight to ten hours examine any colonies, and if pure cultures, plant out as follows: (i) Peptone and salt solution: in twenty-four hours at 37° C., turbid, and gives cholera-red; (ii) Gelatin plates: characteristic colonies with irregular margins; (iii) Gelatin stab: typical funnel-shaped liquefaction; (iv) Agar slope: growth in twenty-four hours at 37° C. must give with anti-cholera serum, agglutination and Pfeiffer's test; (v) A portion of colony should be examined for typical microscopical appearances.

Make films and hanging drops direct from stools. Dunbar diagnoses from two hanging drops, one having added to it an equal quantity of 1–50 normal serum, the other an equal quantity of 1–500 anti-cholera serum. Cholera organisms retain their motility in the first instance, but lose it and agglutinate in the second. The hanging drop is mounted from peptone water in which a piece of mucus has been broken up.

2. *From Water.*—Keep 10 per cent peptone water sterilized. Take 900 c.c. of suspected water, and add 100 c.c. of strong peptone water. Divide into ten flasks, each containing 100 c.c. Incubate at 37° C. In eight to twelve hours make a film and hanging-drop preparation from the surface of each flask. From those flasks showing most similar forms, make subcultures, proceeding as above. If a spirillum conforms to all the above tests, it is probably the true cholera vibrio, but it must be remembered that a certain number of spirilla, although agglutinating to some extent with cholera serum, are sharply differentiated by being multiciliated and hæmolytic.

SPIRILLA OTHER THAN THE CHOLERA SPIRILLUM.

(Often present in water but not necessarily pathogenic).

Sp. Metchnikovi—Is found in a disease resembling fowl cholera in the fæces and blood. It is practically identical with the Sp. choleræ, having a single polar flagellum. Culturally, it fluidifies gelatin twice as rapidly, and grows slightly more luxuriantly. It is sharply distinguished, however, by being very pathogenic to pigeons (doves),

whereas Sp. choleræ is scarcely so. It is negative to Pfeiffer's test, but gives the cholera-red reaction.

Sp. Massaua (Massowah)—Was isolated in a small epidemic of cholera and accepted as the true spirillum, but further study showed that it was negative to Pfeiffer, was very pathogenic to pigeons, and possessed four flagella.

Sp. of Finkler and Prior—Was isolated first from fæces of a case of cholera nostras, and has since been found in water. Morphologically, it is like the Sp. choleræ, though thicker in the centre and more pointed at the ends. It does not give the cholera-red reaction, and liquefies gelatin very rapidly, showing no bubble-like appearance. Grows well on potato, and is negative to Pfeiffer.

Sp. Aquatilis of Gunther—Was found in Spree water, and closely resembles Sp. choleræ, but young colonies have a smooth rim, and it does not give cholera-red reaction, and is negative to Pfeiffer. Does not grow on potato.

Sp. Danubicus.—Cultivated from canal water. Does not give Pfeiffer, and colonies are different; otherwise it closely resembles the cholera spirillum.

Sp. Deneke—Also called Sp. tyrogenum, was isolated from butter and old cheese. It closely resembles the Sp. choleræ, but is thinner and smaller; growth in gelatin similar but more rapid, and does not give the cholera-red reaction. It is very feebly pathogenic, and is usually regarded as a harmless saprophyte.

Sp. Phosphorescens—Gives luminous cultures.

CHAPTER XVI.

SPIROCHÆTES.

THE diseases produced by spirochætes are now referred to as spirilloses or spirochætoses, and fall into (following Muir and Ritchie) two main groups:—(1) The human spirillar fevers, and the corresponding affections of various animals; (2) Syphilis and yaws, and the ulcerative and gangrenous conditions apparently caused by spirochætes (e.g., Vincent's angina). In the first group, blood infection is the rule, and the organisms are, in most cases if not in all, transmitted by blood-sucking ecto-parasites. In the second group, the organisms are primarily tissue parasites, and later show blood infection, and are mainly spread by direct contact. As regards general morphology, staining reactions, and conditions of growth and culture, the various spirochætes present common characters, and their classification with bacteria or protozoa is still a matter of doubt.

Spirillum Obermeieri or Sp. of relapsing fever (Obermeyer, 1873)—Is now usually regarded as a spirochæte, and known as the **Spirochæta recurrentis.** It is found in the blood of patients suffering from "relapsing fever" from shortly before the onset of the pyrexia until shortly before the crisis, and similarly in the relapses. The relationship of the organism to the disease has been proved by the injection of spirochætes into the blood-stream causing the typical attack, both in the human subject and in monkeys; also in white mice and rats, but in these and in monkeys, relapse is rare. Sp. Obermeieri is a delicate spiral thread, 7 to 9 micra long by about 1 micron thick, but the size varies from one-half to nine times the diameter of a red blood corpuscle (7 micra). The windings likewise vary from 4 to 10 or more. It stains with watery basic aniline dyes, somewhat faintly, but best with the Romanowsky stains. It shows a homogeneous cell body

or a few granules, but no division into segments. It is Gram-negative. Flagella have been noted.

Spirochæta Vincenti—Is a delicate spiral-shaped organism, said to be often found in the mouth as a simple saprophyte. It was described by Vincent in 1896, in an inflammatory lesion of the pharynx, since spoken of as Vincent's angina, in association with a fusiform bacillus of large size (3 to 10 micra by 0·5 to 0·8 micron). The curvature of the spirochætes is irregular and the number of curves variable. The relationship of these organisms to the disease is still obscure.

THE MICRO-ORGANISM OF SYPHILIS.

This is variously called the Spirochæta pallida of Schaudinn and Hoffmann, the Treponema pallidum, and the Microspironema pallidum. It was discovered by the two observers named in 1905, in the primary sore and in the adjacent lymphatic glands. It has since been demonstrated in numerous lesions and in the blood, and in congenital syphilis. It has been found in large numbers and in pure culture in the lungs, liver, spleen, pancreas, and kidneys; and in a few cases in the heart muscle. It has also been found in the roseolar spots in the disease, and in blister fluid in an infected person. This shows how the disease can be spread (as at times it has been) by vaccine fluid.

In the bubo, other spirochætes are usually found in association with the Spirochæta pallida. These are much thicker, less undulated, more refractile, and more deeply staining. They are spoken of as Spirochæta refringens. These are said to have an undulating membrane (like the trypanosomes) but no flagella. The term treponema is now reserved for a genus with no undulating membrane, and flagella of some sort at their extremities. To this group the organism of syphilis belongs, and is hence now referred to more strictly as the Treponema pallidum.

The Treponema pallidum is an extremely delicate spiral organism, very slender, about 6 to 14 micra long by less than 0·5 micron thick, showing about a dozen very

regular spiral turns close together, the whole resembling a fine corkscrew. It has a flagellum at each extremity, but no undulating membrane. It multiplies by longitudinal division, the initial stage being shown by the splitting of the flagellum at one end. It can be demonstrated in the living state in a hanging drop, or a ringed-in cover-slip, cutting down the light to a minimum, or better, by using dark-field illumination. In smears, it can be stained by Giemsa's method, of which there are several modifications. A more rapid and simple method is by using India ink. A loopful of secretion from a chancre is mixed with a loopful of ink (Gunther & Wagner's liquid pearl ink), and the mixture made into a smear as for blood. Dry in the air, and examine with an oil-immersion lens. The treponemata appear as white spirals on a dark background. In tissues, ordinary methods do not stain the organisms. Levaditi's method is commonly used, and consists in fixing in formalin for 24 hours, washing out the formalin with water, the traces of which, which might contain formalin, being removed with alcohol; soak in silver nitrate solution for from three to five days, wash and soak in pyrogallol-formalin solution; wash, dehydrate, imbed in paraffin, and section. A shorter method has been devised. The evidence of its pathogenicity is derived from its constant presence in the lesions of acquired and congenital syphilis, and in that it has been communicated to monkeys, producing typical syphilitic course and lesions, from which the treponema was recovered in 70 per cent of the cases examined. It has not yet been successfully cultivated in a pure state, or in that case complement fixation by a pure culture might be an additional proof. It is not regularly recovered from tertiary lesions, which is not surprising. This is analogous to tuberculosis, in which the tubercle bacillus is often not demonstrable by ordinary methods in the chronic lesions. Treponema pallidum does not pass through a filter.

Wassermann Reaction—Is now regularly used in clinical diagnosis. It is described on page 205; its value is discussed on page 209. In a recent research by Calmette, Breton, and Couvrer, it has been practically applied to the diagnosis of syphilis in the newly born child,

with a view to securing early treatment in those cases which would not be diagnosed on ordinary clinical grounds. The mode of procedure was to examine the placental blood taken at the time of delivery, before ligature of the cord. Out of 103 such samples, in 16 a positive result was obtained. Out of the 16, no evidence was got of syphilis by clinical examination or history in the parents or child in 8 cases. Of the remaining 8, there was definite evidence in the parents or child in 5 cases; and in one other the mother had previously borne an anencephalic monster. In every case in which the child showed signs of syphilis at birth, the mother's blood gave a positive reaction.

Immunization.—Though one attack of syphilis usually protects against another infection beginning with a chancre, no success has yet been obtained in attempts to produce either active or passive immunization.

Yaws.—A disease resembling syphilis, and at times regarded as identical with it, which prevails endemically in the West Indies, Brazil, Fiji, Ceylon, the East Indies, and different parts of Africa. It is called variously yaws, frambœsia, bubas, koko, paranghi, and pian. It is highly contagious, but is not hereditary or congenital. It is now believed to be due to Treponema pertenue, first described by Castellani in 1905 as Spirochæta pertenuis, which resembles the Treponema pallidum closely.

CHAPTER XVII.

YEASTS AND MOULDS.

YEASTS and moulds are grouped in the class of fungi to which the bacteria also belong. They are distinguished from the latter (and from one another) by their mode of reproduction. From the bacteria they also differ in being much larger, as a rule. Their biological requirements are also, generally, much less exacting.

Between these groups and the average bacterium, the space is bridged by some forms called the higher bacteria, which resemble the moulds in showing branching. Such are actinomyces (which has been considered immediately after B. tuberculosis), the streptothricæ, etc. These are often grouped as trichomycetes, which is regarded as a subdivision of the true moulds. The whole subject is at present uncertain and confused. Foulerton, in his Milroy Lectures (*Lancet*, 1910, Vol. i., p. 551 on) urges the view that the micro-organisms variously called tubercle bacilli, actinomyces, cladothrix nocardia, oospora, and streptothrix, belong to one family of moulds or hyphomycetes.

In clinical medicine and pathology the term " mycoses " is used (following Virchow) to denote all the affections produced by filamentous and budding fungi, and this term associated with the seat of the lesion has given rise to such terms as dermatomycosis, otomycosis, etc. On the other hand, such terms as actinomycosis, saccharomycosis, blastomycosis, aspergillosis, sporotrichosis, etc., are used.

All the members of these groups may be considered as facultative parasites, parasitic life being unnecessary to their cycle of evolution, as their proper existence is a saprophytic one. The parasitism is, in their case, simply a phenomenon of adaptation.

Being destitute of chlorophyll, they do not need light, and grow luxuriantly in the dark. They vary much in their temperature requirements, a few growing well at

human body-heat, some at 30° to 33° C., and most at air temperatures. Growth at temperatures higher than the optimum, in certain media and anaerobically, results in the production of pleomorphic forms. Most of them die when deprived of air or oxygen; a few are anaerobic. Moisture is absolutely necessary. They grow readily on organic matter of all kinds. The natural media commonly used in their study are bread, sterilized milk, beer wort, potato, carrot, decoctions of fruits, etc. As these media are variable in their composition from time to time, and the problem of pleomorphism has to be faced, artificial media of definite composition and reaction are preferred in scientific work for giving comparable results. Growth is best on solid media, standardized to an acid reaction of + 2 per cent (+ 20 per litre).

YEASTS.

The yeasts are fungi characterized by the mode of multiplication known as "budding" or "gemmation" or asymmetrical fission, and are hence called blastomycetes. From their action in fermenting sugars they have also been called saccharomycetes. Their botanical position as a separate group is not well established, as a large number of intermediate forms relate them closely to the moulds. The usual yeast cell is round or oval in shape, 10 to 20 micra long by 5 to 15 micra across, and occurs singly or in short chains. Each cell is bounded by a cell-membrane composed of cellulose, and of such a thickness (0·5 micron) that it shows a double contour. Within the membrane is contained the protoplasm, in which is a large number of granules, globules, and vacuoles, and in old cultures a nucleus is sometimes seen. When budding, the mother cell throws out a small globular process, which gradually enlarges until it attains nearly the same size as the parent cell. By a gradual narrowing of the isthmus between the mother and daughter cells, the daughter cell finally becomes free. In addition to this mode of reproduction, most yeasts can form spores called "ascospores." This takes place when there is a lack of nourishment or where the conditions of life are otherwise unfavourable. These spores are

formed by endogenous cell-division, and the usual rule is for the protoplasm of one cell to divide into four spores, each with its own cell-membrane, the original cell-membrane persisting and serving as an envelope enclosing the spores.

Yeasts grow more slowly than bacteria, and are hence more difficult to isolate from mixed cultures on the ordinary media. Once isolated they are kept alive by subculture every 2 to 3 months. On glucose agar or plain agar, colonies appear in 3 to 4 days as minute glistening white spots. In stab, the growth is all at the top, forming a heaped-up creamy layer on the surface of the medium. In broth, a stringy gelatinous growth is formed. Growth takes place on gelatin, which is not liquefied. On potato, growth is more rapid. The cultivated yeasts used in brewing and baking processes are capable of fermenting various sugars. This action is due to certain ferments or enzymes elaborated by the yeasts. Two of the enzymes are diffused into the medium in which growth is taking place; one remains closely bound to the yeast cell, and was only isolated by Buchner by rupturing the living yeast cells under great pressure, filtering, and centrifugalizing the filtrate. This filtrate was found to have the power of fermenting glucose and lævulose into alcohol and carbonic acid gas. This endo-enzyme is called "zymase."

$$C_6H_{12}O_6 = 2 \cdot C_2H_5 \cdot OH + 2 \cdot CO_2$$

The other two soluble enzymes are "invertase" and "maltase." The former inverts cane sugar or saccharose into invert sugar (glucose + lævulose), and so renders it susceptible to the action of the "zymase." "Maltase" acts on malt sugar, changing it into glucose, which is then acted on by the "zymase."

$$\underset{\text{Saccharose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{lævulose.}}{C_6H_{12}O_6}$$

$$\underset{\text{Maltose.}}{C_{12}H_{22}O_{11}} + H_2O = \underset{\text{Glucose}}{C_6H_{12}O_6} + \underset{\text{glucose.}}{C_6H_{12}O_6}$$

Saccharomyces cerevisiæ.—This is the name applied to the yeast in common use by brewers and bakers.

It consists of round or oval cells containing a clear fluid and no granules. It is not used older than one week's growth, after which time granules appear in the cells.

When budding very rapidly, delicate mycelial threads are formed. It forms ascospores at 25° C. in thirty hours, and at 12° C. in ten days. In brewing, yeast is added to the beer wort (cooled to 16° C.), and fermentation takes place. A brownish-yellow scum forms on the surface, bubbles of gas (CO_2) escape, forming a foam, and alcohol is formed in the liquid. This is the "high" fermentation with "top" yeast, and takes place in several days.

In some breweries "low" or "bottom" yeast is used, and the fermentation is conducted at 5° C. The yeast cells sink to the bottom as they are formed, and the whole process takes a much longer time (fourteen days).

In baking, the yeast converts the starch into sugar, and then the latter into CO_2 and alcohol. The gas breaks up the glutin into thin-walled cells. The subsequent "baking" or heating kills the ferment, and drives off the CO_2 and alcohol.

Wild yeasts are usually termed *torulæ*. They have oval or spherical-shaped cells, do not produce ascospores, and have only feeble fermentative powers. Some of them have been found to produce a true mycelium.

Torula rosea (Saccharomyces rosaceus) is a pink torula, which, growing on gelatin, agar, etc., produces raised masses with a polished pink surface, similar to a piece of coral. Microscopically, it shows rounded or slightly oval cells, 5 to 8 micra in diameter, and containing a delicate yellow pigment, which in the mass gives the pink shade.

Torula niger (Saccharomyces niger) grows on gelatin as a black heaped-up mass, resembling a piece of black sealing-wax. On potato and bread paste it forms a dull sooty crust, and in milk a black crust. It is met with in the air.

Pathogenic yeasts have been described in connection with (1) Multiple abscesses in bones, lungs, spleen, and kidney, and ending fatally (Busse); (2) An illness simulating diphtheria (Klein and Gordon); (3) Subcutaneous myxomatous tumours (Curtis); (4) Middle-ear disease (Maggiora and Gradenigo); (5) A lupus-like skin disease

(Gilchrist); (6) An intraperitoneal tumour (Blanchard, Schwartz, and Binot), etc.

MOULDS.

The distinguishing feature of the moulds is their growth in long threads or filaments, with seed-bearing branches called hyphæ. Each filament may be a single, simple, multinuclear cell, or a greatly branched one, or may be composed of a row of cells set end to end. The interlacing mass of threads is called the "mycelium." On this basis moulds are divided into two classes: (1) Phycomycetes, or those in which the mycelial threads consist of a single cell; and (2) Mycomycetes, in which the mycelial threads are composed of numerous cells. The two groups also differ in that in the first, reproduction is sexual and asexual; and in the second is by the asexual process only. All moulds prefer an acid to an alkaline medium, and hence are found attacking fruit preserves and similar substances. The spores of moulds are present everywhere, and in the air are in greater numbers than bacteria.

The members of this group of fungi which we shall consider are: Mucor mucedo; Aspergillus; Penicillium; Microsporon furfur; M. minutissimum; Sporotrichum Beurmanni; Oidium albicans; and the moulds of ringworm.

Mucor mucedo is the commonest **mucor** or "**head**" mould, and belongs to the class of single-celled fungi or phycomycetes. It is the common, white, cottony mould which grows on damp bread, rotten fruit, horse dung, etc. There is a finely branched mycelium from which project thicker unbranched hyphæ. Near the end of these hyphæ a septum forms, the terminal portion of the hypha swells, and in it numerous oval spores develop. The globular swelling produced is known as the "sporangium," and is enclosed by a capsule. It ruptures when ripe by the swelling of the gelatinous material in which the spores are imbedded. The end of the hypha projects into the sporangium, and this part of it is called the "columella." This asexual form of multiplication is the more common, but sexual reproduction occurs under conditions not well defined.

In this form lateral branches (gametophores) grow out from two hyphæ close to each other. These gametophores meet by the tips, fuse, and then by septa the central portion is separated off and becomes a "zygospore." The mature zygospore under suitable conditions enlarges and sends out a germ tube or hypha, on the end of which a sporangium may appear. Mucor grows on gelatin plate as round white colonies which soon cause liquefaction. In gelatin stab, it forms a dense white growth spreading over the surface, and sending down penetrating branches—sub-aerial hyphæ; others rise up vertically into the tube—aerial hyphæ. Other mucors are: M. stolonifer (black mucor), M. spinosus (chocolate colour: has spines on the columella).

Aspergillus or "Knob" Mould.—This is a common form of mould, occurring on bread, cheese, oranges, etc. There are several varieties, A. glaucus (blue mould), A. niger (black mould), A. flavus (yellow mould), and A. fumigatus (green turning to grey). The mycelial filaments are composed of numerous rod-like cells joined end to end. They reproduce asexually. Hyphæ arise from the mycelial network, and each hypha terminates in a knob-like expansion, the columella. The surface of the columella becomes studded with flask-shaped organs or cells called sterigmata, and each of these forms spores or conidia, which remain attached in chains like streptococci. The result is a knob with radial projections composed of spores; but having, unlike Mucor, no containing capsule.

Aspergilli grow on gelatin as round white colonies very like those of penicillium. In a few days coloured points appear, denoting spore formation, being blue in glaucus, black in niger, etc. The gelatin is liquefied. In gelatin stab there is a dense felt-like growth (more pronounced than with penicillium), and later liquefaction.

The pathogenic aspergilli include:—

1. *Aspergillus fumigatus*, which has been found on the one hand in a malady simulating pulmonary tuberculosis, but not showing tubercle bacilli in the sputum (at times it is associated with the tubercle bacillus); and on the other hand, causing affections in the external auditory canal, the tympanic cavity, the nasal fossæ, and in wounds.

Such infections mostly occur in those engaged in handling grain, whole or crushed, either as transporters or as feeders of animals or fowls. Many of the cases are among bird fanciers, especially those keeping pigeons, which among birds are specially liable to aspergillosis. Birds and mammals can be fatally infected by intravenous inoculation with aspergillus spores. In birds, infection has been produced by inhalation of spores. In examining sputum for the presence of aspergilli, it is absolutely necessary that the examination should be made immediately after expectoration, since the spores of such moulds may exist in the air in considerable numbers, and falling on to the sputum would germinate there. A film is made on a slide in the ordinary way, dried, fixed by heat or absolute alcohol, stained with carbol-thionin, and examined. The characteristic threads or filaments of the mycelium are seen among the pus cells. It is further necessary to verify the diagnosis by cultivating the fungus and noting its growth and morphology. This should be done on a special medium, such as Raulin's liquid medium, in which the aspergillus grows well. This is composed of water, 1500 grm.; crystallized sugar, 70 grm.; tartaric acid and ammonium nitrate, of each 4 grm.; ammonium phosphate and potassium carbonate, 0·6 grm.; magnesium carbonate, 0·4 grm.; ammonium sulphate, 0·25 grm.; sulphate of iron, sulphate of zinc, potassium silicate, and manganese carbonate, of each 0·07 grm. Take a sufficiency in an Erlenmeyer flask, inoculate by dropping in a small piece of the sputum, and incubate at 37° C. In 3 to 10 days there grows a whitish meshwork, with branches bearing spores, green at first, but in a few days becoming smoke-black. The growth is examined microscopically, and its characters are studied. To verify its pathogenic action, an emulsion of the culture is injected into the ear-vein of a rabbit. The animal dies in several days with a generalized pseudo-tuberculosis.

2. *Aspergillus repens*, found in the auditory canal, producing a false membrane.

3. *Aspergillus flavus*, in chronic ear discharges.

Penicillium, or "Pencil" Mould.—The blue-green variety of this form of mould, Penicillium glaucum, is the

most commonly occurring of all moulds. In this genus, the mycelial threads are septate or many-celled. Hyphæ are given off, and from the end of each hypha, two or more short pencil-like branches arise, and these likewise give origin to other similar branches. These last, or further set of branches, produce spores or conidia, which, remaining attached, form a string of spores. The branches producing the spores are called sterigmata, and the intermediate branches the basidia or conidiophores. The result is not unlike an *x*-ray photograph of the arm, in which the humerus represents the hypha, the radius and ulna—two basidia (omit the wrist), the metacarpus—five sterigmata (say three from the radius and two from the ulna), each sterigma bearing spores—the phalanges. The spores are rounded in shape. Penicillium glaucum grows on bread paste, showing at first a white fluffy growth, becoming either green or blue, as the spores form. It sometimes is covered with little drops of dew-like fluid.

On gelatin plates it grows as small round colonies of hair-like filaments, at first white in colour, but later greenish. The gelatin is liquefied. In gelatin stab a white fluffy layer or scum rapidly forms on the top, and descending branches run into the gelatin, as well as horizontal ones from the stab. The medium becomes bluish or greenish, and liquefaction takes place. Growths on agar and potato have similar characters.

Penicillia have been described as the cause of chronic catarrh of the Eustachian tube, and of gastric hyperacidity.

Microsporon furfur—Is a mould first described in 1846, and found in the skin affection called pityriasis versicolor. It is composed of sinuous hyphæ, 3 micra in thickness, showing right-angle branches. The spores are large, 3 to 5 micra in diameter, and are formed in a manner similar to that in penicillium.

Pityriasis versicolor is seen in persons subject to profuse perspiration, who have been infected with the spores from the air or elsewhere. The fungus grows in the superficial layers of the epidermis, forming a yellowish or coffee-and-milk coloured patch, usually seen on the chest or abdomen or back. Little or no discomfort is caused to

the person affected. The diagnosis is easily confirmed by examining a scale in a drop of liquor potassæ; or the scale may be teased out on a slide in a drop of absolute alcohol, and then stained with eosin. The filaments and the large round spores are readily seen.

Microsporon minutissimum is a mould described as the cause of dhobie's itch or erythrasma, which is a common affection in the tropics. It is mainly seen in the axillæ, the scrotal region, the insides of the thighs, and the submammary folds. Like the Microsporon furfur, it lives a simple saprophytic existence in the epidermis, causing reddish-brown patches with an abrupt edge. When a scale is removed, washed with ether, teased out in acetic acid, allowed to dry, washed with alcohol, and stained with carbol-thionin, the fungi can be seen with the microscope as slender sinuous filaments, formed of short elements, very similar to bacilli, from destruction of parts of the filaments, or non-staining of these parts.

Sporotrichum Beurmanni is a mould composed of a mycelium, the filaments of which branch in all directions. The hyphæ are 1 to 2 micra thick, and at the ends of these oval spores are formed (3 to 5 micra by 1·5 to 3), singly or in grape-like clusters. Spores are also formed around the main filaments, or apparently so. The full life-cycle of the sporotrichon has not yet been worked out, and its exact classification is still a matter of doubt. It was first isolated by Schenk in 1898 from refractory subcutaneous abscesses in man. De Beurmann and Ramond rediscovered it in granulomata in the skin in 1903. Since then numerous cases have been reported in France, and lately two cases have been reported in this country (Ofenhein, *Lancet*, 1911, Vol. 1, page 659; and Norman Walker and James Ritchie, *British Medical Journal*, 1911, Vol. 2, pages 1–5. The latter article is accompanied by a special coloured plate and a short bibliography).

Sporotrichosis is a disease characterized by cutaneous and subcutaneous tumours, firm and indolent. These may ulcerate and discharge a viscid homogeneous pus of a yellowish-grey colour. The tumours have been in the past mistaken for those due to syphilis and tuberculosis, and potassium iodide and tuberculin injections or other treatment

administered. When iodide was given, cure was often effected, and so the diagnosis was apparently confirmed. The ulcers have similarly been treated as syphilitic, as lupus, and as simple pyogenic ulcers. In many cases there is a history of a minor injury, with a spread from this up the line of the lymphatics, with tumour formation and breaking down at various points *en route*. The lymphatic glands are not usually enlarged, the fungus probably not reaching them at an early stage. The affection is usually not a serious one, but there is little tendency towards cure if left untreated. Stimulating local treatment, together with the administration of large doses of potassium iodide (60 to 80 grains, or 4 to 5 grm. daily) is quickly followed by cure. The diagnosis is based on the direct examinations of scrapings, which are usually negative except for spores, oval and 3 to 5 micra long; and by cultivation. Pus from an unbroken abscess (if possible) is inoculated freely, since the parasitic elements are scarce, on broth, glycerin agar, potato, carrot, etc., on all of which it grows well. Growth becomes visible in some days, and gradually increases. On agar, the colonies are first white and cream, and later a dirty grey. On carrot, the colour is first yellow, then grey, and finally quite black. On potato, small, white, woolly spots appear, increase in size, and change to a brownish colour. Further growth results in heaped-up masses likened to cerebral convolutions. "In gelatin stab, an inverted fir-tree growth is got, but no liquefaction. It forms acid specially with inulin in peptone solution, and also with glucose, maltose, galactose, raffinose, saccharose, and mannite; but with lactose, dulcite, inosite, adonite, sorbite, and salicin, the medium remains alkaline. In no case was there any gas formation. No indol formation was observed. The organism was definitely aerobic" (Ritchie, *loc. cit.*). This observer has also studied the organism in hanging-drop agar cultures, and found that the mycelium formation is readily noted at 22° C., but at 37° C. few filaments are formed; instead, large spores (5 micra) from which short stalks sprouted, each bearing a spore, which thus formed a circle round the central body. The latter soon degenerated. This suggests the reason

why filaments are not usually found in the pus or granulomata. The optimum temperature therefore is about 20° C. (15° to 22°). The organism is Gram-positive, but not acid-fast.

Outside the human body, the organism has been found living on decaying vegetable matter. Sporotrichosis has also been described as occurring in dogs, rats, and in the horse. In the last it causes a lymphangitis with superficial granulomata of a benign nature, but important from having a resemblance to glanders, especially as it also at times prevails as an epizootic. Human infection from the horse has been reported in twelve instances in seven or eight years in North Dakota, U.S.A., where sporotrichosis in horses occurs moderately frequently. A case is also reported in a female bitten on both thumbs while holding a rat which had been inoculated with sporotrichosis. Serum agglutination and complement fixation have been found to occur in sporotrichosis, but are not specific, as the serum of patients suffering from other mycotic affections (thrush, actinomycosis) reacts to Sporotrichum Beurmanni, at least in the dilutions tried.

Oidium albicans (sometimes called Saccharomyces albicans) is the cause of thrush (*Gr.* Soor ; *Fr.* Muguet), a localized disease of the mouth and pharynx, but also at times attacking the œsophagus, stomach, small intestine, cæcum, and anus ; besides being occasionally found developing on the vulva, in the vagina, on the prepuce, and the glans penis. On rare occasions it has been found in the bladder, the kidney, the lungs, the brain, and in the blood. The oidium is composed of cylindrical filaments, made up of joints 50 to 60 micra long by 3 to 5 micra in diameter. These give off branches, which bear spores by constriction at their free ends. Budding is also noted when grown in media containing sugars, allying it to the yeasts. Two varieties are described, one which liquefies gelatin and produces spores ; another which does not liquefy gelatin and yields small spores. It grows only in acid media. Like the yeasts, it can ferment sugars, but is not so powerful as they are.

It is easily diagnosed microscopically. A fragment of the white membranous growth from the tongue or mouth

is taken on a slide, teased out in a drop of acetic acid (which renders the epithelial cells almost invisible), and examined. The parasite is clearly seen as described above. The spores are round or oval. A stained specimen may be made by teasing in a distilled water drop, exposing to dry, fixing by heat or absolute alcohol, and staining with thionin. To isolate in culture, inoculate on gelatin, and incubate at a low temperature (15° to 20° C.) for 48 hours. By that time white or creamy colonies appear, which are pure cultures of the thrush fungus. The ordinary microbes of the mouth are unable to develop at that temperature in the same time.

Ringworm Fungi.—The study of these is very complex. The mode of demonstration of them in the various parts affected is the same for all, and is summarized thus by Agasse-Lafont :—

Hairs.—Prepare a solution of caustic potash of 30 per cent strength. Extract some of the diseased hairs, and put them in a drop of this solution on a slide. Put on a cover-glass, and heat *moderately* for several seconds over the flame of a spirit lamp, until the hairs can be crushed by gentle pressure on the cover-glass. Examine directly without staining, with a dry lens and medium light ; or first mount in glycerin or glycerin jelly.

Epidermal Scales.—Tease out with two sterile needles, and treat in the same manner.

Nails.—Reduce to powder with a nail file, and treat as above.

Pus.—Dry on slide, and examine directly without staining.

Favus Crusts.—Tease out, crush between two slides, and thereafter treat as for hairs.

They are best cultivated on Sabouraud's medium Agar, 18 grm. ; peptone, 10 grm. ; maltose, 40 grm ; and water to 1000 c.c. Heat to dissolve, fill into tubes, and sterilize on three successive days. To inoculate tubes, take an infected hair, rinse it for a few seconds in absolute alcohol, and wash thoroughly with sterile water. Then stab it into medium at several places, and grow at 18° C If first growth is not pure, remove plug, and inverting tube

over another, tap it smartly, when spores of the fungus will fall into the other tube and inoculate it.

At 18° C. growth appears in seven days as fine white downy tufts, which increase in size and throw out rays. The surface of the growth becomes covered with a fine white powdery material. If grown on gelatin, liquefaction takes place in twelve to fifteen days. The microsporon fungus gives a more delicate growth than the megalosporon fungus, and also shows microscopically club-shaped ends to some of the filaments, which are not found in the other. In both forms, spores are found on one side of the threads (like the teeth of a crab) or at ends like a bunch of grapes.

TABLE OF THE PRINCIPAL RINGWORM FUNGI (*Agasse-Lafont*).

	Trichophyton tonsurans	Trichophyton mentagrophytes	Microsporon audouini	Achorion schoenleinii
Pathogenic rôle	Ringworm of scalp: *Tinea tonsurans* Body: *Herpes circinatus*	*Tinea tonsurans suppuratæ; Tinea barbæ* or *Sycosis menti*	*Tinea tonsurans, etc.*	Favus of scalp, skin, and nails
Lesions produced	Small: Hairs: broken close to the scalp, or short	Small: Suppurative:	Large: Hairs; broken long, and having at their base a greyish sheath	Sulphur-yellow crusts
Principal characters of fungus	1. Spores large (5μ) 2. In regular lines 3. Inside hair roots	1. Spores vary (2–10μ) 2. In lines 3. Inside and outside hair roots	1. Spores small (2–3μ) 2. In mosaics 3. Spores only on outside of roots	1. Mycelia of varying thicknesses 2. Wavy

Trichophyton tonsurans.—This fungus is the cause of 30 per cent of the ringworm of the scalp in Paris, and almost all the cases in Germany and Italy. In the east end of London, among the Polish, German, and Russian Jews, it is a common cause. It also is found in ringworm of the body, and at times causes an affection of the beard and the eyebrows, dry in character. Rarely, it affects the nails.

It is composed of simple filaments interlaced. In the hair bulbs, the filaments are inside the cuticle (endothrix) and running parallel to the long axis, and are formed of cells or spores, almost square. These spores are 4 to 5 micra long, and are regularly arranged in lines. It is readily grown on Sabouraud's medium, showing in five to six days. It liquefies gelatin. A variety is quite frequently met with in the same places, T. Sabouraudi. It has a more fragile mycelium, and shows round spores.

Trichophyton mentagrophytes is a cause of sycosis menti, a suppurative affection of the beard, and also of ringworm of the body, and a suppurating ringworm of the scalp in infants. The filaments are composed of strings of round spores of varying diameters (2 to 10 micra). The filamentary threads are mostly outside the hair cuticle; (endo- and ectothrix); a few are found inside but towards the periphery.

Microsporon or **Microsporon Audouini** is almost the sole cause of ringworm of the scalp in Scotland, and of 96 per cent of the cases in London among British subjects. It is called the small-spored fungus as compared with the two given above, which are called "megalosporon," or large-spored. The parasite encloses the diseased hair in a whitish case formed of a mosaic of spores (ectothrix). The spores are 2 to 3 micra in diameter, and from pressure against one another in the mosaic pattern, become polyhedral in shape. When stained with carbol-thionin, the filaments are seen in the interior of the hair.

Achorion Schoenleinii is the fungus which causes favus, or honeycomb ringworm, in which the characteristic feature is the formation of cup-shaped crusts of a sulphur-yellow colour. It most commonly attacks the scalp, but also affects the skin of the body and the nails. When attacking the nails, they become yellow and thickened. Besides the characteristic form of attack, the fungus may also produce a moist dermatitis resembling that due to the other ringworm fungi.

In the hair the parasite is seen as wavy lines of mycelia composed of spores. The spores are irregular in size and shape, but mostly polyhedral. Rarely, the mycelium is septate and without spores.

Favus prevails also in mice and cats, and from the latter animals many of the human cases arise.

The Achorion Schoenleinii in culture grows best at 30° to 35° C., and scarcely grows at 10° to 15° C., unlike the other ringworm fungi. It also liquefies gelatin more quickly than they, namely, in three to four days. It forms snowy-white circular or oval colonies, becoming finely powdered over the surface, and wrinkled in old culture. It grows best on *beer-wort agar*. To beer-wort diluted to a specific gravity of 1100 add 1·5 per cent of agar. Heat for two hours until dissolved. Filter, tube, and sterilize. (Avoid overheating.)

CHAPTER XVIII.

SPECIAL
BACTERIOLOGICAL EXAMINATIONS.

BACTERIOLOGICAL EXAMINATION OF WATER.

In all natural unfiltered waters, except when derived from deep wells and springs (in which case filtration has already taken place through the strata) numbers of bacteria are found. The actual content is determined by the accumulated action of the following factors, namely :—

1. Presence or absence of local pollution.
2. Presence or absence of natural purification.
3. The season of the year.
4. The rainfall at any particular period.

The bacteria found in water may likewise be classed under four heads, as—

1. Harmless : the natural water bacteria. Such are the B. fluorescens liquefaciens, B. fluorescens non-liquefaciens, B. prodigiosus, B. violaceus, sarcinæ, and spirilla. These all grow best at room temperature.

2. Unobjectionable : those present from soil washings, as B. subtilis, B. mycoides, and B. megatherium.

3. Objectionable : those derived from sewage, either directly or from sewage-polluted soil. Such are : (*a*) The B. proteus group ; (*b*) B. coli communis and its allies ; (*c*) Streptococci ; (*d*) Staphylococci ; (*e*) B. enteritidis sporogenes.

4. Dangerous : those capable of causing infection by the alimentary canal. Such are the B. typhosus, B. paratyphosus, B. dysenteriæ, and Sp. choleræ.

Samples.—Stoppered sterile glass bottles should be used for sampling, each of at least 250 c.c. capacity (8 to 10 oz.). The bottle should be thoroughly cleansed with soap and water, well rinsed with clean water, and sterilized (inverted and with stopper out) in steamer for one hour.

Allow the sterilizer to cool, then stopper the bottle, and remove and put in case.

In sampling from a tap, run the water to waste for half an hour, and then fill bottle. Stopper, and label with particulars of place, time, and date. Examine at once, or pack in ice to prevent multiplication of organisms.

In sampling from a lake, dip stoppered bottle well below surface; remove stopper, and keep it under water; allow bottle to fill; replace stopper, and bring bottle to surface. Pack in ice.

Examine samples as soon as possible after collection. Keep in ice in meantime.

Dilutions.—If the water is pure, no dilution will be required; if impure, varying dilutions are used according to the degree of impurity. These may be made by the decimal mode of dilution described on page 367; or flasks may be kept ready containing 100 c.c. of sterile water. One c.c. of sample added to such a flask by sterile pipette gives practically a dilution of 1 in 100; 1 c.c. from this flask to another sterile 100 c.c. gives 1 in 10,000; and so on. To get 1 in 10, remove 10 c.c. by sterile pipette, and add 10 c.c. of sample; and from this dilution others are similarly made.

STANDARDS.

	Bacterial Count.		Bacillus Coli Communis.
	Gelatin Plate at 20° C.	Agar Plate at 37° C.	
Deep wells and springs	Should not exceed 50 per c.c.	Should not exceed 10 per c.c.	Should be absent in 100 c.c.
Surface waters:— Shallow wells .. Cultivated lands .. Rivers	Ditto 500 per c.c.	Ditto 50 per c.c.	Ditto in 10 c.c.

METHODS OF WATER EXAMINATION.—These are based on the knowledge that the dangerous organisms in water are usually present from sewage pollution. Inasmuch as some of these forms are not easily isolated from water, the mode of procedure is to enumerate the total bacterial content, and to look for an organism, likewise present from

sewage pollution, but easily found if present. Such an organism is Bacillus coli communis, which is present in enormous numbers in the sewage of man and animals; and is therefore likely to be present in sewage contaminated water, even after great dilution. The B. coli is also a more resistant organism than the dangerous forms, and so serves to indicate pollution at a later stage than these could possibly be found in a water. So far it is not possible to distinguish B. coli of human origin from those of animal origin. It is stated that those of human origin are more pathogenic to animals.

There are various methods in use in this country. A Committee of the Royal Institute of Public Health appointed to consider the "Bacterioscopic Examination of Water," reported in 1904 (*Journal of State Medicine*, vol. xii, p. 471) as follows:—

Minimal Procedure. Unanimous report:—

(*a.*) Enumeration of bacteria present in a water sample, capable of growing on a medium incubated at room temperature (18°–22° C.).

(*b.*) Search for Bacillus coli, and identification and enumeration of this organism, if present.

Majority also recommended:—

(*c.*) Enumeration of bacteria present in sample capable of growing on a medium incubated at blood heat (36°–38° C.).

(*d.*) Search for and enumeration of streptococci.

(*e.*) Do not recommend routine examination for Bacillus enteritidis sporogenes; but in exceptional and special cases advise that it be searched for.

They further report on mode of collection of sample, media to be used in the tests, etc., of which the following is a brief summary:—

Collection of Sample.—The sample should be collected in sterile stoppered glass bottles (minimum quantity 60 c.c., or 2 ounces), and should be packed in ice. At least 10 ounces of sample should be sent, and its examination should be begun within three hours, or it should be left packed in ice.

Enumeration of Bacteria.—The *media* to be used are all to be standardized to have a reaction of +10 (Eyre's scale). Owing to changes in reaction, media should not be more than

three weeks old. For cultivation at room temperature a choice may be made from the following: Distilled-water gelatin, nutrient gelatin, distilled-water agar, nutrient agar, and gelatin agar. At blood heat use agar or gelatin agar. Both agar and gelatin media should be used in any one test. Polluted water gives more colonies on nutrient gelatin than on distilled-water gelatin; unpolluted water gives more with distilled-water gelatin.

The *size* of the plates, the *amount* of medium to be used in plating, and the *amounts* of sample to be added to the media are all specified. Plates should be 10 c.m. in diameter; 10 c.c. of medium should be used; and for ordinary waters, 0·2 c.c., 0·3 c.c., and 0·5 c.c. of sample should be added to gelatin media, and 0·1 c.c. and 1·0 c.c. of sample to agar media. The sample should be thoroughly shaken before removing these amounts, and the tubes should be thoroughly mixed by rotation before plating. Duplicates should be put up. In an unknown water, additional plates of dilutions (ten and one hundred fold) should be made. The colonies should be counted by the naked eye, and preferably by daylight. A lens may be used for doubtful colonies. The *time* of counting should be:

For gelatin plates, at the end of 72 hours (3 days);

For agar plates, after 40 to 48 hours.

The gelatin plates should be inspected daily, in case counting becomes necessary earlier from liquefaction of the medium.

Search for B. Coli.—MacConkey's broth is recommended to be used, the sample to be added directly to the medium, and not first concentrated by filtration.

Isolation of B. Coli.—If indications of the presence of B. coli are got, then the organism must be isolated, cultivated, and identified. This is advised to be done by making surface cultures on plates of either:—

(*a.*) Litmus lactose agar (reaction +10);
or (*b.*) Bile-salt lactose agar (MacConkey's);
or (*c.*) Nutrose lactose agar (Drigalski and Conradi);
or (*d.*) Ordinary nutrient gelatin.

They consider (*c*) to be the best medium of all. Agar media save time.

Identification and Tests.—Having obtained coli-like colonies on plates made from the preliminary cultivations of the water in MacConkey's broth, subcultures must be made to identify the organism. The following subcultures should at least be made:—

(*a.*) Surface Agar, on which the abundant growth enables subcultures, etc., to be easily made if required.

(*b.*) Stab and surface cultures in gelatin. These may be done in the same tube.
(*c.*) Litmus milk incubated at 37° C.
(*d.*) Glucose litmus medium.
(*e.*) Lactose litmus medium.
(*f.*) Peptone water for indol reaction.

The Committee consider an organism to be *typical B. coli* when it conforms to the following characteristics: Small motile bacillus, non-sporing, decolorized by Gram, which grows well at 37° C. and at room temperature; never liquefies gelatin; produces permanent acidity in milk; curdles milk within seven days at 37° C.; ferments glucose and lactose, with formation of acid and gas; grows in smooth thin surface on gelatin (not corrugated); and in gelatin stab grows well to the bottom of stab. This typical B. coli generally also forms indol, gives a thick yellowish-brown growth on potato, changes neutral-red, reduces nitrates to nitrites, and in fermenting glucose half of the gas produced is absorbed by KOH. It sometimes ferments saccharose.

The method here described is that used in the City Bacteriological Laboratory, Glasgow.

I. Enumeration of Bacteria.—Add 0·1 c.c. and 1 c.c. of sample by sterile pipette to 10 c.c. of liquefied gelatin and agar. Mix thoroughly by rotation and plate. Incubate the gelatin plates at 20° C.; inspect daily, and count after three days, unless necessary earlier.

Incubate the agar plates at 37° C., and count after two days.

The ordinary water bacteria grow best on gelatin, whereas the intestinal forms grow best on the agar at blood heat. Hence, in a pure water the gelatin count should be much the greater; and in an impure water the difference between the counts becomes less marked the more impure the water. The ratio between the two counts is also noted. This *ratio* of the number of organisms developing at room temperature to the number at blood-heat = 10 : 1 in pure water and = 10 : 2 or 3 or 5, etc., in polluted waters. The ratio is unreliable in surface waters in tropical countries, because B. liquefaciens, B. fluorescens liquefaciens, and B. fluorescens non-liquefaciens are commonly present, and all grow well at 37° C., and are harmless.

AVERAGE NUMBER OF BACTERIA PER C.C. OF WATER SAMPLE GROWING ON GELATIN AT 20° C.

	Glasgow Tap Water.	Raw Thames Water.
Year 1909 :—		
Highest ..	73 (Dec.)	19,794 (Dec.) river in flood.
Lowest ..	31 (June)	913 (May) river low.
Average ..	54·2	3,818.
Year 1910 :—		
Highest ..	105 (Jan.)	22,939 (Dec.) river in flood.
Lowest ..	19·5 (May)	1,522 (May) river low
Average ..	43·0	7,410.

Dr. A. C. Houston (Director of Water Examination to the Metropolitan Water Board, London), in addition to gelatin and agar media, for enumeration, also uses MacConkey's neutral-*red*, *bile*-salt, *pe*ptone, *lac*tose a*gar* (called rebipelagar for brevity). This is similarly inoculated and plated and incubated at 37°. The colonies are counted on gelatin on the third day, and on agar and rebipelagar after twenty to twenty-four hours.

The following table, culled from Dr. Houston's reports, serves to illustrate the ratios of the various counts :—

RAW RIVER THAMES WATER.

AVERAGE NUMBER OF MICROBES PER C.C. IN COMPARABLE SAMPLES, TESTED ON THREE MEDIA ; WITH RATIOS.

Year.	Gelatin.	Agar.	Rebipelagar.	Ratio. Gelatin. / Agar.	Ratio. Agar. / Rebipelagar.
1908–09	2745	319	38	8 : 1	8 : 1
1909–10	5310	495	63	11 : 1	8 : 1
1910–11	6184	339	20	18 : 1	17 : 1

Gelatin at 20°–22° C. ; colonies counted on third day.

Agar and Rebipelagar at 37° C. ; counted after twenty to twenty-four hours.

This further table (curtailed) from Houston's *Fifth*

Annual Report (page 7) shows the influence of rainfall in the Thames valley on the average daily flow of the River Thames, and on the bacterial content of the raw river water :—

Month.	Rainfall (inches) Thames Valley.	Average daily (natural) flow of the River Thames in million gallons.	Total number of bacteria per cc. in the *raw* Thames Water (gelatin)	Percentage number of samples of *raw* Thames Water containing typical B. Coli in 0·1 c.c.	*Raw* Thames Water. Oxygen absorbed from permanganate test (parts per 100,000)
1910 :					
April ..	2·13	1353	3109	23·7	·1352
May ..	1·96	979	1522	12·5	·1489
June ..	3·64	1149	2721	**50·0**	**·3031**
July ..	2·25	795	2589	**52·6**	·1756
August ..	**2·88**	589	2702	13·7	·1357
September	0·46	501	3035	13·6	·1173
October ..	**3·48**	666	3736	38·1	·1611
November	**3·61**	**1595**	**17932**	**68·2**	**·3249**
December	**5·21**	**5064**	**22939**	**83·3**	**·5253**
1911 :					
January ..	1·21	**2657**	**10438**	**85·7**	·1852
February	1·67	1443	**8035**	**70·0**	·1140
March ..	1·99	2033	**9300**	**78·2**	**·2495**
Sum	30·49				
Averages	2·54	1574	7324	49·4	·2154

Note.—The figures in bold type exceed their respective averages.

II. Search for B. Coli.—For this purpose MacConkey's broth is used of the composition neutral-red, bile-salt, peptone, glucose water,* in single, double, triple and quadruple strengths. The proper quantities are put into suitable sized tubes, and fermentation or Durham's tubes

* Just as neutral-red, bile-salt peptone, lactose, agar has been shortened to rebipelagar (see p. 350), so MacConkey's broth with the various carbohydrates might be written thus :—

Neutral-red,	bile-salt,	peptone,	glucose	water	(aqua)	as rebipegluqua.
,,	,,	,,	lactose	,,	,,	rebipelaqua.
,,	,,	,,	saccharose	,,	,,	rebipesaqua.
,,	,,	,,	dulcite	,,	,,	rebipeduqua.
,,	,,	,,	mannite	,,	,,	rebipemaqua.
,,	,,	,,	adonite	,,	,,	rebipeadqua.
,,	,,	,,	inulin	,,	,,	rebipeinqua.

added, and the whole sterilized. Thereafter, the sample, having been well shaken, is added direct by sterile pipette, and always without concentration by filtration. If the sample is too strong, suitable dilutions are made, and 1 c.c. of the dilution is added. In the case of an unknown water the following tubes would be put up :—

0·0001 c.c. of sample to 10 c.c. of single strength medium.
0·001 ,, ,, ,, ,, ,, ,,
0·01 ,, ,, ,, ,, ,, ,,
0·1 ,, ,, ,, ,, ,, ,,
1·0 ,, ,, ,, ,, ,, ,,
10·0 ,, ,, ,, of double strength.
50·0 ,, ,, 25 c.c. of triple ,,
100·0 ,, ,, 30 c.c. of quadruple ,,

The tubes are put in a nest or basket, and incubated at 37° C. for twenty-four hours. The possible results are *four* :—

(*a*.) Acid and gas.
(*b*.) Acid, no gas.
(*c*.) No acid, no gas ; turbidity.
(*d*.) No visible change.

Interest lies in (*a*) and (*b*), and they are commonly associated ; but the absence of (*a*) from all the tubes should not be held as precluding the necessity for further investigation. Note the tubes showing acid and gas, or acid alone ; say that the tube containing least amount of sample, and which shows acid and gas, is that to which 0·1 c.c. of sample was added, then the result is stated thus : Sample showed acid and gas formation down to 0·1 c.c. As a rule all the higher tubes will show acid and gas too.

Some workers call this the " presumptive B. coli test ; " but as the following paragraphs (from Notter and Firth) show, the test is only a step on the way towards the isolation of B. coli.

MacConkey's Neutral-red, Bile-salt, Peptone, Glucose Water.

Reaction of certain bacteria with :

GROUP 1. *Bacteria producing acid + gas.*

B. coli communis, B. enteritidis (Gaertner), B. paracolon, B. paratyphosus, B. pneumoniæ, B. lactis

aerogenes, B. acidi lactici, B. neapolitanus, B. icteroides, B. psittacosis, B. cloacæ, B. proteus vulgaris, bacillus of hog cholera, bacillus of epidemic jaundice, B. oxytocus perniciosus, and B. capsulatus.

GROUP 2. *Bacteria producing acid, but not gas.*

B. typhosus, B. dysenteriæ, B. choleræ, B. pyogenes fœtidus, streptococci and staphylococci.

The first group can be subdivided into four :—

(*a.*) The proteus group of motile bacilli: *gelatin-liquefying*, form acid and gas in glucose, maltose, *saccharose*, and galactose, but not in *lactose*, lævulose, arabinose, raffinose, mannite, sorbite, dulcite, adonite, dextrin, starch, or inulin ; curdle milk slowly with acid ; commonly produce indol in peptone solutions.

(*b.*) B. coli communis family : motile bacilli, non-gelatin-liquefying, producing acid and gas in all the above except *saccharose*, adonite, starch, or inulin ; curdle milk rapidly with acid, but do not peptonize clot ; form indol.

(*c.*) B. lactis aerogenes group : *non-motile* bacilli, non-gelatin-liquefying, producing acid and gas in all the above except three—dulcite, adonite, and inulin ; curdle milk rapidly with acid ; do not peptonize clot ; form indol.

(*d.*) The paracolon-enteritidis series : motile bacilli, non-gelatin-liquefying, producing acid and gas in all but *lactose*, *saccharose*, adonite, starch, or inulin ; do not clot milk but finally render it alkaline ; *do not form indol.*

III. Isolation of B. Coli.—

(*a.*) From each tube showing acid and gas, and acid alone, make a surface smear with one platinum loopful on a plate of neutral red, bile salt, peptone, lactose, agar (rebi-pelagar) containing 1 part of crystal violet in 10,000. Incubate these plates for twenty-four hours at 37° C.

(*b.*) Examine thereafter. If a plate shows only one kind of colony, inoculate an agar slope from a mixture of these. If more than one form of colony is seen on any plate, inoculate agar slopes from each kind of colony. Incubate the various inoculated agar tubes (properly marked or labelled) at 37° C. for twenty-four hours. This growth on agar gives sufficient material for subsequent steps ; but it is also necessary to revive any fermentative powers

TABLE OF REACTIONS OF SEWAGE AND OTHER ORGANISMS.

	Motility.	Gelatin-liquefying.	Indol.	Litmus Milk. 1–3 days.	Litmus Milk. 3–15 days.	Glucose.	Lactose.	Saccharose.	Dulcite.	Mannite.	Adonite.	Inulin.	Sorbite.
Friedlaender Group:													
B. oxytocus perniciosus (Wyssok) ..	—	—	—	+	+	+	+	+	+	+	+	+	+
B. pneumoniæ (Friedlaender)	—	—	+	A	+	+	+	+	+	+	+	—	+
B. „ „	—	—	—	A	+	+	A	+	+	+	+	—	+
B. rhinoscleromatis	—	—	+	..	+	+	+	+	+	+	+	—	+
B. Neapolitanus (Emmerich)	—	—	+	+	+	+	+	+	+	+	—	—	+
Hueppe Group:													
B. acidi lactici (Hueppe)	—	—	+	+	+	+	+	—	—	+	+	—	+
B. levans	+	+	—	A	+	+	+	—	—	+	+	+	+
B. of Grünthal	+	—	+	A	+	+	+	—	—	+	—	—	+
Coli Group:													
B. coli communis (Escherich)	+	—	+	+	+	+	+	—	+	+	—	—	+
B. mustclæ septicus	+	—	+	+	+	+	+	—	+	+	—	—	+
B. cavicidæ (Brieger)	+	—	+	+	+	+	+	—	+	+	—	—	+
Aërogenes Group:													
B. lactis aërogenes (Escherich)	—	—	+	+	+	+	+	+	—	+	+	—	+
B. capsulatus (Pfeiffer)	—	—	+	+	+	+	+	+	—	+	+	—	+
B. cloacæ (Jordan)	+	+	—	+	+	+	+	+	—	+	—	—	+
B. coscoroba	—	—	+	+	+	+	+	+	—	+	—	—	+
Proteus Group:													
B. proteus vulgaris	+	+	+	+	+	+	—	+	—	—	—	—	—
Food-poisoning and paratyphoid:													
B. enteritidis (Gaertner)	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. of Danycz	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. of Aertryck	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. typhi murium	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. psittacosis (Nocard)	+	—	—	A	Alk.	+	—	—	+	+	—	—	+

B. icteroides (Sanarelli)	+	—	—	A	Alk.	+	—	—	A	+	—	—	+
B. "L" (Hume)	+	—	—	A	Alk.	+	—	—	+	+	+	—	+
B "Gl" (MacConkey)	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. of hog cholera (Smith, Ma.)	+	—	—	A	Alk.	+	—	—	A	+	—	—	+
B. ,, (Smith, Ark.)	+	—	—	A	Alk.	+	—	—	A	+	—	—	+
B. suipestifer	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. paracolon (Day)	+	—	—	A	Alk.	+	—	—	A	+	—	—	+
B. ,, (Le Sage)	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
B. paratyphosus A (Schottmueller) ..	+	—	—	A	A	+	—	—	+	+	—	—	+
B. ,, A (Brion & Kayser) ..	+	—	—	A	A	+	—	—	+	+	—	—	+
B. ,, B (Schottmueller) ..	+	—	—	A	Alk.	+	—	—	+	+	—	—	+
Morgan Group :													
B. No. 1 (Morgan)	+	—	+	..	Alk.	+	—	—	—	—	—	—	—
Typhoid Group :													
B. typhosus (Eberth)	+	—	—	A	Alk.	A	—	—	—	A	—	—	A
Dysentery Group :													
B. dysenteriæ (Shiga)	—	—	—	A	Alk.	A	—	—	—	—	—	—	—
B. ,, (Flexner)	—	—	+	A	Alk.	A	—	—	—	A	—	—	—
Others :													
B. fæcalis alkaligenes	+	—	—	Alk.	Alk.	—	—	—	—	—	..	..	..
B. pyogenes fœtidus	—	—	—	A	Alk.	A	A	A	A	A	—	—	A
B. pseudotuberculosis rodentium ..	—	—	—	—	Alk.	A	—	—	—	A	—	—	—
B. pestis	—	—	—	—	A	A	—	—	—	A	—	—	—

\+ signifies motility, gelatin-liquefying, indol-producing, acid and clot in milk, or acid and gas in carbohydrates.

— ,, absence of these reactions.

A ,, production of acid reaction only.

Alk. ,, production of alkaline reaction.

All the above organisms are Gram-negative.

B. oxytocus perniciosus, B. cloacæ, B. lactis aërogenes, B. levans, and B. capsulatus give the Voges-Proskauer reaction.

possessed by any of the bacteria, as these are found to be depreciated by growth on the plate medium.

(*c.*) From each growth on agar the following subcultures are made: Gelatin stab, litmus milk, peptone water, and seven tubes of MacConkey's broth, containing respectively the following carbohydrates: Glucose, lactose, saccharose, dulcite, mannite, adonite, inulin.

The gelatin is incubated at 20° C. for eight to ten days. The other tubes are incubated at 37° C. for seven days. An organism which does not liquefy gelatin, and which gives acid and clot in litmus milk, indol in peptone water, and acid and gas in glucose, lactose, dulcite and mannite, but not in saccharose, adonite, or inulin, is called **Bacillus coli communis.** The nature of organisms giving varying reactions from these may be established from the preceding table, for which I am indebted to Dr. R. M. Buchanan. Where a reaction here given contradicts that stated elsewhere in the text, it serves to show that different results have been observed.

Houston adopts a different method of arriving at what he calls a "typical B. coli" characteristic of excremental pollution.

He likewise uses MacConkey's glucose broth (rebipegluqua), but incubates at 37° C. for two days (forty-eight hours). If no gas developed, the result was considered negative. On the other hand, if gas developed, he then made subcultures from tubes showing acid and gas on to gelatin slopes, and incubated for two days at 20°–22° C. If no colonies developed resembling in any way that of B. coli, the result was considered negative. If coli-like colonies were present, one of the most typical looking colonies was chosen for subculture into glucose gelatin, and a "shake" culture made in the customary way. After twenty-four hours' incubation at 20°–22° C., if no gas developed, the result was entered as negative; just as if no growth had occurred in the oblique gelatin cultures, or a growth of microbes in no way resembling B. coli.

But if gas production was noted, the result was recorded as positive, and the other biological attributes of the coli-like microbe were studied in neutral-red broth cultures for fluorescence (fl); in lactose peptone cultures for acid and gas formation (ag); in peptone water cultures for indol formation (in); and in litmus milk cultures for acid clotting of the medium (ac). The complete combination of these positive

characters was expressed by the word "flaginac." Since January, 1907, he has modified his method in this wise. Finding that, practically speaking, a "flaginac" B. coli is a B. coli indistinguishable, according to the tests used, from the typical B. coli of excremental pollutions; and that in the great majority of cases a glucose fermenting coli-like microbe will also produce fluorescence (fl) in neutral-red broth cultures; and, further, that lactose fermenting (ag) coli-like microbes nearly always clot milk (ac);—there is, therefore, justification for omitting the neutral-red broth and litmus milk tests, and relying solely on the lactose peptone (ag) and the indol (in) tests. Practically, in the case of the London waters, an "agin" B. coli may be regarded as presumably a "flaginac" B. coli (Report for January, 1907). On this basis he has modified the above method, in order to make it more rapid. The first growth is as before, on MacConkey's glucose broth for forty-eight hours. The secondary cultures are on rebipelagar (instead of gelatin) for twenty-four hours. From red coli-like colonies on this medium, subcultures are made on glucose, lactose, and saccharose, gelatin media and in peptone water. After twenty-four hours gas production in the carbohydrate media is considered positive; and indol is tested for in the peptone water. Fermentation of lactose and production of indol are looked for, "agin" results; if saccharose is also fermented, the result is recorded as a "sagin" one. (For full description see the January, 1907, Report.) In this way Houston claims that "it becomes possible to complete the tests for excremental pollution in water within four days." It will be noticed that Houston accepts as a "type of B. coli" an organism fermenting saccharose. His statement on the subject is as follows:—

"The typical B. coli of the human intestinal tract may be divided into two classes, according to their action on saccharose. The majority do not ferment this sugar, or act on it only to a slight extent. Hence typical B. coli which do not ferment saccharose would seem to be more significant of undesirable pollution than those which do ferment it."

The student should note that Houston uses the term "typical B. coli" on a different basis from many other bacteriologists, who would be inclined to speak of his "typical B. coli" rather as a "coliform organism."

Houston has found that out of every 100 coliform organisms present in raw Thames water, 70 to 80 per cent conform to his "typical B. coli"; whereas the same water after storage and filtration shows a reduction in the coliform organisms

which is more marked among the "typicals," which now number 40 to 50 per cent only of the total coliforms. "This means that the ratio or proportion between the typical B. coli and the B. coli (both typical and non-typical) is altered during the purification processes in the direction of reducing the number of typical B. coli. This constitutes an intrinsic difference between the bacterial flora of the raw and filtered waters, and as the typical B. coli are considered specially significant of undesirable excremental pollution, it is a difference which may mean far more than the actual figures seem to indicate. The processes which affect this modification of the original biological attributes of the raw waters may operate far more powerfully in the direction of eliminating the microbes of epidemic disease" (*Fifth Annual Report*, p. 12).

It should also be noted that Houston uses glucose, lactose, and saccharose *gelatin* media. His statement on this point is as follows: "Particular attention must again be directed to the value of these solid gelatin sugar media. Times without number a microbe which failed to show any visible development of gas in *liquid* sugar media, has been found to give abundant gas when grown in solid gelatin media containing the same sugars."—*First Report on Research Work*, p. 7).

These media are described on page 49 of the *Report on the Metropolitan Water Supply* for January, 1907. They have the following composition:—

Lemco	1 per cent.
Peptone	2 ,,
Gelatin	7·5 ,,
KOH ..	1 c.c. of a 5 per cent solution.

Add 1 per cent of glucose, lactose, or saccharose. The glucose tubes are not tinted, the lactose tubes are tinted with litmus, and the saccharose tubes with neutral-red.

After inoculation, such tubes are placed for exactly three hours in the blood-heat incubator. This melts the gelatin, allows of some multiplication of the organisms to take place, and helps to effect their distribution throughout the medium. The tubes are then placed in the ice chest for half an hour to allow the gelatin to set, and thereafter incubated for twenty-four hours at 20°–22° C. Abundant gas formation (in a positive result) is visible long before the twenty-four hours are completed.

In such a medium liquefaction of gelatin can also be noted.

IV. Streptococci.—To 10 c.c. of MacConkey's glucose broth, *quadruple* strength, add 50 c.c. of sample water, and

incubate at 37° C. for twenty-four hours. Examine a hanging drop, and if cocci are seen, make a subculture by smearing a platinum loopful of culture over a plate of Drigalski and Conradi's medium (nutrose, -lactose, -agar). Incubate at 37° C. for twenty-four hours, and then subculture all the minute colonies into tubes of Houston's Lemco medium (see p. 222), containing 0·5 per cent of lactose, mannite, raffinose, saccharose, and salicin respectively, and also into milk. Incubate for two days at 37° C., and observe results.

From various researches, extending over ten years, into the characteristics of fæcal streptococci (see *Fifth Research Report*, and other references there, p. 13), Houston has found that of 100 " sewage works " streptococci—

All produced acid in lactose and raffinose media.
All clotted milk.
None reduced nitrates to nitrites.
All *but three* produced acid in salicin medium.
Forty-nine produced acid in saccharose medium.
Only *four* produced acid in mannite medium.

These reactions differ from those given by Andrewes and Horder for streptococcus fæcalis (see p. 224).

In 1909, out of 156 samples of raw river water (Thames, Lee, and New River, 52 from each), 28 were found to contain streptococci in 1 c.c.; 1908 subcultures were made, and from these 71 streptococci were isolated, plus 3 from 0·1 c.c. of water, making 71 + 30, or 101 in all, or say 111, so as to overstate any error. That is, 111 lactose-positive streptococci were found in 156 c.c. of raw river water, which amount would yield of all bacteria on gelatin plate over two million colonies; on agar plate about sixty thousand; and on bile-salt agar about six and a half thousand. The ratio, therefore, of streptococci to total bacteria (growing on gelatin plate) in the raw river water works out at one to twenty thousand. The type of streptococcus usually met with in these 156 samples, Houston describes as the la-mi-ra-sac-sal variety (i.e. acid in *la*ctose, clot in *mi*lk, acid in *ra*ffinose, *sac*charose, and *sal*icin media). As noted above, of the fæcal streptococci (100) examined by him, 97 clotted milk, and produced acid in lactose, raffinose, and salicin; and of these 97, 48 produced acid and 49 no appreciable change in a saccharose medium.

In other researches he found that in human fæces of the

streptococci isolated, 15 per cent were of the lamirasacsal variety, whereas in cow-dung 58 per cent of those isolated were of this variety.

Houston gives thus his chief reasons for considering the streptococcus test of value in the examination of water supplies :—

1. That streptococci are superabundant in human fæces.

2. That fæcal streptococci are absent or non-discoverable in a relatively large volume of pure water.

3. That fæcal streptococci do not multiply in pure water.

4. That some fæcal streptococci are of feeble vitality, and that the presence of such in a water, if they could be differentiated from their more robust companions, would seem to indicate pollution of recent and therefore of a specially dangerous nature. Dr. Houston concludes that human fæces usually contain a multitude of streptococci, 100,000 per gramme being an underestimate of the average number.

V. Bac. Enteritidis Sporogenes.—Inoculate 10 c.c. of sample water into 10 c.c. " whole " sterile milk. Heat to 80° to 85° C. for ten minutes. Cool in running water, and incubate anaerobically in a Buchner's tube at 37° C. for forty-eight hours. Examine for coagulation. The curd is almost completely separated from the whey, and is torn by gas formation, part gathering with the cream at the top. The whey is only slightly turbid, and contains numerous bacilli. The growth has the odour of butyric acid, and the B. enteritidis sporogenes is distinguished from the B. butyricus of Botkin only by its pathogenicity when injected into a guinea-pig, which dies twenty-four hours after, with green discoloration and œdema at the seat of entry, and there may be gas formation, gangrene, and a disagreeable odour.

VI. Isolation of B. Typhosus.—This is a very difficult procedure for various reasons. The most important are : The small numbers of B. typhosus usually present ; their rapid disappearance from sewage-polluted water, so that they are often absent when fresh cases arising from a polluted water occur—that is, they die out in about the same time as the incubation period of the disease ; and the

fact that the organism is easily outgrown by the other excremental bacteria, and so is crowded out in most media. When, to help matters, some agent, inhibitory to the others, is added to the medium, the typhoid organism is likewise affected, though to a lesser degree.

From all these causes attempts at isolation of the B. typhosus from water supplies usually fail.

The first step is to concentrate the water. This can be done by :—

(*a.*) Centrifugalization of large quantities, and plating the sediment.

(*b.*) Precipitation by entanglement in a chemical precipitate, such as weak soda solution and ferrous sulphate added to the water, or weak alum and lime-water solutions. Centrifuge, dissolve precipitate in neutral potassium tartrate, and plate on solid media.

(*c.*) Filtration through unglazed porcelain (Pasteur-Chamberland bougie). Wash bougie by brushing with 10 c.c. of sterile water, and use product to smear plates. The candle should filter from without inwards. Bacteria are apt to be lost in the pores of the filter. Some filter from within outwards, add broth, and thus get a primary culture.

(*d.*) Evaporation of the bulk of the water at blood-heat under reduced atmospheric pressure.

The *enrichment* method of Hoffman and Ficker is to add nutrose, caffein, and crystal violet to the sample, to make it a suitable growing medium for B. typhosus. For exact proportions see page 239.

The first method—that is, centrifugalization—is mostly favoured.

One hundred c.c. of the sample are centrifuged. The sediment is plated direct on to rebipelagar, and incubated at 37° C. for twenty-four hours. All the colourless colonies are subcultured on to agar slopes, and incubated, at 37° C. for twenty-four hours. Thereafter subcultures are made into gelatin stab, litmus milk, peptone water, and glucose, lactose, saccharose, dulcite, mannite, adonite, and inulin media. A motile organism, giving negative results to all these reactions except litmus milk, in which it gives acid

Summary of some Results of Water Examination.

Artesian wells and springs, 4 to 100 bacteria per c.c.
Ordinary wells, 100–2000 ,, ,,
Rain and snow, vary greatly, from 4 upwards.
River waters, vary greatly (see table on page 351).

Examination of Various Waters (J. Hume Patterson).

Kind of Water	Germs per c.c.		B. Coli
	Gelatin at 18° C. 3rd Day	Agar at 37° C. 2nd Day	
			Absent in
Upland surface waters — Raw	440	422	50 c.c.
Upland surface waters — Filtered	22	2	100 c.c.
Upland surface waters — Raw	980	15	1 c.c.
Upland surface waters — Filtered	320	5	50 c.c.
Raw	51	12	—
Raw	592	34	—
Waters stored in reservoirs — Raw	402	6	1 c.c.
Waters stored in reservoirs — Filtered	102	4	100 c.c.
Waters stored in reservoirs — Raw	405	16	1 c.c.
Waters stored in reservoirs — Filtered	76	4	10 c.c.
Tap Water from Burghs (different sources)	18	0	100 c.c.
	58	14	100 c.c.
	63	28	50 c.c.
	72	0	10 c.c.
	132	94	1 c.c.
	160	113	50 c.c.
	246	52	50 c.c.
	610	40	1 c.c.
	656	63	1 c.c.
"Burn" water near sewage outfall—			*Present in*
Above outfall	22,300	400	1 c.c.
150 yards below ..	16,800	1,800	0·01 c.c.
600 ,, ,, ...	19,600	1,400	0·1 c.c.
Tributary burn	1,000	40	100 c.c.
Sewage filter effluent entering burn ..	377,000	64,000	0·01 c.c.

and then permanent alkalinity, and glucose and mannite, in both of which it produces acid only, may be considered to be Bacillus typhosus. It should then be tried for agglutination in high dilutions of anti-typhoid serum. Pfeiffer's reaction may also be tried with a known immune serum. (See p. 191.)

VII. Isolation of Spirillum Choleræ. — This has already been described on p. 324.

SUMMARY OF SOME RESULTS : see preceding page.

BACTERIOLOGICAL EXAMINATION OF AIR.

Various methods are employed :—

1. *Exposure of Plates for a Definite Time.*—Incubate ; enumerate ; identify.

2. *Hesse's Method.*—A glass cylinder, 18″ × 2″, is closed at one end by an indiarubber cover, tightly adjusted. The other end is stopped with a close-fitting rubber cork, through which passes a glass tube, attached by rubber tubing to a litre flask filled with water, and which, in its turn, is similarly attached to another litre flask, empty and having its outlet tube clamped. Along the bottom of the cylinder, 50 c.c. of nutrient gelatin are spread, or may be rolled all over the inside of tube. The whole is rested on a tripod stand. Sterilize in the usual way and keep to see if sterile. To test air : pierce a small hole in rubber cover, and open pinchcock on empty litre flask, which place lower than other. Water begins to run from the upper flask to the lower, and aspirates air through the pierced hole over gelatin. By reversing the flasks, another litre of air can be aspirated, and so on. Finally, detach the tubing from cylinder, cover the ends with sterile wool, and incubate for twenty-four hours onwards.

This method has been largely superseded by simpler ones. All the microbes may not be caught on the gelatin surface.

3. *Frankland's Method.*—A tube, 5″ × ¼″, containing two plugs of glass wool. Aspirate a known quantity of air, remove the wool, and add to nutrient gelatin, and plate. The glass wool mixes with the gelatin.

4. *Petri's Method.*—Like Frankland's, only using sand instead of glass wool. There is apt to be some difficulty in distinguishing between colonies and grains of sand.

5. *Sedgwick and Tucker's Method.*—A specially shaped tube is used, in which are placed cotton plugs (3), and cane sugar is packed into a narrow part of the tube. One plug is removed, and air aspirated through the sugar (the whole apparatus having been previously sterilized at 120° C.). The plug is then replaced, the sugar is shaken down into a wider part of the tube, and liquid gelatin is poured in. The sugar melts into the gelatin, and a roll culture is made before the latter solidifies. Incubate at 22° C. and count colonies daily.

Organisms found.—Yeasts, Spores of moulds, Streptococcus brevis, B. coli, B. mycoides, B. enteritidis sporogenes, Streptococcus epidermidis, Streptococcus salivarius.

The last two serve to indicate pollution by particles of skin and saliva. The preceding ones indicate pollution by dirt from the street, brought in on the boots, if the air tested is that of a room ; or it may be blown in.

The effect of stirring dust is to increase the ratio of bacteria to moulds, whereas in still air, bacteria settle down much more rapidly than moulds. The purer the air, the more nearly do the numbers of the bacteria and of the moulds approximate. This is well shown in the figures found by the examination of 1 cubic metre of air (1000 litres or 220 gallons), taken in Paris and the suburbs, in the various seasons.

	Montsouris.		Centre of Paris.	
	Moulds.	Bacteria.	Moulds.	Bacteria.
Winter	145	170	1345	4305
Spring	195	295	2275	8080
Summer	245	345	2500	9843
Autumn	230	195	2185	5665

Particulate pollution of the air—by material from the upper respiratory passages from the skin and from the street—may all be detected by exposing plates filled with broth to the air for a definite time ; incubating anaerobically for forty-eight hours at 37° C., and examining for certain test organisms. (Gordon.)

BACTERIOLOGICAL EXAMINATION OF SOIL.

A quantity of soil may be collected in a platinum or metal spoon, or in a sterile tin trough, or by a Fraenkel's borer. A definite quantity is ground up in a sterile mortar and then mixed with known quantities of sterile water. Cultures are then made from definite quantities of these dilutions, and the number of B. coli, of streptococci, and of spores of B. enteritidis sporogenes, are determined and returned as so many per gramme of the original soil. Houston found 100,000 (of all bacteria) per grm. in sandy uncultivated soil, and 1,500,000 per grm. in garden soil. Spores are determined by adding 1 c.c. of a dilution to 10 c.c. of gelatin; heat to 80° C. for ten minutes to destroy non-sporing bacteria, plate, incubate, and count as late as liquefaction (if present) will allow. The examination for specific pathogenic bacteria is carried out by injecting animals subcutaneously, with some of the material or a dilution of it in normal saline (0·8 per cent).

Organisms Found.—The bacteria found in soil are :—

(*a.*) Putrefactive organisms—
- B. mycoides (earth bacillus).
- B. subtilis (hay bacillus).
- B. megatherium.

(*b.*) Nitrifying organisms or nodule bacteria—
- Nitroso-bacteria, or nitrite formers.
- Nitro-bacteria or nitrate formers.

(*c.*) Pathogenic organisms—
- Bacillus of tetanus.
- ,, of malignant œdema.
- ,, of quarter evil.
- ,, enteritidis sporogenes.

DUST, SEWAGE AND SEWAGE EFFLUENTS, AND EXCREMENTAL MATTERS.

These are examined on similar principles, except that a dry solid, like dust, may be weighed out, crushed in a sterile mortar, and made into a primary dilution with distilled water. Gordon found that in the dust collected at air inlet at House of Commons there were under 10 bacteria per

grm.; in debating chamber 100,000 per grm. (B. coli 1000, streptococci 10, B. enteritidis sporogenes 1000); in the division lobby 1,000,000 per grm. (containing 1000 of each of B. coli, streptococci, and B. enteritidis sporogenes); and in New Palace Yard, 100,000 per grm., of which 10,000 were B. coli, under 1000 streptococci, and 100 B. enteritidis sporogenes).

MILK.

In a healthy cow the milk within the udder is sterile. In the milk ducts and teats a certain number of bacteria may be found, even in healthy animals. From the position of the udder and the mode of milking, it is not possible to collect milk under aseptic precautions, and in the most favourable circumstances freshly taken milk in the pail will yield 100 to 500 bacteria per c.c. Under ordinary conditions the yield is much greater, varying from 2000 to 6000 per c.c where less care is used, and with careless manipulation (the usual method), 30,000 to 100,000 per c.c. With such a bacterial content from the beginning, it is not surprising that at summer temperatures the count in twenty-four hours should be enormous, reaching into millions and even hundreds of millions per c.c. The species found include almost all known varieties. Swithinbank and Newman describe 120 "milk bacteria," apart from organisms of water, soil, etc. The varieties found are there largely because of their presence in the environment, or their power to outgrow other species under the cultural conditions. Pathogenic bacteria are found in milk either (1) derived from the cow, e.g., tubercle bacillus, actinomyces, anthrax bacilli, streptococci, foot and mouth disease germ; or (2) from the introduction into it of infectious material of human origin, e.g., typhoid, diphtheria, germ of scarlatina, cholera; or (3) from the air, when kept in unsuitable conditions. Under (2), the milking of cows, or the handling of milk by persons suffering from disease or by acute or chronic carriers of disease germs, the use of infected water to rinse cans, and the pollution by flies, are the main acting causes. The milk of infected goats contains the germ of Malta fever. Besides these, milk contains many fæcal organisms derived from the

soiled and uncleansed skin near the udder, and from the dust of the byre.

Suggested Bacteriological Standard (Newman).—
(*a.*) Acidity of 100 c.c. + 2 c.c. phth. (0·1 per cent) to be not more than 25 c.c. N/10 alkali.
(*b.*) No excess of blood or pus cells.
(*c.*) No B. coli, B. enteritidis sporogenes, nor B. enteritidis (Gaertner) in 1 c.c.
(*d.*) The milk to be non-virulent.

The New York Milk Commission specify that certified milk shall contain not more than 30,000 bacteria per c.c., and in Philadelphia, the Commission standard is not more than 10,000 per c.c., and little difficulty has been experienced in conforming to this test. Boston has fixed a limit for ordinary milk of 500,000 per c.c., Milwaukee of 250,000, and Rochester, N.Y., of 100,000 per c.c. The average content in this country is 400,000 per c.c., as sold.

Microscopic Examination.—Shows round oil globules and a little epithelium. Abnormal constituents are: much epithelium, pus cells, conglomerate masses and casts of lacteal tubes, and colostrum.

Quantitative Examination.—Decimal mode of dilution. Add 9 c.c. of water to each of a number of test tubes, plug, and sterilize.

Add 1 c.c. of milk sample to No. 1 tube:
dilution = 1–10, or 1 c.c. = 0·1 c.c. milk.
Add 1 c.c. of No. 1 tube to No. 2 tube:
dilution = 1–100, or 1 c.c. = 0·01 c.c. milk.
Add 1 c.c. of No. 2 tube to No. 3 tube:
dilution = 1–1000, or 1 c.c. = 0·001 c.c. milk.
Add 1 c.c. of No. 3 tube to No. 4 tube:
dilution = 1–10,000, or 1 c.c. = 0·0001 c.c. milk.

And so on for any number of dilutions, mixing well each time, with sterile pipette.

Plate 1 c.c. of the various dilutions on gelatin and agar, and count colonies in twenty-four to forty-eight hours. (Begin plating with weakest dilutions, if the same pipette is to be used throughout.)

Qualitative Examination.—

1. For Fæcal Organisms.

Inoculate tubes of MacConkey's bile-salt glucose peptone (10 c.c.) with 1 c.c. from each dilution, beginning with the weakest (1 c.c.=0·000001 c.c. milk, or one-millionth), and continuing in reverse order. Also one tube with 1 c.c. of undiluted milk, and one tube of double strength broth (MacConkey's) with 10 c.c. of undiluted milk. Incubate for two days at 37° C. All the cultures showing acid and gas are subcultured as detailed under Water (page 353).

2. For B. Enteritidis Sporogenes.

Inoculate tubes containing 15 c.c. sterile whole milk, with 0·001 c.c., 0·01 c.c., 0·1 c.c., 1 c.c., 10 c.c. and a tube containing 30 c.c. with 100 c.c. Heat to 80° C. for 10 minutes for the smaller amounts, and 20 minutes for the larger. Incubate anaerobically at 37° C. for 2 days, and observe for the "enteritidis change or reaction" (page 319).

3. For Tubercle Bacilli.

Centrifugalize and take sediment, make a film, fix, clear with ether and alcohol, and stain for acid-fast bacilli. Failure to find them is inconclusive; on the other hand, all acid-fast forms found are not tubercle bacilli. In case of failure, or of success to prove nature, use the animal experiment. Centrifuge 250 c.c. of milk, add sterile water to sediment, and inject into a guinea-pig intraperitoneally. The animal is killed in three weeks (if it has not died before that time from septicæmia or severe infection), and the carcase sought for typical lesions. Among acid-fast organisms, the forms found in hay, butter, etc., are slightly pathogenic, but are easily distinguished by their rapid growth on cultivation. An attempt at cultivation from the lesions in the guinea-pig should therefore be made.

The following excerpt from Dr. J. Hume Patterson's Report for 1910, to the County M.O.H., Lanarkshire. details some experiments on the distribution of the tubercle bacilli in the "fore," "mid," and "strippings" milk:—

Tubercular Disease of the Udder of Cows.—MILK.—During the year (1910) 227 samples of milk from 225 cows with suspected disease of the udder were examined, 53 of which gave positive results, giving an average of 23·5 per cent. From the beginning of February, special attention has been given to the examination of smear preparations, in order that an immediate report might be given, instead of waiting three weeks for the animal inoculation test. Out of 44 specimens so examined, 31 were found positive. The milk in each case was stopped within two days of the taking of sample.

It might here be mentioned in connection with the examination of smear preparations, that an elaborate and complicated process of staining is not necessary, as a good result does not depend so much on the process of staining, as the time spent on the examination of the film. The method now adopted is the ordinary Ziehl-Neelson, the film, after discoloration, being well washed in absolute alcohol to clear it up.

All the samples giving negative smears were subjected to the animal inoculation test.

Twenty-seven samples of milk were obtained from four of the positive cows, for the purpose of ascertaining whether the "fore," "mid," or "strippings" milk contained the most bacilli, also whether the bacilli were more numerous in the cream than in the deposit. The samples were taken by the County Veterinary Surgeon.

In each case the whole of the milk from the affected quarter was drawn off in separate consecutive samples, the last one always containing the last drop of milk procurable.

Each specimen when received was centrifugalized for 15 minutes, and smear preparations made with one platinum loopful of the cream and of the deposit. The loopful was then thoroughly mixed on the slide and spread over an area the breadth of the slide and one inch in length. The films were stained by Ziehl-Neelson's method. The count, which of course was an approximate one, was carried out by running the $\frac{1}{12}$ oil immersion lens straight across the centre of the film, counting the bacilli in each field and multiplying the total by twelve. (The film being an inch in length and the lens used $\frac{1}{12}$). Where the bacilli were few in number, the whole film was examined and counted. The results obtained were as shown in the table on following page.

It will be seen from the results obtained from Cows I., II., and IV., that the "mid" milk was richest in bacilli, and would evidently be the proper part to take when such a milk was to be examined, more especially by smear preparation. No reliance could be placed on the samples taken from Cow No. III., as the milk was too small in amount.

	Specimen	Cream	Deposit
Cow No. I.—			
1		not done	negative
2		,,	,,
3		,,	8 bac.
4		,,	14 ,,
5		,,	negative
6		,,	6 bac.
7		,,	2 ,,
8		,,	8 ,,
Cow No. II.—			
1	Slight cream, $\frac{1}{4}$″ deposit	7 bac.	660 bac.
2	,, $\frac{1}{4}$″ ,,	8 ,,	912 ,,
3	,, $\frac{1}{4}$″ ,,	3 ,,	1,380 ,,
4	,, $\frac{1}{4}$″ ,,	5 ,,	3,816 ,,
5	Very slight cream, $\frac{1}{4}$″ deposit	5 ,,	2,196 ,,
6	,, ,, $\frac{1}{4}$″ ,,	4 ,,	636 ,,
7	,, ,, $\frac{1}{4}$″ ,,	2 ,,	1,104 ,,
8	Very watery. Thin layer of cream, 1″ pus-like deposit	0 ,,	396 ,,
Cow No. III.—			
1	Watery, slight cream, $\frac{1}{2}$″ pus-like deposit	0	168 bac.
3	Ditto ditto $\frac{1}{4}$″ ditto	0	192 ,,
4	Ditto ditto $\frac{1}{4}$″ ditto	0	252 ,,
	(Milk from quarter only amounted to about 2 ozs.)		
Cow No. IV.—			
1	Watery, slight cream, $\frac{1}{4}$″ deposit	120 bac.	5,172 bac.
2	,, ,, $\frac{1}{4}$″ ,,	1,020 ,,	6,804 ,,
3	,, ,, $\frac{1}{4}$″ ,,	1,212 ,,	5,916 ,,
4	,, ,, $\frac{1}{8}$″ ,,	1,584 ,,	14,460 ,,
5	,, very slight cream, $\frac{1}{8}$″ deposit	364 ,,	10,908 ,,
6	,, heavy cream, $\frac{1}{16}$″ deposit	2,532 ,,	5,316 ,,
7	,, ,, $\frac{1}{4}$″ blood-stained deposit	1,824 ,,	4,824 ,,

From the samples taken from Cows II., III., and IV., it was clearly shown that the deposit is the proper part of the specimen to take for the examination, it containing by far the greater number of tubercle bacilli.

4. For Actinomyces.

Make a film from sediment, and stain by acid-fast method. Actinomycosis in the udder of the cow is usually alleged to be rare, but is stated to be more common in the sow. In an article on "The Occurrence of Actinomycosis in Cows' Udders," by Dr. J. Hume Patterson, in the *Journal of Meat and Milk Hygiene* (vol. i,

No. 1, Jan. 1911), the writer cites evidence which goes to show that it may not be so uncommon. Out of fifty specimens from different udders submitted to him for suspected tubercle, in five cases the lesions proved to be actinomycotic. The lesions in each case were indistinguishable by the naked eye from those of tuberculosis. On cutting into the substance of the udder numerous cream-coloured foci, similar to tubercles, were seen, ranging from the size of a pin-head to that of a pea. The part affected was also of a brownish tint, as is so often seen in tuberculosis of this tissue. Smear preparations showed elements of actinomyces in four of the cases; in one, no elements were found, but on making sections typical actinomyces were found. All the others were confirmed by making paraffin sections. In one of the smears both tubercle bacilli and actinomyces were found; and if not on one's guard, such a case could be readily dismissed as tuberculosis without looking for actinomycosis. Dr. Patterson says: "I am confident from my experience in these cases that if each suspected tubercular udder were subjected to a microscopical examination, the percentage of actinomycotic udders would be greater than is generally supposed.

In regard to milk, many of the samples, taken by veterinary surgeons inspecting dairy herds from cows having what appeared to be marked tubercular lesion of the udder, have proved negative even on animal inoculation. Dr. Patterson asks the question: "Might these not be cases of actinomycosis?" and cites the following case:—

"During last winter's inspection (1909–10) a case occurred where the lesion of the udder was markedly nodular and similar to tubercle. A sample of the milk was taken, and a guinea-pig inoculated with negative result. Not satisfied with this result, samples were again taken from all four quarters of the same cow. These samples again proved negative on animal inoculation. Smear preparations from these last samples, made from the deposit of the centrifugalized milk, showed a few acid-fast rod-shaped, and a few fragments of club-shaped, elements suggestive of actinomyces, in those samples taken from both hind quarters and from the left fore quarter. I was unable to procure the udder for further examination, but am convinced that this was a case of actinomycosis. In actinomycosis of the human subject, it is

yet doubtful how infection takes place, as the cereal theory has been partly exploded, through cases arising which had no connection with grain; and I think it is just possible infection may be conveyed by the milk of such a cow as I have quoted, where the elements of the disease were found in the fluid. If that be so, this disease, as affecting the udder of the cow, warrants more attention than is given to it at the present moment in connection with our milk supplies." In this regard the present writer was consulted in 1910 by a young man of about twenty years, who had recently returned to Scotland from Canada. He had been treated in several hospitals for tuberculosis. The history being irregular, his spit was sent to the City Bacteriological Laboratory (Glasgow) for examination with this note: "If you do not find tubercle bacilli, look for something else." Dr. Sutherland, who examined the specimen, found actinomyces, and the diagnosis was confirmed later in the Glasgow Royal Infirmary. He died some months later, but a post-mortem examination was not obtained. The history bearing on the point at issue is this: About two years previously he left his position as an office boy in Glasgow, and went to near Calcary, Alberta, Canada, where he became a farmer's boy. One of the cows he had to milk had a chronic sore on its udder, and he was warned to milk this cow gently, so as not to make the sore bleed. After about one year he took ill at this farm with a sore throat, followed by a swollen right submaxillary gland. The gland was incised and healed well. Then another swelling appeared, and another, and so on. This certainly looked like a milk infection, but verification was not possible.

5. For Johne's Bacilli.

The prevalence of Johne's disease in cattle in Great Britain being now well established, the bacilli may find their way into the milk from the diarrhœal stools in the earlier stages of the disease. The bacilli are shorter than the tubercle bacilli, but are equally acid-fast and alcohol-fast. Twort has cultivated them on egg media.

6. For Streptococci.

Houston advises the use of the medium of Drigalski and Conradi (lactose-nutrose-agar) in plates. The plates are inoculated by smearing over the surface of them 0·1 c.c. of each of the dilutions given above. Incubate twenty-four to

forty-eight hours at 37° C., and subculture the minute colonies formed into broth, and repeat cultivation as to time and temperature. Make films from the broth and examine microscopically, and if found in pure culture, subject the organism to the differential tests for streptococci and to nitrate broth test. According to Houston, 58 per cent of the Streptococci fæcalis of the cow are of the lamirasacsal variety ; that is, clot milk and ferment lactose, raffinose, saccharose, and salicin. It forms short chains, and is not pathogenic to mice. The S. pyogenes does not clot milk, ferments lactose, saccharose, and sometimes salicin, but does not ferment raffinose, and is pathogenic to mice, forms long chains, and does not reduce nitrates.

Leucocyte Test.—In testing for chronic mastitis in cows, Trommsdorff found that the deposit of leucocytes after centrifugalizing the milk in a specially-shaped tube, was a good guide as to the necessity for further investigation. In an enquiry on these lines, he found 20 per cent of chronic mastitis in cows, and it was associated with the presence in very large numbers of capsulated streptococci. Such cows give less milk The milk must be drawn directly from the animal before one can say that the streptococcus is from the udder of the cow.

Centrifugalization of Milk

Is used to precipitate the gross dirt, pus cells and leucocytes, and bacteria. According to Scheurlen, the ordinary milk bacteria in the proportion of 75 per cent of them go into the cream, as do also the organisms of anthrax, typhoid, and cholera. The other 25 per cent of these remain in the separated milk. On the other hand, tubercle bacilli are largely found in the sediment, a few only passing into the cream and separated milk.

Fermentations.

Lactic Acid.—The development of acid and curd occurs normally in milk on keeping It is due to the formation of lactic acid from the milk sugar or lactose, by the action of enzymes produced by microbic growth. Many species of bacteria are able to produce the lactic fermentation, but in milk those most commonly causing it are : (1) Bacillus

lactis aërogenes, and (2) Streptococcus lacticus (Kruse), identical with the B. acidi lactici (Leichmann). Heinemann, who has investigated the subject, states that the two species are ordinarily present in naturally souring milk, the former in abundance at the beginning, the latter in the later stages when the acidity has reached a high degree. The secret of the regularity of the presence of these two species is their power of withstanding a much higher degree of acidity than the other species present at the first. In changing to lactic acid, the lactose is believed to be first hydrolyzed into glucose and galactose.

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6 = 4C_3H_6O_3$$

Coagulation of casein follows on acidification of the milk, the amount of acid necessary to precipitate the casein averaging 0·45 per cent; the terminal amount may reach 0·85 per cent. The casein precipitated by lactic acid formation is never redissolved, because the high acidity inhibits the proteolytic ferments.

Casein precipitation, however, may also be due to a non-acid coagulation caused by bacterial ferments. Casein precipitated in this way may be redissolved by a bacterial trypsin or casease, produced by the same or other bacteria, and the milk hence may become entirely liquid, transparent, and of a yellowish colour.

B. bulgaricus in milk culture produces 2·5 per cent of lactic acid, and 0·05 per cent of acetic and succinic acids, is non-pathogenic, and exerts no putrefactive action upon proteids. Metchnikoff suggested its use in milk cultures as a food to inhibit, by its acid production, the growth in the intestine of the class of bacteria which break up proteids, the bacteria of putrefaction. This is Metchnikoff's bacteriotherapy, which has been extensively practised.

B. bulgaricus is a large, non-motile, non-sporing, Gram-positive bacillus, with square ends (like B. anthracis). It forms short and long chains. It shows little or no growth on ordinary media or below 37° C. Optimum temperature: 42° C. Grows in dextrose-peptone broth, to which calcium carbonate has been added.

Butyric Acid fermentation of milk occurs occasionally in milk from the growth of anaerobic bacteria. It is a

much slower process than the lactic one, and can also be produced by some pathogenic anaerobes, e.g., bacilli of quarter-evil, malignant œdema, B. Welchii and B. enteritidis sporogenes (Klein).

Alcoholic Fermentation of milk occurs spontaneously on rare occasions. The process is due to the natural introduction of yeasts, and once started can be kept going by infecting fresh milk. Koumiss is thus made by the Tartars from mare's milk. Kefir is an effervescent sour milk made from cow's milk by the addition of "kefir grains," little cauliflower-like excrescences whose fermentative power is due to Saccharomyces mycoderma. Mare's milk is more suitable for the preparation, because the lactic fermentation also accompanies the other, and the duration of the double fermentation is conditioned by the amount of sugar, which is 5·5 per cent in mare's milk and 4·8 per cent in cow's. The latter is richer in casein and fat, and both of these constitute disadvantages, so that it is usually diluted in making koumiss. In the making, the milk is stirred constantly by day but rested at night. The amount of alcohol in all these products—koumiss, kefir, and cow's milk koumiss—is under 2 per cent, and the Tartar is capable of consuming three to four gallons of such milk on a hot summer's day without becoming more than hilarious, and with no digestive disturbance. They have been much used as "consumption cures."

Diseases of Milk.

Unusual or abnormal changes in milk are sometimes referred to as "diseases." They are produced by bacteria which have got into milk in various ways. Blue, green, and yellow milks are due respectively to the bacilli cyanogenes, erythrogenes, and synxanthus; and red milk to B. prodigiosus. Bitter milk is due to a number of species, yeasts and diplococci having been isolated. Slimy or ropy milk has been traced to B. lactis viscosus, said to be a water organism. Slimy milk is produced at Edam (Holland) by the use of a streptococcus, for the manufacture of Edam cheese. Soapy milk is due to a micrococcus derived from fodder.

BUTTER.

Butter is made from the cream of milk by churning or agitation, whereby the globules of fat are broken, or rather have their casein envelopes ruptured, and then the fat globules adhere. The cream is first allowed to sour, otherwise the butter will be flavourless. The souring is brought about by bacteria, nearly all of which are lactic acid formers. Tuberculosis is the only infective disease transmitted by butter. Tubercle bacilli have been found alive and virulent in butter after having been kept in a refrigerator for five months. Rabinowitch's acid-fast butter bacillus is easily distinguished culturally from the tubercle bacillus. Foot and mouth disease has been reported as having been transmitted by butter. Typhoid infection is unlikely, and has not yet been definitely traced.

CHEESE.

Cheese is the precipitated casein of milk, the casein or curd being insoluble. In hard cheeses the whey is better expressed than in soft cheeses, and so the subsequent "ripening" which is due to bacterial growth is less in the former than in the latter. In the ripening of the curd, three groups of bacteria are engaged, (1) acid producers, like B. acidi lactici, (2) casein digesters, which break down the curd, and (3) gas producers, which honeycomb it. The actual organisms engaged have been determined in the case of particular cheeses, and are: a bacillus resembling the Bacillus subtilis, a mould (Oidium lactis), a penicillium mould, and yeasts. Tubercle bacilli of the bovine and human types have been found in cheeses.

SHELLFISH.

For Houston's methods, see *Journal of Hygiene*, vol. iv, No. 2, p. 185.

WATERCRESS AND OTHER VEGETABLES.

See *Report to the L.C.C.*, by Houston, 1905.

DISINFECTANTS.

A disinfectant or germicide is a substance which destroys the microbic causes of disease. The same substance in a weaker strength may act as an antiseptic, that is an agent which restrains or checks the growth of bacteria without destroying them. A deodorant is a substance which destroys or masks the offensive effluvia or vapours resulting from bacterial growth, and may or may not have an action on the bacteria themselves. The best example of an agent capable of all these functions is potassium permanganate, which in strengths of 5 per cent and over is a disinfectant, under 5 per cent is an antiseptic, and in all strengths is a deodorant. (Solubility, 1–18 of water; 1–3 boiling.) The mode of action of disinfectants varies. Some, like the strong acids, char or carbonize the bacterial body by oxidizing the other elements present. Others coagulate the bacterial protoplasm; while some, diffusing through the cell wall, exert a poisonous action on the protoplasm. Antiseptics like sugar, probably act by osmotic pressure through the cell wall, causing a flow of water out of the cell, thus drying up the protoplasm and rendering the bacterium inert for the time being.

The chief disinfectants are the metallic salts, acids bases, the halogen elements, oxidizing agents, alcohols phenols, aldehydes, and the essential oils. The salts, acids, and bases act best in watery solution as against alcoholic solutions. Two explanations of this are offered: (1) That the salts, etc., in water dissociate into their ions, and the latter are more active in producing chemical change; in alcohols, dissociation does not take place. (2) That the alcohol hinders the action of the disinfectant by hardening the bacterial cell wall. In favour of the second reason it has been noted that while absolute alcohol is useless as a germicide, added to aqueous mercuric chloride solution in the proportion of 25 per cent it increases the efficiency of the disinfectant. The addition of NaCl, on the other hand, diminishes the efficiency, it is believed by reducing the number of free ions.

The halogens (Cl, Br, I) are efficient in the order given. Chloride of lime liberates free Cl when treated with an

acid. When used simply in solution, oxygen is liberated in the nascent state, and oxidizes any organic matter present ($CaOCl_2 + H_2O = CaO + 2HCl + O$). Peroxide of hydrogen and potassium permanganate act similarly as oxidizers.

Carbolic acid (phenol) and the cresols (lysol and creolin) do not dissociate, and their efficiency is increased by the addition of NaCl and diminished by alcohol. Formaldehyde is not helped by adding salt, but alcohol is harmful.

Standardization. — The efficiency of any disinfectant depends on many factors, namely, the strength used, the solvent, the temperature, the bacterium to which applied, the time allowed for action, the other substances present, the number of bacteria present. For practical purposes the strengths are expressed as percentages; but in comparisons it is more scientific to work with solutions of the molecular weight or multiples thereof in one litre.

Coefficient of Inhibition. — This term is applied to the strength (of a chemical substance) which is able to prevent the growth of a micro-organism; that is, its antiseptic value.

It is determined by making broths or other media containing the chemical substance to be tested, in a range of strengths. Equal quantities of the bacterium used in the test are inoculated into the various tubes (say, one loopful), the contents mixed, and incubated. In the case of solid media, they are poured into plates and the colonies (if any) counted. In broth cultures, look for turbidity, and confirm positive or negative results by making films. The coefficient is expressed in terms of strength and bacterium used. Thus, carbolic acid is said to inhibit the growth of anthrax bacilli when present in a strength of 1 part in 800 of medium. B. typhosus requires 1–400, and Sp. choleræ, 1–600. Of corrosive sublimate, 1–100,000 inhibits B. anthracis, and 1–60,000 inhibits B. typhosus.

Germicidal, Bactericidal, or Disinfectant Strengths. —Koch used anthrax spores dried on silk threads, which he immersed in various strengths of substance being tested, at a definite temperature and for varying times.

The threads were then removed and carefully washed in sterile water to discharge the disinfectant. They were then laid on gelatin and incubated, and the result was noted. This method is faulty, in that it is difficult to remove the disinfectant, some of which clinging to the bacteria inhibits growth. The Rideal-Walker method may be applied here to determine germicidal strength without comparison with carbolic acid.

The results are stated in terms of strength used, time exposed, and bacterium killed: thus *carbolic acid* is bactericidal to anthrax spores at ordinary temperatures in 1–20 dilution in four to forty-five days; at 40°C., in three hours.

To Sp. choleræ at ordinary temperatures, 1–200 is fatal in five minutes, and 1–300 is fatal in two to twenty-four hours.

To B. typhosus at ordinary temperatures, 1–50 is fatal in five minutes.

To staphylococci and streptococci at ordinary temperatures, 1–60 is fatal in five minutes.

Corrosive sublimate is fatal to anthrax spores in 1–2000 in twenty-six hours.

To anthrax and typhoid bacilli and Sp. choleræ, in 1–2000 in five minutes.

To staphylococci and streptoccoci, 1–10,000 to 1–2000 in five minutes.

These tests supply useful data, but cannot be taken as applying to the action of the same disinfectants, when mixed with the body fluids.

Rideal-Walker Test.—In this test carbolic acid is taken as the standard disinfectant, and the results of tests of other disinfectants are expressed in terms of their power, compared to the standard, of inhibiting growth of the same organism in the same time. This is called the "carbolic acid coefficient" of the particular disinfectant.

Process.—A series of accurate dilutions of pure carbolic acid and of the disinfectant are prepared in sterile distilled water. A twenty-four hours' culture at 37° C. in Lemco broth (reaction + 1·5) of B. typhosus is used as the test organism. A standard size of platinum loop is used to make the subcultures. The disinfectant solutions are

arranged in a series of tubes, each containing 5 c.c., and the dilution of the disinfectant is marked on each tube.

To 5 c.c. of a particular dilution, five drops of the filtered culture are added; the tube is shaken and set down. Now repeat with the next dilution, and so on. At the end of 2·5 minutes, a subculture is made from the tube first inoculated, into 5 c.c. of sterile broth, and similarly with all the dilutions, in the proper order. At the end of 5, 7·5, 10, 12·5, and 15 minutes, put up further subcultures thus, making six series of subcultures in all from each tube. The same process is carried out, at the same time, with the same culture, with dilutions of carbolic acid. All the subcultures are incubated for at least forty-eight hours at 37° C., and the presence or absence of growth is noted. From the table of results, the two dilutions doing the same work in the same time are seen. Say that 1–500 of Disinfectant A inhibited growth in 2·5, 5, 7·5 minutes' exposures, and that carbolic acid 1–110 did the same; then the carbolic acid coefficient of Disinfectant A is $500 \div 110 = 4{\cdot}5$.

Since its introduction this test has been much used. It has also been subjected to much criticism. It deals with "naked" bacteria. The other objections are that it must be carefully done, all the dilutions should be accurate, the organism used should be the same throughout, etc. These are not indictments of the test in careful hands, and where the tests on different disinfectants are made by the same individual. Still the results obtained by this test should not be taken for more than they pretend to be, the relative power of different bodies to carbolic acid under the same conditions. The application of such results to ordinary purposes must be made very cautiously.

The "Lancet" Commission Test.—This is a modified Rideal-Walker test, in which the number of dilutions is increased up to nine, and the time periods correspondingly up to thirty minutes; B. coli is used as the test organism; MacConkey's bile-salt litmus glucose peptone water is used as the medium for subcultures instead of broth; platinum spoons are used instead of loops, and the method of calculation is different.

The B. coli used is cultivated for twenty-four hours at

37° C. in a broth made by mincing 1 lb. of fat-free bullock's-heart meat; macerate it in cold water for two to three hours; cook over a small gas flame for two to three hours more; boil; filter; make up to a litre; add 10 grm. each of NaCl and Witte's peptones; and standardize to an acidity of 1·5 per cent to phenolphthalein. To obtain an emulsion from the culture, it is well shaken and then filtered through a double layer of Swedish filter-paper. The carbolic acid dilutions were made at first by taking ac. carbolic B.P. 110 grm. = 100 grm. pure phenol. Later, dry crystalline carbolic acid was taken as 100 per cent phenol. The dilutions were always freshly made, as it was found that otherwise they lost some of their germicidal power, even when kept corked and in the dark. The disinfectant dilutions are made with distilled sterile water, and 5 c.c. of each are put in small glass specimen pots, 2·5 in. × $\frac{7}{8}$ in., arranged in holes on a board. These pots are left uncovered during the experiment, and the results are not vitiated owing to the MacConkey's media used for the subculturing, inhibiting most other organisms than B. coli. Owing to the platinum spoon holding three times the amount in a standard loopful, 10 c.c. are used for the subculture medium. To assist in rapid work a special wheel has been devised, to hold the spoons, when not in use, in a sterilizing flame.

Process.—All the apparatus being ready and to hand, the dilutions of the unknown disinfectant are "seeded," each getting a spoonful of the B. coli emulsion, and being well stirred. The seeding is begun at the strongest dilution and proceeds to the weakest. At the end of 2·5 minutes, a spoonful is removed from the first dilution seeded and added to a tubeful (10 c.c.) of MacConkey's broth, properly labelled. The same process is repeated with all the tubes, and is all gone over again after 5, 7·5, 10, 12·5, 15, 20, 25, and 30 minutes.

The same procedure is followed with dilutions of carbolic acid.

The subcultures are incubated for forty-eight hours at 37° C. A positive result is indicated by the medium turning red, and the formation of bubbles of gas. In some tubes this change will appear in twelve to fourteen

hours as a purple tinge, becoming pink and then red in another two hours. The results are filled into a tabular form, with a plus sign for growth and a zero for no growth.

The coefficient is thus calculated: The weakest dilution of the sample under test, giving no growth at 2·5 minutes, is divided by the weakest dilution of carbolic acting similarly in the same time; the weakest dilution giving no growth at thirty minutes is divided by the same for carbolic acid; the two results are averaged, and the average or mean is taken as the carbolic acid coefficient. Thus, if Disinfectant B gives no growth with 1–220 in 2·5 minutes and with 1–340 in 30 minutes, and if the dilutions for carbolic acid are 1–110 and 1–180, then the coefficient would be:—

$$\left(\frac{220}{110} + \frac{340}{180}\right) \div 2 \text{ or } (2 + 1{\cdot}88) \div 2 = 1{\cdot}94.$$

The Commission prefer to express their dilutions as percentages, and when this is done the mode of division is reversed; that is, the weakest percentage (of carbolic, etc.) is divided by the weakest percentage of the test substance, etc. The above dilutions become for B, 0·454 per cent and 0·294 per cent, and for carbolic, 0·909 per cent and 0·555 per cent respectively. The coefficient therefore is $\left(\frac{0{\cdot}909}{0{\cdot}454} + \frac{0{\cdot}555}{0{\cdot}294}\right) \div 2$ or $(2 + 1{\cdot}85) \div 2 = 1{\cdot}92$. The temperature of the room averaged about 62° to 67° F.

By this method the coefficients ranged from 0·025 to 9·8 for the usual coal-tar disinfectants on the market. Applied to corrosive sublimate, the coefficient at 2·5 minutes was about 2000, and at 30 minutes about 6000, giving a mean of about 4000. Chloride of lime similarly gave figures of 45 and 93, or a mean of 69. For formalin the coefficient was 0·6.

Among the conclusions reached were the following: That results obtained in such bacteriological experiments, although giving a germicidal value to a disinfectant under the most favourable conditions, afford little indication of their germicidal value when used in practical disinfection. That much remains to be done in the solution of such problems (amongst others) as arise, due to: (1) The presence of foreign substances in the material to be disinfected; (2) The temperature at which the disinfecting

process is carried on; (3) The kind of water used for dilution—hard water, soft water, or sea water; (4) The type of micro-organism that has to be dealt with; (5) The nature of the substance to be disinfected and the character of its surface; (6) The time to be allowed for the process. That only when the influence of such factors can be calculated will it be possible to modify any standard co-efficient figure, and thus to obtain data for the preparation of effective and economical dilutions for the practical problem of disinfection.

The inquiry has shown that, so far as emulsions are concerned, those which contain the highest quantity of phenoloids in the finest state of division and having the least tendency to combine with albumins, lime, or other foreign substances in solution (and remain combined), will be found to be the most efficient disinfectants.

The remarkable parallelism between the results of this inquiry (as shown in the figures for the coefficient) and the results of the independent chemical one, is very striking. The chemical commissioners discovered that if they subtracted the carbolic acid equivalent of the bromine absorbed by the percentage of phenoloids present, from the percentage of phenoloids present, and divided the difference by 3, the figures obtained in many instances are the same (or very nearly so) as those assigned as the carbolic acid co-efficient for the same substance by the bacteriological commissioners. In the exceptions the disinfecting fluids did not form an emulsion with water, nor show Brownian movements. (See p. 142.)

$$\frac{P - B}{3} = \text{C.C.}$$

Tables showing the results of the examination (on these principles) of the disinfectants in common use, are given in the *Lancet* for 1909, vol. ii.

APPENDIX.

REGULATIONS FOR THE DIPLOMA IN PUBLIC HEALTH.

I.—The Council, having regard to the terms of Section 18 of the Local Government Act (1888) and of Section 54 of the Local Government (Scotland) Act (1889), and observing that under those sections special privilege is to be accorded to the holders of the diplomas granted under Section 21 of the Medical Act (1886), and therein described as Diplomas in Sanitary Science, Public Health, or State Medicine, thinks it essential to declare, with regard to its own future action under Section 21 of the Medical Act (1886), that it will not consider diplomas to "deserve recognition in the Medical Register" unless they have been granted under such conditions of education and examination as to ensure (in the judgment of the Council) the possession of a distinctively high proficiency, scientific and practical, in all the branches of study which concern the public health; and the Council, in forming its judgment on such conditions of education and examination, will expect the following rules to have been observed:—

RULE 1. The curriculum for a Diploma in Sanitary Science, Public Health, or State Medicine shall extend over a period of not less than nine calendar months.

RULE 2. Every candidate for a Diploma in Sanitary Science, Public Health, or State Medicine shall have produced satisfactory evidence that, after obtaining a registrable qualification, which should be registered before admission to examination for the diploma, he has received practical instruction in a laboratory or laboratories, British or foreign, approved by the licensing body granting the diploma in which Chemistry, Bacteriology, and the Pathology of the Diseases of Animals transmissible to Man are taught.

Note.—The laboratory instruction shall cover a period of not less than four calendar months, and the candidate shall produce evidence that he has worked in the laboratory for at least 240 hours, of which not more than one-half shall be

devoted to practical chemistry. The laboratory course should be so arranged as to lay special stress on practical work which bears most directly on the duties of a medical officer of health.

RULE 3. Every Candidate shall have produced satisfactory evidence—

Either (1) that, after obtaining a registrable qualification, he has during six months been diligently engaged in acquiring a practical knowledge of the duties, routine and special, of public health administration, under the personal supervision of (*a*) In England and Wales, the medical officer of health of a county or of a single or combined sanitary district having a population of not less than 50,000, or a medical officer of health devoting his whole time to public health work ; or (*b*) In Scotland, a medical officer of health of a county or counties, or of one or more districts having a population of not less than 30,000 ; or (*c*) In Ireland, a medical superintendent officer of health of a district or districts having a population of not less than 30,000 ; or (*d*) In the British dominions outside the United Kingdom, a medical officer of health of a sanitary district having a population of not less than 30,000, who himself holds a registrable Diploma in Public Health ; or (*e*) A medical officer of health who is also a teacher in the department of public health of a recognized medical school ; or (*f*) A sanitary staff officer of the Royal Army Medical Corps having charge of an army corps, district, command, or division, recognized for this purpose by the General Medical Council ; or (*g*) An assistant medical officer of health of a county or of a single sanitary district having a population of not less than 50,000, provided the medical officer of health of the county or district in question permits the assistant officer to give the necessary instruction and to issue certificates ;

Or (2) That he has himself held for a period of not less than three years an appointment as medical officer of health of a sanitary district within the British Dominions, and having a population of not less than 15,000.

Note 1.—The certificate for the purpose of Rule 3 (1) must include testimony that the candidate has attended under the supervision of the person certifying on not less than 60 working days. Provided that if the candidate has :—(i) Produced satisfactory evidence that he has attended a course or courses of instruction in sanitary law, vital statistics, epidemiology, school hygiene, and other subjects bearing on public health administration, given by a teacher or teachers

in the department of public health of a recognized medical school; *or* (ii) Produced evidence that he has been a resident medical officer in a hospital for infectious diseases containing not less than 100 beds, during a period of three months—the period during which he has been engaged in acquiring practical knowledge of his duties under this rule may be reduced to three months, to include an attendance on at least 30 working days.

Note 2.—For the districts, commands, and divisions that have been recognized by the Council under Rule 3 (1) (*f*) see below.

RULE 4. Every candidate shall have produced evidence that, after obtaining a registrable qualification, he has attended during three months at least twice weekly the practice of a hospital for infectious diseases at which candidate has received instruction in the methods of administration.

Note 1.—Methods of administration shall include the methods of dealing with patients at their admission and discharge, as well as in the wards, and the medical superintendence of the hospital generally.

Note 2.—In the case of a medical officer of the Royal Army Medical Corps a certificate from a principal medical officer under whom he has served, stating that he has during a period of at least three months been diligently engaged in acquiring a practical knowledge of hospital administration in relation to infectious diseases, may be accepted as evidence under Rule 4.

*** The Rules 2, 3, 4, as to study, shall not apply to medical practitioners registered, or entitled to be registered, on or before Jan. 1st, 1890.

RULE 5. The examination shall have been conducted by examiners specially qualified; it shall have extended over not less than four days, one of which shall have been devoted to practical work in a laboratory, and one to practical examination in, and reporting on, subjects which fall within the duties of a medical officer of health, including those of a school medical officer.

II.—The Council shall, from time to time, appoint an inspector or inspectors of examinations in public health, with special instructions to report to the Council whether the examination of each licensing body does or does not afford evidence, on the part of candidates passing such examination,

of a distinctly high proficiency, scientific and practical, in each and all of the branches of study which concern the public health.

List of the districts and commands that have been recognized by the Council under Rule 3 (*f*) :—

Aldershot.
Salisbury Plain.
Southern and South-Eastern.
Western.
Dublin and Belfast.
Cork.
Chatham and Woolwich.
Home.
Eastern.
North-Eastern and North-Western.
Scottish.
Gibraltar Command.
Malta Command.

The following Indian Divisions, viz. :—
1st (Peshawar).
2nd (Rawalpindi).
3rd (Lahore).
5th (Mhow).
6th (Poona).
7th (Meerut).
8th (Lucknow).
9th (Secunderabad).
Burma.
Quetta

PRESERVATIVES IN MILK AND CREAM.

The Local Government Board, England, in February, 1912, in the exercise of its powers under the Public Health Acts, drafted regulations prohibiting the use of preservatives in milk and defining the conditions under which preservatives may be used in cream.

PRESERVATIVES IN MILK.

Article III.: " 1. No person shall add, or order or permit any other person to add, any preservative substance to milk intended for sale for human consumption. 2. No person shall sell, or expose or offer for sale, or have in his possession for the purpose of sale, any milk to which any preservative substance has been added in contravention of subdivision (1) of this article." The expression " milk " includes separated, skimmed, condensed, and dried milk; but as the traffic in condensed milk would be seriously impeded if the use of sugar were disallowed, it is provided that " neither cane nor beet sugar shall be regarded as a preservative or a thickening substance."

PRESERVATIVES IN CREAM.

" 1. No person shall add, or order or permit any other person to add, (*a*) any thickening substance to cream or preserved cream; (*b*) any preservative substance to cream containing less than 40 per cent by weight of milk fat; (*c*) to cream containing 40 per cent or more by weight of milk fat any preservative substance other than (i.) boric acid, borax, or a mixture of these preservative substances, or (ii.) hydrogen peroxide, in amount not exceeding 0·1 per cent by weight, in any case in which the cream is intended for sale for human consumption. 2. No person shall sell, or expose or offer for sale, or have in his possession for the purpose of sale, any cream to which any thickening substance or any preservative substance has been added in contravention of the provisions of subdivision (1) of this article." Every seller of preserved cream will be required in every invoice, bill, advertisement, trade list, or other document which is used in connection with the sale of preserved cream, to describe the article as (*a*) preserved cream (boracised), or (*b*) preserved cream (peroxidised), as the case may be. (This provision will come into force on

January 1, 1913.) Dealers in cream, preserved in a manner which does not contravene the above regulation, will be required, by means of labels on the receptacles, to declare that the cream is preserved and to state the name of the preservative. In this matter the regulations are precise, laying down in a schedule the size of the label, which varies according to the capacity of the receptacle, and prohibiting the attachment to any receptacle of a label bearing a trade description which would be likely to mislead a purchaser as to the utility of the preservative substance. In tea shops and other refreshment rooms the cream jugs will not be required to be labelled if in each room a conspicuous notice is affixed indicating that the cream supplied is preserved cream, or if a statement to that effect is printed on the bills of fare. The regulations are made under the Public Health (Regulations as to Food) Act, 1907, and any person who wilfully neglects to carry out the regulations is liable to a penalty not exceeding £100, and in the case of a continuing offence to a further penalty not exceeding £50 for every day during which the offence continues. The penalties for offences against the regulations are those for which provision is made by Sub-section (3) of Section 1 of the Public Health Act, 1896, but before the local authority institutes proceedings against any person they will be required to afford him an opportunity of explaining the circumstances in which any irregularity may have occurred. With the exception of the provision relating to the labelling of preserved cream, which takes effect on Jan. 1st, 1913, the regulations take effect from June 1st, 1912.

BOVINE AND HUMAN TYPES OF TUBERCLE BACILLI.*

1. Smith Reaction.

Theobald Smith, in 1896, first drew attention to the existence of two types of tubercle bacilli in mammals, and in 1898 he published a systematic comparative study of bacilli isolated from man and cattle, and pointed out the differences between the two types as summarised on page 277. Pursuing the research further, he found that when grown in slightly acid glycerin broth, the two types give different reaction curves.

Glycerin Broth.—The human type caused at first a lessening of the acidity of the medium until it became nearly but not quite neutral; thereafter the acidity increased until it again approached or slightly exceeded the original reaction.

The bovine type in the early stages of its growth rendered the medium less acid, neutral, or even alkaline; and then it may so remain or become acid again, but never up to the original degree.

The British Royal Commission for this test also used *glycerin litmus milk* (milk freed from cream, plus 5 per cent of glycerin, plus 5 per cent of 5 per cent watery solution of Merck's purified litmus). They found that all human viruses which grew vigorously on this medium at first caused an increase of alkalinity, and later acidity and clotting; if the growth was less vigorous, acidity resulted without clotting. The bovine viruses which grew vigorously in the medium caused acidity finally, but never clotted the milk; the poorly growing viruses left the milk alkaline. They concluded that the reaction curves can be so grouped as to form a scale of the final reactions with complete gradations from one type to the other.

2. Cultural Characters (p. 15).

The results of our work have led to the conclusion that there is no constant qualitative cultural difference between

* Abstracted from Vol. V. of "Collected Studies from the Research Laboratory, Department of Health, City of New York, 1910," by Park and Krumwiede, and others.

the human and bovine types of tubercle bacilli. Quantitatively, and with respect to the effect of glycerin, however, there is a marked difference in the great majority of cultures, so great in fact that almost without exception the type can be determined from cultures alone. This difference is constant in one factor only, viz., amount and rapidity of growth in early cultures. In classifying our cultures according to this characteristic, we can broadly say that all bovine types of bacilli are dysgonic (sparse growth) and all human types of bacilli are eugonic (moderate or luxuriant growth). The question remains, What is the best medium for eliciting this difference? Any medium used must fulfil certain conditions.

1. It must be especially adapted for the growth of the eugonic viruses, so that the best possible growth is obtained.

2. It would be preferable if the growth of the dysgonic virus were somewhat retarded on the medium. This will widen the gap as far as possible.

3. The medium must be nearly uniform in its results; that is, growth should not fluctuate with the different batches of medium used.

We have found that glycerin egg is by far the best medium for this purpose. . . . Cobbett (Royal Commission) made comparisons of the two types of organisms on both serum and glycerin serum. While the human cultures were far more vigorous with the use of glycerin serum, the bovine cultures were either restrained, or if aided by its presence, the increase in growth was slight. This difference he found in early cultures to be of diagnostic value in the separation of the two types. We also noticed that primary cultures of the bovine type repeatedly failed on glycerin egg, whereas the primary cultures of the human type were usually markedly increased in luxuriance. The reading of Cobbett's results led us to adopt glycerin egg as the basic differential medium.

Giving then the results in terms of glycerin, the following general conclusions may be drawn.

(*a*). All cultures* growing luxuriantly on glycerin egg from the start are of the human type.

(*b*). All cultures* growing sparsely, or even not at all, on

* No direct cultures were attempted. Guinea-pigs were inoculated and killed in three to five weeks, and inoculations made from tuberculous lymph nodes and spleen on to plain egg media (Dorset), glycerin egg media (Lubenau), and glycerin potato. Intravenous inoculation into rabbits was used in confirmation of type of culture. If rabbit survived injection of 1 mgr. of culture for 60 days, then human type.

glycerin egg in the first few generations are of the bovine type.

Glycerin egg (Lubenau). Ten eggs are blown into a flask and 200 c.c. of 5 per cent glycerin bouillon (neutral or moderately alkaline) added. The further preparation is as for plain egg medium.

3. Results.

Table of Series of Non-selected Cases of every Type of the Disease, showing Type of Bacillus Isolated.

Form of Tuberculosis	Adults 16 years and over		Children 5–16 years		Children 0–5 years	
	Human	Bovine	Human	Bovine	Human	Bovine
Pulmonary	278	—	8	—	5	—
Adenitis, cervical ..	9	—	19	8	6	12
Do. inguinal and axillary ..	1	—	4	—	—	—
Abdominal	1	—	1	1	—	3
General (alimentary in origin)	—	—	—	—	1	1
General	2	—	1	—	12	4
*General + Meningitis	—	—	—	—	18	1
Meningitis	—	—	1	—	14	1
Bones and Joints ..	1	—	10	—	6	—
Genito-urinary ..	3	1	1	—	—	—
Abscesses	1	—	—	—	—	—
435	296	1	45	9	62	22
	297		54		84	

4. Conclusions (Park and Krumwiede) *loc. cit.* p. 134.

Tubercle bacilli as isolated from man fall into two groups. One of these groups is identical in all its characters with that found in cattle. That is, all tubercle bacilli from man and cattle fall into two groups, which have been designated the human and bovine types.

Each type shows certain differences, the most important for separation being those culturally and in virulence. The great majority of cultures group themselves around two extremes, from which there are a few cultures showing variant characteristics. There is no overlapping of characteristics. The two types are probably different because of residence in different hosts over long periods of time, and as such are stable. The evidence in favour of rapid change of type is incomplete and inconclusive.

* Includes 1 double infection: Mesenteric nodes gave human type, meningeal fluid gave bovine type.

INDEX