Researches on micro-organisms : including an account of recent experiments on the destruction of microbes in certain infectious diseases, phthisis, etc / by A.B. Griffiths.

MICRO-ORGANISMS

A. B. GRIFFITHS

With the Compliments of

A. B. Griffiths.

RESEARCHES

ON

MICRO-ORGANISMS:

INCLUDING AN ACCOUNT OF

RECENT EXPERIMENTS ON THE DESTRUCTION OF MICROBES IN CERTAIN INFECTIOUS DISEASES—PHTHISIS, Etc.

BY

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AUTHOR OF 'THE DISEASES OF CROPS,' ETC.

ILLUSTRATED WITH FIFTY-TWO FIGURES.

LONDON:
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1891.

To

DR. ARMAND GAUTIER,

Professeur à la Faculté de Médecine de Paris, Membre de l'Institut, Ancien Président de la Société Chimique de Paris, etc.,

AND

DR. P. MIQUEL,

Directeur du Laboratoire Micrographique à l'Observatoire de Montsouris (Paris), etc.

(TWO DISTINGUISHED WORKERS IN THE DOMAIN OF BACTERIOLOGY AND PHYSIOLOGICAL CHEMISTRY),

This Work is affectionately Dedicated

BY THEIR SINCERE FRIEND,

THE AUTHOR.

PREFACE.

As a knowledge of bacteriology is of so much importance in therapeutics, pathology, chemistry, and other sciences, I hope the present work may be of some utility to those interested in the study of microbes.

The work gives an account of some of the most important investigations in connection with pathogenic and non-pathogenic microbes.

It does not profess to be a treatise on, or a manual of, bacteriology, but an *exposé* of recent researches in various branches of the subject—especially where such researches throw light on the pathology, therapeutics, etc., of infectious diseases.

Although this work, in a great measure, has a bearing on the *treatment* of certain infectious diseases, I may say that I have had no desire to trespass in the domain of the physician. In the words of Pasteur, 'I am neither physician nor surgeon,' yet the scientist (generally having more leisure for scientific investigations than either the physician or the surgeon) can be of great assistance to the medical profession.

Hence, in presenting this volume to physicians, scientists and others, I hope that it may prove of some utility in pointing out the value of a proper understanding of the new science of bacteriology, and its applications to pathology, medicine, surgery, and other sciences.

I take this opportunity of expressing my sincere thanks to Dr. Armand Gautier and Dr. P. Miquel, for valuable information on the subject of ptomaïnes and micrography respectively, and for the interest they have taken in my own researches.

I am also grateful to Dr. Carl Zeiss (of Jena), Dr. Roux (of the Pasteur Institute), Dr. A. C. Maybury (of London), Dr. J. C. Williams (of Blackpool), Dr. R. Wood (of Bromsgrove), Dr. G. Sée (of Paris), and Messrs. F. E. Becker and Co. (of London), for their generous assistance in various parts of the book.

My obligations are due to the President and Council of the Royal Society of Edinburgh for the loan of certain wood-blocks, illustrating my own papers on microbes, which were originally printed in the Society's publications; also to my sister (Miss Mildred H. Griffiths), for her help in preparing certain drawings for the illustrations.

I may add that I have received the constant assistance of my wife (*née* Frances E. Wright), whose knowledge of botany has been no mean help to me in many ways.

A. B. GRIFFITHS.

EDGBASTON,
November, 1890.

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RESEARCHES ON MICRO-ORGANISMS.

CHAPTER I.

INTRODUCTION.

During the troublous period when the Prince of Orange was at the head of affairs in Holland, Anton Leuwenhoeck, of Amsterdam, employed his spare time in the manufacture of magnifying glasses, which he improved to such an extent that he was capable of seeing some of the smallest organisms. 'In April, 1675, Leuwenhoeck placed beneath his microscope a glass tube full of rain-water, and with great surprise he noticed in the water wonderful forms—little bells which expanded and contracted themselves, and little globules which shot quickly here and there. At first he thought he saw the living atoms which, according to the philosophy of Democritus, composed all bodies, and from whose whirling motion his contemporary, Descartes, believed the world built itself anew. But Leuwenhoeck soon became satisfied that these were animalcules, invisible to the naked eye, but which in varied forms animated the drop of water. Other animal and plant forms were found later in great abundance, in infusions of pepper, hay, and animal and vegetal materials, and for that reason were named *Infusoria*.'

Such was the birth of the subject we are about to

describe in the following pages. 'Leuwenhoeck ("the father of microscopical anatomy," as Sir Richard Owen calls him) was little aware how large a prospect of organic life he was opening to our view when, in 1675, he communicated to his scientific friends* his discovery of the little bell-shaped animalcule, now known as one species of an immense class, and called the *Vorticella convallaria*.'

'Exactly a century after Leuwenhoeck, Müller, an investigator in Denmark, having devoted twelve years of his life to the observation of these smallest of creatures, found, named, and described about three hundred and eighty different forms in the fresh and salt waters of Copenhagen.'

In the next century the number of microscopic observers increased rapidly. With more accurate instruments, Schwann (the author of the cell theory), in 1837, opened new ground, by showing that the phenomena of alcoholic fermentation are connected with the presence and life of certain minute plants, particularly the yeast plant (*Torula cerevisiæ*).

Chemists were at first unwilling to admit the important part played by the *Torula* in fermentation.

Fermentation to them had always been a chemical action. The decomposition of numberless molecules of sugar into alcohol and carbonic acid gas, with *the evolution of heat*, was surely a chemical action. But yeast must always be present before the change takes place, so the chemists assumed the existence of a very obscure physico-chemical phenomenon to which the name of *catalysis*, or action by presence, was given. They fought hard their catalytic theory of fermentation until Dumas, in 1843, and Pasteur, in 1857, clearly explained the *physio-*

* *Philosophical Transactions, Royal Society*, vol. xii., p. 821.

logical function of the living ferment, or yeast. The subsequent researches of Pasteur and others have shown 'that every fermentation has its specific ferment; in all fermentations in which the presence of an organized ferment has been ascertained, the ferment is necessary.'

The first researches on the sterilization of flasks, etc., were made by the Abbé Spallanzani, in the last century; and Schwann (in the present century) first showed that putrefaction was due 'to something suspended in the air which heat was able to destroy.' In 1854 Schröder and Dusch (*Annalen der Chemie und Pharmacie*) introduced the sterilized cotton-wool plug, which in these days plays so important a part in the investigation of microbes.

But the year 1863 will be ever memorable in the annals of medicine, on account of the first discovery of microscopical organisms in contagious diseases. In that year, Davaine (*Comptes Rendus de l'Académie des Sciences*, vol. lvii., pp. 220, 351, 386) observed in the blood of animals suffering from anthrax, or splenic fever, numberless fine thread-like forms, or microbes, which multiplied themselves by division. The microbe of anthrax was designated the *bactéridie du charbon* by Davaine, but was subsequently proved by Cohn to be a bacillus (*Beiträge zu Biol. d. Pflanzen*, vol. ii.). Previous to the researches of Davaine, Drs. Pollender and Brauell, in 1855 and 1858 respectively, had noticed this microbe in the blood of animals dead of anthrax, but they simply recorded the fact; and Davaine subsequently proved its pathogenic nature.

From 1863 down to the present time, numerous workers have raised the study of microbes into a separate science—now called 'bacteriology'; and we find chemists like Pasteur, botanists like Cohn and De Bary, medical men like Koch, Virchow, Klein, Sanderson, and others,

all aiding one another in building up this new science, which is destined to raise 'medicine' from empiricism to that of an exact science.

The connection between microbes and disease is of the utmost importance to medical men and others; but it must not be supposed because the blood and tissues of man and animals (suffering from contagious disease) contain certain microbes, that these microbes are necessarily the cause, or even indirectly the cause, of the disease. Not until the investigator has obtained, by pure cultivations in an artificial sterilized medium, the microbes in a perfectly pure state, and then, by injecting into the blood, etc., of a healthy animal a small portion of the purified culture, the disease is reproduced, can one say that a particular disease is the result of the life-history of a certain microbe.

In fact, Koch (*Die Milzbrand-impfung*, 1883) has laid down the following axioms to ascertain whether a microbe is (directly or indirectly) the *causa causans* of a particular disease:

α. 'The microbe in question must have been found either in the blood, lymph, or tissues of the man or animal which has died of the disease.'

β. 'The microbe taken from this medium (blood, tissues, etc.), and artificially cultivated out of the animal's body, must be transferred from culture to culture for several successive generations, taking the precautions necessary to prevent the introduction of any other microbe into these cultures, so as to obtain the specific microbe, pure from every kind of matter proceeding from the body of the animal whence it originally came.'

γ. 'The microbe, thus purified by successive cultures, and reintroduced into the body of a healthy animal capable of taking the disease, ought to reproduce the disease

in question in that animal with its characteristic symptoms and lesions.'

δ. 'Finally, it must be ascertained that the microbe in question has multiplied in the system of the animal thus inoculated, and that it exists in greater number than in the inoculating liquid.'

It is not the author's object in the present volume more than to allude to the various methods used by bacteriologists in obtaining pure cultivations of microbes, etc., nor is it his intention to describe fully the apparatus, etc., used in these investigations; but before we consider the relationship of microbes to contagious diseases and certain recent researches thereon, a *few details* will be given in the next chapter concerning the apparatus required and the methods used for the study of microbes. This may be some help to those readers whose knowledge of the *technique* of the subject is not very extensive, to understand the methods of investigating the problems described in the following pages.

CHAPTER II.

BACTERIOLOGICAL APPARATUS, ETC.

The Microscope. — Microbes being the smallest, the simplest, and the lowest of known living forms; being, so to speak, the boundary-line of life, beyond which it does not exist, so far at least as present microscopic expedients reach, it is essential that the working bacteriologist should be provided with a first-class microscope with high and low powers. In our experience no better instruments are made than those by Carl Zeiss, of Jena.* Fig. 1 represents one of Zeiss' large microscopes, and when provided with the following objectives:

D, E, J (water immersion), $\frac{1}{12}$ (oil immersion),

is suitable for the complete investigation of the subject. To these should be added the whole of Zeiss' huyghenian eyepieces. Zeiss' lenses give perfect definitions, and everything there is to be seen can be made out with the highest powers.

Zeiss no longer makes the $\frac{1}{18}$ oil-immersion objective; this lens having lately been superseded by the introduction of a new series of objectives—the apochromatic lenses—made of the new glass.

As it is essential to have a brilliant illumination for the examination of microbes, and especially for the examination of stained tissues, the bacteriological microscope

* Sold by C. Baker, 243, Holborn, London.

must be provided with Abbé's substage condenser (Fig. 2), which is also made by Zeiss. This condenser, first described by Professor Abbé, in the *Archiv für Mikr.*

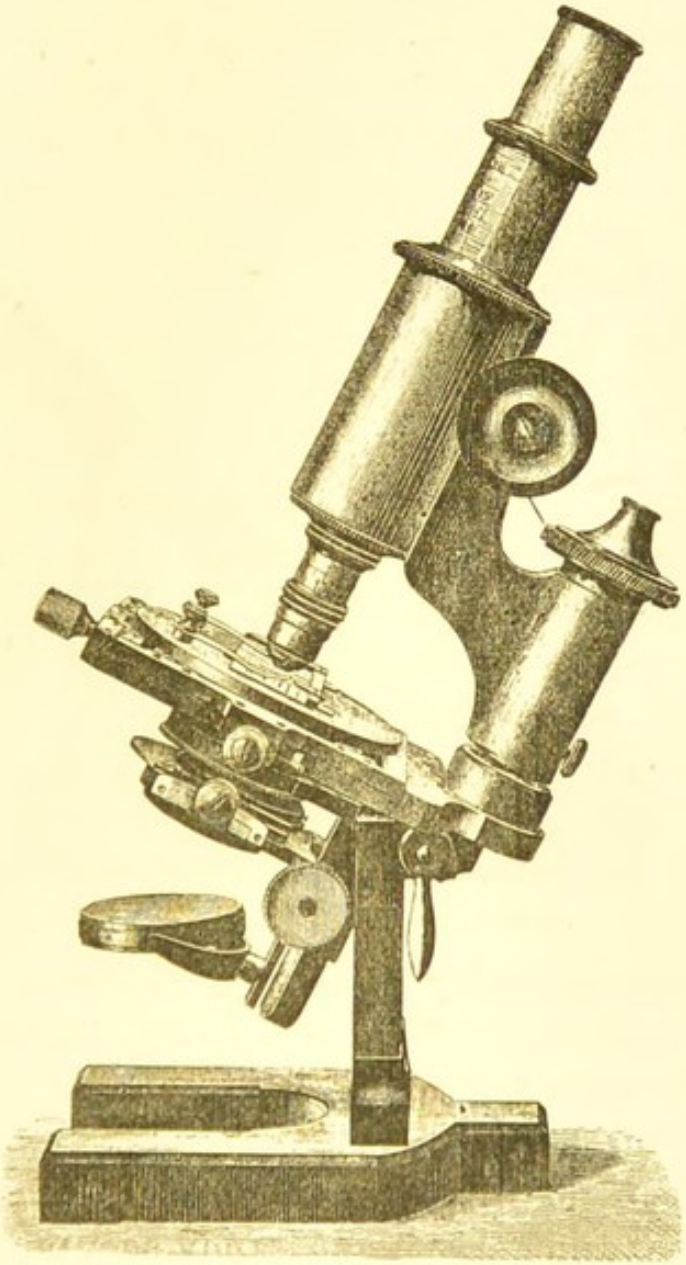


FIG. 1.—ZEISS' MICROSCOPE.

Anat. (vol. ix., p. 496), is of very short focus, and collects the light reflected by the mirror into a cone of rays of very large aperture, and projects it on the object.

The Microtome.—There are several forms in use.

Körting's (*Jenaische Zeitschrift*, vol. xiv.), Reichert's, Schanze's, and Jung's are principally used in France and Germany; while Williams's, Roy's, and Cathcart's are freezing microtomes, and are much in vogue among histologists in this country. Perhaps the most useful form for cutting pathological sections is Jung's microtome, now used in the Pathological Institute of Berlin.

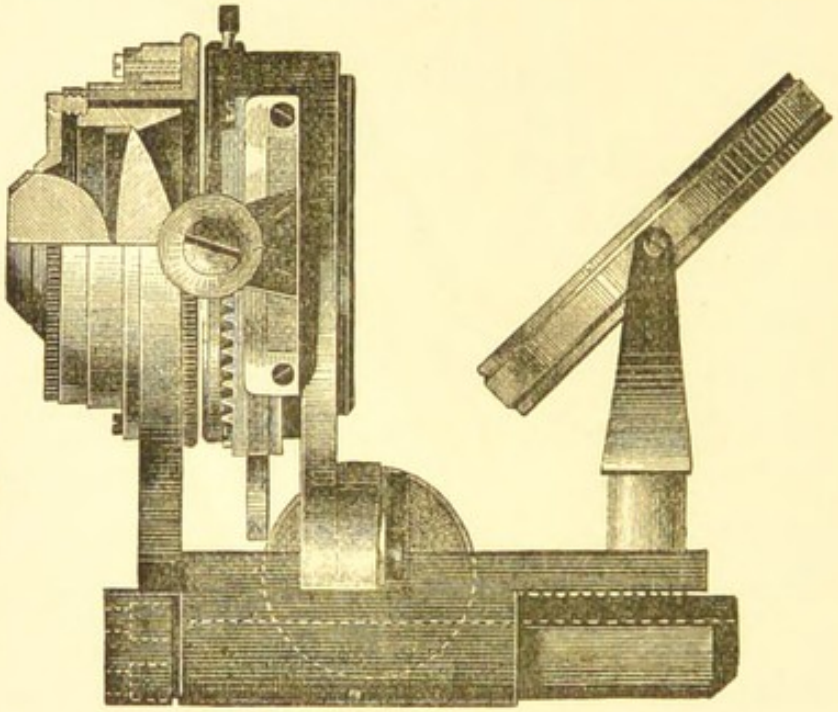


FIG. 2.—ABBÉ'S CONDENSER.

Photo-Micrographic Apparatus. — There are three methods for illustrating microscopic specimens:

i. By using the camera lucida.
ii. Accurate drawings made by hand.
iii. Photography.

Concerning the use of photography as a means of illustrating microbes, either in diseased tissues or isolated, the majority of workers have spoken favourably of the method. Among these may be mentioned Koch, Van Ermengem, Crookshank, and Hauser. On the other hand, Dr. E. Klein, F.R.S. (*Micro-Organisms and Disease*, p. 14), does not attach much importance to photography as a means of illustrating bacteriological preparations.

Zeiss of Jena, Swift of London, and Nachet of Paris, all make excellent photo-micrographic apparatus, and

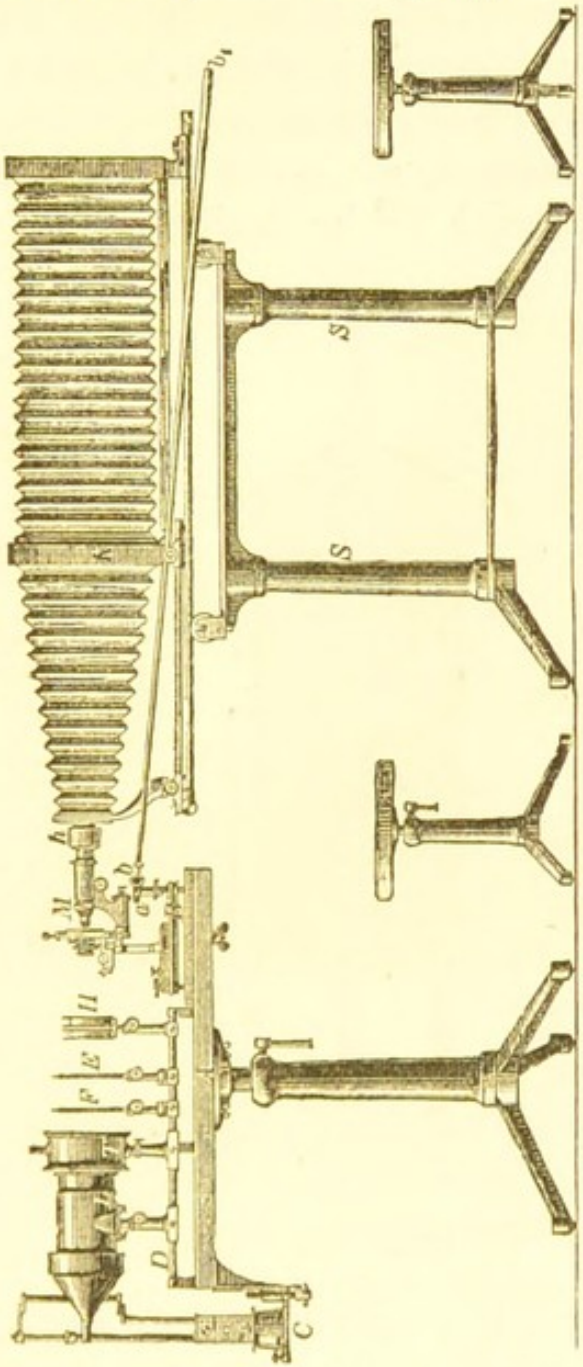


FIG. 3.—ZEISS' PHOTO-MICROGRAPHIC APPARATUS.

Fig. 3 represents the former maker's large apparatus,* which can be used with sun-light, electric-light, lime-

* A and S = tables for microscope and camera respectively.
B = plate to carry microscope (with levelling screws).
E and F = two diaphragm carriers for use with sunlight.
C = electric lamp. H = holder for taking absorption cells.
T = a water chamber for absorbing heat rays. K = camera.
L = collective lens-system for projecting the image of the carbon points on the focussing screen. M = microscope.
a, *b*, *b'* *h* = focussing apparatus.

light, etc. Several authors have recommended the use of the iso-chromatic dry-plates, and no doubt these are most useful (*vide* Van Ermengem in the *Bulletin de la Société Belge de Microscopie*, No. x., p. 170; and Crookshank's *Photography of Bacteria*).

Hardening Reagents, etc.—For a description of the various reagents used for hardening tissues before cut-

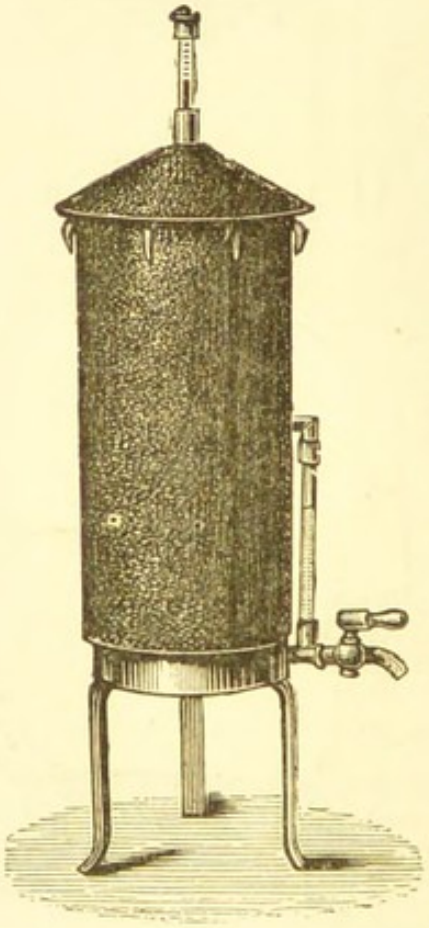


FIG. 4.—STEAM STERILIZER.

ting sections with the microtome, the author refers his readers to manuals specially devoted to bacteriology.

Sterilizers.—The complete sterilization of all vessels, instruments, stoppers, cultivating media, etc., before use is absolutely necessary in a study like bacteriology. Nothing but the utmost care in obtaining pure cultivations of microbes can guarantee satisfactory and success-

ful investigations. For the accomplishment of this object in those laboratories devoted to the study of microbes steam, hot-air, and Bunsen or spirit flames, are chiefly used as sterilizing agents.

Fig. 4 represents Dr. R. Koch's steam sterilizer. This is used for sterilizing test-tubes, flasks containing various cultivating media, and for 'cooking' potatoes. It is a

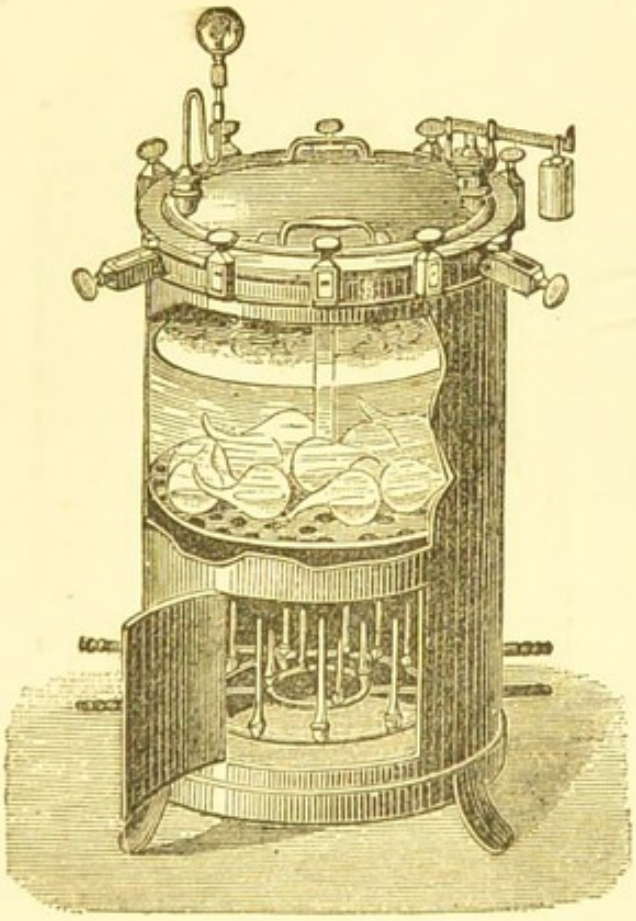


FIG. 5.—STEAM DIGESTER.

cylindrical vessel divided into two chambers. The lower one contains boiling water, while the steam therefrom passes into the upper sterilizing chamber. 'Steaming is kept up for from fifteen to twenty minutes.' This operation is repeated 'on three successive days each time for twenty minutes.'

Another form of steam sterilizer is represented in Fig. 5,

and is used in the laboratories of the French school of bacteriologists. It is made of stout copper, and is used for heating sealed flasks (containing bouillon) under pressure. The cultivating broth or bouillon may by this be exposed to a temperature ranging from 100° to 120° C.

Two forms of *hot-air* sterilizers are illustrated by Figs. 6 and 7 respectively, and are used for the sterilization of test-tubes, flasks, cotton-wool, etc.

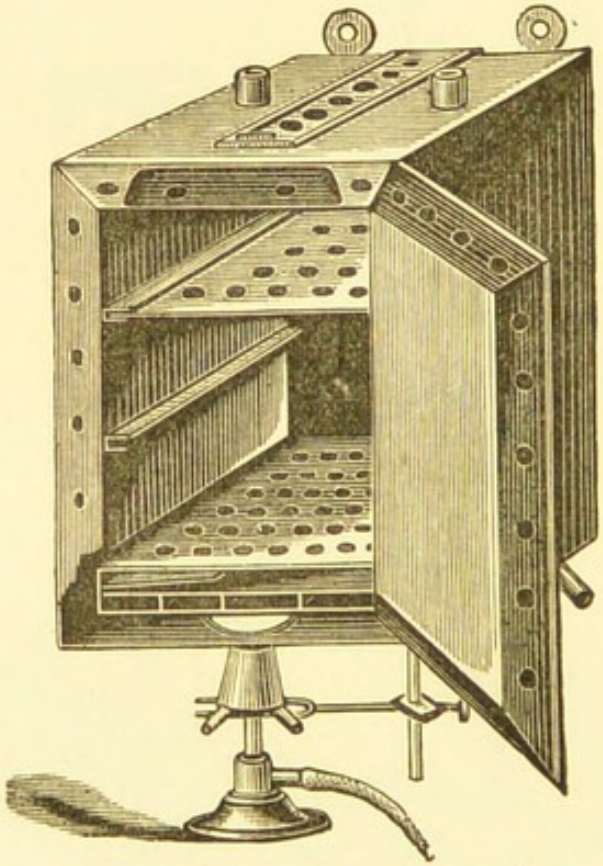


FIG. 6.—HOT-AIR STERILIZER.

The sterilizer Fig. 6 is made to hang against the wall of the laboratory, and consists of a double wall of sheet-iron. It is heated by a gas-burner.

In Fig. 7 the oven is heated by means of a paraffin-oil flame. It is a useful form in places where gas is unavailable. This sterilizer was first described by the author in the *Proceedings of the Royal Society of Edinburgh*,

vol. xiv., p. 105, and can be used with gas when required.

All good hot-air sterilizers should allow the tubes to

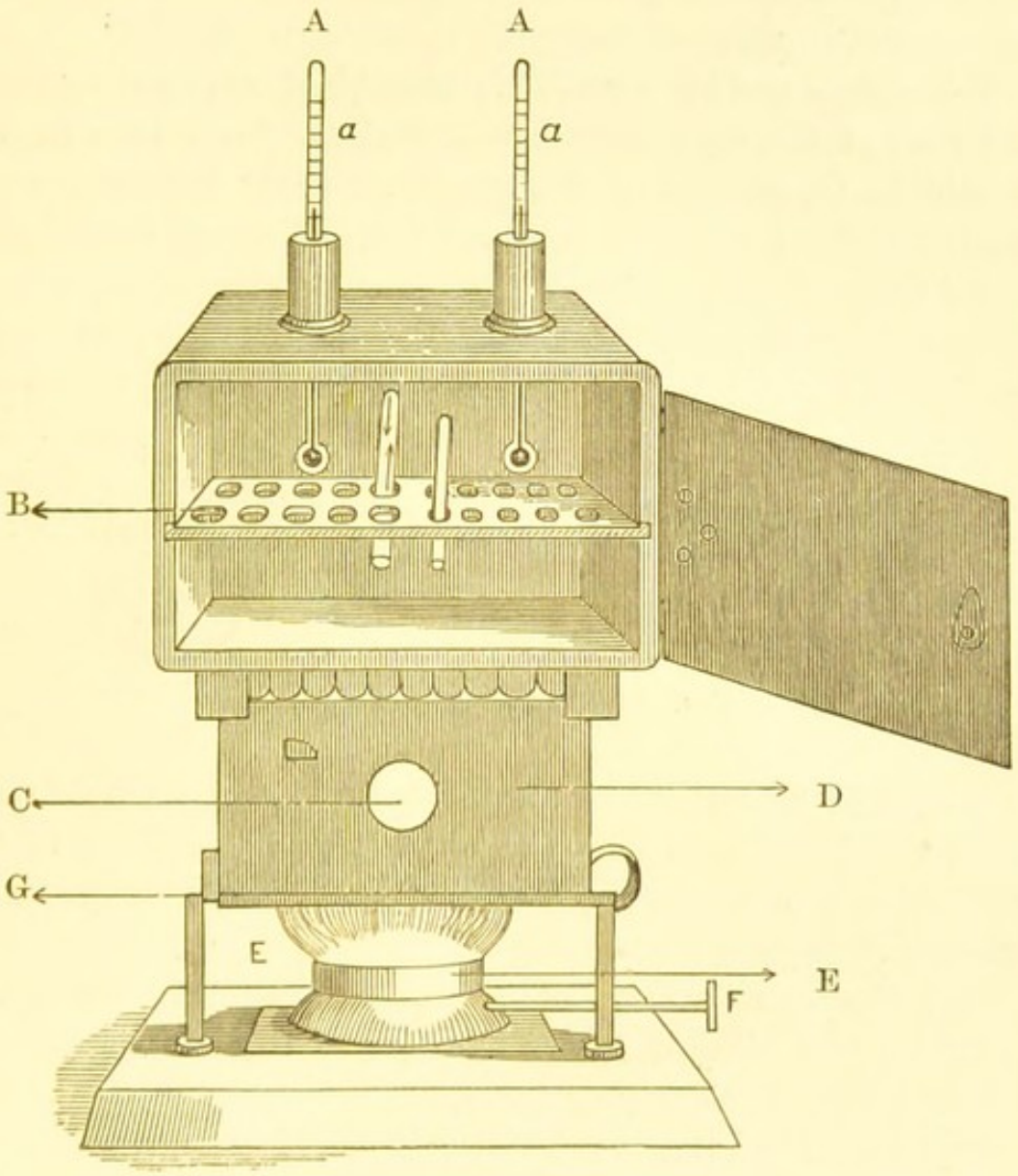


FIG. 7.—HOT-AIR STERILIZER HEATED BY PARAFFIN-OIL OR GAS.
A = thermometers (*a*). B = copper shelf with holes of different sizes.
C = Mica window. D = iron support for oven over flame.
E = paraffin-oil lamp. F = screw to raise wick. G = wire-gauze

be placed in a vertical rather than a horizontal position. By this means the heated air rises in the inverted tubes,

and the current so formed (in each tube) destroys all the organisms and spores present therein. When the sterilizer Fig. 7 is heated by means of a paraffin-oil lamp, the temperature often rises as high as 150° C., and much higher with gas.

Test-tubes (to be sterilized) should be exposed to the full heat of the oven for several hours. After this they should be taken out of the sterilizer while hot, plugged with sterilized cotton-wool, and then reheated for a few hours longer.

Beakers and glass funnels may also be sterilized in the hot-air sterilizer, or by being heated over a Bunsen flame.

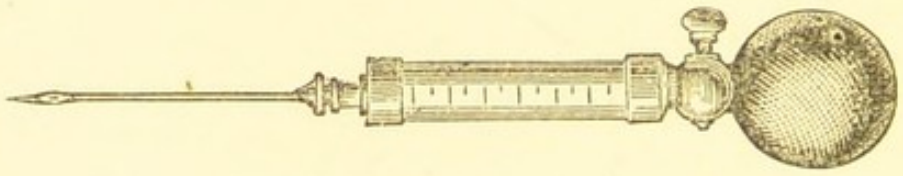


FIG. 8.—INJECTION SYRINGE.

To prepare sterilized cotton-wool, place the wool in a loose condition, and heat it in the hot-air sterilizer to a temperature of about 150° C., for several hours on several successive days.

For the sterilization of platinum needles, forceps, scalpels, and similar instruments, the Bunsen flame is the best way of cleansing them.

But the naked flame is most destructive to the blades of scalpels; to obviate this, Israël's case was devised to overcome the difficulty. It is a sheet-iron box in which the knives, etc., are exposed to a temperature of 150° C. in the ordinary hot-air sterilizer for an hour or so.

Syringes used for hypodermic injections or inoculations should always be sterilized before being used. As the ordinary syringe (Fig. 8) cannot be heated without de-

struction, it is advisable to immerse it in a strong solution of mercuric chloride or mercuric iodide, and after this the syringe should be washed with sterilized hot water.

Dr. Klein uses, for inoculation, a capillary pipette made of glass tubing (about twelve inches long), and 'drawn out into fine points.' This pipette is only made just before it is required for use, and is never used again. 'In this way,' Klein says, 'I am always absolutely safe from any contamination with a previously-used virus, which might possibly adhere to one or other part of a syringe.' And no doubt he is correct, especially when one bears in mind the recent sad death of Dr. Hoffmann, of Vienna. It may not be out of place, in passing, to allude to this case.

On the authority of the *Daily News* (October 29, 1889), a patient died of glanders in the General Hospital of Vienna, and Dr. Rowalski had obtained pure cultivations of *Bacillus mallei* (the microbe of glanders) from the body of the dead man, when Dr. Hoffmann ('an ambitious young physician') expressed his doubts as to whether the bacilli reared artificially had still their virulent properties. Rowalski then handed Hoffmann a pure cultivation of the microbe, which the latter soon found still retained its deadly power.

At the beginning of October, Hoffmann 'caught cold and felt acute pains in his side,' which he tried to cure by injecting morphia. He did this with the syringe which he had used for injecting the glanders virus into the bodies of the animals under experimentation. Although *the syringe had been disinfected by the Bunsen flame*, some particles of the virus must have still been in it, for Hoffmann developed the disease with all its characteristic symptoms, and ultimately his body was covered with ulcers, death occurring on October 22nd.

After death the ulcers were proved to be filled with the bacilli of glanders.

This case shows how important it is that all instruments and vessels used in the study of microbes should be thoroughly sterilized before use.

In continuing the subject of sterilization, the next piece of apparatus to be described is the serum sterilizer

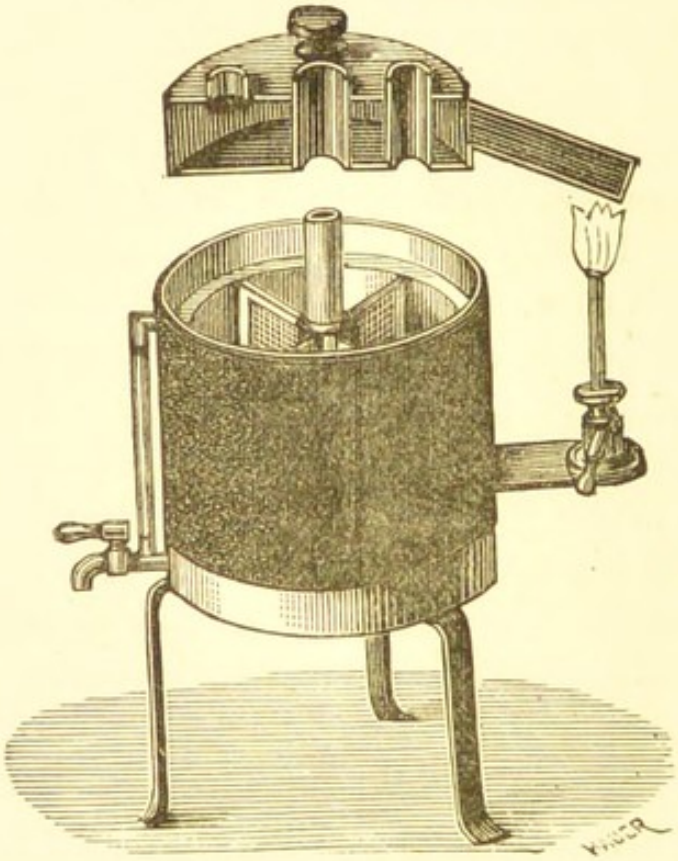


Fig. 9.—Serum Sterilizer.

(Fig. 9). It consists of a double-walled cylindrical case (13 in. high by 11 in. diameter), provided also with a double-walled lid. The whole apparatus, which is divided internally by four partitions, is essentially a 'hot-water jacket.' The water is heated by means of a gas-flame placed beneath the bottom of the sterilizer, while the water contained in the lid is heated by means of a tubular prolongation (see figure). This apparatus is employed in

the sterilization of blood serum, which is used for the cultivation of *Bacillus mallei*, *Bacillus tuberculosis*, and a few other microbes. The temperature of the sterilizer should be maintained for one hour at 60° C. on five or six successive days. 'This completes the sterilization, but to *solidify* the serum it is necessary to arrange the tubes (containing it) in the inspissator (Fig. 10) at the

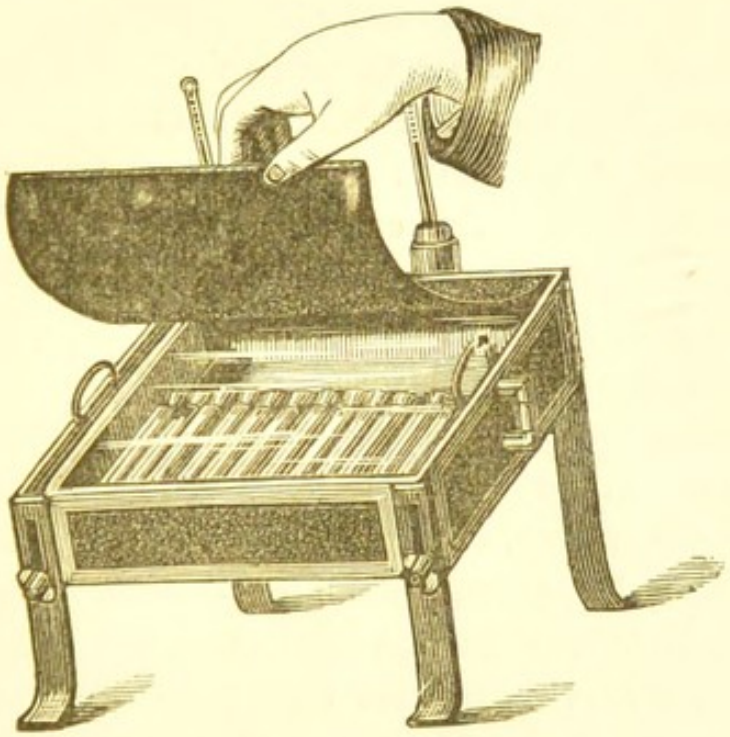


FIG. 10.—SERUM INSPISSATOR.

angle required. The temperature of this apparatus is kept between 65° and 68° C.' (Crookshank).

Incubators.—For the artificial cultivation of microbes in solid or liquid media, it is necessary to maintain them at a suitable temperature (between 20° and 38° C.) for several hours, days, or weeks, as the case may be, or, in other words, until a new growth is visible. For this purpose various forms of incubators or ovens have been devised. Among these may be mentioned Rohrbeck's and D'Arsonval's, which are cylindrical forms, while

those of Pasteur, Babès, Gautier, and Hueppe are more or less rectangular in shape.

The incubator of Pasteur (Fig. 11) is used at L'École Normale, Paris, and L'Institut Pasteur in the Rue Dutot. It is an excellent incubator for large laboratories, and is capable of being heated to 45° C.

Rohrbeck's (Fig. 12) is another good incubator, and is suitable for maintaining at a constant temperature cultures in tubes, flasks, or on plates, etc.

Whatever form of incubator is preferred for the study of microbes, it is essential that it should be provided with a gas regulator, so that it can be maintained at a constant temperature. There are many forms of regulators. Among these may be mentioned Page's (which is one of the simplest), Reichert's, Moitessier's, and Schlösing's, which are all good regulators.

It is not our object to describe the cultivation tubes, flasks, and bulbs of Pasteur, Miquel, Aitken, Lister, Duclaux, Sternberg, Gayon, Lipez, and others, nor the various glass apparatus (such as dishes, plates, funnels, etc.) used in bacteriological laboratories. It only remains for us to say on this subject that all the apparatus used in the laboratories of Koch and Pasteur may be obtained from Messrs. F. E. Becker and Co., 33, Hatton Wall, Hatton Garden, London.

Methods of Cultivation and Cultivation Media.

The artificial cultivation of pathogenic and non-pathogenic microbes necessitates the use of various sterilized nutrient media. Cultivation media are either liquids or solids. The former were first used in France, particularly by Pasteur; while the latter were advocated by Koch and the German school of bacteriologists generally.

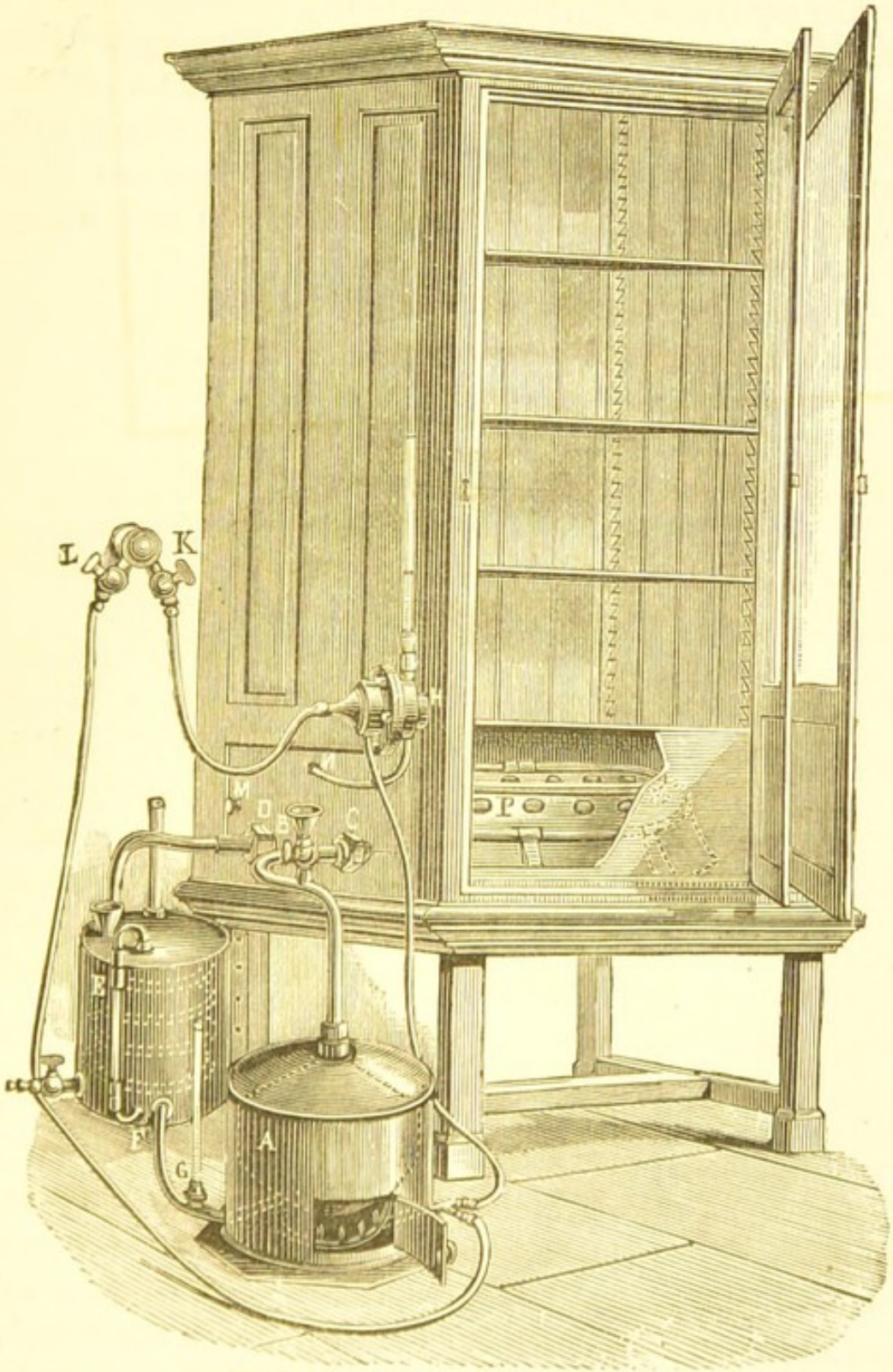


FIG. 11.—PASTEUR'S INCUBATOR.

Both liquid and solid media have their special uses, and no fully equipped laboratory can dispense with either.

Solid Media: (*a*) *Nutrient Gelatine.*—This cultivating medium is prepared in the following way: 1 lb. of lean beef should be cut up, placed in a flask along with 1000 cc. of distilled water and boiled for about an hour. The liquor is strained through fine linen and finally

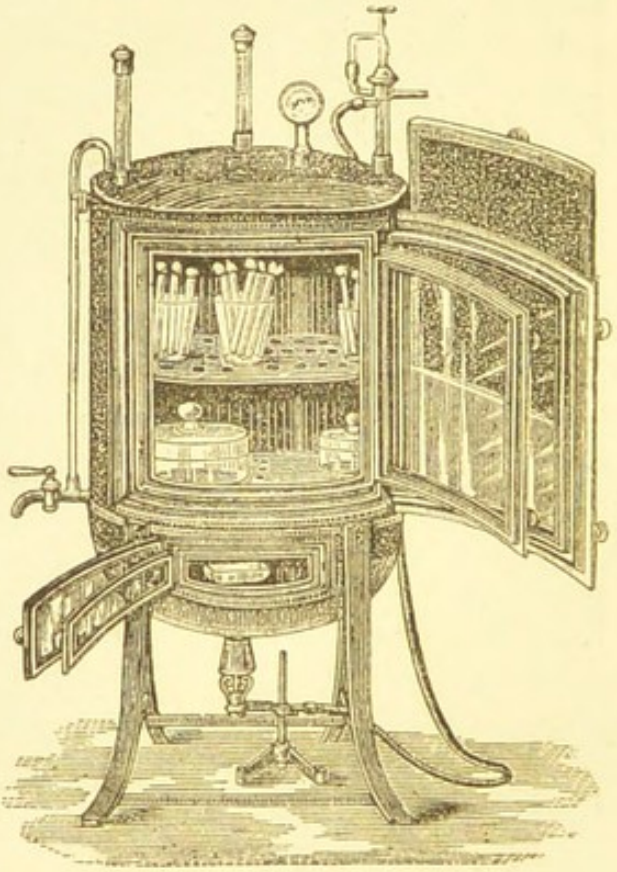


FIG. 12.—ROHRBECK'S INCUBATOR.

filtered through Swedish filter paper. The filtrate is then made up to 1000 cc. by the further addition of water. The filtrate is now placed in a large beaker, and 100 grammes of the best gelatine, 10 grammes of peptone (Savory and Moore's), and 5 grammes of common salt are added. The gelatine is allowed to soften and dissolve gradually by gently heating the mixture in a water-bath.

As infusions of meat are always more or less *acid* (due to the presence of sarcolactic acid), it is necessary to make the infusion slightly alkaline by the addition of a solution of pure sodium carbonate. The growth of many microbes is greatly interfered with by the presence of acids, hence the reason for making the infusion slightly alkaline. After the addition of the sodium carbonate, the infusion should be boiled for three-quarters of an hour, and filtered through a hot filter (Fig. 13) into a sterilized flask plugged with sterilized cotton wool.

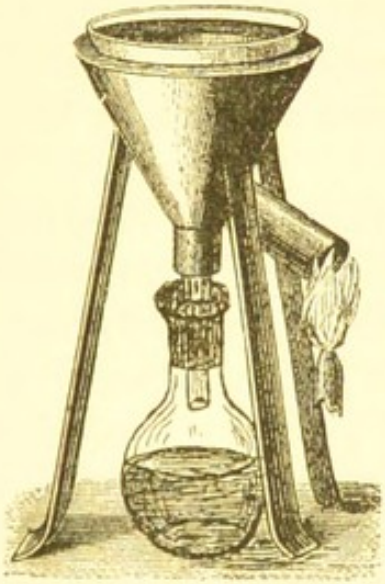


FIG. 13.—HOT-WATER FILTER.

This filtrate is ready for filling sterilized cultivation tubes, flasks, plates, etc., for it solidifies on cooling and remains solid up to 26° C.

The tubes and flasks after being filled with the nutrient gelatine must be sterilized in the steam sterilizer (see Fig. 4) for a quarter of an hour on two or three successive days. If the tubes show no signs of turbidity after about a week's incubation, they may be considered sterile.

(*b*) *Agar-agar.**—As nutrient gelatine melts at about 26° C., it cannot be used for the cultivation of certain microbes which require a higher temperature for their proper growth and development. To overcome this obstacle nutrient agar-agar, or blood serum, is used. The former substance remains solid up to a temperature of about 50° C.; the latter solidifies at 70° C., and always remains solid. Agar-agar, or the *gelose* of French writers, is the so-called Japanese isinglass, and consists of the dried fragments of certain Algæ (*vide* Hueppe's *Die Methoden der Bakterien Forschung*). Sterile nutrient agar-agar is prepared by a similar method to the one already described, with the exception that 20 grammes of agar-agar are used instead of the 100 grammes of gelatine.

(*c*) *Potato Cultivations.*—Sterilized and steamed potatoes (when cut in half) form a good medium for the cultivation of numerous microbes, especially the putrefactive and chromogenic forms. The potatoes are inoculated by means of sterilized platinum needles, after which they are transferred to a moist chamber for incubation.

Sterilized potato, or bread-paste, and white of egg, are used as cultivating media. Bread-paste, after being sterilized in the steam sterilizer, is a very useful medium for the growth of moulds.

Liquid Media.—(*a*) *Bouillon* (broth of beef, pork or chicken) is made according to the process already described for the preparation of nutrient gelatine, with the exception that the gelatine is omitted.

The bouillon should be clear, and may be stored in previously sterilized flasks or tubes plugged with sterilized cotton-wool.

* Agar-agar can be obtained from Christy and Co., 25, Lime Street, London, at 3s. per pound.

(*b*) *Liquid Blood Serum.*—This medium is obtained by collecting the blood of a healthy horse, calf, or sheep, in sterilized flasks, or glass cylinders with stoppers. The flasks or cylinders are placed in an ice-box for about twenty-four hours, when the separation of the clot will be completed. The serum is then transferred, by means of sterilized pipettes, into sterilized test-tubes, the latter being quickly replugged by the cotton-wool stoppers. The tubes are then heated for an hour or two at 60° C. on six successive days.

(*c*) *Urine* is first neutralized by means of sodium carbonate, and then sterilized after the manner described for bouillon.

(*d*) *Milk* is sterilized by being boiled for about thirty minutes on two or three successive days.

(*e*) *Vegetable Infusions* are cultivating media made by boiling water containing chopped hay, turnips, cucumbers, and carrots. The infusion is then filtered, and the filtrate sterilized in the usual way. An infusion of hay is a useful medium for the growth of *Bacillus subtilis*.

(*f*) *Pasteur's Fluid* is a solution containing 10 parts of pure cane-sugar, 1 part of ammonium tartrate, the ash of 1 part of yeast, and 100 parts of distilled water.

(*g*) *The Cohn-Mayer Fluid* contains 1 gramme of ammonium tartrate, half a gramme each of potassium phosphate and magnesium sulphate, 0·05 gramme of tricalcium phosphate, and 100 cc. of distilled water. This fluid, as well as Pasteur's fluid, is sterilized in the usual way. They are useful for the cultivation of certain non-pathogenic microbes and moulds.

Whatever medium is used for the cultivation of microbes, it must, at the outset, be absolutely sterile. By this means alone can pure cultivations be subsequently obtained.

If the original fluid under examination contains different microbes (recognised by the microscope), and it is desirable to separate them, so as to obtain pure cultivations of one or all the microbes present in the original fluid, one of three methods may be used for this purpose. The three methods are the following:

i. Koch's plate-cultivation.
ii. Kleb's fractional cultivation.
iii. Lister's and Naëgeli's dilution method.

The Plate-cultivation Method.—In order to utilize this method, about three tubes containing sterilized nutrient gelatine or agar-agar are placed in a water-bath heated to about 40° C. (or 55° C. for agar-agar), so as to melt the cultivating medium in each tube. The latter are then carefully inoculated with a mere trace of the original microbian mixture. The cotton-wool plugs are replaced and the tubes rolled about so as to distribute the microbes throughout the media. The contents of the three tubes are quickly poured into the lower portion of the same number of Dr. Pétri's double dishes (previously sterilized), which are then placed in a damp chamber.* The damp chamber, with its contents, are removed to an incubator, and remain there for several days at about 23° C., or higher if agar-agar is used (*i.e.*, according to the temperature required for the growth of the microbes).

In a few days or so, each species will have started a separate growth or colony in different parts of the solidified plate of nutrient gelatine, or agar-agar. The individual colonies are recognisable according to certain *macro*scopical appearances, such as colour, shape, liquefaction or non-liquefaction of the medium, and the size

* A damp chamber consists of a ground-glass plate covered over by means of a bell-glass. In the upper portion of the bell-glass is placed a piece of filter paper moistened with a solution of mercuric chloride (1 in 1000).

of the colonies. By plate-cultivation the different species of microbes separate themselves from each other; and from these colonies pure cultivations of each microbe may be obtained by carefully reinoculating a number of tubes containing sterilized nutrient gelatine or agar-agar.

In Dr. Koch's laboratory, in Berlin, sterilized glass plates are used instead of Pétri's double dishes; and after inoculating a tube (*a*) containing liquefied gelatine from the original microbian mixture, a second tube (*b*) is inoculated from tube *a*, and a third from tube *b*: *i.e.*, a first, second, and third attenuation are obtained. The contents of each tube are then poured out upon a glass plate, and the plates finally placed in the damp chamber, etc., as already described.

The Fractional Cultivation Method 'consists in the attempt to isolate, by successive cultivations, the different organisms that have been growing previously in the same culture.' A number of tubes containing various cultivating media (sterilized) are inoculated with a mere trace of the original microbian mixture, and are then placed in an incubator for a couple of days or so. It will then be noticed that the different species of microbes (sown in each tube) will not have increased equally in numbers in all the tubes (due, of course, to the nature of the medium, the temperature, and the period of incubation). It is possible that only one species will have developed, so far, in each tube. With these tubes a similar number of tubes are reinoculated, and so on. By this fractional method of cultivation pure growths are ultimately obtained. For further information concerning this method the reader is referred to Dr. Kleb's paper in the *Archiv für Exper. Pathologie* for 1873.

The Dilution Method consists in greatly diluting a drop of the original microbian mixture with some sterile saline

solution (0·5 per cent.). A series of tubes, containing different cultivating media (sterilized), are each inoculated with a mere trace of the diluted mixture. After about thirty hours' incubation, growths (most likely of one species only) make their appearance in some of the tubes.

By the fractional, dilution, and plate methods, cultures containing many different species of microbes are capable of being separated one from another. Sometimes a combination of the fractional and dilution methods is used for the same purpose.

The Examination of Fresh Tissues and Fluids.

a. Fresh *tissues* for microscopical examination should be teased out with needles in dilute glycerine or salt solution (sterilized), then temporarily mounted, in either liquid, on a glass slide and covered with a thin glass slip. In the examination for micrococci and other small microbes, the tissues should be first treated with acetic acid, and then with a solution of potash, the object being to dissolve and disintegrate fatty and albuminous globules which might be mistaken for microbes. Alcohol and ether are also useful agents for dissolving small globules of fat.

b. Blood, pus, urine, saliva, culture-fluids, and other liquids containing microbes, are easily examined microscopically by placing a drop of the liquid on a glass slide and covering it with a glass slip.

c. When microbes for examination are growing on plates (*i.e.*, plate-cultivation), a small portion of the culture should be taken up on a sterilized needle and placed in a drop of sterilized water on a glass slide.

After 'thinning,' the preparation is covered with a glass slip and examined under the microscope.

Drop Cultures.

When it is necessary to watch the growth and multiplication of microbes under the microscope, the most useful method is that known as 'drop-culture.'

For this object a glass ring ($\frac{3}{4}$ in. diameter and $\frac{1}{8}$ in. high) is cemented to a clean glass microscopic slide, forming a kind of cell. The cell is thoroughly cleansed with alcohol and ether, and the upper edge of the ring is moistened with olive oil. The cell is covered over by means of a thin glass slip, the lower surface of which contains the drop of bouillon or other nourishing medium, along with the microbes for examination. A drop of sterilized water should be deposited at the bottom of the cell (*i.e.*, upon the upper surface of the glass slide). This arrangement forms a miniature moist chamber in which the growth, etc., of microbes may be watched under the highest powers. When oil immersion lenses are used (such as Zeiss' $\frac{1}{12}$), a drop of cedar oil is placed on the lens, which is then lowered until it touches the upper surface of the glass slip (cover-glass). After the examination of the glass cell and its contents, it may be removed to an incubator until it is required again for examination.

Instead of using an incubator, warm stages like those of Schäfer, Israël, Ranvier, etc., are often used upon the fixed stage of the microscope. These devices keep up a constant temperature round the drop-culture, which may be examined from time to time without removal to an incubator.

Staining and permanently mounting Cover-Glass Specimens and Sections.

Only *general* methods are given in this section, because those used for the special staining of certain microbes will be alluded to under the detailed description of such microbes.

To prepare a cover-glass preparation for staining, a sterilized cover-glass is smeared with the microbian matter (solid or liquid), or with blood, pus, etc., by means of a sterilized needle or capillary pipette. The excess of material is squeezed out by means of an additional cover-glass placed over the original one. The two glasses are then separated, each bearing a small portion of the microbian matter. After drying for a few minutes, they are passed rapidly (three or four times) through a Bunsen flame.

To stain the preparations, they are allowed to float (with the prepared side downwards) on the surface of an aqueous solution of methyl violet, gentian violet or magenta, for a short time. After this the cover-glasses are washed with water, then spirit, and finally with distilled water. They are then drained, dried, and mounted in Canada balsam.

To stain Tissues containing Microbes.—'Place them in either of the above-mentioned solutions, and allow them to remain for some hours. When deeply stained, wash in water to remove the excess of the stain, and then lay them out flat in methylated spirit, and let them remain until no more colour comes away. Transfer them to absolute alcohol, and then oil of cloves, and mount in Canada balsam' (Dr. Gibbes).

To double-stain Bacilli which produce Spores. — The

cover-glass preparation should be floated for half an hour on the surface of a small quantity of hot magenta-and-aniline stain. The magenta is discharged from the bacilli by washing in water, in alcohol, or weak nitric acid, according to the species. The preparations are then treated (for three or four minutes) in a solution of methylene blue, and finally washed with water, drained, dried, and mounted in Canada balsam. By this method the spores are stained red, while the bacilli are blue.

Gram's Method for staining Microbes in Tissues.—The sections containing the micrococci, bacteria, or bacilli, are soaked in absolute alcohol for twelve minutes, and then placed in an aniline-gentian-violet* staining solution for about three minutes. The sections are then placed in a solution of iodine (in potassium iodide) for several minutes, or until they are of a brown colour. After this they are transferred to absolute alcohol until decolourized; they are then placed in oil of cloves, and finally mounted in Canada balsam.

As Gram's method only gives a faint colour to the tissues, they may be stained a deeper colour by immersing the sections (after decolourizing with alcohol) in an aqueous solution of vesuvin, eosin, or Ranvier's picro-carminate of ammonia. They are finally washed in alcohol and mounted as already described.

Ehrlich-Weigert Method.—This is another method for staining microbes *in situ*. The tissues are placed in a warm solution of aniline-methyl-violet, and then decolourized with nitric acid (1 in 2). The tissues may be stained brown by immersing them in an aqueous solution of Bismarck brown or vesuvin. In this case the microbes are blue and the tissues brown. Other

* Excellent bacteriological stains may be obtained from Messrs. F. E. Becker and Co. of 33, Hatton Wall, Hatton Garden, London.

aniline colours may be used, but the decolourizer is nitric acid.

Gibbes' Rapid Double-Staining Method.—This process is applicable for staining sections as well as cover-glass preparations. No decolourizing agent is used, while the double-staining process is performed in one operation. The preparations are allowed to remain in a warm aniline-magenta-methyl-violet solution for five minutes (in the case of sections for several hours), and then they are washed in methylated spirit until no more colour comes away. The preparations are now dehydrated in absolute alcohol, dried and mounted in Canada balsam.

For delicate tissues Gibbes' method is far superior to that of Ehrlich-Weigert, as the nitric acid of the latter method often destroys the tissues. Gibbes' method is most useful for staining sputum in cases of phthisis. The tubercle bacilli are stained red, while the putrefactive bacteria and micrococci are blue.

From the above remarks it will be seen that microbes 'have a great affinity for certain dyes, especially aniline dyes, and, therefore, these are used with great success to demonstrate their presence, and to differentiate in many instances morphological details which in the unstained condition are not discernible.'

CHAPTER III.

GENERAL REMARKS CONCERNING MICROBES.

At a meeting of the Academy of Sciences of Paris on March 11th, 1878, a prolonged and animated discussion took place as to the grouping of micro-organisms. Some naturalists were in favour of placing them in the *Microzoa* (small animals), and others in the *Microphyta* (small plants). As neither the zoologists nor the botanists would give way, the late Dr. Sédillot suggested that these organisms should be known under the name of *microbes*, which simply means *a small living being*, the grouping of these small beings remaining an open question. Sédillot's suggestion was immediately adopted by the Academy, and from 1878 to the present day, these organisms have been known under the general name of microbes or micro-organisms.

But the adoption of the word 'microbe' did not settle the question as to whether they belong to the animal or vegetal kingdom. For some time microbes vacillated between the two kingdoms, until Haëckel united the *Microzoa* and *Microphyta* into one group under the name of *Protista* (primitive beings).

According to Haëckel's grouping, a protist is, therefore, a unicellular organism, whether truly vegetal or animal, or doubtful, in its affinities.

This grouping, however, did not satisfy phylogenists,

and the discussion was reopened, but ultimately terminated in the vegetal nature of microbes being well established. That they are minute vegetal cells or plants is now the unanimous verdict of biologists, although their true position among cryptogamic plants still remains (more or less) a moot point.

Microbes differ from animal cells by having a cellulose covering, and by being able to derive their nitrogen from salts of ammonia and nitric acid—that is, nitrogenous compounds in a very stable state of chemical equilibrium. Animal cells, on the other hand, cannot decompose such compounds as ammonia or nitric acid, but derive their nitrogen from albuminous or organic matter. In these compounds the nitrogen is in an unstable state of chemical equilibrium, and they have a somewhat similar composition to the protoplasm of animal cells: for this reason they are readily assimilated by the latter.

Microbes (although vegetal cells) differ from the higher plants in being unable to decompose atmospheric carbonic acid gas. This is due to the absence of chlorophyll, or green colouring matter. In this respect they are closely allied to the fungi.

Microbes, as already stated, consist of an external covering of cellulose ($n\ C_6H_{10}O_5$), and an internal living substance called protoplasm ('the physical basis of life.')

According to Nencki, the protoplasm of some microbes is devoid of sulphur and phosphorus, and thereby differs from the protoplasm of the higher plants, which may be represented by Lieberkühn's formula: $C_{72}H_{112}N_{18}O_{22}S$. Nencki's protoplasm* has been named mycoprotein, and contains 14·75 per cent. of nitrogen.

There is little doubt that the nitrogenous body varies considerably in different species. The author has found

* See also Würtz's 'Traité de Chimie Biologique,' p. 84.

the presence of sulphur in the nitrogenous body of *Bacillus tuberculosis;* and it has been stated that 'the living substance' of *Bacillus anthracis* does not give the mycoproteic reactions.

If the protoplasm in all microbes were of identical composition, there would not be the varied reactions with staining agents. For instance, phthisical sputum, stained by the Ehrlich-Weigert method, only the tubercle-bacilli remained stained, while all the other microbes present are discoloured. Then, again, Dr. Baginski demonstrated before the Berlin Physiological Society, on December 2nd, 1887, that both *Bacterium lactis* and *Bacterium coli* (in the absence of air) destroy the colour of methylene blue, while certain other microbes had not this power.

These reactions, as well as others, show the varied nature or composition of microbian protoplasm.

Besides the protoplasm, certain microbian cells contain other bodies. Among these may be mentioned that *Beggiatoa alba* and *Beggiatoa mirabilis* contain sulphur granules, while *Bacillus butyricus* contains starch, and *Beggiatoa roseopersicina* a violet-coloured pigment.

Many species of microbes are capable of surrounding themselves with a protoplasmic sheath; and when this sheath surrounds a number of microbes (*i.e.* embedding them), the latter are said to be in the zoogloœan or resting stage. There is little doubt that this protoplasmic sheath is exuded or secreted by the cells, and in certain cases it is stated to have a similar composition to mycoprotein.

Microbes vary considerably in *form*, being either round, oval, rod-shaped, spiral, or filamentous. The round, or oval-shaped microbes, are termed *micrococci;* the rod-shaped, *bacteria, bacilli,* and *vibriones;* the spiral forms, *spirilla;* and the filamentous forms are generally termed *leptothrix* (straight), and *spirochæta* (wavy).

Some microbes are provided with flagella ('lashing tails'), while others are devoid of these appendages.

Since the time of Leuwenhoeck, 'these minute beings have afforded histologists a subject for controversy and dispute. Existing as they do upon the very borderland of the vegetal and animal kingdoms, not only have they been transferred from one to the other, but the question has even been raised whether the smaller forms should be considered as living beings at all.'

Because they are so small that millions of them may swim through the eye of a needle, we have no right to exclude them from the organic world. There is plenty of evidence to show that even the smallest microbe has come into the world for the same object as the rest of animated nature—namely to live, reproduce, and die They are, therefore, part and parcel of the organic world, and not isolated from it.

'Rightly viewed, no meanest object is insignificant; all objects are as windows, through which the philosophic eye looks into Infinitude itself.'—CARLYLE.

And may the microscopist not hope that his magnifying lenses will yet be improved so that he will be able to discern living beings far smaller than the smallest micrococcus?

To give the reader some idea of the magnification of the highest lenses, the oil-immersion system of Zeiss gives a magnifying power of from 3,000 to 4,000 diameters; and could we view a man under such a lens, he would appear from three to four miles in height, or as high as Mount Blanc, Mount Ararat, or even Chimborazo. But even under this colossal magnification the smallest microbes do not appear larger than the points and commas of ordinary print.

As a general rule the dimensions of microbes vary

from about 0·0005 mm. to 0·05 mm. in length or diameter, as the case may be. No wonder that M. Pouchet called them 'les infiniment petits.'

Not only have the size of microbes been ascertained but their approximate *weights* have likewise been computed. Dr. Ferdinand Cohn says: 'If we call the specific weight of one bacterium (*B. termo*) equal to that of water, which cannot be far from the truth, it appears that a single rod will weigh 0·000,000,001,571 milligramme, or that six hundred and thirty-six milliards of bacteria would weigh one gramme, or six hundred and thirty-six thousand milliards a kilogramme.'

Being so light in weight, microbes are always present (more or less) in the atmosphere, and are carried over thousands and thousands of miles by air currents, without losing their vitality. This is not surprising when one bears in mind that Rome has been showered with the sands of Sahara, France with South American diatoms, and that the volcanic dust from Cotopaxi fell thousands of miles away from the seat of the eruption.* If sands and volcanic dust† (which are far heavier than the largest microbe) are capable of being carried enormous distances, it is hardly irrational to suppose that microbes may travel from planet to planet, especially the anaërobic forms, and even those which are aërobic are capable of being dried up without losing their vitality. Be this as it may, microbes are everywhere present—in the atmosphere, in soils and waters; and it is possible that they have existed from the earliest period of geological time.

We know that fungi allied to *Peronospora* lived in the

* Professor J. W. Judd, F.R.S., kindly informed the author that 'Krakatoã dust fell, at least, a thousand miles away from the volcano.'

† Dr. Bonney, F.R.S., calculated that the volcanic dust from Cotopaxi 'would take from 4,000 to 25,000 particles to make up a grain in weight.'

Carboniferous Age; and if fungi lived in that remote period, why not microbes? In the words of Mr. W. G. Smith, F.L.S., 'Although two fungi (*Peronospora* and *Protomyces*) have been detected in Palæozoic rocks, it must not be concluded that they are the simplest known forms of primal fungi. . . . In those far-off times the primordial plant was probably *a mere microscopic cell*, or thin sac, resting on the moist surface of the earth. It probably increased by division and redivision; each of the four parts soon becoming distinct, and each segment speedily reaching the original size and form.'

M. Béchamp believes that he has isolated living microbes from the chalk of the Secondary Epoch. If this be true, it points to an extremely long period of desiccation, which certain microbes are capable of enduring without destruction.

Microbes propagate by fission (*i.e.*, division) and by spore-formation.

(*a*) *Micrococci* multiply 'always by simple division, never by any other means, *e.g.*, gemmation and spores.' If the division takes place in one direction only, the resulting form (if the two cells remain together) is a diplococcus, dumb-bell, or colon. The diplococcus may again divide, without separation, forming a streptococcus or chain, which may become curved or even twisted in appearance. Sometimes the division of micrococci is in two directions, resulting in four cocci (forming a kind of double colon called a merismopedia), or in three directions, forming a sarcinacoccus or sarcina. After a micrococcus has divided, the two halves are not perfectly spherical: they are flattened (more or less) on their internal sides. After a short time they become rounded, and finally separate.

(*b*) *Bacteria* are small, rod-like microbes, with rounded

ends. They multiply by fission or bi-partition, growing until they reach about double their original size. When this stage is reached they constrict themselves in the middle like a figure eight, and then separate.

In a short time each half divides again, and on account of the rapidity of this process the observer usually finds them multiplying, either constricted in the centre, or hanging together in pairs. In this latter condition it is sometimes difficult to distinguish them from the diplococci. A bacterium (from *βακτήριον*), being a small *rod*, 'must have at least two sides parallel.' Bacteria are capable of moving rapidly. This is due to one or more flagella. Nearly all bacteria possess two different modes of life, one of motion and another of rest. They are excessively motile where there is a favourable temperature, plenty of nourishment, and the presence of oxygen; under unfavourable conditions they are motionless.

(*c*) *Bacilli* are longer than bacteria, with either round or square ends. They propagate by fission and by spore-formation. When the former mode of reproduction takes place, bacilli are often to be seen in short or long chains, and sometimes in long filamentous masses.

Bacillus subtilis, *Bacillus anthracis*, *Bacillus butyricus* are among those which produce spores. When spore-formation is about to take place, the protoplasm becomes granular, and at certain points the cell gives rise to one or more oval-shaped spores. When fully mature they burst the parent cell and are free.

In some bacilli spore-formation only takes place when the microbes are growing in contact with oxygen (air); and a low temperature is unfavourable for the formation of spores.

According to Cohn (*Beiträge zur Biologie der Pflanzen*, vol. ii.) the spores of bacilli (especially *B. subtilis*) are

capable of withstanding the action of boiling water for several minutes, although this treatment kills the bacilli as well as other microbes. This resisting action is due to the fact 'that the substance of each spore is enveloped in a double sheath, an internal sheath probably of a fatty nature, and an external one probably of cellulose; both are very bad conductors of heat' (Klein).

Some bacilli (*e.g.*, *Bacillus subtilis*) are provided with flagella, while others (*e.g.*, *Bacillus tuberculosis*, *Bacillus anthracis*) are devoid of these appendages.

Bacilli, like all microbes, require for their nutrition and growth oxygen, carbon, nitrogen, certain salts, and water. Although some microbes are anaërobic, they require *oxygen*, which is obtained from the carbohydrates and albuminoids of the medium in which they live or from the free oxygen which may be dissolved in that medium.

(*d*) *Vibriones* are curved, or more or less wavy rods. They are provided with flagella and propagate by fission and spore-formation.

(*e*) *Spirilla* are spiral-shaped microbes, and multiply by fission and spore-formation. They are motile.

(*f*) *Spirochætæ* are filamentous and wavy microbes.

Although some microbes propagate at certain times by spore-formation, fission or simple division is the chief mode of reproduction.

The warmer the air the faster proceeds this division, and the stronger the multiplication. In the space of an hour a bacterium divides into two parts, then again in another hour into four, after three hours into eight, etc. 'After twenty-four hours,' says Cohn, 'the number exceeds sixteen and a half millions (16,777,220); at the end of two days this bacterium will have multiplied to the incredible number of 281,500,000,000; at the end of

three days it will have increased to forty-eight trillions; and after a week the number can only be expressed by figures of fifty-one places. In order to make this number comprehensible, we will reckon the mass which may result from the multiplication of a single bacterium. A single individual of the most common species of rod bacteria (*Bacterium termo*) has the appearance of a short cylinder of a thousandth of a millimetre in diameter, and perhaps one five-hundredth of a millimetre in length. Let us now think of a cube, the side measuring a millimetre (cubic millimetre): six hundred and thirty-three millions of bacteria will completely fill this cavity without leaving an empty space. The fortieth part of a cubic millimetre would perhaps contain the bacteria that proceed from one single little rod in twenty-four hours; but at the end of the following day the bacteria would fill a space equal to 442,570 such cubes, or what is the same thing, perhaps, half a litre, or forty-four and a half cubic centimetres. Take the space which is occupied by the seas of the world at about two-thirds of the terrestrial surface, say with a mean depth of a mile, the collective contents of which would be nine hundred and twenty-nine millions of cubic miles; by continued progressive multiplication the bacteria which spring from one germ would in less than five days fill completely the whole of the seas; the number can only be expressed by figures of thirty-seven places.

'We do not consider such computations idle play; they alone can make the immense work executed by the bacteria comprehensible to us. They also depend only on such hypotheses as Nature herself presents to us; should, for example, the continuance of the process of fission be in truth somewhat longer than that stated by us, the numbers would agree in a few hours or days later. Certainly if in

limited space this quantity is at no time reached, it perhaps does not signify that the power of multiplication in the bacteria falls below the calculation, but rather depends altogether on the limited nutrition. It is self-evident that bacteria do not generate the material which forms their bodies, but take it in from without as food, and therefore no more can be formed than there is food provided for. It follows that other plants and animals are assigned to the same food, and they on their side strive for existence. The fierce combat concerning life, according to the old usage of the extermination of the weaker, holds the increase of the bacteria, as of all other beings, in limitation; and it is only where they hold the upper hand that they are able to keep off the rivals which at the same time are their deadly enemies.'

Microbes may be divided into pathogenic, chromogenic (pigment-forming), septic (putrefactive), and zymogenic (fermentive) forms. They have been classified by numerous scientists, but the one suggested by Cohn is still the best, that is, from the bacteriologist's point of view. This classification divides microbes into five genera:

(1) Spherobacteria or Micrococci.
(2) Microbacteria or Bacteria.
(3) Desmobacteria or Bacilli and Vibriones.
(4) Spirobacteria or Spirilla.
(5) Spirochætæ.

From the time of Müller (1773) down to 1841 microbes were classed as *Infusoria*. Davaine and Cohn placed them among the *Algæ* (notwithstanding the absence of chlorophyll), while Naëgeli considered them to belong to the *Fungi*, and Sachs gave them a place in his group—the *Thallophytes* (which embraces both the *Algæ* and *Fungi*).

It appears from the recent researches of Zopf, Van Tieghem, Cienkowski, Lankester, and others, on the

subject of pleomorphism, that the best of classifications must only be provisionally accepted.

These observers have noticed that certain microbes pass through various phases before arriving at their ultimate form. That is, a certain microbe may pass through the stages of a micrococcus, a bacterium, and a bacillus before it becomes a spirillum; and Büchner believes that a non-pathogenic microbe is capable of being transformed into a pathogenic one (*e.g.*, that *Bacillus subtilis* is transformed into *Bacillus anthracis*).

Then, again, Chauveau (*Comptes Rendus*, vol. cix.) has shown that *Bacillus anthracis* loses its virulence when submitted to the action of compressed oxygen; but it does not lose its vaccinal property after this treatment. This new character is said to be maintained by suitable cultivation. Although *Bacillus anthracis* may lose its virulence under such abnormal conditions as already alluded to, it does not become a saprophytic microbe of ordinary fermentations, for it still preserves one of the most essential attributes that indicate the infectious nature of the pathogenic microbe (viz., its vaccinal property).

Besides, Chauveau has further shown that the non-virulent *Bacillus anthracis* may be revivified by degrees when grown in suitable cultivating media. These researches do not point to any transformation of *B. anthracis* into a non-pathogenic species, but simply show that oxygen *under pressure* is capable of modifying the microbe's pathogenic power.

It appears from this that Pasteur (*Comptes Rendus*, 1881) was probably correct when he stated that 'it is the oxygen of the air which attenuates and extinguishes the virulence of a virus,' and this may also account for the spontaneous cessation of epidemics.

Büchner, as already stated, believes that he succeeded in transforming a non-pathogenic microbe into a pathogenic one.

Sattler believed that he transformed the non-pathogenic *Bacillus subtilis* into a pathogenic form, capable of producing infectious ophthalmia, by simply cultivating the microbe in an infusion of the seeds of *Abrus precatorius* (jequirity seeds); and finally Gravitz believed he had transformed the non-pathogenic moulds—*Aspergillus glaucus*, *Penicillium glaucum*—into pathogenic forms by cultivating them in alkaline media at about 40°C.

Klein, Koch, and others do not accept the theory of pleomorphism, or the transformation of microbes; and Klein in his *Micro-Organisms and Disease* (pp. 207-231), ably argues, as well as experimentally proves, that the researches of Büchner, Sattler, Grawitz, and others on the transformation of microbes are erroneous. He says, in conclusion: 'That some definite micro-organisms, although, as a rule, existing and growing in various substances of the outside world, have the power when finding access into the body of a suitable animal, to grow and thrive here also, and to induce a definite pathological condition. But this power they have *ab initio*. Those that do not possess this power cannot acquire it by any other means whatever. Just as there are species of plants which act as poisons to the animal body, and other species of plants which, although belonging to the same group and family, and very much alike to the others, have no such power, and cannot acquire such a power by any means, so there are micro-organisms which are pathogenic, while others are quite harmless. The latter remain so, no matter under what conditions and for how long they grow.'

Some microbes are pathogenic, others non-pathogenic;

the former are the cause (directly or indirectly) of certain diseases, while the latter cause various changes in organic matter, etc.

To the physician the pathogenic microbes are of special interest, and will be fully discussed in the following pages; but it is 'impossible by localizing one's knowledge to pathogenic species to thoroughly understand the life-history of these particular forms, or to be able to grasp and appreciate the various arguments and questions that arise in comparing their life-history with progress of disease.'

There is little doubt that the study of microbes in general is of the utmost importance; so much so that this study is destined to give a full scientific explanation of all the various changes which occur in contagious diseases. But it is only by bacteriological and microchemical methods that the pathologist is, or will be, able to investigate such far-reaching problems as the causation of diseases, and thereby the methods of treating them.

Speaking of the importance of a proper study of bacteriology, Sir William Aitken, F.R.S., says: 'Could we but climb some pathological Pisgah, and be allowed to stand as Moses stood, when he was permitted to view the promised land, we might rejoice in the bright and certain prospect that there lies before us a great and glorious future for pathology, and for the science and practice of our art.'

CHAPTER IV.

THE DISTRIBUTION OF MICROBES, ETC.

THE study of microbes is not only of the greatest value to the pathologist and physician, but concerns the brewer, manufacturer, hygienist, and agriculturist; in fact, it concerns all, for there is not a moment of our lives in which we are not in contact with 'the unseen mist of organic forms.' It may be said that microbes are omnipresent, that they are always present in air, water, and the soil; they attach themselves to the surface of all firm bodies, but develop in masses only where decomposition, putrefaction, fermentation, or contagious diseases are present. As an example, *Bacillus subtilis* is always (more or less) present in the atmosphere, and it only requires the investigator to place a previously sterilized nutrient medium in contact with the air to obtain growths of this microbe as well as others.

Bacterium termo, one of the putrefactive microbes, is also present in natural waters. A glass of spring water, if allowed to stand a short time, gets a coating on its surface. This coating consists of numberless microbes. Dr. P. Miquel, of the Observatory of Montsouris, estimated that one gramme of the soil of Montsouris contained an average of 750,000 microbes.

From the above one concludes that microbes are always present in air, water, and soil. Their numbers

vary with the nature of the air, water, and soil; the time of the year and various climatic conditions also influence their rate of increase.

Although the majority of microbes found in air, water, and soil are of the non-pathogenic or harmless kind, there is plenty of evidence to show that pathogenic microbes lurk about in the air as well as in water and the soil. The evidence may be more or less of an indirect nature, yet this does not render the statement invalid.

Pathogenic microbes, or the microbes of disease, are to be found in *the air* of hospitals for consumption, fevers, small-pox, etc., and Dr. Klein (*Micro-organisms and Disease*, p. 217) has shown that the instruments, etc., in a laboratory where anthrax experiments have been carried on contain upon their surfaces spores of *Bacillus anthracis*, these spores being carried from place to place in the atmosphere. The author of the present volume, during his researches on *Bacillus tuberculosis*, has experimentally proved that tubercle-bacilli are to be found in the atmosphere of a laboratory where experiments are being performed with phthisical sputum, especially when the sputum is in a dried condition.

Dr. Klein has proved that infectious pneumo-enteritis of the pig (swine-fever) is due to specific bacillus, and the contagium (which is highly infectious) is transmissible from pig to pig *through the air*.

Because there is little direct evidence to show that pathogenic microbes have been directly obtained from the atmosphere, we have no right to conclude that they do not exist there.

Why has the search for pathogenic microbes in the air been unsuccessful? It is due to the fact that whereas one pathogenic microbe exists, countless numbers of non-

pathogenic forms are to be found; and it is of the utmost difficulty—yea, almost impossible—to find pathogenic microbes among such an overwhelming number of non-pathogenic forms. We may compare this search to the finding of a small fragment of a diamond in a sack of white sand. If the experimenter passes slowly two, five, ten, twenty, or even fifty gallons of air (in a certain locality) through his aëroscope, and subsequently finds that no pathogenic microbes have developed, this surely does not prove that there were no pathogenic microbes in the atmosphere (of the locality) at the time of the experiment. It may be that none of these forms passed through the instrument, or, if they did, it is possible that the non-pathogenic forms (being in the largest numbers) prevented the development and growth of the pathogenic microbes.

The indirect evidence is in favour of the existence of pathogenic microbes in the atmosphere. If not, how can we account for epidemics passing from town to town, and from one nation to another? If the endemic area (India) of cholera is somewhat limited, its epidemic area is very vast—covering the whole of the southern part of Asia, the whole of Europe as far north as the sixtieth parallel of latitude, and nearly the whole of South America, as well as the United States and Canada. Therefore, if cholera gets a footing, say, in India, it may pass through Persia into the very heart of Europe. How can this and similar epidemics travel over thousands of miles unless the pathogenic microbes are carried in the atmosphere or in waters? Need there be any wonder at this, considering the extreme lightness of these microbes, and their power, within certain limits, of resisting the extremes of atmospheric temperatures?

As the greatest number of microbes in the air are of a

harmless nature, the same remarks apply to those found in waters. 'As a matter of fact, however, pathogenic forms can and have been discovered in waters by the process of plate-cultivation; thus the comma-bacillus, which is regarded by many authorities as the cause of Asiatic cholera, was found by Koch in some tank-water in India; and the bacillus which with more or less probability is identified with typhoid fever has by Chantemesse and Widal been discovered in the drinking-water which had been consumed by persons suffering from that disease.'

It is said on good authority that 'cholera follows the course of rivers, this probably being due to the fact that the riparian areas possess soil saturated with water and decaying organic matter. There can be no doubt, too, that cholera spreads most rapidly in countries having an alluvial or tertiary soil.' Therefore it is possible that if a *moist wind* is blowing, cholera may be spread over a considerable area.

Although a very low temperature (say —18° C.) is capable of destroying most microbes, many forms may be embedded in ice (at 0° C.) without losing their vitality. The author has often obtained artificial cultures of *Bacillus subtilis* from pond ice although the surrounding temperature registered three or four degrees of frost.

Dr. T. M. Prudden, of New York, has also shown that certain bacteria are capable of being frozen for thirty-seven days without losing their vitality; and recently Dr. Dornil, of Paris, has discovered that ice is often a medium for transmitting infectious diseases, and particularly typhoid fever.* According to this authority, ice

* M. Cassederat (*Comptes Rendus*, vol. cx.) has recently found the typhoid fever bacillus in the Marseilles drinking-water.

obtained from ponds or rivers is as dangerous as the water itself.

The number of microbes in any water depends, among other conditions, upon the amount of organic matter present. A water rich in organic matter always contains a larger number of microbes (in a given volume) than a water almost free from such matter. M. Miquel obtained the following results, among numerous others, concerning the average number of microbes in one litre of certain waters about Paris :

Rain-water - - - -	64,000
Seine water (from Bercy, above Paris) -	4,800,000
Seine water (from Asnières, below Paris)	12,800,000
Sewer-water (from Clichy, north of Paris)	80,000,000

These investigations show that the larger the amount of organic matter in a water, the greater will be the number of microbes present.

Not only are microbes found in the atmosphere and in waters, but also in soil. The majority of microbes found in soils (like those found in air and water) are of the harmless or non-pathogenic kind. Yet at the same time pathogenic or disease-producing microbes have been found in soils. *Bacillus malariæ*, which has been proved to be the cause (directly or indirectly) of malarial fever, was found by Klebs and Tommasi-Crudeli (*Archiv für Experimental Pathologie*, 1879-1880) in the soils of the Roman Campagna: in fact, this microbe has been called 'an earth-born poison' (Felkin).

Pasteur (*Bulletin de l'Académie de Médecine*, 1880) stated that the casts of *Lumbricus terrestris* may contain the microbes of splenic fever, at the same time possessing all their original virulence. Both Klein and Koch dispute Pasteur's statement concerning the presence of

anthrax-bacilli in worm-casts. Yet there is every reason to believe that anthrax is spread, to a certain extent, by the soil.

It has been found that the *spores* of *Bacillus anthracis* are capable of retaining their vitality for several months in ordinary drinking-water as well as on moist soils.

The yellow fever (so prevalent on the east coasts of South and Central America) microbe (*Streptococcus*) 'clings to the *ground*, and its diffusion may be barred by streams, walls, and, some say, by much travelled thoroughfares, and it does not appear that the water-supply of cities aids its spread. A certain saturation of the atmosphere is an essential condition for an epidemic of yellow-fever. It is probable that it does not occur until a high dew-point, the minimum being upwards of 74, exists, and it is certain that epidemics cease before the dew-point descends to 58.'

The largest number of microbes present in the air, in waters, and in soils are of a non-pathogenic nature; but there is also a considerable amount of evidence to show that the pathogenic forms may retain their vitality in the same media.

Although air, water, and soil are means of spreading or distributing pathogenic microbes, the great factor in the distribution of most infectious diseases is that of human intercourse. In the words of Cohn, 'probably with the increase of commerce the visitation of that human scourge, the epidemic, has grown more frequent during the last few years, on man and animals. It wanders with undetainable progress from city to city, from land to land, stopping at one place but a short time, then, as if exhausted, disappearing in order to carry on its work in a new locality, and usually after an interval of time turning back again. Only too often the physician's

skill and knowledge are exercised in vain to wrest the victim from the devastating power of these diseases, or to limit their course by rules of precaution. As various as are the different forms of disease, yet all epidemics—cholera, typhoid, diphtheria, variola, scarlatina, hospital gangrene, and the like—have certain features in common. These diseases originate nowhere of themselves, neither from internal nor external causes, but are introduced from another place where they have been prevalent, by means of a diseased person, or through material which has been in contact with such: they spread only through contagion. When the infection has taken place, hours or even days may pass before the symptoms appear outwardly. After a certain time of incubation the disease breaks out through a powerful disturbance of the normal action of all the organs, from the brain to the digestive system; the diseased person appears as if he were under the influence of a poison which had penetrated into his blood; and as he himself is infected by the virus, he spreads it further by the breath, by the perspiration, by the excretions, even by his clothing or his ablutions. In many diseases the contagious material collects in a concentrated form in peculiar pustules or blisters, whose clear humour, in the slightest quantity, is sufficient to infect a sound person as soon as it has been received into his circulation, and to place him under the same appearances of disease as the originator of the poison. A breeze is sufficient to poison every open wound by the poison that adheres to the knife of the surgeon or anatomist. In anthrax it is proved that a fly may convey the poison from a diseased to a sound animal.'

At this point it is our intention to describe a little more fully the details concerning the microbes of the air, soil, and water.

Pasteur, during his researches on spontaneous generation, was the first investigator who made a systematic study of the presence and distribution of microbes in the atmosphere ; but it was not until 1879 that MM. Miquel and De Freudenreich (*Annuaire de l'Observatoire de Montsouris*, 1880) attempted the quantitative estimation of aërial microbes.

The method subsequently devised proved of the utmost value in the hands of these distinguished *savants*.

This method consists in aspirating a known volume of air through tubes containing previously sterilized plugs of glass-wool ; *i.e.*, the air is filtered through glass-wool.

The glass-wool plugs containing the microbes are then introduced into flasks containing sterilized bouillon or liquefied nutrient gelatine.

In the flasks colonies form which can be easily counted with the aid of a magnifying lens.

Dr. Pétri (*Zeitschrift für Hygiene*, vol. iii., 1887) and Dr. P. F. Frankland (*Phil. Trans. Royal Society*, vol. clxxviii., p. 113) have both used filters of powdered glass, sand, or sugar along with glass-wool. The filter-plugs containing the microbes from a known volume of air are each introduced into separate flasks containing liquefied nutrient gelatine ; ' with this the plug is agitated until it becomes completely disintegrated.' The gelatine is then allowed to solidify, forming a thin film over the inner surfaces of the flasks, and the flasks finally placed in an incubator. After a few days ' the colonies derived from the organisms, which were collected by the plug, make their appearance and can be counted and further studied. Now if the plug has been properly constructed, the flask into which the second, or more impervious plug has been introduced will be found to remain quite sterile, clearly showing that the first plug has arrested all the microbes

suspended in the aspirated air.' Fig. 14 represents Frankland's filtering-tube. The aspirator, or air-pump, is attached to the tube at B, while the air enters at A. The first filter-plug (*a*) consists of glass-wool, the second (*b*) of glass-wool and powdered glass or sugar, and finally a third plug (*c*) of cotton-wool to protect plug *b*. It is stated that 'this method yields results which agree not only very closely amongst themselves, but also with those obtained by Hesse's method, if the experiments are made in still air, which is the condition necessary for an accurate result being obtained with a Hesse tube.'

Although MM. Miquel and De Freudenreich were the first to use tube-filters containing plugs of glass-wool or asbestos, they subsequently found it expedient to substi-

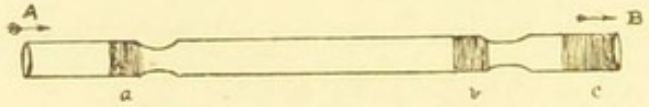


FIG. 14.—FRANKLAND'S TUBE (*one-third natural size*).

tute soluble filtering media for the insoluble glass-wool, asbestos, sand, etc. When insoluble media are employed the colonies form in large numbers, and the experimenter, according to M. Miquel, finds himself unfortunately compelled to make a *premature* estimation of the fructifying germs, before all the microbes or moulds have had time to reveal their presence by the formation of bacterial spots.

The introduction into water, or gelatine, of *insoluble* filtering substances, presents several disadvantages. Among these may be mentioned: (*a*) It is possible that certain germs may become imprisoned among the fibres of the glass-wool or asbestos; (*b*) The sand (when sand is used as a filtering medium) in gelatine may also hide some colonies, or prevent others from developing.

To obviate these disadvantages *soluble* media have come into use, and are largely used by the French school of bacteriologists.

In 1885, Dr. H. Fol (*La Nature*, 1885) recommended powdered marine salt as a filtering medium. Common salt would also be a useful medium if it were not so

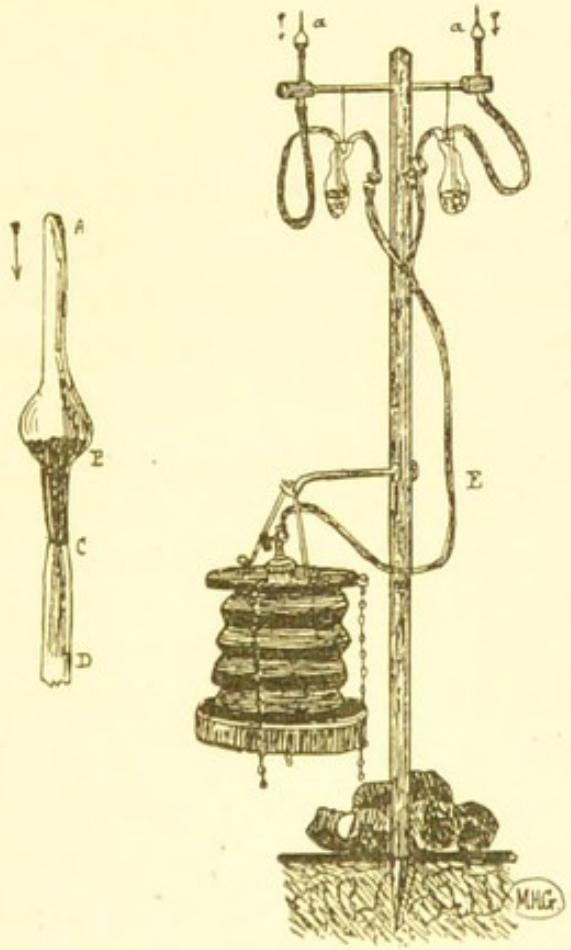


FIG. 15.—GAUTIER'S FILTERING APPARATUS: A—D (a and a) = Filter-tube. C = A plug. E = Aspirator. B = Sodium Sulphate.

deliquescent, and if one had the certainty that the germs fixed by the liquid which covers the crystals of moist sodium chloride did not suffer from the caustic action of this saturated water.

In 1886, Dr. Armand Gautier (*Revue Scientifique*, 1886) used for the same purpose de-hydrated sodium sulphate. This substance and powdered sugar are

perhaps the best soluble media for the filtration of aërial microbes. Fig. 15 represents Gautier's filtering apparatus. Anhydrous sodium sulphate is introduced into a pointed bulb-tube, and forms a layer at B. The sodium sulphate rests upon a plug, C, and the air is introduced by aspiration at D. After a known volume of air has passed, the sulphate is dissolved by allowing a certain volume of sterilized water to pass through the bulb in the same direction as the air was aspirated.

This water is collected in sterilized vessels, and is then ready for the study of the mixture of atmospheric microbes which it contains. The water is portioned, be it by the method of fractionation, of plate-cultivation, or by the mixed process of fractionation in gelatine.

FIG. 16.—MIQUEL'S FILTER.

M. Miquel (*Annuaire de l'Observatoire de Montsouris*, 1889) now uses an open tube, 8 inches long by $\frac{1}{4}$ inch diameter, provided with a cap (Fig. 16), instead of one drawn out to a capillary point; and without doubt this is one of the best (if not the best) forms to use for the microbian filtration of air. A and B are plugs, C the soluble filtering medium, D the cap, and air is aspirated from D to A.

According to Miquel the soluble substance used in the various tubes for the filtration of air ought to be unalterable and infusible at 170°-180° C. It ought to have *weak* antiseptic power, and to be as soluble as possible in pure water. Among soluble filters may be mentioned sugar, sodium sulphate, magnesium sulphate, sea-salt and sodium phosphate.

Crystalline salts should be deprived of their water of

crystallization by being heated in a platinum or iron dish. Although sea-salt has marked antiseptic properties, Miquel has shown that when employed in the proportion of not more than five to seven grammes per 1000 cc. of bouillon, it raises the nutritive power of the bouillon in regard to microbes. In the same way a small quantity of either sodium or magnesium sulphate and sodium phosphate introduced into bouillon favours the multiplication of microbes.

Whatever substance is chosen it must be pulverized in a mortar before use; and after the introduction of the filtering medium into the tube already containing the glass-wool plugs (A and B), the tube is heated to 170° or 180° C. in an air sterilizer, and after cooling it is ready for use.

As a rule, the French school of bacteriologists use liquid cultivating media, while the German and English schools prefer solid media. It has been said that ' the advantages possessed by solid over fluid media are very great; for whereas in fluid media, such as broth, the organisms are in no way restricted in their movements, and their mutiplication can take place indiscriminately throughout the entire liquid: on the other hand, if they are introduced into gelatine-peptone which has been first melted, they can be evenly dispersed throughout the culture-material by gentle agitation, and by subsequently allowing it to solidify they are not only isolated, but rigidly confined to one spot. Thus each individual organism becomes a centre round which extensive multiplication takes place, and in a few days definite points of growth are visible to the naked eye, which are appropriately described as *colonies*. Although each colony consists of many thousands, or even millions of individual microbes, yet as in the first instance they owe their origin

to a single organism or indivisible group of organisms, it is correct to regard the number of colonies as representing the number of microbes. These colonies have often very beautiful and characteristic appearances, and it is exceedingly remarkable how constant and distinct for one and the same organism these appearances are. In many cases they give rise to magnificent patches of colour—deep orange, chrome yellow, brown, various shades of red, green, black, etc. Often, under a low magnifying power, they are seen to spread over the surface of the gelatine, producing tangled networks of threads; sometimes they resemble the petals of a flower, sometimes the roots of a tree or its branches; in fact, one is constantly startled by the novelty and beauty of their modes of growth.'

In 1881 Dr. Koch (*Mittheilungen aus dem kaiserlichen Gesundheitsamte*, vol. i.) devised a method for the estimation of aërial microbes, by using a solid medium for their growth. Koch's apparatus consists of a glass jar about six inches high, plugged at the neck with sterilized cotton-wool, containing a glass capsule. The jar and its contents must have been sterilized at 150° C. before the glass capsule is filled with melted nutrient gelatine from a stock-tube. After the solidification of the gelatine, the jar is exposed to the atmosphere for a definite period of time, when the cotton-wool plug is replaced, and the jar removed to a warm place for the development of colonies.

A better method than the one of Koch is that devised by Hesse, and described by him under the title of 'Ueber Quantitative Best der in der Luft enthaltenen Mikroorganismen' (*vide Mittheilungen a. d. kaiserlichen Gesundheitsamte*, vol. ii.). Hesse's method consists in aspirating a known volume of air through a glass tube, 28 inches

(70 centimetres) long and $1\frac{3}{8}$ inch (3·5 centimetres) in diameter, which has previously been coated internally with a film of nutrient gelatine. 'The microbes, owing to the property they possess of rapidly subsiding in the absence of disturbing influences, fall on the surface of

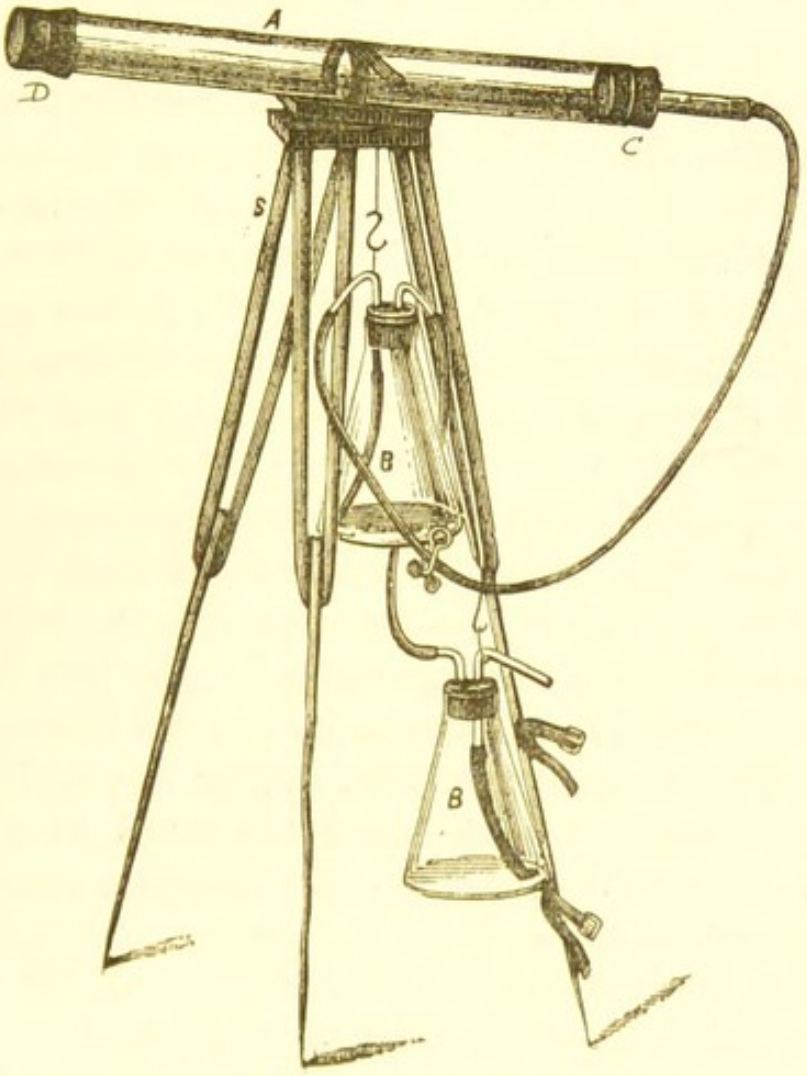


FIG. 17.—HESSE'S APPARATUS.

the gelatine, and give rise to colonies.' Hesse's apparatus is represented in Fig. 17. At *C* is an indiarubber stopper, perforated to admit a small glass tube, plugged with cotton-wool: and at the opposite end is a perforated indiarubber cap, which is covered by an unperforated cap (*D*) of the same material. The small glass tube is

connected by indiarubber tubing with an aspirator, consisting of two flasks (each 1,000 cc. capacity). The tube and flasks (*B*, *B*) are supported by means of a tripod stand (*S*).

Before introducing the nutrient gelatine, the tube, caps, and plug are sterilized by means of a solution of mercuric chloride, and finally with alcohol. After this treatment, 50 cc. of melted nutrient gelatine are poured into the tube. The tube and its contents are then sterilized in a steamer for an hour on three or four successive days.

After being heated for an hour on the third or fourth day the tube is rotated, so that the gelatine may form (after cooling) a thin film over its entire surface.

The tube is now ready for use, except that the outer cap and the cotton-wool plug must be removed, and the apparatus attached to the aspirator, the upper flask of which contains water. The water flows from the upper to the lower flask. When the upper one is emptied, the lower flask becomes the upper one, *i.e.*, the two flasks are reversible. By this means a known volume of air is made to pass through the tube. After the aspiration is completed the outer cap and cotton-wool plug are replaced; the tube is then removed to a warm situation for colonies to develop.

By the methods of Miquel, Frankland, Hesse, and others important results have been obtained concerning the number of microbes contained in the air at different places and altitudes, and under various climatic conditions. Among these results may be mentioned the following:

In a paper* read before the Royal Society of Edinburgh on March 18, 1889, the author of the present volume gave the results of his examination of the air of Lincoln,

* See *Proc. R.S.E.*, vol. xvii.

Paris, and London respectively. The methods used for estimating the number of microbian colonies in a known volume of air were those of Hesse and Frankland. The latter method is practically the same as that of Dr. Pétri (*Zeitschrift für Hygiene*, vol. iii.). Before August 6th, 1888, Hesse's method was used, while after that date the Frankland-Pétri method was substituted for that of Hesse.

(*a*) The Air of Lincoln.

Average number of colonies in 3 gallons.

Place.	Year, 1887.											
	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.
(1) Top of hill ... (near Cathedral)	3	6	14	16	19	25	34	—	30	28	12	4
(2) Base of hill ... (Broadgate)	18	26	30	41	50	62	65	—	59	57	19	17

(*b*) The Air of Paris.

Average number of colonies in 3 gallons.

Place or Part of Paris.	Situation in Paris.	August, 1887.
(1) Cimetière du Père la Chaise ...	E.	96
(2) Boulevard Saint-Germain ...	Centre.	104
(3) Forest of Ville d'Avray	S.W.	81
(4) Rue de Rennes	Centre.	99
(5) Palais du Trocadéro	W.	50
(6) Park of Versailles (near Palace)	S.W.	78
(7) St. Cloud (near Palace ruins) ...	S.W.	82
(8) Boulevard Voltaire	E.	100
(9) Cimetière Montparnasse ...	S.	98
(10) Cimetière Montmartre (near tomb of Offenbach)	N.	95
(11) Parc des Buttes Chaumont ...	N.E.	80

(*c*) The Air of London.

Average number of colonies in 3 *gallons.*

Place or Part of London.	July, 1888.	August, 1888.
(1) Forest Gate (Essex)	64	79
(2) City (near Bank)	85	110
(3) West End (Piccadilly)	80	96
(4) East End (near Mint)	88	106

The conclusions drawn from these investigations are the following :

(1) There are a larger number of microbes in the atmosphere during the summer than either the spring or winter. They appear to reach a maximum during the month of August.

(2) The number of microbes found in the atmosphere decreases the higher one ascends. Hence near the Lincoln Cathedral there are fewer microbes in the atmosphere (on any given day) than in the valley of the Witham (*Table a*). The same remark also applies to the number of microbes found in the atmosphere at the top of the Trocadéro Palace, Paris, where there are fewer microbes than in a low-lying but crowded thoroughfare like the Boulevard Saint-Germain (*Table b*).

(3) There are a larger number of microbes in the atmosphere of crowded centres than in less densely-populated districts.

(4) By gradually passing from a large city towards the country the number of aërial microbes decreases; *e.g.*, there are fewer microbes in the atmosphere of the Forest of Ville d'Avray, the Park of Versailles, and the village

of St. Cloud, than in the principal thoroughfares of Paris and London.

Dr. Fischer (*Zeitschrift für Hygiene*, vol. i.) and MM. Miquel and Moreau (*La Semaine Médicale*, 1885) have obtained important results concerning the number of microbes in *the air at sea*. The first-named observer 'found that beyond a distance of 120 miles from land microbes were generally absent. And, inasmuch as microbes are abundantly present in sea-water, it thus appears that no microbes are communicated to the air from the water even when the latter is much disturbed. Moreover, as might have been anticipated, this freedom from microbes was attained even in close proximity to land, provided the wind had passed over the above-mentioned distance of sea.'

'The *air of sewers* has been shown by Carnelley in this country and by Pétri in Berlin to be remarkably free from microbes, the number being almost invariably less than in the outside air. That this should be the case is only natural when the moist nature of the walls and the absence of dust in these subterranean channels is borne in mind, and although their liquid contents is teeming with bacterial life, there is no reason why the latter should be carried into the air provided no effervescence or splashing takes place. On the other hand, if the contents of the sewer enter into fermentation, and bubbles of gas become disengaged, minute particles of liquid with the living matter present may be carried to great distances, and it must not, therefore, be too hastily concluded, because sewer air is generally remarkably free from microbes, that therefore a visit to the sewers should be attended with such beneficial results as a trip to the sea or the ascent of a mountain summit!'

Besides the results already given, Drs. Miquel and

Frankland have published interesting statistics concerning the number of microbes in the atmosphere of Paris and London respectively.

The experiments of Dr. Miquel on the atmosphere of Paris have been published in the *Annuaire de l'Observatoire de Montsouris* from 1877 to the present time. In the last number of the *Annuaire* (for 1889) elaborate tables are given concerning the number of microbes in the air of Paris during the year 1888.

(*d*) The Air of Montsouris.

The figures given in the table (*d*) express the mean weekly results obtained simultaneously at Montsouris and at the Place St. Gervais during the year 1888.

Month.	Number of Weeks.	Microbes per Cubic Metre of Air.		Barometric Pressure (*mm.*).	Temperature (C.).	Hygrometric State.	Rainfall (*mm.*).	Wind.		Ozone.
		IVth Arrondissement of Paris.	Montsouris.					Direction.	Velocity (*km.*).	
Jan., 1888.	1	670	100	755	2·8°	89	7·9	S.W.	15	2·2
,,	2	1250	170	770·7	3·7	84	0·8	N.E.	11	3·0
,,	3	6000	490	766·2	−1·2	74	4·0	N.E.	16	1·6
,,	4	1400	500	760·8	3·4	82	3·8	S.W.	16	2·3
,,	5	1750	78	754·6	−3·1	78	5·8	N.E.	14	2·3
Feb., 1888.	6	720	100	753·2	6·5	85	6·0	W.	15	1·2
,,	7	4050	48	748·9	2·2	82	30·9	N.W.	21	2·2
,,	8	4400	92	746·1	−3·0	75	0·7	N.E.	16	1·5
,,	9	3470	210	757·0	−2·2	72	0·3	N.	14	1·7
March, 1888.	10	2730	85	754·7	5·8	67	15·3	S.W.	21	2·1
,,	11	2030	50	741·4	6·5	70	22·3	S.W.	19	2·6
,,	12	1480	80	750·7	0·5	76	15·8	*var.*	17	2·6
,,	13	8070	165	737·4	7·6	67	34·0	S.W.	24	3·1
April, 1888.	14	11100	150	752·7	3·7	51	4·6	N.E.	19	2·2
,,	15	2750	160	753·9	6·0	66	8·7	N.W.	13	1·8
,,	16	5830	91	750·7	10·8	67	14·6	S.W.	15	2·6
,,	17	5580	140	751·9	10·2	66	13·0	S.W.	18	2·6
,,	18	13100	285	754·7	12·2	58	16·7	S.W.	17	2·6
May, 1888.	19	7400	87	763·2	12·2	49	—	N.	14	2·5
,,	20	27800	98	750·7	16·3	52	14·2	*var.*	15	2·1
,,	21	10000	210	759·4	14·8	46	—	N.E.	20	3·5
,,	22	5800	225	754·9	14·5	58	3·5	S.W.	13	2·6
June, 1888.	23	11700	—	753·0	20·2	55	5·7	S.W.	16	2·0
,,	24	8550	—	754·0	15·1	57	13·0	W.	14	2·3
,,	25	4200	—	751·1	14·3	73	21·3	N.	13	2·1
,,	26	6000	—	751·1	17·9	73	39·6	S.	14	2·1
July, 1888.	27	5100	—	751·1	15·6	69	39·5	S.W.	17	2·2
,,	28	4550	—	755·6	15·1	67	12·6	N.W.	14	2·0
,,	29	7860	—	748·8	17·2	71	4·1	S.W.	14	2·1
,,	30	6700	—	751·7	18·1	68	13·3	S.	20	2·0
,,	31	7600	—	754·9	15·9	67	8·2	W.	16	2·2
August, 1888.	32	5000	—	758·8	19·5	66	9·5	S.W.	13	2·1
,,	33	27100	—	756·5	17·9	65	11·2	N.	16	—
,,	34	26000	—	753·3	17·5	65	22·7	S.	12	1·4
,,	35	88000	—	758·2	15·9	64	2·4	W.	12	1·5
Sept., 1888.	36	20500	—	758·6	15·7	61	0·5	W.	9	2·3
,,	37	8000	—	761·3	15·1	57	0·0	N.W.	10	2·6
,,	38	25100	—	758·5	17·1	60	—	N.E.	14	2·1
,,	39	10000	—	755·8	18·0	74	21·2	S.	9	1·1
Oct., 1888.	40	7780	260	746·5	9·1	65	13·7	W.	14	1·5
,,	41	3630	98	756·6	7·3	71	6·3	N.W.	14	1·5
,,	42	14500	—	761·7	8·3	61	—	N.E.	13	2·6
,,	43	7830	144	762·4	8·8	66	—	S.E.	10	2·0
Nov., 1888.	44	8600	143	753·9	11·9	77	19·8	S.W.	14	1·5
,,	45	6200	341	749·8	6·7	80	10·1	E.	13	1·4
,,	46	6140	136	756·4	9·7	85	7·1	S.	12	1·6
,,	47	7110	330	761·7	10·2	76	2·2	S.W.	21	2·8
,,	48	5470	121	748·3	8·7	80	10·1	S.W.	19	2·6
Dec., 1888.	49	7330	245	761·3	4·8	75	0·1	S.E.	11	1·5
,,	50	7430	260	752·1	1·8	85	1·2	N.E.	11	1·4
,,	51	2170	122	753·7	2·4	87	5·7	S.E.	11	0·6
,,	52	4200	68	750·6	5·5	85	14·2	S.W.	16	0·4

The figures in the column headed *barometric pressure* give the mean height of the barometer at Montsouris, the normal height being about 755 mm.

Under the head of *temperature* are given the weekly means (maxima and minima) indicated by the thermometer placed in the shade and sheltered, but near to the place where the aëroscopic experiments were conducted.

The *hygrometric state* is calculated from 9 a.m. to 3 p.m. in the vicinity of the aëroscopic experiments.

The eighth column gives the total *rainfall* (in millimetres) for each week. Columns nine and ten give the *direction* and the mean weekly *velocity* of the wind. The velocity is given in kilometres per hour.

The last column gives the weekly mean of ozone per 100 cubic metres of air at Montsouris, expressed in milligrammes and fractions thereof.

From the results of Dr. P. Miquel given in the preceding table, it will be noticed that in January, 1888, the number of atmospheric microbes was relatively high in the park of Montsouris, and during the third and fourth weeks of that month two remarkable maxima were registered. In February they diminished considerably, and increased in April and May. From June to September no micrographic results were recorded at the Observatoire de Montsouris. In October the aërial microbes were rare; they increased again in November, and lessened in December.

The next table gives the mean annual results (for eight years, 1881-88) of the number of microbes contained in one cubic metre of the air of Montsouris:

January . .	228	July . . .	676
February . .	170	August . .	628
March . .	255	September .	470
April . .	358	October . .	332
May . .	379	November .	239
June . .	448	December .	189

Dr. Miquel, in the *Annuaire* for 1889 (p. 388), compares the mean quarterly results of 1888 with those of a normal year. For instance, the mean number of microbes for the four seasons of 1888 and those of a normal year are given below:

Seasons.	Year, 1888.	Normal Year.
Winter	171	218
Spring	210	395
Summer	400	591
Autumn	185	253
Annual means	242	364

From these results Miquel concludes that during the year 1888 the air in the vicinity of Montsouris was poorer in microbes than a normal year.

As to the nature of the microbes collected at this well-known observatory, they may be divided as follows:

$\frac{3}{4}$ Micrococci.
$\frac{1}{10}$ Bacilli.
$\frac{1}{6}$ Bacteria.

The statistics of microbes gathered in the vicinity of the Hôtel de Ville and the Place St. Gervais (*i.e.*, fourth arrondissement) gave results numerically higher than those published in former years. This was due to the fact that many of the experiments were prolonged for twenty-four hours, at the same time soluble filters being used. In January the figure was very low; it increased in February and March; in May a remarkable maximum of 27,800 germs was noticeable, which con-

siderably raised the monthly mean of that month. In June and July the aërial microbes were moderately high, and at the end of the summer the number increased considerably, but decreased slowly from the commencement to the end of the autumn.

The following table gives, among other matter, an exact idea of the variations of the different microbes collected; and in the last column are also given the number of moulds collected in one cubic metre of the air at the Hôtel de Ville in 1888:

(*e*) The Air at the Hotel de Ville.

Mean monthly results.

Month.	Microbes.	Nature of Microbes.			Moulds.
		Micro-cocci.	Bacilli.	Bacteria.	
January	2330	79	7	14	480
February ...	2730	79	8	13	520
March	3560	78	10	12	515
April	6320	78	10	12	750
May	12820	72	12	16	1620
June	7610	69	15	16	2040
July	5050	68	16	16	3140
August	14900	72	14	14	3070
September ...	15900	76	13	11	3040
October	8430	76	11	13	2410
November ...	6700	75	12	13	2300
December ...	5280	71	16	13	1980
Mean ...	7720	75	15	15	1820

Contrary to what was observed at Montsouris, the figure of the moulds collected in the centre of Paris (Hôtel de Ville, 1820) is much less than the figure of microbes. The reason is that Paris has numerous centres producing microbes, and fewer centres producing moulds. But even in the centre of the town a place was found in

which there were a larger number of moulds (1820)—in the same volume of air—than at the Observatory of Montsouris (215); this was accounted for by the fact that the mucor growths of the country are added to the mucors developed in the town or found in the interior of the houses.

Dr. Miquel again compared the mean quarterly results obtained at the Hôtel de Ville during the year 1888 with those of a normal year. The results were as follows:

Seasons.	Year, 1888.	Normal Year.
Winter	2870	2960
Spring	8920	5120
Summer	12280	5450
Autumn	6800	3640
Annual means	7720	4290

The mean annual results (for eight years, 1881-88) of the micrographic analysis of air made in the centre of Paris are now given in the subjoined table:

January .	2,310	July . .	5,200
February .	3,140	August . .	5,640
March . .	3,420	September .	5,510
April . .	4,340	October .	4,335
May . .	5,950	November .	3,700
June . .	5,070	December .	2,885

If one compares the microbian curve represented in the diagram (Fig. 18) by the shaded spaces to the curve of the mortality caused by zymotic diseases expressed in the diagram by a black line, it will appear that there is a general coincidence in the configuration of the lines.

The mortality per weekly period varied little during the winter of 1888, but decreased during the early spring, and then increased along with the microbian curve.

At the commencement of the summer and during the month of July the microbian curve considerably diminished with the total deaths from epidemic diseases. It will be observed that in August there was a considerable increase of microbes in the atmosphere, coinciding with an elevation in the rate of mortality. In the autumn the

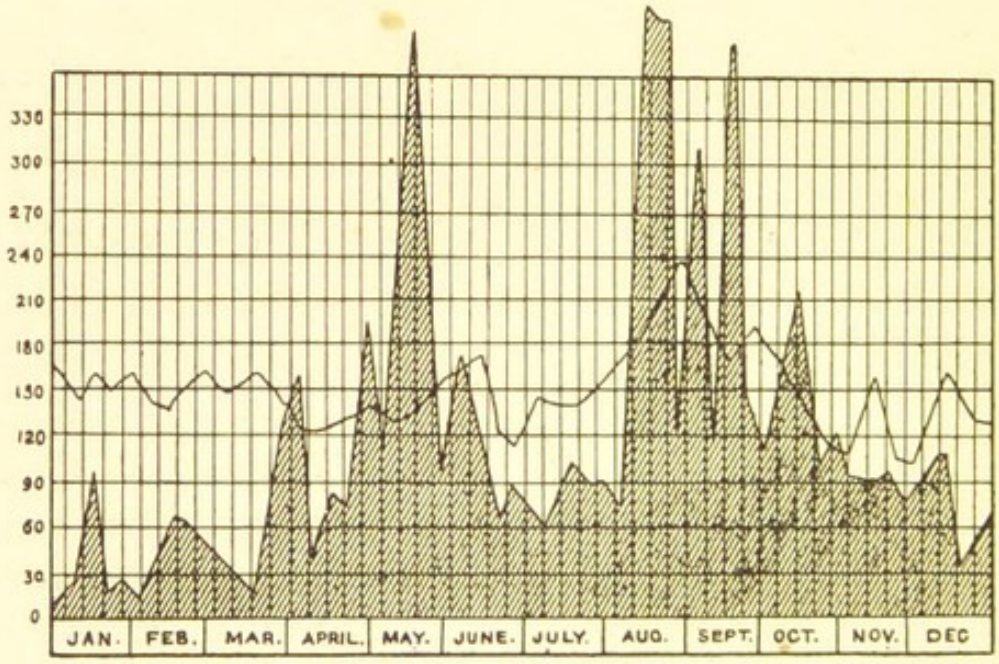


FIG. 18.

number of deaths diminished as the number of aërial microbes diminished.

Dr. P. F. Frankland (*Proceedings, Royal Society*, 1885 and 1886) obtained the following results by using Hesse's method:

One series of observations ' was made in 1886 on the roof of the Science Schools, South Kensington, London, in order to trace the seasonal variations in the number of micro-organisms present in the air of one and the same place. The following are the averages obtained for each month during which these observations were made:

(*f*) The Air of London (*S. Kensington*).

(*Number of colonies in* 10 *litres*—2 *gallons*).

January	4	July	63
February	—	August	105
March	26	September	43
April	—	October	35
May	31	November	13
June	54	December	20

'From these figures it will be seen that it is during the summer that the largest number of micro-organisms are found in the air, whilst the smallest average number was recorded in the month of January.'

'As regards the air at higher altitudes, experiments have been made,' says Dr. Frankland, 'on the dome of St. Paul's, in London, and on the spire of Norwich Cathedral, which show that even in ascending to such modest elevations in densely-populated centres the number of micro-organisms suspended in the air undergoes very marked diminution.

'Thus, on the top of Norwich Cathedral spire, at a height of about 300 feet,' Frankland found in 'ten litres (two gallons) of air only seven micro-organisms, and on the tower, about 180 feet high, he found nine, whilst at the base of the Cathedral, in the Close, eighteen were found. These results are fully confirmed by another series of experiments made at St. Paul's Cathedral. In this case the air examined from the Golden Gallery yielded in the same volume eleven, that from the Stone Gallery thirty-four, whilst in the churchyard there were seventy micro-organisms present.

'The contrast between town and country air, and even between the air of the London parks and streets, is also

exceedingly sharp. In Hyde Park—the place selected for the experiment being as far removed from roads and traffic as possible—Frankland found eighteen, whilst on the same day, June 7th [1886], the air in the Exhibition Road, South Kensington, yielded as many as ninety-four. On the following day, however, when the traffic was very great and the air was consequently heavily laden with dust, the number rose to 554. This is in marked contrast to the microbial condition of country air, for on the Surrey Downs in the same volume only two micro-organisms were found, and in the case of an extensive heath near Norwich only seven.

'Within doors we find that the number of micro-organisms suspended in the air depends, as we should have expected, upon the number of people present and the amount of disturbance of the air which is taking place. Thus, on examining the air in the large entrance-hall of the Natural History Museum (London), it was found to yield under ordinary conditions from fifty to seventy organisms in the same volume (two gallons), but on Whit-Monday, when an immense number of visitors were present in the building, as many as 280 were found. Again, on a paying-day at the South Kensington Museum, about eighteen micro-organisms were found, but on the Saturday, when no entrance-fee is charged, there were as many as seventy-three in the same volume of air.'

The author of the present volume has observed, on many occasions, that during and after a thunderstorm the atmosphere (both in the country and in towns) is almost free from microbes. It appears that atmospheric electricity is detrimental to the life of aërial microbes.

It has been stated that during a fog there are a greater number of microbes in the atmosphere than on either a moist or a dry day.

The Microbes of the Soil.

To study the microbes contained in soils, both liquid and solid media are used. There are two methods: (*a*) A small quantity of the sample of dried earth is added to a stock-tube containing liquefied nutrient gelatine. The earth or dried powder is distributed throughout the gelatine; after which the latter is poured out upon a glass plate, forming a kind of plate-cultivation. (*b*) The second method consists in triturating a sample of dried earth with sterilized cold water. A small quantity of the water is then sprinkled on the surface of a gelatine plate.

When liquid media (*e.g.*, beef-broth) are used, the earth is first triturated with water, and then a drop of the water is transferred to a flask containing sterilized beef-broth.

Certain pathogenic microbes, or their spores, have been isolated from soil. For instance: the spores of *Bacillus œdematis maligni* have been found in the upper strata of cultivated soils. Dr. Nicolaier (*Deut. Med. Woch.*, 52) obtained, by artificial cultures, a bacillus from soil, which is said to produce tetanus in rabbits, mice, and other animals.

According to recent researches, the microbes of the soil play an important part in the nutrition of plants. By the agency of certain microbes (among these may be mentioned *Bacillus tardecrescens*, *Bacterium ureæ*, *Bacillus fluorescens*, *Micrococcus cereus*) the organic matter and ammonium compounds present in soils are oxidized to nitric acid, which forms nitrates, with bases like lime, potash, soda, etc., contained in the soil. The nitrates are then taken up in solution by the rootlets of plants. In fact, the process of nitrification, so ably

investigated by MM. Schlösing and Müntz (in 1878), Mr. R. Warington, F.R.S. (*Journal Chemical Society*, 1884-88), Dr. P. F. Frankland (*Chemical News*, vol. lxi., p. 135),* and others, is the result of the life-histories of certain microbes found in soils.

How and where *leguminous* plants obtain their supplies of nitrogen has been a problem which has occupied the attention of botanists and scientific agriculturists for a number of years. Due to the excellent researches of Hellriegel and others, this problem has been solved. From these investigations it is evident that leguminous plants are provided with nitrogenous nutriment by certain microbes of the soil, and that these enter into a partnership, or symbiotic relationship, with the leguminous plant for mutual advantage.

M. Berthelot (*Comptes Rendus de l'Académie des Sciences*, vol. cviii.) has succeeded in establishing by certain proofs that a fixation of atmospheric nitrogen really takes place principally in certain vegetable soils, giving rise to the formation of complex organic compounds, similar to the albuminoids, whilst it does not take place in the same soil if sterilized. These phenomena, along with others, tend to refer the fixation of nitrogen to *microbes* contained in the soil. The fixation of nitrogen, according to Berthelot, does not take place exclusively by means of the lower plants — properly speaking, moulds, fungi, algæ, etc., which may be developed on the surface of the soil. On the contrary, the surface layer is often poorer in nitrogen than the entire mass.

There is little doubt that the majority of microbes contained in a soil play an important part in bringing about

* See also a paper by Dr. and Mrs. P. F. Frankland, which was read before the Royal Society on March 13th, 1890.

various changes in dead organic matter, which results in the production of plant-foods. The smallness of these microbes for such work as the processes of nitrification and the fixation of atmospheric nitrogen in the soil need not cause the least surprise when one bears in mind the exceedingly large number of microbes contained in the soil. One gramme of the latter may contain as many as two and a quarter millions of microbes.

The Microbes of Water.

For the bacteriological examination of waters two principal methods are used. One is known as the plate-cultivation process, introduced by Dr. R. Koch in 1881 (*see Mittheilungen aus dem Kaiserlichen Gesundheitsamte*, vol. i.); and the other as 'fractionnement dans le bouillon.'

(1) The gelatine or plate-cultivation method consists in taking a known small quantity (one to twenty drops) of the water, and mixing it with melted nutrient gelatine, contained in a stock-tube. After shaking the mixture, it is rapidly poured out upon a sterilized glass plate, then allowed to solidify, and finally placed in a damp chamber, kept at about 22° C. After a few days' incubation, colonies make their appearance on and in the layer of gelatine. The colonies are counted by means of the eye or lens, with the aid of Wolffhügel's counting apparatus, which consists of a glass-plate, ruled by vertical and horizontal lines into centimètre squares, which are often sub-divided. The cultivation-plate is placed on a black background, and the ruled glass plate placed over the former, without touching the colonies. 'If the colonies are very numerous, the number in some small divisions is counted; if less, in some large ones, and an average is obtained

from which the number of colonies on the entire surface is calculated.'

If the original sample of water is very impure (or, in other words, rich in microbes), it may be necessary to first dilute it with sterilized water.

In the Laboratoire de l'Observatoire de Montsouris, Dr. Miquel employs (to a certain extent) a conical flask (12 cm. diameter at the base, 5 cm. high, with a narrow neck, and provided with a glass cap, containing a sterilized plug of cotton-wool at the upper extremity). From 30 cc. to 40 cc. of melted nutrient gelatine are introduced into the flask, which is ultimately sterilized at 110° C. The flask is then allowed to cool, after which the gelatine is just melted, and the water under examination is introduced into the flask with all the necessary precautions.

After gentle agitation and allowing the gelatine to solidify, the flask is placed in an incubator at 18°—20° C. Dr. Miquel notes every twenty-four hours the number of colonies developed, until the day when one or more of the colonies overlap one another, or until liquefaction of the gelatine destroys the solid *substratum*, and with it 'the collective seed-bed of microbes.' To remedy this grave inconvenience, Miquel, as well as other bacteriologists, dilutes the sample of water before mixing it with the nutrient gelatine.

(2) The method of 'fractionnement dans le bouillon' is largely used by the French bacteriologists. It consists of two operations: (*a*) the dilution process; (*b*) the distribution of the water diluted.

To dilute the water Miquel employs flasks of a capacity between 30 cc. and 2,000 cc. These flasks have their necks covered with caps containing sterilized cotton-wool plugs. They are half filled with a known volume of distilled water, which is sterilized in a digester for

more than an hour at 110° C. In the digester these flasks do not lose any appreciable weight by the vaporation during sterilization.

The water under investigation ought always to be agitated before dilution. After agitation and dilution one gramme of the water is taken up by means of a sterilized capillary pipette (capable of discharging about twenty-five drops to the gramme), which is dipped four times into the water at different points of the liquid mass to obtain the above-mentioned quantity. By this means a fair sample of the water is obtained.

In the laboratory of M. Miquel, thirty-six small flasks of fifteen cc. capacity are each half filled with sterilized beef bouillon. These flasks, having each a glass cap containing a sterilized cotton-wool plug, are placed in a divided box. Each flask receives one, two, or three drops of the sample of water, as the case may be; all the flasks are placed in an incubator at 30°—35° C. during a period of at least fifteen days, when the microbian colonies are counted.

By the dilution and 'fractionnement' method in liquid media, there is no over-lapping of the colonies as when Koch's method is used. There are other advantages which the former method possesses. (*a*) As only a comparatively low temperature (20° C.) can be used with solid media, many microbes do not develop. With liquid media the temperature may be increased at 35° C. (*b*) The accidental contamination with aërial microbes is reduced to a minimum when the 'fractionnement' method is used. But, with these few drawbacks, Koch's plate method has well been described in these words:

'Cette méthode est simple et très élégante; elle rend surtout de réels services quand il importe de séparer les

microbes les uns d'avec les autres; elle permet le triage rapide des organismes contenus dans les eaux.'

Whichever method is used, the water collected from a river, stream, well, etc., must be preserved (until required) in a refrigerating apparatus at 0° C., in order to render the microbes inactive.

Dr. P. F. Frankland (*Proc. Roy. Soc.*, 1886) and Mr. Meade Bolton (*Zeitschrift für Hygiene*, vol. i.) have shown 'that many of the microbes found in natural waters are capable of the most abundant multiplication in the absence of practically any organic matter whatever;' and this multiplication occurs when the sample of water is thoroughly protected from air contamination. Dr. Frankland gives the following figures as an illustration of the rapid increase of microbes in a sample of water kept for only three days:

Number of microbes obtained from 1 *cc. of water.*

	Day of Collection.	Standing 1 Day at 20° C.	Standing 3 Days at 20° C.
Sample of water from Kent Co 's deep well in the chalk	7	21	495000

From these observations it is hardly necessary to point out that the sample of water should be examined immediately, or preserved (as already mentioned) in a refrigerating apparatus containing melting ice.

The average number of microbes contained in a cubic centimetre (cc.) of a sample of water from the river Witham during 1887 was ascertained by the author to be the following:

Number of microbes in 1 *cc. of water from river Witham at Lincoln.*

January	2,016	July	10,184
February	3,488	August	—
March	10,287	September	4,110
April	11,692	October	9,621
May	11,923	November	10,211
June	12,000	December	9,787

These figures (monthly means) give a yearly mean of 8,665 microbes per cubic centimetre, or quarterly means as follows:

Spring	11,300
Summer	11,092
Autumn	7,980
Winter	5,097

From these results (obtained by Dr. Koch's method) the greatest number of microbes in the Witham were found during the spring and summer.

Dr. P. Miquel (*Annuaire de l'Observatoire de Montsouris*, 1889) has from time to time made accurate micrographic analyses of various waters in and around Paris.

Paris takes its supplies of water from the Seine and Marne. There are three water-works belonging to the former, and one belonging to the latter river. During 1888 Miquel obtained the following results with the Paris water supply from the two rivers:

Months.	Seine.			Marne.
	Ivry.	Austerlitz.	Chaillot.	St. Maur.
January	6260	13040	18550	8370
February	17860	83000	166300	82500
March	18930	28570	35710	121800
April	105000	150000	114700	33750
May	20000	21750	73580	14800
June	16700	26500	95000	12060
July	8190	17550	81500	12800
August	6950	13100	90000	5690
September	3100	10350	36500	1560
October	15300	8320	45550	6770
November	56800	34500	125000	67300
December	95000	95000	120000	40000
Yearly means ...	30840	41805	79325	33950

The above results represent the monthly means of the number of microbes obtained from 1 cc. of water by the 'fractionnement' method.

The quarterly means are given in the next table:

Seasons.	Seine.			Marne.
	Ivry.	Austerlitz.	Chaillot.	St. Maur.
Winter	14350	41536	56854	70990
Spring	47234	66084	94425	20204
Summer	6080	13666	69333	6683
Autumn	55700	45940	96850	38029

Dr. Miquel gives the number of microbes contained in sewer-water (collected at Clichy and St. Ouen, Paris) as follows:

Number of microbes in 1 *cc. of sewer-water.*

Monthly Mean (1888).	Clichy.	St. Ouen.
January	8570000	7140000
February	—	21430000
March	15810000	14280000
April	7500000	50000000
May	8400000	4000000
June	16000000	14660000
July	23600000	10400000
August	4170000	8340000
September	4120000	7200000
October	3100000	900000
November	5200000	2350000
December	18200000	8500000
Mean	10420000	11425000

The above results give quarterly means (Clichy *plus* St. Ouen) as follows:

Winter . . .	14,780,000
Spring . . .	16,760,000
Summer . . .	9,638,000
Autumn . . .	6,375,000

It appears from the observations of Miquel that the greatest number of microbes found in river and sewer waters is during the spring months.

'From numerous investigations made by means of gelatine plate-cultivations, it appears that whilst surface-waters, such as rivers, contain an abundance of microbial life, waters which, like those from springs and deep wells, have undergone filtration through porous strata of the arth have been deprived of those microbes which they contained whilst at the surface. This removal of microbes (see P. F. Frankland's paper in the *Proceedings Royal Society*, 1885) from water also takes place in a very

marked manner when it is submitted to some kinds of artificial filtration, such as that through very finely-divided coke or charcoal, as well as in the filtration of water on the large scale through sand. The process of filtration, however, which absolutely removes microbes with the greatest degree of certainty is that introduced by Pasteur, in which the water is forced through porous porcelain. It is especially noticeable that the efficiency exhibited by these various materials in removing microbes stands in no sort of relationship to their chemical activity, *i.e.*, power of removing organic matter from water. Thus the porous porcelain produces practically no change whatever in the chemical composition of the water, whilst it deprives it entirely of microbes.'

Dr. Frankland says that 'the relative abundance of bacterial life in surface-water, in deep-well water, as well as in surface-water after filtration through sand on the large scale, is well illustrated by the following results:

'Thus the average number of microbes obtained during the past year (1887) from a cubic centimètre (about 20 drops) of the raw water as abstracted from the rivers Thames and Lea by the metropolitan water companies was 21,500 and 13,200 respectively. The same water, however, after having undergone storage and filtration, contained on an average respectively 500 and 450 microbes in 1 cubic centimètre. It is at once apparent, therefore, what striking results can be obtained by sand filtration as at present carried out, and there is no doubt that with the introduction of fresh improvements and increased care an even greater reduction will be effected.

'In deep-well water obtained from the chalk, which has undergone no artificial filtration, we find the remarkably low number of eighteen as the average for the year.

Thus the artificial filtration through sand is far surpassed by the exhaustive filtration through vast thicknesses of porous strata.'

A thorough study of the distribution of contagious (epidemic, endemic, etc.) diseases by the agency of air, soil, and water is of great importance to the physician and others interested in the prevention and cure of those diseases which periodically attack mankind.

There is little doubt that air, soil, and water are all-important factors in the distribution of most contagious diseases.

As examples, we will allude to: malarial fevers, Asiatic cholera, Oriental plague, yellow fever, and dysentery.

(1) The various kinds of malarial fever are all produced by the same microbe (*Bacillus malariæ*). Concerning the distribution of the disease, moisture and air have much to do with it; for the disease is more abundantly developed in wet than in dry years. Moisture in the soil is essential for the production of malaria, while clayey, loamy, and marshy soils favour its development. In marshy districts, the larger the amount of organic matter present in a soil the greater will be the prevalence of malaria. The disease is more prevalent the lower the level of the country, although in Central Africa a height of 2,500 feet is not free from it. Both air and water may convey the disease, and there is little doubt that it finds an entrance into the system by means of air, potable water, and food.

From these facts it will be seen that air, soil, and water are potent agents in the distribution of malaria.

(2) *Cholera*, as already stated, 'follows the course of rivers.' Moisture in the atmosphere and the soil is needed for its distribution. Moist winds spread it, but

the great factor in the distribution of cholera is human intercourse.

'Persons suffering from the disease, though it may be only in a latent form, undoubtedly convey the poison for long distances; and it is a well-known fact that troops, pilgrims, and emigrants have spread it far and wide. It is necessary, however, for an epidemic of cholera to arise, that the poison conveyed into a district should find there a fitting soil for its growth. What that fitting soil is, it is impossible yet to say.'

(3) *Oriental plague* is a violent form of blood-poisoning, doubtless due to a specific living virus. It is attended by bubo of the inguinal or other glands, and occasionally carbuncles may appear on the limbs.

Human intercourse spreads this disease. It has no connection with the soil, and, unlike malaria, the disease does not lurk about in low-lying places; in fact, moderately high situations are more prone to be affected by Oriental plague than low-lying ones, although the plains are not free from it.

This is a disease which, as far as is known, is not distributed by air, soil, and water.

(4) *Yellow fever* is distributed (within certain areas) by moist winds and human intercourse. Water and the geological characters of the soil have nothing to do with the spread of this disease, although it is a disease which 'clings to the ground,' hence one of the reasons of its endemic nature. It is always prevalent in the plains near the sea-coast, and 'along the courses of the great rivers.'

(5) *Dysentery* is a specific disease due, most probably, to a microbe. The disease is often epidemic, and sometimes sporadic. Moisture in the air favours its development, and spreads it. Contaminated drinking-water

frequently spreads the disease, but there is no connection between the disease and the geological nature of the soil.

From these remarks the reader will note that air, soil, and water* play a most important part in the distribution of diseases.

Reverting once more to the bacteriological examination of water, 'it is often urged that such an examination of water is of little practical importance, inasmuch as the microbes found are not necessarily prejudicial to health, and that the method of examination does not aim at the detection of harmful forms. A little more mature consideration, however, will show that the actual detection of harmful or pathogenic forms is a matter of very little importance; and if methods of water purification are successful in removing microbes in general, and more especially those which find a suitable home in natural waters, there can be no serious doubt that they will be equally successful in removing harmful forms, which are not specially adapted for life in water. Could it be, for instance, reasonably contested that a method of purification which is capable of removing *Bacillus aquatilis* from water would be incapable of disposing of the *Bacillus anthracis* when suspended in the same medium? The supposition is, on the face of it, absurd, and not a particle of experimental evidence can be adduced in its favour. It is, therefore, only rational to conclude that those methods of water purification, both natural and artificial, which succeed in most reducing the total number of microbes, will also succeed in most reducing the number of harmful forms should they be present.

* Dr. R. T. Thorne, F.R.S., has shown that typhoid fever was disseminated by *water* at Caterham and Redhill (Reports, Medical Officer of Local Government Board).

'Of much more importance,' says Dr. P. F. Frankland, 'than the discovery of pathogenic organisms in particular waters is the problem of ascertaining the fate of pathogenic forms, when these are introduced into waters of different kinds. A considerable amount of work has been done in this direction with a number of typical pathogenic forms,* and some very remarkable results have been obtained. Thus it has been found that the bacilli of anthrax do not survive many hours on being introduced into ordinary drinking-water; their *spores*, however, are not in any way affected by such immersion, and even in distilled water the latter retain their vitality for practically an indefinite length of time. In polluted water, such as sewage, on the other hand, not only do the bacilli not succumb, but they undergo extensive multiplication. Similarly Koch's "comma bacillus" was found to flourish in sewage, being still present in very large numbers after eleven months' residence in this medium. In deep-well and filtered Thames water, on the other hand, although the "comma bacilli" were still demonstrable after nine days, they were only present in small numbers. Much less vitality is exhibited by the micrococcus of erysipelas when introduced into waters of various kinds, for even in sewage this organism was not demonstrable on the fifth day. In fact, all the pathogenic micrococci which have been experimented with in this manner exhibit but little vitality under similar circumstances.

'From these experiments it appears, therefore, that whilst ordinary drinking-water does not form a suitable medium for the extensive growth and multiplication of

* Frankland in Proc. Roy. Soc., 1886; Wolffhügel and Riedel in *Arbeit. aus dem kaiserlichen Gesundheitsamte*, 1886; Meade Bolton in *Zeitschrift für Hygiene*, vol. i.

those pathogenic forms which have hitherto been made the subject of investigation in this respect, yet that, *in the condition of spores*, they are extremely permanent in any kind of water, however pure, and that even those of which no spores are known may often be preserved for days or even weeks.

'Thus the investigations which have hitherto been made on the microbes of air and water, by the light which they throw on the behaviour of microbes in general in these media, the manner in which they may be preserved, and the manner in which they may be removed, are of great service in indicating how the spread of zymotic diseases through these media is to be avoided.

CHAPTER V.

ANIMAL ALKALOIDS, ETC.

ORGANIC chemistry has long known of an important class of compounds occurring in plants, which have many properties in common. These compounds, which are produced during the life of plants, have been called alkaloids, indicating that they possess alkaline properties; and in many ways they have similar reactions to the alkalies of mineral or inorganic chemistry.

Not only are alkaloids found in the vegetal kingdom, but they are also present in the animal kingdom.

The first animal alkaloid was discovered by Dr. Armand Gautier in 1872, and since that date a large number of alkaloids from the animal kingdom have been isolated, and their properties investigated.

Gautier has divided the animal alkaloids, etc., into three classes, as follows :

(1) Ptomaïnes.
(2) Leucomaïnes.
(3) Extractives.

The ptomaïnes* were first discovered in decomposing animal tissues, as their pseudonym of *cadaveric* alkaloids implies. Their presence in these dead tissues introduced a new factor in the *post-mortem* search for poisons in

* From $\pi\tau\tilde{\omega}\mu\alpha$.

suspected cases. But a more important result of their discovery has been the explanation of the cases of poisoning by decayed animal foods, such us sausages, fish, 'tinned' and putrid meats, in which they have been found.

The ptomaïnes are due to the action of microbes (or soluble zymases produced by microbes) on the albuminous substances of dead tissues.

The majority of ptomaïnes are produced during the process of *putrefaction* of animal substances. By the direct or the indirect action of putrefactive microbes the albuminoid molecules are disintegrated with the formation of ptomaïnes among other products. Most likely a certain amount of carbon, hydrogen, nitrogen, etc., of these molecules are required to sustain the life of the microbes, and the residue from these albuminoid molecules consists of ptomaïnes among other substances.

Not only have putrefactive microbes the power of giving rise to ptomaïnes, but certain pathogenic microbes (if not all) yield ptomaïnes.

Ptomaïnes have been isolated from the following diseases: anthrax, tetanus, typhoid fever, scarlet fever, cholera, rabies, etc.

Recent researches, especially those of Gautier, have brought to light the fact that similar bodies of an alkaloidal nature may be produced within, and by, the living organism. In this case they may be considered as of 'vital origin,' the products, that is, of the metabolism of protoplasm; or they may, in some cases, be the result of the decomposition of albuminoid bodies; in both cases the term 'leucomaïnes' has been given to them by Gautier. On this point he says, in a paper published in the *Bulletin de l'Académie de Médicine* (1886):

'Vu leur origine albuminoïde, et pour distinguer cette

nouvelle classe de celle des alcaloïdes cadavériques ou *ptomaïnes*, j'ai donné aux alcaloïdes qui apparaissent durant la vie dans les tissues des animaux le nom de *leucomaïnes* (de λεύκωμα, *blanc d'œuf*), nom qui se borne à rappeler que ces alcaloïdes dérivent tous des substances albuminoïdes.'

The leucomaïnes are, therefore, nitrogenous compounds, produced normally or abnormally during life. For example, not only do leucomaïnes exist in a minimum proportion in normal human urines, but they augment very notably during the course of certain infectious diseases. Many ptomaïnes and leucomaïnes have been isolated, purified, and obtained in the crystalline condition, as well as their chemical and physiological actions noted.

There is still a third class of bodies allied to the ptomaïnes and leucomaïnes, which Gautier believes are elaborated in the living animal economy. These nitrogenous compounds, known as 'extractives,' are uncrystallizable bodies, which have not been determined.

The various members of the three classes of animal 'alkaloids' are all poisonous, but the most violent are the extractives.

'The greatest source of danger from microbes is now believed to exist in the poisonous products which they manufacture; and it is in the artificial cultivations in flasks and tubes that the poisonous products of the pathogenic activity of the microbes are to be sought for.'

'It has been shown that many pathogenic microbes manufacture poisonous products; the microbes of typhoid fever, of Asiatic cholera, of blue pus, acute experimental septicæmia, and of diphtheria, all belong to this class.'

It appears that the real cause of many infectious diseases is due to the formation of certain poisonous

alkaloids by microbes, and that the microbes are the means of spreading infection.

Before discussing the nature, origin and properties of various animal alkaloids, a short historical account of the subject may not be out of place.

In 1820 Kerner was the first experimenter who appears to have given an account of the origin of a poisonous alkaloid from the decomposition of albuminous matter; and he noted the resemblance between the toxic effects of poisonous sausages and those of atropine ($C_{17}H_{23}NO_3$).

In fact, Kerner went so far as to state that he believed that tainted sausages contained a poisonous substance (analogous to atropine), produced from the decomposition of albumin.

In 1822 Gaspard and Stick observed the poisonous nature of certain cadaveric extracts.

In 1849 Liebig, and afterwards Pettenkofer, discovered creatinine in the urine of man and of the dog. It was the first body of an animal origin obtained with strong alkaline properties; but certain *à priori* theories prevented the two distinguished chemists from following up this discovery in what has since proved to be a most fertile field of research.

After creatinine ($C_4H_7N_3O$), creatine ($C_4H_9N_3O_2$) was discovered in muscles and animal tissues. Liebig believed that creatinine was produced in the animal economy by the dehydrating action of certain acids, salts, or heat on creatine, and he erroneously explained the presence of creatinine in urines by supposing that the alkaloid resulting from the reaction on creatine passed from the muscular tissues into the bladder. Liebig (*Annalen der Chemie und Pharmacie*, vol. lxii., p. 278) also stated that creatine did not possess any of the properties which are characteristic of organic bases! Liebig's

mind (like a great many others since his day) was influenced by a false theory, still prevalent, that animals do not furnish any nitrogenous compounds, other than those of an amidic nature, or at the outside of methylamine and trimethylamine (Gautier).

In 1856 the Danish physiologist, Panum (*Bibliotek for Laegen*), showed that putrid animal matter contains a poisonous substance of a chemical nature, which causes inflammation of the intestines.

This substance, which is soluble in water and alcohol, is very poisonous; from five to six centigrammes are sufficient to kill a dog. Panum's researches on the cause of 'putrid infection' were subsequently confirmed by Hemmer, Schweninger, Müller, Raison, Schmitz, and others.

In a paper published in the *Medic. Centralblatt*, 1868, p. 497, Drs. Bergmann and Schmiedeberg extracted from putrid beer a crystallizable nitrogenous substance which they named sepsin.

In 1869 Liebreich discovered betaine ($C_5H_{11}NO_2$) in human urine. Betaine is therefore not only a product of the vegetal (as has been long known), but also of the animal kingdom. In the same year, Zuelzer and Sonnenschein (*Berlin Klin. Wochenschr.*, 1869, No. 2) discovered an alkaloid (resembling atropine in its physiological actions) in decomposing animal matter. Like atropine, this substance dilates the pupil, increases the heart's action, and paralyzes the muscular fibres of the intestines. The alkaloid of Zuelzer and Sonnenschein 'has also been found in the bodies of persons suffering from typhus fever.'

In 1870, Dr. Armand Gautier commenced his important researches on the albuminoids, and in 1872 discovered several alkaloids of an animal origin. About the same

time the late Professor F. Selmi, of Bologna, began to work on the same subject; and on December 6, 1877, he presented a paper to the Académie de Bologne, in which he detailed the isolation of the two new alkaloids produced during the putrefaction of pure albumin.

In 1875 Dr. B. W. Richardson (*Lancet*, April 3, 1875) isolated an alkaloid from the sero-sanguineous fluid obtained from the peritoneal cavity of a patient suffering from pyæmia. This alkaloid (septine) forms poisonous salts with acids.

Dr. G. Pouchet, in a paper before the Medical Faculty of Paris (1880), announced that he had extracted from human urine, not only allantoin—regarded since by Baeyer* as a ureide—but also carnine ($C_7H_8N_4O_3$) —already known as a product obtained during the putrefaction of flesh—and an alkaloid which was subsequently found to have an empirical formula of $C_7H_{14}N_4O_2$. This alkaloid possesses all the general properties of a ptomaïne.

During the year 1882, Dr. C. Bouchard presented to the Société de Biologie (August 5) two remarkable papers on the origin and nature of certain pathogenic and non-pathogenic alkaloids present in human urines and fæces.

The conclusions Bouchard arrived at may be summarized as follows:

(1) That alkaloids are always present in the urine in certain infectious diseases (*e.g.*, typhoid fever). These alkaloids have an intestinal origin (*Revue de Médecine*, 1882, vol. ii., p. 825).

(2) The same alkaloids were found in normal urines.

(3) The same alkaloids were found in normal and abnormal fæces. Some of these nitrogenous compounds

* *Récherches sur le groupe urique.*

are soluble in ether, while others are soluble in chloroform.

(4) When present in normal urine, the alkaloids are produced in the intestines, from the decomposition of albuminoids by the agency of putrefactive microbes; and after having been more or less absorbed by the mucous membranes, they are eliminated by the kidneys.

(5) Although present in small quantities in normal human urines, these alkaloids greatly increase during the course of certain infectious diseases.

(6) The five or six different alkaloids present in urine have been divided into a narcotic and a convulsive group (*Comptes Rendus*, 1884); and the toxic effects vary in different individuals.

(7) Bouchard, in the *Gazette Hebdomadaire* (1886), defines 'a *toxic*, or a unit of poison, as that amount of poison required to kill one kilogramme of living matter, *e.g.*, of rabbit. The *urotoxic* is that quantity of urinary alkaloids capable of killing a rabbit weighing one kilogramme.'

The extraction of alkaloids (ptomaïnes) from urines by means of ether in a certain number of pathological cases has also been made by MM. Lépine and Guérin in 1884, and since that date by MM. Lépine and Aubert (*Revue de Médecine*), and others, who have generalized these researches, and shown that in the course of the same disease abundant poisonous products can be extracted from the urines until the crisis is reached, when these bodies again diminish and ultimately disappear.

The poisonous products contained in urines are different in different diseases. Thus the urotoxic alkaloid in cases of typhoid fever is a different compound from the one in pneumonia.

Among recent works on the subject of animal alkaloids

are the following: (*a*) Dr. O. Bocklisch (*Berichte der deutschen chemischen Gesellschaft*, vol. xx., p. 1441) found that *Vibrio proteus* produced in contact with sterilized beef cadaverine ($C_5H_{14}N_2$), which had been proved by Dr. Ladenburg (*Berichte der deut. chem. Gesellschaft*, vol. xix., p. 2585) to have all the chemical and physical properties of pentamethylenediamine ($C_5H_{14}N_2$). This alkaloid produced by *Vibrio proteus* (Finkler's bacillus) is non-poisonous. As this microbe has been found to be the cause (directly or indirectly) of sporadic cholera, or cholera nostras,* it appeared peculiar that the product of its life-history should be non-poisonous. Bocklisch, however, remembered that the human intestine was never free from putrefactive microbes, and this led him to try a further series of experiments. Ultimately he found that when *Vibrio proteus* was allowed to live upon sterilized beef along with putrefactive microbes, besides cadaverine, a very poisonous base—methylguanidine [$CN_3H_4(CH_3)$] —was the chief product formed by the microbes. There is little doubt that this substance is directly the cause of cholera nostras.

(*b*) Brieger (*Berichte*, vol. xix., p. 3119) has succeeded in isolating four alkaloids from pure cultivations of the bacillus which causes tetanus. The first, *tetanine* ($C_{13}H_{30}N_2O_4$), produces tetanus in animals. The second alkaloid is *tetanatoxine*, which produces tremor and paralysis, followed by violent convulsions. A third, *spasmotoxine*, produces tonic and clonic convulsions. The fourth alkaloid (which has not been named) causes tetanus accompanied with a flow of saliva and tears.

Therefore tetanus is most likely due to the above poisons manufactured by the tetanus bacillus, producing

* See Finkler and Prior in *Ergänzungshefte zum Centralblatt für Allgemeine Gesundheitspflege*, 1885.

their effects after getting into the blood, by virtue of some selective action on certain parts of the motor nerve centres.

(*c*) Although it has not been isolated, Pasteur believes that the virus of hydrophobia is a microbe, and that it produces an alkaloid. Dr. Anrep (*British Medical Journal*, 1889, p. 319) has isolated a toxic ptomaïne from the brain and medulla oblongata of rabbits suffering from rabies. This ptomaïne produces all the characteristic symptoms of rabies.

(*d*) Although many distinguished pathologists have not accepted Koch's evidence of the bacillary nature of Asiatic cholera, there can be no doubt, after the important and extensive researches of Drs. Macleod and Milles (Proceedings, Royal Society of Edinburgh, vol. xvi. [1889], pp. 18-35), that the comma bacillus of Koch is the cause (directly or indirectly) of Asiatic cholera.

Brunton, Lewis and Cunningham, Klebs and Cantani, and others, have all obtained indications of a poison or alkaloid in cholera dejecta. In 1885 Pouchet obtained a base, of an oily nature, from cholera stools. This base belongs to the pyridine series, and is most probably one of the alkaloids manufactured by the comma bacillus. Brieger has also obtained two alkaloids from pure cultivations of the same bacillus in beef-broth. Since the researches of Pouchet and Brieger, M. Villiers (*Comptes Rendus*, vol. c., pp. 91-93) described the properties of an alkaloid, extracted by Stas' method, from the intestines and kidneys of two patients who had died from cholera. The alkaloid is a liquid with an odour of hawthorn, and it combines with hydrochloric acid, forming a hydrochloride which is soluble in water. When six milligrammes of the latter substance, dissolved in half a cubic centimètre of distilled water, were injected

(hypodermically) into the thigh of a guinea-pig, the following symptoms were observed :

(1) Violent tremblings of the limbs. (2) The animal refused nourishment. (3) Marked periodic variations in the muscular contractions of the heart. (4) Death in four days after the injection. It appears from the researches of Pouchet, Brieger, and Villiers that the comma bacillus produces a number of different alkaloids. As these experimenters did not work under exactly the same conditions, it is possible that the cholera microbe is capable of manufacturing various substances, according to the nature of the medium in which it lives. Bocklisch (*Berichte der deutschen chemischen Gesellschaft*, 1887) has shown that certain bacilli ' may under altered conditions produce ptomaïnes of dissimilar chemical constitution and physiological action.'

(*e*) It was shown by Duclaux (in his work *Ferments et Maladies*) that when the ptomaïne produced by *Bacterium choleræ gallinarum* (which has narcotic properties) is separated, by filtration through a Chamberland filter (of porous porcelain), from its microbe, it does not produce fowl cholera, but causes a passing sleep, which does not generally end fatally.

From this fact, the conclusion may be drawn that in fowl cholera the microbe is essentially the active agent in producing the disease.

(*f*) The cause of typhoid fever has been stated by Eberth (*Virchow's Archiv*, vols. lxxxiii. and lxxxvii.) to be due to the *Bacillus typhosus*. Klebs (*Archiv für Exper. Pathol.*, vol. xii.) found similar bacilli in the spleen, mesenteric glands, lungs, larynx, and in the inflamed Peyer's patches, in fatal cases of typhoid fever.

Whether the *Bacillus typhosus* is the real cause of typhoid fever is still doubted by some writers. A pure

cultivation of the microbe yielded, in the hands of Brieger, a small quantity of a base (typhotoxin) which dilates the pupil, produces diarrhœa, and rapidly kills animals. The quantity of typhotoxin obtained varied considerably, sometimes being a mere trace. This was due to the nature of the cultivating media, for the microbe grows better in some media than in others. If the contagion is conveyed by potable water, milk, the atmosphere, sewage, sewer gas and bad drains, one can account for numbers of persons in an infected neighbourhood not taking the disease while others are attacked. Although the contagion may enter the bodies of those who do not become diseased, the microbe does not find there a suitable soil for its development and multiplication.

Dr. L. Brunton says, in regard to typhoid fever, that 'the symptoms do not point so much to the formation of a poison affecting the body generally, as to the local action of the microbes upon the intestines, although in some epidemics of typhoid the intestinal symptoms are but slightly marked, while bronchial irritation is due to the action of a microbe or to a ptomaïne produced by it on the bronchial mucous membrane.'

As Brieger only obtained a *small* quantity of the base, typhotoxin,* from pure cultivations of the microbe, may not this be due to the absence of certain micrococci in his cultures, which are always present in the intestines in typhoid fever? It may be that a different alkaloid to Brieger's base is produced by the joint action of the bacilli and micrococci in the human body.

(*g*) The disease known as malignant pustule or anthrax is due, without doubt, to a microbe—*Bacillus anthracis*. This microbe flourishes in the blood, spleen, etc., of man, sheep, and other animals. It has been stated that the

* See also De Blasi in *Gazzetta Chimica Italiana*, vol. xviii., p. 521.

anthrax bacillus produces a ptomaïne called anthracin; but the researches of Pasteur, as well as those of Nencki, Gautier, and others have shown that if the anthrax bacilli produce a ptomaïne or a ferment (see Chapter VI.) at all, the latter are incapable of producing the disease, although an injection of the filtered fluid,* from a pure cultivation of anthrax bacilli, has been shown by Pasteur, Perdrix, and Wooldridge to render an animal proof against subsequent inoculation.

Dr. Paul Bert believed that after destroying the bacilli by compressed oxygen the fluid was still virulent, but Pasteur proved that the *spores* of the microbe had not been destroyed by the compressed gas.

From these facts the *Bacillus anthracis*† is certainly the *contagium vivum* of anthrax.

The masterly researches of Gautier and Brieger have shown that many of the animal alkaloids are analogous in their chemical constitution to certain alkaloids contained in plants. For instance: during the putrefaction of fish, Brieger met with a soluble base having the same composition and the same action as muscarine (obtained from *Agaricus muscarius*) or oxynevrine. The putrefaction of cheese furnished Brieger with neuridine (which he had previously isolated from flesh), a base analogous to isophenylethylamine.

But it appears also, from the researches of the German chemist, that the same species of microbes give different products according to the nature of the medium on which they live. Certain putrefactive microbes living on the flesh of mammals produce nevrine; while on fish they produce muscarine.

The researches of Gautier and Etard have thrown the

* Filtered through a Chamberland filter.

† For other chemical products of the growth of *B. anthracis* see a paper by Dr. S. Martin in *Nature*, vol. xlii., p. 118.

most light on the properties and the chemical composition of the ptomaïnes, especially those produced during the putrefaction of animal tissues. Many of the ptomaïnes produced by putrefactive bacteria have been referred by MM. Gautier and Etard to the pyridine and hydropyridine series of organic bases.

The putrefactive bases, or ptomaïnes, are, as a rule, colourless, oily liquids, extremely alkaline, neutralizing the most powerful acids. Some of these ptomaïnes have the power of absorbing atmospheric carbonic acid. The ptomaïnes belonging to the previously mentioned series of organic bases are not amides, as some authors maintain.

The ptomaïnes devoid of oxygen emit a penetrating and tenacious odour, resembling hawthorn, musk, or syringa. This odour is so persistent that Dr. Gautier found it in the products of prehistoric putrefactions (transformed into guano and phosphate of lime), and met with in certain bone caves of the Stone Age and of the Age of the Cave Bear.

In union with acids the ptomaïnes yield crystallizable salts, very changeable in the presence of an excess of the mineral acid, which colours them rose and red, and then precipitates them in the form of a brown resin. All the ptomaïnes appear very oxidizable and very unstable. Their crystalline platinochlorides are sometimes soluble, and at other times only slightly soluble, the colour varying from yellow to a deep rose. As the platinochlorides are soluble in an excess of platinic chloride, it is necessary to quickly extract the latter by washing with cold water, and drying the crystals *in vacuo* over sulphuric acid. The crystals must not be exposed to light if they are to be preserved without alteration.

All the ptomaïnes (cadaveric) are soluble in alcoholic

ether. Many of them dissolve in chloroform and amylic alcohol.

The general reagents which precipitate the ptomaïnes are the following:

Myer's and Nessler's reagents, a solution of iodine in potassium iodide, the iodide of bismuth and potassium, and the phospho-molybdate of sodium. Mercuric chloride sometimes precipitates, and sometimes does not precipitate, the ptomaïnes; but it generally forms with them a double crystallizable chloride deposited from boiling water.

Auric chloride often gives a yellow precipitate, soluble in water, or generally a very soluble aurochloride which rapidly dissolves.

Picric acid forms slightly soluble picrates.

Tannin produces insoluble, as well as very slightly soluble, tannates.

The principal characteristic colouring reactions of the ptomaïnes were specially studied by the late Professor Selmi. They are as follows:

Sulphuric acid diluted with a very small quantity of water produces a red-violet colour.

Hydrochloric acid alone, or, better, mixed with sulphuric acid, gives a red-violet colour which heat develops.

The ptomaïnes are all very oxidizable in air, and consequently have a powerful reducing action. In fact, they reduce solutions (either cold or warm) of auric chloride, chromic acid, iodic acid, silver nitrate, silver bromide and ferric chloride—the latter reagent producing Prussian blue with potassium ferricyanide.

This last reaction, observed by Selmi as well as by MM. Brouardel and Boutmy, was considered a characteristic reaction of the ptomaïnes. But Gautier (*Bulletin de l'Académie de Médecine*, 2nd series, vol. x., p. 621) has

shown that apomorphine and muscarine give the same reaction; and Tanret, Brouardel and Boutmy state that aconitine, amorphous ergotinine, eserine, liquid hyoscyamine, and morphine give the same reaction with ferric chloride and potassium ferricyanide as the ptomaïnes.

Prussian blue is also produced with phenylic bases, naphylamine, the pyridine and hydropyridine bases, as well as those of the allylic and acetonic radicles.

From the researches of Pouchet and Brieger among the oxidized ptomaïnes, some give this reaction while others do not. Many of the 'extractive' matters, formed during putrefaction and accompanying the ptomaïnes, also reduce ferric chloride instantaneously. Therefore this reaction, although generally negative for the ordinary vegetable alkaloids, is not reliable as a means of differentiating the ptomaïnes with certainty.

Having given some of the *general properties* of the putrefactive ptomaïnes, we now describe recent researches concerning the properties and isolation of certain ptomaïnes:

Researches of Drs. Gautier and Etard.

Parvoline ($C_9H_{13}N$).—This base was isolated from mackerel and horse-flesh after putrefaction (*i.e.*, bacterial putrefaction).

It is an oily yellow base, with the odour of hawthorn, and it boils at 188° C. It is slightly soluble in water, but very soluble in alcohol, ether, and chloroform. Parvoline changes, in contact with air, to a brown substance of a resinous nature. Its aurochloride is a soluble salt; and its platinochloride, of a carnation colour, rapidly changes to a rose colour in contact with the air. It is only slightly soluble.

The analysis of the platinochloride gave the following figures:

	Found.	Calculated for $(C_{19}H_{13}N,HCl)_2PtCl_4$.
Carbon	31·8	31·8
Hydrogen	4·0	4·1
Nitrogen	5·1	4·1
Platinum	29·3	28·5

HYDROCOLLIDINE ($C_8H_{13}N$) AND COLLIDINE ($C_8H_{11}N$).—The ptomaïne extracted by chloroform from the bacterial fermentation of mackerel corresponds to the formula of hydrocollidine. According to Gautier and Etard, it is the base formed in the greatest abundance during the putrefaction of the flesh of horse and of cattle. It is a constant and definite product of the various fermentations of albuminoid matters, whatever be their origin, and the means by which their putrid destruction is commenced and terminated. In fact, it is the principal base produced by those bacteria which are the strongest and most active in the struggle for existence.

Hydrocollidine is a nearly colourless liquid, of a penetrating and persistent odour of syringa, having a density of 1·0296 at 0° C. Exposed to the air, it absorbs carbonic acid, and thereby becomes a brown-coloured and viscous liquid.

Its hydrochloride is very soluble in water and alcohol, crystallizing in fine white needles as well as other forms. This salt is neutral to test-papers, and possesses a bitter taste. An excess of acid (HCl) reddens it, besides converting it into a resinous-like mass. The aurochloride of the base is tolerably soluble, and its solutions are slowly reduced in the cold, but rapidly when heated. Its platinochloride is a pale yellow salt tinged with pink, slightly soluble, which fuses on the application of heat, and crystallizes in needles on cooling. This alkaloid is not altered when heated to 210° C.

The analyses of the platinochloride of hydrocollidine gave the following figures :

	Found.			Calculated for
	I.	II.	III.	$(C_8H_{13}N,HCl)_2PtCl_4$.
Carbon	30·1	29·9	29·76	29·3
Hydrogen ...	3·8	3·7	4·58	4·2
Nitrogen ...	5·7	—	4·07	4·2
Platinum ...	29·1	—	29·00	29·7

Analyses Nos. I. and II. were made on the alkaloid extracted from the flesh of fish, while analysis No. III. was made on the alkaloid extracted from horse-flesh.

Nencki's collidine* must not be confounded with hydrocollidine, as the two bodies are entirely distinct.

Base x ($C_{17}H_{38}N_4$).—In the mother-liquors of the platinochloride of the preceding base, Gautier and Etard found a soluble platinochloride crystallizing in yellow needles. It can be dried *in vacuo* without decomposition, but when heated to 100° C. it slowly decomposes and emits the odour of syringa.

An analysis of the platinochloride gave the following results :

Carbon	28·73
Hydrogen	5·81
Nitrogen	7·19
Platinum	27·93
Chlorine	30·50
	100·50

This body does not contain oxygen, and, from the above analysis, corresponds with the formula ($C_{17}H_{38}N_4$, 2HCl)$PtCl_4$, which contains carbon 28·81, hydrogen 5·70,

* *Ueber die Zersetzung der Gelatine und der Eiweisser bei der Fäulniss mit Pancreas*, 1876.

nitrogen 7·91, platinum 27·55, and chlorine 30·08 per cent.

Researches of the Author.

In the author's paper published in the *Proceedings of the Royal Society of Edinburgh*, vol. xv., p. 40, a description was given of a new putrefactive bacterium found upon decomposing onions. This microbe (*Bacterium allii*), whose life-history will be alluded to later in the volume, forms an alkaloid from albuminoid molecules.

When pure cultivations of *Bacterium allii* are allowed to grow on previously sterilized nutrient agar-agar for several days, an alkaloid corresponding to the symbolic formula $C_{10}H_{17}N$ is obtained.* The results of the analyses of this substance were as follows :

	Found.			Calculated for
	I.	II.	III.	$C_{10}H_{17}N$.
Carbon	79·47	79·50	79·48	79·48
Hydrogen ...	11·26	—	11·24	11·25
Nitrogen ...	9·27	—	—	9·20

The alkaloid† was extracted by Gautier's and Brieger's processes, with same results, from a considerable number of cultivation-tubes. It is a white solid, soluble in warm water, alcohol, ether, and chloroform. It crystallizes

* See Dr. A. B. Griffiths' paper in the *Comptes Rendus de l'Académie des Sciences*, vol cx. (February 24, 1890) ; abstracted into *Chemical News*, vol. lxi., p. 145 ; and *Nature*, vol. xli., p. 432.

† This alkaloid is precipitated by the general reagents used in testing for alkaloids : A solution of iodine (in KI) produced a brown precipitate. Nessler's reagent gave a brownish-yellow precipitate. Tannic acid produced a brown precipitate. Picric acid produced with this base a yellow precipitate which is slightly soluble. Auric chloride produced a dense yellow precipitate which was soluble in water. Sulphuric acid (slightly diluted) produced a red-violet colour with this base. Sodium phosphomolybdate gave a white precipitate. Platinic chloride produced a yellow crystalline precipitate which is insoluble in alcohol, and only slightly soluble in cold water.

from water in microscopic needles belonging to the prismatic system (Fig. 19). These crystals are extremely deliquescent, and have the odour of hawthorn, especially

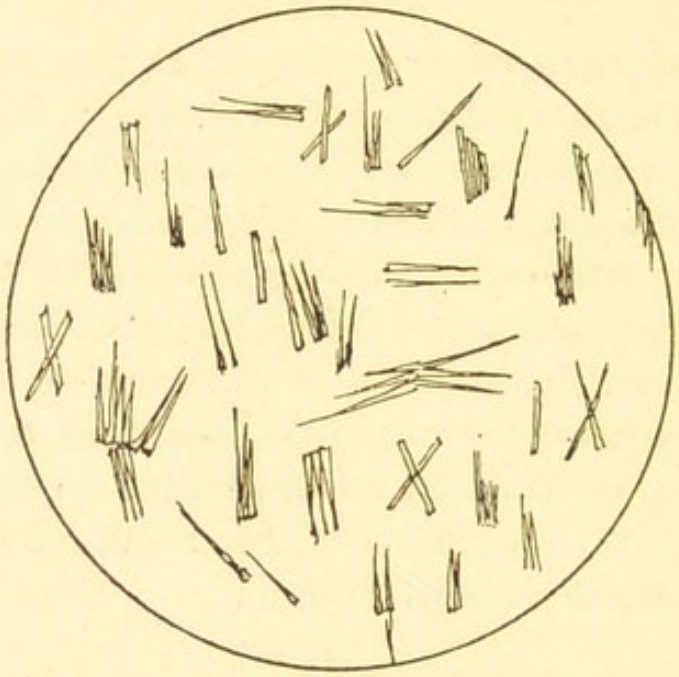


FIG. 19.—THE ALKALOID PRODUCED DURING AN ARTIFICIAL CULTIVATION OF BACTERIUM ALLII.

when heated. The alkaloid forms a platinochloride, which gave the following results on analysis :

	Found.			Calculated for
	I.	II.	III.	$(C_{10}H_{17}N,HCl)_2PtCl_4$.
Carbon ...	33·72	33·77	33·80	33·75
Hydrogen ..	5·10	5·08	5·18	5·06
Nitrogen ...	4·12	3·99	—	3·93
Platinum ...	27·21	—	27·25	27·28
Chlorine ...	—	—	29·99	29·95

The platinochloride of this base, therefore, may be represented by the formula $(C_{10}H_{17}N,HCl)_2PtCl_4$. It is a yellow-coloured crystalline compound, slightly soluble in cold water, but soluble in hot water and insoluble in alcohol.

There is little doubt that this new compound is related to the pyridine, or the $C_nH_{2n-5}N$ series of organic bases. Most likely this base is *hydrocoridine*. If Gautier's hydrolutidine* and hydrocollidine are compared with lutidine and collidine, and the new base $C_{10}H_{17}N$ with coridine, the analogy is remarkable.

Thus :

Pyridine Series.	Hydropyrid'ne Series.
Lutidine (C_7H_9N).	Hydrolutidine ($C_7H_{11}N$).
Collidine ($C_8H_{11}N$).	Hydrocollidine ($C_8H_{13}N$).
Parvoline ($C_9H_{13}N$).	Hydroparvoline (unknown).
Coridine ($C_{10}H_{15}N$).	Hydrocoridine ($C_{10}H_{17}N$).

Concerning the origin of this alkaloid, there can be no doubt that it is a product formed by the chemical disintegration of albuminoid molecules derived from the nutrient agar-agar during the life-history of the microbe in question. The alkaloid did not exist in the agar-agar before the cultivation of *Bacterium allii* in that medium, nor was it formed by the action of the reagents used in extracting process, as some authors suppose who have written upon the subject.

This alkaloid is undoubtedly the product of the putrefaction of albumin by *Bacterium allii*.

In the author's paper (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 40) it was stated that this microbe (when growing in nutrient agar-agar, etc.) produced 'small quantities of sulphuretted hydrogen gas.' The H_2S gas offers further proof that the microbe causes certain changes in albuminoid molecules—the albumin

* Obtained from cod-liver oil.

($C_{72}H_{112}N_{18}O_{22}S$) providing the sulphur required for the formation of this gas.

From the above there appears to be direct evidence of a putrefactive microbe producing an alkaloid from the medium in which it lives.*

Researches of Drs. Guareschi and Mosso.

Coridine ($C_{10}H_{15}N$).—Drs. Icilio Guareschi and Angelo Mosso (*Archives Italiennes de Biologie*, vol. ii., p. 367, and vol. iii., p. 241; and also *Journal für Praktische Chemie*, 1883) have extracted from putrid fibrin (beef) a base answering to the formula $C_{10}H_{15}N$. In fact, it is a pyridine base, the next higher homologue of parvoline.

It is an oily liquid, having a slight odour of pyridine and conicine, very alkaline, slightly soluble in water, and easily made into a resinous-like body even *in vacuo*.

Phosphomolybdic acid and phosphotungstic acid give with it yellowish-white precipitates. Potassium ferricyanide gives rise to a bluish precipitate, which, on the addition of ferric chloride, yields Prussian blue. Both picric acid and tannin precipitate this base with the formation of amorphous compounds; while mercuric chloride produces a white precipitate. Auric chloride gives with its hydrochloride a crystalline precipitate, which becomes reduced to the metallic state. Platinic chloride produces a copious crystalline precipitate of a flesh-colour, which is not decomposed at 100° C.

The hydrochloride crystallizes in fine laminæ, which are colourless and slightly deliquescent. This base was extracted during the putrefaction of flesh by Gautier's method.

* Dr. Griffiths' paper, read before the Royal Society of Edinburgh on March 18, 1889.

It appears that the ptomaïnes just described belong to or are derivatives of the pyridine series of organic bases.* These compounds (metameric with aniline and its homologues) form with each other a homologous series. Beginning with pyridine, the various members are as follows:

Pyridine (C_5H_5N).	Parvoline ($C_9H_{13}N$).
Picoline (C_6H_7N).	Coridine ($C_{10}H_{15}N$).
Lutidine (C_7H_9N).	Rubidine ($C_{11}H_{17}N$).
Collidine ($C_8H_{11}N$).	Viridine ($C_{12}H_{19}N$).

Some of these bases have been met with during the putrefaction of albumin, while others have not yet been isolated from that source. It is stated that the lowest member of the series, pyridine, may be regarded as benzene (C_6H_6), where one of the CH groups has been replaced by nitrogen.

'The other bases derived from it by substitution of CH_3, C_2H_5, etc., for one or more of the hydrogen atoms, admit of isomeric modifications depending on the orientation of the replacing radicles. Picoline, $C_5H_4(CH_3)N$, occurs in three such modifications, but very little is known of the isomerides of the higher bases.'

It is possible that a certain putrefactive microbe may produce one or more of the isomerides of a given base, according to the nature of the medium in which it lives. If this be so, the same microbe may produce very different products (different in physiological action as well as in chemical constitution); in fact, the latter depend more on the nature of the nourishing medium than on the microbe which is primarily instrumental in their formation.

Dr. Guareschi (*Annali di Chimica e di Farmocologia*,

* With the exception of the base $C_{17}H_{38}N_4$

vol. lxxxvii., p. 237) has recently obtained another compound from putrid fibrin.

The putrid mass was made alkaline with baryta in the cold, and then extracted with ether and chloroform. From the chloroform extract a compound having the formula $C_{14}H_{20}N_2O_4$ was obtained. It is believed to be an amido-acid. It forms beautiful shining plates, which melt at about 250° C.; they are soluble in chloroform. The aqueous solution is neutral or feebly acid, and gives all the general reactions for ptomaïnes. It forms a platinochloride.

Researches of M. Pouchet.

G. Pouchet extracted from putrid products two oxygenized bases of which the soluble platinochlorides can be separated one from the other by the addition of alcohol, and then by ether. One of these platinochlorides, insoluble in strong alcohol, crystallizes in prismatic needles; the other is tolerably soluble in alcohol, and can be separated from it by the addition of ether. The analyses of these salts have led to the formulæ $(C_7H_{18}N_2O_6,HCl)_2PtCl_4$ for the compound insoluble in alcohol, and $(C_5H_{12}N_2O_4,HCl)_2PtCl_4$ for the compound which is precipitated from the ethereal alcohol. These bases resemble the *oxybetaines*. The silky crystalline hydrochlorides of these bases are alterable in an excess of HCl. The base $C_7H_{18}N_2O_6$ forms microscopic prisms of a brown colour. The base $C_5H_{12}N_2O_4$ forms loose hair-like needles. Both bases are very poisonous.

Researches of Dr. Brieger.

Brieger* has recently isolated a number of different bases during the various stages of putrefaction. About

* 'Ueber Ptomaïnes' in *Berlin Klin. Wochenschrift*, 1886 and 1887.

the second day appeared a non-poisonous base, which Brieger named neuridine ($C_4H_{14}N_2$), together with choline ($C_5H_{15}NO_2$), a base already known and derived from lecithin (a compound of choline with glycerophosphoric acid and fatty acids*). This compound entirely disappears after the fourteenth day of putrefaction. It can be separated by picric acid, which gives a picrate, insoluble in cold water, but soluble in warm water. The next stages of putrefaction yield cadaverine ($C_5H_{14}N_2$), putrescine ($C_4H_{12}N_2$),† saprine ($C_5H_{16}N_2$), and mydaleine (not determined). Cadaverine, putrescine, and saprine are non-poisonous.

Mydaleine (which contains C = 10·83; H = 2·23; Pt = 38·74) is a very poisonous diamine, causing paralysis and death. The four last-named bases Dr. Brieger obtained from human corpses. Together with these bases two others have been isolated. One is very poisonous, while the other is non-poisonous.

Choline, neuridine, putrescine, and saprine, treated with potassium ferricyanide and ferric chloride, do not produce Prussian blue.

To isolate these bases, Brieger precipitated them in the state of mercuro-chlorides (chloro-mercurates), and separated them by their differences of solubility. The aurochloride of putrescine is only very slightly soluble in water. Its hydrochloride is deposited from hot alcohol in prismatic needles.

The aurochloride of cadaverine is very soluble, while the platinochloride of the same base forms crystals very readily.

The hydrochloride of mydaleine is the most soluble of all, as it remains in the alcoholic mother liquors.

* See Würtz's *Traité de Chimie Biologique*, p. 616.
† Tetramethylenediamine.

During the putrefaction of fish, Brieger met with a soluble base having the same chemical composition and physiological action as muscarine ($C_5H_{15}NO_3$) or oxynevrine.

From the *Comptes Rendus de l'Académie des Sciences*, vol. cvi., pp. 858-861, it appears that Dr. O. de Coninck has confirmed Brieger's work, and has isolated collidine ($C_8H_{11}N$) and coridine ($C_{10}H_{15}N$) from the muscular tissues of *Sepia officinalis* (the cuttle-fish) after putrefaction. Both these bases had been previously isolated by Nencki and Guareschi and Mosso respectively from putrid animal tissues.

Therefore it appears that the pyridine bases are constant products of the putrefaction of muscular tissues, not only of the *Vertebrata*, but also of the *Invertebrata*.

In addition to the above-mentioned alkaloids obtained by Brieger, 'a number of poisons have been got by other workers from decomposing articles of food or from dead bodies, and even from portions of healthy animal bodies.'

These poisons, or *extractives*, have not been isolated in a state of purity, and therefore their chemical composition is unknown. Nevertheless, these extractives must not be lost sight of in future research. What part do these extractives play in the physiology and pathology of man is an important question, which requires the attention of the bacteriologist.

Researches of MM. Brouardel and Delèzinier.

The ptomaïne discovered by Brouardel, and described by him as both chemically and physiologically analogous to veratrine (*Moniteur Scientifique*, 3rd series, vol. x., p. 1140), has recently been studied by Delèzinier (*Bulletin de la Société Chimique de Paris*, 3rd series, vol. i., p. 178);

and he finds that the body in question is only analogous to veratrine when it has been exposed to the air. Possibly Brouardel's compound was an oxidized product of the real base.

Delèzinier has studied the various reactions of this base in an atmosphere of nitrogen, and gives the following composition of it as the results of his analyses:

Carbon	89·41
Hydrogen	7·30
Nitrogen	3·03
	99·74

From these figures the formula $C_{32}H_{31}N$ is deduced. Therefore it differs considerably from veratrine, which is an oxygenized base.

This ptomaïne is a colourless oily liquid, having a hawthorn-like odour. It is insoluble in water, but alcohol, ether, toluene, and benzene dissolve it readily. It is extremely oxidizable, and forms salts which are very deliquescent.

This alkaloid appears to be a secondary monamine.

Extraction of Ptomaines, etc.

We now propose to indicate some of the methods used for the extraction of animal alkaloids or ptomaïnes:

Stas' Method.—Selmi, Guareschi, Mosso, Giglioli, and others belonging to the Italian school of physiological chemists, have employed for the extraction of ptomaïnes the method of Stas slightly modified.

The putrid viscera are treated with twice their weight of alcohol, slightly acidulated with tartaric acid and heated on a water-bath. The mixture is then filtered, and the filtrate evaporated at 32° C. in a current of

hydrogen or *in vacuo*. The alcoholic acid extract is then treated with ether, which dissolves, besides the fatty matters, a small quantity of the ptomaïnes, which are extracted from their aqueous solution by adding baryta and again treating with ether. The original dry alcoholic extract, after having been previously washed with ether and made alkaline with baryta, bicarbonate of soda, or ammonia, is successively treated (1) with ordinary ether or petroleum ether; (2) with cold chloroform, which is afterwards heated; (3) with cold amylic alcohol, which is likewise subsequently heated. These successive extracts, treated with water acidulated with hydrochloric acid, give a liquor, when evaporated, which allows the experimenter to apply the various reagents so as to distinguish the alkaloids.

According to Gautier, this method does not yield the alkaloids in a pure state, for they are always mixed with the extractives which accompany them in putrid animal substances.

Gautier's First Method.—Dr. Armand Gautier gives, in the *Comptes Rendus de l'Académie des Sciences* (vol. xciv., p. 1357; *ibid.*, p. 1598; vol. xcvii., p. 263; *ibid.*, p. 325), an account of his apparatus and method of obtaining putrid liquors from various animal substances (after bacterial fermentation).

The putrid liquors, separated from the oils after slight acidulation and agitation with very dilute sulphuric acid, are then distilled *in vacuo* at a low temperature. This liberates ammonia, phenol, indol, skatol, etc. The residual syrupy liquor, separated from the crystals which are formed, is made alkaline by the addition of baryta, then filtered, and the filtrate agitated many times with chloroform, which dissolves the bases. The chloroform solution is distilled at a low temperature either *in vacuo*

or in a current of carbonic anhydride; and to the liquor remaining water is added and tartaric acid, which separates a brown resinous-like body and a liquor. The liquor is collected and treated with a dilute solution of potash, which produces a strong odour of certain carbylamines, and, at the same time, the bases are liberated. Gautier now agitates the liquor with ether, which dissolves the bases. The ethereal solution is evaporated in a current of carbonic anhydride under slight pressure; and then under a bell-glass, in the presence of caustic potash to prevent them (the bases) being carbonated by the air (*Comptes Rendus*, vol. xciv., p. 1600; and vol. xcvii., p. 264).

The ptomaïnes (bases) are separated by fractional precipitation with platinic chloride, or by distillation *in vacuo*.

Gautier's Second Method.—In this process Gautier avoids the use of sulphuric acid. To the warm alkaline liquor of putrefaction oxalic acid is added until the liquor is decidedly acid. By this means fatty or oily liquors are liberated, and float on the surface of the liquor. After separating the fats, the liquor is filtered. The filtrate is distilled, when pyrrol, skatol, phenol, indol, the volatile fatty acids, and a portion of the ammonia, are driven off. Gautier then adds lime (until alkaline) to the portion which has not been distilled, separates the precipitate which forms, and which contains the greater portion of the fixed fatty acids, and he then distils the alkaline liquor to dryness *in vacuo*, taking care to condense the vapours in weak sulphuric acid. The bases are then distilled with ammonia. After the distillation is completed the distillate is neutralized, then evaporated nearly to dryness, when ammonium sulphate deposits in the crystalline condition. This is separated and rejected.

Concentrated alcohol is now added to the mother liquor (after separating ammonium sulphate), which dissolves the sulphates of the ptomaïnes. After evaporating off the alcohol, a small quantity of caustic soda solution is added. This solution is successively treated with ether, petroleum ether, and chloroform (*i.e.*, three different extracts are obtained).

As to the product remaining in the retort with the excess of lime which had served to separate the bases, it should be treated (after desiccation and trituration) with ether at 36°C., which, under these conditions, dissolves the fixed bases. By the addition of a small quantity of acidulated water the bases are separated from the ether, and are then easily precipitated by the addition of an alkali.

Pouchet's Method.—In 1880 Dr. G. Pouchet extracted toxic alkaloids from urines by the following method: The alkaloids are precipitated from a slightly alkaline solution by an excess of tannin. The tannates so formed are decomposed by plumbic hydrate in the presence of alcohol. The alcoholic solution is then evaporated. The syrupy mass which remains is then dialyzed, and the alkaloids, found in the portion dialyzed, are then extracted by ether, petroleum, or chloroform.

M. Gautier does not recommend the use of tannin, for it oxidizes and alters in the presence of the alkaloids, and only precipitates them very imperfectly, especially in the presence of air.

Brieger's Method.—The method of Professor Brieger consists in first allowing the albuminous substances to ferment for several days in an incubator. To extract the putrefactive alkaloids, which are formed after the coagulation of the juices by heat, Brieger precipitates by means of plumbic acetate, extracts the excess of lead by

sulphuretted hydrogen, evaporates the liquor to the consistency of syrup, and then dissolves the residue in amylic alcohol. The amylic solution, being evaporated, is treated with water, concentrated by evaporation, then acidulated with sulphuric acid, and washed several times with ether, which frees it from the oxy-aromatic acids.

The aqueous-acid liquor is then concentrated to a quarter of its volume. After standing twenty-four hours, the precipitate which forms is dissolved in boiling water, and decomposed by sulphuretted hydrogen. In concentrating the liquors, Brieger crystallizes at once various mineral or organic salts which are rejected, then the dried residue is treated with absolute alcohol, which, after concentration, deposits the hydrochlorides of the putrefactive bases in the crystalline condition. The various hydrochlorides are now separated by the difference of their solubility or by fractional precipitation with picric acid, platinic chloride, auric chloride, etc. (see *Ueber Ptomaïne*, 1885; and *Weitere Untersuchungen über Ptomaïne*, 1885).

Luff's Method.—Dr. A. P. Luff, F.C.S. (*British Medical Journal*, 1889, p. 193), has used the following process for the extraction of ptomaïnes contained in abnormal urines: (*a*) A considerable quantity of the urine is made alkaline by a solution of sodium carbonate, and then agitated with half its volume of ether. (*b*) The ethereal solution (after standing) is filtered and agitated with a solution of tartaric acid. The tartaric acid combines with any alkaloids present, forming soluble tartrates, and the solution of tartrates forms the lower layer of the liquid mass. (*c*) The tartaric acid solution (after being separated from the ether) is also made alkaline by the addition of sodium carbonate, and is once more agitated with half its volume of ether. (*d*) The ethereal solution

(after standing) is separated, and the ether allowed to evaporate spontaneously. (*e*) The residue (after drying over sulphuric acid) is finally examined for alkaloids (ptomaïnes).

Physiological Action of the Ptomaïnes, etc.

The ptomaïnes, isolated from their salts, have, as a rule, a cadaveric or a urinary odour; and those which are most oxygenized possess a poisonous odour analogous to that of conicine or of pyridine. Sometimes they emit tenacious but agreeable odours, resembling orange blossom, hawthorn, syringa, rose, cinnamon, and musk.

They possess, for the greater part, a *piquant* taste, which benumbs the tongue—a sensation followed by a feeling of strangulation when they have been taken in too large a quantity. Some animal alkaloids are manifestly bitter.

Ptomaïnes in the isolated condition are more poisonous than their salts, especially those extracted by ether.

Gautier gives the following as the principal phenomena observable when a small quantity of an aqueous solution of a ptomaïne is injected into a frog and dog respectively:

Frog.	Dog.
(1) Dilation of the pupil, followed by contraction.	(1) Irregular pupil, followed by contraction.
2) Tetanic convulsions, followed by muscular flaccidity.	(2) Remarkable injection of the vessels of the concha of the ear, through paralysis of the vaso-motors.
(3) Diminished beats of the heart, rarely an increase.	(3) Respiration very diminished.
(4) Absolute loss of cutaneous sensibility.	(4) Somnolence, followed by convulsions and death.
(5) Loss of muscular contractibility.	(5) Loss of muscular contractibility.
(6) Death.	

The loss of muscular contractibility, even under the influence of electrical excitants, is very remarkable. It distinguishes the ptomaïnes from the poisonous alkaloids of fungi,* etc.

M. Gautier has shown that a small quantity (·007 grm.) of the hydrochloride of hydrocollidine injected into a bird killed it in fifty-eight minutes. In fact, this base is nearly as poisonous as the venom of the cobra.

Many of the bases of Brieger are also very poisonous. Mydaleine causes diarrhœa, vomitings, intestinal inflammations; and after death the heart is in diastole, and full of blood. Nevrine, itself, is very poisonous; a few milligrammes suffice to kill a cat. It causes salivation, excessive nasal secretion, a copious flow of tears, diarrhœa, increased respiration, convulsions, and death.

According to Dr. A. M. Brown, poisoning by ptomaïnes† and leucomaïnes is accompanied by hypothermia.

'Poisoning by the extractives is attended by hyperthermia. A combination or succession of hyperthermic and hypothermic phenomena may become manifest, according to the combination or alternation of poisoning by the deleterious physiological products, or their antagonistic action.'

'Many of these symptoms, described above, occur in men in consequence of poisoning from decomposing food, or from disease; and it is possible that the occurrence of some symptoms, and not of others, may be due to the occurrence in disease of alkaloids allied to mydaleine, although not identical with it, or to the presence of two or more alkaloids, which partially neutralize each other's effects.'

* See Gautier's paper in *Comptes Rendus du Congrès International d'Hygiène de Paris*, vol. ii., p. 266; and his *Traité de Chimie appliquée à la Physiologie* (tome i.).

† Although a ptomaïne, mydaleine is hyperthermic in action.

It is 'impossible to doubt that poisonous alkaloids are formed in the alimentary canal; that when excretion is seriously diminished, they must be in some degree absorbed; and that, mixing with the blood, and entering the tissues, they must produce some sort of injurious effects, determined by the rate of absorption and the amount absorbed.'*

Yet these alkaloids (as Bouchard has shown) are produced from the decomposition of albumin '*by the agency of putrefactive microbes*'; and according to Dr. Lauder Brunton, F.R.S., the alkaloids formed in the body in disease are very probably of a different character to those formed during health, and possibly they vary with the disease. May not pathological microbes, either with or without the help of putrefactive bacteria, produce poisonous substances which cause the various symptoms in particular diseases? Bocklisch obtained the non-poisonous base—cadaverine—from an artificial cultivation of Finkler's bacillus, the supposed cause of sporadic cholera; but when the bacilli grew in the same medium along with putrefactive microbes, the poisonous base methylguanidine was produced besides cadaverine. The author of the present book repeated Bocklisch's experiments with the same results, and he found that the particular putrefactive microbes employed did not produce methylguanidine unless Finkler's bacilli were also present.

Therefore, it is most probable that infections or microbian diseases are caused by the presence of some particular pathogenic microbe, which acts independently of, or in conjunction with, certain non-pathogenic microbes, and is thereby the means of giving rise to the formation of certain poisonous alkaloids (morbid leucomaïnes) from

* Sir A. Clark, in *Proc. Medic. Soc.*, vol. xi., p. 55.

albuminoids, and these substances produce the various symptoms which characterize certain infectious diseases.

If the characteristic symptoms of any particular infectious disease are due to toxic animal alkaloids, these alkaloids must be elaborated somehow. They are either the products formed from albuminoids by microbes (pathogenic or non-pathogenic, or both combined), or the products formed from albuminoids by 'vital physiological processes,' and when they are not eliminated from the system produce disease.

In our opinion the most feasible explanation is that these alkaloids are products formed by the action of microbes upon albuminous substances.

Sir W. Aitken says that pathogenic microbes 'are unable to settle in a perfectly healthy body: they can only develop when the physico-chemical constitution of the tissues is morbidly altered so as to correspond with their requirements.'

But would the 'morbidly-altered' conditions alone produce a particular infectious disease (with all or a portion of its symptoms) without the action of a particular microbe? If not, the microbe is in reality the cause of the disease.

The same authority says: 'On the one hand, therefore, the microbes must be endowed with certain vital properties of a special kind; and, on the other, there must be a predisposition of the system in a certain physico-chemical constitution of the tissues, so that the micro-organisms may find within the body, and in proper combinations, all the conditions necessary for their growth and development.'

No doubt all microbes are 'endowed with certain vital properties of a special kind,' for we find that some microbes will grow in certain media and not in others;

and, according to Bocklisch, the same microbe may, under altered conditions, produce entirely different alkaloids.

Dr. A. Binet (*The Psychic Life of Micro-Organisms*) has shown that microbes have a psychological history, and have also powers of selection. For example :

(*a*) 'Microbes are capable of discriminating between bits of albumin and particles of coal. Microbes do not nourish themselves indiscriminately, nor do they feed blindly upon every substance that chances in their way.' They exercise a choice ; and as Dr. G. J. Romanes, F.R.S., has observed, *the power of choice* may be regarded as the criterion of psychical faculties.

(*b*) When putrefactive bacteria (aërobic) 'are put in a drop of water containing no oxygen, but in which have been placed grains of chlorophyll, nothing happens in the first instant ; but if the preparation be illuminated, so as to allow the chlorophyll to act, the bacteria are seen to exhibit very rapid movements, and to proceed, all together, towards the point of the preparation where the generation of oxygen is taking place ; that is to say, about the grains of chlorophyll. If the preparation be darkened the bacteria cease assembling about the chlorophyll grains, which, hid from the light, cease to disengage oxygen.'

(*c*) Microbes are capable of discriminating between their own kind and other microbes, for they generally live in colonies.

Therefore, alkaloids are most likely produced, in the majority of cases, by the action of microbes (using their powers of selection or choice) upon albuminoid molecules.

Again, if pathological microbes, singlehanded or in conjunction with putrefactive microbes, manufacture morbid alkaloids, we can account for the crisis and de-

cline of any particular infectious disease. When a certain quantity of the alkaloid has been produced, and is present in the blood, tissues, etc., the microbian action ceases in the individual or individuals infested. It is well known from the researches of Wernich and others that the products formed by the action of microbes on albumin are detrimental (after a certain quantity has been formed) to the life of these microbes; the microbes not manufacturing the alkaloids for their own consumption. In fact, the alkaloids form the residua from the decomposition of albumin by the selective action of microbes; the latter extracting, for their own nutrition, certain elements from, or rather a portion of, this complex molecule. For example: certain elements—carbon, hydrogen, nitrogen, oxygen, and sulphur, or certain compounds containing them—are essential for the growth and multiplication of *Bacterium allii*, and these are extracted from albuminoid molecules; while the residue, which is rejected by the particular microbe, is represented by the formula $C_{10}H_{17}N$, or the alkaloid which we have named hydrocoridine.*

The Leucomaïnes.

The leucomaïnes are another class of animal alkaloids, but they are said to differ from the ptomaïnes, being elaborated by the vital energy of the *cellular tissues*, and are produced in health as well as in disease. According to Dr. Gautier (who first distinguished them as a separate class), they are excretory products (like urea, carbonic acid, etc.) formed by 'vital physiological processes' from albuminous substances, consequently they must be eliminated from, or destroyed in, the system, or disease will be the result of their poisonous action. We resist, there-

* The residue also contains a pigment and sulphuretted hydrogen.

fore, incessant auto-infection by two distinct mechanisms: the elimination of the leucomaïnes by means of the excretory organs, and by the destruction of the leucomaïnes by means of the oxygen contained in the blood.

Although the leucomaïnes appear to be excretory products formed physiologically, there is a considerable amount of evidence to show that microbes may have some action in their formation. Gautier, Bouchard, and others have shown that certain leucomaïnes increase considerably in quantity during the progress of several infectious diseases; and alkaloids of definite characters have isolated from infectious diseases.

Dr. Luff has extracted a leucomaïne from urine in cases of typhoid fever. This leucomaïne is said to have reactions entirely different from all known animal alkaloids. In fact, it is peculiar to typhoid fever, and is most likely formed from albuminoids by the action of *Bacillus typhosus*, either alone or in union with other microbes.

If putrefactive microbes are capable of giving rise to certain alkaloids within the alimentary canal, etc., there is every reason to infer that pathogenic microbes also produce within the living body poisonous compounds from albuminous substances.

Like the ptomaïnes, the leucomaïnes are produced from the decomposition of albuminoids; in some cases by 'the vital action of the cells themselves,' but in others, most likely, by the aid of pathogenic and other microbes.

Although Gautier makes an important distinction between the two classes of animal alkaloids, this distinction 'does not support the view that the ptomaïnes and leucomaïnes are distinct, or opposed to each other' (Aitken).

The Leucomaïnes derived from Muscle.

Gautier has extracted certain important and definite leucomaïnes from the muscular tissues of mammifers by the following process: Thirty kilogrammes of beef-flesh (of good quality) were cut into small pieces, then put to infuse in 60 kilogrammes of tepid water, with 0·25 gramme of oxalic acid added, and 1 cc. of commercial oxygenized water per litre — these precautions being taken to prevent fermentation. At the end of twenty-four hours it was boiled, then filtered through linen, and the residue strongly compressed, then boiled again, and filtered through paper. The filtrates were then evaporated *in vacuo* at 50° C.

After evaporation there remained a viscous residue of a brown-yellow colour, very acid, and having the odour of roast beef. This residue was treated with alcohol (90%), and gave an alcoholic solution, and a brown residue (No. 1), which is thick, and very rich in inorganic salts. The alcoholic solution, on being evaporated *in vacuo*, left a residue, which was again treated with warm alcohol (99%). The second alcoholic solution was filtered, and allowed to stand twenty-four hours, when it produced a second deposit, having the odour of broth. The liquor was now decanted, again filtered, and to the filtrate ether was added, as long as a precipitate was produced. The mixture was allowed to stand twenty-four hours, and then decanted, when a clear amber-coloured liquor (ethero-alcoholic) was obtained. The amber-coloured liquor was distilled over a water-bath, and finally *in vacuo*. Only a small quantity of residue was obtained, from which were extracted a very small quantity of ptomaïnes, having a hawthorn-like odour.

Gautier then treated in the same manner (except alcohol of 99 per cent. was used in the first instance) 'extract of American meat.' The precipitate obtained after the addition of ether to the concentrated alcoholic liquors was found to contain the new bases or leucomaïnes. This precipitate (of an amber colour, thick, and slightly bitter) separated itself (after keeping a short time) into a mass of crystals, mixed with a syrupy liquid. To this mixture Gautier added a small quantity of absolute ether, and after standing for a few days the syrupy liquid (of an amber colour) was separated, as much as possible, from the green-coloured fluorescent crystals (*a*). The remaining portion of the syrupy liquid was removed from the crystals by washing with alcohol (99%).

The crystals (*a*) were then treated with boiling alcohol (95%). The alcoholic liquor obtained was partly evaporated, and produced on cooling: (1st) a quantity of crystals (*b*) of a citron-yellow colour, which to the touch resemble talc; (2nd) mother liquors from which were deposited new crystals (*c*).

The crystals (*a*), when treated with boiling alcohol (95%), left a crystalline residue (nearly insoluble) of a whitish-yellow colour. The crystalline residue on being dissolved in boiling water deposited a small quantity of a whitish-yellow compound, crystallized in brilliant oblique rhombic prisms (*d*). In continuing to concentrate the mother liquors another crystalline substance (of an orange colour) (*e*) was obtained.

In obtaining the above crystals, Gautier only used alcohol, ether, and water as extracting agents. These crystals are new bases (leucomaïnes); some are neutral to test-papers, while others blue-red litmus. Their hydrochlorides and nitrates are all neutral salts, and perfectly crystallized.

The first of these bases to be described is **Xanthocreatinine** ($C_5H_{10}N_4O$). This is the most abundant of these bases, and corresponds to the crystals (*b*) already mentioned. It is a substance of a citron-yellow colour, crystallizing in rectangular tables. These crystals have a soapy feel, a slightly bitter taste, and a slight cadaveric odour.

When dissolved in alcohol, xanthocreatinine produces the odour of acetamide. Its crystals are very soluble in water, and soluble in warm alcohol (99%), from which they crystallize. Xanthocreatinine, when warmed, gives off the odour of roast beef; and when partially carbonized it emits an ammoniacal odour, as well as that of methylamine. It has an alkaline reaction, and the hydrochlorides, platinochlorides, and aurochlorides of it have been isolated. This base resembles, in all its properties, creatinine ($C_4H_7N_3O$), from which it differs by CH_3N.

A solution of zinc chloride* precipitates creatinine from a solution of xanthocreatinine. Silver nitrate produces (in the cold) a flocculent precipitate, or, from a warmer solution, a crystalline precipitate is produced. Mercuric chloride produces a whitish-yellow precipitate. This reagent ($HgCl_2$) Gautier used to separate this alkaloid from the alkaline chloride (KCl), with which it is associated, and which dissolves even in nearly absolute alcohol.

Oxalic and nitric acids do not give any precipitates, even in concentrated solutions of this substance.

Copper acetate does not produce any precipitate; this fact distinguishes xanthocreatinine from the group of bases of which xanthine and hypoxanthine are important members.

Sodium phosphomolybdate precipitates it in yellowish

* See Monari's paper in *Gazetta Chimica Italiana*, vol. xvii., 1887, p. 360.

masses. Tannin makes a solution of this base turbid after a time.

Gautier gave this compound the name of xanthocreatinine on account of its yellow colour and its great analogy with creatinine, which accompanies it in the extracts from muscular tissues.

Xanthocreatinine is poisonous even in small quantities. It produces, in animals, faintings, repeated vomitings and extreme fatigue.

Crusocreatinine ($C_5H_8N_4O$).—This base is obtained as the orange-coloured crystals (*e*) already alluded to. It is slightly alkaline, and produces a non-deliquescent, crystalline, and soluble hydrochloride. It forms a soluble platinochloride, crystallized in prisms, as well as a slightly soluble aurochloride which crystallizes in small grains. Crusocreatinine does not reduce zinc from a solution of its acetate, nor mercuric oxide from its nitrate, but in the cold it precipitates alumina from a solution of alum. Crusocreatinine only differs from xanthocreatinine by two atoms of hydrogen. It possesses, in common with xanthocreatinine, the general properties of creatinine, which it resembles in its crystalline form and its alkalinity, and from which it differs by the group CNH (hydrocyanic acid).

Zinc chloride, when added to a solution of this base, produces a precipitate according to the following equation:

$$2C_5H_8N_4O + ZnCl_2 = 2HCN + (C_4H_7N_3O)_2ZnCl_2.$$

That is, it produces the chloro-zinc compound of creatinine.

Oxalic and nitric acids do not form salts with crusocreatinine, therefore it is not related to urea or guanidine.

Copper acetate does not produce a precipitate, therefore crusocreatinine does not belong to the xanthines and analogous bodies.

Gautier named this base crusocreatinine because of its

golden colour and the relationship which it holds to creatinine.

Amphicreatine ($C_9H_{19}N_7O_4$).—This body corresponds to the crystals (*d*) already alluded to in the process of extraction.

This base, which crystallizes in whitish-yellow, oblique rhombic prisms, has a very slight bitter taste. When it is heated to 100° C. it decrepitates slightly, and becomes an opaque white at about 110° C. without visibly changing its form. Potash does not liberate ammonia in the cold.

Amphicreatine is a feeble base. Its hydrochloride is crystallizable and non-deliquescent; its platinochloride is soluble in water, insoluble in alcohol, and forms flat tables; and its aurochloride is very soluble, crystallizing in microscopic hexahedra, tetrahedra, and other forms.

Copper acetate and mercuric chloride do not precipitate this base or its hydrochloride.

Sodium phosphomolybdate forms, with its hydrochloride, a yellow precipitate.

When treated with nitric acid, then by ammonia and potash, it does not give any of the characteristic reactions of the derivatives of uric acid or xanthine. In fact, its general properties resemble completely those of creatine.

Analysis of this base gave the following results:

	Found.		Calculated for $C_9H_{19}N_7O_4$.
	I.	II.	
Carbon	36·89	36·97	37·37
Hydrogen	6·98	6·86	6·57
Nitrogen	34·27	34·11	33·91
Oxygen	—	—	22·15

These figures correspond to the formula of $C_9H_{19}N_7O_4$.

According to Gautier, this body appears to be formed from creatine and a base having the formula $C_5H_{10}N_4O_2$, which only differs from creatine by the addition of HCN. And, as already stated, crusocreatinine has the same relationship with creatinine. For instance:

$$(1)\quad \underset{\text{[Crusocreatinine]}}{C_5H_8N_4O} = \underset{\text{[Creatinine]}}{C_4H_7N_3O} + HCN.$$

$$(2)\quad \underset{\text{[Amphicreatine]}}{C_9H_{19}N_7O_4} = 2\underset{\text{[Creatine]}}{C_4H_9N_3O_2} + HCN.$$

Dr. A. M. Brown states 'that the most terrible poison, cyanhydric acid (HCN), forms the chemical skeleton of the cellular nucleus which is the most active phenomenon of vitality.' Or, as Dr. P. W. Latham puts it, 'Ammonium cyanate is a type of living, and urea of dead nitrogen, and the conversion of the former into the latter is an image of the essential change which takes place when a living proteid dies.'

Pseudoxanthine ($C_4H_5N_5O$).—The alcoholic mother liquors from the preceding compounds, first deprived of alcohol *in vacuo*, are treated with water and copper acetate in slight excess. This yields a yellowish precipitate, which is washed and decomposed by sulphuretted hydrogen. In filtering the precipitate in the presence of an excess of water (hot), a sulphur-yellow powder is obtained. This powder (formed of microscopic crystals) dissolves in hydrochloric acid, forming a very soluble hydrochloride, which closely resembles hypoxanthine in crystalline structure and solubility. It is precipitated (like xanthine) by mercuric chloride and silver nitrate. Like xanthine, this compound is not precipitated by lead acetate.

When treated with nitric acid, then evaporated and finally treated with dilute potash, a beautiful orange-red

colour is developed (in this respect it also resembles xanthine).

With the exception of its solubility being a little greater and its crystalline form better indicated, this body shows all the physical and chemical properties of xanthine, with which it has been often confounded.

Analyses of this body gave the following results:

	Found.		Calculated for $C_4H_5N_5O$.
	I.	II.	
Carbon	30·77	30·59	30·97
Hydrogen ...	3·18	3·20	3·22
Nitrogen ...	44·07	44·26	45·16
Oxygen	—	—	20·65

Although the two compounds (pseudoxanthine and xanthine) are entirely distinct, Gautier gave $C_4H_5N_5O$ the name pseudoxanthine because of its many resemblances to xanthine.

Bases $C_{11}H_{24}N_{10}O_5$ and $C_{12}H_{25}N_{11}O_5$.—These complex compounds differ one from another by the group HCN.

The first base was obtained from the mother-liquors of xanthocreatinine and the second from those of crusocreatinine.

The base $C_{11}H_{24}N_{10}O_5$ crystallizes in colourless, tasteless, rectangular tables, having a slight alkaline reaction. It forms a hydrochloride and a sulphate, both of which crystallize in prismatic needles. Its crystalline platinochloride is a soluble, but not a deliquescent salt. Heated in sealed tubes to 180° to 200° C., this base loses ammonia and carbonic acid, and produces a new crystalline base which has not been examined.

Analyses of this base (dried at 110°C.) gave the following results:

	Found. I.	Found. II.	Calculated for $C_{11}H_{24}N_{10}O_5$.
Carbon	35·01	35·14	35·10
Hydrogen ...	6·84	6·65	6·40
Nitrogen ...	37·15	37·20	37·23
Oxygen	—	—	21·27

The compound $C_{12}H_{25}N_{11}O_5$ forms fragile, silky, rectangular tables, having much analogy with those of xanthocreatinine and the preceding substance. It is also a weak base, yielding crystalline salts. After repeated crystallizations from alcohol, and finally dried at 110° C., it gave the following results on analysis:

	Found. I.	Found. II.	Calculated for $C_{12}H_{25}N_{11}O_5$.
Carbon	35·58	35·55	35·73
Hydrogen ...	6·39	6·43	6·21
Nitrogen ...	—	38·72	38·21
Oxygen	—	—	19·85

The properties of these two last substances are analogous to those of creatinine and creatine. Further investigations are certainly necessary to establish their constitution. But however complex the formulæ appear at first sight, the composition of these substances resembles those already alluded to, and creatinine, which has been known for many years. It is remarkable that these bases only differ from each other by the group HCN.

These six new alkaloids (leucomaïnes) discovered by Gautier are endowed with an action more or less powerful upon the nervous centres, producing sleep, fatigue, sometimes vomitings and purgings; but they are not so active as the ptomaïnes. They are very oxidizable, and

are accordingly eliminated from the system by the oxygen of the blood.

The following classification of the *leucomaïnes* is based on that given by Dr. A. M. Brown in his *Treatise on the Animal Alkaloids* :*

Classification of the Leucomaines.

I. THE CREATININE GROUP.

All these compounds are oxygenized bases, and are said to be related to the ureides.

(*a*) *Creatinine* ($C_4H_7N_3O$ or $HN=C\begin{cases}NH\text{——}CO\\ \quad\quad\quad\ |\\ N(CH_3)\text{—}CH_2\end{cases}$ —methylglycocyamidine) crystallizes in rhombic prisms. This body is largely formed in uræmia.

(*b*) *Xanthocreatinine* ($C_5H_{10}N_4O$) crystallizes in yellow rectangular tables.

(*c*) *Crusocreatinine* ($C_5H_8N_4O$) crystallizes in golden yellow prisms. It differs from creatinine by the addition of the HCN group.

(*d*) *Amphicreatine* ($C_9H_{19}N_7O_4$) strongly resembles creatine ($C_4H_9N_3O_2$), and may be looked upon as two molecules of creatine *plus* the group HCN.

(*e*) *Bases* $C_{11}H_{24}N_{10}O_5$ and $C_{12}H_{25}N_{11}O_5$. They are allied to creatinine and creatine.

(*f*) *Methylhydantoïn* ($C_4H_6N_2O_2$ or $CO\begin{cases}NH\text{——}CO\\ \quad\quad\quad\ |\\ N(CH_3)\text{—}CH_2\end{cases}$) was obtained by Guareschi and Mosso in 1883 from the flesh of a calf. It is related to creatinine, but Dr. Gautier doubts its alkaloidal nature.

* Published by Messrs. Baillière, Tindall, and Cox.

II. THE URIC GROUP.

(*a*) *Betaine* ($C_5H_{11}NO_2$—trimethylglycocine) was first isolated from urine by Liebreich in 1869. It is related to choline ($C_5H_{15}NO_2$) and nevrine ($C_5H_{13}NO$), hence it is also called oxycholine and oxynevrine.

(*b*) *Carnine* ($C_7H_8N_4O_3$) obtained from flesh by Dr. Weidel in 1869.

(*c*) *Adenine* ($C_5H_5N_5$) was discovered by Dr. Kossel in the pancreas, while Schindler (*Zeitschrift für Physiologische Chemie*, vol. xiii., p. 432) discovered it to the extent of 2·278 per cent. in the spermatozoa of the carp, and to the extent of 1·919 per cent. in the calf's thymus.

Dr. G. Thoiss (*Zeitschrift Physiol. Chem.*, vol. xiii., p. 395) has shown that adenine and hypoxanthine contain a group ($C_5H_4N_4$) called adenyl.

Adenine ($C_5H_4H_4NH$) is adenylimide, while hypoxanthine ($C_5H_4N_4O$) is adenyl oxide. Adenine contains a hydrogen-atom, which is replaceable by acid radicles—for methyl and benzyl substitution products of this base have already been prepared.

(*d*) *Guanine* ($C_5H_5N_5O$), found in the flesh, the organs, etc., of the mammalia, birds and fishes. Also in the excretory products of the *Invertebrata**.

(*e*) *Hypoxanthine* (sarcine—$C_5H_4N_4O$) found in the animal organism, crystallizes in needles, which are only slightly soluble in water.†

(*f*) *Xanthine* ($C_5H_4N_4O_2$) is found widely distributed in the animal organism. It is a white amorphous com-

* See Dr. A. B. Griffiths' researches in *Proceedings, Royal Society of London*, 1885-1888 ; *Proceedings, Royal Society of Edinburgh*, 1885-1890.

† See Dr. G. Bruhn's paper in the *Berichte*, vol. xxiii., pp. 225-229.

pound (see Würtz's *Traité de Chimie Biologique*, pp. 707-716).

(*g*) *Pseudoxanthine* ($C_4H_5N_5O$), extracted from muscular tissues, is related to xanthine and hypoxanthine.

III. AN UNCLASSIFIED GROUP.

(*a*) *Samandarine* ($C_{34}H_{60}N_2O_5$), obtained by Zalesky from the venom of the salamander.

(*b*) *Protamine* ($C_9H_{21}N_5O_3$), obtained by Miescher and Picard from the seminal fluid of animals.

(*c*) *From the urine.*—An alkaloid having the formula $C_7H_{14}N_4O_2$ has been extracted from urine.

(*d*) *From the saliva.*—Gautier, in 1881, obtained an alkaloid from human saliva. It has narcotic properties.

(*e*) *From the breath.*—Anthropotoxine has been isolated from the breath by Du Bois-Raymond.

(*f*) *From the spleen.*—A crystalline alkaloid having a paralyzo-motor action has been isolated from the spleen by M. Morell.

(*g*) *From the testicle.*—Wooldridge (*Proc. Roy. Soc.*, 1886) prepared an alkaloid from the testicle. When injected into the veins it produced thrombosis.

(*h*) *From the intestines.*—A pyridine base has been isolated from cholera dejecta.

(*i*) *From the blood.*—Certain alkaloids have been isolated from this source.

(*j*) *From venoms.*—According to Gautier (*Bulletin de l'Acad. de Méd.*, vol. x., p. 947), the venoms of snakes contain certain alkaloids.

(*k*) *Mytiloxin* ($C_6H_{15}NO_2$) has been isolated from certain fishes by Dr. Brieger.

(*l*) *From cod-liver oil.*—Drs. Gautier and Mourgues*

* *Comptes Rendus*, vol. cvii., pp. 110, 254, 626 and 740; also *Bulletin de l'Acad. de Médecine*, 1890.

have recently extracted a miscellaneous series of bases, etc., from cod-liver oil. These bases, etc., are as follows:

Butylamine ($C_4H_{11}N$).	Dihydrolutidine ($C_7H_{11}N$).
Amylamine ($C_5H_{13}N$).	Aselline ($C_{25}H_{32}N_4$).
Hexylamine ($C_6H_{15}N$).	Morrhuine ($C_{19}H_{27}N_3$).

Morrhuic acid ($C_9H_{13}NO_3$).

The Extractives.

These bodies have been called 'the *x*, *y*, *z*'s of morbid anatomy and pathology,' for very little is known about them. They are very poisonous, uncrystallizable, nitrogenous compounds of a non-basic character. They have been extracted from human urine, 'decomposing articles of food, or from dead bodies, and even from portions of healthy animal bodies.'

All these nitrogenous compounds (ptomaïnes, leucomaïnes, and extractives) have been derived from the decomposition of albumin or albuminoids. Albumin is the compound which is capable of nourishing pathogenic and other microbes, therefore it would be better if we knew a little more about its constitution and its chemical and biochemical decompositions than are known at the present time. Several years ago Loëw and Bokorny (*Berichte der Deut. Chem. Gesellsch.*, vols. xiv. and xv.; and *Pflüger's Archiv für Physiologie*, vol. xxv.) endeavoured to prove that *living* albumin (protoplasm) was an aldehyde, or contained an aldehydic group of elements.* Albumin may contain an aldehydic group of elements, but we can hardly classify it amongst the aldehydes, for, according to numerous investigations bearing on this subject, 'albumin is a compound of cyan-alcohols united to a benzene nucleus, these being derived from the

* See also the author's paper in the *Chemical News*, vol. xlviii., p. 180; *Journal Chemical Society*, 1884, p. 202; *Journal Royal Microscopical Society*, 1884, p. 249.

various aldehydes, glycols, and ketones, or that they may be formed in the living body by the dehydration of the amido-acids; that from a body so constituted all the different substances may be obtained which have been extracted from albuminoid tissues; that lactic acid is obtained in two ways, either from $C_2H_4\begin{cases}OH\\CN\end{cases}$, or from changes and condensation in $CH_2\begin{cases}OH\\CN\end{cases}$, with the simultaneous development of CO_2, a result which is brought about when a muscle contracts or when it dies; and that urea may be obtained from one series of cyan-alcohols with the production of a cyan-alcohol higher in the series.

'Taking this view, then, of the constitution of albumin, the following may be given as a summary of nutritive changes.

'The amido-acids glycocine, leucine, tyrosine, etc., in passing from the alimentary canal to the liver are dehydrated, forming a series of cyan-hydrins or cyan-alcohols attached to a benzene nucleus, and then pass into the circulation. In the tissues these cyan-alcohols, partly by condensation, partly by hydration and oxidation, give rise to the various effete products which are eliminated from the system chiefly in the form of carbonic acid and urea.'—*Latham.*

Therefore, if albumin is a compound of cyan-hydrins united to a benzene nucleus, we can account for the formation of the various alkaloids from this body by the agency of microbes, etc.

Ptomaïnes from Certain Infectious Diseases.

Dr. A. P. Luff (*British Medical Journal*, 1889, p. 193) extracted two new alkaloids from the urine in cases of

typhoid fever and scarlet fever respectively.* These alkaloids are not contained in normal urines.

(*a*) *From typhoid fever* the alkaloid is a white crystalline substance. It forms a hydrochloride, an aurochloride, a white precipitate with phosphomolybdic acid, a yellowish-brown precipitate with tannic acid, a dense yellow precipitate with picric acid, and it is also precipitated by iodine solution and mercuric-potassic iodide. It was extracted by the ether-tartaric acid method already described.

(*β*) *From scarlet-fever* the alkaloid is a white semi-crystalline body, soluble in water. It has a faint alkaline reaction. It forms a hydrochloride, an aurochloride, a yellowish-white precipitate with phosphomolybdic acid, a white precipitate with phosphotungstic acid, a yellow precipitate with picric acid, and it is also precipitated by iodine solution and mercuric-potassic iodide.

The investigations of the author of the present book entirely confirm and extend those of Luff. An alkaloid having all the properties described above was extracted (in small quantities) by the author from a cultivation of *Micrococcus scarlatinæ* in nutrient gelatine. Whether this alkaloid is capable of reproducing all the characteristic symptoms of scarlet fever remains to be seen.

(*γ*) *From rabies*.—Dr. Anrep isolated a poisonous ptomaïne from the brain and medulla oblongata of rabbits suffering from rabies. This ptomaïne reproduced all the characteristic symptoms of the disease, and it is stated that a 'gradual habituation of the animal to small doses of the ptomaïne produced a certain degree of immunity.'

(*δ*) *From mumps*.†—In a case where the kidneys were involved, and the parotid and sub-maxillary glands

* The patients had not been taking any alkaloid or antipyretics.

† See Dr. A. B. Griffiths' paper in *Chemical News*, vol. lxi., p. 87.

were both affected, an alkaloid has been extracted from the urine by the ether-tartaric acid or the Luff method. This alkaloid crystallizes in white prismatic needles, which are soluble in water, ether, and chloroform. It has a neutral reaction and slightly bitter taste. This base forms a yellow crystalline platinochloride, a pale yellow aurochloride, and a white crystalline hydrochloride.

It combines with phosphomolybdic acid, forming a golden yellow precipitate. This alkaloid produces a white precipitate with phosphotungstic acid, a slight yellow precipitate with mercuric-potassic iodide, a brown precipitate with iodine solution, and a flocculent precipitate with picric acid.

Analyses of the alkaloid in question gave the following results :

	Found.			Calculated for $C_6H_{13}N_3O_2$.
	I.	II.	III.	
Carbon ...	45·34	—	45·29	45·28
Hydrogen ...	8·22	—	8·20	8·17
Nitrogen ...	26·39	26·42	—	26·41
Oxygen ...	—	—	—	20·12

The above figures correspond with the formula $C_6H_{13}N_3O_2$.

When boiled with mercuric oxide this base yields creatine (methylglycocyamine) and finally methylguanidine and oxalic acid :

(1) $C_6H_{13}N_3O_2 + 3O_2 = C_4H_9N_3O_2 + 2CO_2 + 2H_2O$.

(2) $C_4H_9N_3O_2 + O_2 = CN_3H_4(CH_3) + C_2H_2O_4$.

Therefore it is related to creatine (an animal product) or methylglycocyamine as well as guanidine. It may possibly be *propylglycocyamine :*

$$HN = C<\begin{matrix}NH_2\\N(C_3H_7).CH_2.CO_2H.\end{matrix}$$

If the alkaloid is propylglycocyamine we have the basis of an important homologous series of oxygenized bases related to the ureides. Thus :

$CH_3N_3O_2$—(?).
$C_2H_5N_3O_2$—(?).
$C_3H_7N_3O_2$—Glycocyamine.
$C_4H_9N_3O_2$—Methylglycocyamine (creatine).
$C_5H_{11}N_3O_2$—Ethylglycocyamine (?).
$C_6H_{13}N_3O_2$—Propylglycocyamine (the new base).

The new animal alkaloid is poisonous, and produces nervous excitement, cessation of the salivary flow, convulsions, and death.

The alkaloid in question is not found in normal urines, therefore there is no doubt that it had been produced within the system during the course of the disease (which is highly infectious).

It is difficult to say how this alkaloid is formed ; it may be a morbid leucomaïne (a product of vital physiological, or rather pathological, processes), or it may be formed from the decomposition of albuminoid molecules, by the agency of pathogenic* and other microbes.

(ε) *From diphtheria.*—Drs. Fraënkel and Brieger have recently isolated an alkaloid (toxalbumin) in cases of diphtheria.

This alkaloid is a white crystalline compound, and when injected into an animal's throat produces all the characteristic symptoms of diphtheria.

Toxalbumin has also been extracted from various media containing pure cultivations of *Micrococcus*† *diphtheriticus*. This fact proves conclusively that the microbe manufactures the alkaloid from the medium in which it lives.

* According to M. Trouessart, mumps, or *parotitis* (the *oreillons* of French writers), is due to a pathogenic microbe ; but the author's friend, Dr. Armand Gautier, doubts the microbian nature of the disease.

† Since described as a bacillus by Klein.

(ζ) *From cystinuria.*—Drs. Udránszky and Baumann (*Zeitschrift Physiol. Chemie*, vol. xiii., p. 562) have obtained cadaverine ($C_5H_{14}N_2$—pentamethylenediamine) and putrescine ($C_4H_{12}N_2$—tetramethylenediamine), and other compounds, from the urine of patients suffering from cystinuria.

It is stated that if the formation of these compounds in the alimentary canal, etc., of patients suffering from cystinuria is found to be constant, it may ultimately turn out that cystinuria is an infectious disease. The bacteria, however, differ from most pathogenic microbes in their prolonged existence in the same individual.

Stadthagen and Brieger (*Arch. Pathol. Anat.*, vol. cxv.) have also found similar alkaloids in cases of cystinuria.

From recent investigations it appears that in some cases of infectious diseases it is an alkaloid which is *directly* the *causa causans* of disease; in others various microbes are solely responsible for the disease; and in others, again, may not infectious diseases be due to the combined action of the microbes and the alkaloids?

Many microbes which appear to be pretty constant in certain infectious diseases, when cultivated in artificial media and subsequently injected into animals, do not produce all the characteristic symptoms of those diseases. They may produce some of the symptoms, but not all—something appears to be wanting. May not this something be the alkaloids produced from albuminoids by pathogenic microbes alone or in conjunction with putrefactive bacteria? It is possible that the microbes, *plus* the alkaloids, would give all the characteristic symptoms of the diseases.

It must not be supposed that the alkaloids are the all-important agents of infection, for in some diseases they play a very insignificant part. For example, Duclaux

separated (by means of a Chamberland filter) the ptomaïne of fowl cholera from the *Bacterium choleræ gallinarum*, and found that it did not produce fowl cholera, but only a passing sleep. The microbe, and not the alkaloid, reproduced the disease.

Therefore, 'it is wrong to think that one cause only is operative in the production of disease. There are instances in which but one agent seems to be at work, as, for example, in the acute specific fevers; but in reality this is not so, for the virus must often enter the body of an individual without giving rise to any morbid process. From this fact alone it is clear that other causes, besides the chief cause, must be acting and co-operating to develop the disease. Hence we distinguish exciting or obvious causes from predisposing or secondary causes. In scarlatina it is usually necessary for the individual to be young, not to have had the disease before, and to be exposed to the scarlatinal poison. The last-mentioned is the exciting cause; the age and the not having had the disease previously would be spoken of as predisposing causes.

'What has just been remarked holds good, not only of actual diseases, but also of the signs, symptoms or modes of manifestation of disease. For symptoms may be present when their causes are present, as they will certainly be absent when the conditions on which they depend are wanting. Hence we may see how in some cases of phthisis cough or expectoration may be wanting altogether for a long period of time. In such cases, it is incumbent on us to suppose that some conditions, on which cough and expectoration are dependent, are absent or else suppressed by some other circumstance. And in the rational treatment of symptoms, the problem is to find out the conditions on which the symptom

depends. As a rule,' says Dr. A. Money, 'this is not difficult, and then rational treatment will suggest the removal of one or more of the casual factors, and thus our patient may be benefited, and the objectionable symptom caused to disappear.

'It is of the greatest practical importance *to find out every circumstance in the causation of disease* in any case which may come before us, for it is only by so doing that we can hope to cure the patient of his disease by rational and scientific treatment.'

CHAPTER VI.

SPECIAL OR SOLUBLE FERMENTS.

It has been stated, on good authority, that certain microbes give rise to special or soluble ferments (enzymes)—'products of living protoplasm.'

What have these special ferments produced by microbes to do with infectious diseases? Before the formation of alkaloids, is a special ferment excreted by each microbe? Is it possible that these ferments (with the aid of the microbes) cause the chemical disintegration of albuminoid molecules, with the ultimate formation of alkaloids? These problems require the earnest attention of scientific workers.

'The soluble ferments, or enzymes, have always aroused the deepest interest, partly from the mystery which enshrouded their mode of action, partly from the importance of the processes with which they are associated. The peculiar power which each possesses of decomposing apparently unlimited quantities of a specific medium, without itself being used up in the process, has occasioned the confusion of enzyme action with processes truly vital in their nature. Although this action had only been demonstrated as subserving an alimentary function, its aid was invoked to explain many of the obscure phenomena of biology. The series of decompositions which carbohydrates may undergo,

known as the alcoholic, lactic and butyric fermentations, were long ascribed to it. Even when Pasteur had proved that these processes were always correlated with a vital effect—the growth and multiplication of living cells—Traube,* Hoppe-Seyler, and Liebig† still contended that these might act only indirectly by the formation of soluble ferments. The analogy between fermentation and the infectious processes is so striking, that the latter have long been grouped together under the term 'zymotic diseases,' and these we are every day coming to recognise more and more as parasitic diseases conditioned by microbes. Here again, however, many tend to regard the microbe as not acting directly, but through the production of soluble ferments. The consideration of enzyme function in the lower organisms has accordingly another interest than that which attaches to it, as throwing light upon the processes of digestion in the higher animals. Upon the view which we take as to its origin and meaning will depend the standpoint from which we regard many important physiological and pathological questions.'‡

Concerning soluble ferments, it is well known that vegetable diastase (produced in the first instance from albumin by the action of living cells) is capable of converting starch into dextrose; and cane-sugar into dextrose and levulose:

(α) $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$.

(β) $C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6$.

These substances are of a definite chemical composi-

* *Theorie der Fermentwirkungen*, Berlin, 1858.

† *Ueber Gährung, Quelle der Muskelkraft und Ernährung*, Leipsic, 1870.

‡ See Dr. G. E. C. Wood's paper in *Proc. Roy. Soc. Edinburgh*, vol. xvii., p. 27.

tion, and formed by the action of diastase (a soluble ferment).

It has been shown from the researches of Dr. Musculus (*Berichte*, 1874, and *Comptes Rendus*, vol. lxviii.) and Dr. Sheridan Lea, F.R.S. (*Journal of Physiology*, 1883 and 1885) that *Micrococcus ureæ* does secrete a ferment which causes the ammoniacal fermentation of urea :

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3.$$

The ferment was isolated (in aqueous solution), and was found capable of converting urea into ammonium carbonate.

Dr. H. Bitter, in 1887, ' furnished rigorous proof that bacteria produce enzymes separable from the organisms which form them. He managed to kill the organisms by sterilization at 60° C. without materially destroying their products, and in this way demonstrated that two organisms, when grown on gelatine, produced enzymes which were able, apart from the organism, to liquefy gelatine and peptonize albumin.'

Drs. Roux and Yersin (*Annales de l'Institut Pasteur*, 1888) have recently obtained a ' soluble poison ' (enzyme) from a cultivation of *Micrococcus* diphtheriticus*. This poison produces all the symptoms of diphtheria. It is not an animal alkaloid, but a special ferment, for boiling water destroys its action.

If *Micrococcus diphtheriticus* produces a special ferment which, when separated from the microbe, produces all the characteristic symptoms of the disease, we may safely say that the micrococcus is the cause of the disease.

It has been stated that *Bacillus anthracis*† produces a

* Since described as a bacillus by Klein.

† See also Dr. S. Martin's paper in *Nature*, vol. xlii., p. 118.

ferment; but it has been proved that the ferment is incapable of producing the disease.

Pasteur has shown that when the blood of animals suffering from anthrax is filtered through a Chamberland filter, the filtered blood (free from bacilli) did not produce anthrax when injected into various animals. Nencki has since confirmed Pasteur's investigations.

Dr. Schiavuzzi, of Pola, Istria (*Atti della R. Accademia dei Lincei*, 1886), confirmed Klebs' and Tommasi-Crudeli's* discovery of *Bacillus malariæ*, and that it is the real cause (directly or indirectly) of malarial fever. Schiavuzzi also finds that in the blood of animals suffering from the disease, the red corpuscles undergo similar alterations as Marchiafava and Celli (*Fortschr. d. Med.*, vol. iii.) have shown to be characteristic of malarial fever; and he considers these changes in the blood corpuscles to be caused by a 'pathological' *ferment* of a different nature to *Bacillus malariæ*. Most probably this ferment is a soluble enzyme secreted by the microbe itself.

In an important paper read before the Royal Society of London (April 4, 1889) Drs. Lauder Brunton and A. Macfadyen have shown that certain microbes have the power 'of manufacturing a ferment suited to their needs.' The microbes used were: Koch's spirillum, Finkler's spirillum, a putrefactive micrococcus, scurf bacillus, and Welford milk bacillus (Klein), and the results obtained were as follows:

(1) The microbes which liquefy gelatine do so by means of an enzyme.

(2) This enzyme can be isolated, and its peptonizing

* *Archiv für Experimental Pathologie*, 1879; and also Tommasi-Crudeli's memoir: 'Der *Bacillus malariæ* in Erdboden von Seliunte und Campobello,' in the *Archiv für Exp. Pathol.*, 1880.

action demonstrated, apart from the microbes which produce it.

(3) The most active enzyme is that formed in meat-broth.

(4) Acidity hinders, alkalinity favours, its action.

(5) The microbes which form a peptonizing enzyme on proteid soil can also produce a diastatic enzyme on carbohydrate soil.

(6) The action of the diastatic enzyme can be demonstrated apart from the microbes which produce it.

(7) The diastatic enzyme has no effect on gelatine, and *vice versâ*.

(8) The microbes, for purposes of nutrition, can form a ferment adapted to the soil in which they grow.

(9) The putrefactive micrococcus gave negative results.

Bitter and Steinberg have both obtained similar results with Koch's bacillus—although Koch's cholera bacillus, Deneke's cheese bacillus, Finkler's cholera nostras bacillus, and Miller's bacillus exhibit in all directions a most striking similarity.

Dr. G. E. C. Wood (*Proc. Roy. Soc. Edinburgh*, vol. xvii., p. 29) has proved 'that the enzymes of these organisms are, in each case, distinct, and that the enzymes, even in the same organism, are not alike as regards their capacity of acting under different conditions.'

According to the same authority 'an enzyme is to be looked upon as a function which has undergone a high degree of differentiation—indeed, as a property which is able to exist and act apart from the protoplasm. As each organism is adapted to special conditions, we should expect the enzymes also to act best under these conditions. The enzymes of cholera—Deneke, Miller, and Finkler—exhibit a varying susceptibility to acid reaction,

precisely as the organisms themselves do. This does not indicate that the organisms are more susceptible to acidity, according as their enzymes are more sensitive to its presence, but that the protoplasm as a whole, and with it the enzymes, is adapted to a certain set of conditions—its usual environment.'

But perhaps the most important account of these special ferments, or enzymes, elaborated by microbes is given by the distinguished Professor of Chemistry in the University of Naples (Dr. Italo Giglioli), in his work *Fermenti e Microbi*, to which the reader is referred for a detailed account of the subject.

According to the investigations of Lauder Brunton and Macfadyen, Finkler's spirillum secretes a ferment, and Bocklisch has shown that the same microbe produces an alkaloid.

It would be important to know if the ferment (isolated from the microbe) were capable of producing the alkaloid or alkaloids when placed in a similar medium in which the alkaloids were originally produced by the microbe.

It must be borne in mind that alkaloids are not secreted by microbes, but the special ferments are secreted by them.

From recent investigations, it appears that the real cause of infectious disease is due, in some cases, to a microbe or a ferment, and in others to an alkaloid.

Whether the pathological symptoms, in any special disease, are directly due to an enzyme or an alkaloid, or not, there is plenty of evidence to show that pathogenic microbes have their full share in producing those symptoms, and in the majority of cases produce poisonous alkaloids as well as special ferments.

'Different species of bacilli may vary greatly in their power of producing an alkaloid or secreting a ferment,

just as the elaboration of pigment is much more marked in some species than in others; thus, it need not follow that the number of microbes bears any relation to the virulence or activity of the substance they produce.'

Therefore it may be that the various changes in the blood, organs and secretions in disease 'are produced by the presence and growth of the organisms, as truly as, in the alcoholic fermentation of sugar, the alcohol produced is a result of the presence of the yeast; this change is only in so far a product of the organism as this, in its multiplication, assimilates some molecules of carbon and hydrogen, which it abstracts from the sugar, and in consequence of which the sugar yields alcohol; but it is not, as it were, a secretion of the organism, a special ferment. But it is likewise possible,' says Dr. E. Klein, F.R.S. (*Micro-Organisms and Disease*, p. 252), 'that the organism elaborates a special ferment, which after a certain amount has been produced sets up the particular pathological changes. From these considerations it follows that the virus cannot be considered independent of the organism; we cannot assume that the two can have a separate existence; for . . . the most feasible assumption, and the one borne out by observation, is that, owing to the multiplication of the organisms, certain chemical changes are produced in the blood and tissues, or that a special ferment is created, which sets up the anatomical changes characteristic of the particular disease.'

Whatever may be the outcome of future researches in connection with special ferments and alkaloids, there is every reason to believe that the various symptoms which characterize certain infectious diseases will be shown to be due to the action (partially or wholly) of poisonous alkaloids and special ferments manufactured by pathogenic

and other microbes. It is not improbable that each (microbe, ptomaïne, and special ferment) may produce different symptoms, and that the sum of these different symptoms characterizes the disease. We find, in some cases, certain symptoms entirely absent or imperfectly developed: this may be due to the soil (in which the microbes, for the time being, are living) not being of such a nature as to allow the special ferments, etc., to come into full play, for it has already been shown that acidity, *e.g.*, hinders their action.

CHAPTER VII.

VARIOUS SUBSTANCES PRODUCED BY MICROBES.

In the last two chapters an account has been given of two products (*viz.*, alkaloids and enzymes) formed by the agency of microbes from albuminous substances; in the present chapter a summary will be found of various products formed by different microbes from the media in which they live.

The "microbe" which has been the longest known, and most closely observed, is the *Torula cerevisiæ* (the alcohol ferment). Its small oval globules were first observed by Leuwenhoeck in beer, but in 1837 were recognised by Cagnard Latour as the cause of the fermentation of sugar; nevertheless we are indebted to Pasteur for a complete investigation of the subject. Pasteur proved that the *Torula* was composed of carbon, oxygen, hydrogen, nitrogen, and a number of mineral substances, and that it obtained these substances from the saccharine liquor in which it lived for its own maintenance, growth, and reproduction. The alcohol, carbonic acid gas, and a small quantity of glycerol and succinic acid left (after fermentation) form the residue from the sugar. The yeast-plant does not make the alcohol for its own consumption, for in large quantities it is poisonous to the *Torula*. In fact, alcohol is a product formed by the yeast-plant from sugar, while the alka-

loids are formed from albuminoids by the agency of microbes.

The *Torula* is 'not a bacterium, but belongs to an altogether different order of fungi—the *Blastomycetes*.' It multiplies chiefly by gemmation, but it can also produce spores. When it is suddenly deprived of nourishment, spores are formed. Possibly this is the same with pathogenic microbes: at the end of a disease, when the supply of food is diminished, or when the microbes require a new soil, spores are formed.

Other fermentations are caused by microscopic organisms. Alcohol is a medium in which *Bacterium aceti* lives its life-history; and from this medium acetic acid is produced by the microbe.

Although Pasteur maintained that *Bacterium aceti* was the cause of the acetic fermentation, and Cohn (*Biol. d. Pflanzen*, vol. ii., p. 173) observed the microbe largely in sour beers, yet not until the commencement of 1886 could anyone say with certainty that this microbe was the real cause of the acetic fermentation.

In that year, Mr. Adrian J. Brown, F.C.S. (*Journal Chemical Society*, 1886, p. 172), prepared *pure* cultivations of *Bacterium aceti*, and found that the well-known reaction,

$$C_2H_5OH + O_2 = H_2O + CH_3.COOH,$$

is produced by *Bacterium aceti* (*Mycoderma aceti*).

The author of the present work entirely endorses the correctness of Brown's observations. After obtaining pure cultivations of the microbe by the fractional and dilution methods, it was found that these cultivations, when used to inoculate sterilized ethyl alcohol (6 per cent.), gave acetic acid in abundance.*

* Dr. Griffiths' paper in *Proceedings Royal Society of Edinburgh*, vol. xv., p. 46.

Sweet milk often becomes sour. This is due to the action of a microbe (*Bacterium lactis*) on the sugar of milk. The microbe requires certain elements from milk sugar for its own nourishment, and the residue is lactic acid.

This microbe, with others, plays an important part in the preparation of sauerkraut ; and recently Dr. Baginski has shown that *B. lactis* produces a powerful reducing action in pure cultivations, where the nutrient fluid was coloured with methylene blue.

Putrefaction is not simply a chemical action as formerly supposed, but is the result of the action of numberless microbes. It appears almost self-evident that every body from which life has departed submits to corruption ; and still this is known, that without the life-energy of microbes all bodies after death would retain their form, etc., as well as the Egyptian mummies, or the giants sunk in the Danish moors, or the mammoth and rhinoceros corpses which remained frozen in the Siberian ice for unnumbered thousands of years, and which still retain their skin and hair uninjured, But as soon as the ice melts these last remains of an extinct animal world sink in a few days to corruption. The cause of this is easily comprehended: the life-energy of the microbes is suspended in the neighbourhood of the freezing-point, while they, in a somewhat higher temperature, immediately multiply and excite putrefaction. In the bog and in mummies, it is the chemical mixture which hinders the development of the microbes.

Putrefaction is brought about by the action of many different genera and species of microbes, but perhaps the most important is *Bacterium termo*.

Some of the products formed by putrefactive microbes have already been mentioned.

The butyric fermentation is produced by *Bacillus butyricus*. This microbe causes the rancidity of butter and the ripening of cheese. It decomposes cellulose, and hence it is of great 'importance in the digestive process of herbivorous animals, in whose stomachs and intestines it is very common.'

Micrococcus ureæ is the principal agent in the conversion of urea into ammonium carbonate in the fermentation of urine :

$$CH_4N_2O + 2H_2O = (NH_4)_2CO_3.$$

And there is little doubt that various microbes play an important part in the conversion of organic nitrogen into nitrates. The process of nitrification* which goes on in all cultivated soils is due to the action of microbes.

Although certain microbes form the nitrates of the soil, others reduce them to nitrites, etc. In 1886 Gayon and Dupetit (*Ann. de la Science Agronomique*, 1886, p. 226) stated that a large class of microbes reduce only to nitrites, while others reduce to nitric oxide and nitrogen. They isolated two microbes from sewage, which they named *Bacterium denitrificans* α and β; 'they reduce nitrates to nitrogen gas, nitrites being formed under most circumstances as a stage in the reaction.' It has recently been stated by Pétri (*Centr. Backteriologie und Parisitenkunde*, vol. v.) that the comma bacillus reduces nitrates to nitrites.

The above forms an outline of the various substances produced by different microbes during the processes of fermentation, putrefaction, and nitrification. These substances are formed from the media in which the microbes live.

Let $a_3b_4c_5d_3$ represent the composition of the medium

* See Dr. P. F. Frankland's paper in *Chemical News*, vol. lxi., p. 135.

in which certain microbes live, and let $a_2b_2c_3d_1$ represent the food extracted (from such a medium) by the microbes for their nourishment; it therefore follows that $a_1b_2c_2d_2$ will represent the residue, or the products, of the microbian action—be it fermentation, nitrification, or the production of ptomaïnes, pigments, etc.

Having alluded to such common products of microbian action as alcohol, acetic, lactic, butyric and nitric acids, etc., we come now to consider certain special products formed from various media by the action of microbes.

The Decomposition of Albumin.

Nencki (*Monatshefte für Chemie*, vol. x., p. 506) has recently investigated the decomposition of albumin (serum) by three anaërobic bacilli, namely, *Bacillus liquefaciens magnus*, *Bacillus spinosus* and the *Rausch-brand bacillus*. He obtained scatolactic acid, phenyl-propionic acid, and parahydroxyphenylpropionic acid, as well as certain gaseous products.

The bad-smelling gas evolved during the putrefaction of albumin by the first-named microbe contained 97·1 per cent. of carbonic acid, sulphuretted hydrogen, and other gases absorbable by potash, and 2·63 per cent. of free hydrogen.

Nencki states that the putrid smell during the putrefaction of albumin by *Bacillus liquefaciens magnus* is due to the presence of methyl mercaptan, for he has proved that it is evolved during the putrefaction of flesh by the *Emphysem bacteria*.

Cellulose formed by certain Microbes.

In the *Journal of the Chemical Society*, 1886, page 432, Mr. Adrian J. Brown, F.C.S., describes an acetic ferment, called by him *Bacterium xylinum*, which forms

cellulose—the substance of the membranous growth of the so-called 'vinegar-plant,' or the 'Essighautchen' of Dr. Zopf.

In 1886 Dr. E. Freund, of Vienna, working in Professor Ludwig's laboratory, isolated cellulose from the organs and blood of tuberculous persons. The cellulose extracted from tuberculous material (lungs, spleen, peritoneum with miliary tubercles and blood) had the following reactions :

(1) By the action of strong sulphuric acid it was converted into dextrose.

(2) It yielded a collodion-like mass by the action of nitric acid and ether.

(3) It was transformed into a blue compound by the action of iodine in the presence of strong sulphuric acid or a solution of zinc chloride.

(4) A violet colour was produced by the action of a-naphthol when dissolved in strong sulphuric acid (Molisch's reaction).

(5) The tuberculous cellulose is soluble in an ammoniacal solution of cupric hydroxide.

This cellulose (in the lungs and other organs) is a pathogenic product formed by *Bacillus tuberculosis*. It is entirely absent in the normal tissues and in the following diseases: Emphysema, pneumonia, and pulmonary gangrene; as well as in carcinomatous, sarcomatous, lupoid, syphilitic and other non-tuberculous granulations.

Not only is cellulose present in the blood and organs of persons suffering from tuberculosis, but also in the sputum.

The author (*Proc. Roy. Soc. of Edinburgh*, vol. xv., p. 36) has extracted small quantities of cellulose from *sputum* in certain cases of acute general phthisis. This

substance answers to all the characteristic reactions of cellulose, and when submitted to analysis gave the following results :

	Found.			Calculated for $C_6H_{10}O_5$.
	I.	II.	III.	
Carbon ...	45·00	44·92	44·81	44·74
Hydrogen...	6·20	6·18	—	6·17
Oxygen ...	—	—	—	49·09

It appears that cellulose, and not an alkaloid, is the product formed by *Bacillus tuberculosis* from albuminoids.

According to Udránszky and Baumann (*Zeit. Physiol. Chem.*, vol. xiii., p. 562), ptomaïnes are entirely absent in the stools of patients suffering from tubercular ulceration of the intestines. About seven years ago M. Pouchet extracted sugar (glucose) from the lungs of patients who had been suffering from bacillary phthisis. Is it possible that the glucose is formed first, and then becomes dehydrated with the formation of cellulose?

Dr. E. Kramer (*Monatshefte für Chemie*, vol. x., p. 467) has recently shown that *Micrococcus viscosus* (Pasteur), *Leuconostoc mesenterioïdes* (Prazmowsky) and *Ascococcus billrothii* (Cohn) are not the cause of the mucous fermentation.

The mucous fermentation is brought about by the agency of *Bacillus viscosus sacchari* and *Bacillus viscosus vini*. Both of these microbes produce *cellulose*, which is precipitated from the fermented liquid by alcohol, by basic lead acetate, and by baryta-water, in the form of a white, insoluble, amorphous, stringy mass, which has a specific rotatory power of $[a]_D = + 195°$.

From these remarks it will be noticed that certain microbes produce cellulose from the medium in which they live, while others (*e.g.*, *Bacillus butyricus*) decompose cellulose.

Pigment-forming Microbes.

Certain microbes have the power of forming various coloured pigments from the media in which they live.

The largest number of chromogenic microbes are not associated with disease. Among those which are also pathogenic may be mentioned *Micrococcus pyogenes aureus*, *Micrococcus pyogenes*, *Micrococcus citreus*, and *Bacterium Neapolitanum*.

The majority of the pigment-forming microbes may be cultivated in the ordinary media used for the cultivation of microbes, but they grow best on boiled potatoes, bread-paste, boiled carrots, and boiled egg albumin. Each chromogenic microbe always produces the same pigment.

Very little is known concerning the chemical composition of the various pigments by microbes.

According to Dr. Gessard, *Bacillus pyocyaneus* produces a greenish pigment of a definite composition,* which has been called 'pyocyanin.' Pyocyanin can be extracted from pus by means of chloroform.

Dr. J. Kunz (*Monatshefte für Chemie*, vol. ix., p. 361) has grown *Bacillus pyocyaneus* in nutrient gelatine kept for three or four days at the ordinary temperature, and then for seven days at 35° C. The microbe liquefies the gelatine, which shows a green fluorescence and has the specific smell of blue pus. Kunz extracted from the liquefied gelatine pyocyanin and pyoxanthose, but the liquid still showed a green fluorescence due to a distinct colouring matter, which is only soluble in water and alcohol, and is not destroyed by boiling. Concentrated solutions of this colouring matter transmit red and green light only, but dilute solutions have no absorptive power.

* See *De la Pyocyanine et de son Microbe*, 1882.

According to Kunz, pyocyanin contains nitrogen and sulphur. The green pigment which is formed when this bacillus is grown in nutrient gelatine is most probably produced by the oxidizing action of the air on a chromogen which is formed by the bacillus, as the pigment is not contained in the bacillary cells. In gelatine solutions, the green colour disappears gradually at the ordinary temperature in ten to fifteen weeks, giving place to a dark, reddish-brown colour, and the reaction

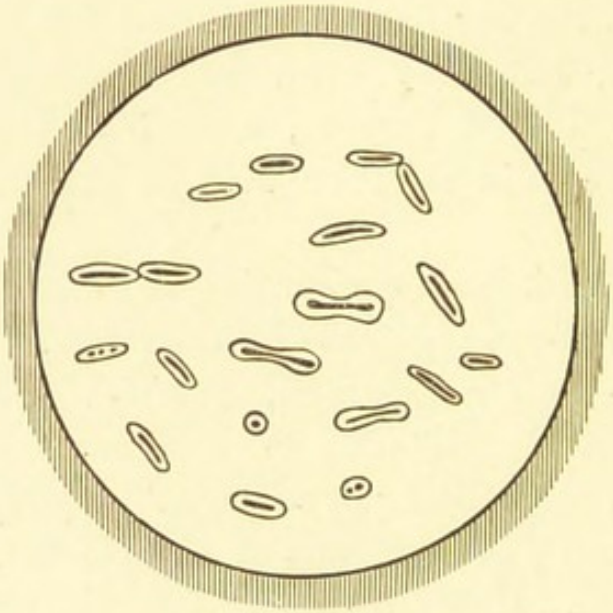


FIG. 20.—BACILLUS OF INDIGO FERMENTATION.

becomes strongly alkaline. *B. pyocyaneus* grows in milk, and produces a yellowish-green solution, which becomes intensely green when ammonia is added.

Dr. E. Alvarez (*Comptes Rendus de l'Académie des Sciences*, 1887, vol. cv.) discovered the microbe which is the cause of the indigotic fermentation and the production of indigo-blue. This microbe is an encapsulated bacillus (Fig. 20), similar in appearance to the bacillus of Rhinoscleroma (Cornil and Alvarez).

Indigo-blue (indigotin) is the product of several species

of plants belonging to the *Indigofera* and other genera. 'It does not exist in these plants ready-formed, but is produced by the decomposition of a glucoside ($C_{26}H_{31}NO_{17}$) called indican.'

By the action of Alvarez's bacillus, indican yields indigo-blue (C_8H_5NO) and indiglucin ($C_6H_{10}O_6$):

$$C_{26}H_{31}NO_{17} + 2H_2O = C_8H_5NO + 3C_6H_{10}O_6.$$

The bacillus of the indigo fermentation has been shown to possess pathogenic properties, and occasions in animals a transient local inflammation, or death, with visceral congestion and fibrinous exudations.

Indican is sometimes found in urine.* It is often present in large amount in intestinal obstruction and ulceration, and in granular kidney. Dr. Lauder Brunton, F.R.S. (*Gulstonian Lectures*, 1889) 'looks upon much indican in urine as an indication for a mercurial purgative. It shows that microbes are active in the small intestines, and that albuminous matters are undergoing rapid decomposition there.'

Dr. A. Kast (*Zeit. Physiol. Chemie*, vol. xi., p. 501) has shown that the blue colour of the sweat in chromidrosis is due to microbes.

But, as already stated, the chemistry of the microbian pigments is a subject which has been very little investigated. They are undoubtedly products formed from the decomposition of albuminoids by the agency of microbes.

The following table gives a number of chromogenic microbes and the colour of the pigments produced:

* For the detection and estimation of indican in urine, see a paper by W. Michailoff in *Journal of the Russian Chemical Society*, 1887, p. 326.

Microbe.	Found in.	Colour of Pigment produced.
Micrococcus pyogenes ...	Acute abscesses	Pale brown
Micrococcus pyogenes aureus	Abscesses in osteomyelitis	Orange-yellow
Micrococcus pyogenes citreus	Pus	Yellow
Micrococcus prodigiosus	Air	Red
Micrococcus cereus flavus	Pus	Lemon-yellow
Micrococcus citreus conglomeratus	Air and blennorrhœic pus	Lemon-yellow
Sarcina lutea	Air	Yellow
Sarcina ventriculi ...	Vomit	Greenish-yellow.
Micrococcus cyaneus ...	Air	Blue
Micrococcus aurantiacus	Air	Orange-yellow
Micrococcus chlorinus ...	Air	Yellowish-green
Micrococcus violaceus ...	Air	Violet
Micrococcus rosaceus ...	Air	Rose
Micrococcus hæmatodes	Human sweat ...	Brick-red
Bacterium pseudo-pneumonicum	Pus	Grayish-white
Bacterium Neapolitanum	Cholera dejecta ...	Yellowish-brown
Bacterium oxytocum perniciosum	Sour milk	Yellow
Bacterium cavicida ...	Human fæces ...	Yellow
Bacterium hyacinthi ...	Slime from decomposing hyacinths	Yellow
Bacterium allii	Slime from decomposing onions ...	Green
Bacterium xanthinum ...	Air	Lemon-yellow
Bacterium indicum ...	Air	Scarlet
Bacterium brunneum ...	Rotting infusion of maize	Brown
Bacterium æruginosum	Green pus	Green
Bacillus pyocyaneus ...	Pus	Green
Bacillus ianthinus ...	Water	Violet
Bacillus cyanogenus ...	Milk	Blue
Spirillum tyrogenum ...	Old cheese	Brownish-green

The pigment-forming microbes *generally* grow in the state of zoogloœa upon the surface of the substances which furnish them with nutriment. According to Cohn, microbian pigments offer the greatest diversity as to chemical action, and by spectroscopic analysis, etc.; but

each microbe cultivated in the most diverse media produces always the same colouring matter.

Many of the chromogenic microbes are found in the atmosphere, and often give rise to patches of colour upon various articles of food (raw or cooked). In fact, during the year 1843 the military bakehouses of Paris were attacked by an unusual prevalence of *Micrococcus prodigiosus*, which gave the bread a red colour. This microbe sometimes produces a blood-red colour in milk.

It has long been a saying that suddenly, from time to time, a drop of blood would form on food, and especially on bread, and so increase that it would spread over wide surfaces. This was observed in ancient times, and it was held that it was a sign of threatened disaster, that it showed the anger of God, disclosed secret guilt, and called for bloody atonement; and history records numberless sacrifices which fell to the superstition as often as the 'blood-wonder' was seen on food, but especially if on the consecrated wafer.

With a century of enlightenment the 'blood-wonder' gradually ceased; but only within recent times do we know that the wonderful account had foundation in a fact of science. It was Ehrenberg who first investigated this appearance of blood, and he found that the red slime was composed of numberless minute spherical bodies, which are now known by the name of *Micrococcus prodigiosus*. They nourish themselves on the albumin contained in the food on the surface of which they develop, decompose the same, and generate thereby the red colouring matter which, as Drs. Erdmann* and Schröter† have shown, possesses a striking resemblance to certain aniline colours.

* *Journal für Praktische Chemie*, 1866.
† *Beiträge zur Biologie der Pflänzen*, vol. i., pp. 109-126.

Bacterium xanthinum, also discovered by Ehrenberg, is the cause of 'yellow milk.' The pigment formed by this microbe is said to be 'similar to yellow aniline colours both spectroscopically and in ordinary sections.'

The *litmus* so much used by chemists is obtained from certain lichens (*Rocella tinctoria* or *Lecanora tartarea*) by macerating them in water, and allowing the mixture to ferment in contact with the air until a purple or blue colour is developed. The production of this colour is due to the action of putrefactive microbes. It has been experimentally proved that when putrefactive microbes are placed in a solution containing ammonium acetate and cream of tartar, a colouring material exactly similar to litmus is the result of the microbian action.

On the authority of Magnin, microbian pigments may be divided into two categories, according as the pigment is soluble or insoluble in water. Thus the red pigment formed by *Micrococcus prodigiosus* is insoluble in water and soluble in alcohol, while the yellow pigment formed by *Bacterium xanthinum* is soluble in water and insoluble in alcohol.

The red colouring matter ('bacterio-purpurin') formed by *Beggiatoa roseo-persicina* is insoluble in water and alcohol, and is allied to chlorophyll (Engelmann).

The whole subject of the formation, composition, properties, and physiological action of microbian pigments is worthy of the deepest study and research on the part of physiological chemists and bacteriologists.

Sulphur-forming Microbes.

Certain microbes play an important *rôle* in the elimination of sulphur and the disengagement of sulphuretted hydrogen.

Most of the sulphur-forming microbes are found in

certain waters, and many of the natural sulphurous waters are due to the action of microbes on alkaline sulphates and organic matter present in such water. The decomposition of calcium sulphate—*e.g.*, by the sulphogenic microbes—may be represented by the following equations:—

(*a*) $3CaSO_4 + H_2O = S_2 + H_2S + 3CaO + 5O_2$.

(*b*) $2CaSO_4 = S_2 + 2CaO + 3O_2$.

Sulphogenic microbes are capable of decomposing animal and vegetal albumin with the liberation of sulphur.

Amongst the sulphogenic or sulphur-forming microbes may be mentioned the following:

(*a*) *Sarcina litoralis* was discovered in sea-water containing putrefying organic matter. Each cell contains from one to four granules of sulphur.

(*b*) *Beggiatoa mirabilis* also occurs in sea-water, 'forming a white gelatinous scum on decomposing algæ, etc.' The cells of this microbe contain sulphur granules.

(*c*) *Beggiatoa alba* is found in marshes and sulphur springs. The cells of *B. alba* contain sulphur granules. According to Cohn and Cramer, these granules consist of *crystalline* sulphur, which is highly refractive (*Beiträge zur Biologie der Pflänzen*, vol. i.).

When these crystalline granules are 'disintegrated' and examined under the highest powers of the microscope, they are seen to be composed of a number of rhombic (octahedral) crystals.

(*d*) *Beggiatoa roseo-persicina*, or the 'peach-coloured bacterium' of Lankester, is a sulpho-chromogenic bacillus. It occurs 'on the surface of marshes, or on water in which algæ are rotting; and sometimes these bacilli are in such quantity that whole marshes and ponds may be coloured blood-red by them.' These microbes contain dark-coloured sulphur granules, the dark colour being

due to the pigment (bacteria-purpurin) formed by the microbes.

Phosphorescent Microbes.

Many of the lower animals have the power of rendering themselves phosphorescent; and Dr. Kunz (*Monatshefte für Chemie*, vol. ix., p. 361) has recently studied the cells of *Bacterium phosphorescens*, which are almost circular (from 1·3 to 1·9 μ long and 1·1 to 1·7 μ broad); each cell is mobile and surrounded by a clearly perceptible zooglæan membrane. This microbe grows slowly at the ordinary temperature in peptonized gelatine, or in peptonized gelatine containing 2 per cent. of glucose, but only at the surface, and the property of emitting light seems to be dependent on the presence of oxygen.

It grows well in 2, 3 and 4 per cent. solutions of sea-salt, containing 0·25 per cent. of peptones. These solutions are very phosphorescent, far more so than any inorganic substances or an alkaline amylic alcohol solution of lophin; on shaking, the phosphorescence becomes more clearly apparent, but on cooling to 0° C. its intensity is slightly diminished. The phosphorescence disappears when the solution is heated at 35° C. for a few minutes, but reappears on cooling; it is, however, completely destroyed by heating at 35° C. for fifteen minutes.

After two or three weeks, the culture solutions become yellowish, and gradually lose their phosphorescence; after several weeks phosphorescence ceases entirely, but the microbes do not die. The phosphorescence is most probably caused by some vital process, as it is destroyed by all reagents which kill the protoplasm of the cells.

Bacterium phosphorescens grows in 3 per cent. solu-

tions of sodium chloride, magnesium sulphate, or sodium sulphate, containing 0·25 per cent. of peptones, and the solutions show intense phosphorescence. This microbe also grows in urine and milk.

In a paper read before the Royal Academy of Sciences of Amsterdam, on February 22, 1890, Dr. Beyerinck gave the results of his researches on the luminous food and the plastic food of phosphorescent microbes. Of the six species of phosphoresent microbes known, four—*viz.*, the alimental gelatine non-melting *Bacterium phosphorescens* and *Bacterium Pflügeri* of luminous fish, and the Baltic phosphorescent microbes, *B. Fischeri* and *B. balticum*—require, besides peptone, a second carbonic combination, as glycerol, glucose, or asparagine, for their complete nourishment; *i.e.*, to 'phosphoresce' and grow. They may be called peptone-carbon-bacteria. The gelatine quick-melting phosphorescent bacteria from the West Indian Sea and the North Sea, *Bacterium indicum* and *B. luminosum*, can phosphoresce and grow on peptone alone. They are, therefore, peptone-bacteria. Again, other bacteria can derive their nitrogen either from amides, the amide-bacteria, or from ammonia, the ammoniac-bacteria. *B. Pflügeri* does emit light with peptone and glucose, but not with peptone and maltose, while *B. phosphorescens* emits light both with glucose and maltose. Now, if one mixes some starch in a phosphorescens-peptone-gelatine, obtained by mixing this gelatine with a large number of *Bacterium phosphorescens*, and place upon this some ptyalin, pancreas-diastase, or urindiastase (nefrozymase), fields of light make their appearance; if, however, one places these same sorts of diastase on a Pflüger-peptone-starch-gelatine, then no fields of light appear, which proves that in this instance no glucose whatever is formed, as

was lately believed to be the case. The development of luminosity is constantly accompanied by the transition of peptones into organized, living matter, under the influence of free oxygen, with or without the concurrence of another carbonic combination.

A large number of the substances formed by zymogenic, saprogenic, and pathogenic microbes have already been alluded to, therefore they do not require any further description in the present chapter.

CHAPTER VIII.

THE ACTION OF HEAT, LIGHT, ELECTRICITY, GASES, ETC., ON MICROBES.

HEAT, ETC.—Many microbes, and especially the spore-bearing forms, are capable of withstanding (within certain limits) the action of either a high or low temperature.

In 1881 Drs. Koch and Wolfhügel (*Mittheilungen aus dem kaiserlichen Gesundheitsamte*, 1881), having tested the value of hot air as a disinfectant, came to the following conclusions in regard to the action of heat on certain microbes :

'Sporeless microbes at a little over 100° C. are destroyed in an hour and a half.

'Spores of bacilli require three hours at 140° C., and spores of fungi require one and a half hours at 110-115° C.'

The action of heat (hot air and steam) upon microbes has been recently studied by Drs. H. F. Parsons and E. Klein.* The infective materials employed in these experiments were as follows :

(1) Blood of guinea-pig dead of anthrax, containing anthrax-bacilli without spores.

* *The Annual Report of the Medical Officer of the Local Government Board* (1884).

(2) Pure cultivation of *Bacillus anthracis* in rabbit broth, without spores.

(3) Cultivation of *Bacillus anthracis* in nutrient gelatine, with spores.

(4) Cultivation of *Bacillus of pneumo-enteritis of the pig* in pork broth.

(5) Tuberculous pus, from an abscess in a guinea-pig which had been inoculated with tubercle.

The experiments on the action of dry heat were mostly made in a copper hot-air bath, or in one improvised of flower-pots, and furnished with a Bunsen's regulator; those with steam were made in a felt-covered tin cylinder, through which passed a stream of steam from a kettle beneath.

The mode of procedure in exposing the materials to heat was as follows: Strips of clean flannel were steeped in the respective infective fluids, dried in the air, wrapped separately and loosely in a single layer of thin blotting-paper, and suspended in the centre of the apparatus in company with a thermometer, so placed that its bulb was close to the packets of infected material.

The following were the results of the experiments with *dry air*:

Anthrax bacilli without spores were sterilized in five minutes by an exposure to a dry heat varying between 100° and 103° C. Spore-bearing cultivations of the same bacillus, on the other hand, did not lose their vitality by a two hours' exposure to 104° C., but were sterilized by an exposure for four hours to 104° C., or one hour to 118° C.

According to Parsons and Klein, a rabbit inoculated with swine-fever virus which had been exposed to a dry heat varying between 100° and 103° C. for an hour remained well; but one inoculated with virus which

had been exposed to a similar heat for only five minutes died of swine fever after nineteen days, the usual time of death after inoculation being between five and eight days.

Guinea-pigs inoculated with tuberculous pus which had been exposed for five minutes to 104° C. remained well.

Therefore, it appears that the spores of *Bacillus anthracis* lose their vitality after an exposure for four hours to a temperature a little over the boiling-point of water, or for one hour to a temperature of 118° C.

The non-spore-bearing bacilli of anthrax and of pneumo-enteritis of the pig were destroyed after an hour's exposure to a temperature of 100-103° C.

These experimenters conclude that as none of the infectious diseases, for the extirpation of which measures of disinfection are in practice, are known to depend upon the presence of bacilli in a spore-bearing condition, their contagia are not likely to retain their activity after being heated for an hour to 105° C. (220° F.).

In the experiments of Parsons and Klein with steam, the results were conclusive as to the destructive power of steam at 100° C. upon all the contagia submitted to its action.

These results are in accordance with those of Drs. Koch, Gaffkey, and Löffler (*Mittheilungen a. d. kaiserlichen Gesundheitsamte*, 1881), and it may be considered established that the complete penetration of an object by steam heat for more than five minutes is sufficient to destroy all microbes and their spores.

Dr. Klein found that boiling in water for only one minute was sufficient to render inert the spores of *Bacillus anthracis*, although it is known that some of the spore-bearing non-pathogenic bacilli are only destroyed by prolonged boiling, or by a moist temperature above the boiling-point.

In fact, Klein says, in that excellent little book of his, *Micro-Organisms and Disease*, p. 56: 'Most bacteria are killed by heat below the temperature of boiling water, many of them when exposed for several hours to a temperature above 50° to 60° C. Exceptions are the spores of bacilli, which in some instances (spores of hay bacillus, Cohn) require exposure to the heat of boiling-point for as long as half an hour. By raising the boiling-point above 100° C., it does not require more than a few minutes to kill them (Sanderson).'

Although most microbes are destroyed in a few hours

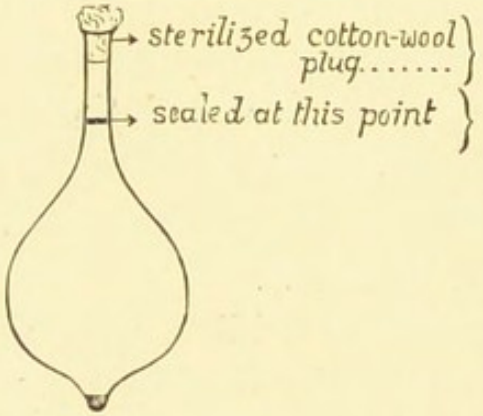


FIG. 21.

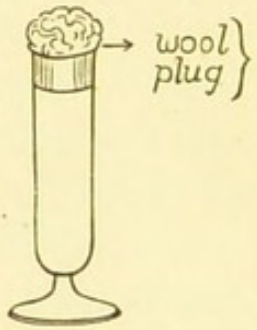


FIG. 22.

by being heated to 50° to 100° C., many are capable of withstanding a *dry heat* of 32° C. for several months.

The author (*Proceedings Royal Society of Edinburgh*, vol. xv., pp. 42-44) has shown that tubercle-bacilli are capable of being dried up for three or four months at a temperature of 32° C. without losing their vitality.

The experiments were conducted in the following manner: A small quantity of sputum was mixed with calcium sulphate and calcium carbonate (previously sterilized at a temperature of 135° C.), and the mixture placed in twelve sterilized tubes (Fig. 21), and the latter were then hermetically sealed.

Each tube contained about ten grammes of the mixture.

Twelve dry sterilized tubes (Fig. 22), not hermetically sealed, also contained the same quantity of the mixture of sputum, calcium sulphate, and calcium carbonate (these mineral substances constituting the principal ingredients contained in the dust of the atmosphere). The twenty-four tubes were kept at a dry heat of 32° C., from one to six months.

Two of the hermetically-sealed tubes and two of the open tubes were opened after being exposed to the above temperature for one month; and four tubes, containing sterilized blood serum, were inoculated from the contents of the tubes. In the two inoculated from the open tubes growths of *Bacillus tuberculosis* (proved by staining, microscopical appearance, etc.) made their appearance in sixteen days after the inoculation.

Growths of *Bacillus tuberculosis* also made their appearance in the two tubes inoculated from the contents of the sealed tubes after nineteen days' incubation.

Four more tubes were opened after being exposed for two months at the temperature already mentioned. Inoculations from two *open* tubes revealed the vitality of *Bacillus tuberculosis* after twenty days' incubation; and inoculations from two sealed tubes proved the vitality of the bacilli after the lapse of twenty-three days' incubation.

The remaining tubes were examined in a similar manner after the lapse of three, four, five, and six months respectively. After being exposed to the dry heat for three and four months, this microbe and its spores were *not* destroyed. But after being heated for five and six months *B. tuberculosis* and its spores were completely destroyed; for no growths made their ap-

pearance in sterilized blood-serum kept at a temperature between 37° and 39° C. for nearly two months.

From these experiments it appears that *Bacillus tuberculosis* is capable of being dried up in the dust of the atmosphere for several months without its vitality being impaired.

A similar series of experiments were performed, by the author, with other microbes (the final inoculations being made in different media so as to suit each case).

The results* were as follows :

	After an Exposure at 32° C. (dry heat) for Months.							
	1	2	3	4	5	6	7	8
Micrococcus rosaceus	L†	L	L	L	—	D	D	D
Micrococcus prodigiosus ...	L	L	—	L	—	D	D	D
Micrococcus chlorinus ...	L	L	L	L	L	L	—	D
Bacterium allii	L	L	L	L	L	L	D	D

From these experiments it appears that various microbes are capable of being dried up in the dust of the atmosphere for several months (at 32° C., or 89° F.) without losing their vitality.

M. Duclaux (*Comptes Rendus*, vol. c., pp. 119 and 184) proved that the germs of certain species of *Tyrothrix*, especially *Tyrothrix scaber*, are not destroyed by at least three years' exposure in a dry state to air of a tropical

* Dr. Griffiths' paper read before Royal Society of Edinburgh, March 18, 1889.

† L = living ; D = dead.

temperature. The same authority found the *Sterigmatocystis migra* retained its vitality for over two years in a warm, dry atmosphere, but was dead after the expiration of three years.

It has already been stated in this chapter that Koch found that the spores of fungi required one and a half hours at 110-115° C. before their vitality was destoyed. Although a temperature of 110° C. destroys the spores of most fungi, the author* has shown that they are capable of being heated to 35° C. (dry heat) for several months without losing their vitality. For instance, a quantity of the spores of *Peronospora infestans* was taken from a crop of diseased potatoes. These spores were placed in a porcelain mortar, with about five grammes of a mixture of calcium sulphate and calcium carbonate, and thoroughly (but gently) mixed together. This mixture was then placed in a small oven kept at a temperature of 35° C. (dry heat).

After the spores had been dried up with the mineral substances for two months, their appearance, when mounted in a drop of water and examined under the microscope, was little different from the fresh spores. These spores after two months' desiccation were sown on the leaves of a potato-plant, kept in a warm, moist atmosphere. In the space of three days after sowing, the spores began to penetrate into the mesophyll of the leaves of the host-plant, through the stomata. On the fifth day after sowing there were mycelia (which had ramified through the tissues of the inoculated leaves), and conidia-bearing branches, the latter making their appearance through the stomata of the leaves.

* *Chemical News*, vol. liii., p. 255 ; *Proc. Royal Society of Edinburgh*, vol. xv., p. 410 ; *Journal de l'Agriculture*, 1889, p. 106 ; *Bulletin de la Société Chimique de Paris* (3rd series), vol. ii., p. 667 ; *Journal Chemical Society*, 1884, p. 1070.

After six months of dry heat, the spores of *Peronospora infestans* had not lost their vitality, for after inoculating potato-leaves with the desiccated dust, there was a rapid development of hyphæ, mycelia, etc., in the space of seven days.

After a desiccation for ten months the spores of this fungus had completely lost their vitality, for they did not germinate upon the leaves of *Solanum tuberosum* after being in contact with the leaves for a month or six weeks.

The author has also shown that the fungus (*Ustilago cucumeris*) which he discovered on the roots of *Cucumis sativa* is capable of being desiccated for four months without losing its vitality (see *Proc. Roy. Soc. Edinburgh*, vol. xv., p. 403; and *Journal de l'Agriculture*, 1889, p. 103).

Like certain microbes and their spores, the spores of fungi are capable of withstanding the action of a dry heat of 32° to 35° C. for several months.

In the animal kingdom, many of the lower forms of the *Invertebrata* are also capable of being completely desiccated for long periods of time without losing their vitality. For instance—it was shown by Dr. Kühn (*Ueber die Wurmkrankheit des Roggens*) that *Tylenchus devastatrix*, one of the *Anguillulidæ*, retained its vitality for two years in a state of complete desiccation.

Mr. W. Carruthers, F.R.S. (of the Botanical Department of the British Museum), states that the vitality was restored in some 'eelworms' (*Tylenchus*) after they had been in the National Collection for over thirty years!

Sir Richard Owen, K.C.B., F.R.S. (*Comparative Anatomy and Physiology of Invertebrata*, p. 54), records the fact that the Abbé Spallanzani and others revived rotifers after four years' desiccation.

From this it will be gathered that the lower forms of animal as well as vegetal life are capable of withstanding complete desiccation for long periods of time.

These facts have an important bearing on the distribution of microbes and fungi by means of the atmosphere.

Cold.—At the freezing-point of water the life energy of most microbes is suspended, and in some cases destroyed. Prudden has shown that certain microbes are capable of being frozen for thirty-seven days without losing their vitality.

In a paper read before the Royal Society of Edinburgh (March 18, 1889), the author detailed the results of certain experiments he had made with freezing mixtures on the vitality of microbes.

The microbes in these experiments were: *Bacillus tuberculosis*, *Bacillus subtilis*, *Bacterium allii*, *Spirillum tyrogenum*. They grew in tubes containing suitable media for their development and growth. These tubes were then placed in vessels containing the following freezing mixtures:

Mixtures.*	Proportions by Weight.	Minimum Temperatures recorded.
Ice	2	—18° C.
Salt	1	
Water	1	—15° C.
Ammonium nitrate	1	
Sodium sulphate	8	—17° C.
Hydrochloric acid	5	

* The tubes containing the microbes were removed from time to time if the temperature of the freezing mixtures in the vessels recorded a higher temperature than those given in the third column. After removing, the tubes were again placed in freezing mixtures newly made.

After the microbes had been exposed to the above temperatures for *several* days, a number of tubes containing sterilized blood-serum, agar-agar, etc., were inoculated from the contents of tubes which had been exposed to the action of the freezing mixtures.

From these experiments the following results were obtained :

	At −18° C.			At −17° C.			At −15° C.		
	For 1 day.	For 3 days.	For 14 days.	For 1 day	For 3 days.	For 14 days.	For 1 day.	For 3 days.	For 14 days.
Bacillus tuberculosis ...	L*	D	D	L	D	D	L	D	D
Bacillus subtilis	L	D	D	L	L	D	L	L	D
Bacterium allii	D	D	D	D	D	D	L	L	D
Spirillum tyrogenum ...	L	L	D	L	L	D	L	L	D

It appears that certain microbes are capable of withstanding the severity of a low temperature, although their vitalities (proved by longer periods of incubation) are impaired.

Electricity.—The action of the electric current upon the vitality of various microbes has been very little studied; therefore, the following notes may be of interest.

Details of the author's experiments will be found in the *Proceedings of the Royal Society of Edinburgh* (vol. xv., pp. 45, 46).

The experiments were performed on pure cultivations of microbes growing in various media. Fig. 23 represents the *general* arrangement of the apparatus; and the results were as follows :

* L = living ; D = dead.

(1) *Bacillus tuberculosis*, growing in previously sterilized fluid blood-serum (slightly alkaline) was killed by an E.M.F. of 2·16 volts.*

(2) *Bacterium lactis*, growing in previously sterilized milk, was killed by an E.M.F. of 2·26 volts.

(3) *Bacterium aceti*, growing in previously sterilized alcohol (7 per cent.), was killed by an E.M.F. of 3·24 volts.

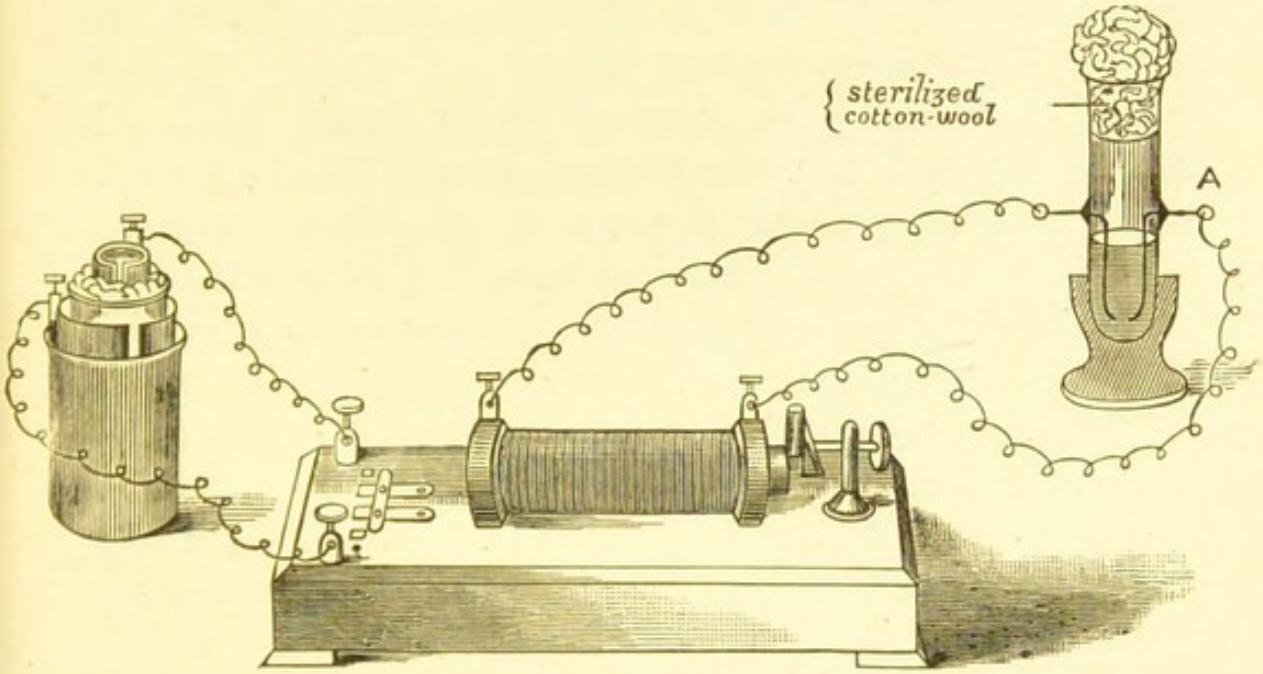


FIG. 23.—ELECTRICAL EXPERIMENTS ON THE VITALITY OF BACILLUS TUBERCULOSIS AND ITS SPORES, ETC.
A = a tube containing growing bacilli in sterilized fluid blood-serum slightly alkaline.

The temperature of the laboratory was 16° C., and the current was allowed to pass for ten minutes in each case. Ten tubes containing sterilized fluid blood-serum were inoculated from the 'electrified' tubercle-bacilli, and after being kept at a temperature of 38° C. for twenty-five days, *no growths* made their appearance in any of the tubes.

A similar number of tubes containing sterilized sweet

* See any good work on electricity.

milk were inoculated from the 'electrified' lactic-bacteria, with *no results* after twenty-five days' incubation.

Seven tubes containing the purest ethyl alcohol and ordinary filtered tap-water* (the mixture containing six per cent. of alcohol) were inoculated with the 'electrified' acetic-bacteria, with negative results.

A further series of experiments on the same lines as those just alluded to were recorded in the author's paper read before the Royal Society of Edinburgh on March 18, 1889. The results were as follows :

(1) *Bacterium allii*, growing in previously sterilized pork-broth (neutral), was killed by an E.M.F. of 3·3 volts.

(2) *Bacillus subtilis*, growing in previously sterilized pork-broth (neutral), was killed by an E.M.F. of 2·72 volts.

(3) *Bacillus tuberculosis*, growing in previously sterilized fluid blood-serum, was killed by an E.M.F. of 2·16 volts.

The temperature of the laboratory was 17° C., and the current was allowed to pass for ten minutes in each case.

The 'electrified' microbes were then transplanted to a certain number of tubes containing the above cultivating media. After an incubation for twenty days at 35° C. *no growths* made their appearance in any of the tubes.

It appears that the electric current has a detrimental action on the growth of microbes. In Chapter IV. it was stated that atmospheric electricity 'is detrimental to the life of aërial microbes.' There are always fewer microbes in the atmosphere after a thunderstorm than at any other time.

Light.—Concerning the action of light on microbes very little is known. As the majority of pathogenic and anaërobic microbes live in the absence of light, it is probable that a powerful light would hinder their action.

* Tap-water was used in preference to distilled water, on account of the mineral matter which it contains—the microbes requiring small quantities of mineral matter.

According to M. Duclaux (*Comptes Rendus de l'Académie des Sciences*, vol. c.), *Tyrothrix scaber* is killed by exposure to direct sunlight for a few weeks.

The microbe called by Dr. T. W. Engelmann (*Pflüger's Archiv*, vol. xxx., p. 95) *Bacterium photometricum* is influenced by the action of light. In fact, its movements are stated to depend on light. This microbe produces a red pigment, but the amount of the pigment formed varies with the action of light.

Since Engelmann published his first account of *B. photometricum*, he has made a number of observations on different varieties of microbes which produce a red pigment. All belong to the class of 'sulphur-bacteria' (*Botanische Zeitung*, 1887, Nos. 31-37)—that is, bacteria which in the presence of free hydrogen-sulphide oxidize sulphur, forming sulphuric acid. All these bacteria are, moreover, coloured by a purplish-red pigment diffused through their protoplasm—*i.e.*, the bacterio-purpurin of Ray Lankester—and they are all also influenced by light like *Bacterium photometricum*. This last point distinguishes them from certain *colourless* sulphur-bacteria. Different coloured lights affect the bacteria differently, the most powerful being the ultra-red, the yellow, and part of the green. These are the places in the absorption-spectrum of bacterio-purpurin where the greatest absorption of light occurs. According to Engelmann, absorption and physiological effects are closely related to one another. This fact suggested a comparison between this pigment and chlorophyll; and it was then found that bacterio-purpurin is a chromophyll, absorbing carbonic anhydride, and giving out oxygen *in the presence of light*. Sunlight produces this action most readily, but the ultra-red works only a little less efficiently than mixed light.

If *Beggiatoa roseo-persicina*, or any other sulpho-

chromogenic microbes are placed in a drop of water on a slide under the microscope, and a spectrum thrown on to the slide by means of Dr. Engelmann's micro-spectral objective (Fig. 24),* the microbes always move or congregate near the red end of the spectrum.

There is little doubt that light is beneficial to some microbes, and detrimental to others. Possibly the

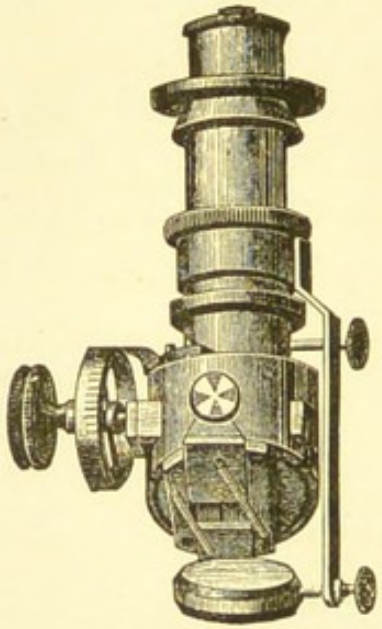


FIG. 24.—ENGELMANN'S MICRO-SPECTRAL OBJECTIVE.

chromophyllic microbes are capable of manufacturing albumin from such materials as carbonic anhydride, water, sulphates, and ammonia. Be this as it may, the researches of Engelmann have shown that these microbes

* Engelmann's micro-spectral objective is used for observing and measuring the effect of the colours of the spectrum on microbes and other objects (*Botanische Zeitung*, 1882; *Pflüger's Archiv*, vols. xxvii. and xxix.). The slit mechanism, collimator lens, Amici prism, and projection objective are combined in a tube about 77 millimètres (about three inches) in length, which *fits below the stage* concentrically with the axis of the microscope, so as to project a real spectrum upon the preparation under observation.

The edges of the slit are moved symmetrically by a screw with two reversed threads, so that the middle of the slit remains unaltered in position; the divided head of the screw (see Fig. 24) shows the width of the slit as adjusted in 100ths of a millimètre; the length of the slit may be shortened on both sides by two slides acted upon by screws.

are capable of decomposing carbonic anhydride in the presence of light.

GASES.—The next point to consider is the action of various gases on microbes.

(1) *Oxygen.*—'Some microbes require free access of oxygen, and are called aërobic (Pasteur); others grow without free oxygen, and are called anaërobic (Pasteur),' but all require oxygen in some form or other.

The action of compressed oxygen on microbes has been investigated by various bacteriologists. According to Paul Bert, this agent 'kills all living things (!), but that infective materials in solution, such as scorpion venom, vaccine matter, whether liquid or dried and re-dissolved, resist the action of compressed oxygen; and from further experiments he was led to infer that the active agent in vaccine and in glanders is not a living micro-organism (!). He also exposed anthracic splenic blood to the action of compressed oxygen; the blood retained its virulent properties intact, as proved by inoculation; but in no instance did the blood contain bacilli.'

But Pasteur subsequently proved that the *spores* of *Bacillus anthracis* are not destroyed by compressed oxygen, and in M. Bert's experiments the disease was reproduced from the spores which had resisted the compressed gas.

M. Chauveau (*Comptes Rendus*, 1889) has recently investigated the action of compressed oxygen on *B. anthracis*, and finds that the virulent power of the microbe is greatly reduced by the gas (under pressure), but can be revivified when the microbe is grown in suitable media.

Pasteur has stated in the *Comptes Rendus* (1881) that it is the oxygen of the air which attenuates the virulence of a pathogenic microbe, and according to the view of

Pasteur dry air is the chief cause of the attenuation of the virus of rabies.

(2) *Hydrogen.*—Dr. P. F. Frankland (*Proc. Roy. Soc.*, vol. xlv., p. 292) has shown that hydrogen gas had very little effect on *Bacillus pyocyaneus*, Koch's bacillus, and Finkler's spirillum.

(3) *Sulphuretted Hydrogen.*—The author (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 51) proved that H_2S completely destroys the vitality of *Bacillus tuberculosis* (see later in this volume).

P. F. Frankland (*loc. cit.*) found that sulphuretted hydrogen was detrimental to the vitality of *Bacillus pyocyaneus*, Koch's bacillus, and *Spirillum Finkleri.*

Although sulphuretted hydrogen is 'poisonous' to the majority of microbes, some (especially the sulpho-chromogenic bacteria) have the power of oxidizing this gas, forming sulphuric acid :

$$H_2S + 2O_2 = H_2SO_4,$$

while other microbes produce this gas by decomposing albumin or the alkaline sulphates (see preceding chapter).

(4) *Chlorine, Bromine, and Iodine.*—Chlorine gas and the vapours of bromine and iodine are powerful antiseptics, easily destroying most microbes. According to the author's investigations, the disinfecting power of the three halogen elements is inversely as their atomic weights (Cl. = 35.5 ; Br. = 80 ; I. = 127)—*i.e.*, chlorine is the best disinfectant, then bromine, and finally iodine. In fact, the disinfecting power of these elements coincides with their chemical affinities.

The inhalation of volatilized iodine is of great value as a therapeutic agent in pulmonary phthisis, for it cleanses the throat, larynx, trachea, and the large bronchi.

(5) *Iodoform Vapour.*—In a paper read before the Royal Academy of Sciences of Amsterdam on May 25,

1889, Dr. Forster stated the tubercle-bacilli and cholera-bacilli do not develop under the influence of iodoform vapour.

(6) *Carbonic anhydride.*—The investigations of Frankland (*loc. cit.*) have shown that carbonic anhydride destroys the vitality of Koch's bacillus and Finkler's spirillum. It arrested the growth of *Bacillus pyocyaneus*, but on exposure to air growth recommenced.

(7) *Carbon Monoxide and Nitrous Oxide.*—Frankland proved that both of these gases destroy the vitality of microbes.

(8) *Sulphurous anhydride.*—MM. Dubief and Bruhl (*Comptes Rendus*, 1889), in their researches on the disinfection of hospitals, dwellings, etc., have shown that sulphurous anhydride has a most destructive effect on aërial microbes, especially when saturated by aqueous vapour; that it acts mainly on the germs of bacteria, and that when employed in the pure state for a prolonged period it may prove fatal to germs in the dry state.

P. F. Frankland (*loc. cit.*) has proved that sulphurous anhydride destroys *Bacillus pyocyaneus*, Koch's bacillus, and *Spirillum Finkleri*.

According to the researches of Baumann, a pupil of Liebig in the Apotheke Verein, sulphurous anhydride destroys *Bacillus tuberculosis*, and he advises persons suffering from bacillary phthisis to live in rooms where one or two drachms of sulphur are ignited on a hot stove. The first ten days bring increased cough and irritation, then these cease and the patients improve rapidly. Baumann has cured several patients in the early stages of phthisis by means of sulphurous anhydride obtained from burning sulphur.

From the above investigations, sulphurous anhydride

is detrimental to the growth and development of microbes.

(9) *Ozone.*—The author has proved, in the following manner, that this gas is a germicidal agent of great power :

A Siemens' induction tube for ozonizing oxygen was taken, and the terminal portion of the tube was placed in the upper part of a cotton-wool-plugged tube containing a pure cultivation of the microbe under investigation. The ozone produced by the apparatus was allowed to enter the culture tube, and remained in contact with the microbes for thirty minutes. After this several tubes containing sterilized media (suitable for the growth of the microbe under investigation) were inoculated by the ' ozonized ' tube. By this device one is capable of studying the effect of ozone (almost pure) on various microbes. The following microbes were completely destroyed by this gas :

Bacillus subtilis.
Bacterium allii.
Sarcina lutea.
Micrococcus indicus.
Bacillus butyricus.
Micrococcus rosaceus.
Micrococcus aurantiacus.

By a modification of the above device, plate-cultivations, microbes growing on solid media in small capsules, etc., may be submitted to the action of the gas in question. If a plate-cultivation is placed under a small bell-glass, and the terminal portion of a Siemens' ozonizing tube fitted into the neck of the bell-glass by means of a previously sterilized cotton-wool plug, the action of the ozone on the microbes may be easily studied.

By this device ozone proved fatal to the following microbes :

Bacillus tuberculosis.
Micrococcus chlorinus.
Micrococcus violaceus.
Micrococcus prodigiosus.
Spirillum Finkleri.
Bacillus cyanogenus.

From the investigations, it appears that ozone has a powerful effect on microbes. It may be due to this fact that the air at sea (containing an appreciable amount of ozone) is almost free from microbes

CHAPTER IX.

IMMUNITY AND VACCINATION.

In the last chapter we considered the action of certain physical and chemical phenomena on microbes; in the present chapter another phenomenon which acts upon certain pathogenic microbes comes under consideration, viz., the phenomenon of immunity.*

Immunity is the condition of being insusceptible to an infective disease. It is well known that if a man or animal is attacked by some (although not all) infective diseases (*e.g.*, small-pox, yellow fever, splenic fever, etc.), he acquires a new property—namely, that of being thereafter, either for a longer or shorter period, or for the length of his entire life, inaccessible to the same diseases. We have seen that 'contagia are living things, which demand certain elements of life just as inexorably as trees, or wheat, or barley; and it is not difficult to see that a crop of a given parasite may so far use up a constituent existing in small quantities in the body, but essential to the growth of the parasite, as to render the body unfit for the production of a second crop. The *soil is exhausted*, and, until the lost constituent (or constituents) is restored, the body is protected from any further attack of the same disorder.'

If a particular microbe requires organic and inorganic

* Prof. E. Ray Lankester, F.R.S., proposes the word 'mithradatism' as a substitute for immunity (*Nature*, vol. xl., p. 149).

substances represented by *abcdefg*, and *c* or *cd* have been previously used up by the microbe, the soil is unsuited for the subsequent growth of the same microbe.

This is one of the theories which explains the phenomenon of immunity, and is known as the *theory of exhaustion*. Such a theory of non-recurrent diseases naturally presents itself to a thorough believer in the germ theory of disease. 'To exhaust a soil, however, a parasite less vigorous and destructive than the really virulent one may suffice; and if, after having by means of a feeble organism exhausted the soil without fatal result, the most highly virulent parasite be introduced into the system, it will prove powerless. This, in the language of the germ theory, is the whole secret of *vaccination*.' The attenuated virus constitutes a sure protection against the virulent virus. 'It so exhausts the soil that the really fatal contagium fails to find there the elements necessary to its reproduction and multiplication.'

The *theory of exhaustion*, however, does not explain all the facts concerning certain infective diseases, and from his important researches on anthrax and swine-plague, Dr. Klein says that 'there is no reason whatever for assuming that, if after one attack of illness the blood and tissues become an unfavourable soil for a second invasion of the same organism, this should be due to the exhaustion of some necessary chemical compound.'

If the *theory of exhaustion* only partially explains (in certain cases) the phenomenon of immunity, Kleb's *antidote theory* explains it far more fully.

According to the latter theory, it is supposed that the microbes produce directly or indirectly some substance which is poisonous to the same microbes if they enter the body on a subsequent occasion. There is a certain amount of direct evidence which materially substantiates the *antidote theory*. Dr. Raulin, for example, has shown

that *Aspergillus niger* produces, directly or indirectly, a substance which is prejudicial to its own growth 'in the absence of iron salts in the nutrient soil.'

The poisonous substances produced, according to this theory, may be ferments, or alkaloids, or other substances not yet isolated.

Immunity may be divided into two classes—natural or acquired; and acquired immunity may be subdivided into immunity acquired by acclimatization, and immunity acquired by protective inoculation.

It is well known that certain pathogenic microbes do not produce disease in some animals, whereas the same microbes produce disease in other animals.

Bacillus anthracis is innocuous to cats, dogs, pigs, and other animals, but to sheep, man, etc., it is a pathogenic microbe of great power.

Bacillus cuniculicida is innocuous to guinea-pigs and white rats; but rabbits, mice, and birds are very susceptible to the attacks of this microbe.

Bacillus of septicæmia is pathogenic in house-mice, but field-mice have an immunity.

Where an animal is insusceptible to an infective disease, that animal is said to have a natural immunity.

In certain cases, if an animal has had an infective disease, it is not liable to have a second attack of the same disease—such a condition therefore comes under the head of acquired immunity.

'Immunity may be acquired by acclimatization, for the inhabitants of tropical climates are less susceptible to the diseases of the country—malarial fevers, for instance—than strangers.'

The immunity acquired by vaccination or protective inoculation plays an important part in the well-being of all civilized communities. Vaccination or protective inoculation is nothing more than the introduction into

the system of an attenuated virus, or a virus of a milder or weaker form than the one which produces the disease with all its characteristic symptoms.

The general problem, of which Jenner's discovery was a particular case (viz., small-pox), has been grasped by Pasteur in a manner and with results which a few years ago were simply unimaginable. Therefore it is to Pasteur* that science and medicine are indebted for the generalization of the Jennerian method, and for an explanation which bids fair to render possible the protective treatment of many infectious diseases.

Although protective inoculation is useful in certain infectious diseases, in others (according to the present state of science) it is impotent in protecting man and animals. Among the diseases where vaccination is either protective or non-protective may be mentioned the following :

Vaccination is Protective.	Vaccination is Non-Protective.
Small-pox, or variola.	Tuberculosis.
Yellow fever.	Pneumonia.†
Anthrax, or splenic fever.	Gonorrhœa.
Fowl cholera.	Syphilis.
Hydrophobia, or rabies.	Erysipelas.
Swine fever.	Malarial fevers.
Cattle plague.	Dengue.
Diphtheria. (?)	Relapsing fever.
Acute septicæmia.	

According to Cornil, in erysipelas, pneumonia, and gonorrhœa a first attack is so far from warding off a second attack of the disease that it creates a favourable field for relapses.

* Pasteur has formulated the following law : 'When an animal has been inoculated with a diluted poison, and the organism has overcome it by its resistance, an inoculation with the condensed poison produces but insignificant effects.'

† See *Report of Departmental Committee on Pleuro-Pneumonia*, 1888.

It may consequently be assumed, *a priori*, that protective inoculation in such cases would do more harm than good. It is the same with tuberculosis, syphilis, etc., all diseases by which the same individual may be attacked several times, and at varying intervals of time —a clear proof that the first attack has created no *immunity* against subsequent attacks.

Dr. Germain Sée has proved that the ordinary methods of vaccination are incapable of inoculating tuberculosis; and further proof has been obtained by using the blood-serum of a cat dead of tuberculosis as a nutrient medium for the growth of *Bacillus tuberculosis*. This medium did not destroy or hinder the growth of the bacilli.*

It has been proved that *Bacillus tuberculosis* does not produce an alkaloid,† although it manufactures cellulose from albuminoid substances; but it has not been proved whether *B. tuberculosis* secretes an enzyme or not. Therefore it may be possible that in sterilizing the serum (in the above case) the heat destroyed any ferments, if present, in the blood.

Be this as it may, it does not alter the fact that tuberculosis is a disease in which immunity plays no part.

Protective inoculation is of the utmost value in certain infectious diseases, as the antiseptic treatment of other diseases is also of equal value.

Concerning protective inoculation, Dr. Domingos Freire (*Comptes Rendus*, 1889) has recently published certain statistics showing the value of vaccination against yellow fever. From 1883 to 1889 there were 10,524 persons inoculated in Brazil, and the mortality was 0·4 per cent. The deaths through non-vaccination during the fever

* MM. Charrin and Roger (*Comptes Rendus*, 1889) have experimentally proved that the serum of vaccinated and diseased animals (in the majority of cases) is adverse to the growth of microbes.

† Udránszky and Baumann (*Zeit. Physiol. Chem.*, vol. xiii., p. 562).

epidemics were over 6,500. Dr. Freire's method of prophylactic vaccination against yellow fever is much the same as the one employed for the vaccination of cattle against anthrax.

Since Pasteur discovered the method of protective inoculation against anthrax, no fewer than 1,700,000 sheep and about 90,000 oxen have been inoculated, and in 1888 269,599 sheep and 34,464 oxen were treated. The mortality, which before the introduction of the protective treatment was in the case of sheep 10 per cent., was, after the adoption of the method, reduced to less than 1 per cent. In France sheep and oxen are now always vaccinated against anthrax.

In regard to rabies, vaccination against the disease is an accomplished fact. At the Pasteur Institute in Paris, during 1887, no less than 1,770 patients (who had been bitten by rabid dogs and wolves) were treated by the Pastorian system of inoculation, and the mortality was 0·73 per cent. In 1888 1,622 patients were treated, and the mortality was 0·55 per cent.

The practical value of vaccination or protective inoculation against small-pox, anthrax, rabies, and yellow fever is well illustrated by the following table:

	Deaths before Vaccination (B).	Deaths after Vaccination (A).	Absolute Preservative Power of Vaccination (*i.e.* B÷A).
Small-pox (deaths in 1000)	500	23	21·73
Anthrax „ „	120	5	24·00
Rabies „ „	160	7	22·85
Yellow fever „ „	100	10	10·00

From the recent investigations of Drs. Roux and Yersin (*Annales de l'Institut Pasteur*, 1888), it appears possible that the method of protective inoculation may also be applied to diphtheria.

The Pastorian methods of protective inoculation against anthrax and rabies (especially the latter) have been objected to by numberless writers, and it has been ironically stated that 'the majority of people treated at the Pasteur Institute had not been bitten by mad animals at all (!). Never had anyone dreamt of the existence of so many victims of rabid dogs. Never, too, had anyone suspected the existence of such a large number of atrophic bone affections in the course of locomotor ataxy before Charcot first pointed them out; and people still remember that the same objection was urged against Sir Spencer Wells, Mr. Lawson Tait, Dr. Keith, and others; and yet it must have been very difficult for them to invent ovarian tumours. They simply were the right men, able to treat the affection successfully, and the patients took care to find them out. So also M. Pasteur for rabies.'

Rabies is no longer the much-dreaded disease of days gone by, for the unhappy sufferer is now snapped from a certain and horrible death by the method of inoculation invented by Pasteur.

In speaking of the value of Pasteur's discoveries, Professor Huxley says: 'They fully balance the ransom of £200,000,000 paid by France to Germany after the war of 1870-71.'

But perhaps the most important fact which proves the value of the Pastorian method of inoculation is that 'Pasteur Institutes' are now established in Odessa, St. Petersburg, Rio Janeiro, Moscow, Buenos Ayres, Constantinople, Palermo, Naples, Havannah, and other places.

What is the virus of rabies? is it an alkaloid, a ferment, or a microbe? If the saliva of a rabid dog is diluted with a small quantity of sterilized water (distilled), and then heated to 90° C. for a few hours, the saliva loses its virulent power. This proves that no alkaloid was present, because it would not have been destroyed on the application of heat.

Dr. Gamaleia, of Odessa, believes that the virus of rabies is not a dead chemical substance, but actually the living but weakened germs. As to the mode of action of the Pastorian inoculations, it is believed that 'the amœboid white blood-corpuscles absorb and digest those live germs, and their power of absorption for germs is trained and increased by the progressively stronger inoculations, so that finally the virus deposited by the rabid animal can also be absorbed and destroyed. The whole process is carried out, therefore, in the lymphatic system.'

'This theory of *acquired immunity* against virulent diseases by destruction of the infecting germs by *phagocytes* was first put forward by Professor Metschnikoff, of St. Petersburg' (*Fortschrift der Medicin*, 1885).

The next points for consideration before closing the chapter are the methods which may be employed for obtaining various attenuated viruses suitable for protective inoculation. These methods may be enumerated as follows:

(1) By successive cultures.
(2) By the action of heat.
(3) By the action of chemicals.
(4) By the passage of a virus through various animals.
(5) By the action of dry air (desiccation).

(1) *By successive cultures.*—Pasteur used this method

in obtaining an attenuated virus suitable for protective inoculation in fowl cholera. The microbe of this disease was passed from culture to culture (in fresh media), a sufficient number of times to render it impossible that the least trace of the virulent matter from which it originally started should still exist in the last cultivation. By this means a virus or an enfeebled microbe was obtained which produced only a slight disorder, but rendered the animal proof against subsequent attacks.

In this case, Pasteur believes that it is the oxygen of the air which attenuates the virus, and gives a certain amount of experimental proof in support of this idea.

(2) *By the action of heat.*—By keeping cultivations of *Bacillus anthracis* at about 43° C., Chauveau (*Comptes Rendus*, vol. xcvi.) found its virulence disappeared in twenty-four hours. After exposing anthrax virus to 47° C. for three hours, it is suitable for inoculation, and renders animals proof against subsequent attacks.

(3) *By the action of chemicals.*—Drs. Chamberland and Roux (*Comptes Rendus*, vol. xcvi.) have shown that it is possible to obtain an attenuated virus of anthrax by the action of small quantities of carbolic acid and potassium bichromate on pure cultivations of the microbe.

There is no doubt that many of the germicidal agents when in very dilute solutions have the property of reducing the virulent power of pathogenic microbes, and possibly the thus enfeebled microbes might be used for protective inoculations.

(4) *By the passage of a virus through various animals.*—Pasteur found that by passing the virus of rabies from the dog to the monkey, and subsequently from monkey to monkey, the virus grows weaker at each passage.

'The small number of passages from monkey to monkey suffice to bring down the attenuation to a point at which the virus injected hypodermically in dogs never gives rise to rabies in them. Intracranial inoculation itself, the never-failing means of communicating rabies, may now remain without effect, whilst, however, creating a refractory state in favour of the inoculated animal.

'Successive passages from rabbit to rabbit, and from guinea-pig to guinea-pig, increase the virulence of rabies virus. The exalted virulence comes to a fixed maximum in the rabbit. If now transferred to the dog it remains exalted, and shows itself to be much more intensely virulent than the virus of ordinary street rabies. So great is this acquired virulence, that the new virus injected into the blood-system of a dog unfailingly gives rise to mortal madness.

'A logical application of the results just indicated gives us the means of easily rendering dogs refractory to rabies, for we can prepare and keep at our disposal *a set of attenuated viruses of different strength*, some, not mortal, preserving the animal economy against the ill effects of more active ones, and these latter against the effects of mortal ones.'

In 1883 Pasteur and Thuillier (*Bulletin de l'Académie de Médecine*, 1883) used the same principle for attenuating the virus of swine erysipelas. The virus passed through rabbits gives immunity to pigs.

(5) *By the action of dry air.*—The method now in use at the Pasteur Institute for the attenuation of rabies virus consists in suspending portions (a few centimètres in length) of the spinal cords of inoculated rabbits in a dry atmosphere (*i.e.*, the marrows are desiccated in bottles of one litre capacity by means of caustic potash). By this method the virulent power generally diminishes,

and finally disappears. By using attenuated viruses of varying intensities (prepared by this method), Pasteur has successfully treated numberless animals and human beings which are now refractory to rabies.

Pasteur has shown that if the rabid marrow (rabbit's) be put, while still moist, into an atmosphere of carbonic anhydride (devoid of microbes) its virulence can be preserved intact for several months. The rabid marrow may also be preserved in pure and neutral glycerine at 30° C. (Roux).

In concluding the chapter, we may say that 'there are not wanting objectors to the protective inoculation of animals and man. They will do good by opposition, if it be founded upon truth and experiment, particularly on animals. Probably the stamping out of infectious diseases by isolation of cases and germs might be preferable to *general* inoculation. But antidotes—true medicines—are wanted for most of the virulent diseases, and it is in their discovery that the *chemical method* of investigating disease will, in the future, meet with its greatest successes' (Thudichum).

CHAPTER X.

GERMICIDES AND ANTIPARASITIC THERAPEUTICS.

In the present chapter we intend to discuss various *chemical agents* which destroy or hinder the growth and multiplication of pathogenic and other microbes.

The substances which destroy the vitality of microbes may be called antiparasitics, necrophytes, germicides and disinfectants; while those which simply retard or hinder the growth of microbes are generally spoken of as antiseptics.

It must be borne in mind that this is only a conventional classification or division, for a germicide may become an antiseptic by simply reducing its strength; and, conversely, an antiseptic (as a general rule) may become a germicide by increasing its strength. For example: carbolic acid is a germicide, but in *weak* solutions it has only antiseptic properties, and in that state MM. Chamberland and Roux have used it to obtain an attenuated virus of splenic fever.

It has been known for some years that certain *chemical agents* have the power of destroying the vitality of microbes, and there is no doubt that the application of these facts to medicine would be of the utmost importance.

The physician cannot dispense with the chemist—as subsequent work (especially on the subject of alkaloids,

germicides, etc.) requires the chemist's modes of investigation.

Speaking of the importance of CHEMISTRY as applied to pathology, Sir W. Aitken, F.R.S., says: 'The influence which chemistry has exerted on the science of pathology during the past fifty years cannot be overrated, and points to the conclusion that the microscopist must give way, and share or divide his work with the chemist. The processes of chemistry are now far in advance of microscopical revelations, so that henceforth the results of microscopic work, especially as regards micro-organisms, must be more controlled or checked by the chemist than they have hitherto been. Hence it is by *chemical* combined with biological and bacteriological methods, that we must look for the discovery of the many factors in the causation of diseases, and for the power of preventing or removing diseases.'

Bearing in mind that many chemical substances entirely destroy pathogenic microbes, it would be invaluable to the physician if he were able to apply successfully (say by hypodermic injections) certain germicidal agents in cases of infectious diseases.

Certainly, progress must be reported in this particular line of research, for a considerable amount of work has already been accomplished.

The object of these researches is to find some germicidal agent or agents capable of destroying the microbes of disease, which have been proved to reside in the blood, and are the cause (directly or indirectly) of certain infectious diseases. At the same time an aqueous solution of such an agent, while destroying the microbes of disease, must have very little or no detrimental action upon the blood and tissues. Having discovered a substance or substances, the rationale (in the majority of

cases) is to hypodermically inject a solution of the microbe-destroyer directly into the blood. By so doing, the destruction of the pathogenic microbes *in situ* would be the result, the disease would be at end, and Nature would then have a chance of restoring to its 'normal standard the lowered vitality which enabled the microbes to get a footing.'

If infectious diseases are caused by poisonous alkaloids and special ferments manufactured by microbes, the use of necrophytic or germicidal medicines would destroy the latter and thereby prevent further accumulation of the former in the system.

If the special ferments produced by living pathogenic microbes are the real cause of disease, the hypodermic injection method steps in, for many substances are known to interfere with the action of special ferments.* The destruction of the microbes prevents the formation of special ferments or alkaloids; and any given infectious diseases (under these circumstances) would be at an end.

Before any chemical substance may be said to be a germicidal agent, certain conditions must be fulfilled.

Dr. E. Klein, F.R.S., says†: 'In order to pronounce a certain substance a germicide in the strict sense of the word, it is necessary to place the organisms in this substance for a definite time, then to remove them thence, and to place them in a suitable nourishing medium; if they then refuse to grow, the conclusion is justified that the exposure has injured or destroyed the life of the organisms. In the case of pathogenic organisms, a

* Dumas in *Comptes Rendus*, vol. lxxv., p. 295; Bouchardat in *Annales de Chimie et de Physique* (3rd series), vol. xiv., p. 61; Griffiths in *Proceedings, Royal Society of Edinburgh*, vol. xiii., p. 527, vol. xiv., p. 97, and vol. xv., p. 33.

† *Micro-Organisms and Disease*, p. 259.

substance, to be pronounced a germicide, must be shown to have this power, that when the organism is exposed to the substance, and then introduced into a suitable artificial medium, it refuses to grow; and it must also be shown that when introduced into a suitable animal it is incapable of producing the disease which the same organism, unexposed to the substance in question, does produce.'

If any substance answers these conditions (the canons of Klein), we may pronounce it a germicidal agent; and such an agent may be of the greatest therapeutic value.

The science of antiparasitic therapeutics is certainly in its infancy, but it has undoubtedly a great future before it; and it behoves the physician, chemist, and bacteriologist to study and investigate with the utmost zeal the action of various chemical substances on pathogenic and other microbes. By so doing medicine will be raised from empiricism (more or less) to the level of an exact science.

At this point we consider in detail the various necrophytic and antiseptic agents.

Mercuric Salts.

(1) *Mercuric chloride.* — Dr. R. Koch, in his paper, 'Ueber Desinfection' (*Mittheilungen aus dem K. Gesundheitsamte*, vol. i. [1881], p. 234), gives the result of an important and extensive series of experiments with mercuric chloride, and comes to the conclusion 'that a single application of a very dilute solution (1 to 1,000, or even, to 5,000) is sufficient to destroy the most resistant organism in a few minutes.' He further states that, 'with longer exposure, it only begins to be unreliable when diluted beyond 1 to 20,000.'

Dr. Klein has shown that mercuric chloride (0·1 per

cent.) solution does not destroy the *spores* of *Bacillus anthracis*, even when the latter are exposed to the action of the chloride for several hours, for animals inoculated with these bacilli died of typical anthrax and the microbes were found in the blood. Klein has found that this substance (1 to 10,000) kills the bacilli after an exposure for thirty minutes; he, however, agrees with Koch that mercuric chloride is an efficient germicide, but maintains that the German bacteriologist has overrated its necrophytic properties.

Dr. Sims Woodhead, F.R.S.E. (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 246), who has recently published some important investigations on the necrophytic properties of certain mercuric salts, says: 'It appears to be possible that Klein's lower results, as compared with those of Koch, in the use of bichloride of mercury as an antiseptic, may have some such explanation as the following:

'The peptones, like other albuminoids, are coagulable by bichloride of mercury; hence a large proportion of the salt may be rendered completely inactive. It may be pointed out that, in Klein's experiments, a single drop of the fluid in which the micro-organisms had been cultivated was drawn into a pipette, and then 100 (or these proportions) of the sublimate solution. It will be evident that, under these conditions, all the albumin in the cultivation medium would be coagulated immediately, and would so remain, for there is an excess of the mercury salt, not of the albumin. In such a case it is quite possible that there is actually *a coating or pellicle of albuminate of mercury formed at an early stage around the spores or micro-organisms* which, protecting them against the action of the added sublimate solution, is only dissolved when the organisms with their pellicles

are again introduced into a nutrient fluid in which there is sufficient albumin to form an excess, and so to dissolve the pellicle and set the organism free to flourish in its new surroundings.'

Koch found that a solution of mercuric chloride (1 to 1,000) kills the spores of *Bacillus anthracis* in ten minutes.

Dr. P. Miquel (*Semaine Médicale*, 1883) proved that five centigrammes of mercuric chloride per litre of bouillon prevents putrefaction.

As already stated, Koch has found that mercuric chloride solution only begins to be *unreliable* as a germicide when diluted beyond 1 to 20,000. In fact, H. Schulz (Pflüger's *Archiv*, vol. xlii., p. 517) has recently shown that a solution of mercuric chloride when diluted to about 1 to 500,000 increases the activity of *Torula cerevisiæ*.

(2) *Mercuric iodide*.—There are several objections to mercuric chloride as a necrophytic agent—among these may be mentioned that it combines with albumin (of the cultivating media, blood, etc.), forming mercuric albuminate, which 'greatly detracts from its potency' as a germicide.

Dr. Sims Woodhead (*loc. cit.*, p. 237) has found that mercuric iodide dissolved in potassium iodide is a valuable germicide, and has many advantages over the chloride of that metal. He has arrived at the following conclusions:

(*a*) Mercuric-potassic iodide 'is not so poisonous (as the chloride), hence the risks of poisoning by absorption are not so great.'

(*b*) 'It does not form an albuminate, consequently the whole of the salt is available as an antiseptic.'

(*c*) 'It may be used with either acids (especially

vegetable acids) or alkalies, neither of which appear to interfere immediately with its antiseptic properties.'

(*d*) 'It is not necessary that the solution should be made with distilled water; all that is necessary is a slight excess of iodide of potassium.'

(*e*) 'The mercury from this solution is not deposited on the surface of the skin, or on instruments, or the deposit is exceedingly slight, so slight, indeed, that it will not injure the most delicate instrument.'

(*f*) 'The exact strength of the solution is always known, as its properties remain constant.'

The strength of the solution used in these experiments was '1 gramme of mercuric iodide with a slight excess of potassium iodide in 1,000 cc. of distilled water.'

Although insoluble albuminates are formed when mercuric chloride is used, it must be borne in mind that a small quantity of sodium chloride (common salt) and other alkaline halogen compounds, as well as acids, dissolve the albuminates.

From the above, Sims Woodhead comes to the conclusion 'that all the mercuric salts which can be kept stable and in solution have powerful antiseptic and germicidal properties, varying (*a*) according to the quantity of mercury they contain, and (*b*) according to the acid or halogen with which they combine.'

Dr. A. Edington (*British Medical Journal*, 1889), after having performed a large number of experiments with the utmost care, and under the new conditions as indicated in Woodhead's paper, has come to the conclusion that the maximum germicidal action of mercuric chloride 'lies very nearly at 1 in 4,800, while the minimum is 1 in 10,000.' He has found that mercuric chloride dissolved in water (rendered acid) in the proportion of 1 part in 1,000 destroys the spores of *Bacillus anthracis* in

fifteen minutes, for the spores after this treatment, and subsequent washing in sterilized water, refused to grow on nutrient agar-agar. *Bacillus subtilis* (also a spore-bearing microbe), *Bacterium innominatum*, *Staphylococcus pydogenes aureus*, and *Sarcina lutea*, were all destroyed by the same reagent.

The author has found that *Micrococcus tetragonus*, *Micrococcus prodigiosus*, *Bacterium allii*, and *Micrococcus violaceus*, are all destroyed by one-tenth per cent. solution of mercuric chloride containing a small quantity of sodium chloride.

The *bacilli of syphilis* which were found by Lustgarten,* De Giacomi,† Doutrelspont and Schütz‡ in various syphilitic products are destroyed by an acid solution of mercuric chloride. This is an important fact, bearing in mind that subcutaneous injections of mercuric chloride are of great value in the treatment of syphilis.§

Although mercuric chloride is said to destroy *Bacillus tuberculosis* (which is doubtful),‖ it is not the necrophytic medicine to be employed in pulmonary phthisis. Dr. Hiller tried the parenchymatous injection of this germicidal agent, 'the dose being two centigrammes in solution. In all three cases he was obliged to suppress the treatment. This want of success in the treatment of phthisis by mercurials is perhaps surprising, if we take into account the marvellous results obtained from mercuric chloride in surgery, midwifery, and lately in ophthalmic practice, as a preventive and curative agent in the ophthalmia of new-born children.'

* *Med. Jahrb. der k. k. Gesellschaft d. Aerzte*, 1885.
† *Correspondenzblatt für Schweizer Aerzte*, 1885.
‡ *Deut. Med. Woch.*, 1885.
§ See *The Treatment of Syphilis with Subcutaneous Sublimate Injections*, by Dr. Lewin, of Berlin (English edition).
‖ See Dr. Williams's paper in *Proceedings Royal Society*, 1884.

Iodine and its Compounds.

(1) *Iodine.*—Davaine was the first experimenter who proved the germicidal power of iodine.* He ascertained that seven milligrammes of iodine suffice to kill anthrax-bacilli in 1,000 cc. of the liquid; and he says that 'if we take blood from a carbuncled fowl, and dilute it to 1,000, or even 10,000, and then place in contact with it, during fifty or sixty minutes, a very weak solution of iodine, the guinea-pigs that are inoculated with from two to four drops of this mixture continue to live, whilst other animals inoculated with similarly diluted blood, without iodine, infallibly succumb.'

Certain French experiments have proved the curative properties of the following solution of iodine in cases of anthrax: 25 centigrammes of iodine, 50 centigrammes of potassium iodide dissolved in 1,000 grammes of distilled water. This mixture when injected into the œdematous skin of patients suffering from anthrax proved to be a curative agent.

In regard to *Bacillus tuberculosis*, Dr. C. T. Williams (Physician to the Brompton Hospital for Consumption) has shown that iodine (1 part in 12 of water) greatly reduced the number of bacilli, and prevented spore-formation (*Proc. Roy. Soc.*, 1884).

It has been shown that 'we can inject with impunity into the blood of a dog, for each kilogramme of body weight, 2 to 3 centigrammes of free iodine, dissolved with twice the quantity of sodium iodide. This would be for a man of ordinary weight (70 kilogrammes) a quantity of from 1·4 to 2·1 grammes.

* 1 milligramme of iodine in 100 cc. of nutrient broth destroyed the vitality of *Sarcina lutea* (Griffiths in *Proc. Roy. Soc. Edinburgh*, vol. xv., p. 37).

Beyond these limits iodine is poisonous, and causes death within twenty-four hours.

The inhalation of volatilized iodine, which sometimes causes tracheal irritation and troublesome cough, is nevertheless a valuable necrophytic medicine in cases of pulmonary phthisis.

In a particular case of pulmonary phthisis (see *Proc. Roy. Soc. Edinburgh*, vol. xv., p. 54), in which the author was interested, it was proved that the inhalation of iodine vapour was of great therapeutic value. Although the iodine may not reach very deeply into the lungs, it cleanses the throat, larynx, trachea, and the large bronchi.

Then, again, it has been stated that in all *asthmatic* forms of dyspnœa iodine exercises a double action—that is, as a modifier of bronchial secretions, and as a respiratory medicament. The mucosities become more abundant, but also less consistent, so that the obstructed respiratory tubes become more permeable to inspired air. The only inconvenience which iodine is likely to give rise to is hæmoptysis.

After absorption, iodine is eliminated by the mucous membranes, and is consequently of great value in the treatment of lung diseases.

After an injection of iodine, it is readily found in the urine, sweat, tears, saliva, sputum, milk, etc.

(2) *Potassium iodide.*—Dr. Fournier recommends in cases of syphilis from 4 to 8 grammes of this salt daily. And about 1·5 grammes of the same salt has been prescribed for phthisis, unless there is hæmoptysis, when it should not be used.

Potassium iodide is very rapidly absorbed, and penetrates with great facility into all the tissues. Its action (like that of iodine) is of an oxidizing nature; and there-

fore both potassium iodide and iodine possess denutritive properties, 'which is shown by the atrophy of a large number of morbid products, tissues, and healthy glands;' but these substances aid general nutrition—possibly iodine augments the appetite and digestion.

(3) *Sodium iodide.*—Sodium iodide is a far safer salt than potassium iodide, although the latter salt, when used in small doses, has no detrimental action in the least.

It may be well, at this point, to remind the reader that Grandeau and others (*Archiv für Physiologie*, vol. ii., p. 49) have shown that when *potassium* salts are injected into the blood, they exert a paralyzing action on the heart and striated muscles; but this is only when they have been used in large doses.

It may be asked, What is the dose of sodium iodide which can be used with safety? It must be borne in mind that sodium iodide (pure) contains 84 per cent. of iodine, while potassium iodide (pure) contains only 76 per cent. Although sodium iodide contains a larger amount of iodine, its diffusive power is far less than the potassium salt; consequently it is necessary to use a large dose of sodium iodide. According to the researches of Bochefontaine, the dose of the sodium salt should be double that of the potassium salt.

(4) *Ethyl iodide.*—When pure, this liquid contains 81 per cent. of iodine, and it has proved to be a useful therapeutic agent in the treatment of phthisis, as well as in asthma. The vapour of ten to fifteen drops of ethyl iodide should be respired several times a day. It appears to penetrate throughout the respiratory tracts, and is certainly absorbed into the system. The author has detected iodine in the urine and saliva after the respiration of ethyl iodide.

It possesses germicidal properties, and readily destroys *Bacillus tuberculosis*.

(5) *Potassium iodate.*—This salt contains 59 per cent. of iodine, and is a powerful germicidal agent.

The author (*Proc. Roy. Soc. Edinburgh*,* vol. xv., p. 37) has experimentally proved that a solution containing 0·5 per cent. of potassium iodate destroys several microbes. Among these experiments may be mentioned the following:

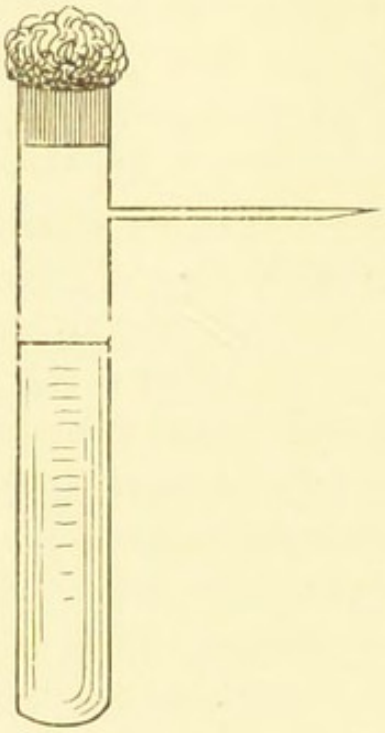


FIG. 25.

Several Aitken's tubes (Fig. 25) containing sterilized beef-broth (neutral) were treated as follows: Tube No. 1 was inoculated with the chromogenic saprophyte *Sarcina lutea* (from a pure cultivation in nutrient agar-agar), and kept at a temperature of 40° C. The microbe grew rapidly, and after four days formed a yellow pellicle upon the surface of the broth. Tubes

* Also a paper read before the Royal Society of Edinburgh on March 18th, 1889 (*P. R. S. E.*, vol. xvii., p. 257).

Nos. 2 and 3 each contained, in addition to the sterilized beef-broth, potassium iodate (0·5 per cent.). These tubes were inoculated with *Sarcina lutea* from the same source as tube No. 1. No growths made their appearance after the lapse of twenty-eight days, although the tubes were kept at the most favourable temperature for the development of this microbe. After twenty-eight days' incubation, sterilized platinum needles were carefully dipped into tubes Nos. 2 and 3, and the contents of four tubes containing sterilized nutrient agar-agar were inoculated with them. The four inoculated tubes remained in the incubator at 40° C. for twenty-one days without any growths making their appearance.

In a similar manner experiments were performed upon *Micrococcus prodigiosus*, *Micrococcus tetragonus*, *Bacterium allii*, and *Bacillus tuberculosis;* and all these microbes succumbed. There is no doubt that potassium iodate* is a powerful germicidal agent.

(6) *Iodoform.* — This compound, analogous in its chemical constitution to chloroform, contains nearly 97 per cent. of iodine. It is a yellow, crystallizable substance, nearly insoluble in water, but dissolves in alcohol and ether.

'In medicine it is employed in moderate doses, say 20 to 50 centigrammes daily, in the form of pills, and with the addition of a deodorant, as coumarine; in larger doses than 1·5 grammes daily, iodoform may become toxic.'

Iodoform has been successfully used in the treatment of phthisis, syphilis, scrofula, and malaria.

In 1878 Dr. Moleschott was the first who used the

* Dr. W. D. Spanton, F.R.C.S.E. (*Chemical News*, vol. lxi., p. 166), has proved that calcium iodate possesses remarkable germicidal properties.

germicidal agent in phthisis, and his observations were taken up by some of the most prominent of Italian physicians, and subsequently by Drs. Schnetzler and Drasche, of Vienna.

In Italy, iodoform has been used in the form of pills (4 to 7 centigrammes of iodoform with extract of gentian), or inhaled, or in the form of a liniment.

It is stated that iodoform is a curative agent in the early stages of phthisis; and is also useful in advanced cases, for it lessens the formation of sputum and the paroxysmal cough. It prevents caseation, and laryngeal pain and dysphagia entirely disappear. Iodoform, when inhaled, greatly modifies tuberculous laryngitis. Kowalski considers iodoform a valuable therapeutic, especially when it is in contact with tubercular surfaces.

Perhaps the best method of administering iodoform is in the form of pills.

Dr. Drasche, of Vienna, treated seventeen patients suffering from phthisis with iodoformic pills (containing 2 centigrammes of iodoform and extract of gentian). Eight of the patients treated rapidly increased in body-weight, and were ultimately cured.

On the other hand, Dr. Schnetzler obtained negative results with capsules, containing cod-liver oil and iodoform, and also with inhalations of alcoholic and ethereal solutions of iodoform.

M. Miquel has shown that iodoform (25 to 60 centigrammes per litre) sterilizes beef-broth; and recently Dr. Forster has proved that under the influence of iodoform vapour tubercle-bacilli and cholera-bacilli do not develop.

The researches of Marchand (*Archiv für Path. Anat. Berlin*, vol. xciii. [1883]) have proved that iodoform

prevents the formation of certain elements of tubercular cells (*e.g.* giant cells are not formed).

Various Halogen Elements and their Compounds.

(1) *Bromine.*—Dr. Hiller (*Deut. Med. Woch.*, vol. viii.) has shown that the vapour of bromine, when diluted with air, has little or no action on the bacilli of tuberculosis, and their number does not diminish in the sputa. In the undiluted state* bromine (even in small quantities) cannot be breathed with impunity. It irritates the mucous membranes and becomes toxic.

(2) *Ethyl bromide.*—Professor Giuseppe Sormani (*Atti dell' Istituto Lombardo*, 1887) has proved that ethyl bromide completely destroys *Bacillus tuberculosis;* but its general therapeutic effects have not been studied.

(3) *Chlorine.*—The same remarks apply to chlorine as already given under the head of bromine.

(4) *Sodium chloride.*—In a remarkable paper, read before the Royal Academy of Sciences of Amsterdam on May 25, 1889, Dr. Forster stated that sodium chloride (salt) destroys cholera-bacilli; but the typhoid and pyogenetic microbes, tubercle-bacilli, and the microbe of cattle-plague, may remain for months buried in common salt without losing their powers of growth and reproduction. The salting of butchers' meat may, therefore, in some cases prove ineffectual.†

In phthisical patients, sodium chloride (used medicinally) causes an increased elimination of urea and uric acid, and is apt to produce hæmoptysis (Voit).

* In the undiluted state both bromine vapour and chlorine are powerful germicides.

† See also Foster's paper read before the Royal Academy of Sciences (Amsterdam) on April 25, 1890.

Sulphur and its Compounds.

Sulphurous anhydride, the alkaline sulphides and hyposulphites, and sulphuretted hydrogen gas, are all powerful germicides (see Chapter VIII.).

Dr. Bergeon (*Comptes Rendus*, 1886, p. 176; and *British Medical Journal*, December 18, 1886) has used, with marked success, anal injections of sulphuretted hydrogen gas (diluted with CO_2) in the treatment of pulmonary phthisis. Full details will be given of Bergeon's method in a subsequent chapter.

The author (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 51) has proved that, in Bergeon's method, sulphuretted hydrogen gas is a destroyer of tubercle-bacilli, and is also 'curative of local lesions.'

M. Nièpce also recommends sulphuretted hydrogen gas in the treatment of bacillary phthisis. His treatment comprises the inhalation of the gas, 'sulphur baths,' and the internal use of the thermal sulphur waters. Nièpce says that 'tuberculosis may be modified or cured by the therapeutic agency of sulphuretted hydrogen.'

Bergeon's method is, however, preferable to that of Nièpce. In the former method the gas is absorbed into the venous system, and is speedily got rid of by the lungs; whereas in Nièpce's method the gas passes into the arterial system, and only small quantities of this gas can be inhaled without toxic effects.

It was the illustrious Claude Bernard who first demonstrated that certain gases were poisonous when they passed into the arterial system through pulmonary inhalation, but innocuous when merely present in the venous system by intestinal absorption.

Oxidizing Compounds, etc.

Under this heading are included: oxygenated water, potassium permanganate, ozone, and turpentine. All these substances have germicidal and antiseptic properties, due (directly or indirectly) to the liberation of nascent oxygen.

(1) *Ozone.*—The antiparasitic action of this gas has already been alluded to, namely, in Chapter VIII. Possibly this action is of an indirect nature, and may be due to the decomposition of ozone in contact with organic matter, and the liberation of oxygen in the nascent or atomic state.

The higher strata of the atmosphere contain a larger percentage of ozone than the lower; consequently it is freer from microbes, and this may account for the therapeutic value of the air of high altitudes (*e.g.*, the Swiss mountains for phthisical patients).

(2) *Oxygenated water.*—This substance (H_2O_2) has a similar action to ozone. It has a powerful action on most microbes.

(3) *Potassium permanganate.*—This substance, under the name of 'Condy's fluid,' is too well known to need description. It has an oxidizing action, but its antiseptic and disinfecting powers are inferior to many other well-known agents of a similar nature.

(4) *Turpentine oil.*—Air saturated with the vapours of this compound has germicidal properties, and forms a valuable therapeutic agent.

It destroys the vitality of *Bacillus subtilis*, *Sarcina lutea*, *Bacillus tuberculosis*, and other microbes.

The action of turpentine oil was formerly supposed to be due to the conversion of the atmospheric oxygen, which it readily absorbs, into ozone; but according to

Kingzett, the oxidizing compound is an organic peroxide, $C_{10}H_{14}O_4$, which, when heated with water or aqueous vapour, is resolved into hydrogen dioxide and camphoric acid :

$$C_{10}H_{14}O_4 + 2H_2O = H_2O_2 + C_{10}H_{16}O_4.$$

In addition to the above organic peroxide, hydrogen dioxide is the disinfecting or antiparasitic body, being easily decomposed with the liberation of nascent oxygen.

Moist air impregnated with turpentine vapours has been used by Dr. Skoda in the treatment of pulmonary gangrene ; and he states that this treatment destroys the putrefactive microbes. This treatment ' suppresses the effects of putrid mucus on the respiratory organs ; and, further, it slows the pulse and respiration.'

Moist air impregnated with turpentine vapours might possibly be beneficial in the treatment of pulmonary phthisis.

Oil of Mustard.

Oil of mustard, or allyl thiocarbimide, $N(CS)C_3H_5$ (and possibly other essential oils), possesses strongly marked germicidal properties.

Dr. Babès (*Virchow's Archiv*, 1885) has shown that oil of mustard is an excellent preservative from cholera. ' If a drop of this oil is put at the bottom of a bell-glass which covers a culture of Koch's comma-bacilli, it arrests their development and destroys them within forty-eight hours.'

Quinine.

The salts of quinine destroy certain non-pathogenic, as well as pathogenic microbes.

Dr. C. T. Williams (*Proc. Roy. Soc.*, 1884) has proved that solutions of quinine sulphate (2 grains to 10 grains in

an ounce of water) destroy the vitality of tubercle-bacilli, for 'the bacilli in the sputum after being mixed with the quinine salt could not be cultivated even in beef-broth.'

Quinine salts also destroy *Bacillus malariæ;* and quinine hydrochlorate has been recently recommended in combating malarial fever. It should be used in large doses—1 gramme or upwards—and dissolved in dilute alcohol.

Sailors arriving at unhealthy ports (such as that of Tandjong Priok, in Java) are recommended by eminent medical authorities to receive the first day each one gramme of quinine dissolved in gin, repeating the dose on the 8th, 12th and 16th days, and on the 10th and 14th days half a gramme.

It must be borne in mind that the ordinary doses of quinine are useless for combating the malaria of tropical climates. Dr. G. Schweinfurth,* whilst botanizing in the African swamps, took half a gramme three times daily; and Mr. H. M. Stanley† took doses of three and three and a half grammes of quinine.

Naphthols.

There are two naphthols ($C_{10}H_7OH$) which have germicidal properties in a marked degree, and are suitable as antiseptic medicines.

These isomeric compounds are the α-naphthol and the β-naphthol. Both compounds are only slightly soluble in water, but readily soluble in alcohol and ether. Although of the same empirical formula, they differ in physical as well as chemical properties. Among the former may be mentioned the following:

* *Artes Africanæ.*

† See also *In Darkest Africa*, vol. ii., p. 31.

	Crystalline Forms.	Melting Points.	Boiling Points.
α-Naphthol ...	Monoclinic prisms	94° C.	279° C.
β-Naphthol ...	Rhombic tables	122° C.	286° C.

M. Bouchard (*Comptes Rendus*, vol. cv., p. 702) proved recently that 0·33 gramme of β-naphthol in 1,000 cc. of various cultivating media prevents the development of several species of microbes, including those of:

Bacillus anthracis,
Bacterium pneumonicum agile,
Bacillus typhosus (weak cultivations),
Bacillus tuberculosis (retards the development),
Bacterium choleræ gallinarum.

It prevents the ammoniacal fermentation of urine (destroying *Micrococcus ureæ*), and the production of putrefaction by fœcal matter. Putrefying organic substances mixed with β-naphthol in the proportion of 0·2 gramme per 1,000 cc. cease to putrefy, and soon lose their fœtidity.

Experiments have proved that β-naphthol may be introduced into the stomach of a rabbit to the extent of 3·8 grammes per kilogramme of body-weight without producing death. The fatal dose of a man of 65 kilogrammes* would, therefore, be more than 250 grammes, and it is only slightly more poisonous when injected subcutaneously.

Dr. J. Maximovitch (*Comptes Rendus*, vol. cvi., p. 1441) confirms, and further extends, Bouchard's investigations. He has shown that α-naphthol in the pro-

* 143 lb. (over ten stone).

portion of 5 grammes per 1,000 cc. destroys most microbes growing in nutrient broths and on gelatine or agar-agar.

The author completely endorses the investigations of Bouchard and Maximovitch concerning the germicidal properties of the two isomeric naphthols; and he has found that 0·5 gramme of β-naphthol, and 0·3 gramme of α-naphthol, per litre of various cultivating media, destroy the following microbes:

Micrococcus tetragonus,
Micrococcus prodigiosus,
Bacterium allii,
Bacillus tuberculosis.

After the microbes had been in contact with the above reagents for several hours, a number of sterilized tubes containing different nutrient media were inoculated from each naphtholized tube, but no growths made their appearance in any of the tubes after an incubation which lasted from ten to twenty days.

Both naphthols are germicides of considerable power, and it has been proved that the β modification is capable of being injected into the system *without* toxic effects. There is little chance of the operator injecting a poisonous quantity, for β-naphthol is only soluble to the extent of 0·25 gramme in 1,000 cc. of water.* From 20 to 30 minims of the above solution may be hypodermically injected (at one time) into the system without any ill effects.

It is possible that these two isomeric naphthols would prove of great value in antiparasitic therapeutics,

* 1 litre of water alone dissolves 0·25 gramme of β-napthol.
,, ,, +0·1% alcohol dissolves 0·33 gramme of β-napthol.
,, ,, +5·0 ,, ,, 1·00 ,, ,,
,, ,, +20·0 ,, ,, 2·00 ,, ,,

especially for those diseases in which protective inoculation plays no part.

Hydronaphthol, manufactured by Messrs. Seabury and Johnson,* is a name given to a compound derived from β-naphthol. It is β-naphthol ($C_{10}H_7OH$) where one atom of hydrogen is replaced by a semi-molecule of hydroxyl. In fact, 'hydronaphthol' is the dihydroxy-naphthalene ($C_{10}H_6(OH)_2$, of the chemist; and is a derivative, like α- and β- naphthols, of naphthalene. It is non-poisonous, non-corrosive, and odourless; and 'hydronaphthol' is soluble in the proportion of 1 to 1,100 parts of cold water. This solution readily destroys *Bacillus subtilis*, *Micrococcus tetragonus*, *Micrococcus prodigiosus*, *Bacillus anthracis* and its spores, *Sarcina lutea*, and other microbes; for they cannot be cultivated after its action. The minimum *antiseptic* action of this important compound lies very nearly at 1 in 7,200, while the maximum lies between 1 in 2,500 and 1 in 3,000.

'Hydronaphthol' is of great value as an antiseptic agent in surgical operations, etc., as a saturated aqueous solution of it (1 in 1,100) may be used with impunity; and it has been reported that doses of 15 grains have been administered (internally) without any ill effects.

For further information concerning the germicidal and antiseptic properties of 'hydronaphthol,' the reader is referred to Dr. A. Edington's paper in the *British Medical Journal* of May 11, 1889.

Arsenical Compounds.

The germicidal properties of these compounds is still an open question; but their action upon the system has been carefully studied by several observers.

* 46, Jewin Street, London, E.C.

Arsenical compounds diminish the production of urea and carbonic acid; that is, they lessen oxidation, and have been consequently spoken of as 'medicines of economy.'

Arsenic acts as a depressor of arterial circulation; and according to Böhm and Unterberger 'the tension is often so much diminished that circulation is insufficient.' The use of arsenic and its compounds diminishes the red corpuscles of the blood, as the following analyses show:

	Before Treatment with Arsenic.		After Treatment with Arsenic.	
	I.	II.	I.	II.
Blood corpuscles ...	12·014%	12·210%	9·314%	9·425%
Hæmatin	0·412%	0·423%	0·246%	0·216%

And Delpeuch first proved that arsenic diminished the absorbing power of blood for oxygen.

(1) *Arsenious acid.*—'The therapeutic dose of this acid should not exceed 5 milligrammes; gradually and by exception it may be increased up to 1 centigramme.' It is always the best to administer arsenious acid in solution rather than in the form of granules.

To a certain extent this acid has anti-fermentative and antiparasitic properties; and Büchner says it has a preservative action against microbes, 'by fortifying the living tissues' and thus depriving the microbes of a nutrient soil.

According to the observations of Dr. Leyden, arsenious acid does not possess any anti-microbian property, 'and enjoys, at most, favourable action on nutrition.'

Dr. C. T. Williams (*Proceedings, Royal Society*, 1884) has shown that arsenious acid '*exercised no destructive*

influence on the bacilli' of phthisis; but Büchner* believed that he had discovered in arsenic a prophylactic against tuberculo-bacillary infection; that the administration of small doses of arsenious acid rendered the system proof against the attacks of tubercle-bacilli as well as other pathogenic microbes. He administered 2 cc. of an aqueous solution of arsenious acid (1 in 2,000) in six cases of pulmonary phthisis; and he concluded that this compound acts against the diffusion of the poison in the system, against hectic fever, and finally against the destruction of the lungs.

But the important researches of Dr. Stintzing have clearly shown that 'the effects of arsenic in phthisis may be considered as *nil*;' for 'in no case was the morbid process arrested.'

(2) *Sodium arsenite.*—Dr. Thilenius of Soden has used (daily) doses of 2 or 3 milligrammes of this compound in the treatment of phthisis, and has found that it produces an amelioration of the general condition, but it is not a curative agent. It has no action on the bacilli of phthisis, for Köbner, as well as others, obtained no advantage by using it in the form of hypodermic injections.

(3) *Potassium arsenite.*—A solution of this compound is known as 'Fowler's solution,' and contains 1 part of arsenious acid in 120 of water. The dose should not exceed 15 drops.

(4) *Arsenic acid.*—This acid and its salts (sodium and potassium arseniates) are less powerful than arsenious acid and the arsenites.

There is little doubt that arsenic and its compounds are only feeble antiseptic agents;† so much so that Dr.

* *Eine neue Theorie über Erzielung v. Immunität gegen Infectionskrankheiten* (1883).

† That is, in quantities suitable for medicinal purposes.

P. Miquel, of the Observatoire de Montsouris, found that he 'had to use from 6 to 9 grammes of these substances per litre' to preserve bouillon and other cultivating media from putrefaction. Therefore, if arsenical preparations are useful therapeutic agents in certain infectious diseases, their action is not of a necrophytic or antiparasitic nature.

Boric Acid.

To a certain extent, boric or boracic acid is a germicidal agent, and was recommended by Dr. Schech (*Deut. Med. Woch.*, 1884) in the treatment of laryngeal phthisis; but it has been proved by Dr. C. T. Williams* (of the Brompton Hospital for Consumption) that boric acid 'exercised no destructive influence on the bacilli' of phthisis. In fact, he says that 'they increased abundantly in solutions containing boric acid, and displayed many groups of 2, 5, 7, and 10. Rods in couples, arranged at an angle of 45°, were common. They were of fair length, and many contained spores. Multiplication by division was proceeding.' In concluding his paper, Dr. Williams says that 'the tubercle-bacillus is characterized by great durability of structure, as evidenced by its not being destroyed by the strong acids used in the various processes for its detection,† and by its little tendency to decomposition. It does not multiply in distilled water, but does so largely in beef solutions. Arsenic, boric acid, and mercuric chloride do not interfere with its development, but rather promote it.'

A warm saturated solution of boric acid was recommended by Pasteur as an antiseptic in cases of puerperal fever; but it has been superseded by mercuric chloride in the lying-in hospitals of Paris.

* *Proceedings, Royal Society*, 1884.

† See methods for staining this microbe, Chapter II.

Aromatic Compounds.

Many of the derivatives of benzene* and its homologues are powerful germicidal agents. Among these compounds may be mentioned the following :

(1) *Benzoic acid.* — An aqueous solution containing a quarter per cent. of this acid forms a germicidal agent for a large number of microbes ; and the above quantity of benzoic acid preserves bouillon from putrefaction.

The action of benzoic acid on pathogenic microbes has been little studied. Koch† found that its antiseptic properties, in connection with the spores of *Bacillus anthracis*, were inferior to those of mercuric chloride.

Benzoic acid checks the abnormal formation of uric acid, and consequently is of great therapeutic value in the treatment of gout and allied diseases.

'In gout, the uric acid is the result of modified innervation of the liver, or exhaustion of the hepatic cells, and so there are non-transformation of the glycocine and the consequent formation of uric acid.'

Benzoic acid acts upon the glycocine (whether derived from the bile poured out into the duodenum or formed elsewhere in the body) with the formation of hippuric acid :

$$\underset{\text{[Benzoic acid]}}{C_6H_5COOH} + \underset{\text{[Glycocine]}}{CH_2(NH_2)COOH} = \underset{\text{[Hippuric acid]}}{C_9H_9NO_3} + \underset{\text{[Water]}}{H_2O}.$$

By this means the formation of an abnormal amount of uric acid is prevented, while the glycocine is eliminated as hippuric acid.

* Dr. T. Carnelley (*Proc. Chem. Soc.*, 1890, p. 90) has shown that (as a rule) the para-compounds of *benzene* (*i.e.*, its derivatives) are 'more antiseptic than the corresponding ortho- and meta- compounds.'

† *Mitth. a. d. k. Gesundheitsamte*, 1881.

Dr. Garrod (*Lancet*, 1883, p. 673) has successfully used benzoic acid and its salts in the treatment of gout and gravel.

(2) *Benzoates.*—The alkaline benzoates are also useful germicides. Buchholz (*Archiv für Exp. Pathologie*, vol. vii.) has shown that sodium benzoate (which is more soluble than benzoic acid) in proportion of 1 in 2,000 preserves bouillon from putrefaction.

Like benzoic acid, 'sodium benzoate diminishes the uric acid secretion,'* and has a favourable action on the mucous membranes in disease.

(3) *Sodium benzenesulphinate.*—This compound is readily obtained by dissolving benzoic acid in a concentrated solution of sodium sulphite.

According to M. Heckel (*Comptes Rendus*, vol. cv., p. 896), sodium benzenesulphinate possesses germicidal and antiseptic properties more efficient than phenol (carbolic acid), and ranks with mercuric chloride and iodoform. It is very soluble in water, and has no injurious effects even in somewhat large doses. It may be applied (as an antiseptic for wounds) in the form of a solution containing from four to five grammes per litre.

(4) *Salicylic acid.*—This acid occurs in meadow-sweet, winter-green, violets,† pansy,‡ tulip,§ yucca,§ hyacinth,§ birch,‖ and other members of the vegetal kingdom. It is also prepared artificially by heating sodium phenate in a stream of carbonic anhydride, and subsequently de-

* Dr. Noel Paton in *Journal of Anatomy and Physiology*, 1886, p. 26.

† See Prof. K. Mandelin's paper in *Pharmaceutical Journal and Transactions*, vol. xii., p. 627.

‡ A paper by E. C. Conrad and A. B. Griffiths in the *Chemical News*, vol. l., p. 102.

§ Dr. Griffiths' paper in *Proceedings, Chemical Society*, 1889, p. 122.

‖ Trimble and Schröter in *Pharm. Journal and Transactions*, vol. xx., p. 166.

composing the sodium salicylate by means of an acid. Salicylic acid prepared from sodium phenate often contains cresotic, parahydroxybenzoic, and hydroxyisophthalic acids, etc. Cresotic acid is the most important impurity, as, apart from its obscure physiological action, it presence is very objectionable, and for medicinal purposes the *natural* salicylic acid should alone be used. Its germicidal and physiological actions are entirely different from those of the artificial salicylic acid, however much the latter may have been dialyzed and purified.

Professor W. N. Hartley, F.R.S. (*Journal, Chemical Society*, 1888, p. 664), has tried to prove spectroscopically that 'recrystallized commercial salicylic acid can be obtained in a state of great purity, and identical with that from oil of winter-green.' Chemically they may be identical, but bacteriologically, as well as physiologically, they are quite distinct substances.

To preserve beef-broth from putrefaction, *natural* salicylic acid must be added, so as to form a 3 per cent. solution;* whereas, with purified artificial salicylic acid, the broth must contain at least 5 per cent. of acid.

Dr. P. W. Latham (*The Croonian Lectures*, 1886) says that in the treatment of gout and allied disorders 'the true salicylic acid obtained from the vegetable kingdom must alone be employed.'

In the treatment of phthisis by the hypodermic injection method, natural salicylic acid will alone give beneficial results.†

It appears from the above observations that the *natural* variety possesses properties which are not found in artificial salicylic acid. These facts support Pasteur's

* The acid is far more soluble in broths than in distilled water.

† See the author's paper in *Proceedings, Royal Society of Edinburgh*, vol. xv., p. 33.

theory (*Revue Scientifique*, 1884), that organic compounds prepared by *synthesis* are not altogether identical with the natural compounds. 'Life' brings into play asymmetrical molecular forces, while in the mineral kingdom, and also in our laboratories, only symmetrical molecular forces come into play. Pasteur's theory is summed up by M. Wyrouboff* in these words :

'Ces théories sont fondées sur une première hypothèse, qui suppose les phénomènes naturels soumis à deux sortes d'actions ; les unes symétriques, les autres dissymétriques ; es premières président à la minéralité et aux synthèses de nos laboratoires, les secondes appartiennent à la vitalité.'—*Bulletin de la Société Chimique de Paris* [2nd series], vol. xli., p. 210.

Fig. 26 represents microscopical preparations of pure salicylic acid crystals deposited from alcohol and ether :

The author has shown that natural salicylic acid prepared from oil of winter-green (*Gautheria procumbens*) is a powerful germicidal agent. Among these experiments may be mentioned the following : First, several Aitken's tubes containing sterilized beef-broth (neutral) were treated in the following manner :

Tube No. 1 was inoculated with *Sarcina lutea* (from a pure cultivation in nutrient agar-agar), and kept at a temperature of 40° C. The microbe grew rapidly, and after four days' incubation formed a yellow pellicle upon the surface of the broth.

Tubes Nos. 2 and 3 each contained, in addition to the sterilized broth, salicylic acid (3 per cent.). These tubes were inoculated with *Sarcina lutea*. No growths made their appearance after an incubation which lasted twenty-

* Author of *Manuel Pratique de Cristallographie*, and well known as the author of valuable contributions to the science of crystallography.

eight days. Four tubes containing sterilized nutrient agar-agar were inoculated with the contents of tubes

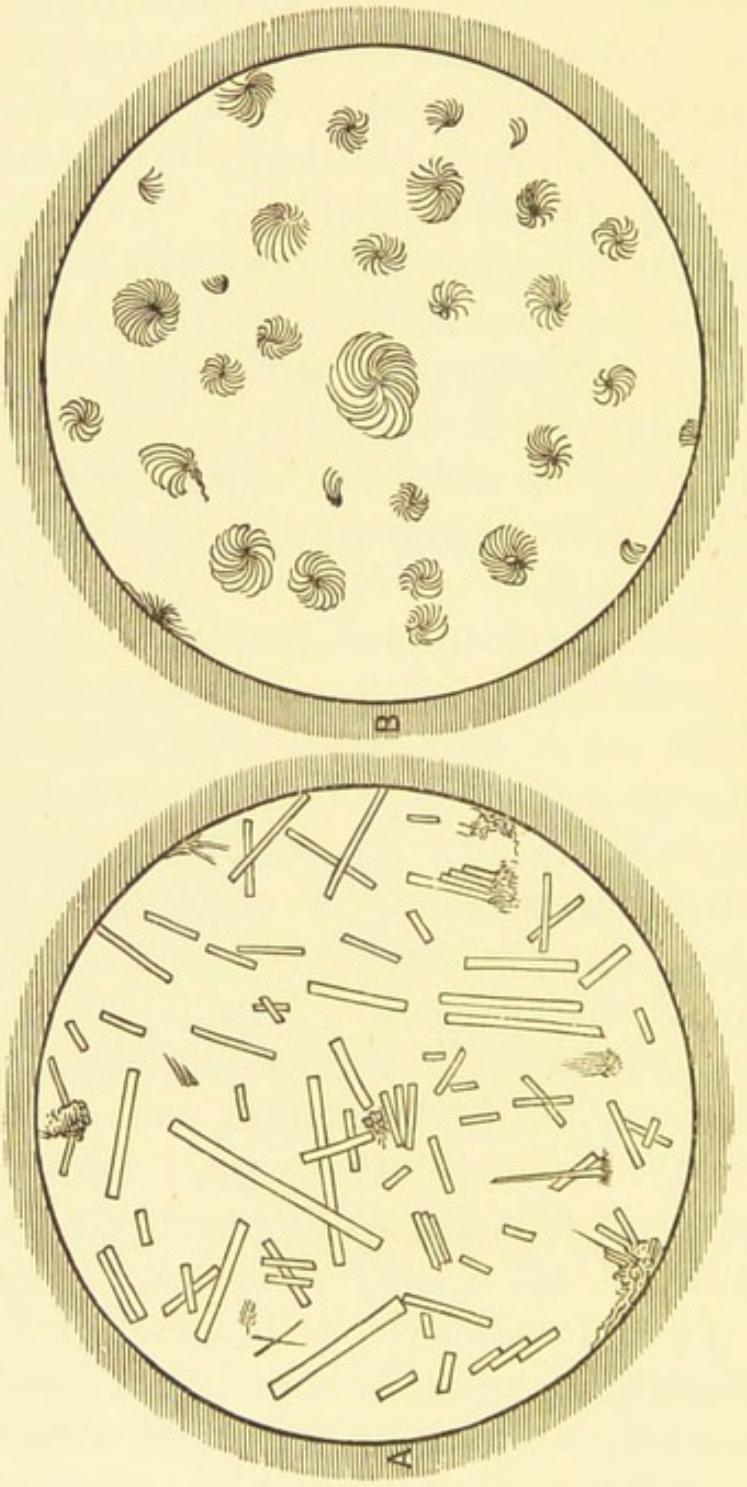


FIG. 26.

SALICYLIC ACID CRYSTALS, CRYSTALLIZED FROM ETHER. (×ABOUT 95.)

SALICYLIC ACID CRYSTALS, CRYSTALLIZED FROM ALCOHOL. (×340.)

Nos. 2 and 3, but after an incubation at 40° C. for twenty-one days no growths made their appearance in any of the tubes.

In a similar manner (using the most suitable media in each case for the growth of the various microbes) experiments were performed upon *Micrococcus prodigiosus*, *Bacillus tuberculosis*, *Bacterium allii*, *Micrococcus tetragonus*, and all these microbes were destroyed by salicylic acid.* The author† has also shown that salicylic acid destroys *Bacterium lactis*, *Bacterium aceti*, *Micrococcus aurantiacus*, *Micrococcus in diarrhœa*, *Bacillus subtilis*, *Leptothrix buccalis*, *Bacterium æruginosum*, *Micrococcus ureæ*, *Bacillus butyricus*, etc. Since the publication of the author's papers, Dr. Pierre Grosfils, working on *Bacillus butyricus*, has confirmed the germicidal properties of salicylic acid (*Soc. d'Encouragement de Vervier*, 1887).

From the author's investigations (*Proc. Roy. Soc. Edinburgh*, vol. xiii., p. 527) it appears that salicylic acid prevents the hydrating action of soluble enzymes.

Yeast was added to a solution of cane sugar containing salicylic acid (0·2 gramme in 1,000 cc.); no hydration took place according to the following equation:

$$C_{12}H_{22}O_{11} + H_2O = C_6H_{12}O_6 + C_6H_{12}O_6,$$

and fermentation was entirely absent—although the mixture was allowed to stand for two or three days to a temperature most favourable to engender alcoholic fermentation. To make sure that the cane sugar had not been hydrated, the mixture was tested for glucose sugars by means of Fehling's solution, but with negative results.

The acid (in the above proportions) does not prevent the fermentation of glucose.

From the above the acid in question acts upon the

* *Proceedings, Royal Society of Edinburgh*, vol. xv., p. 37.
† *Ibid.*, vol. xiii., p. 527, and vol. xiv., p. 97.

enzymes secreted by the *Torula*, if they are secreted at all when the microbe is in the acid mixture. According to Dr. Schulz (*Pflüger's Archiv*, vol. xlii., p. 517), salicylic acid (in *small* quantities) has the power of raising the activity of the yeast cells.

Salicylic acid solution also prevents the hydration of starch by ptyalin. And as Dr. Wagner* has successfully used salicylic acid in the treatment of diphtheria, it is possible that hypodermic injections of salicylic acid (15 minims of a saturated solution, twice daily) would be beneficial in the treatment of that disease, more especially now that Drs. Roux and Yersin have isolated an enzyme from a cultivation of the microbe of diphtheria. The enzyme should *a priori* be destroyed by salicylic acid.

The author has shown (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 53) that a solution of salicylic acid destroys *Bacillus tuberculosis*, and that pulmonary phthisis, especially in its early stages, is capable of being cured by repeated subcutaneous injections of a saturated aqueous solution of this acid. The treatment of phthisis by the injection of salicylic acid and other antiparasitic medicines will be fully described in a special chapter devoted to the subject. From 5 to 20 minims of a saturated solution of salicylic acid has no detrimental action on the blood, and has the power of completely curing muscular rheumatism of the kind which often accompanies phthisis. That is, salicylic acid prevents the abnormal formation of uric acid, by seizing upon or eliminating the glycocine from the system — glycocine being an antecedent of uric acid. The reaction may be represented by the following equation :

* *Journal für Praktische Chemie*, vol. xi., pp. 57 and 211.

$$C_6H_4(OH)COOH + CH_2(NH_2)COOH =$$
[Salicylic acid] [Glycocine]

$$C_6H_4\begin{cases}OH\\CO.NH-CH_2-COOH\end{cases} + H_2O.$$
[Salicyluric acid] [Water]

The salicyluric acid so formed is far more soluble than uric acid, and passes off in the urine.

Not only does salicylic acid destroy *Bacillus tuberculosis*, and prevent the abnormal formation of uric acid, which causes phthisico-rheumatic pains, but it ameliorates the various functional troubles which ensue during the course of the disease.

(5) *Salicylates.*—Although their solubility is greater, the salicylates have far less antiseptic and germicidal properties than salicylic acid *per se*. According to Dr. Miquel ten, or even twelve, times the quantity of sodium salicylate is required to preserve bouillon from putrefaction.

(6) *Carbolic acid.*—Koch has found that a 3 per cent. solution of carbolic acid completely destroyed the spores of *Bacillus anthracis* in seven days, while a 5 per cent. solution destroyed them in two days. A 1 per cent. solution easily destroyed the sporeless bacilli, but in a ·5 per cent. solution they were not destroyed. Koch's *modus operandi* was to impregnate silk threads with anthrax spores, which were then placed in bottles containing solutions of carbolic acid of various strengths. Threads were taken of each solution and transferred to nutrient gelatine, and the results noted.

As an antiseptic agent, carbolic acid, in the shape of dressings and lotions, and as a spray, in surgical operations, is of the greatest value; but, according to Jalan de la Croix (*Archiv für Experim. Pathol.*, vol. xiii.), its

germicidal properties are inferior to those of salicylic acid.

As an antiseptic, 'carbolic acid used with the steam spray is undoubtedly an efficient agent, if proper care and assistance be at hand; but in hospital practice very many circumstances, in some cases, combine to render futile all attempts at antisepsis in this way. As the result of careful observation,' says Edington, 'the carbolic spray is most likely to render efficient service in private practice, when it and its surroundings can be kept under the personal supervision of the surgeon himself, but should be dispensed with in hospital practice if a more stable substitute can be readily obtained.

'The use of carbolic acid for washing out wounds is at best a dangerous method of procedure. It has already been shown that, as the result of the action of 1 in 20 carbolic lotion upon muscular tissue and blood, there is formed a viscous or glue-like mass, and thus, if applied to a wound, there is formed on the surface of it a distinct layer of necrosed material. This, like most dead tissues, forms a suitable nidus for the growth of microbes. Supposing, then, that carbolic acid irrigation be efficient in destroying the microbes in a wound, this necrotic area has still to be cast off, and contributes in this way a form of suppuration, during the progress of which excessive care will have to be taken in order to prevent the entrance of fresh microbes. But as it happens that such a proceeding is hardly likely to be successful, the surgeon using this method simply makes matters worse, in that, while he does not destroy sepsis, he ministers directly to it by giving the microbes pabulum on which to feed. Thus we see that carbolic acid irrigation, instead of tending in the direction of the cure of sepsis, predisposes indirectly to pyæmia and septicæmia.'*

* *British Medical Journal*, 1889.

Carbolic acid (phenol) has been successfully employed in disinfecting the infection of cattle plague. There is little doubt that the acid destroys the streptococci of the disease.

(7) *Phenates or carbolates.*—As antiseptic and germicidal agents, these compounds are far less powerful than carbolic acid.

(8) *Thymol.*—This compound is allied to phenol (*i.e.*, it is a 'ten-carbon phenol,' having the formula $C_{10}H_{13}OH$). It exists in the essential oils of thyme (*Thymus serpyllum*), horse-mint (*Mentha silvestris*), and other plants; and it crystallizes in large transparent plates. Thymol has powerful antiseptic properties, and according to De la Croix (*Archiv Exp. Path.*, vol. xiii.) it readily sterilizes culture broths.

Concerning the aromatic compounds, Dr. E. Klein says: 'The presence of phenol, thymol, salicylic acid, etc., restrains even when in great dilution the growth of micro-organisms.'

Alcohols.

The antiseptic properties of these compounds are well known. Probably ethylic, amylic, and propenylic alcohols have the greatest power.

Ethylic, or ordinary alcohol, arrests the development of microbes and the spores; but the spores (*e.g.*, the spores of *B. subtilis*) are capable of withstanding the action of strong alcohol for two or three months. The preservative action of alcohol against putrefaction is well shown in the preserved specimens in biological, pathological, and other laboratories.

Amylic alcohol is not so powerful in its action as ethylic alcohol. Fourteen grammes of amylic alcohol

are said to be necessary to preserve a litre of bouillon from putrefaction.

Propenylic alcohol, or glycerol (glycerine) has considerable antiseptic powers, and has been used in preserving the rabid marrows (for inoculation against rabies) during their transit from France to various foreign countries.

Various Antiparasitic Medicines.

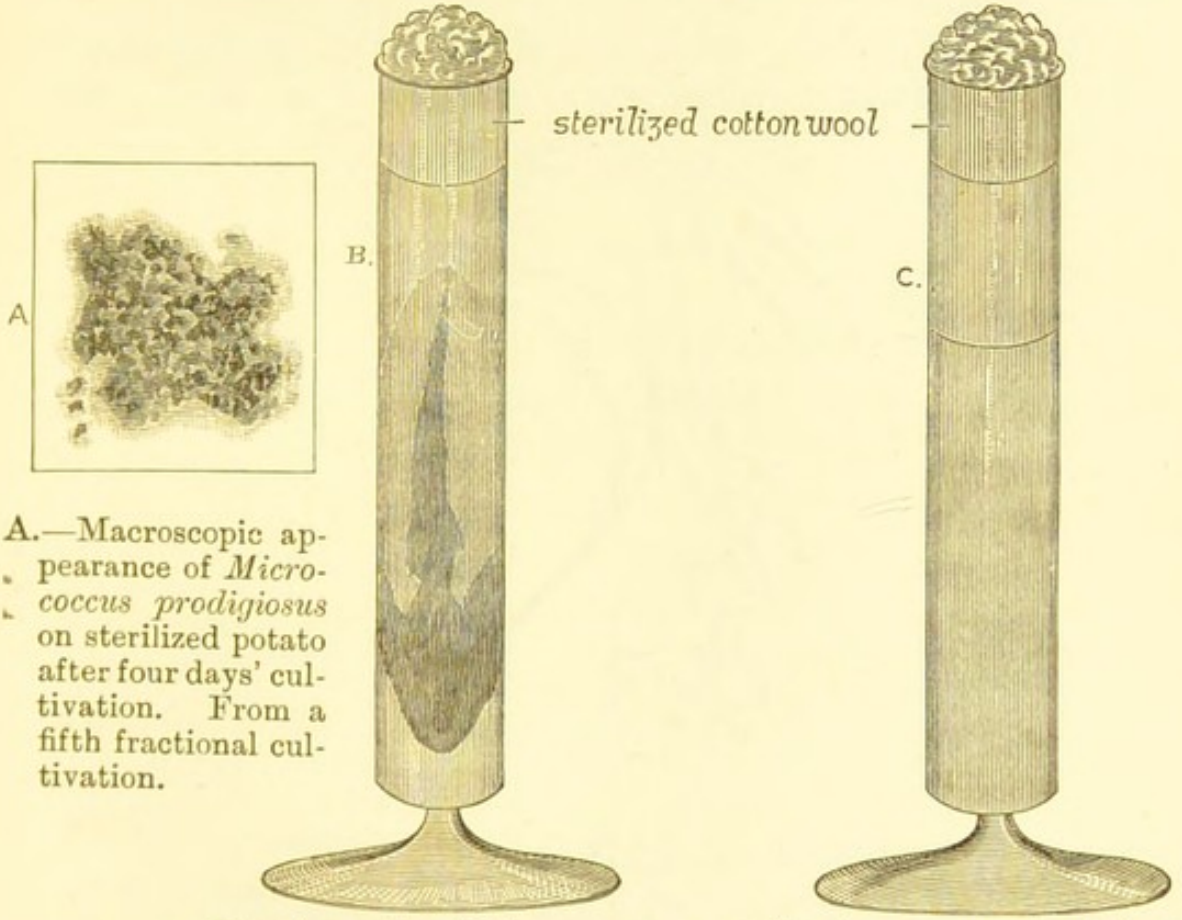
(1) *Ethyl and amyl nitrites* are both germicidal agents of considerable power. Giuseppe Sormani (*Atti dell' Istituto Lombardo*, 1887) has shown that ethyl nitrite is effective in destroying tubercular virus; and amyl nitrite has a similar action. It is probable that both of these compounds might be employed in the treatment of phthisis with considerable success.

Amyl nitrite has long been used as a 'remedy' in *angina pectoris*. It is antispasmodic and 'though it dilates the arterioles, it does not prove that spasm of the arterioles is the pathology of all cases, for it may relieve spasm of the heart.'

(2) *Camphorated chloral* is also stated by Giuseppe Sormani (*loc. cit.*) to destroy *Bacillus tuberculosis*.

(3) *Sodium fluosilicate* is a very powerful germicide. According to Mr. W. Thomson, F.R.S.E. (*Chemical News*, vol. lvi., p. 132), who has used it for that purpose, it is non-poisonous and odourless; and a saturated aqueous solution contains 0·61 per cent. of the salt. Sodium fluosilicate does not irritate wounds, and it has 'greater antiseptic power for animal tissues than one part of mercuric chloride in 1,000 of water, which is a stronger solution than that which can be generally employed for surgical purposes without producing poisonous effects.'

The author of the present volume (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 37) has experimentally proved the antiseptic and germicidal properties of sodium fluosilicate. Among these experiments may be mentioned the following: After preparing a series of tubes containing sterilized nutrient agar-agar (with and also without the germicidal agent), they were all inoculated (the utmost care being observed) with pure cultivations of *Micrococcus prodigiosus* (Fig. 27). Tubes Nos. 1 and 2 were inoculated with a sterilized platinum needle from



A.—Macroscopic appearance of *Micrococcus prodigiosus* on sterilized potato after four days' cultivation. From a fifth fractional cultivation.

B.—A growth of *Micrococcus prodigiosus* in nutrient agar-agar.

C.—Appearance of nutrient agar-agar (inoculated) in each tube after the addition of the antiseptic, and also after twenty days' incubation at 34° C.

FIG. 27.—MICROCOCCUS PRODIGIOSUS.

a potato cultivation of the microbe. After five days' growth at 34° C. in an incubator, the appearance was similar to the growth in Fig. 27 B. Tubes Nos. 3 and 4 (each containing 0·4 per cent. of sodium fluosilicate) were inoculated with the same microbes, but no growths made their appearance in the tubes after twenty-five days' incubation at 34° C. After this period had elapsed, sterilized platinum needles were plunged into each tube,

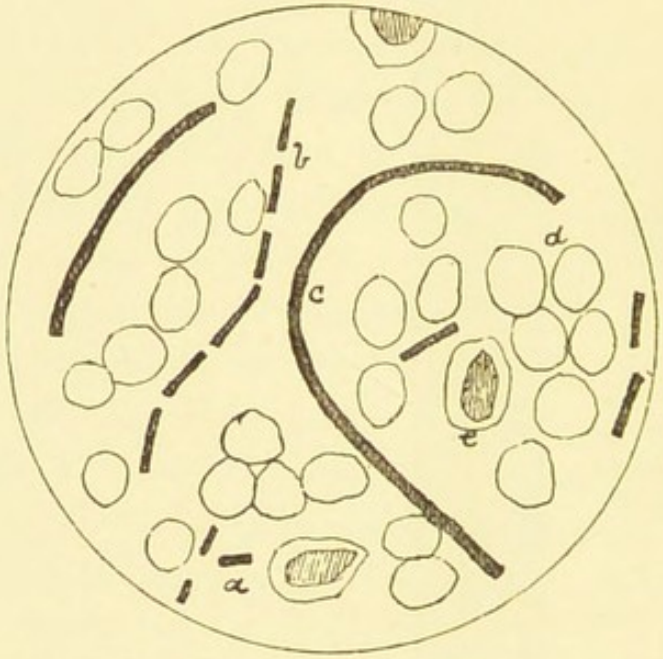


FIG. 28.—BACILLUS ŒDEMATIS MALIGNI.

a = Single bacilli; *b* = bacilli in chains; *c* = leptothrix; *d* = blood corpuscles; *e* = white blood corpuscles. (×745.)

which were then transferred to two tubes containing sterilized nutrient agar-agar. No growths made their appearance in these tubes (Fig. 27 C) after an incubation which lasted for three weeks.

Bacillus Œdematis Maligni.

This bacillus (Figs. 28 and 29), obtained from soil, is a pathogenic microbe. Mice, rats, cats, etc., inoculated with a pure cultivation of this microbe die in a few hours.

B. œdematis maligni grows well on the surface of a neutral solution of Liebig's extract of meat at 36° to 38° C., or in nutrient agar-agar. It is an *anaërobic* microbe, but lives its life-history in an atmosphere of carbonic acid gas (Fig. 29).

This microbe is destroyed by sodium fluosilicate (0·4 per cent.).

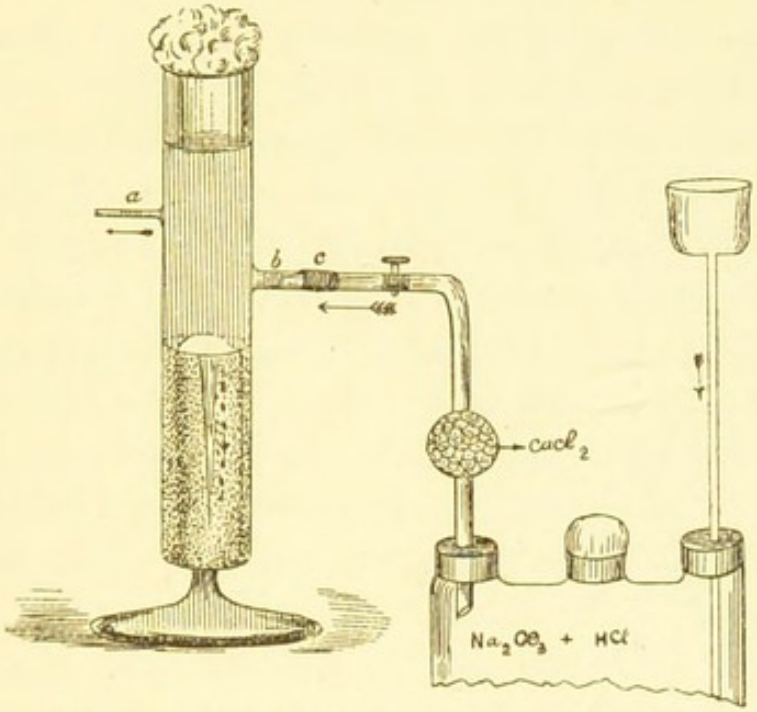


FIG. 29.—BACILLUS ŒDEMATIS MALIGNI.

The microbe growing on a solution of Liebig's extract of meat in an atmosphere of carbonic acid gas.

a and *b* = sterilized cotton-wool plugs; *c* = india-rubber tubing connecting the gas-generator with the cultivation tube.

The author has also proved that sodium fluosilicate has the power of destroying *Bacillus tuberculosis*, *Micrococcus tetragonus*, *Bacterium allii*, *Spirillum tyrogenum*, *Bacillus subtilis*, *Sarcina lutea*, *Micrococcus candicans*, *Bacterium indicum*, and other microbes.

In this chapter an account has been given of certain germicidal and antiseptic agents of more or less value to

the physician, surgeon, and pathologist; but it must be borne in mind that 'the chemical substances which serve to nourish one form may be poisonous to another; thus, a solution of potassium arseniate enables one species to vegetate, but becomes poisonous to a parasite of superior order. In solutions of quinine at $\frac{1}{20}$, colonies of moulds and microphytes are developed, whilst weaker solutions are sufficient to disinfect liquids containing other microbes. A question suggests itself on this subject: Are pathogenic species fixed, or may they combine and create varieties? Certainly, new diseases have appeared, and others are extinct: the plagues of the Middle Ages have disappeared; diphtheria, recurrent fever, are of recent date; but in reality this fatal family of microphytes dates from afar, and remains immovable and immutable; there are true species which are reproduced indefinitely by culture whose morphology may be similar, but whose vital pathogenic action differs totally. That is why all antiseptics do not enjoy specific necrophytic properties.'

But as Professor E. M. Crookshank, M.B.,* says: 'Such knowledge (of necrophytics) must necessarily prove of the greatest importance to the sanitarian, who is concerned in preventing the spreading of disease and in the disposal of putrefactive matter; to the surgeon, who is anxious to exclude micro-organisms during surgical operations, and to arrest the development in wounds of bacteria which have already gained an entrance; to the physician, in the treatment of micro-parasitic diseases. The sanitarian and the surgeon must profit directly by such experiments, for in the disinfection of clothes and the sick-room by the one, and in the application of antiseptic dressings and lotions by the other, the micro-

* *Manual of Bacteriology*, p. 151 (second edition).

organisms are encountered, as in the test experiments, apart from the living body. The physician, on the other hand, is principally concerned in dealing with micro-parasites when circulating in the blood, or carrying on their destructive processes in the internal tissues.' And there is no doubt that for certain infectious diseases necrophytic medicines will, in the long-run, play an important part in the scientific and rational treatment of such diseases.

CHAPTER XI.

THE BIOLOGY, ETC., OF CERTAIN MICROBES.

We now propose to discuss the biology, etc., of certain microbes, more particularly of those which are associated with, or are the cause of, disease.

'In a normal state the bodies of men and animals contain different parasites; the mouth and digestive canal always, but the blood and urine only in certain morbid conditions, principally in the case of infectious diseases. These specific diseases are absolutely distinct from those caused by mineral or vegetable poisoning, which, whether in the acute or chronic state, have always effects proportional to the dose of the poison. In the former the type of malady depends on the microbe from which it is derived.'

Micrococcus Endocarditicus.

Endocarditis ulcerosa is a malignant form of endocarditis, and there is little doubt that the disease is due to a pathogenic microbe (*i.e.*, *M. endocarditicus*, 1 μ to 0·5 μ diam.). This microbe has been found by numerous observers in masses and chains in the granulations, bloodvessels, the valves and muscles of the heart, in this disease. *M. endocarditicus* is capable of assuming the zooglœan state, and no doubt when in this state it gives

rise to embolism. The same microbe has been found in the spleen, kidneys, and urine.

Micrococcus in Rabies.

Rabies, or hydrophobia, is most likely due to a microbe which flourishes in the medulla and spinal cord of dogs and other animals.

According to Fol,* Babès,† and Dowdeswell,‡ the microbe is a micrococcus, and it has been observed in microscopical sections of the spinal cord of animals dead of rabies. This microbe has not yet been isolated and cultivated apart from the body.

The incubation of rabies appears to take a long time, usually not less than four to six weeks, and sometimes longer. Rabies is *not* a disease of the blood, for the supposed microbe is not found in the blood system, and when the *blood* of a rabid animal is injected into animals it does not reproduce the disease.

If rabies is a micro-parasitic disease (which is most likely) the microbe is located in the nervous system, especially the medulla oblongata.

Micrococcus in Yellow Fever.

Micrococci (0·6 to 0·7 μ diam.) have been found in the kidney, spleen, and liver during the course of yellow fever. They form rosaries and masses, which greatly distend the bloodvessels and give rise to hæmorrhages. The origin of yellow fever seems to be connected with heat, 'for although it occurs in sporadic and epidemic forms in all seasons in the tropical part of the yellow fever zone, the disease is greatest and takes an epidemic

* *Comptes Rendus de l'Académie des Sciences*, 1885.
† *Les Bactéries* (Cornil and Babès), 1886.
‡ *Journal, Royal Microscopical Society*, 1886 ; and *Lancet*, 1886.

spread at the hottest period of the year, a temperature of at least 70° F. (21° C.) being required for its production. Frost puts an end to an epidemic at once, and storms, heavy rains, or cold weather check its progress.'

The period of incubation of yellow fever is variable; on an average it is from twenty to one hundred hours, but sometimes it may take several weeks.

Micrococcus Tetragonus.

Micrococcus tetragonus (Fig. 30) is found in the sputum of patients suffering from phthisis.

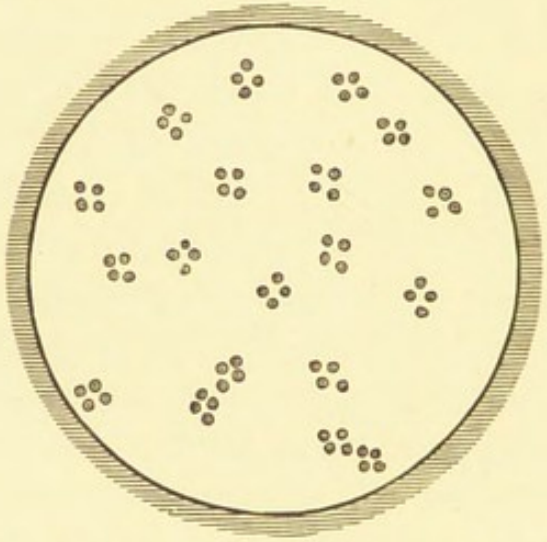


FIG. 30.—MICROCOCCUS TETRAGONUS, STAINED WITH GENTIAN VIOLET (MUCH ENLARGED).

According to the most reliable sources, *Micrococcus tetragonus* (1 μ diam.) is only saprophytic in man, but pathogenic in animals. Mice inoculated with a small quantity of this microbe die in a few days, the microbe afterwards being found in the various organs of the body.

This microbe grows tolerably well in nutrient agar-agar, and is easily stained with gentian violet. It is chiefly found in groups of four (hence the specific name of *tetragonus*), 'surrounded by a hyaline capsule.'

Micrococcus Erysipelatosus.

The micrococci of erysipelas* (0·4 μ diam.) occur in the lymphatic vessels of the skin. These microbes grow on agar-agar, nutrient gelatine, and solid blood-serum. Orth (*Archiv für Exp. Pathol.*, vol. i.) and Fehleisen (*Die Aetiologie des Erysipels*, 1883) have both reproduced the disease, with all its symptoms, in animals and man, from artificial cultivations of the microbe. There is little doubt that *M. erysipelatosus* is the cause of erysipelas. The incubation of the disease is from three to ten days.

Fehleisen has found that a 3 per cent. solution of phenol destroys this micrococcus; and a 2·5 per cent. solution of natural salicylic acid has a similar action.

Micrococcus Variolæ et Vacciniæ.

Micrococci (0·5 μ diam.) have been found in the lymphatics of the skin (in small-pox,† and in cow-pox and sheep-pox‡) in the vicinity of the pocks. The same micrococci were found by Cohn (*Virchow's Archiv*, vol. lv.) in the lymph of vaccina and variola. No doubt they are the active agent in small-pox and cow-pox, for if the lymph is filtered through a Chamberland filter, the filtrate loses its infectious properties.

The author has shown that a solution of salicylic acid acts upon vaccine lymph, and deprives it of the power of inoculation.§ *Micrococcus vacciniæ* occurs singly, in pairs, chains and colonies.

According to Klebs, variola is equally transmissible by air and by inoculation of its micrococci, ' which accumu-

* From ἐρυθρὸς (red) and πέλλα (skin).
† Weigert (*Med. Centralblatt*, 1871).
‡ Klein (*Philosophical Transactions of Royal Society*, 1874).
§ Griffiths in *Proc. Roy. Soc. Edinburgh*, vol. xiv., p. 97.

late especially in the little areolar cavities of the Malpighian layer at the place of the pustules.'

Micrococcus Pneumoniæ.

This microbe is present in pneumonia,* or inflammation of the lungs.

The microbe was discovered by Friedländer (*Virchow's Archiv*, vol. lxxxvii.), and occurs in the sputa of pneumonic patients, either singly or as diplococci, short chains, and in the zoogloœan state. Sometimes the microbes are free, while at other times they are encysted in the lymphatic cells.

They are oval, encapsulated microbes, and have been cultivated in blood-serum, nutrient gelatine, bouillon, and on boiled potatoes. When the artificially-cultivated microbe is inoculated in the tissue of the lungs, it produces in animals all the characteristic symptoms of pneumonia; the lungs become red, solid, and enlarged, and pieces of them sink in water. The composition of the *blood* is considerably altered, as shown in the following analyses:

	In Pneumonia.	In Health.
Water	839·848	780·150
Fibrin	9·152	2·104
Albumin	100·415	65·091
Globulin	38·517	133·003
Hæmatin	1·800	3·961
Fat	2·265	3·740
Extractive matter and salts	8·003	11·951
	1000·000	1000·000

* From πνεύμων.

The microbes have not only been found in the sputum, but also in the blood during the course of the disease.

Rats, mice, dogs, and rabbits (Salvioli) are susceptible to pneumonia; and 'inhalation experiments by spraying the cocci diffused in water into mouse-cages succeeded in producing pneumonia and pleurisy in three out of ten mice.' This explains the fact that in a house where one inmate is suffering from pneumonia, others of the same household are successively attacked by the same disease.

According to Drs. Nolen and Poels (*Centralblatt für d. Med. Wiss.*, 1884), pleuro-pneumonia of cattle and human pneumonia are produced by the same microbe. They have artificially cultivated the microbe of human pneumonia, and also the microbe of pleuro-pneumonia, and found that when either is injected into cattle it produces pleuro-pneumonia, with all its characteristic symptoms.

It may be mentioned in passing that Dr. Klein does not accept these statements without reservation.

Pleuro-pneumonia, or the contagious pneumonia of cattle, is an epidemic disease, and is transmissible by contact and inoculation.

'The general history of pleuro-pneumonia points to its introduction into the United Kingdom about the year 1840; but, unfortunately, no accurate account of the earlier outbreaks can be discovered, as the official records only go back to 1869.' From 1869 to 1887, '74,552 have been returned as attacked, 59,599 killed as diseased, 7,480 have died, and 7,470 recovered.' 'The spreading of disease can be clearly traced to the movement of infected cattle through fairs, markets, and auction marts, and their unguarded and reckless admission into the cowsheds of towns.'

The micrococci contained in pneumonic sputa are

easily stained by Gram's and other methods described in Chapter II.

Micrococcus in Whooping-cough.

Whooping-cough is undoubtedly a contagious disease, and, according to Bürger (*Berlin Klin. Woch.*, 1883), oval-shaped micrococci are always present in the pearly phlegm ejected by persons suffering from this disease. They have not yet been cultivated.

Micrococcus in Measles.

Dr. Keating of Philadelphia (*Phil. Medical Times*, 1882), and subsequently Cornil and Babès (*Les Bactéries*, 1885), have observed the presence of micrococci (singly and as diplococci) in the capillary vessels of the skin, in the catarrhal exudations, and in the blood of persons suffering from measles. The same microbe has also been found in the urine during the course of the disease.

The period of incubation of this disease is from ten to fourteen days; and it has been stated that 'the contagion of measles is less persistent, but spreads with greater rapidity than does that of scarlatina or small-pox.'

Micrococcus Gonorrhœæ.

Dr. Neisser (*Centralblatt für d. Med. Wissensch.*, 1879) and others, since 1879, have described micrococci (0·83 μ diam.) in the urethral discharge and the pus of gonorrhœa. These microbes (Fig. 31) occur singly, as diplococci, as tetrads, and in zoogloean groups. They have been artificially cultivated by Bockhart, who proved their pathogenic character by inoculation.

A similar micrococcus is often found in the purulent ophthalmia of newborn infants; and it is possible that

such ophthalmia is, in the majority of cases, of gonorrhœal origin.

A dilute solution of silver nitrate destroys the microbe; hence the value of this salt in the treatment of the disease.*

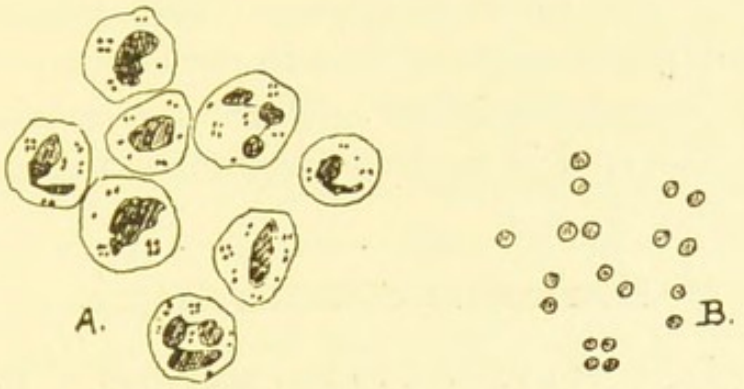


FIG. 31.—MICROCOCCUS GONORRHŒÆ.

A.=Cells of gonorrhœal pus containing nuclei and micrococci. (×640 diam.) B.=Micrococci. (×2020 diam.—*i.e.* Zeiss' $\frac{1}{18}$ homog., Oc. 5).

Micrococcus gonorrhœæ is readily stained with a solution of magenta.

Micrococcus Scarlatinæ.

This microbe has been found in the blood, the exudations and tissues of the ulcerated throat, and in the desquamating epidermic cells of this disease. *Micrococcus scarlatinæ* has also been observed in *the urine* of patients suffering from scarlatina.

This microbe is capable of existing in milk, for Dr. Klein (*The Times*, May 28, 1887) has proved the presence of *Micrococcus scarlatinæ* in the milk of cows suffering from certain diseases of the udders and teats.

The period of incubation of scarlatina is from one to six days; occasionally twenty-one days.

* See *On Gonorrhœal Infection in Women*, by Dr. W. J. Sinclair.

Micrococcus in Gangrene.

Small oval-shaped micrococci have been found in gangrene of the lungs. They are small microbes living in colonies, and form zoogloœa. These microbes grow on nutrient gelatine, giving rise to the characteristic, but offensive, gangrenous odour. The same microbes have also been observed in various gangrenous tissues.

Micrococcus of Cattle Plague.

Cattle plague is ascribed to the presence of micrococci in the blood and lymphatic glands. This microbe grows in beef-broth and other cultivating media at 37° C. According to Semmer and Archangelski (*Centralblatt für d. Med. Wissensch.*, 1883), calves inoculated from a pure cultivation of this microbe died in seven days with all the typical symptoms of cattle plague.

By successive cultivations the microbe of cattle plague loses its virulence (*i.e.*, becomes attenuated), and in this weakened form has been used for the protective inoculation of sheep and cattle.

Micrococcus of Foot-and-Mouth Disease.

According to Dr. Klein, the microbe of this disease occurs singly, as diplococci, and in curved chains. 'It grows well in milk, in alkaline peptone broth, in nutrient gelatine, and in agar-agar.'

It has been observed 'in the vesicles of sheep suffering from foot-and-mouth disease.'

Klein says that this microbe 'is highly sensitive towards antiseptics.'

Micrococcus in Cerebro-spinal Meningitis.

Micrococci (singly, diplococci, chains, and zooglœa) have been observed by Leyden (*Centralblatt für Klin. Med.*, 1883) in the pus found at the base of the brain after death, as well as in the kidneys.

Micrococcus in Puerperal Fever.

According to Heiberg (*Die Puerperalen und Pyämischen Processe*), micrococci have been found in the form of chains and zooglœa (but chiefly the latter) in all organs affected in this disease* (*i.e.*, endocardium, lung, spleen, kidney, cornea, brain, etc.).

Heiberg's micrococcus has not yet been artificially cultivated. If it is capable of being cultivated in artificial media, it would be important to ascertain whether the microbe produces an alkaloid or alkaloids in the medium in which it is cultivated. Bourget isolated several poisonous bases from the viscera of a woman who had died of puerperal fever; and subsequently proved the existence of the same bases in the urine of patients suffering from the same disease.

According to Pasteur, the different symptoms classed under the name puerperal fever are all due to the invasion of microbes, 'which develop themselves on the surface of wounded parts, and from thence spread themselves, in one form or another, by the medium of the blood or of the lymphatics, over different parts of the body. Here the various morbid symptoms are determined by the nature of the parasite and the general constitution of the patient.' Pasteur is convinced that

* Puerperal fever is highly infectious. Recently a midwife carried the contagion to *five* different women, all of whom died of the disease (see the report of the 'Limehouse Epidemic' in the *Echo* of September 17, 1889).

'with the possible exception of cases where, by the presence either of internal or external abscesses, the body before confinement contains microbes, the *antiseptic treatment* ought to be infallible in preventing puerperal fever from declaring itself. The employment of carbolic acid may be of great service, but its smell, and often the melancholy association of ideas which it awakens, might render it unsuitable for women in labour. There is not the same objection to concentrated solutions of *boric acid*, which, at the ordinary temperature, contain from thirty to forty grammes of acid per litre of water' (see *Bull. de l'Acad. de Méd.*, vol. ix.).

A solution of mercuric chloride (1 in 1,000) is now used in the Maternity Hospital of Paris, as it gives excellent results and keeps off all danger.

Micrococcus in Pernicious Anæmia.

According to Dr. Frankenhäuser (*Centralblatt für d. Med. Wissensch.*, 1883) the blood of pregnant women suffering from pernicious anæmia contains a large number of micrococci which appear to be of a pathogenic character. The microbes are comparatively of large size ('about one-tenth of the broad diameter of a red blood-corpuscle'), but they have not been cultivated.

Micrococcus Pyogenes Aureus.

This chromogenic microbe is pathogenic in man and animals. According to Schüller,* Rosenbach,† and Becker,‡ it is always present in the pus from patients suffering from acute infectious osteomyelitis.§

* *Centralblatt für Chirurgie*, 1876.
† *Ibid.*, 1884.
‡ *Deutsche Med. Woch.*, 1883.
§ See also a recent paper in the *Comptes Rendus*, vol. cx. [1890].

M. pyogenes aureus grows on boiled potatoes, nutrient gelatine or agar-agar, and blood-serum, giving rise to orange cultures.

According to Becker, 'when a small quantity of a cultivation was introduced into the jugular vein after previous fracture or contusion of the bones of the leg, the animal died in about ten days, and abscesses were found in and around the bones, and in some cases in the lungs and kidneys.'

Death from acute peritonitis is the result of an injection of this microbe into the peritoneal cavity of animals.

Micrococci in Pyæmia and Septicæmia.

A considerable number of micrococci have been found in various organs, etc., in pyæmia and septicæmia in the lower animals and in man.

Bacterium Choleræ Gallinarum.

This microbe is found in large numbers in the blood and organs of fowls dead of this disease. Pasteur describes the symptoms of fowl-cholera in the following words:

'The bird which is attacked by this disease is without strength, staggering, the wings drooping. The ruffled feathers of the body give it the shape of a ball. An overpowering somnolence takes possession of it. If forced to open its eyes, it appears as if it were awakened out of a deep sleep. Very soon the eyelids close again, and generally death comes without the animal changing its place, or without any struggle, except at times a slight movement of the wings for a few seconds.'

B. choleræ gallinarum is easily cultivated in chicken-

broth (neutral) at 25°-35° C., and when fowls are inoculated with a drop of this culture they always die with the characteristic symptoms of the disease. If a culture of the microbe is kept for two or three months, its virulence is lessened. An attenuated virus has been successfully used by Pasteur in the protective inoculation of fowls against this disease.

This microbe is pathogenic in rabbits as well as fowls, but guinea-pigs have an immunity. *B. choleræ gallinarum* is aërobic (Pasteur), and is cultivated in contact with sterilized air or in aërated liquids. In fact, 'its toxic effect has been supposed to be due to the abstraction of oxygen from the blood producing asphyxia.'

Bacterium Allii.

During the year 1887 the author (*Proc. Roy. Soc. Edin.*, vol. xv., p. 40) discovered a new microbe in the greenish slime of diseased or putrefying onions and allied plants.

The cells of this microbe are about 0·005 to 0·007 mm. long, and about 0·0025 mm. in width. It belongs to the *Bacteriaceæ*, and was named *Bacterium allii* because it was originally found on *Allium cepa* (the onion).

Bacterium allii grows tolerably well on nutrient agar-agar, and produces a bright green pellicle on the surface of the nourishing medium (Fig. 32).

It has already been stated that this microbe produces an alkaloid from albuminoid molecules (see Chapter V.), the alkaloid answering in chemical composition to hydrocoridine ($C_{10}H_{17}N$).* Besides this base small quantities of sulphuretted hydrogen gas are liberated from the media on which the microbes are living. The sulphuretted

* See Dr. Griffiths' paper in *Comptes Rendus de l'Académie des Sciences*, vol. cx., p. 418.

hydrogen was proved by the black stain (PbS) produced upon paper impregnated with a solution of lead acetate, and also by the yellow stain (CdS) produced on cadmium paper ($CdCl_2$).

This microbe (Fig. 33) forms zoogloœa, and is best stained with gentian violet.

Therefore *Bacterium allii* produces a pigment, an

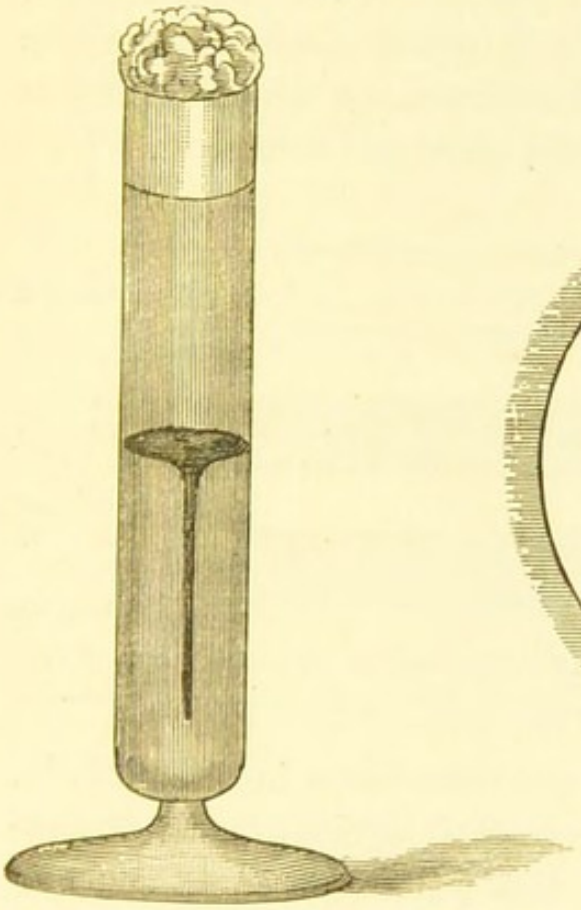


FIG. 32.—BACTERIUM ALLII (A NEW MICRO-ORGANISM) GROWING ON NUTRIENT AGAR-AGAR.

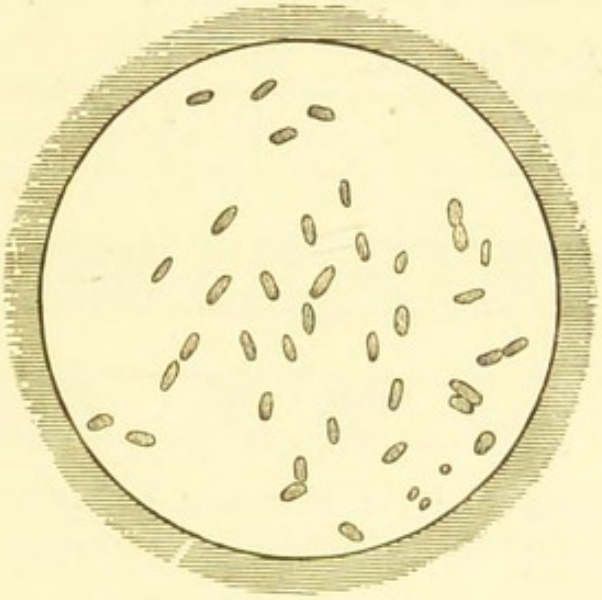


FIG. 33. — BACTERIUM ALLII (UNDER HIGH POWER), STAINED WITH GENTIAN VIOLET.

alkaloid, and a sulphur-gas from the medium in which it lives.

The green pigment is soluble in alcohol, and the alcoholic solution gives an absorption spectrum (Fig. 34), consisting of a band extending from the extreme violet to the blue part (nearly to the Fraunhofer line—F) of the spectrum.

There is also an absorption band in the green, and one in the yellow, part of the spectrum. The end of the band in the yellow is exactly in the same position as the D line in the solar spectrum. It will also be seen from Fig. 34 that the spectrum produced by this pigment differs from chlorophyll, although both solutions were of the same intensity of colour and nearly the same thickness when placed in front of the slit of the spectroscope.

The microbe in question is quite distinct from the *bacillus* (giving a green fluorescence) which Heraëus (*Zeitschrift für Hygiene*, 1886) obtained from soil.

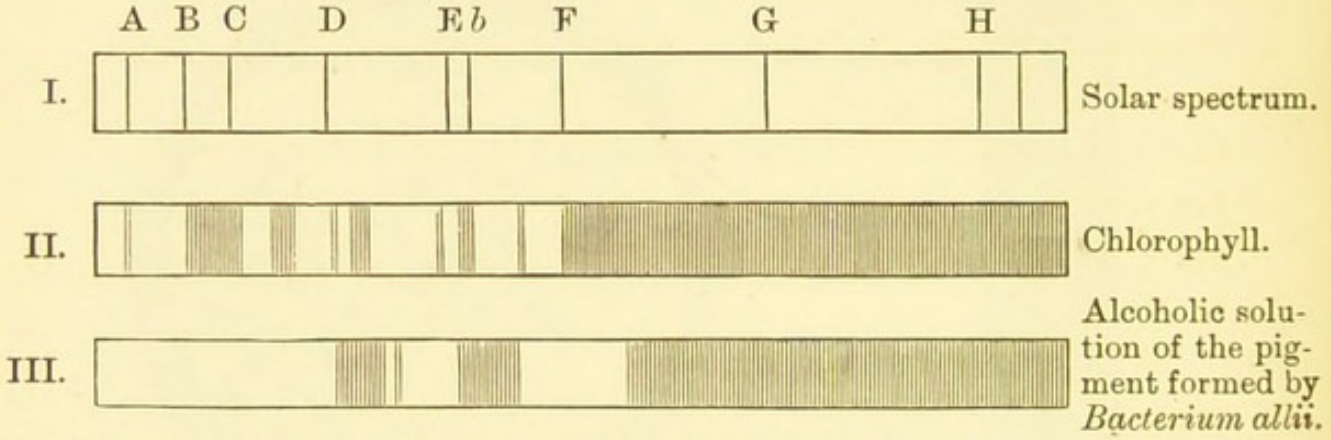


FIG. 34.—ABSORPTION SPECTRUM OF AN ALCOHOLIC SOLUTION OF THE GREEN PIGMENT FORMED DURING THE LIFE-HISTORY OF BACTERIUM ALLII.

The bacillus of Heraëus converts urea into ammonia, while *Bacterium allii* has no such action, for it decomposes albuminoids (vegetal and animal) with the formation of a ptomaïne among other products.

Experiments are still wanting to ascertain whether *Bacterium allii* is pathogenic or otherwise in animals.

Bacteria of Septicæmia.

Certain bacteria have been described by Koch, Davaine, and others in the blood of various animals suffering from septicæmia.

Bacillus Diphtheriæ.

According to the investigations of Drs. Roux and Yersin (*Ann. Inst. Pasteur*, 1888), diphtheria is due to a soluble poison (enzyme), most probably produced by the *Bacillus diphtheriæ* which has been recently investigated by Dr. E. Klein, F.R.S.*

Two microbes were originally isolated by Klebs and Löffler from human diphtheritic membranes; but Klein has shown that the Klebs-Löffler bacillus No. 1 'is not constant in diphtheritic membranes, does not grow on solid gelatine at 19-20° C., and does not act pathogenically on animals; the other species, Klebs-Löffler bacillus No. 2, is constant in diphtheritic membranes—in fact, is present even in the deeper layers of the membranes in great masses and almost in pure culture, acts very virulently on animals, and grows well on gelatine at 19-20° C.'

This bacillus (No. 2) 'acts very virulently on guinea-pigs on subcutaneous inoculation; at the seat of the injection a tumour is produced, which in its pathology and in microscopic sections completely resembles the diphtheritic tissue of the human subject. In human diphtheria the diphtheria bacillus is present only in the diphtheritic membrane, but neither in the blood nor in the diseased viscera; the same holds good for the experimental guinea-pigs. In subcutaneous inoculation with artificial culture, though it causes in these animals acute disease and death—the lungs, intestine, and kidney are greatly congested—the diphtheria bacillus remains limited to the seat of inoculation.'

Klein has shown that this microbe (Klebs-Löffler bacillus No. 2, which is the true pathogenic microbe of

* A paper read before the Royal Society on May 22, 1890.

diphtheria) also attacks the cat and cows, as well as man and the guinea-pig. But, unlike human diphtheria, the disease locates itself in the lungs of the cat, *i.e.*, 'the lung is the organ in which the diphtheritic process in the cat has its seat.'

Klein has also shown that a definite disease can be produced in the cow by the *Bacillus diphtheriæ*, 'consisting of a diphtheritic tumour at the seat of inoculation with copious multiplication of the diphtheria bacillus, a severe pneumonia, and necrotic change in the liver; the contagious nature of the vesicular eruption on the udder and excretion of the diphtheria bacillus in the milk prove that in the cow the bacillus is absorbed as such into the system.'*

According to Talamon (*Bulletin de la Soc. Anat. de Paris*, vol. lvi.), diphtheritic membranes contain the mycelia and conidia of some fungus, but it is not likely that Talamon's fungus has anything to do with the disease in question.

Bacillus Typhosus.

The microbe of typhoid fever has been found in Peyer's glands, the spleen, larynx, lungs, liver, and in the lymphoid follicles of the intestines in fatal cases. Sometimes the microbe is present in the kidneys and urine. *Bacillus typhosus* ($50 \times \cdot 2\ \mu$) has rounded ends, and spore-formation occurs at the extremities of the rods. These microbes (Fig. 35) have also been observed in the blood obtained from living patients.

They grow on nutrient gelatine,† boiled potatoes (at 37° C.), and blood-serum.

According to Fraënkel and Simmonds (*Die Aetiologische*

* For further information see Dr. Klein's paper in *Nature*, vol. xlii., p. 113.

† Gaffky, *Mitth. aus dem k. Gesundheitsamte*, 1884.

Bedeutung des Typhus-bacillus, 1886), this microbe is the cause of typhoid fever, for they have reproduced the disease, by inoculation, from a pure cultivation of the microbe.

Many other microbes (especially micrococci*) 'appear in the intestines when the disease is approaching its end, but the *bacillus* in question is the only one found in the blood and internal organs [as well as in the roseolous eruption], so that it is really characteristic of the disease.'

FIG. 35.—BACILLUS TYPHOSUS.

A = Bacilli in blood. (×1,500.) B = Spore-formation. (×2,500.)

During the course of typhoid fever the composition of the *blood* is considerably altered, as the following analyses show :

	IN TYPHOID FEVER.	IN HEALTH.
Water	801·00	780·150
Fibrin	2·30	2·104
Albumin	64·40	65·091
Globulin	121·81	133·003
Hæmatin	2·69	3·961
Fat	1·52	3·740
Extractive matter and salts ...	6·00	11·951
	1000·00	1000·000

* Klein, *Reports of Medical Officer of the Privy Council*, 1875.

The period of incubation of typhoid fever is from ten to fourteen days.

The eruption appears on the seventh or eighth day; and the duration of the disease varies from twenty to thirty days.

As the stools* of typhoid fever patients are highly infectious, they should always be disinfected before being thrown away. Several authors have recommended carbolic acid or mercuric chloride for this purpose; but iron sulphate, according to Jalan de la Croix, is far more powerful than carbolic acid, and is only slightly inferior to mercuric chloride: besides, iron sulphate is a cheap disinfectant, non-poisonous and inodorous, and therefore may safely be recommended for the purpose of disinfecting the stools of patients suffering from typhoid fever and other infectious diseases. The author has proved, in a series of papers, the high value of iron sulphate (*i.e.*, ferrous sulphate) as a germicidal and fungicidal agent;† and this compound readily destroys *Bacillus typhosus*.

Dr. Proust (*Traité d'Hygiène*) has used, for a number of years, iron sulphate to disinfect the stools in cases of typhoid fever.

It will hardly be out of place to remind the reader that the late Dr. W. Budd, F.R.S.,‡ disinfected the sewers of Bristol against the cholera-microbe by using iron sulphate; and Dr. E. Divers, F.R.S., of Tokio, informs the author that this salt has been used for a similar purpose in Japan.

* *Bacillus typhosus* can be isolated from the stools of typhoid fever patients by fractional cultivation.

† *Proceedings Royal Society of Edinburgh*, vol. xv.; *Bulletin de la Société Chimique de Paris*, 1889, p. 667; *Journal Chemical Society*, 1883-87; *Chemical News*, vols. xlvii.-lvi.; *The Diseases of Crops and their Remedies* (G. Bell and Sons).

‡ *The Cholera Microbe, and How to Meet It*, p. 25, by Dr. C. Cameron, M.P. (Baillière, Tindall, and Cox).

Bacillus Malariæ.

This microbe (2 to 7 μ long), when grown on gelatine, gives rise to a well-developed growth: and the researches of Klebs and Tommasi-Crudeli (*Archiv für Exper. Pathol.*, 1879) have shown that when a drop of the culture is inoculated in rabbits, it reproduces malarial fever, with all its characteristic symptoms, 'and in the spleen and marrow the threads and spores of the bacilli were found in abundance.'

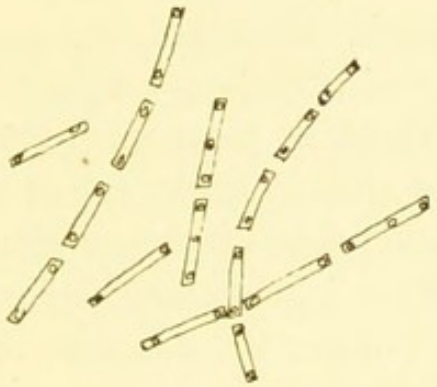


FIG. 36.—BACILLUS MALARIÆ.

Bacillus malariæ (Fig. 36) produces spores either at the ends or in the centre of the cell.

This microbe grows also in albumin, urine, and other media, and was originally discovered in the soil of the Roman Campagna. The bacillus is abundantly found in the blood of patients suffering from malaria. The microbe is inhaled into the blood by way of the lungs,* and perhaps it may enter through the stomach and skin also.

The microbe flourishes in marshy districts, in deltas, on alluvial soils, and on the banks of tropical rivers—in

* Mr. H. M. Stanley (*In Darkest Africa*, vol. ii., p. 33) says: 'Might not a respirator, attached to a veil or face-screen of muslin, assist in mitigating malarious effects when the traveller finds himself in open regions?'

fact, a proper degree of porosity, of temperature, and of humidity of soil favour the growth of this microbe. For this reason, *B. malariæ* has been called 'an earth-born poison.'* This microbe is said to be heavier than most gases, 'and scarcely floats six feet above the ground; it may be wafted some distance by winds, but mountains hold it back, and belts of trees, especially the eucalyptus, destroy its efficacy.' This microbe is common in the malarious parts of Italy, and is found in abundance in the sweat of the forehead and hands of people inhabiting those parts.

Laveran, Richard, Marchiafava, and Celli have discovered amœbiform organisms allied to the flagellated *Protozoa*, in the blood of patients suffering from malaria. These organisms have been called *Hæmoplasmodium malariæ*, and are capable of giving rise to 'intermittent fever in man after intravenous injection. The blood corpuscles of a person so infected again contain the hæmoplasmodia.'

It is very probable that several microbes are the cause of the disease which is known by the name of malarial fever; hence the reason of the different types of this fever—tertian, quartan fever, etc.

Bacillus Mallei.

The microbe of glanders or farcy is very similar to the bacillus of phthisis, but it is readily distinguished from the latter microbe by staining reagents.†

Bacillus mallei has been found in the lungs, liver, spleen, and nasal membranes of horses and sheep 'dead or dying from glanders.'

* See Dr. R. W. Felkin's paper in *Proceedings, Royal Society of Edinburgh*, vol. xvi., p. 269.

† See *Revue Médicale Française*, 1882.

The same microbe was found in human glanders by Babès and Havas in 1881, and by Wassilieff in 1883. The sad death of Dr. Hoffmann, of Vienna, in 1889 (see Chapter II.), is a standing proof of the infectious nature of this microbe, and its being the cause of the disease known as glanders.

B. mallei grows on solid blood-serum (at 38° C.), sterilized potatoes (at 37° C.), and in neutral solutions of extract of beef (at 37° C.). Horses, asses, cats, rabbits, guinea-pigs, and mice inoculated with a few drops of a *pure* cultivation of this microbe have died with the characteristic lesions of glanders (glanderous ulcers and nodules in the internal organs, and on the nasal septum).

The tissues containing the microbes of glanders are best stained by the method of Schutz (*Deut. Med. Woch.*, 1882)*—that is, with an aqueous solution of methylene-blue, followed by washing with dilute acetic acid.

Bacillus Lepræ.

'There seems to be no doubt now that leprosy is both contagious and hereditary, and that it is caused by the *Bacillus lepræ*' (Felkin).

The bacilli of leprosy are from 4 to 6 μ long, and about 1 μ wide; they are pointed at both ends, and 'occur in masses within the large leprosy-cells of the leprous tubercles' of the skin, spleen, liver, testicles, kidneys, lymphatic glands, 'and of the mucous membrane of the mouth, palate, and larynx.'

These microbes are sometimes motile and produce

* The sections are placed (for twenty-four hours) in a mixture containing equal parts of potash solution (1 in 1000) and concentrated alcoholic solution of methylene-blue. They are then washed in very dilute acetic acid, dehydrated in alcohol, clarified in oil of cloves, and mounted in Canada balsam.

spores. They grow on blood-serum and extract of meat; and according to Damsch (*Virchow's Archiv*, vol. xcii.), the disease is produced in cats inoculated with leprous tissues.

The disease is very rare in Europe, but common in Egypt, Morocco, Madagascar, Cape Colony, Southern Asia, Brazil, Guiana, Argentine Republic, in certain of the Pacific Ocean islands (especially Hawaii), and other parts of the world.

'Two types of leprosy are described—the tubercular and anæsthetic varieties; the first variety is more frequently seen in temperate climates, the latter in the tropics.'

Dr. J. Hutchinson, F.R.S.,* has suggested that leprosy depends in a great measure—that is to say, the bacillus had its origin—upon eating fish, especially decomposed fish, by persons in a low state of health. In the Middle Ages leprosy was prevalent over nearly all Europe, and *salted* fish† formed in that time almost the only food during the winter months.

It may be remarked that Sir Morell Mackenzie‡ is of opinion that in cases of *genuine* leprosy 'medicine is impotent in the matter of cure, and can at most give some occasional relief to the more distressing symptoms.'

Bacillus of Syphilis.

In 1885, Dr. S. Lustgarten, of Vienna, discovered certain bacilli (3 to 4 μ long and 0·8 μ in width) in the nucleated cells of various syphilitic products (for instance, 'in the discharge of the primary lesion, and in hereditary affections of tertiary gummata'). According

* President of the Royal College of Surgeons.

† See the remarks concerning salt (sodium chloride) in Chapter X.

‡ *Leprosy of the Air-passages.*

to Lustgarten this bacillus is the virus of syphilis; and De Giacomi, Doutrelepont, and Schütz have fully confirmed Lustgarten's observations.

Syphilis is divided into three stages. The first, or primary stage, lasts for a few weeks, and about a fortnight after the introduction of the virus the Hunterian chancre makes its appearance, but at the same time 'indurated buboes or glands may be detected in the groins.'

After this the blood becomes tainted; the virus, greatly interfering with the functions of the blood and tissues, produces the varied morbid phenomena known as secondary and tertiary syphilis.

Syphilis leaves 'no tissue untouched.'

Shakespeare* well describes the disease in the following words:

> 'Consumptions sow
> In hollow bones of man; strike their sharp shins,
> And mar men's spurring. Crack the lawyer's voice,
> That he may never more false title plead,
> Nor sound his quillets shrilly: hoar the flamen,
> That scolds against the quality of flesh,
> And not believes himself: down with the nose,
> Down with it flat; take the bridge quite away
> Of him that, his particular to foresee,
> Smells from the general weal: make curl'd-pate ruffians bald;
> And let the unscarr'd braggarts of the war
> Derive some pain from you: plague all;
> That your activity may defeat and quell
> The source of all erection.'

According to Astruc and Van Swieten, syphilis plays an important *rôle* in the production of phthisis. 'The syphilitic patient often dies tubercular. One finds then in the lung, syphilitic gummata by the side of tubercular granulations, which are very difficult to distinguish, and

* *Timon of Athens*, act iv., sc. iii. Timon says the above words to Phrynia and Timandra (mistresses to Alcibiades) after their remark: 'Believe 't, that we'll do anything for gold.'

which cannot be recognised except by the presence of the bacillus. Further, the syphilitic sufferer often gives birth to a tubercular infant: is that on account of a cachectic state which is not constant, or following the transmission of one specific agent which favours the development of another?'

Sections of syphilitic tissues containing the bacilli may easily be stained by immersing them in an aqueous solution of gentian-violet (1 per cent.), and then using an after-stain of safranin.

Bacillus Tuberculosis.

This organism was discovered by Dr. Koch* in 1882 as the specific microbe of phthisis.† Numerous observers in England, France, and Germany have confirmed Koch's important researches.

There is no doubt that phthisis is an infectious disease, and that the virus is Koch's bacillus. This microbe has been found in the sputum, in the cells of tubercles, in the blood, tissues, urine, fæces, etc. It is also found 'in the different forms of tubercular lesions, whether localized in the lungs or disseminated at the same time in the other organs. It is to be found in the lesions called granulations and miliary tubercles, as well as in the destructive phases of caseation or ulceration. In all these cases its favourite, but not exclusive, habitat is in the tubercular elements called epithelioid cells and giant cells.'

Bacillus tuberculosis (2 to 8 μ long) has been cultivated artificially; and it has been proved that the strength of its virulence is not lessened by successive cultivations. When inoculated into various animals it always produces tuberculosis.

* *Berliner Klin. Wochenschrift*, vol. xv.

† From $\phi\theta\acute{\iota}\omega$, to consume.

It grows on solid blood-serum, agar-agar, gelatine and glycerol (Hammerschlag), or in beef-broth containing glycerol, mannitol, glucose (or glycogen), or in a solution of 2 parts of peptone, 6 parts of glycerol, and 1 part of mineral salts in 100 parts of distilled water (at 37° to 39° C.).

Dr. A. Hammerschlag (*Monatshefte für Chemie*, vol. x., p. 9) submitted a cultivation of the bacillus of phthisis to chemical analysis, and gives the following as the results of his determinations :

If it is considered that the whole of the nitrogen present, after treatment with alcohol, is in the form of albumin (containing 16 per cent. of nitrogen), the composition of the dried bacillus may be taken as—

Matter soluble in alcohol - -	27·0
Albumin - - - -	36·9
Cellulose - - - -	28·1
Ash - - - - -	8·0
	100·0

Bacillus tuberculosis differs considerably from other bacilli in containing both a large amount of substance soluble in alcohol and ether ; its power of forming cellulose from the medium in which it lives ; and its characteristic reactions with staining agents.

According to Hammerschlag, the tubercle-bacillus (in the dried condition) contains a ' powerful poison ' (?). The author could not detect any such poison, after exhausting the dried bacillus with alcohol (96% and 65%), ether, chloroform, and petroleum ether.

The bacilli consist of delicate sheaths with protoplasmic contents, and often contain rounded, refractive

bodies (spores). These spores are produced endogenously.

Ray Lankester (*Nature*, 1884) and others have stated that *B. tuberculosis* 'never gives rise to spores by endogenous formation;' while other observers believe that the small granular bodies within the cells are formed from the protoplasm by 'the treatment they are subjected to in making a microscopical preparation.' But these observers forget that sometimes the microbes, after staining, show no granular bodies; and it is most probable that 'the grains' (which exhibit the same colour reactions as *B. tuberculosis*) found in stained preparations of the cells of tubercles are in reality the *spores* of this microbe in an isolated state.

The presence of *B. tuberculosis* in the sputa of patients supposed to be suffering from phthisis (even in its masked and latent forms) is a certain means of diagnosis. Hence the importance for the physician and medical practitioner to possess a thorough knowledge of the methods used for staining this microbe contained in phthisical sputum.

Therefore some details of the methods employed for staining tubercle-bacilli may not be out of place.

(1) *Koch's method.*—Cover-glass preparations of the sputum are placed in a solution containing: 1 part of a concentrated solution of methylene-blue, 2 parts of a potash solution (10%), and 200 parts of distilled water. The preparations remain in the solution (heated to 40° C.) for twenty-four minutes. They are then washed in water, and placed in an aqueous solution of vesuvin for two or three minutes; again washed, and subsequently treated with alcohol, oil of cloves, and finally mounted in Canada balsam. This method stains the bacilli blue and the nuclei, etc., brown.

(2) *Ehrlich's method.*—Cover-glass preparations are

made to float (with the prepared face downwards) in a solution of fuchsine made in the following manner : 5 cc. of aniline oil and 100 cc. of distilled water are mixed together and filtered. To the filtrate is added a concentrated alcoholic solution of fuchsine. The preparations remain in this solution for fifteen minutes; they are then washed in nitric acid (1 part nitric acid to 2 parts distilled water) and rinsed in distilled water. An after-stain of methylene-blue or vesuvin gives the nuclei, etc., a blue or brown colour, while the tubercle-bacilli are stained red.

The elegance of this method is that *Bacillus tuberculosis* impregnated with fuchsine resists the action of nitric acid, whilst the saprophytic microbes (contained in phthisical sputum*), nuclei, etc., are immediately decolourized by the acid.

Koch's and Ehrlich's methods are also applicable for staining tubercular tissues, etc.

(3) *Baumgarten's method.*—Cover-glass preparations of sputum are placed in a very dilute solution of potash, and after being slightly pressed on the microscopic slides they are ready for examination.

By this method the bacilli are seen in the unstained condition.

The Ehrlich-Weigert method, as well as the one devised by Dr. Gibbes, have already been described in Chapter II.

Bacillus tuberculosis attacks other animals besides man, but it does not attack all animals equally. Arranging them in order of respective liability to tuberculosis, they are as follows :

* *Micrococcus tetragonus, Micrococcus lacteus faviformis, Bacterium crassum sputigenum*, etc.

Man.	Goats.
Cows.	Sheep.
Fowls.	Horses.
Rodents.	Carnivora (dogs,
Pigs.	cats, etc.).

From this it appears that the microbe grows most readily in those animals which are omnivorous and herbivorous.

Dr. V. Galtier (*Comptes Rendus*, vol. civ.) has shown that whey and cheese from the milk of tuberculous cows often contain the bacilli of phthisis. He has also demonstrated that swine and poultry fed upon dairy produce of this character often develop the disease. Their flesh may then in turn impart the disease to man.

The expectorations of phthisical patients are highly infectious, even after being desiccated for several months.

When susceptible animals are fed upon food mixed with tuberculous matter, they become infected with the disease.

The author* has shown that when three fowls were allowed to feed upon Indian corn mixed with human sputum (which had been obtained from an advanced case of general phthisis), one of the fowls died of typical tuberculosis, while the other two resisted the action of the virus.

The fowl dead of tuberculosis was examined immediately after death, when a large number of bacilli were found in the liver (Fig. 37), which was greatly enlarged, and of a mottled appearance. The lungs also contained tubercles filled with the bacilli.

These experiments prove (1) that susceptible animals

* A paper read before the Royal Society of Edinburgh on March 18, 1889.

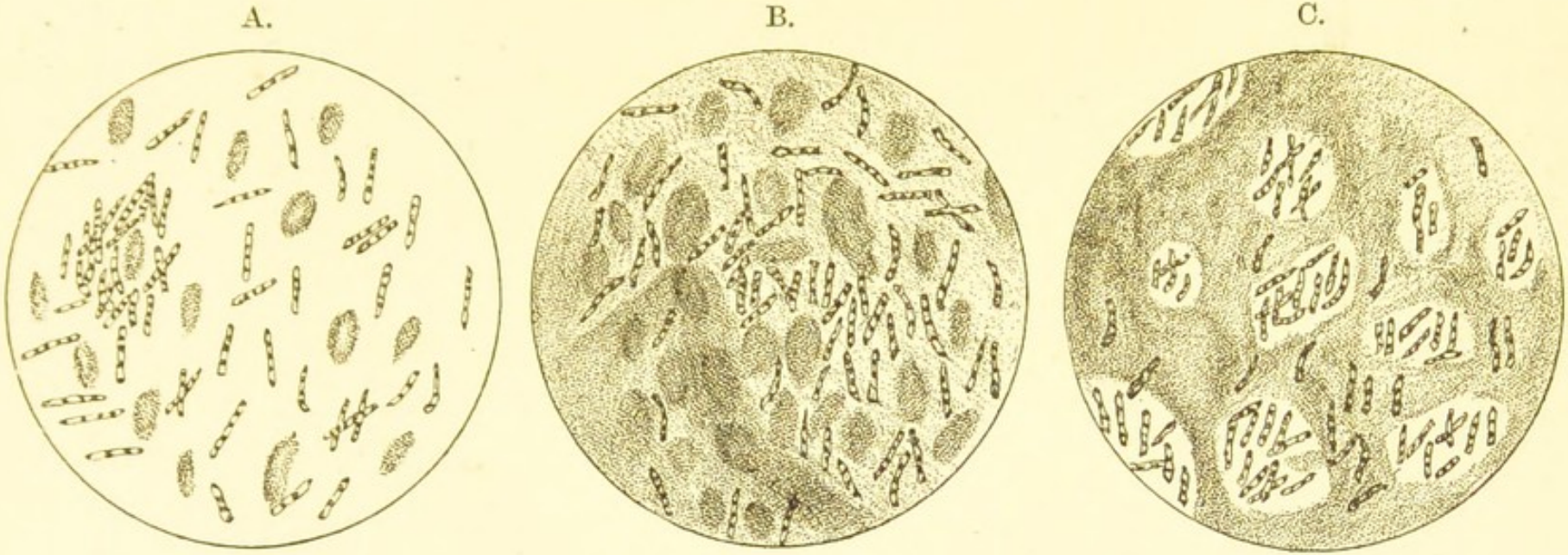


FIG. 37.

A = Examination of the sputum before the above experiments. (×1,515 diam.)

B = A microscopical preparation of pus from tubercles of hen's lung. The preparation shows numbers of bacilli. (×1,515 diam.)

C = Section of hen's liver, showing numbers of bacilli. (×1,515 diam.)

fed on tuberculous matter contract the disease; (2) that *Bacillus tuberculosis* lives (to a certain extent) in the blood, for the microbes must have passed into the blood before finding their way to the liver and other organs. Dr. Weichselbaum (*Wiener Med. Blätter*, 1884) found that in human tuberculosis the blood contains a large number of tubercle-bacilli.

Dr. Babès (*Centralblatt für d. Med. Wissensch.*, 1883, p. 145) proved the existence of *Bacillus tuberculosis* in the urine, as well as in the blood of phthisical patients.

The author has also shown that the saliva* and sweat of patients suffering from advanced phthisis contain tubercle-bacilli.

From the above facts it is advisable for physicians attending phthisical patients to insist upon nurses and others disinfecting the urine, fæces, sputa, etc., so that there may be no chance of infection. It would not be a difficult task for nurses attending consumptives to immerse all handkerchiefs after use in water containing carbolic acid or some other disinfectant.

It should be borne in mind that *Bacillus tuberculosis* has been proved to enter the body in the following ways:

(1) *Inhalation* into the air-passages and lungs.

(2) *Swallowing* into the alimentary canal. This is more difficult, as shown by the author's experiments on fowls. One out of three became infected.

(3) *Direct introduction* into the sub-cutaneous or sub-mucous tissue, by means of a scratch, or cut, or sore in the skin or mucous membrane.

(4) *Heredity*.

The flesh of tubercular animals (*e.g.*, cattle, fowls, pigs, etc.), and the milk from tubercular cows, have been

* *Proceedings, Royal Society of Edinburgh*, vol. xv., p. 44.]

stated to give rise to tuberculosis in human beings.* It may be remarked in passing that 'boiling always destroys the virulence, even when the milk contains bacilli, which is the case when the udder of the affected cow is itself tuberculous.'

Dr. Klein does not accept the statement that the bacilli of bovine tuberculosis are identical with those of human tuberculosis. He finds (*Micro-Organisms and Disease*, p. 170) 'that in the two diseases their (*i.e.*, bacilli) morphological characters and distribution are very different. The bacilli of human tuberculosis are conspicuously larger than those of the tuberculosis of cattle, and, in many instances, more regularly granular.' He further says that 'the bacilli in the tuberculous deposits of cattle are always contained in the cells; the larger the cell, the more numerous the bacilli. . . . But in the human tubercles the bacilli are always scattered between the cells.'

There is little doubt that the differences in the dimensions and the distribution of the tubercle-bacilli in the human and bovine disease respectively are due to the difference of the soil in the two cases, and not to any difference in the nature of the microbe.

Therefore, farm animals are capable of giving the disease to man, and *vice versâ*.

The risk of infection is greatly diminished, if not abolished, when meat from tuberculous cattle is thoroughly cooked. It is the opinion of eminent veterinarians that bovine tuberculosis has become of so much importance, both in its effect on the health of the human race and on that of cattle, especially of the highly-

* Dr. Sims Woodhead and others. See also the *Annales de l'Institut Pasteur*, vol. iv., p. 185; and Dr. Gasperini's paper in the *Giornale d. R. Soc. Ital. d'Igiene*, 1890, p. 5.

bred stock, that they desire tuberculosis to be placed among the other scheduled diseases, such as pleuro-pneumonia and anthrax, with power for compulsory slaughter and State compensation.

While realizing the danger of infection arising to man from tuberculous food, that from tuberculous human beings is infinitely greater. Young people whose family history shows a marked consumptive tendency should not be placed in such a position as to bring them frequently into contact with patients suffering from phthisis.

As a rule phthisis is a disease of slow growth, but, nevertheless, it is highly infectious. The breath of a phthisical patient is capable of giving rise to growths of *Bacillus tuberculosis* on sterilized blood-serum; therefore, it would be well for persons suffering from phthisis to sleep apart.

Many delicate persons (susceptible to disease) have perished through sleeping with friends or relatives suffering from this disease.

Phthisis, as already stated, is a disease of slow growth, and 'the slow progress of the disease explains the cases of spontaneous cure effected by the expulsion of the microbe in the sputum, or by the tubercles passing into a cretaceous condition, which causes the destruction of the bacilli encysted in them.' Hence, also, the fact that all the causes which weaken the constitution—bad food, overwork, deficiency of oxygen by bad ventilation, inflammatory diseases, pregnancy, prolonged lactation, etc. —hasten the end of phthisical persons.

In the human body tubercle-bacilli, as is well known, completely disintegrate the various organs in which a tubercle can be developed. Phosphates and albuminous compounds have been largely found in the sputa and urine of phthisical patients.

Dr. Panoff (*Med.-Chi. Acad., St. Petersburg*, 1888) has recently shown that the sputa of patients suffering from phthisis contain a much larger amount of nitrogen than the sputa obtained from any other lung disease.

The following analyses give the average composition of human expectorations in health and in disease:

		Pulmonary Mucus in Health.	Phthisical Sputum.
	Water	95·55	94·31
Solid organic constituents	Mucin	2·37	2·28
	Extractive matters	0·80	2·01
	Albumin and fats	0·46	1·20
Ash or solid inorganic constituents	Sodium chloride, phosphates, potash, etc. ...	0·82	1·30
		100·00	100·00

Not only are phosphates, nitrogen, etc., lost by the expectoration, but 'it has been clearly shown that consumptives lose phosphates by the urine (Teissier); the diminution in body weight corresponds to the augmentation of calcium phosphate in the urine. Phosphates diminish in the urine after the ingestion of fatty materials and carbohydrates; cod-liver oil, without any doubt, reduces the phosphatic loss (Hanot); it is the same with feculent substances, which are so useful to phthisical patients.'

According to Quinquaud (*Comptes Rendus*, vol. lxxii., p. 487), the hæmoglobin of the blood gradually diminishes during the course of tuberculosis (*i.e.*, from 13·16 per cent. [normal] to 4·8 per cent. in the last stages of the disease).*

* See Würtz's *Traité de Chimie Biologique*, p. 370; also Quinquaud's *Traité Technique de Chimie Biologique avec Applications à la Physiologie.*

We have spoken at considerable length of the bacillus of phthisis and its activity within the system, but further remarks concerning various methods of treating the disease—by directly attacking the bacilli—will be given in a subsequent chapter.

Bacillus Anthracis.

'Woolsorters, tanners, hide-dressers, and others who deal in skins, are liable to a remarkable form of blood-poisoning, due to inoculation of some part of the body with the *Bacillus anthracis*—a microbe which flourishes in the blood and tissues of the body of man, sheep, and other animals. The spores of this bacillus wonderfully resist the action of heat and drying. The skin or any part of the mucous tracts may be the point of inoculation. On the skin this organism sets up at the site of inoculation a sort of hard boil with a black centre; if this malignant pustule is cut out further infection may be prevented. But the lymph and blood streams may be invaded and the bacilli develop therein, causing œdema, congestion, and ecchymoses, partly by mechanical obstruction, the bacilli being cultivated, so to speak, in the capillaries.'

Bacillus anthracis (5 to 20 μ long and 1 to $1\frac{1}{4}$ μ broad) is the actual cause of anthrax, splenic fever, or malignant pustule.

This microbe, often appearing in masses of filamentous threads (Fig. 38), produces oval-shaped spores; and when either the microbe or its spores are hypodermically injected into mice, guinea-pigs, rabbits, etc., they die with all the characteristic symptoms of anthrax. According to Dr. Klein, 'all rodents and herbivorous animals are susceptible to anthrax; rats are, however, infected with

difficulty; pigs are very insusceptible, and so are dogs and cats. Infection of animals can be produced by inoculation into the skin and subcutaneous tissue, intravascular injections, and by inhalation of spores.'

The microbes have been found in the blood, spleen, and other organs; also in the urine and fæces of animals suffering from or dead of anthrax.

Bacillus anthracis grows in nutrient gelatine, agar-agar, bouillon (neutral), and on boiled potatoes 'at all temperatures between 15° and 43° C., best between 25°

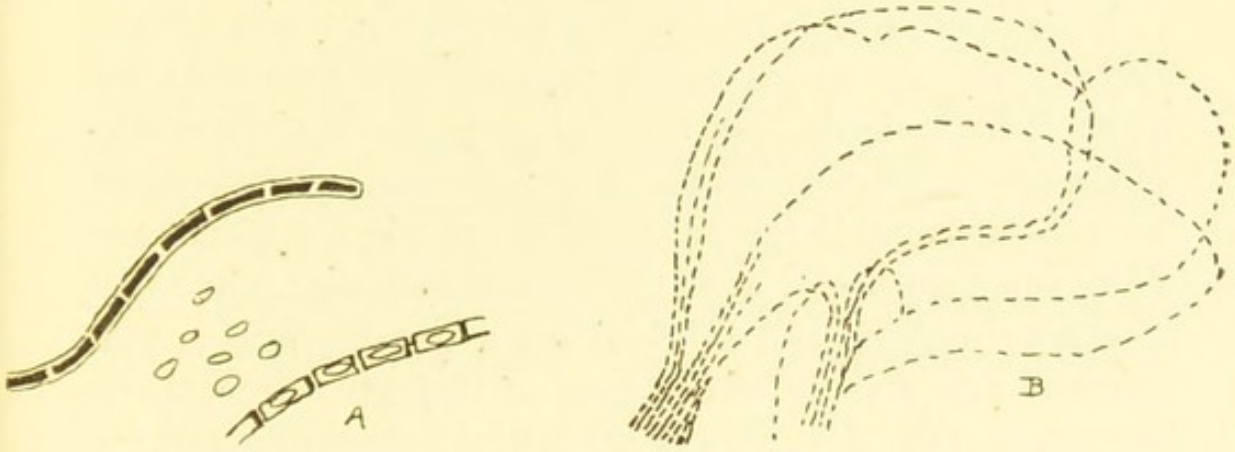


FIG. 38.—BACILLUS ANTHRACIS.

A = Chains of bacilli with spores (× about 1,200 diam.). B = Convolutions of bacillary threads (× 320 diam.).

and 40° C.' Free access of air (oxygen) is essential for *Bacillus anthracis* to produce spores, no spores being formed when the microbes grow in the depth of a liquid medium; and in such circumstances they often degenerate. Successive cultivations of anthrax-bacilli do not weaken their power (*i.e.*, they retain their full virulence).

As already stated, woolsorters and others are liable to the attacks of this microbe. The mode of infection is either by the inhalation of spores, or by direct inoculation of a wound or scratch on the hands or face.

In regard to cattle, it has been shown that farm animals may be inoculated through the bite of flies; and Pasteur (*Bulletin de l'Académie de Médecine*, 1880) states that the casts of *Lumbricus terrestris* may contain the spores of splenic fever, at the same time possessing all their original virulence, and therefore animals grazing on the land are liable to infection.

If this be so, the burial of animals dead of anthrax should be prohibited, as it becomes a means of spreading the disease, the best method for the disposal of the dead carcases of diseased animals being cremation.

Both Klein and Koch do not accept Pasteur's idea of the manner in which cattle may become infected; but there is little doubt that sometimes pastures are a source of danger to farm animals. For instance, anthrax-bacilli have been found in the urine, fæces, and in the discharges from the mouth and nose of diseased animals; and 'they find a nourishing soil in decaying vegetable and animal matter, and having free access of oxygen from copious spores, so that the grass is extensively contaminated.'

Bacillus anthracis and its spores are readily stained in the following manner:

Cover-glass preparations of anthracic blood, etc., are floated in a *hot* alcoholic solution of fuchsine for thirty minutes. They are then decolourized in weak hydrochloric acid, and after-stained with methylene-blue. By this method the spores are stained red and bacilli blue.

Sections of tissues may be stained by the methods of Gram, Weigert, etc. (see Chapter II.).

As already stated, *successive* cultivations do not weaken the virulence of *Bacillus anthracis;* but if the microbe is cultivated in bouillon (neutral) at 42° or 43° C. for *twenty days*, an attenuated virus is obtained. This attenuated virus ('premier vaccin') protects animals against the

disease. To make the animals perfectly refractory, they are inoculated a second time with a vaccine of less strength ('deuxième vaccin').

The practical value of Pasteur's method for the protective inoculation against anthrax has already been alluded to in Chapter IX.; but the immunity or 'mithradatism' is not of a permanent character; for in 1883 it was proved that the duration of the immunity generally lasted about a year. 'It is, however, prudent to vaccinate every year, and to select for performing the operation a period when splenic fever has not yet become developed —in March and April. If the vaccinating is postponed until the fever is in the sheep-folds, there is the risk of attributing to vaccination the losses which in reality belong to the natural disease. Just as human vaccination cannot preserve from small-pox a patient who is already under the influence of small-pox, so the splenic vaccinations are powerless against a fever already in process of incubation.'

Although the duration of immunity against anthrax only lasts twelve months, this is about a third of the duration of a sheep's life (*i.e.*, considered from a commercial and economic point of view). In regard to vaccination against small-pox, the average duration of the immunity is ten years, or about one-seventh of a man's life. From these facts protective inoculation against anthrax is of the utmost value to the sheep-breeder and farmer.

Attenuated viruses for the protective inoculation against anthrax have also been obtained by exposing the bacilli to a temperature of 55° C., or to an aqueous solution of carbolic acid (0·5 to 1 per cent.), or sulphuric acid in a diluted form, as well as other chemicals.

According to Dr. E. Klein, F.R.S., the virulence of

Bacillus anthracis is also altered by passing it through different species of animals.*

Bacillus of Swine-fever.

This microbe (2 to 3 μ long), unlike *Bacillus anthracis*, is actively motile, but, like the latter, produces spores. It is the cause of swine-fever, swine-typhoid, or swine-plague. The bacillus of swine-fever has been found in the lungs, spleen, liver, intestines, and the serous membranes of pigs dead of the disease.

Dr. Klein† has shown that inoculations from pure cultivations of this microbe always produce the fever in susceptible animals (pigs, rabbits, mice, pigeons), with all the characteristic symptoms of the disease; but 'in rabbits, after several transferences, the virus becomes attenuated, and with this a mild form of the disease can then be produced, protecting the animal thus operated upon from a subsequent severe attack.'

The bacillus of swine-fever has been artificially cultivated in bouillon and hydrocele fluid, at a temperature between 30° and 42° C.; and pigs inoculated from such cultures are rendered proof against a fatal attack (Klein).

Bacillus Choleræ Asiaticæ.

After the important researches of Drs. Macleod and Milles (*Proc. Roy. Soc. Edinburgh*, vol. xvi., p. 18), there is little room for doubt that Koch's 'comma-bacillus' is the real cause of Asiatic cholera.

This curved bacillus, from 1·5 to 2·5 μ long, is aërobic, motile, and is reproduced by fission.

* *Report of Medical Officer of Local Government Board*, 1882.
† *Ibid.*, 1877-78.

Nearly all observers of this microbe have described what are called involution forms. These are characterized by irregularities of shape, so that it requires reinoculation and growth under ordinary favourable conditions to determine that the specimens having these appearances are indeed pure cultivations of comma-bacilli.

'Ceci and others have described certain spore-like appearances in involution forms, but they are now regarded as dying or dead parts of the rods, such parts staining very slightly or not at all.'

The microbe has been found in the 'rice-water' stools formed by the desquamation of the mucous membrane of the intestines. It has also been found in the intestinal follicles and in the sub-epithelial spaces, and probably in the kidneys and urine.

Comma-shaped bacilli 'have been discovered in other diseases of the alimentary canal, in the fluid of the mouth of normal persons (Lewis); in old cheese (Denike),' etc.; but Drs. Macleod and Milles (*loc. cit.*) have shown that these microbes are entirely different from Koch's bacillus.

The microbe is always present (especially in the collapse stage) in Asiatic cholera, and it has not been found apart from this disease, and disappears from the body with the disease. Its habitat is the intestinal canal.

Nicati, Rietsch, Koch,* Ermengen, Watson Cheyne, and others, have reproduced the disease in dogs and guinea-pigs.

Koch maintains that he has reproduced the disease, but his experiments are not as yet regarded in this

* See his new experiments, 'Etiology of Cholera,' in Laycock's *Microparasites and Disease.*

country as sufficiently conclusive, and the objections urged are:

(*a*) 'The symptoms produced in the guinea-pig are not those of cholera in man, there being no purging, vomiting, or cramps.'

Dr. Macleod says: 'There seems to be little doubt that Pasteur has produced hydrophobia both in dogs and rabbits by material taken from the disease in man; but there seems to be as little doubt that the symptoms of that disease in man, the dog, and the rabbit, do not correspond in any two out of the three. Is it to be expected that cholera in the guinea-pig will present the same symptoms as in man? Will anyone maintain that a given case in man without vomiting, purging, or cramps is not one of Asiatic cholera? Such cases are certainly met with in man, and what is here the exception may be the rule in the guinea-pig.'

(*b*) 'It is objected that death and the *post-mortem* appearances in the animal experimented with may be the result of some other cause than cholera.'

Dr. Macleod (*Proc. Roy. Soc. Edinburgh*, vol. xvi., pp. 27-35) answers this objection by showing that he has obtained in guinea-pigs all the characteristic symptoms of cholera. The *post-mortem* examination of animals dosed with comma-bacilli revealed the following: 'The blood was fluid, thicker and darker than natural; the tissues of the thoracic and abdominal walls were markedly dry; the small intestine was throughout distended, congested and paralyzed-looking, and occupied a much larger proportion of the abdominal cavity than usual. The cæcum was distended with fluid or semi-fluid contents, . . . mucous flakes were abundant . . . and the comma-bacilli were demonstrated microscopically, and by cultivation, as in man.'

'On floating the bowel in water, the stripping of the epithelium could be well demonstrated.'

(*c*) 'The strongest reason for not admitting this kind of bacillar relation to the disease is this, *that no bacilli exist in the blood* or any other tissue of patients suffering from cholera.* Possibly, the blood is *not* the most suitable soil for the growth of the microbe in question; and certainly 'the first manifestations of the disease are in the *alimentary canal*, and are only followed by the general constitutional disturbances, while a marked departure from the normal condition is met with in the small intestine and its contents after death' (Macleod).

Drs. Macleod and Milles conclude their important paper (*loc. cit.*) with the following remarks:

(1) 'The comma-bacillus of Koch is invariably present, and associated with certain changes, in the small intestine in cases of Asiatic cholera.

(2) 'There is no evidence to show that it is a *normal* inhabitant of the human alimentary canal, and therefore no proof for the assertion that it is a result of the disease.

(3) 'The means used to introduce the comma-bacillus into, and those used to lessen the peristalsis of, the small intestine of the guinea-pig, cannot be regarded as causing appearances like those of Asiatic cholera, or as causing the death of the animal, far less a mortality of over 60 per cent.

(4) 'Pure cultivations of the comma-bacillus introduced into the stomach under the precautions described are pathogenic to the guinea-pig.

(5) 'Injected with similar precautions, the contents of

* Klein and Gibbes in the *Government Report of Cholera Investigation in India*, 1885, p. 32; and *The Bacteria in Asiatic Cholera* (Klein).

the ileum from those animals killed by injections of pure cultivations of the bacilli act in the same manner as pure cultivations of that organism.

(6) 'The organism multiplies in the small intestine of the animal, and there is associated therewith changes similar to those in man in Asiatic cholera.

(7) 'As there are conditions which favour the passage alive of the bacillus through the stomach of the guinea-pig, and also conditions which favour its multiplication

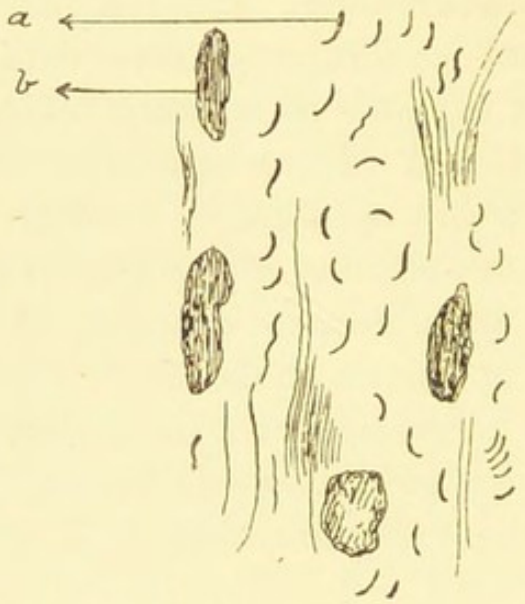


FIG. 39.—KOCH'S COMMA-BACILLUS.
From contents of a cholera intestine. *a* = bacilli ; *b* = remains of epithelial cells (× 600 diam.).

in the small intestine of that animal ; so in man, as there cannot be a doubt that the organism finds conditions favourable to its multiplication in his small intestine, it must have found conditions favourable to its entrance alive, through, in all probability, the mouth and the stomach.

(8) 'There is strong evidence, therefore, for regarding the comma-bacillus of Koch as the cause of Asiatic cholera.'

The comma-bacillus (Fig. 39) grows well in neutral

bouillon, milk,* nutrient gelatine (slightly alkaline or neutral), agar-agar, and on boiled potatoes at 16° to 40° C.

Cold does not kill it, for it has been ascertained that the microbe is still alive at – 10° C.

Dr. Cornil has shown that potable water 'can serve as its vehicle, but does not supply sufficient nutriment, so that it soon disappears.' The microbe, however, cannot live in stagnant or distilled water.

It has already been stated that the composition of the blood is considerably altered in cholera, *i.e.*, it often may be 'dark and tarry-looking.' This is possibly due to the indirect action of the microbe, as the microbe aids in formation of one or more highly poisonous alkaloids (see Chapter V.). The composition of the blood in cases of cholera† is represented in the following table :

	In Cholera.	In Health.
Water	740·000	780·150
Fibrin	11·000	2·104
Albumin	110·420	65·091
Globulin	124·460	133·003
Hæmatin		3·961
Fat	...	3·740
Extractive matter and salts	14·120	11·951
	1000·000	1000·000

The comma-bacillus of Koch is readily stained by the following methods :

(1) The fluid containing the microbe is spread and dried on a cover-glass ; then stained with an aqueous solution of fuchsine, washed with water, dried, and mounted in Canada balsam.

* See Heim's paper in *Arb. a. d. k. Gesundh.*, vol. v., p. 294.

† See also Würtz's *Traité de Chimie Biologique*, p. 382.

(2) The hardened sections of the intestines are placed in a strong aqueous solution of methylene-blue for twenty-four hours, and finally treated in the usual way.

Spirillum Obermeieri.

This microbe (16 to 40 μ long) is the cause of relapsing fever (the jungle fever of India), and was first discovered by Obermeier (*Centralblatt für Med. Wissensch.*, 1873), in the blood of patients suffering from this disease. Carter* reproduced the disease in monkeys, in whose blood they (the microbes) were found in great numbers.

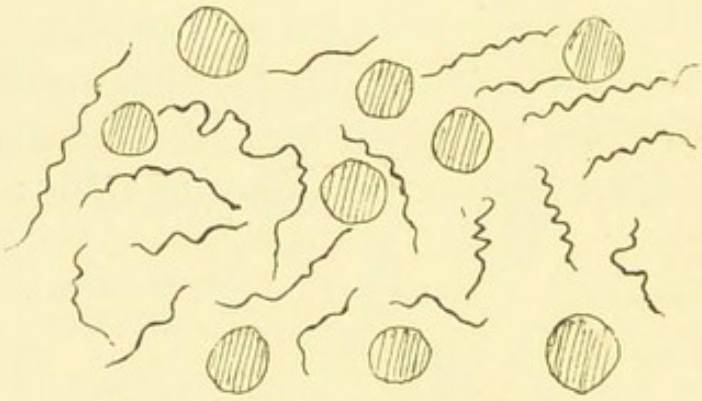


Fig. 40.—Spirillum Obermeieri.

They have also been found in the lungs, liver, kidney, etc. (Carter).

These microbes (Fig. 40), which are motile, exhibit spiral forms, and, according to Albrecht (*St. Petersb. Med. Woch.*, 1879), produce spores. They have been artificially cultivated by Koch.†

'The microbes only occur during the relapses, and are absent during the non-febrile intervals.'

The period of incubation of relapsing fever is about a week. The invasion is sudden, with a severe rigour and prostration.

* *Lancet*, vol. i., p. 84, and p. 662.
† *Deutsche Med. Woch.*, vol. xix.

'The symptoms of the disease are those of high fever with bilious vomiting, epigastric pain and tenderness, and swelling of the spleen and liver. Jaundice is common. Crisis ends the first attack at the end of a week; the relapse usually occurs on the fifth day after the crisis, and lasts about five days. Many relapses may occur.

'The chief complications are bronchitis and pneumonia, rheumatic pains, diarrhœa, dropsy, and ophthalmia. Pregnant women abort.'

Spirillum Obermeieri is easily stained with fuchsine, gentian-violet or Bismarck-brown.

Bacillus in Tetanus.

This microbe, which is most likely the cause of tetanus, is about 1 to 1·2 μ long. It produces spores, and inoculations in mice and rabbits reproduce the disease. It has been cultivated on blood-serum.

According to Professor Sormani (*Atti d. Reale Istituto Lombardo di Scienze e Lettere*, vol. xxii.), the bacilli and spores may be drawn into the respiratory passages by inhalations, or even injected into the bronchial tubes, without producing tetanus. The bacillus is anaërobic, and is unable to develop in the presence of oxygen. The tetanus called rheumatic is thought to be of traumatic origin really, the wound being slight, and but little of the virus introduced. Tetanus is most common in Northern Italy; its maximum being in Lombardy and Emilia, where people frequently work in the hot season with bare feet.

They are attacked in the proportion of 100 males to 30 females; and the mortality in the hospitals is about 44 per cent. of those attacked.

Oidium Albicans.

The fungus *Oïdium albicans*, or *Saccharomyces albicans*, is the cause of thrush. It is found on the mucous membrane of the mouth of infants, causing whitish-looking patches on the tongue, gums, and soft palate. Like the higher fungi, this plant (Fig. 41) is composed of hyphæ and spores, which take root in the mucous lining of the mouth. The spores are produced by the division

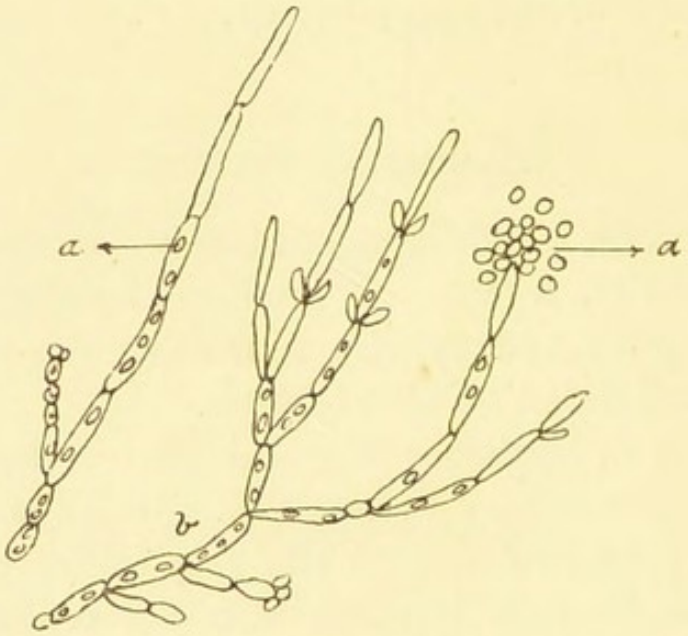


FIG. 41.—OÏDIUM ALBICANS.
a = spores ; *b* = branched hyphæ.

of the terminal cells, or sometimes by endogenous formation within the hyphæ.

In concluding the chapter, it must be ' admitted that the action of pathogenic microbes on the system is complex, and may be analyzed as follows : (1) The action of a living parasite, which is nourished and multiplies at the expense of the fluids, gases, etc., of the system ; (2) the formation by the parasite of a poisonous substance

(ptomaïne), of which the elements are derived from the invaded organism, and it acts as a poison on this organism.'

It must be borne in mind that 'the theory of ptomaïnes *without* microbes is, however, inconsistent with an impartial study of facts. It is true that a suitable filtration will separate the ptomaïne from its microbe; . . . but when this microbe is separated from the original liquid, and transferred (successively) to nourishing media, so as to purify it from every foreign element, it continues to produce its characteristic ptomaïne [or ptomaïnes], which is manufactured completely at the expense of the culture fluid. There is no ptomaïne without its special microbe, any more than there is ergotine without *Claviceps purpurea*, or vinegar without *Mycoderma aceti*' (Trouessart).

CHAPTER XII.

RECENT EXPERIMENTS ON THE DESTRUCTION OF MICROBES IN CERTAIN INFECTIOUS DISEASES.

We have already seen (Chapter X.) that certain reagents are capable of destroying a large number of pathogenic as well as non-pathogenic microbes; therefore, it is only reasonable to infer that these substances might be useful in the treatment of certain infectious diseases—especially those diseases where the microbes have been found to reside in the blood.

In such cases a rational and scientific method of treatment is found in the introduction of germicidal agents directly into the blood by means of hypodermic injections. By so doing the microbes, *a priori*, would be destroyed, and the disease would be at an end. As these ideas have been tested practically—more particularly in connection with PHTHISIS and its microbe—we propose to give the results, etc., of our experiments and observations.

Phthisis.

Before describing the proposed treatment of phthisis, certain introductory remarks are given concerning the disease.

Phthisis, or tuberculosis, is known by various names, according to the parts of the body the disease may

happen to attack, or according to the kind of lesions it produces, or, finally, according to its general effect on the body. Thus, it is commonly called consumption, cheesy inflammation of the lungs, caseous pneumonia, tubercular pleurisy, caseous broncho-pneumonia, consumption of the intestines, tabes mesenterica, scrofula,* tubercular meningitis, etc.

For many years most of these conditions were supposed to be different diseases; we now know for certain that they are all forms of one and the same process, and caused by a microbe—*Bacillus tuberculosis*—which, growing in the blood and tissues, gives rise to tubercles, and which, by reason of its being thrown off from the diseased person or animal in quantity, renders the disease an infectious one.

Each tubercle or nodule is made up of cells, and in typical examples three forms of cells are present—at the periphery, small round cells, resembling the white corpuscles of the blood; next, a zone of cells, some two or three times the size of the former; and in the centre, one or more giant cells, which are protoplasmic masses, each containing twenty to a hundred nuclei. In addition to these elements, there are the tubercle-bacilli, found chiefly in the giant cells, which constitute the most important character of tuberculosis. The cell-element or elements, among which the microbes lie, are simply the expression of resentment on the part of the tissues affected, and evidence of resistance to the growth and development of the bacilli. Sometimes the tuberculous lesion is localized, as for instance in the lungs, the primitive effect of the microbe being purely local. But *local* tuberculosis may give rise to *general* tuberculosis,

* In all its local forms, scrofula contains the characteristic *Bacillus tuberculosis.*

for 'the bacilli may be spread either along the lymphatic spaces and vessels, or, finding their way into the blood, they may infect tissues at a great distance from their original source.'

Although phthisis (in all its forms) is essentially the result of the action of *Bacillus tuberculosis*, there are certain factors which render man and animals liable to contract the disease and receive the poison. These may be divided into *external* and *internal* causes.

Among the external causes are :

(1) Deficiency of oxygen by bad ventilation. The 'overcrowding of human beings, as in barracks, shops, schools, prisons ; the air under such conditions is both confined and microphytic.' As the air of towns is not so pure as that of the country, the mortality from phthisis is greater in the towns than in the country (Colin).

(2) Certain foods have been asserted to favour the development of tuberculosis. We know from the experiments recorded in the last chapter that phthisical matter taken into the system as food may give rise to phthisis. Thus, the milk* and flesh of tuberculous cows (if not thoroughly boiled and cooked) may lead to tuberculosis in man.

Among the *internal* causes which render man liable to the attacks of the tubercle-bacilli are the following :

(1) There are certain diseases which favour the development of phthisis. The phthisogenic or phthisis-producing diseases are :

Syphilis.	Anthracosis (miner's lung, etc.).
Diabetes.	Whooping-cough.
Measles.	

Ischæmia.

* See a paper entitled 'Changes in Milk by Udder Tuberculosis,' by Dr. V. Storch, in *Biedermann's Centralblatt für Agricultur-Chemie*, vol. xix., p. 105.

(*a*) As already stated, 'syphilis plays an important part in the production of tuberculosis.'

(*b*) According to Dr. Leyden, *Bacillus tuberculosis* has been found in the lungs and sputa of diabetic patients; and on the authority of Dr. Griesinger, forty-three per cent. of diabetic patients become tubercular, and develop the usual symptoms of the disease. The blood of diabetic patients appears to be a better medium for the growth of the microbe than pure blood.

(*c*) According to the observations of Barthez, Rillet, and others, ten per cent. of the patients who have suffered from measles become phthisical.

(*d*) Anthracosis* is a disease produced by the continued respiration of air containing mineral and other solid particles. These particles 'provoke pulmonary engorgements, metallic or mineral infiltrations, pneumonia of mechanical origin,' which may lead to phthisis (see Dr. Proust's *Traité d'Hygiène*).

(*e*) Whooping-cough, being more dangerous than measles, is decidedly phthisogenic in its action.

(*f*) Ischæmia and aneurisms are also phthisis-producers.

Besides the above pathological causes tending to pave the way for bacillary phthisis, there are physiological and local causes.

All individuals have not equal power of resisting the attacks of microbian diseases. Some are *predisposed* to disease—that is, the system is of a lower standard than usual; and this lower standard may be inherited or acquired. Predisposition to phthisis may be *acquired* through insufficient nutrition, deficiency of oxygen by bad ventilation, etc.

* Miners', potters', and knife-grinders' phthisis.

(2) Among the physiological causes which are phthisogenic are the following:

Pregnancy.
Inheritance.
Starvation.

(*a*) If the patient is predisposed, 'pregnancy aids powerfully in the development of phthisis; and it is only by masking symptoms that pregnancy can appear to arrest its progress. The puerperal stage starts the process of tuberculization into singular activity; further, after the confinement, tuberculization often attacks the genital organs.'

(*b*) 'No individual circumstance plays such an important part in the development of phthisis as inheritance; but this must be made to comprise all forms of tuberculo-bacillosis, and not be limited only to the pulmonary form of phthisis.'*

While it is undeniable that phthisis runs through certain families, 'there is considerable doubt as to whether this is simply because the tissues are especially favourably disposed to nourish the tubercle-bacillus, or whether the bacillus is actually contained in the ovum or spermatozoon, and so becomes a constituent part of the embryo and fœtus, and develops within the uterus. In favour of the latter, it may be said that Baumgarten has actually, in the rabbit, observed the bacillus within the ovum, and, further, that the bacilli have, by different observers, frequently been seen mingled with active spermatozoa. In one striking case, found by Professor Johne, of Dresden, an unborn calf of seven months' intra-uterine growth was discovered to present numerous tubercles in its lungs,

* For a full exposition of the theory of heredity and the facts which support that theory see Darwin's *Origin of Species*, and *The Descent of Man*.

showing that if the ovum had not been inoculated, it had received the virus through the placenta, which amounts practically to the same thing. Similar intra-uterine infection has been shown to be more than probable in the human being.'

It is possible that the tissues, etc., of a person born of a phthisical parent or parents form a suitable soil for the subsequent growth and development of the tubercle-bacillus; and Professor August Weismann's researches on heredity* substantially support such an idea. He says: 'Fertilization is merely a union of the *hereditary tendencies* of two individuals; tendencies which are bound up with the matter of nuclear loops; the cell-body of the ovum and spermatozoon is indifferent in this connection, and plays merely the part of a nutritive matter which is modified and shaped by the dominant idioplasm of the nucleus in a definite way, as clay in the sculptor's hand. . . . There certainly is *a material carrier of heredity in the ovum;* it certainly can be transported from nucleus to nucleus; it certainly can be modified in the process, or can remain the same; and even the supposition that it is able to stamp its own character on the cell contains nothing which seems to us impossible and non-existent; on the contrary, we are able now to state that it is so, even if we do not understand in what wise it happens.'

It has been stated, on reliable authority, that from 10 to 14 per cent. of the deaths among human beings are due to the activity of the tubercle-bacilli; and about 50 per cent. of the deaths from phthisis are hereditary. The mortality among women is 12 per cent. greater than

* See *Nature*, vol. xli., p. 317; and the English edition of Dr. Weismann's *Essays*.

that of men; and 'the disease is more often inherited by women than by men.'

Pseudo-heredity.—'It has been contended that many cases called hereditary are really examples of contagion.' For instance: Minnie, a tuberculous patient, aged 22 years, who had inherited phthisis, was an intimate friend of Charlotte, aged 24 years, who was untainted with the disease (either hereditary or acquired), and at the same time had never had any of the phthisogenic diseases. Charlotte slept with Minnie for months at a time, and over a number of years without becoming tubercular. However, Charlotte (at the above age) contracted a severe cold, which, unfortunately, was neglected, and thereby her constitution, becoming of a lower standard than usual, offered a fertile soil for the growth and development of *Bacillus tuberculosis*. By breathing an infected atmosphere, Charlotte* became distinctly tubercular, and died of pulmonary phthisis at the age of 26 years. Minnie, who has since taken a sea voyage, is now better—the phthisical complaint being entirely cured. The above is an authentic case of direct infection, for there was no hereditary predisposition, or phthisis in a latent form.

Dr. Debove,† of the Hôpital de la Pitié, Paris, gives the following facts concerning the *infectious* nature of phthisis: 'Jean, a tuberculous patient, was married to Antoinette, a young woman with no previous tendency to tuberculosis. Jean died, and his wife became phthisical.‡ She was remarried to Louis, who had likewise no phthisical taint; Louis and Antoinette both

* Charlotte's mother is still living, but her father died of *angina pectoris* at the age of sixty-two years.

† *Leçons de Clinique Médicale* (1883).

‡ See also Harvey's book: *The Fœtus in Utero as inoculating the Maternal with the Peculiarities of the Paternal Organism*.

died of phthisis.* The niece of the latter, equally without phthisical taint, contracted the disease in nursing her aunt, then married, and her husband was, in his turn, attacked by phthisis. All these people resided in a place in which it was easy to verify the absence of hereditary taint.'

'A young woman without hereditary taint nursed a phthisical patient and contracted phthisis. She returned home, and communicated the disease to the six sisters with whom she lived. One sister survived, but she was not living with her family.'

'A soldier became phthisical while with his regiment, and was therefore discharged, and returned to his family. His father, mother, two brothers, and a neighbour who nursed them, became phthisical. Yet none of them were predisposed by hereditary taint' (Debove).

From these facts it will be perceived how easy it is to pronounce such and such a case as hereditary, when it is one of undoubted infection.

(*c*) Another physiological cause tending to render the body a suitable soil for the growth of the tubercle-bacilli is *starvation*, 'since it causes degeneration of the tissues, and diminishes thereby their resistance to the growth of the parasite microbes.' It goes without saying that such diseases as anorexia nervosa, malignant stricture of the œsophagus, etc., are phthisogenic.

Of the *local* causes tending to pave the way for bacillary phthisis, a *sedentary life* is one of the most powerful, especially if a sedentary person has a badly-formed or contracted chest.

Besides the physiological and local factors, and certain diseases (already alluded to) which are phthisogenic in

* See Weber's work: *The Communicability of Consumption from Husband to Wife.*

action, there are other diseases which *may* possibly give rise to phthisis. Among these Dr. G. Sée gives the following:

Asthma and emphysema.
Syphilis of lung.
Cancer of lung.
Bronchiectasis—Dilatation of bronchi.*
Engorgement of bronchial glands.
Hydatid cysts.
Pneumothorax.

Of course, these diseases may simulate *other* diseases besides phthisis; therefore, 'in most of these cases the best and only diagnostic method is the microscopic examination of the expectoration.'

The modes in which tubercle-bacilli enter the body have been mentioned in Chapter XI. They are—inhalation, swallowing, direct inoculation, and heredity. But we intend to speak a little more fully concerning the first three modes in which the virus of phthisis becomes parasitic in man.

(1) *Inhalation.*—This is the commonest mode of infection. Koch and others have shown that animals after a few inhalations of tubercular sputum, disseminated in a spray, readily become infected—giving rise to miliary tubercles, caseous tubercles, etc.; and even such insusceptible animals as cats, dogs, etc., become infected with bacillary phthisis.

Dr. A. Ransome (*Proc. Roy. Soc.*, 1882) has isolated tubercle-bacilli from the breath of certain cases of advanced phthisis; and the author† of the present work has fully confirmed Ransome's researches.

* Germicidal or antiseptic inhalations, such as terebene, iodine, eucalyptus, 'sanitas,' and stimulant expectorants, are most valuable in the treatment of the disease.

† A paper read before the Royal Society of Edinburgh on March 18, 1889.

The dust from dried phthisical sputa is also capable of transmitting phthisis; as already stated, dried sputum retains its virulence for months. Therefore it is self-evident that a handkerchief used by a phthisical patient is a source of danger, the microbes and their spores being inhaled by nurses and others;* although it must not be forgotten that 'the influence of habit or acclimatization in enabling us to resist respiratory contagion is marked.'†

Cohabitation, as already stated, is a source of infection. 'There are many well-established cases of matrimonial contagion, but it is probable that the mode of transmission was by contamination of air with bronchial secretions;' for genital contagion has hardly been demonstrated with any degree of certainty.

(2) *Swallowing.* — Rabbits, guinea-pigs, fowls, pigs, etc., become tubercular when fed upon tubercular tissues, sputum, saliva, milk, pure cultivations of the tubercle-bacillus, etc. Herterich has recorded the case of a healthy widow with two children, who married a second husband who had phthisis, by whom she had three children. She herself became phthisical, and her two youngest children developed deep yellow-coloured ulcers on the mouth and fauces, and ultimately general tuberculosis. *The children had been fed on food which the mother had previously chewed.*

Reich records ten cases of tubercular meningitis in a country village, occurring within fifteen months in the practice of a phthisical midwife, who was in the habit of sucking the mucus from the mouths of the newborn infants, and of blowing air into their lungs.

* It may be mentioned that Brown-Séquard and D'Arsonval (*Comptes Rendus*, vols. cvii.-cix.) have stated that the *breath* exhaled by patients suffering from pulmonary tuberculosis contains a poisonous substance or substances (!); but the author doubts this assertion.

† See *Pulmonary Consumption*, by Drs. C. J. and C. T. Williams.

Although there is a certain amount of danger through ingesta—this mode of infection cannot be compared with the danger arising from the inhalation of finely-divided phthisical matter.

(3) *Direct inoculation.*—When tubercular matter is introduced subcutaneously, the disease is reproduced with great certainty. At first the disease is local, *i.e.*, an inflammatory swelling makes its appearance at the seat of infective inoculation. The bacilli causing this swelling (tubercle) grow along the lymphatic vessels, and finally reach the nearest glands. 'These become diseased, and from them the microbes pass through the large lymphatic vessels, which subsequently discharge into the veins, so that the virus is distributed throughout the body, and the disease, at first local, becomes general, affecting most of the organs—especially the lungs.'

Some experimentalists have introduced various irritant substances (of a non-tubercular origin) into the lymphatic system as well as into the peritoneum, and state that they have produced granular nodules similar to those in tuberculosis. But Drs. Toussaint and Martin and others have shown that these nodules '*never* give rise to tuberculosis, and have not the power of producing even local inflammation. Thus the *specificity* of tubercle is demonstrated despite the anatomical similarity of the common lesions.'

It has been recorded that man has become affected with phthisis by the bacilli entering the system 'through a scratch or sore on the hands which have been brought in contact with tubercular sores or secretions.' And it is not improbable that the common house-fly may disseminate the virus of phthisis by inoculating open sores on the hands and face. Drs. Spillman and Haushalter (*Comptes Rendus*, vol. cv.) have observed the common

house-fly in hospitals for consumption upon the expectorations of the patients. Some of these flies were caught and placed under bell-glasses, and it was subsequently found that their excrements contained numbers of the tubercle-bacilli.

As already stated, the sweat of phthisical patients often contains tubercle-bacilli; and a case is recorded by Dr. Debove (*loc. cit.*) where the clothes of a girl, who had contracted phthisis while at school and died of that disease, 'passed to her sister, who died of the same disease. A third sister died under like conditions.'*

This is not very wonderful, when the author (*Proc. Roy. Soc. Edinburgh*, vol. xv., p. 44) has shown that phthisical *saliva* maintains its virulence after drying; for the envelopes moistened by phthisical patients are capable of giving rise to growths of *Bacillus tuberculosis* in sterilized blood serum.

From the above remarks it will be gathered that phthisis may be contracted by direct infection, and that the mode of infection is not always the same.

We now offer a few remarks concerning the diagnosis of phthisis. On the authority of Dr. Germain Sée† (the distinguished professor of clinical medicine in the Faculty of Paris), phthisis presents itself under *four* forms:

(1) '*Latent phthisis*.—It remains latent by physical signs, by functional symptoms, and is only revealed by a micro-chemical examination of the expectoration.' Let it be borne in mind that although phthisis 'varies in its forms, its course, periods, and manifestations, it is always the same phthisis.' The tubercle-bacilli are always pre-

* It may be remarked that there was no hereditary taint in this case.

† *Bacillary Phthisis* (English translation by Dr. Weddell).

sent in genuine cases of tuberculosis, 'and not in any other disease which may simulate it.'

(2) '*Distinct phthisis*.—Distinct phthisis is recognised by auscultation and percussion, but it is often confirmed only by an examination of expectorated matters.'

Therefore, the search for tubercle-bacilli is of the utmost importance to the practitioner, who should be as skilled in using the microscope as the stethoscope or midwifery forceps.

Drs. Fräntzel and Palmers (*Berl. Klin. Woch.*, 1882-83) have shown that 'in 120 cases of distinct phthisis, bacilli were found 120 times, whilst they were totally wanting in other patients attacked with pulmonary affections.' 'The matters expectorated by the phthisical patient, whatever be the period of his malady (often even at the start) will always, like tuberculous matter itself, contain bacilli, which constitute the most important element of tuberculosis. These materials, then, are unchallengeable witnesses of the malady, and constitute its characteristic. It is, so to speak, the signature of tuberculosis; and this is so true, that if you find it in the expectoration, or rather in any morbid product whatever, you may be certain by the presence, well and duly established, of bacilli, that you have to deal with a tubercular patient, and in the particular species with pulmonary phthisis' (Sée).

(3) '*Masked phthisis*. — This category comprises phthisis which takes the mask (*a*) of another pulmonary disease (bronchitis, pneumonia, congestion, emphysema); (*b*) or of an extra-pulmonary thoracic affection (laryngitis, pleurisy, fever, and circulatory troubles); and, lastly (*c*), of an extra-thoracic lesion, as genito-urinary or intestinal tuberculosis.'

'In the presence of pseudo-phthisis, that is pleurisy,

nephritis, spasmodic cough, hæmoptysis, which have given good reason to believe in the existence of tuberculosis, the *absence* of bacilli will enable us to give a positive opinion against tubercle. On the other hand, says Dr. Weddell, 'we see in Sée's clinical observations phthisis simulating typhoid fever, metroperitonitis, simple pleurisy with friction. Nothing would have made us suspect the tuberculous nature in such complex cases. The presence of bacilli alone permits the affirmation.'

(4) '*True and false cavernous phthisis*.—The last group which comprises phthisis arrived at complete development presents, nevertheless, a doubtful semeiology. In other words, although characterized by the positive signs of induration and pulmonary excavation, it often resembles indurations of another nature—tumours, and simple bronchial cavities. Reciprocally the same maladies may simulate extensive and cavernous phthisis; this double cause of error will justify the terms true and false cavernous phthisis.'

In advanced phthisis other lesions are produced besides those of a strictly tubercular nature; for 'it is not solely tubercle which results from the action of the bacillus (*i.e.*, *Bacillus tuberculosis*). There are also apparently simple inflammations, fatty degenerations, amyloid transformations, hæmorrhage with infarctus,* thrombosis, emboli or dropsy; failure everywhere, denutrition in all its forms, in all organs. Here is the balance-sheet of phthisis.'

'The pathological scale commences by functional trouble, and finishes by the most grave anatomical alterations. The same pathological series is found for all the organs, the heart and its coverings, for the

* From *farcio*, I stuff.

kidneys, liver, spleen, for the nervous system. Everywhere lesions of organs are linked to *chemical changes* —that is, oxidation.'

We have already alluded to the fact that the composition of the blood, urine, etc., is greatly altered in phthisis. This alteration, along with the various lesions and symptoms of the disease, is the result of the action of numberless tubercle-bacilli. Hence the following excellent definition given for phthisis by Dr. Germain Sée: 'Phthisis is a virulent malady, due to a special microbe, which is specific, always inoculable to animals, transmissible from suffering men to healthy men by way of direct contagion, but much more frequently by heredity, very frequently localized in a single organ, *and thus to be cured without compromising the rest of the body*. The bacillus, on the contrary, whilst it lives, or whilst it invades all the economy, whilst it multiplies there, constitutes the danger; it will continue its ravages.'

Having spoken of the nature of phthisis, its various lesions, the diseases which favour its development, and the different modes in which the microbe attacks man, we now proceed to discuss certain scientific methods (*recently* introduced) for treating the disease by directly destroying the microbes *in situ*. Before coming to his own experiments and observations, the author intends to allude to the work of others in the same direction.

Bergeon's Method.

'. . . Diseases, desperate grown,
By desperate appliances are relieved.'
(*Hamlet*, iv. 3).

In the *British Medical Journal* of December 8th, 1886, there appeared an article from the pen of Dr. J. H. Bennet (of Paris), describing Dr. Bergeon's treatment of

pulmonary phthisis by means of anal injections of a mixture of *pure* sulphuretted hydrogen and carbon dioxide gases. Bergeon* found that these gases were absorbed by the intestines without any poisonous effects. It will be remembered that the illustrious physiologist, Claude Bernard, more than thirty years ago discovered that certain gases could be injected into the intestines without toxic effects. Bergeon repeated these experi-

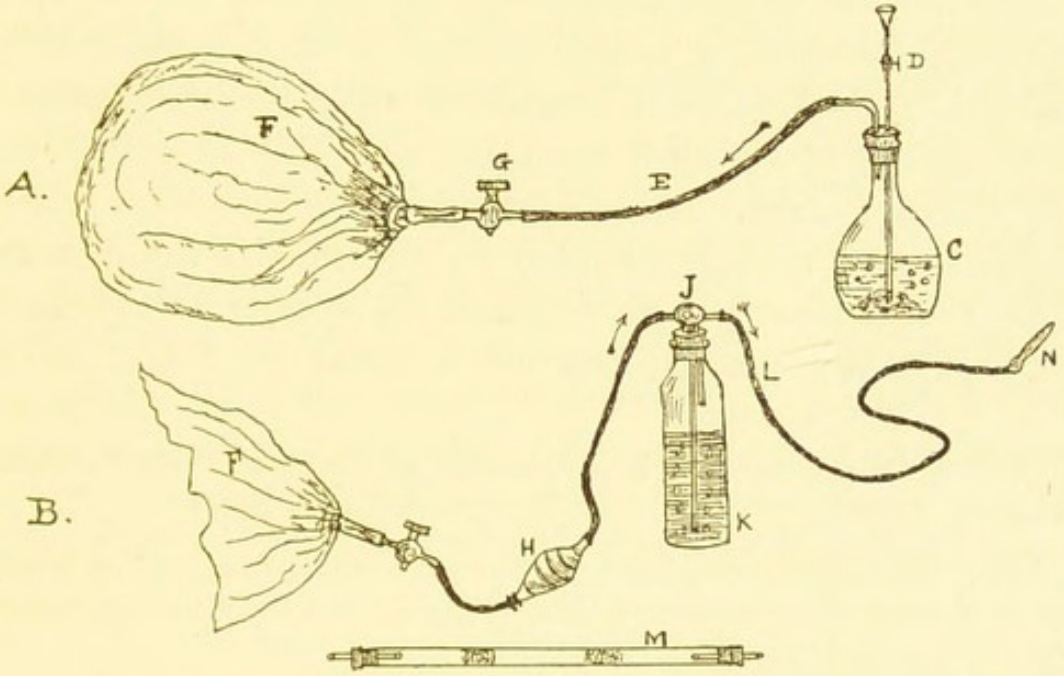


FIG. 42 (A and B).—THE BERGEON-MOREL APPARATUS.

ments on animals (rabbits, etc.) with the same result. He then tried the injection of large quantities of carbonic acid gas in human beings, to the extent of two, three, or four litres, two or three times in the twenty-four hours, with absolute immunity from any toxic effect. 'The gas is expelled by the mouth in the course of a few minutes after injection, without oppression, dyspnœa, or intestinal disturbance. The carbonic acid gas appears to be innocuous, but without any decided

* Professor in the School of Medicine, Lyons.

medicinal effect. It is the sulphuretted hydrogen, a powerful germicide (see Chap. X.), which appears to exercise the therapeutic influence. Dr. Bergeon, having obtained an innocuous medium in carbonic acid, began a series of experiments with various medicinal agents, which would take up too much space to enumerate, stopping at last at sulphuretted hydrogen, as evolved from natural mineral waters. He found the most efficacious to be that of the *Eaux Bonnes* in the Pyrenees. By passing the carbonic acid gas through a bottle charged with this water (Fig. 42 B),* it becomes impregnated with the sulphuretted hydrogen gas which it contains, and this is well borne by the intestines. It is absorbed by the intestinal venous system, and rapidly exhaled by the mouth through the lungs. In two or three minutes, on applying the nose to the patient's mouth, the air emitted is found to be

* Bergeon's apparatus is obtainable at La Pharmacie Centrale, 7, Rue de Jouy, Paris, and may be described as follows:

(1) *Preparation of* CO_2 *gas* (Fig. 42 A).—About 3 tablespoonfuls of sodium bicarbonate are placed in the flask C, and dilute sulphuric acid (1 in 4) is added by means of the funnel D. The gas evolved passes to an india-rubber or gutta-percha balloon (which *must* be empty of air). When the balloon is filled the tap G is turned off.

(2) *Preparation of mixed gases* (Fig. 42 B).—The balloon filled with CO_2 is then connected to a gutta-percha tube provided with a pressure sac H, and then to one end of the tube J, called a *barboteur*, which is introduced into the bottle K containing the mineral water. To this is attached the anal tube L N. If carbon disulphide is used, instead of the mineral water, it is placed on cotton-wool in a tube M. This tube is then fastened to the tube that fastens the *barboteur* with the anal tube.

When the mineral water (*Eaux Bonnes*) is used, about 6 oz. of hot water should be put into a large bottle and the half-bottle of *Eaux Bonnes* water added, so as not to quite fill the bottle. This is to warm the gas. When no mineral water is used, the carbonic acid gas (CO_2) is passed through warm water and then to the carbon disulphide (bisulphide of carbon).

We may remind the reader that any surgical instrument maker could easily make an efficient form of Bergeon's apparatus, and certainly at much less cost than the one obtainable in Paris. The price of the latter is about sixty francs.

tainted by the sulphur gas. Twenty minutes is the time prescribed for the slow, gradual injection of four litres' (7·04 pints). Bergeon performs this operation two or three times daily. It is stated that the abdomen becomes greatly distended, but without pain or discomfort, unless atmospheric air or *impure* gases are, from imperfect manipulations, injected with the medicated gas. If this be the case, griping pains (tormina) are the result of careless manipulation. 'Within half an hour after ceasing the injection, all the gas is absorbed and expelled through the lungs and mouth, the abdomen regaining its usual shape and softness.'

It has already been stated that the injection of four litres of the medicated gas produces no toxic effects; but one would expect this immense amount of gas thrown into the venous blood would interfere with retrograde nutrition, with the transformation and elimination of used-up nutritive material. This does not appear to be the case, as all the functions of life, of retrograde nutrition and elimination, are said to take place normally, although the treatment may have been used consecutively for several weeks. Dr. Bergeon claims great therapeutic effects for this medication. He has applied it, for the last few years, in more than *two hundred* cases, and the results have been successful to a degree that has surprised and astonished him. He says that in 'early phthisis, even in general acute phthisis (a form of the disease nearly always fatal), in two or three weeks there is generally an arrest, and in a few months a cure. Even in advanced, incurable phthisis great amelioration is obtained. The pulse is lowered, the temperature falls, the night sweats cease, the appetite returns, the expectoration rapidly diminishes, losing its purulent character, and the cough becomes less harassing and frequent. The

amelioration is also rapidly obtained in advanced laryngeal phthisis, when all local or constitutional treatment has failed to give ease, or to arrest the ulcerative process.'*

It may be mentioned that Dr. McLaughlin (Physician to the Philadelphia Hospital), in 1887, reported the cure of thirty patients, in the last stages of phthisis, by using Bergeon's method.

Bergeon's observations and experiments prove that sulphuretted hydrogen prepared from any other source than *Eaux Bonnes* mineral water, or carbon disulphide, gives negative results. But in the case of Mr. John Snodgrass, jun., of Glasgow (see later in this chapter), carbon disulphide gave much better results than the natural mineral water; for it is difficult (in this country) to obtain the latter fully charged with the gas.

Bergeon 'does not propose his method as a microbicide treatment, but merely as one that succeeds,' and that 'the injection of sulphuretted hydrogen is decidedly antiseptic and curative of local lesions.'

Dr. Bergeon not having given any details of the action of his medicated gaseous enemata on the vitality of *Bacillus tuberculosis*, the author† (in 1887) performed the following experiments:

A four-litre bag was filled with pure carbon dioxide

* Concerning the literature on Bergeon's method see the following publications:

Dr. Bergeon's papers in the *Comptes Rendus*, 1886, p. 176; and *Bulletin de l'Académie de Médecine* [second series], vol. xvi.

Dr. Morel's book: *Nouveau Traitement des Affections de Voies respiratoires d'après la Méthode du Dr. Bergeon.*

Dr. Dujardin-Beaumetz's paper in the *Bulletin de Therapeutique*, 1886.

Dr. Coghill's paper in the *British Medical Journal*, 1887.

Dr. Bennet's paper in the *British Medical Journal*, 1886.

Dr. Griffiths' paper in *Proceedings Royal Society of Edinburgh*, vol. xv., p. 49.

† *Proceedings Royal Society of Edinburgh*, vol. xv., pp. 51-53.

gas (prepared from pure sodium bicarbonate and dilute sulphuric acid). The gas was passed slowly through a half-bottle of *Eaux Bonnes* water (thoroughly impregnated with the H_2S gas), and then allowed to pass into a pure cultivation of *Bacillus tuberculosis* (Fig. 43). After

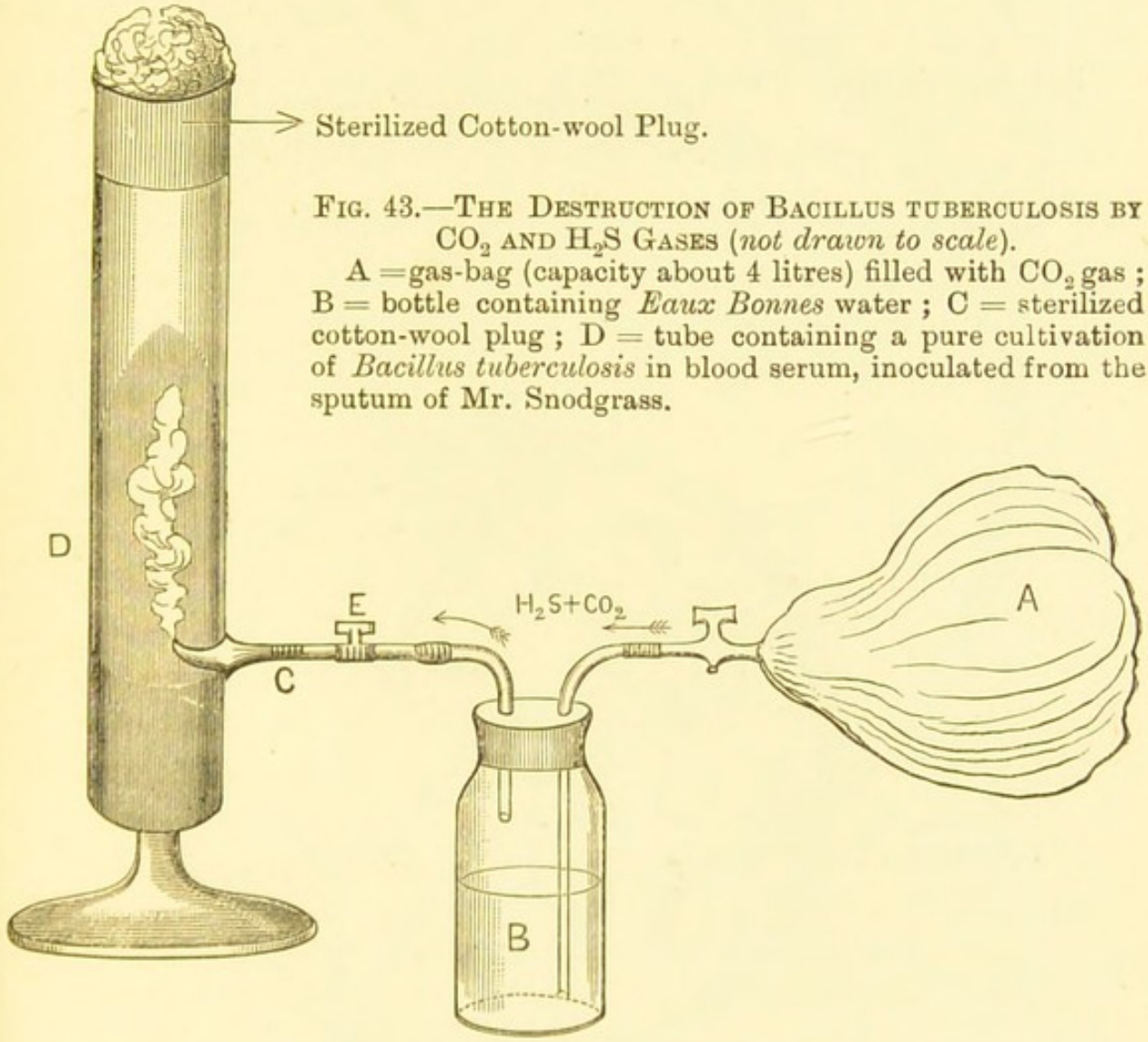


FIG. 43.—THE DESTRUCTION OF BACILLUS TUBERCULOSIS BY CO_2 AND H_2S GASES (*not drawn to scale*).
A = gas-bag (capacity about 4 litres) filled with CO_2 gas; B = bottle containing *Eaux Bonnes* water; C = sterilized cotton-wool plug; D = tube containing a pure cultivation of *Bacillus tuberculosis* in blood serum, inoculated from the sputum of Mr. Snodgrass.

all the gases had passed through the cultivation, the tap E (Fig. 43) was turned off. Ten tubes containing sterilized blood serum were inoculated with the growths which had been submitted to the action of the gases. The tubes so inoculated were subsequently placed in the

incubator at a temperature of 37° C. After forty days' incubation, no signs of any growths made their appear-

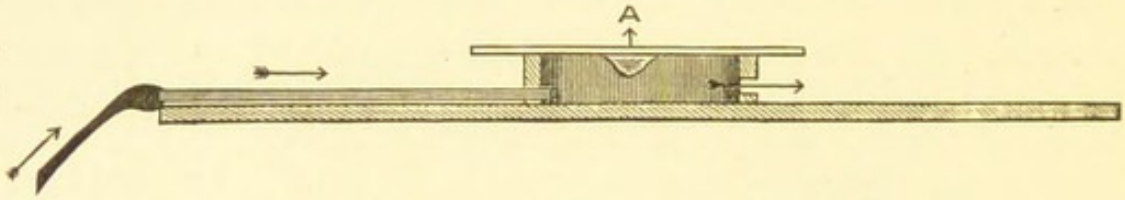


FIG. 44.—ACTION OF H_2S AND CO_2 GASES DIRECTLY UPON THE BACILLI IN FRESH HUMAN SPUTA.

A = a drop of human sputum adhering to the cover-glass.

ance in any of the tubes. These experiments were repeated a second time with similar results.

Another device was used to test the action of the gases upon the microbes. The gases were allowed to pass for

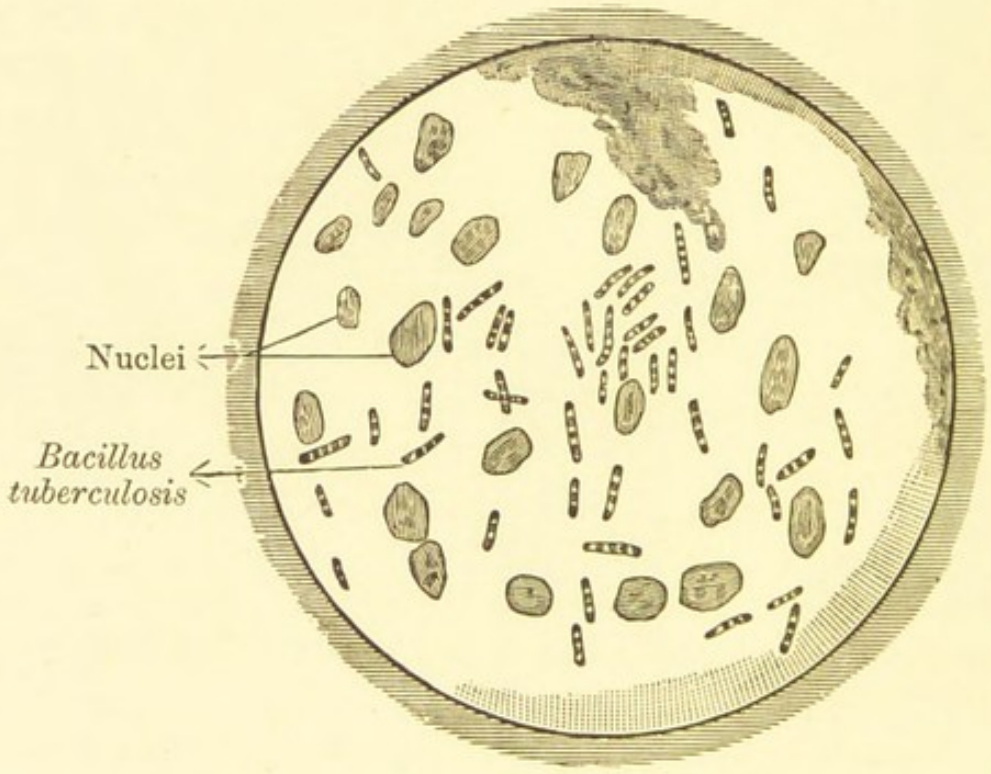


FIG. 45.—BACILLI IN SPUTUM.

Case of Miss Green-White. Stained by the Koch-Ehrlich Method (×750).

fifteen minutes into a small glass cell (Fig. 44), containing a drop of phthisical sputum upon the internal surface of

the cover-glass (A). After allowing the gases to pass through the cell, the cover-glass was then transferred to sterilized blood serum, and after an incubation of twenty-six days *no* growths of *Bacillus tuberculosis* (or putre-

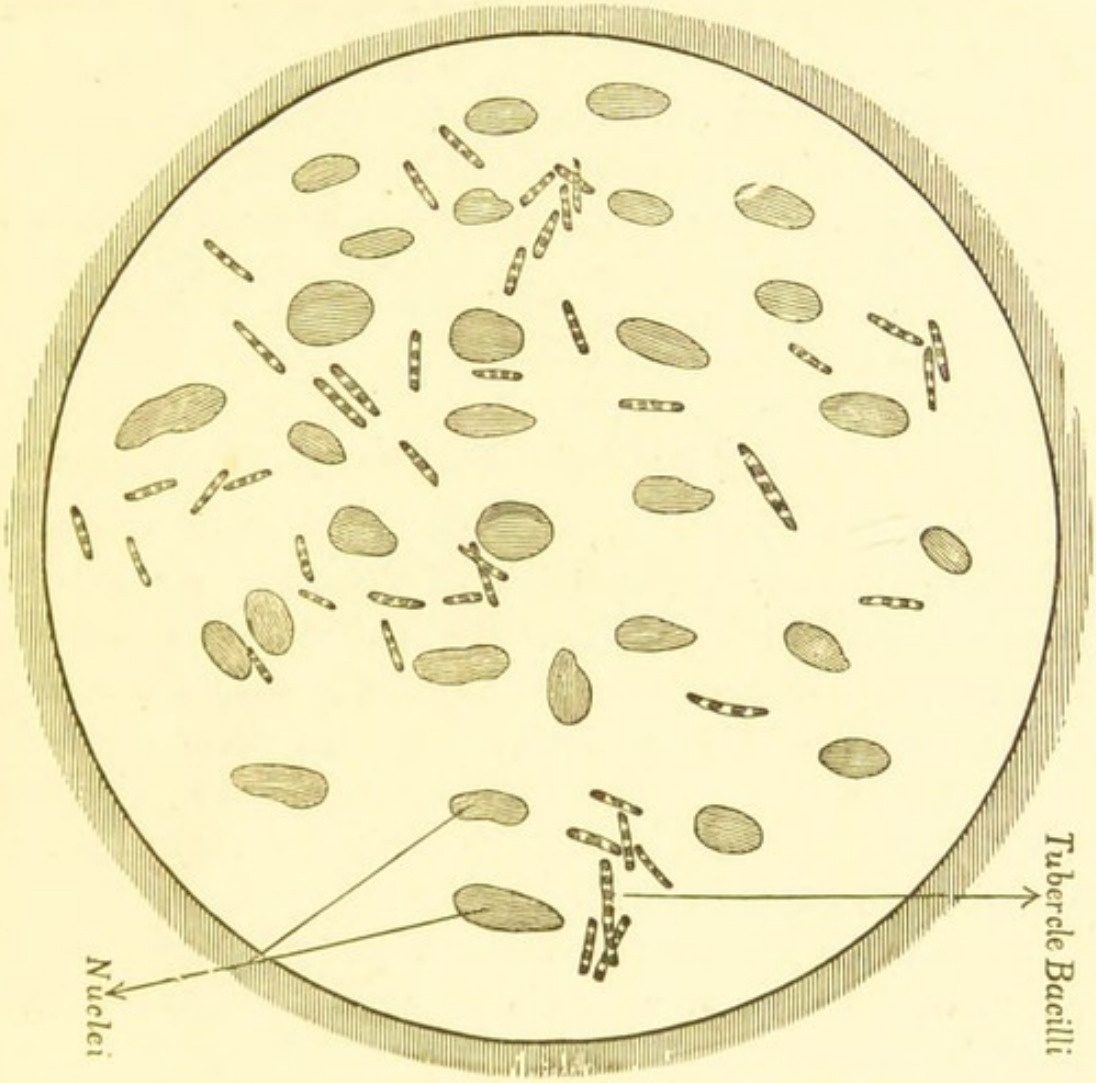


FIG. 46.—BACILLUS TUBERCULOSIS IN ACUTE GENERAL PHTHISIS.

From sputum of Mr. John Snodgrass, jun. Stained by the Weigert-Ehrlich method (× about 1400).

factive microbes) made their appearance. This was repeated in duplicate with the same result.

The sputa used in the above experiments were obtained from Mr. Snodgrass and Dr. Wood,* of Bromsgrove.

* R. Wood, M.D., L.R.C.P., M.R.C.S., L.S.A., etc

Dr. Wood's tube came labelled: '*Expectorated May* 29, 1887. *Girl named Miss Green-White. Incipient phthisis; night sweats, and harsh breathing under the clavicles.*' An examination of this specimen of sputum proved the presence of a large number of bacilli (Fig. 45).

The sputum received from Mr. Snodgrass also revealed the presence of the microbes of phthisis (Fig. 46).

From the above experiments there is every reason to conclude that Bergeon's gaseous enemata destroy *Bacillus tuberculosis* and its spores.

Kolischer's Method.

During the year 1887, Dr. Kolischer read a paper before the Society of Physicians of Vienna on a proposed method for treating phthisis. He started on the assumption that tuberculosis occasionally heals naturally owing to the tubercles being 'calcined,' and hit upon the idea of causing artificial 'calcination' by means of *hypodermic injections* of 'calcium phosphoricum.' In every case the experiments turned out successful.

There is little doubt that calcium phosphate (or phosphate of lime) is a valuable therapeutic agent in the treatment of phthisis.

Kolischer's method is of value, and, in certain stages of the disease, ought to be tested by medical authorities. It may be remarked that the tubercles have a fatal tendency to caseous or calcareous degenerations. 'These caseous collections, which are easy to distinguish by their yellow colour, their granular and fragile consistence, come either from lobular pneumonia or from lobular inflammations fused into a single mass. They are in reality products which have become fatty and dry; also perhaps mixed

with *calcareous salts* capable of transforming the collection into a stony mass. *It is a sort of cure starting from this calcification; they are only inert bodies in the lungs.* It may also happen that around this pulmonary concretion are formed centres of softening, which allow the stone to be detached and to be expelled.'

Ball's Method.

Dr. Ball (*Bulletin de l'Académie de Médecine*, 1887) considers that phthisis can be cured by *injections* of eucalyptus oil under the skin; that this germicidal agent destroys the bacilli, and is curative of local lesions.

In a case in which the author was interested—namely, that of Mr. John Snodgrass, jun.,* of Glasgow, who had been suffering from general acute phthisis—the *inhalation* of volatilized eucalyptus oil proved 'very irritating' and had to be abandoned.†

Weigert's Method.

Professor A. Visconti (*Atti dell' Istituto Lombardo*, 1889) has recently recorded certain results obtained from Dr. L. Weigert's therapeutic treatment of pulmonary phthisis.

Seven patients in various stages of phthisis were subjected to this treatment for the purpose of testing its efficacy.

Weigert's method consists in administering superheated dry air (150° to 180° C.), which is inhaled through a specially prepared apparatus. According to Weigert the superheated air destroys the tubercle-bacilli. In the

* The translator of Heine's *Religion and Philosophy in Germany*, also *Wit, Wisdom, and Pathos, from the Prose of Heinrich Heine* (Trübner and Co).

† See the author's paper in *Proc. Roy. Soc. Edinburgh*, vol. xv., p. 56.

incipient stages of the disease satisfactory results were obtained in some respects, such as relief of the cough, greater freedom of respiration, less profuse perspiration, and increased appetite. But, says Visconti, 'it was doubtful whether the germ itself was killed,' while in the advanced stages the malady continued its normal development without being perceptibly arrested by the treatment.

It is a well-known fact that *Bacillus tuberculosis* only grows between the temperatures of 30° and 41° C. (86° to 105·8° F.); therefore it is possible that in Weigert's treatment the microbes, if not actually destroyed, become inactive.

Griffiths' Method.

The author has been, for some years, engaged in researches on microbes (from time to time submitted to the Royal Society of Edinburgh), with the view of ascertaining if there are any germicides which will kill the microbe of phthisis without injuring the patient, and at the same time possessing curative properties.

Having discovered such a germicide, the author had the good fortune to meet with a distinguished literary man—Mr. John Snodgrass, jun., of Glasgow—who had been suffering from phthisis, and who was most anxious to try the proposed method of treatment, having read an abstract of the author's paper (read before the Royal Society of Edinburgh on January 31, 1887) on the subject in the *Glasgow Herald*.

His case was that of lung disease of thirteen years' standing, which became distinctly tubercular several years ago. Ever since the discovery of Koch's bacillus, Mr. Snodgrass tried various devices (of his own) for destroying the microbes in his own lungs. Among these

experiments he used volatilized iodine, using the apparatus illustrated in Fig. 47. The apparatus explains itself, and is very simple in construction.

According to Mr. Snodgrass, the patient should, if possible, inspire gently by the mouth (from the mouthpiece D) and expire by the nose, taking as full and as deep inspirations as possible. He considers that although the iodine may not reach very deeply into the lungs, it will cleanse the throat, larynx, trachea, and the large

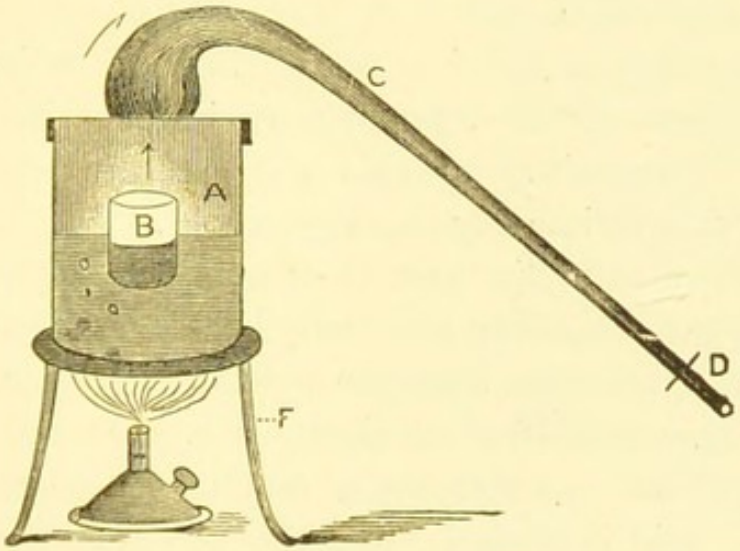


FIG. 47.

A = a tin vessel containing water (which is kept near its boiling-point); B = a small vessel capable of floating in water. This vessel contains tincture of iodine ($\frac{1}{2}$ oz. tincture of iodine and $\frac{1}{2}$ oz. of water are used every time); C = tube (of indiarubber); D = mouthpiece; E = spirit-lamp; F = a tripod stand.

bronchi. Concerning the value of his device of inhaling volatilized iodine, he says: '*The inhalations of iodine have certainly put the hand back on the dial in my case for nearly two years.*'

Mr. Snodgrass (already such an enthusiast in the bacillary nature of phthisis) asked his physician to adopt the author's method of treatment in his case. The latter (*i.e.*, the physician) willingly acquiesced, and much good was done to the patient.

The treatment consists in injecting subcutaneously an aqueous solution of *natural* salicylic acid (see Chapter X.).

From 5 to 20 minims of a warm saturated solution of the acid should be injected, if possible, twice a day; but as far as the number of injections to be made (say weekly), the physician himself is the best judge, as one cannot lay down hard and fast rules for the treatment of a disease like phthisis, which has so many different phases in its work of destruction.

Natural salicylic acid has no detrimental action on the blood and tissues,* and appears to be curative of local lesions. It certainly arrests the development of the microbes, and ultimately destroys them.

During the salicylic acid treatment the night sweats disappear, the breathing gradually becomes less laboured, the appetite returns, and the sputum becomes thinner and thinner, and ultimately disappears. The body-weight increases, as well as the measurement of the chest.

Salicylic acid *injections* have the power of completely curing muscular rheumatism of the kind which often accompanies phthisis.

In the case of Mr. Snodgrass, the author constantly reported the microscopical appearances of specimens of sputa received from him, and it was surprising to note from time to time the *decreasing* number of bacilli present. Not only do the bacilli decrease, but also the quantity of cellulose in the sputum, showing the inactivity of the bacilli present. In fact, their pathological power appears to be proportional to the quantity of cellulose found in the sputum.

Mr. Snodgrass adopted Dr. Bergeon's† as well as the

* See the author's paper in *Proc. Roy. Soc. Edinburgh*, vol. xiv., p. 97.

† At the author's suggestion.

author's hypodermic injection method; and we have permission to make free use of his letters, in which he describes the experiments performed and results obtained. As Snodgrass's letters are of value, they will be included in the Appendix to this volume.

Mr. Snodgrass (and his physician) firmly believed in the value of both methods, and much good was done by using them. When he first wrote to the author, in February, 1887, he was apparently a dying man. He greatly improved by using the methods; in fact, so much so that he was able to leave Glasgow and spend the summer of that year in the Kyles of Bute (see Appendix).

Although he obtained the greatest relief from his sufferings, the disease was already too far advanced, and had thoroughly undermined his constitution; therefore a permanent cure in his case was hopeless. Nevertheless, according to his own account, his life was prolonged for fifteen months by the injection methods—the disease proving fatal on May 24, 1888.

But Mr. Snodgrass's case is not the only one in which the author's method has been used—several cases of cure have already been recorded, while others are in progress. Among these may be mentioned the following:

(*a*) Dr. J. Cobden Williams, L.S.A., M.R.C.S., etc., of Blackpool (a stranger to the author), reported the following cases on November 14, 1889:

'I have treated (so far) four patients with your method. The first patient, Miss B——, who has a decided phthisical family history, and who had suffered from loss of flesh, night sweats and hæmoptysis, with short, irritating cough, was under my care from the end of June until September, when I discharged her as cured. She gained weight from the commencement. The chest

troubles have all disappeared, and I called to see her the other day, and was surprised to find her so altered; she said she had never felt better in her life.

'Miss B——'s weight at the commencement of the treatment was 6 stone 6½ lb.; at the end of the treatment, 8 stones 8½ lb., and is now much heavier. The chest measurements were as follows: 29 inches before treatment and 30½ inches after treatment. I may say that the only other medicament used was one bottle of Oppenheim's "Cream of Malt."

'The second patient, Miss W——, whose chest troubles had been in progress for two and a half years, is now gaining weight, and the symptoms on auscultation have disappeared; but she still has the irritable cough (without expectoration), and the last attack (in August) of hæmoptysis was only very slight. There is no doubt that she has greatly improved by your treatment.

'The third patient, Mrs. J——, comes here (Blackpool) from Manchester. There is, at present, only a slight improvement in her case, but I attribute this to the *great advance* made by the tubercle, and also to her having to travel to and from Manchester.

'The fourth patient has only been under my care for one month. She is pregnant, and this may have a beneficial effect for a time, so I cannot say whether the great improvement she has made is due to the treatment or not.'

(*b*) Dr. R. Wood, L.R.C.P., M.R.C.S., etc., of Bromsgrove, reports that he has cured a girl suffering from phthisis, by using the author's salicylic acid treatment. He says, in a letter dated September 2, 1888: 'I have been injecting salicylic acid twice a week on a girl for one year, and she is now better.'

Dr. Wood is now injecting warm solutions of salicylic acid into the blood of another phthisical patient. He recently sent the author a bottle containing sputum for examination. The bottle was labelled: 'Sputum of a girl, Webb; phthisical night sweats; age 14. She has expectorated blood. I am injecting a saturated solution of salicylic acid daily.—R. Wood, M.D.

The above specimen of sputum contained a considerable number of tubercle-bacilli (Fig. 48).

By using the salicylic acid *injection* method, the operator need not fear injecting too large a quantity,

FIG. 48.—BACILLI IN SPUTUM.

Case of girl, Webb. Stained by the Weigert-Ehrlich Method.

for the *natural* acid is only soluble to the extent of 1 part of acid in 600 parts of cold water, but it is somewhat more soluble in water at the temperature of the blood.

It may be remarked that only a small quantity of the acid gets into the blood by the hypodermic injection method, yet it is capable of destroying the bacilli, as proved by their diminished number in the sputa, etc., also by the general improvement and ultimate cure of the patients under the treatment.

It is well known that salicylic acid and sodium salicylate have been, and are, used in the treatment of rheuma-

tism, gout, ulcerative endocarditis, tabes mesenterica, etc., but they are taken into the system by the mouth and not by hypodermic injections. The latter method of administering salicylic acid and other germicides is the most rational and scientific method of attacking those infectious diseases whose microbes live in the blood and tissues.*

If salicylic acid is administered by the mouth, a very small percentage of the dose is absorbed into the blood, for the largest proportion of the dose is found in the fæces, as the following analyses (performed by the author) show :

No. of Experiment.	Dose of Salicylic Acid taken.	Salicylic Acid found in Fæces.
1	10 grains.	9·65 grains.
2	8 ,,	7·11 ,,
3	10 ,,	9·54 ,,
4	10 ,,	9·71 ,,
5	15 ,,	14·87 ,,

And this may be the case with other medicines and germicides administered by the mouth. The medicines have to pass through a considerable portion of the alimentary canal before they are absorbed into the blood. They may become changed (before absorption takes place) by the various secretions pouring into the alimentary canal.†

The more soluble sodium salicylate cannot be used

* See the researches of Dr. Limbourg on the action of bile acids in *Zeit. Physiol. Chem.*, vol. xiii., p. 196.

† The author's paper in *Proc. Roy. Soc. Edinburgh*, vol. xiv., p. 97. In the case of a germicide it may be rendered inactive.

instead of salicylic acid, because the former has little or no germicidal properties (see Chapter X.).*

It must be borne in mind that the *natural* salicylic acid obtained from the oil of winter-green is the only one which will give satisfactory results.

Although salicylic acid is a powerful germicide and a useful medicine in the treatment of phthisis, it must not be understood that by its use cod-liver oil, milk, fats, butter, and other moderators of denutrition are to be dispensed with. Salicylic acid is a germicide, and appears to be curative of local lesions, but it does not directly strengthen the parts attacked; therefore it should be used in conjunction with cod-liver oil, etc.

The internal administration of cod-liver oil, Kepler's 'malt' and 'malt and oil,' and the various patented emulsions, is of great service generally in the treatment of phthisis.

'Fats and starchy foods must be prescribed to phthisical patients in sufficient quantity to repair the waste of carbon; from 60 to 100 grammes of fat, and 500 to 600 grammes of feculents, as bread, pastry, or dry decorticated vegetables, etc. To obviate loss of nitrogen, 120 grammes of nitrogenous food will be sufficient. It must consist of meat, game, or fish, and be made as palatable as possible. To preserve the appetite it is really indispensable to vary the forms of diet indefinitely, and to rigorously maintain the quantity of carbohydrates in excess of that of the nitrogenous food. It must be noted that we have in view the patient who is relatively in good health, and whose digestive functions are regular.'

Not only are foods, etc., rich in carbohydrates necessary for phthisical patients, but also phosphorus (in the

* In the treatment of rheumatism and gout, Dr. P. W. Latham says: 'Give the acid (*i.e.*, salicylic acid) without any alkali or base.'

form of hypophosphites and phosphates) is essential; for large quantities of phosphates are lost in the sputum and urine during the course of the disease. Therefore, such preparations as Scott's and Mellin's Cod-liver Oil Emulsions, with hypophosphites* (sodium and calcium), are useful adjuncts in the treatment of phthisis.†

It is well known that a change of air, generally to a warm seaside place—such as Bournemouth, Ventnor, Torquay, Mentone, or the Riviera—is also beneficial for patients suffering from phthisis and other lung complaints.

'Climate is a medicament composed of the common elements—oxygen, temperature, light, movements of air, and barometric condition. All these things constitute a climate which is but a physico-chemical mixture, to be studied with regard to physiological effects, and especially curative action. We add a second kind of element, which will in future serve the educated physician who is careful of his scientific dignity—that is, the vital composition, if we may so express it, of the atmosphere he is about to prescribe. Is it pure—that is to say, exempt from microbes in general? Is it hostile to the life and the multiplication of bacilli, which have already invaded the bodies of those whose care is confided to us? These are the questions which start up in the therapeutics which we may call climatic.'

It is not our object to discuss the therapeutic value of *warm* maritime climates, or the climates of altitudes which are necessarily *cold*, for the reader will obtain full information on the subject by referring to the works,

* See also Thorowgood's *Consumption and its Treatment by the Hypophosphites* (Baillière, Tindall and Cox).

† In France a preparation called 'Sirop de Dusart' is sold for a similar purpose. It is phosphate of lime dissolved is lactic acid (the chief acid of the gastric juice).

etc., of Williams,* Cullimore,† Weber, MacCormac, and others too numerous to mention.

But it should be borne in mind that *Bacillus tuberculosis* only grows and multiplies between the temperatures of 30° and 41° C. (86° to 105·8° F.); that the air of seaside resorts,‡ as well as the air at high altitudes, is comparatively free from microbes (see Chapter IV.); that ozone (see Chapters VIII. and X.) is present in marked quantity in the air of warm seaside places and of mountains; and that certain bromo-iodated saline compounds are also present in the atmosphere of the former.

In concluding our remarks concerning phthisis and its treatment, it may be mentioned that it is upon the lines indicated in the present chapter that the physician in the future must look for a scientific method of treating those infectious diseases whose microbes reside in the blood.

Asiatic Cholera.

The native habitat or the endemic area of this terrible disease is in India (especially in the delta of the Ganges).

Since the commencement of the present century a certain number of epidemics of the disease have occurred in Europe, as well as in South America.

In 1887 there was a violent outbreak of cholera in the province of Cordova, in the Argentine Republic, the papers giving full accounts of 'human beings dying in

* *The Treatment of Phthisis by Residence at High Altitudes;* and *Pulmonary Consumption: Its Etiology, Pathology, and Treatment.*

† *The Medical Press and Circular*, 1889; and *Consumption as a Contagious Disease: the Merits of the Air of Mountains and Plains* (Baillière, Tinda and Cox).

‡ Excepting the microbes of malaria, which may be present in the air of seaside places, especially in Northern Italy.

heaps.' Is there no cure for cholera? Is there no agent that will destroy Koch's bacillus in the human body?

In passing, it may be stated that Mr. T. F. Agar (Consul-General for the Argentine Republic in Scotland) presented a copy of the author's paper (*Proc. Roy. Soc. Edinburgh*, vol. xiv., p. 97), on 'Microbes and their Destruction in certain Cases of Infectious Diseases,' to his Government at Buenos Ayres, with the view of introducing a modified form of the author's injection method in cases of cholera.

Salicylic acid has been used in South America during cholera epidemics. The solution taken consisted of 25 grammes of salicylic acid to a litre of rum or cognac, and 'three teaspoonfuls of the mixture to be taken between meals in coffee or tea.'

If salicylic acid should prove useful in destroying Koch's comma-bacillus, or even as a means of preventing the severer attacks of cholera, would not anal and hypodermic injections of solutions of the acid* be the best method of combating the disease? By these injections we should meet the growths of the microbe in the intestines, and also those that may possibly have passed into the blood-system by absorption.

If this method proved useless, possibly Bergeon's medicated gaseous enemata would destroy the microbes.

'Among other substances unfavourable to the development of the microbe, and thus constituting a preventive of cholera up to a certain stage, we may mention calcium sulphate, which acts by producing sulphuretted hydrogen gas; also carbolic acid, thymol, and oil of mustard. All these substances constitute excellent antiseptics in an epidemic of cholera.'

* Koch has stated that *acids* in general are the greatest hindrance to the development of the cholera bacillus.

Diphtheria.

We have already alluded to the fact that certain diseases are antagonistic to other diseases (*e.g.*, scarlatina and tuberculosis).

Dr. Babtchinsky has recently* communicated to a number of St. Petersburg medical journals evidence which seems to show that the microbia of diphtheria (Fig. 49) and erysipelas (Fig. 50) are mutually antagonistic. Patients attacked with both complaints there-

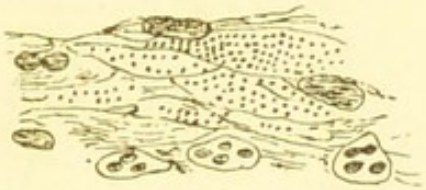


FIG. 49.—DIPHTHERITIC MEMBRANE.
(After Klein.)

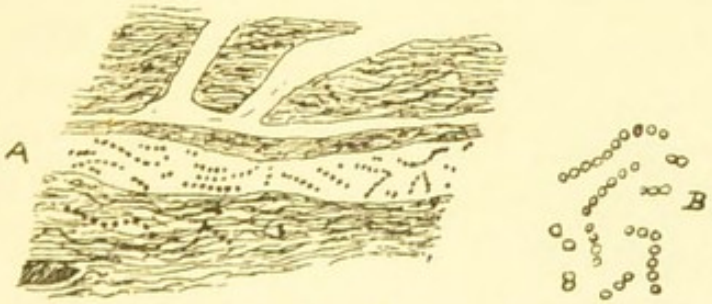


FIG. 50.

A = Section of Skin in Erysipelas; B = Microbes isolated and enlarged.

fore recover. Encouraged by an accidental observation in the case of his own son, Dr. Babtchinsky has in twelve cases of grave diphtheria inoculated with pure cultures of the microbe of erysipelas, and in each case saved the life of the sufferer.

At the present moment test experiments are being carried on at the Pasteur Institute in Paris.

* December, 1889.

Hydrophobia, or Rabies.

Rabies is a canine disease, which is communicated by a bite, and the inoculation of man and other animals by the saliva. The exact nature of the microbe of this disease is not yet known (see Chapter XI.); but whether the microbe is a *micrococcus** or some other form, it does not reside in the blood, its habitat being the nervous tissues.

From the researches of Pasteur, it appears that the microbe of rabies has a very different life-history from other microbes.

(*a*) The principal seat of the virus is in the central nervous system. Even after the injection of the poison into the blood-system, 'the spinal marrow is the region first attacked, the virus locating itself and multiplying there before spreading to other parts.'

(*b*) Pasteur has shown that the period of incubation of the disease varies considerably. It varies with the different modes of inoculation, being longer when the virus is injected into the muscular than the nervous system. Hence the reason that at the Pasteur Institute the rabbits, after trepanning, are always inoculated with the virus on the surface of the brain.

(*c*) The symptoms of rabies are very varied, depending upon the nature of the region in the nervous system—encephalon or spinal cord—where the virus locates itself.

The virus of rabies is found in every part of the encephalon (brain, cerebellum, medulla oblongata, pons, and peduncles).

From these details it appears that the medium in which the microbe of rabies resides is essentially nervous matter† and not in the *blood* system.

M. Galtier, of Lyons, found that *salicylic acid* injected

* Pasteur in the *Comptes Rendus*, 1884.

† Hence the reason that Brown-Séquard called rabies 'an ascending neuritis.'

hypodermically was quite inefficient in preventing the development of the disease. As the microbe or virus of rabies resides in the nervous, and not in the blood system, the injection of salicylic acid or any other germicide would be useless as a means of preventing or curing rabies. Hence the reason of Galtier's failure with salicylic acid.

There is no doubt that Pasteur's system is the only means (at present known) of combating this terrible disease with anything like success; but it must be distinctly borne in mind that protective inoculation is also useless for many infectious diseases, and in such cases necrophytic medicines are of the greatest value.

To illustrate the value of Pasteur's system we abstract certain statistics from the *Annales de l'Institut Pasteur* (vol. iv., p. 131) which have been sent to us by Dr. Roux, of Paris.

The following table gives the results of the antirabic treatment at the Pasteur Institute during the past four years:

Year.	A*			B*			C*			Total.		
	No. of patients treated.	Deaths.	Mortality per cent.	No. of patients treated.	Deaths.	Mortality per cent.	No. of patients treated.	Deaths.	Mortality per cent.	No. of patients treated.	Deaths.	Mortality per cent.
1886	231	3	1·30	1926	19	0·99	514	3	0·58	2671	25	0·94
1887	357	2	0·56	1156	10	0·86	257	1	0·39	1770	13	0·73
1888	402	6	1·49	972	2	0·21	248	1	0·40	1622	9	0·55
1889	346	2	0·58	1187	2	0·17	297	2	0·67	1830	6	0·33
	1336	13	0·97	5241	33	0·63	1316	7	0·53	7893	53	0 67

* Class A = patients who had been bitten by animals demonstrated to have been *mad* by inoculation experiments.

,, B = patients certified by veterinary surgeons to have been bitten by *mad* animals.

,, C = patients bitten by animals suspected of having been mad.

The above table shows that there have only been 53 deaths per 7,893 patients treated, representing a mortality of 0·67 per cent. It will also be observed, by referring to the last column of the table, that the mortality has been reduced from 0·94 in 1886 to 0·33 per cent. in 1889, due, no doubt, to the better skill in the application of the treatment.

Concerning the nationalities of the patients treated at the Pasteur Institute, the largest number during the four years were from France (6,350); next comes England (308), followed by Belgium (230), Spain (229), Russia (188), Italy (155), Portugal (116), Austria (84), etc.

During three years (1887-89) the largest number of French patients treated came from the following departments: Seine (1,018), Rhône (187), Bouches-du-Rhône (179), Seine-et-Oise (169), and Hérault (107).

The next table gives the number of patients, from 20 departments, treated per 100,000 of inhabitants:

No.	Department.	Treated per 100,000.	No.	Department.	Treated per 100,000.
1	Seine	47·0	11	Savoie	18·4
2	Bouches-du-Rhône	32·6	12	Isère	17·8
3	Seine-et-Oise ...	31·6	13	Loire	17·6
4	Rhône	27·6	14	Lot-et-Garonne ...	17·6
5	Hautes-Pyrénées	25·4	15	Drôme	17·4
6	Hérault	24·0	16	Lot	16·7
7	Pyrénées-Orient.	21·7	17	Haute-Savoie ...	15·3
8	Aude	21·7	18	Tarn-et-Garonne	15·0
9	Garde	19·3	19	Puy-de-Dôme ...	14·8
10	Basses-Pyrénées ...	19·3	20	Oise	14·7

The highest figure being in the small department of the Seine, which includes Paris; while of the remaining twenty departments (of France) the lowest figure is 0·6 per 100,000 from the department of Sarthe, in the west of France.

It may be remarked that the departments of the north, east, and west of France have, so far, sent the fewest patients to Paris to be treated by Pasteur's system.

For further details the reader is referred to the *Annales de l'Institut Pasteur* (vols. i. to iv.).

Although the microbe of rabies has not been isolated with anything like satisfactory results, the virus has daily been attenuated, and the greatest good done to suffering humanity. Pasteur did not wait until the microbe was isolated, or his important prophylactic treatment would not have been given to the world at the present moment. In such researches one should always bear in mind the words of Lavoisier: 'A man would never give anything to the public if he waited until he had reached the goal of his undertaking, which is ever appearing close at hand, and yet ever slipping farther and farther as he draws nearer.'

Concluding Remarks.

For those microbian diseases where preventive inoculation is useless there is undoubtedly a great future for the treatment of such diseases (phthisis, malarial fevers, etc.) by chemical methods, the most rational and scientific method being the injection of germicides.

The author, in concluding one of his papers (*Proc. Roy. Soc. Edinburgh*, vol. xiv., p. 97), said: 'We have reason to conclude that it may be, with a more extended study of the action of salicylic acid upon disease "germs" and their organisms, we have the most rational mode of treating those infectious diseases whose seat of energy is in the blood.'

Perhaps a better germicide than salicylic acid may be

discovered, yet the principle of the method of treatment is certainly on the right track. In every case of disease produced by microbes residing in the blood and tissues, the most scientific method of treating such diseases would be to destroy the microbes *in situ* by injections; and by so doing Nature would have a chance of repairing the damage done. It is this object which forms the basis of the researches recorded in this chapter, and of the author's proposed method of treating certain infectious diseases.

Taking the above circumstances into consideration, we cannot conclude our remarks better than by quoting from the Harveian oration given by Dr. P. W. Latham before the Royal College of Physicians on October 18, 1888: 'It seems that with increasing knowledge of the chemical changes in the blood and tissues we are on the threshold of most important discoveries, and of a very marked advance in the science of medicine. We might even hope to find that the action and growth of the bacilli [of phthisis] might be inhibited by certain substances, and then by injecting these substances into the blood, disease might be prevented; or if disease existed it might be arrested or cured.'

APPENDIX.

I.—MR. SNODGRASS'S LETTERS.

THE following are abstracts of Mr. Snodgrass's letters (see Chapter XII.), and were originally published in the *Proc. Roy. Soc. Edinburgh*, vol. xv., pp. 58-63:

(1) *Letter of February* 28, 1887.—'Some time since I obtained remarkable results by a simple process I devised for the inhalation of the volatilized vapour of iodine, with satisfactory results.'

(2) *Letter of March* 3, 1887.—'The result of inhalation of iodine three days ago has been to cleanse the lung, and to bring away the débris found in the sputum this forenoon. This is, of course, a good result. The iodine inhalation caused headache and considerable depression of heart.'

(3) *Letter of March* 8, 1887.—'Since I last wrote to you I have twice injected a 15 minim solution of the acid. The strength was $2\frac{1}{2}$ grains of salicylic acid to a fluid drachm of water; but the acid was mixed with an equal quantity of borax. My immediate reason for injecting the solution (which would roughly contain $\frac{5}{8}$ of a grain of the acid) was a severe attach of rheumatism, of the kind that often accompanies phthisis. The rheumatism disappeared almost entirely. . . . I may mention that before making the injections there was a large deposit of uric acid on the urine. This has quite

disappeared—at least, to the naked eye. That the salicylic acid passed through the system I am perfectly certain, as I had the usual headache which follows taking it by the mouth, and the taste—that unmistakable taste—was very apparent next morning on the tongue and palate. I made the injections in the calf of the leg, near a large vein. . . . I had (it appears) rightly assumed that a cavity was forming in the lung about the time I first wrote to you. About forty-eight hours after inhaling volatilized iodine a considerable quantity of matter came away, with the usual discoloured blood-clot. This débris, on examination, contained an abundance of lung fibre. . . . One most important part of your paper is that which deals with the action of the gastric juice on medicines. I swallow a great deal of sputum at times—for it is impossible always to eject the whole. Yet I have every reason to suppose that the bacilli thus swallowed in large numbers *pass harmlessly through the alimentary tract without getting into the blood.* In fact, it happens with me and *Bacillus tuberculosis* as it happened with M. Bochefontaine* and the *Comma bacillus.* This thoroughly bears out your most important remark, that "there is no doubt the *acid* properties of the gastric juice . . . had acted upon these micro-organisms,' etc.† In cases where consumption of the intestines follows upon pulmonary consumption, the inference will be that the gastric juice is either weak or imperfectly secreted. With reference to the salicylic acid (without borax), what I think of doing is to dissolve a part of the acid in a small quantity of hot water; then, if the water takes up the acid in the proportion of 20 to 1, by injecting 15 minims of the solution before it is cold—say at the

* *Comptes Rendus*, 1884.

† See Dr. Griffiths' paper in *Proc. Roy. Soc. Edinb.*, vol. xiv., p. 97.

temperature of blood-heat—I shall get into the system about $\frac{3}{4}$ of a grain of the acid. Now, the medium dose by the mouth being 10 grains, $\frac{1}{3}$ of this would be reckoned safe, or at least not dangerous by injection, consequently I am much within the line of safety.'

(4) *Letter of March* 11, 1887.—'This forenoon I tried the injection of salicylic acid, and after injecting 5 or 6 minims into the tissue of the left thigh, I had to stop, owing to the pain caused by the acid. Judging by the pain that immediately followed the injecting of the drops of fluid, the solution must have been of considerable strength. The only question is, whether the heat of the body is sufficient to dissolve any crystals that remained in the fluid. I suspect that this is the great fallacy of administering, say, 10 grain doses of salicylic acid by the mouth. Possibly very little of the acid passes into the blood system, the greater part being carried away in the fæces as insoluble. From this, I am sure your method is on the right lines. The micro-organisms *must be reached*, and *must be destroyed*.'

At this point Mr. Snodgrass used Dr. Bergeon's method along with the salicylic acid injections.

(5) *Letter of March* 28, 1887.—'Dr. Bergeon's instrument (the only one in Scotland) has been seen by my doctor. It is very elaborate; we think needlessly so. Briefly, the mode of obtaining the mixed gas is to pass the carbonic acid gas through *Eaux Bonnes* water. Now, it seems that during five or six trials on a patient at the Western Infirmary (Glasgow) no sulphuretted hydrogen could be detected being emitted through the mouth, showing that the gas did not permeate the lungs. My notion is that too little *Eaux Bonnes* water was used, that a fresh supply should from time to time have been put into the jar in which the CO_2 passed through the

water. Your suggestion of the proportion of three volumes of CO_2 to one of H_2S is very valuable.'

Mr. Snodgrass and his doctor constructed a much simpler apparatus for this gaseous injection than that of Bergeon.

(6) *Letter of March* 31, 1887.—'At this point, I may say extensive damage has been done to the throat, for large ulcers are seen on the back of it, by merely pressing down the tongue. Two litres of gas (CO_2 and H_2S) were injected, and the sulphur smell was distinctly perceptible five minutes after beginning the injection. *Eaux Bonnes* water was not used, but bisulphide of carbon was employed, instead of the mineral water. Some rather disagreeable symptoms followed the injection: severe headache, colic pains, weakness, and slight pain of the heart. You will be glad to learn that (now for the third time) relief from rather severe rheumatism followed two injections of salicylic acid.'

(7) *Letter of April* 5, 1887.—'After the last injection of gas I again suffered from severe toxic effects, and during the night was much pained with the unabsorbed gas, and with violent and incessant purging. . . . . Many persons would be far too weak for Bergeon's method, and for them your salicylic acid treatment would be invaluable, for I have not the least doubt that you have discovered an efficient bacillus-destroyer.'

(8) *Letter of April* 10, 1887.—'To-day I have injected 10 minims of salicylic acid solution. It certainly "bites" pretty sharply, so it must have been strong enough. I find it of advantage to inject it tepid, as it is then more quickly absorbed, the swelling going down in a few minutes. There is no doubt that I am much better, as far as phthisical disease is concerned. I can now speak without distress, and breathing is much less laboured.

Last night I slept seven hours, a thing that has not happened for months, my usual sleep being a broken half-hour several times a night, and in all not more than three hours in the twenty-four. I firmly believe that in many cases Dr. Bergeon's system will not be applicable, and in these cases your treatment would be valuable.'

(9) *Letter of April* 13, 1887.—'Yesterday I again injected the gases. They produced much pain in the left lung, and also in that small portion of the right lung that is impaired. My doctor thinks your observations of immense importance.'

(10) *Letter of April* 27, 1887.—'For the last ten or twelve days I have suffered a great deal of pain. Large quantities of uric acid are being secreted. I inject salicylic acid occasionally, which has the effect of checking the formation of uric acid.'*

In the month of June Mr. Snodgrass was well enough to travel to the Kyles of Bute from Glasgow. He wrote from there as follows:

(11) *Letter of June* 17, 1887.—'My lung trouble has of late *very greatly* improved; but the abdominal mischief has been very severe. During the last two days, however, a marked improvement has taken place. . . . . I have the greatest faith in yours and Dr. Bergeon's systems. But in my case the disease has apparently gone too far, and the condition of the large bowel is such that there is great risk in applying Bergeon's method.'

(12) *Letter of September* 19, 1887.—'You will recollect that you were able to make a most favourable report on the sputum sent to you after the salicylic acid treatment and eight or nine injections of gas by the Bergeon mode

* See 'On some Points in the Pathology of Rheumatism, Gout, and Diabetes,' by Dr. P. W. Latham (*The Croonian Lectures for* 1886).

of treatment. As far as the lung was concerned, a *great improvement* took place, and for more than two months —certainly during the whole of June and July—I could *not have sent you a typical specimen of sputum.* Indeed, during that time expectoration almost entirely ceased (as did also the cough), and what there was, was merely mucous phlegm, such as might be present in a slight attack of bronchial inflammation. Otherwise, however, matters were very bad; the large bowel was severely ulcerated, and adherent in the ileo-cæcal region to the wall of the abdomen. I continued to inject salicylic acid after ceasing the Bergeon treatment, but this, too, had to be discontinued on account of the disordered state of the whole system. One remarkable thing, however, has occurred—*ever since the salicylic acid injections I have had no attack of muscular rheumatism.*

'About a month ago I again began the Bergeon treatment, but very cautiously, and the operations have been continued. There is undoubtedly some improvement, but, as before, *uric acid deposits took place*, and I have not ventured to use the treatment on more than two days consecutively.

'I am afraid it must be admitted that (with me, at least) there are certain dangerous symptoms caused by the CO_2. Amongst these are: obstinate constipation, great difficulty in expelling the unabsorbed residuum of carbonic acid gas in the intestines, and certain rather alarming head symptoms.

'About June 23 last, Dr. Coghill, of Ventnor, communicated an account of his experience of the Bergeon treatment to the *British Medical Journal.* He there stated that the results, both in his hospital and in his private practice, were not merely remarkable, but astonishing. In the same number of the *British*

Medical Journal, however, a physician of one of the London hospitals says that not only negative results, but very imperfect results, were obtained from experiments in the hospital he represents. But this scarcely surprised me, after the failure at the Western Infirmary (Glasgow). Most likely *Eaux Bonnes* natural mineral water was used. I found it quite inefficient. Again, in the same journal, another communication gives, as a formula from which excellent results had been obtained, the following: A saturated solution (aqueous) of washed sulphuretted hydrogen; ½ to 2 oz. being added to 12 oz. of pure water in the bottle through which the stream of carbon acid gas is passed.'

It will be observed from the experiments of Mr. Snodgrass and those of the author:

1. That inhalation of iodine vapour has the property of cleansing the lungs, etc., of bacilli, débris, and Freund's cellulose.

2. That both the salicylic acid injection method and Dr. Bergeon's treatment are capable of preventing the growth and multiplication of *Bacillus tuberculosis*.

3. That Bergeon's process has a tendency to greatly increase the formation of uric acid in the urine.

4. That salicylic acid injections lessen the abnormal formation of uric acid in the urine.

5. That in severe cases of phthisis it is difficult for the whole of the gases (in Bergeon's treatment) to be absorbed by the intestines — the unabsorbed gases causing an ulcerated state of the intestines.

6. That salicylic acid *injections* have the power of completely curing muscular rheumatism of the kind which often accompanies phthisis.

II.—MAGNIFYING POWER OF OBJECTIVES AND EYE-PIECES.

(1) The following table shows the magnifications of Zeiss' objectives and eye-pieces (oculars) with a tube of 155 millimètres in length—*i.e.* the Continental microscope with a *short* tube :

	Eye-pieces.	I.	II.	III.	IV.	V.
Dry objectives.	a_1	7	11	15	22	
	a_2	12	17	24	34	
	a_3	20	27	38	52	
	a		4–12	7–17	10–24	
	aa	22	30	41	56	75
	A, AA	38	52	71	97	130
	B, BB	70	95	130	175	235
	C, CC	120	145	195	270	360
	D, DD	175	230	320	435	580
	E	270	355	490	670	890
	F	405	540	745	1010	1350
Water immersion objectives.	G	260	340	470	640	855
	H	320	430	590	805	1075
	J	430	570	785	1070	1430
	K	570	760	1045	1425	1900
	L	770	1030	1415	1930	2570
Oil immersion objectives.	$\frac{1}{8}$	260	340	470	640	855
	$\frac{1}{12}$	380	505	695	950	1265
	$\frac{1}{18}$	605	810	1110	1515	2020

Zeiss' J objective is equal to an English $\frac{1}{15}$ in., while his B, C, D, DD, E, and $\frac{1}{12}$ oil immersion are said to be equal to an English 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{6}$, $\frac{1}{8}$, and $\frac{1}{30}$ in. respectively. Oil immersion lenses are taking the place of water immersion high powers in histological and bacteriological researches, as they need no correction for the thickness of the cover-glass, and are consequently much easier to use; 'the only drawback is that the essential oil (*e.g.*, cedar oil*) used will dissolve Canada balsam, Dammar varnish, and many of the other sealing fluids, and it is necessary to cover them with Hollis' glue, which is not acted on by cedar oil.' Of Zeiss' high powers the $\frac{1}{12}$ oil-immersion lens is the best, and may be thoroughly recommended for bacteriological research. Oil immersion lenses possess far greater brilliancy and definition than the water and dry lenses.

(2) The magnifications of the English objectives and eye-pieces with a ten-inch tube (*i.e.*, the English microscope with a *long* tube) are given in the following table:

Objectives.	Eye-pieces.			
	A	B	C	D
4 inch.	10	14	28	40
3 „	20	27	40	52
2 „	30	40	60	75
1 „	60	80	120	150
$\frac{2}{3}$ „	75	100	150	190
$\frac{1}{2}$ „	100	133	200	250
$\frac{4}{10}$ „	170	227	350	440
$\frac{1}{4}$ „	250	333	500	625
$\frac{1}{6}$ „	270	360	540	675
$\frac{1}{8}$ „	450	600	900	1125
$\frac{1}{10}$ „	500	666	1000	1225
$\frac{1}{12}$ „	700	940	1350	1640

* From *Juniperus virginiana.*

The above will serve as approximately correct tables for ordinary work, but if the *exact* magnifying power of any objective is required it must be specially tested.

Concerning Zeiss' objectives, the following remarks are rather important. The medium objectives are issued in two different forms, with a greater or less aperture according to the purpose for which they are required. Those with a larger aperture (distinguished by double lettering) possess with equally perfect definition a considerably higher resolving power, and permit of greater magnification being obtained by the use of the stronger eye-pieces. Nevertheless, the working distance in BB, CC, DD, although relatively large, is perceptibly less than in the corresponding series of smaller aperture, and the former are more sensitive to differences in thickness of the cover-glass and object than the latter. Therefore, B, C, and D are recommended as the more suitable for working glasses in histological and anatomical research, particularly when the next stronger dry lens is available for higher magnification.

Thickness of Cover-glasses.—All Zeiss' objectives in fixed mounts are corrected for a cover-glass of medium thickness (between 0·15 and 0·20 mm., or 0·006 and 0·008 in.). In the higher series from CC upwards the thickness of the cover-glass consistent with the most perfect correction is indicated on the side of the mount by small figures (mm.). As a rule it is sufficient for ordinary work to use cover-glasses of an estimated medium thickness.

Oil-immersion objectives are within wide limits independent of the thickness of the cover-glass. But considerable variations in the thickness of the cover-glass may be compensated for—by slightly lengthening the body-tube for thinner cover-glasses; by slightly shortening the body-tube for thicker cover-glasses.

Zeiss makes a good tester (Fig. 51) suitable for the exact measurement of the thickness of cover-glasses. The measurement is effected by a clip projecting from a box; the reading is given by an indicator moving over a

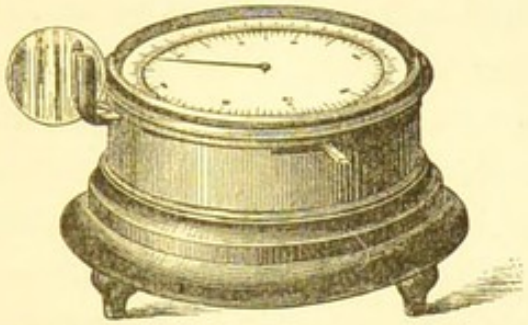


FIG. 51.—ZEISS' COVER-GLASS TESTER.

divided circle on the lid of the box. The divisions show hundredths of a millimètre, and the instrument is capable of measuring up to 5 millimètres.

III.—USEFUL DATA.

(*a*) *Conversion of Thermometer Degrees.*

C.° to F.°, multiply by 9, divide by 5, then add 32.

F.° to C.°, first subtract 32, then multiply by 5, and divide by 9.

(*b*) *Weights and Measures, etc.*

To reduce grammes to grains, multiply by 15·432.

To reduce grains to grammes, multiply by 0·0648.

To reduce kilogrammes to pounds, multiply by 2·2046.

To reduce ounces to grammes, multiply by 28·349.

To reduce inches to mètres, multiply by 0·0254.

To reduce inches to centimètres, multiply by 2·540.

To reduce centimètres to inches, multiply by 0·3937.

To reduce pints to cubic centimètres, multiply by 567·936.

To reduce litres to gallons, multiply by 0·22.

To reduce gallons to litres, multiply by 4·548.

1 grain = 0·064799 gramme.
1 gramme = 15·43235 grains.
1 millimètre = 0·03937 inch.
1 litre = 1·76077 pints.
1 minim = 0·91 grain of water.
1 line = $\frac{1}{12}$ inch.
1 μ = one-thousandth of a millimètre, or 1 micromillimètre, or 0·001 mm.
1 oz. (*Avoir.*) = 28·34954 grammes.
1 oz. (*Troy*) = 31·10349 grammes.
1 cc. of water at 4° C. = 1 gramme.
30 in. (barometer) = 761·986 millimètres.

IV.—VOLTMETER.

Fig. 52 represents a useful form of voltmeter for testing the electro-motive force (E.M.F.) of the cells, etc., used

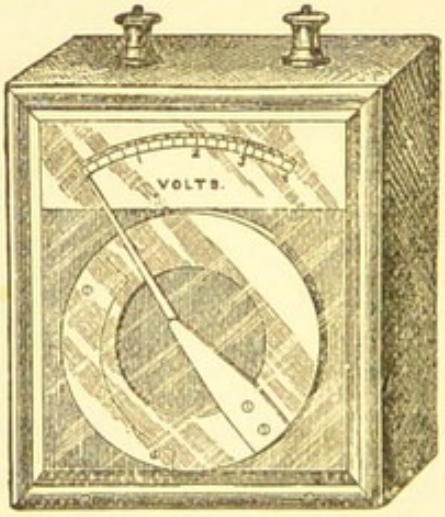


FIG. 52.—VOLTMETER.

in electrical experiments on microbes (see Chapter VIII.). The instrument is calibrated in fifths and tenths of a volt, and may be obtained from Messrs. P. Harris and Co., of Birmingham.

With the continued advance of medical electricity, an explanation of such terms as volt, ohm, ampere, etc.,

will not be out of place in the appendix to the present volume. The above terms are used by physicists, etc., to perpetuate the names of celebrated scientists, and stand for the principal *units* of electricity. The *volt* is the unit of electro-motive force or electrical pressure.* The *ohm* is the unit of electrical resistance. The *coulomb* is the unit of electrical quantity. The *ampere* is the unit of electrical current, or rate of flow. The *watt* is the unit of electrical power. The *joule* is the unit of electrical work. One unit of electrical pressure acting on one unit of quantity performs one unit of work, thus:

1 volt × 1 coulomb = 1 joule.

The unit rate of flow at the unit pressure gives the unit of power, or rate of doing work, thus:

1 volt × 1 ampere = 1 watt.

The unit of pressure against the unit of resistance gives the unit rate of flow, thus:

1 volt ÷ 1 ohm = 1 ampere.

Conversely, the unit rate of flow (or current) through the unit resistance gives the unit difference of pressure across the resistance, thus:

1 ampere × 1 ohm = 1 volt.

Thus, again, unit pressure, sending unit rate of flow against unit resistance, expends unit power:

1 volt × 1 ampere × 1 ohm = 1 watt.

This power expended for unit time does unit work:

1 volt × 1 ampere × 1 ohm × 1 second = 1 joule.

Unit rate of flow for unit time gives unit quantity:

1 ampere × 1 second = 1 coulomb.

It will be seen that although for the sake of simplicity the mechanical equivalent of the joule and watt only have been given above, the equivalents of the other units can be easily ascertained.

* The volt is equal to 0·926 of a standard Daniell cell.

V.—USEFUL BOOKS ON MICROBES, PATHOLOGY, AND ALLIED SUBJECTS.

The following is not a full list, but merely contains the names of certain books useful in the study, etc., of the subjects treated in the foregoing pages:

Klein's *Micro-Organisms and Disease.*
Crookshank's *Manual of Bacteriology.*
Brown's *Treatise on Animal Alkaloids.*
Aitken *On the Animal Alkaloids.*
Würtz's *Traité de Chimie Biologique.*
Gautier's *Traité de Chimie appliquée à la Physiologie à la Pathologie et à l'Hygiène.*
Duclaux's *Ferments et Maladies.*
Macé's *Traité Pratique de Bactériologie.*
Cornil et Babès' *Les Bactéries et leur Rôle dans l'Anatomie et l'Histologie pathologiques des Maladies infecteuses.*
Banti's *Manuale di Tecnica Batteriologica.*
Lauder Brunton's *Pharmacology, Therapeutics, and Materia Medica.*
Ziegler's *Text-Book of Pathological Anatomy* (English edition).
Hamilton's *Text-Book of Pathology.*
De Bary's *Lectures on Bacteria* (English edition).
De Bary's *Comparative Morphology and Biology of the Fungi, Mycetozoa, and Bacteria* (English edition).
Magnin's *Bacteria* (American edition).
Sachs' *Text-Book of Botany* (English edition).
Giglioli's *Fermenti e Microbi.*
Garnier's *Ferments et Fermentations.*
Cullimore's *Consumption as a Contagious Disease.*
Thorowgood's *Consumption and its Treatment by the Hypophosphites.*
Colman's *Section Cutting and Staining.*

Creighton's *Bovine Tuberculosis in Man.*
Crookshank's *Photography of Bacteria.*
Williams' *Pulmonary Consumption: Its Etiology, Pathology, and Treatment.*
Gamgee's *Text-Book of Physiological Chemistry.*
Gibbes' *Practical Histology and Pathology.*
Hill and Cooper's *Syphilis and Local Contagious Disorders.*
Lewis's *Physiological and Pathological Researches.*
Powell's *Diseases of the Lungs and Pleuræ.*
Microparasites and Disease (New Sydenham Society's publication).
Bramwell's *Diseases of the Heart.*
Schäfer's *Essentials of Histology, Descriptive and Practical.*
Johne's *Die Geschichte der Tuberculose.*
Tyndall's *Essays on the Floating Matter of the Air.*
Miquel's *Les Organismes Vivants de l'Atmosphère.*
Hueppe's *Methods of Bacteriological Investigation* (English edition).
Eisenberg's *Bakteriologische Diagnostik.*
Germain Sée's *Bacillary Phthisis of the Lungs* (English edition).
Smith's *Tubercular Consumption in its Early and Remediable Stages.*
Trouessart's *Microbes, Ferments, and Moulds* (English edition).
Woodhead and Hare's *Pathological Histology.*
Flügge's *Die Micro-Organismen.*
Klein's *Bacteria in Asiatic Cholera.*
Gautier's *Les Alcaloïdes de l'Huile de Foie de Morue.*
Koch's *Etiology of Cholera* (English edition).
Francotte's *La Diphthérie.*
Gautier's *Sur les Alcaloïdes (Ptomaïnes et Leucomaïnes).*

Foster's *Text-Book of Physiology.*

Drysdale's *On the Germ Theories of Infectious Diseases.*

Hoppe-Seyler's *Handbuch der Physiologisch- und Pathologisch-Chemischen Analyse.*

Claude Bernard's *Leçons sur les Propriétés physiologiques des Liquides de l'Organisme.*

Kühne's *Lehrbuch der Physiologischen Chemie.*

Kühne's *Practical Guide to the Demonstration of Bacteria in Animal Tissues.*

Lehmann's *Handbuch der Physiologischen Chemie.*

Hayem's *Recherches sur l'Anatomie normale et pathologique du Sang.*

Claude Bernard's *Leçons de Physiologie expérimentale.*

Annuaire de l'Observatoire de Montsouris (from 1877 to present time).

Fabre-Domergue's *Analyse micrographique des Eaux.*

Eimer's *Organic Evolution as the Result of the Inheritance of Acquired Characters according to the Laws of Organic Growth* (English edition).

De Coninck's *Nouvelles Recherches sur les Bases de la Série pyridique et sur les Bases de la Série quinoléique.*

Kingzett's *Animal Chemistry.*

Schmitt's *Microbes et Maladies.*

Brouardel and Boutmy's *Des Ptomaïnes.*

Binet's *Psychic Life of Micro-Organisms* (English edition).

Hugounenq's *Les Alcaloïdes d'Origine animale.*

Millican's *The Evolution of Morbid Germs.*

Zeissl's *Pathology and Treatment of Syphilis* (English edition).

Billroth's *General Surgical Pathology* (English edition).

Buist's *Vaccinia and Variola.*

Cheyne's *Antiseptic Surgery.*

Bumm's *Mikro-Organismus der Gonorrhoischen Schleimhaut-Erkrankungen 'Gonococcus'-Neisser.*

Certes' *Analyse micrographique des Eaux.*
Cabadé's *Leçons sur les Maladies Microbiennes.*
Carter's *Spirillum Fever.*
Charles's *Physiological and Pathological Chemistry.*
Cornil and Ranvier's *Pathological Histology* (American edition).
Jaccoud's *Pulmonary Phthisis.*
Yeo's *Pulmonary Consumption.*
Jessop's *Asiatic Cholera.*
M'Kendrick's *Text-Book of Physiology.*
Cooper's *Syphilis and Pseudo-Syphilis.*
Béchamp's *Microzymas et Microbes.*
Gorup-Besanez's *Traité d'Analyse Zoo-chimique qualitative et quantitative.*
Brieger's *Ueber Ptomaïne.*
Brieger's *Weitere Untersuchungen über Ptomaïne.*
Casali's *Sulla Natura Chemica delle Ptomaïne del Selmi.*
Vallin's *Traité des Désinfectants et de la Désinfection.*
Baumgarten's *Lehrbuch der Pathologischen Mykologie.*
Flügge's *Handbuch der Hygiene und der Gewerbe Krankheiten.*
Mayer's *Lehrbuch der Gährungschemie.*
Laveran's *Traité des Fièvres Palustres.*
Heiberg's *Die Puerperalen und Pyämischen Processe.*
Coze and Feltz's *Les Maladies Infectieuses.*
Griffiths' *The Diseases of Crops.*
Eckhard's *Beiträge zur Anatomie und Physiologie.*
Valentin's *Lehrbuch der Physiologie.*
Claude Bernard's *Leçons Professées au Collège de France.*
Schmidt's *Charakteristik der Epidemischen Cholera.*
Liebermeister's *Handbuch der Pathologie und Therapie des Fiebers.*
Debove's *Du Traitement de la Phthisie pulmonaire par l'Alimentation forcée.*

Wagner's *Handwörterbuch der Physiologie.*
Fournier's *Leçons sur la Syphilis Vaccinale.*
Pettenkofer's *Cholera: How to Prevent and Resist it* (English edition).
Semple's *Diphtheria: Its Causes, Pathology, Diagnosis, and Treatment.*
Lithgow's *Heredity and Disease.*
Gordon's *Hydrophobia.*
Dolan's *Whooping-cough: Its Pathology and Treatment.*
Cheyne's *Selected Essays on Micro-Parasites in Disease.*
Trousseau's *Lectures on Clinical Medicine* (English edition).
Beale's *The Microscope in its Application to Practical Medicine.*
Aitken's *Science and Practice of Medicine.*
Pidoux's *Études générales sur la Phthisie pulmonaire.*
Schützenberger's *On Fermentation* (English edition).
Peter's *Leçons de Clinique médicale.*
Musgrave-Claye's *Étude sur la Contagiosité de la Phthisie pulmonaire.*
Laënnec's *Traité de l'Auscultation médiate.*
Koch's *Die Ætiologie der Tuberculose.*
Koch *On Bacteriology and its Results.*
Jaccoud's *Curabilité et Traitement de la Phthisie pulmonaire.*
Hérard and Cornil's *De la Phthisie pulmonaire.*
Empis' *De la Granulie ou Maladie granuleuse.*
Damaschino's *Rapports de la Scrofule, et de la Tuberculose.*
Charcot's *Pathological Anatomy of Pulmonary Phthisis.*
Chauveau's *Contagion de la Tuberculose.*
Brouardel's *De la Tuberculisation des Organes genitaux de la Femme.*
Bouchard's *Leçons sur les Maladies infectieuses.*

Ziegler's *Ueber Tuberculose und Schwindsucht.*

Louis' *Recherches anatomiques, pathologiques, et thérapeutiques sur la Phthisie.*

Harvey's *On the Fœtus in Utero.*

Sutton's *General Pathology.*

Brouardel and Boutmy's *Sur un Réactif Propre à distinguer les Ptomaïnes des Alcaloïdes végétaux.*

VI.—TABLES OF ATOMIC WEIGHTS

(*after Lothar Meyer and Seubert*). $O = 16$.

Element.	Symbol.	Atomic Weight.	Element.	Symbol.	Atomic Weight.
Aluminium ..	Al.	27·10	Molybdenum ..	Mo.	96·2
Antimony	Sb.	119·9	Nickel	Ni.	58·7
Arsenic	As.	75·09	Niobium	Nb.	93·9
Barium	Ba.	137·20	Nitrogen	N.	14·046
Bismuth*	Bi.	208·0	Osmium	Os.	195·0
Boron	B.	10·9	Oxygen	O.	16·0
Bromine	Br.	79·952	Palladium	Pd.	106·6
Cadmium	Cd.	112·0	Phosphorus ..	P.	31·04
Cæsium	Cs.	133·0	Platinum¶ ..	Pt.	195·5
Calcium	Ca.	40·02	Potassium	K.	39·136
Carbon	C.	12·00	Rhodium	Rh.	104·3
Cerium	Ce.	141·5	Rubidium	Rb.	85·4
Chlorine	Cl.	35·454	Ruthenium ..	Ru.	103·8
Chromium‡ ..	Cr.	52·13	Scandium	Sc.	44·1
Cobalt	Co.	58·7	Selenium	Se.	79·07
Copper	Cu.	63·34	Silicon**	Si.	28·399
Didymium ..	Di.	145·4	Silver	Ag.	107·93
Erbium	E.	166·4	Sodium	Na.	23·053
Fluorine	F.	19·1	Strontium	Sr.	87·52
Gallium	Ga.	70·1	Sulphur	S.	32·06
Glucinum	Gl. or Be.	9·1	Tantalum	Ta.	182·7
Gold§	Au.	197·324	Tellurium	Te.	128·0
Hydrogen	H.	1·0024	Thallium	Tl.	204·2
Indium	In.	113·7	Thorium	Th.	232·5
Iridium	Ir.	193·0	Tin	Sn.	117·6
Iron	Fe.	56·02	Titanium†† ..	Ti.	48·08
Lanthanum ..	La.	138 9	Tungsten	W.	184·00
Lead	Pb.	206·9	Uranium	U.	240·5
Lithium	Li.	7·02	Vanadium	V.	51·2
Magnesium† ..	Mg.	24·37	Ytterbium	Yb.	173·0
Manganese‖ ..	Mn.	55·00	Yttrium	Y.	89·8
Mercury	Hg.	200·3	Zinc‡‡	Zn.	65·37
			Zirconium	Zr.	90·6

* and † = Marignac.
§ = Thorpe and Laurie.
¶ = Dittmar and McArthur.
†† = Thorpe.
‡ = Siewert.
‖ = Dewar and Scott; Marignac.
** = Thorpe and Young.
‡‡ = Marignac and Baubigny.

N.B. These atomic weights (of 9 elements) are of recent determination, and are not after Meyer and Seubert.

VII.—LIST OF FIRMS WHERE BACTERIOLOGICAL APPARATUS, ETC., CAN BE OBTAINED.

Microscopes, etc.:

C. Zeiss, Jena, Germany; or Zeiss' agent, C. Baker, 244, High Holborn, London.

Incubators, Sterilizers, etc.:

F. E. Becker and Co., 33, Hatton Wall, London.

Chemical Apparatus and Chemicals:

J. Orme and Co., 65, Barbican, London.

Staining Solutions, etc.:

F. E. Becker and Co., 33, Hatton Wall, London.

Agar-agar and Gelatine:

(1) Christy and Co., 25, Lime Street, London.
(2) J. F. Shew and Co., 89, Newman Street, Oxford Street, London.

Microtomes:

(1) Cambridge Scientific Instrument Co.
(2) C. Zeiss, Jena, Germany.

Dissecting Knives, etc.:

C. Baker, 244, High Holborn, London.

Every description of apparatus for the bacteriological laboratory may be obtained from Muencke, 58, Louisen Strasse, Berlin.

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THE END.

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