Microbes & toxins / by Étienne Burnet ; with a preface by Élie Metchnikoff ; tr. from the French by Charles Broquet and W.M. Scott.

Contributors

Burnet, Etienne, 1873-1960.
Broquet, Charles.
Scott, W. M.

Publication/Creation

New York : G. P. Putnam, 1912.

Persistent URL

https://wellcomecollection.org/works/qcj4c7tu

License and attribution

Wellcome Collection
183 Euston Road
London NW1 2BE UK
T +44 (0)20 7611 8722
E library@wellcomecollection.org
https://wellcomecollection.org

MICROBES AND TOXINS

Mr. HEINEMANN'S MEDICAL PUBLICATIONS

THE PROLONGATION OF LIFE.
Optimistic Studies. By ÉLIE METCHNIKOFF. Translation Edited by P. CHALMERS MITCHELL. One vol. Demy 8vo. 12*s.* 6*d.* net.

THE NEW HYGIENE.
Three Lectures on the Prevention of Infectious Diseases. By ÉLIE METCHNIKOFF. With Preface by E. RAY LANKESTER. One vol. Fcap. 8vo. 2*s.* 6*d.*

METABOLISM AND PRACTICAL MEDICINE.
By CARL VON NOORDEN. English Issue under the Editorship of J. WALKER HALL. 3 vols. Roy. 8vo. £2 12*s.* 6*d.* net.
I. THE PHYSIOLOGY OF METABOLISM. By ADOLF MAGNUS-LEVY.
II.—III. THE PATHOLOGY OF METABOLISM. By CARL VON NOORDEN, FR. KRAUS, AD. SCHMIDT, W. WEINTRAUD, M. MATTHES, and H. STRAUSS.

THE NUTRITION OF MAN.
By RUSSELL H. CHITTENDEN. Demy 8vo. Illus. 14*s.* net.

PHYSIOLOGICAL ECONOMY IN NUTRITION.
With special reference to the Minimum Proteid Requirement of the Healthy Man. An Experimental Study. By RUSSELL H. CHITTENDEN. Demy 8vo. 14*s.* net.

THE NATURAL HISTORY OF CANCER.
With Special Reference to its Causation and Prevention. By W. ROGER WILLIAMS. Roy. 8vo. 21*s.* net.

MEDICINE AND THE PUBLIC.
By S. SQUIRE SPRIGGE. Cr. 8vo. 6*s.* net.

SCIENTIFIC NUTRITION SIMPLIFIED.
A Condensed Statement and Explanation for Everybody of the Observations of Chittenden, Fletcher, and others. By GOODWIN A. M. BROWN. Supplementary Chapter by J. SVEN, M.D. Cr. 8vo. 2*s.* 6*d.* net.

THE EFFICIENT LIFE.
By LUTHER H. GULICK, M.D. Cr. 8vo. 3*s.* 6*d.* net.

HAY-FEVER, AND ITS SUCCESSFUL TREATMENT. By W. C. HOLLOPETER, M.D. Cr. 8vo. 3*s.* 6*d.* net.

HINTS ON THE MANAGEMENT OF THE COMMONER INFECTIONS. By R. W. MARSDEN, M.D. Demy 8vo. 3*s.* 6*d.* net.

SELF-HELP FOR NERVOUS WOMEN.
Familiar Talks on Economy in Nervous Expenditure. By JOHN K. MITCHELL, M.D. Cr. 8vo. 2*s.* 6*d.* net.

THE FASTING CURE.
By UPTON SINCLAIR. Cr. 8vo. 2*s.* 6*d.* net.

THOSE NERVES.
By GEORGE LINCOLN WALTON, M.D. 2*s.* 6*d.* net.

WHY WORRY?
By GEORGE LINCOLN WALTON, M.D. Small Cr. 8vo. 2*s.* 6*d.* net.

PASTEUR

When a pupil at the École Normale, 1843–46

Pencil drawing by Lebayle from a daguerreotype in the possession of M. Vallery-Radot

MICROBES & TOXINS

By Dr. ETIENNE BURNET

OF THE PASTEUR INSTITUTE OF PARIS

WITH A PREFACE BY

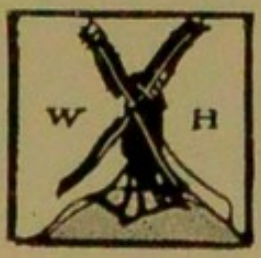
ÉLIE METCHNIKOFF

Translated from the French

BY

Dr. CHARLES BROQUET and W. M. SCOTT, M.D.

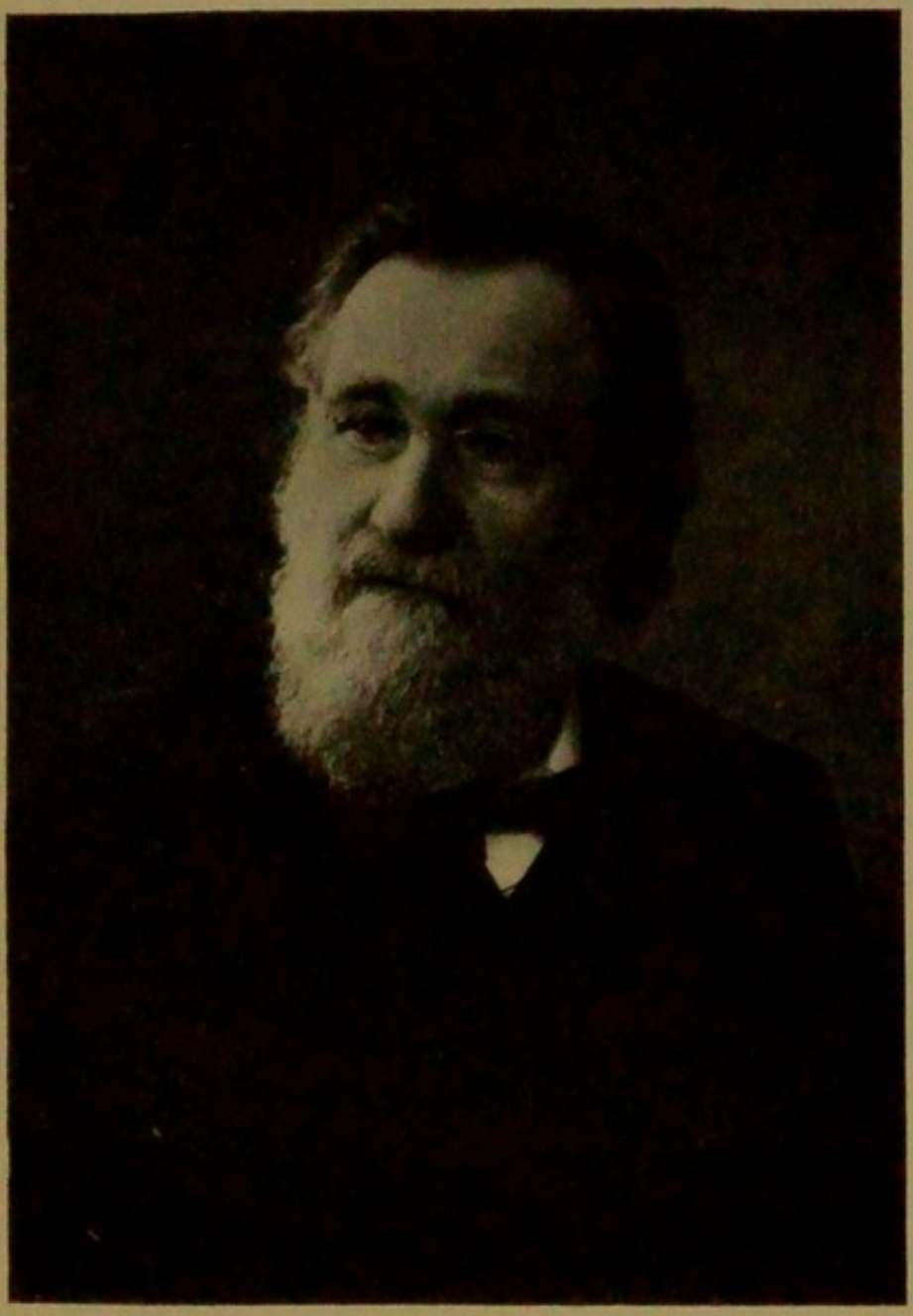
ILLUSTRATED



LONDON:

WILLIAM HEINEMANN

1912



Photo] [*Henri Manuel, Paris.*

ÉLIE METCHNIKOFF

TO

DR. ÉMILE ROUX

Director of the Institut Pasteur

INTRODUCTION

THE publication in the *Bibliothèque de Philosophie Scientifique* of a volume dealing with micro-organisms was entirely indicated, for microbiology is taking every day a larger and larger place in the realm of knowledge and philosophy. Although discovered more than two hundred years ago, microbes were long neglected, and it was only during the second half of last century that their true rôle was ascertained.

Ehrenberg in the middle of the nineteenth century had already perceived the importance of microscopic organisms in the evolution and life of our planet. The discovery of the fossil remains of Diatoms and Foraminifera led him to appreciate the great part these minute creatures have played in the building up of the earth's crust.

There were not lacking men of science disposed to attribute to micro-organisms an important action in the phenomena of fermentations and of disease, but it was only after the labours of Pasteur that this truth was definitely established and became part of our common heritage of knowledge.

Relations had long been perceived between the animal kingdom and the vegetable, animals furnishing carbonic acid and nourishment to plants, while these, on their side, nourish the animals with their organic matter and provide them with oxygen. Later it was recognised that between these two kingdoms there lies the domain of the microbes. It is the

microbe which transforms the animal material supplied by dead bodies and dejecta into simpler substances, nitrates, and salts of ammonia, capable of assimilation by those plants which supply us with food. Further, it is the microbe which renders pleasant to the taste certain animal and vegetable food-products, as, for example, the juice of the grape, the extract of malt, cabbages, apples, and milk, transforming these respectively into wine, beer, sauerkraut, cider, kephir, various kinds of cheese, etc.

Thanks to Pasteur the activity of micro-organisms was established in every case of putrefaction and fermentation; and, with this fact to start from, it became more easy to tackle the problem of infectious diseases.

Putrefaction and suppuration have been recognised for centuries as being phenomena of the same order. Decomposing pus, fæcal matter smelling of putrefaction, urine issuing from the bladder in a state of decomposition, all indicated that an illness, a state of suffering, was equivalent to an infection of the body.

Although certain microbes, such as the bacteridium of anthrax, had been observed before Pasteur's great discoveries, it was only as a consequence of these discoveries that the fundamental rôle of microscopic organisms in disease was understood. The labours of Lister in surgery, and of Davaine and Koch on the "black blood of anthrax" first authorised the application of Pasteur's doctrine to surgery and medicine.

Pasteur himself with his pleiad of disciples was in the midst of this activity, an activity which in a very few years revolutionised medical science and endowed medicine with more than one preventive vaccine such as those against anthrax and rabies.

The Pasteur school in France and the school of Koch in Germany have succeeded in elucidating many medical problems of the highest importance and have drawn valuable practical conclusions from these.

Thanks to all this work, work which has increased during these last years in extraordinary fashion, a universe of micro-

organisms, beneficent and mischievous, has been revealed to humanity; and it is this new knowledge which has so largely contributed to the diminution in disease and death at the present day and which holds out to man the hope of a more happy future.

The micro-organisms inhabiting our bodies have set going there a poison factory, which cuts short our existence, and by secreting poisons which penetrate all our tissues, injures our most precious organs, our arteries, brain, liver, and kidneys.

Man balked of his full term of life feels himself unhappy and is ready to accept any solution to the problem of gaining happiness. And the progress of microbiology leads us to hope that this science will one day liberate man from his fear of the grave and permit him to attain the true object, the true conclusion of life.

It is time for bacteriological science to leave the laboratory and the lecture theatre, and to take its place before the great public, in order that its benefits may receive the widest and readiest application.

It was very natural for the creator of this "Library of Scientific Philosophy" to apply to the Pasteur Institute for an account of the actual position of science with regard to microbes and toxins. Not only was the movement started from Pasteur's laboratory and continued in the Institute bearing his name, and still sheltering one of his most illustrious collaborators in the person of Dr. Roux, but it is in this Institute that every branch of microbiology is undergoing active study. To take colloids and the physico-chemical laws which govern their activity, we have at the *Institut Pasteur* studies on ferments and fermentations as well as on the chemical processes which lie at the root of life and of recovery from disease. In this Institute also there are zealous workers in the field of infective microbes and the means of combating them.

Several laboratories are specially set apart for researches on tropical diseases, and finally the Pasteur Hospital has been created for patients suffering from all sorts of infectious maladies.

If Pasteur were to see his Institute again, he would be

CONTENTS

CHAPTER I

PAGE

THE GENERAL FUNCTIONS OF MICROBES—THE TRANSFORMATION CYCLES OF CARBON AND NITROGEN 1

CHAPTER II

MICROBES IN THE HUMAN BODY—LIFE WITHOUT MICROBES—THE INTESTINAL FLORA 25

CHAPTER III

FORM AND STRUCTURE OF MICROBES 50

CHAPTER IV

PHYSIOLOGY OF THE MICROBES 72

CHAPTER V

PATHOGENIC MICROBES—INFECTION 107

CHAPTER VI

INFLAMMATION AND PHAGOCYTOSIS 130

CHAPTER VII

THE PATHOGENIC PROTOZOA: FILTER-PASSING VIRUSES . . 141

CHAPTER VIII

PAGE

THE TOXINS 157

CHAPTER IX

TUBERCULIN AND MALLEIN—ANIMAL TOXINS—VENOMS . . 173

CHAPTER X

IMMUNITY 187

CHAPTER XI

IMMUNITY 210

CHAPTER XII

ANAPHYLAXIS 233

CHAPTER XIII

APPLICATIONS OF BACTERIOLOGY 250

CHAPTER XIV

VACCINES AND SERA 265

CHAPTER XV

CHEMICAL REMEDIES 285

CHAPTER I

THE GENERAL FUNCTIONS OF MICROBES—THE TRANSFORMATION CYCLES OF CARBON AND NITROGEN

The circulation of matter; anabolism and catabolism—Views of Lavoisier—The transformation cycles of carbon and nitrogen—The rôle of micro-organisms: I. Maturation of the soil and formation of arable land. II. Fermentation of vegetable matter—Decomposition of starch and manure—Hypotheses on the formation of coal. III. Putrefaction of albuminous materials. IV. Nitrification and denitrification: in agriculture; in the biological purification of sewage. V. Fixation of atmospheric nitrogen in the soil: by bacteria alone; by bacteria in association with algæ; by the nodule bacteria of Leguminosæ—Some ideas about the useful micro-organisms: Fermentations in connection with food production and in various industries.

ALMOST all living matter is made up of water, that is to say, of oxygen and hydrogen, and of compounds of carbon and nitrogen. Other elements may enter into the tissues of animals and vegetables, for example, sulphur, iron, arsenic, boron; but by following the circulation of carbon and nitrogen, it is possible to have a general view of the movements of exchange between matter and life.

The living creature restores to nature what it has absorbed, and eventually its own body, by its excretions during life and by its decomposition after death. The elements set free are recombined into organic bodies, and these exchanges and this circulation form the essence of life. It is an abuse of our subjective attitude to consider as two opposites life and death. At most, life is the opposite of inertia. Death is a special kind of accident, life being the all-embracing phenomenon. The first of the workers in the great cycle of life are the microbes,

and the decompositions and re-combinations of living matter depend entirely upon them.

Life without microbes is not conceivable at the present day. That does not mean that they were the first living beings to appear on the surface of the earth. It is the more difficult to get an idea of their origin, since in all probability they have undergone evolution, and have not always had the appearance they have at present. It is possible that under forms that we can hardly guess at, life appeared long before the existence of microbial forms; but microbes have been the chief agents in the spread and extension of life throughout the world.

In the world of to-day, the building up and breaking down of organic substances are functions of cells, conducted by an infinite number of diastases. These cells include the cells built up into animal and vegetable tissues, and the isolated cells, the microbes. It would not be right to contrast too much the microbial cells with the cells of tissues, merely because the former are separate individuals, whereas the latter are arranged in systems. In nature, micro-organisms can exist as solitary individuals, but are seldom actively at work in this condition. For example, in the soil, the bacteria which fix nitrogen act rather like a wide-spread tissue. The active agents are in any case invariably cells.

To describe in a few words the cycle which organic matter follows, one cannot do better than quote the celebrated page of Lavoisier, "Plants draw from the circumambient air, from water and in general from the mineral kingdom, the substances necessary for their own organisation. Animals feed either on plants or on other animals, which have themselves fed on plants, so that eventually the matter building them up is always derived either from the air or from the mineral kingdom.

"Finally, fermentation, putrefaction and combustion are continually restoring to the atmosphere and to the mineral kingdom the elements which plants and animals have borrowed.

"By what rocesses does nature effect this wonderful

circulation between the two kingdoms? How does nature succeed in producing substances which are combustible, putrescible and capable of fermentation from combinations which have none of these properties? Here are impenetrable mysteries. One may perceive, however, that since combustion and putrefaction are the means which nature employs to return to the mineral kingdom what has been drawn from it in the building up of plants and animals, the latter process must be the converse of the former."

Green plants get their carbon from the carbonic acid of the air. In virtue of their chlorophyll activity they build up this carbon into starch, cellulose, sugars and fats. This carbohydrate synthesis then represents the accumulation of energy. A green plant kept in the dark burns up its hydrocarbons, returning them to the atmosphere. A dead plant returns its hydrocarbons after a series of decompositions, in the form of carbonic acid and water. A plant which has been eaten by an animal supplies the animal with glycogen and fats which are consumed in the course of muscular work and respiration. Dying animals like dead plants return their hydrocarbons to nature. Plants without chlorophyll and all animals spend and dissipate the energy accumulated by the chlorophyllous plants, energy derived entirely from the rays of the sun.

Nitrogen exists in the atmosphere, from which it enters the soil. It exists in the dejecta of living animals and in the bodies of plants and animals rotting on the surface of the earth. It accumulates there in the mould or humus: plants take up the nitrogenous matter of the soil in the form of salts of ammonia and nitrates, and build up from them the vegetable proteins. Animals which eat plants produce from these the animal proteins. Animals and plants in decomposition, and the excretions of animals in general, return their nitrogen to fertilize the soil.

Animals are in a certain sense parasites of plants, since they are unable to build up by themselves, starting from mineral elements, their hydrocarbons and proteins. Even in plants

the formation of the protein compounds is dependent on the chlorophyll activity; without this it would be impossible for them to build up the carbohydrates, and these are indispensable in the employment and elaboration of the nitrogenous elements of the soil. The chlorophyll activity is itself dependent on the sunlight, so that life is one great pæan to the sun.

In the transformation of carbon and nitrogen all the operations are not carried out by microbes, for these latter do not count at all in chlorophyll assimilation nor in animal digestion. But it is the bacteria which keep up the supply of organic matter which forms the source of animal and vegetable life; it is the bacteria which restore to circulation those elements which were for a moment arrested in the bodies of animals and plants: they restore to life matter which had ceased to live. It is they also which prepare the soil for vegetation and cultivation: they accumulate in the soil the nitrogen which we are to eat in the form of cereals.

It is they which carry on the impenetrable mystery referred to by Lavoisier.

They prepare the primary material of life. Their activity is as universal as that of water, as that of light.

The discoveries of Pasteur have not only revolutionised medicine, they have filled with new life the science and practice of agriculture and husbandry.

I.—*Maturation of the soil and formation of arable land.*

It was not the bacteria which shattered, splintered and powdered the rocks which formed the first crust of the cooling globe. But as soon as in this chaos there appeared water and alkaline phosphates, algæ and bacteria could establish themselves and with the help of carbonic acid continue the first formation of soil.

In those rocks known as "rotten rock," Müntz found nitrifying microbes: the nitrifying ferment has also attacked the Faulhorn, a mountain of the Bernese Oberland near

Grindelwald, which is composed of a calcareous schist, black, friable, and crumbling down. There is therefore a sort of putrefaction of rocks (alkalis and alkaline earths). According to Fausto Sestini the carbonic acid produced in the respiration of plant roots hastens the breaking down of feldspars.

Mosses, lichens, algæ and bacteria prepare the way for the highest plants. The more there is growth of plants, the more there is decomposition. In this way, soil or humus has little by little spread over all the earth.

II.—*Fermentation of Carbohydrates.*

These fermentations restore to circulation the hydrocarbons of animals and plants.

For the decomposition of sugars, starches, fats, glucosides, and celluloses, several series of fermentations are necessary, each supplementing another in the work. The anaerobic organisms break down the large organic molecules, whereas the aerobes carry out, in particular, oxidations. When the bacteria are insufficient, the moulds continue the work. Finally, nothing is left but carbonic acid and water. All the operations which in our laboratory analyses we distinguish and conceive in terms of abstract formulæ, are combined and intermingled in nature, each succeeding and limiting the other. The yeasts transform sugars into carbonic acid and alcohol. Alcohol attacked by the acetic ferments is turned into acetic acid. Finally, the *Bacterium aceti* can split the acetic acid of vinegar into carbonic acid and water. The *Mycoderma vini* destroys and oxidises both alcohol and acetic acid, producing again carbonic acid and water. The sugar contained in Raulin's fluid (*v. infra*), for example, is attacked by *Aspergillus niger*, the products including oxalic acid. Various moulds and bacteria turn starch into sugar; it is the moulds in particular which complete the oxidations, producing carbonic acid and water.

Milk left to itself ferments, *i.e.*, produces lactic acid, which, meeting with an alkaline carbonate, furnishes calcium lactate.

Pasteur discovered the transformation of the lactate into the butyrate by the butyric vibrio; the butyrate can be completely consumed in its turn by the moulds.

The glucosides (compounds of sugar with an organic body, an alcohol or phenol) are decomposed into their two elements. A diastase, tannase, secreted by the *Aspergillus*, splits tannin into two molecules of gallic acid, from which other moulds produce again carbonic acid and water.

Just as there is not one starch but several, so there are several celluloses, which resemble starches but are more stable: $(C_6H_{10}O_5)_n$. They form the walls of vegetable cells, and make up one-third of the weight of the straw, which is the principal component of farmyard manure. If the celluloses

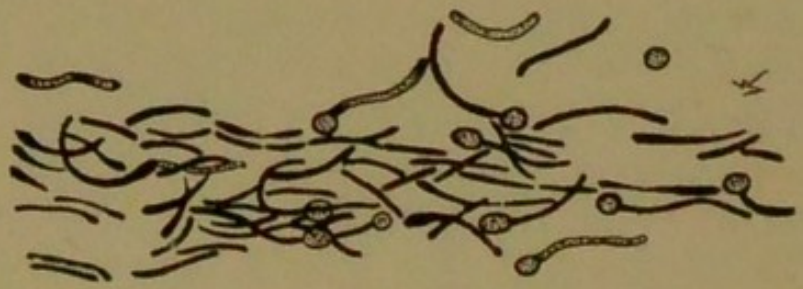


FIG. 1.—The microbe which ferments cellulose, described by Omeliansky: bacilli with spores.

were not decomposed and restored to circulation, the earth would soon be cumbered with useless refuse material. But from the beginning moulds establish themselves on the outer skin of the living plant; when it dies they invade its tissues, attacking first the sugar and then the cellulose, the latter being hydrolysed, transformed into sugars and consumed. The *B. Amylobacter* of Van Tieghem, an anaerobe, produces from cellulose hydrogen, carbonic acid and butyric acid. Omeliansky has demonstrated two methods of anaerobic fermentation in cellulose, one with production of hydrogen, the other with production of methane. Those ferments which in a tube in the laboratory decompose the cellulose of Berzelius' paper, act in precisely the same way in manure heaps. The aerobic fermentation of cellulose is carried on by moulds, by fungi

more highly organised, and by the nitrifying and denitrifying bacteria.

Pectose is a hydrocarbon associated with cellulose in the membranes and interstices of plant cells; the rust which attacks hemp and linen is a fermentation of the pectose, transforming it into pectic acid, then to sugar, by the *B. amylobacter* and by the *Granulobacter* of Fribes and Winogradsky. In the manure heap, aerobic fermentation proceeds at the surface and raises the temperature up to nearly 80° C. In the depths of the heap the temperature is low, and there the anaerobic ferments attack the cellulose. The bubbles which rise and burst on the surfaces of ponds are signs of the anaerobic fermentation which is decomposing organic debris at the bottom. Manure kept in a latticed box with free access of air heats up without there being destruction of the cellulose; loss of nitrogen takes place. Manure heaped in a closed box or kept corked in a large carboy, liberates methane from the decomposition of the cellulose. It is possible to collect the gas and by means of an exit tube to make it furnish a light.

It was a natural step to attribute to the ferments of cellulose the formation of peat, lignite and coal. Microbes certainly are at work in the decomposition of vegetable matter in the peat-bogs, and coal is supposed to be the product of more complete fermentations, two varieties at least of bacteria being in activity in succession; the first dissolves the central membranes of the cell walls, the others attacking the cellulose, more or less pure, which constitutes the thick parts of the wall. "The bacterial activity," says the most convinced defender of this theory, "produced a de-hydrogenation and de-oxidation, the final result being the production of carbon. We do not know if the final limit of this process has ever been reached, but figures show that the more geologically ancient the fuel, the more carbon it contains" (Bernard Renault). Fuel of less age, lignite, and peat, for example, contains besides bacteria, amœbæ and infusoria. In the lignites, the cannel coals, and the boghead coals, we find fungi and algæ, which do not occur in coal proper. Bacteria alone exist in all the fuels; they are

much less altered than the structures which surround them: therefore it is supposed that they must have survived tissues which they destroyed.

The presence of bacteria in coals derived from organic matter should not astonish us. But it appears difficult to believe that bacteria have been the only agents in the formation of coal. The study of their form is extremely difficult; fine particles of iron pyrites and little crystals often imitate bacterial forms in the thin sections of coal, so difficult to prepare and examine by transmitted light under the microscope. On the other hand the fermentation of cellulose does not explain the frequent impregnation of the debris by a blackish, bituminous material, nor perhaps does it really explain the enrichment in carbon of the deposits; further, there have been produced from the fermentation of fatty bodies, resins and similar volatile substances, phenol bodies like those found in coal (E. Duclaux).

There is nothing surprising in the presence of algæ in the *Bogheads*, for these are precisely the coals derived from algæ and often named algal coals. Their formation was due to luxuriant algæ growths rapidly developed on the surface of stagnant pools, which then sank to the bottom carried down by a coagulum in the peaty water; this mass contained bituminous substanceswhich must have come from elsewhere, for there is no indication that they took their origin on the spot by an alteration of the algæ. It is these bitumens which have produced the carbon enrichment of the mass, so that on the whole instead of destruction a preservation process has taken place. In other cases, instead of this enormous growth of algæ, clouds of pollen and spores from the primeval forests have been deposited: these "rains of sulphur" also sank in the peaty water and became saturated with bitumen, hence the so-called spore and pollen coals.

We ought not to be too ready to reject the bacterial theories of the formation of the various coals, especially should it turn out to be impossible to explain without microbes the formation of the bitumen which impregnates them. The small lower algæ are rich in fats and capable of yielding petroleum bodies

by distillation. The problem of the origin of coal has therefore some relation to the problem of the origin of petroleum. The decomposition of fatty substances commences with their saponification, which splits them into fatty acids and glycerine, and glycerine is a good food medium for various bacteria.

The moulds decompose fatty acids into carbonic acid and water. Fats resist decomposition longer than other carbohydrates, and longer than nitrogenous substances; it is from this cause that the proportion of fat increases in a cheese which has been kept, or in a dead body which is decomposing. The '*adipocere*' is the final stage in an animal body left entirely to nature.

III. *Putrefaction of albuminous materials.*

Putrefaction returns to the soil the nitrogen which makes up 15 per cent. of the proteins of animal tissues.

Since plants draw their nitrogen from the soil in the form of nitrates and salts of ammonia, it is necessary for the complex protein molecule to be broken down so as to supply finally

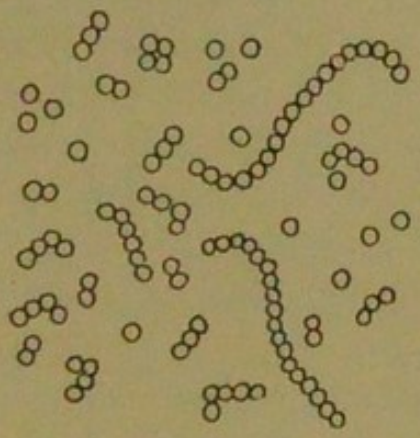


FIG. 2.—Ammoniacal fermentation of urine: *Urococcus* of Pasteur.

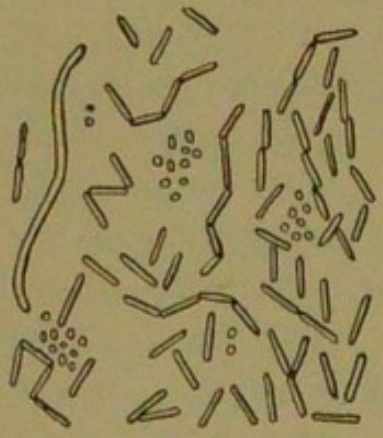


Fig. 3.—*Urobacillus* of Duclaux.

nitrogen in the form of ammonia and nitric acid. Later green plants and finally animals, raise this mineral nitrogen to the level of organic nitrogen.

Already during life animals discharge nitrogen with their excretions in the form of urea, uric acid and hippuric acid. The Urobacteria (there are about a hundred species known)

transform the urea, by means of a urease which they secrete, into carbonate of ammonia. Hippuric acid is transformed into benzoic acid and glycocoll, and finally into ammonia.

The putrefactions of albuminous materials in nature are never simple, that is to say carbohydrates almost always accompany the proteins ; even meat contains a little sugar. Putrefactions therefore are almost always mixed fermentations.

Pasteur thought that all putrefaction was the work of anaerobes. He discovered the *vibrion septique*, an anaerobic bacillus capable of decomposing proteins. Later, the anaerobes were neglected and it was thought that various aerobes, in particular *Proteus*, were the principal agents of putrefaction. But after the study of fetid suppurations had drawn attention to the presence of anaerobes (Veillon), the idea rose again that they too must play a part in putrefaction, and the methodical study of this subject was recommenced, chemical analysis going hand in hand with bacteriological examination.

In their experiments, Tissier and Martelly followed for months the events which took place in flasks into which meat had been put and allowed to putrefy either with or without access of air. Meat taken from the slaughter-house as fresh as possible contains all the germs necessary and sufficient for putrefaction, and, as it contains carbohydrates, it is a mixed putrefaction which occurs.

There are two phases. In the first the sugar and the proteins are attacked by mixed ferments, that is to say, by microbes which decompose at the same time both proteins and sugar, *proteolytic* and *saccharolytic*—(peptolytic is the term applied to the ferments which attack protein only after its reduction to peptone). In the second phase the protein and its products are attacked by ferments which are proteolytic or peptolytic pure and simple, not saccharolytic.

But between these two phases a critical turning-point occurs: the decomposition of the sugars produces an acidity sufficient to stop putrefaction. The "antagonistic force" which Bienstock observed in his investigations on a putrefactive

bacterium the *B. putrificus*, is nothing but this acidity. A piece of meat, as is well known, keeps excellently in unboiled milk, because the milk on fermenting produces lactic acid, which protects the proteins of the meat from putrefaction. That is why the housewife when she salts down meat, does not forget to add some vinegar, *i.e.*, acid. It would seem then that putrefaction ought to cease after the action of the mixed ferments.

If it does not stop, it is because there is not sufficient sugar. The *limit of acidity* for the pure proteolytic ferments has not been reached. Further, owing to the decomposition of the albumins already begun, there appears a base, ammonia, which neutralises the acidity, and the pure proteolytic ferments can begin their action. It is the anaerobes which break down the protein molecule and produce putrefaction; but they cannot do without the auxiliary aerobes; thanks to the aerobes which hasten the production of ammonia and the alkalinisation of the medium, putrefaction passes successfully through the crisis of the antagonistic acidity.

In the putrefaction of milk, two analogous phases succeed each other. Milk being richer in sugar than meat, the acidity developed in the first phase is greater and the crisis more difficult to surmount. To surmount it, more powerful ferments are required than the aerobes which produce the ammonia in the putrefaction of meat: these are the fungi (*Oïdium lactis*, *Rhizopus nigricans*) and the yeasts, which destroy the acids, consume the milk-sugar, and attack the casein. The fungi prepare the way for the pure ferments, which then carry out the second phase of putrefaction.

When an animal dies, all the microbes necessary for its putrefaction are present in its intestine. They invade the tissues and carry out on a larger scale what we see on a small scale in the experimental flasks. The worm of the grave is an old poetical image long out of date. The real destroyer is the microbe.

It is a fact well established to-day, that in a normal intestine, the bacteria of putrefaction are capable of vegetative existence,

and that our digestion is accompanied by a commencement of putrefaction,—and of intoxication. The aerobic bacteria of the *Proteus* group *can* produce putrefaction there, but it is chiefly the anaerobes, as was Pasteur's opinion, which do this. There have been found in the human intestine all the most important of these, the *B. putrificus*, the *B. sporogenes*, and the *B. perfringens*. The anaerobic flora of the intestine does not differ much from the anaerobic flora of the putrefying meat in Tissier's experiments. These microbes produce poisons which are the true source of auto-intoxications (Metchnikoff). For life in general, putrefaction is necesssary to permit of the circulation of nitrogen in nature: but our own particular interest demands that putrefaction should not begin too soon, *i.e.*, in our intestine, and mask itself under an appearance of perfect health.

To combat the intestinal putrefaction, it is necessary to adopt a diet capable of producing in us that *limit of acidity* which induces the crisis separating the two phases of putrefaction of meat or milk, and of arresting decomposition in our bodies at the end of the first phase. Therefore, we ought to eat carbohydrates and sugars, and so alter the conditions in our intestine as to favour the lactic ferments.

IV.—*Nitrification and Denitrification.*

Nitrates represent the form of nitrogen preferred by the plants, and it has long been known that the ammonia set free in putrefaction becomes oxidised in the soil, the ammonium salts being transformed into nitrates. This is the process of *nitrification* and is carried on by bacteria.

It was long thought that the ammoniacal salts were oxidised in contact with the soil by the direct action of atmospheric oxygen, in the same way that certain chemical combinations can take place on contact with porous substances. But chalk and sand, which ought to act as porous substances, cannot take the place of earth. Pasteur perceived with his peculiar intuition that the lower plant must play a part in nitrification.

That nitrification is the work of living creatures has been proved by the celebrated experiment of Schlœsing and Müntz. If a cylinder is filled with cultivated soil, ammonia poured on the top appears at the bottom as nitrate of lime. But this transformation no longer occurs if the earth is previously heated to 100° C., or if it is impregnated with the vapour of chloroform or of carbon bisulphide, *i.e.*, if the living organisms that it contains are killed or paralysed. The soil recovers its activity when the paralysing vapours have been removed by passing through a current of air.

Nitrification is a process of two stages, and is carried out by two species of bacteria, each with its own function. In the first phase, the ammoniacal salts are transformed into nitrites by the microbes known as the nitrous ferment, *e.g. Nitrosomonas* of Europe, *Nitrosomonas* of Java, and the *Nitrosococcus* of America (Mexico and Brazil). In the second phase the nitrites are turned into nitrates by the nitro-bacterium or nitric ferment, the *Nitrobacter* of Winogradsky (figs. 4–6).

Neither the nitrous nor the nitric ferments develop in presence of organic matter. The activity of the nitrous ferment is arrested by 0·3 per cent. of glucose, peptone or asparagin. The nitric ferment, less sensitive, is stopped by 0·3 per cent. of glucose, 1·25 per cent. of peptone or 1 per cent. of asparagin. The former is inhibited by 1·5 per cent., the latter by 3 per cent. of sodium acetate.

Now we are accustomed to the idea that bacteria live on organic matter. Yet the bacteria of nitrification must, as we have seen, get their nourishment elsewhere. They differ from other bacteria in being capable of taking up carbon by decomposing carbonates, in being aerobic, and in behaving like chlorophyllous plants. They are not saprophytes in the same sense as the majority of bacteria.

In the laboratory, the two phases of nitrification can be demonstrated separately by pure culture. But in nature they are simultaneous. Under laboratory conditions ammonia exerts an inhibiting action on the nitric ferment, whereas in nature both the actions can occur in presence of quantities of

ammonia frequently very considerable. Schlœsing has shown that in the soil ammonia does not prevent the nitric ferment from acting. These apparent contradictions between theory and practice are capable of explanation. Ammonia prevents the *development* of the nitric ferment but scarcely acts at all on the same ferment in the adult state. The soil in nature being populated by the adult ferment, the inhibiting action of ammonia is much more limited than in an experimental flask (Boullanger and Massol).

The nitrous ferment will stand neither an excess of ammonia nor an excess of its own product, the nitrite of magnesium. The nitric ferment ceases to act when too much nitrite has

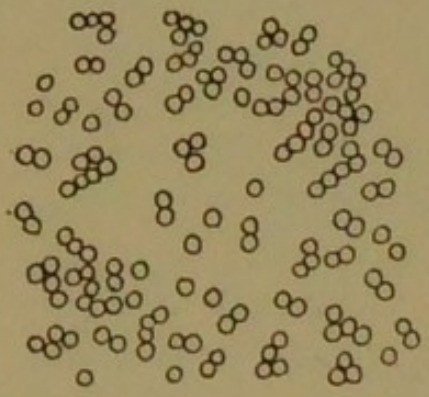


FIG. 4.—Nitrous ferment from Gennevilliers.

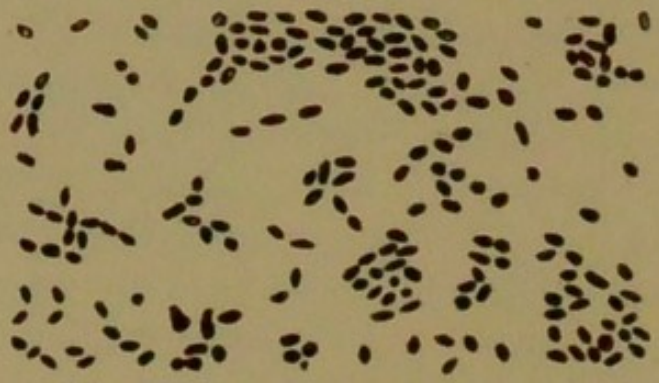


FIG. 5.—Nitric ferment : the nitro-bacterium from Quito (after Kayser).

been produced around it and when it has itself developed a certain quantity of nitrates. Thus each demands suitable proportions both of the primordial material and of the products. It is, no doubt, to maintain these favourable conditions that we have the denitrifying microbes which partially undo the labour of the nitrifying organisms. They return the nitrates and nitrites to the condition of ammonia, liberating protoxide of nitrogen, dioxide of nitrogen, or simply nitrogen. Wheat straw, the straw of maize and of lucerne and oilcakes contain denitrifying bacteria. Animal excrement also contains them, for soil to which cow dung is added loses part of its nitrates. When soil is treated with nitrate of soda too soon after receiving farmyard manure it loses nitrogen. The denitrifying organisms act best in presence of the excess of organic matter

which is so prejudicial to nitrification. Probably the two functions balance and regulate each other. The nitrogen liberated by the denitrifying bacteria is, however, not lost ; it may be taken up by the bacteria which fix nitrogen. It is nevertheless true that farmers ought to be on their guard against the denitrifying organisms and avoid putting on the soil farmyard manure, especially if fresh, along with nitrates.

Formerly in the manufacture of saltpetre cultivated soil was

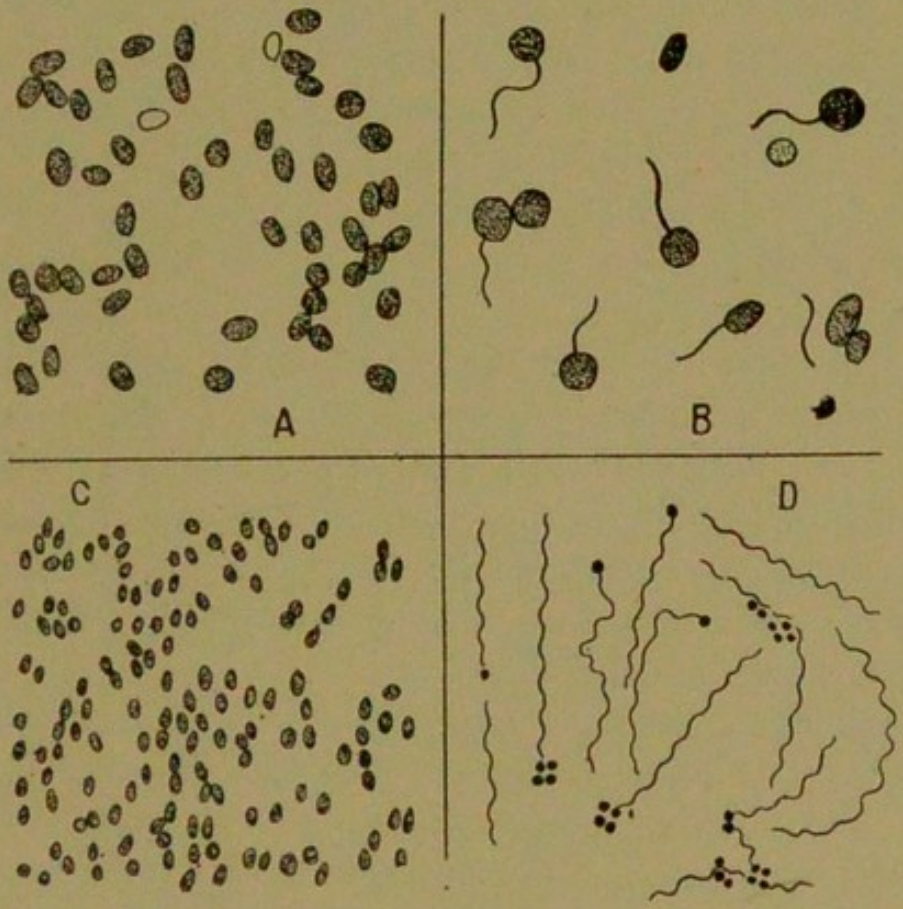


FIG. 6.—Nitrifying bacteria (after Winogradsky).

A. Nitrous ferment from Zürich.
B. The same in the motile form with flagella.
C. Nitrous ferment from Kazan.
D. Nitrous ferment from Java, motile cells and groups of cells.

freely sprinkled with urine. If the deposits of nitrate in Chili and Peru should some day be exhausted, it would not be impossible to make it with the help of bacteria (not to speak of the electrical methods which have already been employed). Saltpetre is produced everywhere when suitable conditions of moisture and organic matter are given. The saltpetre of

cellars is nitrate of lime which has risen from the soil through the walls by capillarity and has undergone evaporation.

Long ago Müntz prepared artificial nitre beds which furnished eight grams of nitrate daily; in recent experiments he and Laîné have obtained such a yield that they find themselves capable of preparing by means of nitrifying organisms all the saltpetre for the powder necessary to defend the nation in war. Distributed over beds of peat of two metres thickness on the top of a layer of clinker, the nitrifying bacteria are capable of producing, for each 25 acres of area, 1,500 tons of nitrate per day, after a starting period of one month at most to get the beds into working order; in five days, that is, 7,500 tons, or the annual requirement of powder for the army. It is easy to calculate what 750,000 acres of peat bogs in France could produce if necessary.

The purification of sewage is one of the greatest tasks which burden the hygienists of large towns. Broad irrigation followed by cultivation demands much land and is not quite safe except when employed solely for the growth of forage, not for market-gardening. Hence it is gradually giving place to the biological method of purification, an intensive process carried on in small space, and here again by bacteria.

We shall not enter here upon the details of its application. In principle, complete biological purification goes on in two phases: a phase of anaerobic fermentation in the septic tank and a phase of aerobic fermentation in the bacterial beds.

In the septic tank, into which the sewage must be run gently—so as not to carry in the air which would favour aerobic fermentation—in which it must be allowed to circulate quietly, and to stay at least twenty-four hours, the disintegration takes place both of hydrocarbons and proteins under the agency of legions of bacteria which secrete all sorts of diastases. The sludge dissolves and the resultant product can be submitted to the action of the nitrifying agents.

The experimental control of the ferment activity in the septic tank can be carried out by comparing the action on coagulated egg-white, meat, raw or cooked, fats, paper, &c.

In six weeks 100 grams of egg-white leave only one gram of residue, whereas in stagnant sewage there remain 76 grams, and in running water 83 grams. In three weeks meat loses almost 50 per cent. of its weight, and in six weeks 96 per cent. The body of an animal, immersed in it is for long protected by the layer of subcutaneous fat; but cleaned cartilage and tendon lose in five weeks 65 to 99 per cent. of their weight; even wool and feathers decompose.

As regards hydrocarbons, the fats are slowly split into fatty acids and glycerine. Cabbages and potatoes are almost completely destroyed in six weeks. A hempen rope which, after five weeks of immersion in stagnant sewage or running water, could still bear the weight of 12 kilograms, broke under 15 grams after the same period of immersion in the septic tank. After three weeks newspaper begins to dissolve liberating bubbles of gas. It is quite wrong to consider the septic tank as operating like a simple settlement tank: it is rather a sort of crucible in which the powerful microbes melt and disintegrate the most resistant organic matter. The septic tank liberates various gases, methane, hydrogen, nitrogen and carbonic acid, a cubic metre of sewage furnishing from 40 to 170 litres of gas.

The aerobic phase of the purification is accomplished by *bacterial beds*, into which the organic matter, already dissolved and transformed into ammoniacal compounds is discharged. What takes place in them is an intense *nitrification* carried on by the same microbes which in arable soil transform the ammonia into mineral salts, nitrites and nitrates.

The bacterial bed consists of a thick layer of clinker or slag, and is filled with sewage for periods of an hour and a half to two hours separated by intervals of four to six hours. *Contact* may be renewed if necessary two or three times by passing the effluent through a second or third bacterial bed. During contact the organic matter fixes itself on the clinker, while during the aeration period it is oxidised by the ferments which take up the necessary oxygen from the air.

The nitrification is balanced in the bacterial beds by a

denitrification as in cultivated soil. Nitrifying and denitrifying ferments can live side by side; the latter only interfere with the object desired when there is present an excess of hydrocarbons.

A town of 500,000 inhabitants, furnishing 6 million gallons of waste water, could replace the 682 acres required for broad irrigation, or the 15 acres required for the bacterial beds with slags and clinker, by 2·5 acres of bacterial bed built on peat (Calmette).

The biological purification returns the nitrogen to nature in the form of nitrate, although a certain quantity is lost in the form of gas. There are other bacteria, however, capable of taking up nitrogen from the air and re-introducing it into the cycle of animal and vegetable life.

V.—*Fixation of Atmospheric Nitrogen in the Soil.*

The life of both animal and vegetable species depends on the stock of nitrogen retained by the soil. Although the earth acquires nitrogen from putrefying processes it loses nitrogen also, discharged in the gaseous condition; some is lost also during denitrification, and in percolating water which robs uncultivated soil of as much as 40 kilos. of nitrogen per acre per annum. Floods also carry off the nitrates, and after the floods of 1896, Schloesing calculated that the Seine carried off about 5 milligrams of nitric acid per litre, at the rate of 800,000 litres per second; the total nitric acid lost amounted to 350,000 kilos. per twenty-four hours, equal to 650,000 kilos. of saltpetre. The rivers pour this nitrogen into the sea.

And yet in spite of these losses the soil retains its nitrogen. Nay more, it accumulates it. The soil of forests is never manured and the woodcutters carry off a great quantity of nitrogen with the wood; yet the soil there remains fertile. In the hill pastures, flocks are feeding all the summer and furnishing us with nitrogen in the form of milk, cheese and meat: and yet the soil of these natural fields contains quantities of nitrogen greater than is found in soil ploughed and copiously

manured, *i.e.*, from 5 to 9 milligrams of combined nitrogen per kilo. of soil. Finally, the crops remove from the soil much more nitrogen than the manuring supplies: the difference varying with the rotation of the crops from 1·5 to 400 kilos. per acre. Where does this nitrogen come from?

It can only come from the inexhaustible reservoir of the atmosphere.

Rain water carries into the earth the ammonia which has evaporated from it and the oxidised compounds of nitrogen which form during thunderstorms. But these gains—about 1·5 kilos. per acre per annum—are quite insufficient to compensate for the losses occasioned by drainage and cultivation. Plants must take up not only the ammonia and the nitrates of the atmosphere supplied by rain, but also uncombined nitrogen, the free nitrogen of the air.

Cultivated soil, kept moist and exposed to the air, fixes atmospheric nitrogen (Berthelot). This fixation does not occur if the earth has been sterilised by heating to 120° C. Living creatures must therefore be at work in this.

These workers are the *bacteria* of the soil which are found to a depth of one foot. They are also found in the sea, especially in the neighbourhood of algæ. They are more abundant in soil, the better it is aerated and cultivated. They include anaerobes (*Clostridium pasteurianum*) and aerobes (genus *Azotobacter: Pseudomonas leuconitrophilus*). Their organic food in soil as in laboratory cultures is carbohydrate, glucose, saccharose, levulose, dextrine, mannite and other sugars; butyrates, lactates and acetates; their mineral food consists of salts of lime and phosphates. The *Azotobacter chroococcum* will only develop in soil containing at least 0·1 per cent. of lime.

It is not exactly known how bacteria fix nitrogen. They build it up into their substance and liberate it when they are destroyed. Doubtless also they build up nitrogenous compounds which are taken up by the nitrifying bacteria.

Nitrogen can only be thus combined on condition that the bacteria are supplied with energy in the form of carbohydrate

food. No hydrocarbons, no bacterial activity, no fixation of nitrogen. To fix a gram of nitrogen, experiments show, 100 to 200 grams of glucose are required.

Just as we do not know all about a disease when we know its microbe, so it is not sufficient to have simply the bacteria which fix free nitrogen in order to enrich at will a soil with nitrogen. Nature is not the laboratory. The inoculation of poor soil with these beneficent bacteria produces scarcely any increase in the crops. To excite bacterial multiplication it would be necessary to distribute sugars over the soil—14 kilos., it is calculated, to fix the nitrogen to which corresponds 1 kilo. of nitrate. Nitrogen at such a price is far from cheap. Besides, the hydrocarbons favour the denitrifying action of the microbes which results in a loss of nitrogen. The useful germs are already present in good soil; it is literally the soil itself which must be altered, by adding marl, by tilling, drainage, and all the operations which change its physical properties.

There exist certain moulds which fix the nitrogen of the air, for example the *Penicillia* and the *Sterigmatocystes*. The algæ of the *nostoc* group, *Chlorella*, *Stichococcus* and *Cystococcus*, fix nitrogen only when in symbiosis with the fixing bacteria: it is then the bacteria which fix nitrogen; the algæ, as green plants, nourish their associated bacteria by means of the hydrocarbons which they synthesize in virtue of their chlorophyll activity. According to Beijerinck's experiments the *Cyanophyceae* are capable of taking nitrogen into their tissues independently just as they do carbon. But if all the algæ possessed this double function they would be so powerfully adapted for life that they must have long ago invaded the whole universe.

There is a third group of microbes which fix nitrogen; but in this case they do not live free in the soil, but are confined to the roots of Leguminosæ.

The soils which are found to be most rich in nitrogen are those in which there have been longest grown crops of this family. Georges Ville in 1852 thought that the leguminosæ took up oxygen from the air. He sowed leguminosæ in sand which had been washed and calcined, thus being sterile and

freed from nitrogen compounds ; he kept them in an atmosphere similarly freed from all compounds of nitrogen ; when the young plants had overcome these rather unfavourable conditions they contained at the end of the experiment more nitrogen than was present in the seeds.

Malpighi observed long ago—in 1687—little nodules on the roots of Leguminosæ whose function has only in modern times been discovered thanks to Pasteur. These little nodules are in fact crammed with bacteria the function of which is to fix the atmospheric nitrogen. The plant suffers from a malady which is actually beneficent (Hellriegel and Wilfarth).

Grown in a sterile soil and protected from the germs of the

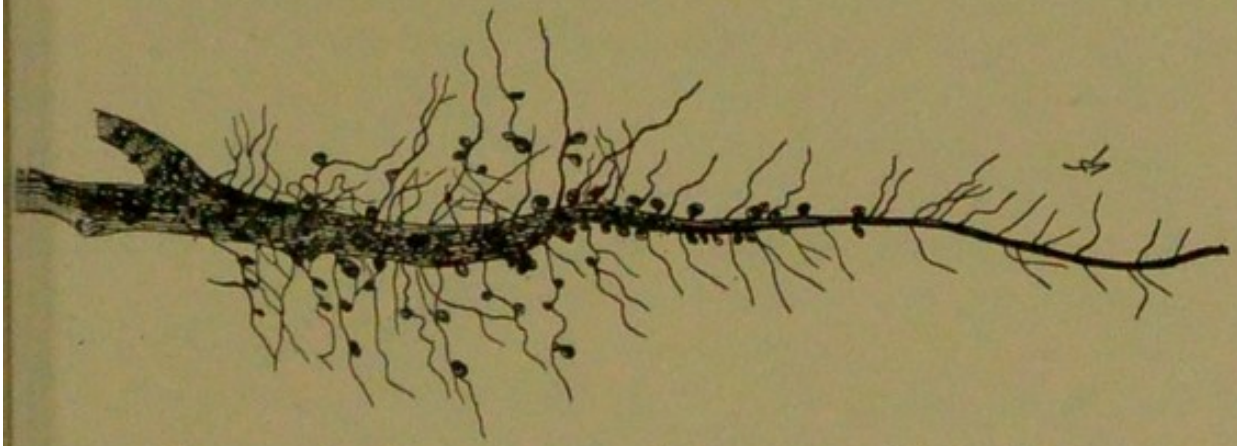


FIG. 7.—Nodules on the roots of Leguminosæ (*Vicia faba*).

air and of the soil, the roots of Leguminosæ never present these nodosities. If this soil is watered with a suspension of the nodules or of earth in which Leguminosæ have grown, or is simply sprinkled with garden soil, the Leguminosæ grow in it possessing nodules, but if these suspensions are boiled for a sufficient time they lose this property.

It is possible to inoculate the plant with the infection by simply pricking with a needle first a nodule on the root of a normal pea, then the root of a pea which has been grown aseptically without nodules ; under such conditions the roots which were free from nodules develop them.

The bacteria of the nodules have an irregular and peculiar shape, and have been given the name of bacteroïdia. The best known is the *B. radicicola* of Beijerinck. Two groups

have been made : those of the vetches, clovers, and peas, and those of the beans and lupines. Mazé distinguishes those adapted to calcareous soil, the 'calcicolæ' from those adapted to acid soils, the 'calcifugæ.' They are aerobic, and pure cultures can be obtained. They require carbohydrate food, and are not fond of nitrates. Grown in pots, the nodules are encouraged by the addition of chromium, manganese, nickel, and cobalt. About 100 grams of sugar are consumed for each gram of nitrogen fixed. The bacteroïdia only act well when their food supply is regulated in quantity as well as in quality. The broth for the cultures should contain 1 per 10,000 at

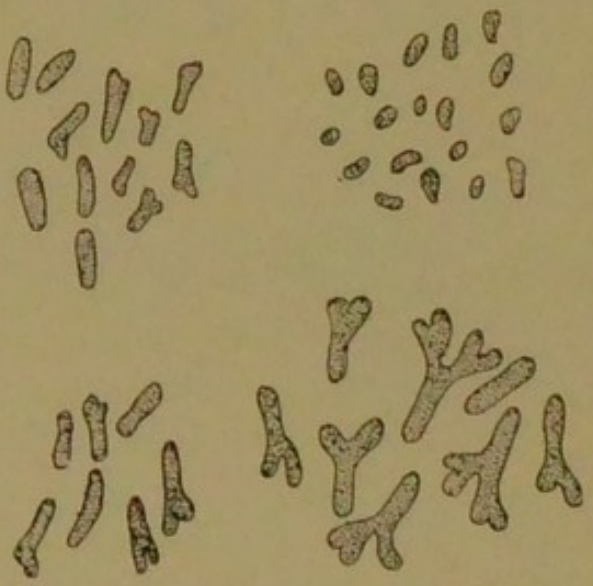


FIG. 8.—Bacteria and Bacterioidia from the nodules of Leguminosæ (after Beijerinck).

least and 1 per 3,000 at most of combined nitrogen ; the proportions of saccharose should lie between 2 and 6 per cent.

The virulence of the bacteroïdia varies ; those which have undergone several passages in the roots attack the new roots more easily. It seems that the weaker bacteria induce a certain immunity in the rootlets towards the more virulent. A root already bearing nodules does not produce new ones except when inoculated with very virulent bacteroïdia. On the same plant the recent nodules of the lateral roots contain bacteria much more virulent than those of the main root.

The bacteroïdia scattered through the soil are attracted towards the young roots by the vegetable carbohydrates: there is a positive chemiotaxis.

The bacteroïdia and the plant are mutually beneficial. The plant supplies carbohydrate food to the bacteroïdia, which in their turn supply the plant with nitrogen. In culture a viscous glairy jelly appears, which is present also in the nodules, but only in their early stage. The sap rapidly carries it into the body of the plant, and it is this jelly probably which contains the nitrogenous food product. Some diastase probably of the root liberates the nitrogen compound from the cells of the bacteroïdia.

Nitrogenous manures are expensive, and hence the idea has arisen to improve leguminous crops by planting them in prepared soil, or by adding soil which has already grown leguminous plants. Afterwards the attempt was made to inoculate the soil with artificial cultures of the nodule bacteria. The process is analogous to the injection into an animal of a food substance or a drug: it is agricultural *bacteriotherapy*.

In observing nature, not from the point of view of any single living species, man or animal, but throughout the whole of her operations, one sees that the pathogenic bacteria are less prominent than the useful ones—one may say even that all bacteria are useful and are only injurious by accident. They all have their rôle in the cycle of matter and only destroy one existence to prepare for another.

To perceive the function of the useful bacteria, it has been necessary to study all the fermentations in relation to both industry and food supply: wine, beer, cider, vinegar, cheese, bread, *sauer-kraut*, tobacco, and the leather of our shoes are all more or less the result of bacterial operations. Life as a whole could not continue without bacteria; they do not create life, but they supply it with the necessary material.

We may ask ourselves now if, with nature thus provided with nutritive material, the life of a particular organism might be possible although it did not contain microbes in itself.

Logically it is possible. An animal without microbes, an *aseptic* creature, might take advantage of the general activity of microbes without itself being open to these peculiar fermentations which we call diseases.

This then would be at once the first principle of hygiene, and, as it were, its paradox.

CHAPTER II

MICROBES IN THE HUMAN BODY — LIFE WITHOUT MICROBES — THE INTESTINAL FLORA

Microbes in and on the bodies of animals and man : skin, mucous membranes, mouth, stomach, intestine.
Life without microbes — Pasteur's ideas — Aseptic breeding — The example of the bat, Pteropus.
The intestinal flora of man : quantity, species, and variations of the microbes.
Intestinal putrefactions — The flora and diet — Products of putrefaction and auto-intoxication.
Associations and antagonisms — Cholera — Experiments of Metchnikoff, Bienstock, Tissier — Principles of intestinal bacteriotherapy — Sour milk ; culture, pure and mixed, of the lactic ferments.
Is the large intestine useless? — Is the intestine permeable to microbes? — Investigations on anthracosis and tuberculosis — The defensive powers of the mucous membrane.

The Microbial Flora in Man and Animals.—We have seen what innumerable legions of bacteria are at work in the universe. Let us now proceed from the contemplation of the macrocosm to the microcosm, as represented by our own bodies.

There is scarcely a living being which is not crowded with microbes. Living as we do in a universe where they swarm, how could we avoid having them both within and without?

Normally, we enter the world free from microbes. But from the first moment after birth they begin to settle on our skin and on our mucous membranes. Our mother's first touch communicates them to us, even before she has given us the first drops of her milk. They penetrate the nose, the mouth, and

the lungs with our first respiratory movements and our first cries; they are deposited on our skin from our first bath and our first swaddling-clothes. Even four hours after birth and invariably between the tenth and the seventeenth hour of life —they have already reached the intestine.

Our skin is inhabited chiefly by round bacteria, such as streptococci, and especially staphylococci; these latter are most abundant, and there are several species of them, some commoner than others. They do not inhabit so much the smooth surface of the epidermis as the hair follicles and their adnexa, the sebaceous glands, which are regular dens of bacteria. Even the cleanest persons, however little oily their skins, have only to press between the fingers a little fold of skin on the end or at the side of the nose, to squeeze out what looks like a little worm, but which is nothing else than a colony of staphylococci.

As a matter of fact, however healthy the skin, it is more or less *inhabited* by bacteria, which, however, in general, remain harmless; the skin is not *infected*. But given at some point a lesion, a boil or pustule, where bacteria have multiplied, the microbes of these foci spread themselves over all the cutaneous surface, even to the most distant parts. "On the surface of healthy skin, the diffusion of germs takes place around the original lesion for a great distance, and with an abundance and continuity such as is only paralleled by the law of conservation of widely distributed species. An analogous case is that of plants covering square miles of country round with their pollen or their winged seeds" (Sabouraud). Hence, the best way to avoid cutaneous infections is to preserve intact the epidermis, and not to weaken by the abuse of antiseptics its protecting cells, which endeavour to maintain the integrity of the skin against bacteria.

The mucous membranes, warmer and moister than the skin, form a better soil for microbes. There is in the conjunctiva of the eye, among others, a little bacillus, resembling the bacillus of diphtheria, which is found from the first hours of life (Morax). In the cavities of the nose and pharynx, there are

not only the common bacteria, the streptococci and staphylococci, but also pathogenic bacteria, which take shelter there and remain latent until an opportunity offers to multiply; examples are the diphtheria bacillus, the microbe of pneumonia, and that of cerebro-spinal meningitis.

In the deeper respiratory passages, there are few microbes; there they may be numbered by units. But it is proved that the tubercle bacillus can penetrate with the inspired air right to the bottom of the pulmonary alveoli.

The mouth, the vestibule of the digestive tube, contains already a large proportion of the microbial species which populate the stomach and intestine, *e.g.*, staphylococci and streptococci, resembling those of the skin, bacilli, aerobic and anaerobic, resembling those found both in the healthy intestine and in the intestine and appendix in disease, and finally a whole flora peculiar to the mouth which plays a part in dental caries.

The stomach being acid suits moulds and yeasts better than bacteria. However, about thirty species of bacteria have been described in it (Coyon), several of which have attracted special attention because of the idea that they might favour or inhibit the penetration of certain pathogenic bacteria into the intestine.

All the cavities and recesses of the human body deserve study from the point of view of their flora. Many species have been seen but are not yet well known, because we do not yet know how to cultivate them artificially.

The flora hitherto most studied is that of the intestine. It is also the most important. The intestine is the great laboratory of digestion, and at the same time, unfortunately, of putrefactions, the products of which are absorbed by the body. Hence one may say that man, like other animals, is dependent on his belly, and no system of therapy is justified in neglecting it. Just as the soil outside of us is, in nature, the great microbial reservoir, so in us the great reservoir of microbes is our intestine.

Under the impulse of Metchnikoff, the intestine has with justice become the great field of study and experiment in those

problems of nutrition which affect most of all the general health, the individual development, and the evolution of man. That is why we pay special attention here to the intestinal microbes as being the most important of all those which inhabit the bodies of man and animals.

The first problem in this question is the possibility of life without microbes.

Life without Microbes.—Life in general, as we know it on the surface of this earth of ours, is impossible without the action of the micro-organisms. As some philosophers have well said, "life is but a little mould growing on the surface of a rather moist planet." But is it possible for certain individuals to live free from bacteria, although of course depending on the conditions produced by bacteria, and paying tribute to bacteria by reason of the fermentations to which they owe their origin and nourishment and by which one day they are doomed to be dissolved?

Plants have bacteria both around their roots and in their tissues. Animals have thousands of millions in their digestive tubes. The microbial world not only surrounds them but is in them. In our intestine there proceed fermentations from which we can absorb the products. Is this to our advantage or is it not?

It seems that plants derive only benefit. The bacteria prepare for them their food. A sterile seed made to sprout in a sterile nutritive fluid, such as milk, can use neither casein nor sugar nor starch; it secretes neither rennin nor casease, nor sucrase, nor amylase. There is amylase, it is true, in the cotyledons of the germinating seed, but its action does not extend into the surrounding medium. Plants thus prosper by the help of bacteria, and the aim of cultivators is to provide for each plant the microbes most favourable for the yield desired by mankind. But it is to be noted that these useful microbes are not *within* the plant.

The problem is quite otherwise in the case of animals. As in so many other cases, the question was first stated by Pasteur in connection with Duclaux's experiments on the nourishment

of plants under sterile conditions and their rational culture with the help of selected microbes. Pasteur expressed an opinion which he had not time to submit to experiment, but which has been taken up by others, the idea namely that an animal cannot dispense with the bacteria living within it. He suggested that a young animal should be fed from birth with pure sterile food.

"I do not conceal the fact that, had I time to undertake this study, I should do so with the preconceived opinion that life under such conditions would become impossible. Given that such experiments are capable of being gradually simplified, it might be possible to study digestion by adding systematically to the sterile food of which I speak various individual bacteria or different bacteria together, each of definitely known species.

"The hen's egg lends itself without serious difficulty to experiments of this kind. Previously freed externally from every sort of living impurity just before hatching, the chick should be immediately put into a chamber free from every sort of bacterium, so that it could be supplied with pure air and with sterile food, easily introduced from without in the shape of water, milk, and corn.

"Whether the result were positive, confirming the preconceived idea which I am putting forward, or negative, or even absolutely the opposite, *i.e.*, that life without bacteria is easier and more vigorous, in any case the experiment would be full of interest."

The experiment *has* been tried and the programme is still a long way short of completion. One thing only has to be altered in Pasteur's statement. The experiments are far from being without "serious difficulty."

Two currents of opinion have developed. The first, following literally Pasteur's idea, maintains that animals are like plants and cannot nourish themselves with the sole help of their own digestive juices but require also the intestinal microbes. We do not know, say its supporters, if in the beginning of life an animal organism has ever developed free from micro-organisms; it is not very probable; what is certain is that for centuries and

centuries there have been microbes within living beings, and there must have become established a sort of understanding, or adaptation between the intestinal flora and the intestine. Just as plant roots absorb from the soil the fluids elaborated by the bacteria, so the intestinal villi absorb the juices prepared by the microbes of the intestine. These microbes accumulate and their presence in masses stimulates the intestinal muscles. Further, the normal intestinal flora opposes an invasion by foreign bacteria which might be or might become pathogenic. Consequently the intestinal bacteria both nourish and protect; they are useful, salutary, and providential.

The opposite opinion is maintained by Metchnikoff. The digestive ferments prepare the nutritive materials without any of this problematical assistance from the microbes. The intestinal flora is injurious (taking of course the point of view which interests us most, that of mankind), because long before the food stuffs leave the body, the bacteria induce in them fermentations the products of which, absorbed by the mucous membrane, are for the most part poisonous.

From the point of view of nature, it is quite normal for the albuminous excreta of our food to putrefy and thus return into the general circulation of matter; but it is regrettable when this takes place in our bodies, for phenols, skatol, and indol, among other products, penetrate into our circulation and affect the cells of our arteries and brain. It would be to our advantage if the food-stuffs were expelled immediately after useful digestion, and before the terminal phase, the putrefaction, begins in the waste products. And since in the part of the intestine which properly speaking is the digesting part, the small intestine, there are practically no bacteria, and since on the other hand they swarm in the large intestine where there are scarcely any digestive ferments, it is evident that, on the whole, the intestinal flora is injurious. The ideal condition would be to live free from bacteria while the world remained populated by them. Is a life of such *purity* possible?

Aseptic breeding.—There are, it has been said, certain Arctic animals, both birds and mammals, without intestinal

microbes. But the recent polar expeditions failed to find a single animal of this kind.

Nevertheless, such have been found among the burrowing larvæ which excavate galleries in the thickness of leaves and live shut off from the external world by a transparent wall of epidermal cells. The truth of this can be demonstrated by extracting them with a sterile needle through a litttle perforation made in the epidermis after sterilisation with peroxide of hydrogen. According to Portier's experiments, the *Lithocolletis* caterpillars are aseptic in about a third of the cases ; those of the *Nepticula* of rose-trees always. These burrowing larvæ dispose of their excreta by stowing them aseptically in their closed tunnels, whereas *Zischeria*, which discharges its dejections externally, through a little hole made in the leaf, is always contaminated.

Finding experiments on these small invertebrates easy, Bogdanoff introduced the previously disinfected eggs of flies into sterile meat and found that the larvæ hatched and kept under these conditions develop less well than the control larvae fed on putrefying meat rich in bacteria. When he added to the sterile breeding ground a digestive ferment capable of attacking meat, *i.e.*, trypsin, the sterile larvæ flourished equally with the others. Bogdanoff would have concluded that the bacteria play the necessary part which in the latter case was performed by the ferment, had he not found some sterile broods, without addition of ferment, quite as vigorous as in ordinary breeding. Larvæ can thus develop without the aid of microbes. Wollmann using an excellent technique has finally proved this : he succeeded in breeding from the egg sterile flies. " During the first days of life the sterile larvæ develop more slowly than the contaminated controls, probably because the digestive glands are not yet in full activity and find the sterilised meat difficult to attack. Later these differences disappear and the sterile larvæ attain the weight and size of normal adults."

Experiments on aseptic breeding of vertebrates, such as Pasteur desired, have been carried out, with a patience worthy of all praise, by Nuttall and Thierfelder, who took young

guinea-pigs from their mothers by Cæsarean section, by Schottelius and Cohendy on the chick, by Mme. Metchnikoff and by Moro on tadpoles.

The little guinea-pigs appeared to develop and increase in weight in normal fashion, but Schottelius has questioned the interpretation of these experiments; he maintains that the increase in weight was due to food swallowed but not digested. The tadpoles developed badly without microbes. The chickens of Schottelius were '*weaklings*' and lived seventeen days at most, becoming increasingly thin and feeble. Their digestive juices were apparently insufficient for digestion, and they only recovered when there was added to the sterile food certain bacteria, among which was the bacillus coli.

But it is impossible to conclude with certainty from these experiments that life is impossible without microbes; these new-born animals were placed under conditions too different from the natural; their intestine was not yet secreting enough ferment, and the food they were getting, sterilised as it was at a high temperature, was not adapted to the hereditary disposition of their alimentary canal.

It would therefore be necessary to study adult animals free from microbes, but we know that this condition hardly exists.[1]

There are, however, animals which approach it somewhat, namely, the large fruit-eating bats of the tropics (Pteropus medius) recently studied by Metchnikoff. These animals have a large intestine very limited in size; there is no reservoir in which to accumulate the waste products of their food, and they begin to evacuate fæces one hour after the ingestion of the food from which these are derived. They are obliged to eat a great deal and to evacuate in proportion. They have, properly speaking, no intestinal flora, simply a few bacteria conveyed by the food and changing with the diet.

[1] The intestine of the scorpion is almost always free from microbes; and the same is the case with the intestine of certain maggots provided with digestive juices sufficiently powerful to digest seeds, wool, and even such resistant microbes as the bacillus of tubercle.

The juices of their small intestine have no power of killing bacteria such as has been alleged in the dog and the cat, so the poverty of their intestinal flora cannot be thus explained. The digestive tube being almost free from bacteria in its whole length, the digestion of the food cannot be attributed to microbes.

The fruit-bat above-mentioned digests the cellulose of bananas, yet it is precisely for the digestion of cellulose among the herbivora that the necessity of auxiliary microbes has been maintained; the reason is that cellulose-digesting microbes are known, whereas no ferment has been separated from the digestive tube capable of doing this. But the bacterial ferment has not been isolated either: the question of cellulose digestion is one on which our ignorance is great and which demands fresh investigations. Finally, in the dejecta of the fruit-eating bat the putrefactive poisons phenol, skatol, and indol do not occur, although these are constant among all other mammals including the herbivora. This absence of putrefaction corresponds with the extreme shortness of the large intestine and the poverty of the intestinal flora. We have then in this animal a good example of an adult animal capable of digesting its food, under normal conditions, by its own digestive juices without the help of bacteria.

The Intestinal Flora of Man.—Man differs from the bat. He harbours a very abundant intestinal flora in his large intestine which is highly developed and in which the food-stuffs begin to putrefy.

It is almost impossible to calculate the number of bacteria in our intestine. Gilbert and Dominici have calculated that we discharge daily 11,725 millions, without counting the anaerobes, of which there is a prodigious number. Klein speaks of 9 billions of bacteria discharged every twenty-four hours, of which 100,000 millions are still alive. Strassburger, using a different method of counting, calculates the quantity of bacteria at one-third of the normal dried fæces; in twenty-four hours a man evacuates 120 billions of bacteria. It would

be too much to say that these figures are imaginary, but they are certainly only approximate. It is sufficient to state that the real figure is enormous and certainly approaches the magnitude stated.

The small intestine is less populous, so much so that a bactericidal action even has been ascribed to it. Yet the pancreatic juice which is poured into the small intestine is invaded by microbes when collected and left outside the body. The auto-sterilisation of the small intestine seems to be due to the combined action of the two principal digestive juices, the pancreatic and the intestinal. The flora of the small intestine like that of the intestine in general varies with the diet.

It is more important to know the nature of the microbes than their quantity. In 1898 Mannaberg counted only twenty-seven species in the intestine ; but there are now many more described and the improvement in our methods of cultivation is leading daily to the discovery of new ones. MacFadyean, Nencki and Mme. Sieber have isolated fourteen from the small intestine alone.

A child is born with a sterile alimentary canal, but from the tenth to the twentieth hour of life it begins to be inhabited.

FIG. 9.—*Bacillus bifidus* (Tissier) : 10 day culture.

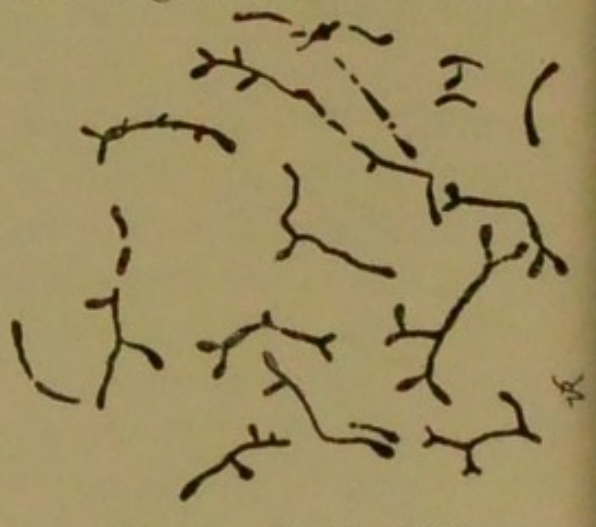


FIG. 10.—*Bacillus bifidus* (Tissier) : 15 day culture.

From the first to the third day in the *breast-fed* child there appear Streptococci, Bacillus coli, *B. perfringens* (= Welch's Bacillus), B. III of Rodella, *B. lactis aerogenes*, Sarcinae,

enterococci, *mesentericus* and an *acidophilus*. After the third day the flora becomes more simple, being dominated by one of the last to appear, the anaerobe *B. bifidus* of Tissier, which is characteristic of the flora of the healthy breast-fed infant; along with it persists the Bacillus coli, the enterococcus and the B. lactis aerogenes. Thanks to the presence of *B. bifidus* and the lactic bacilli, which produce acids from the carbohydrates (human milk is rich in sugar especially after the tenth day), the putrefying bacteria (*B. perfringens*, for example) are kept in check or even entirely eliminated. Proceeding from the stomach to the rectum the species which predominate in turn are the B. coli, the *B. lactis aerogenes*, the *enterococcus*,

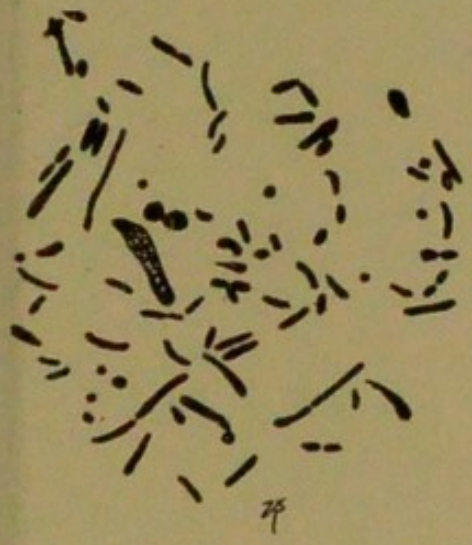


FIG. 11.—Bacteria in the fæces of a normal child of 19 months.

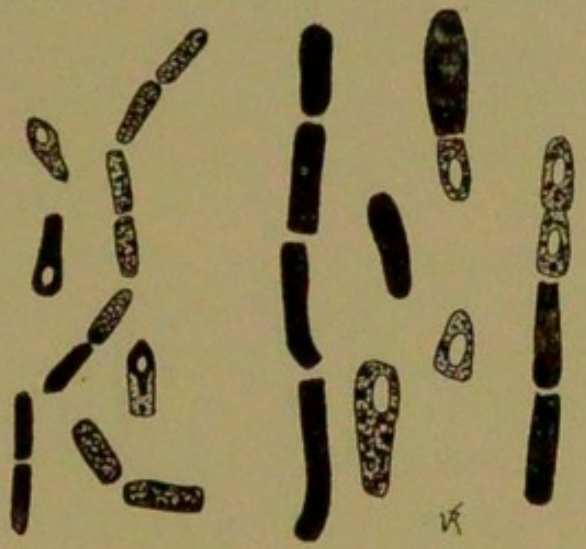


FIG. 12.—*Bacillus sporogenes* (Metchnikoff).

the *B. exilis*, the *B. acidophilus* and lastly the *B. bifidus*. The distribution and proportions may be altered in the case of the infant not breast-fed. The chemical surroundings being different, the fermentations also differ.

From the age of one to five years and in particular after weaning, the flora has added to it several new species, while still retaining the species found in the infant, which may be regarded as the fundamental flora. Tissier calculates the proportion of this fundamental flora in the child brought up on a vegetable diet at 90 per cent. of all the bacteria and $\frac{4}{5}$ of

this are made up of B. bifidus. The proportion is less (70%) in the child receiving a fair quantity of animal food.

In the human race three microbial species seem to be chiefly capable of provoking the putrefaction of albuminous material in the intestine; namely the three anaerobes: *B. putrificus* of Bienstock, *B. sporogenes* and the *B. of Welch* also called *B. perfringens.*

Not only are these three anaerobes putrefying organisms but they seem also to be true pathogenic organisms. *Putrificus* has been found in peritoneal suppuration, in appendicitis and in various intestinal disorders. *B. sporogenes* has been found in many cases of diarrhœa; *B. perfringens* is common in acute and chronic suppurations, and in infantile diarrhœa: it is also the cause of crepitating gangrene or gaseous phlegmon. The

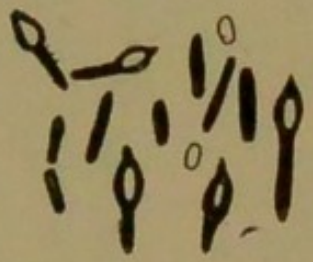


FIG. 13.—*Bacillus putrificus* (Bienstock).

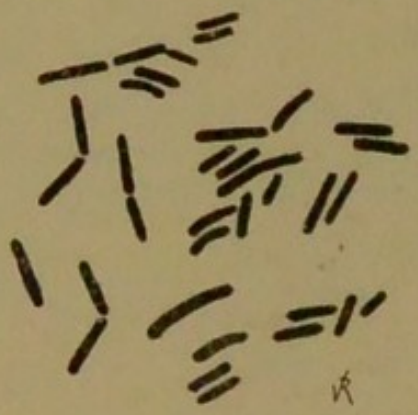


FIG. 14.—Welch's bacillus (*B. perfringens*).

study of their virulence and toxicity by experiments on small laboratory animals and monkeys has hardly been begun. They indicate well how delicate is the distinction between a simple saprophyte of wide prevalence and a pathogenic organism properly speaking.

The B. coli, the commonest and most abundant bacterium in the intestine, though it was considered by Schottelius, in his experiments on the aseptic chick, as an indispensable adjunct to nutrition and though it has even been employed as a remedy for constipation, is nevertheless prejudicial to health.

It does not, it is true, attack the genuine albumins but it

breaks down the peptones, producing indol, phenol, mercaptan and sulphuretted hydrogen. It is precisely these bodies, putrefaction products, which enter the blood and produce the chronic poisoning which induces premature old age.[1] Among the bacteria of the intestine, *B. lactis aerogenes*, *B. perfringens*, *B. sporogenes*, *Staphylococcus pyogenes* and *Proteus* produce indol. *B. coli* produces both indol and phenol, and there is experimental proof that indol, phenol, and their sulpho-compounds can produce auto-intoxications of the body. With indol and potassium phenyl-sulphate, aortic atheroma has been produced in rabbits in about 60 per cent. of those tried, whereas spontaneous atheroma does not occur in more than 6 to 10 per cent. Monkeys treated with paracresol presented arterial lesions in the brain and kidney. By combining the phenols with sulphur, the body manufactures "sulpho-compounds" which are less injurious than the pure phenols, but which are still chronic poisons (Metchnikoff).

Intestinal Putrefaction.—The study of the putrefactive bacteria being in its infancy and beset with great difficulties besides, it is not surprising that certain authorities maintain that intestinal putrefaction is harmless to man.

It is true, they say, that our instinct leads us to reject putrefying food and that common sense has always connected putrefaction with disease. Yet we see the Indo-Chinese, the Malays, Polynesians, and the Greenlanders regaling themselves with decayed fish, meat, or eggs! Meat which has gone bad frequently determines diseases resembling acute poisoning; but it is not because it is putrefying; it is because it contains pathogenic bacteria of the family of *B. typhosus*, or bacilli like the B. of botulismus, which produces a violent toxin. Extracts of putrefied meat have been injected into different animals without result. Further, how many people exist in perfect health in spite of the putrefaction going on normally in their intestine! It recalls the dictum of Malvoz: "Tout ce qui pue ne tue

[1] The intestinal flora of the dog does not differ from that of man. Little rabbits eight days old have not a rich flora. The flora of parrots, young and old, is also scanty (five species in the ileum). The flora of the alligator is much less rich than that of man.

pas, tout ce qui tue ne pue pas." (What stinks does not kill, what kills does not stink.)

A Belgian physiologist, Falloise, has shown that the contents of the large intestine where the putrefications are going on are much less toxic than those of the small intestine which is free from putrefaction. The toxicity of fæcal matter left in the incubator, with all its contained bacteria, diminishes instead of increases. Hence the toxicity is not in proportion to the putrefaction.

What then causes those gastro-intestinal disorders, in particular infantile diarrhœa, with symptoms resembling typhoid fever or cholera? It must be poisons of some kind, but poisons which are the result not of bacterial decomposition of the intestinal contents but of abnormal metabolism of the food.

This theory of non-microbial intoxication, is opposed to that which regards microbial putrefaction as the cause of the poisoning. Finkelstein, for example, says that there is no specific infecting organism in infantile diarrhœa and that the same symptoms appear with very different intestinal flora; according to Nobécourt and Rivet, the intestinal flora depends on the diét and the disorders of digestion, its rôle being secondary and rather an effect than a cause. The principal rôle is played by those poisons which are produced directly in the digestion of the food-stuffs.

But though it is true that the study of intestinal putrefaction is not far advanced, the opponents of this theory must acknowledge that still less is known about the auto-intoxications. Nothing definite has been established in connection with the ptomaines and toxalbumins which have been incriminated. Besides, the argument of Falloise on the slight toxicity of the fæcal matter is not justified, for he forgets that the fæces do not represent actually the intestinal contents, these latter having lost before discharge precisely those poisons which have been absorbed by the mucous membrane.

The phenols, for example, which are produced in our intestine are absent in the fæces, but appear in the urine. The volatile fatty acids also are in greater quantity in the urine than in the

fæcal matter. As to the poisons found in the small intestine, these are nothing but the digestive juices themselves, from the pancreas and intestinal mucous membrane ; the fact of their toxicity when injected into the veins of a rabbit does not prove in the least that they are noxious to the animal producing them. It is, in fact, a very rough way of studying the toxicity of fæcal matter to inoculate extracts of it into animals. The biological and chemical study of the intestinal bacteria in their capacity as ferments is the necessary and proper introduction to the problem of intestinal putrefaction.

The diminution of toxicity which Falloise discovered on incubating fæces represents only the primary phase of the phenomena which occur under such conditions, namely, a preliminary fermentation which produces acid and suspends the putrefaction ; it is only after the third day that true putrefaction can begin, the reaction having then become alkaline.

Finkelstein's argument was the speedy effects of change of diet on an intestinal disorder ; but this action may equally well be explained by the intervention of microbes ; the flora also varies with the diet. In the intestine, as in a culture flask, different cultures can be got with the same microbes when the media originate on the one hand from meat and on the other from vegetables.

In studies so difficult as these, no good result can be got by treating fæcal material and injecting it in quantity. It is necessary to proceed analytically, isolating patiently the bacterial species of the intestines, studying them in a condition of purity, as every ferment ought to be studied, studying secondly their actions in association, and studying finally the poisons they produce, phenols, indol, skatol, etc. It is by this method that the action of *B. putrificus*, *B. perfringens*, and *B. sporogenes* in intestinal decomposition has been rendered certain as has been the rôle of *B. proteus* in infantile diarrhœa.

Mutual Assistance and Mutual Antagonism.—These legions of microbes do not escape the law of competition which prevails throughout life. They have not all the same demands

or the same appetites; one requires more alkalinity, another requires a certain degree of acidity. The unused residue of the food of one may provide excellent nourishment for another. Their actions on elementary waste products have to follow each other in a regular and chemically definite order. Hence we get agreements and antagonisms, one species, by reason of its nature and the food of its host, being towards another either a help or a hindrance.

This idea was first expressed in connection with the bacteria of the intestine by the experiments of Metchnikoff on cholera and its vibrio. Cholera is due to the multiplication in the intestine of a spirillum, which rarely penetrates into the blood, but elaborates in the intestine poisons which diffuse and kill the patient. Cholera is an acute, toxic, specific enteritis. Koch's discovery of the spirillum or "comma bacillus" of cholera had to contend with an obstinate scepticism, because cholera could not be produced with it at will in laboratory animals, unlike anthrax or fowl-cholera. Whether by inoculation under the skin, or in the peritoneum, or by the mouth, the result was the same. Even when several savants swallowed cultures of it, the results of these experiments "*in anima nobili*" were very inconstant. In this, typhoid fever resembles cholera, the disease being very difficult to reproduce experimentally; it is only quite recently that experimental typhoid fever has been successfully produced by employing the higher apes, the nearest congeners of man.

It cannot be a matter of indifference to the cholera vibrio what is the nature of the flora of the alimentary canal into which it penetrates. By a very simple method of experiment, namely, by inoculating together on gelatine plates vibrios and various other microbes along lines which crossed each other, Metchnikoff was able to prove that the association of certain bacteria favoured the growth of cholera while others inhibited it. Round the colonies of a favouring bacterium a swarm of little cholera colonies developed. Various favouring and inhibiting bacteria were discovered in the air, among animals, and—most interesting of all—in the human stomach.

But here we were still only dealing with growth on plates, *i.e.*, under very artificial conditions. The importance of the experiments increased when it was found possible to transmit to an animal, with certainty, a genuine attack of cholera. Neither the cat nor the guinea-pig nor the adult rabbit takes the disease. Metchnikoff had the idea that their resistance might be due to the presence in the alimentary canal of inhibiting bacteria, and he experimented accordingly on little sucking rabbits, which, while they are at the breast—a period of some weeks—present a very scanty intestinal flora. Little rabbits of one to four days old, made to swallow a culture of the cholera vibrio, died in about half the cases ; they were thus capable of taking cholera. But further, when to the culture of the vibrio other bacteria were added which had been recognised as being favouring in the gelatine experiment, the little rabbits took cholera without exception. They were refractory, on the contrary, when the inhibiting bacteria were added.

The question naturally arose whether during an epidemic man might not increase his resistance to a cholera infection by ingesting cultures of these inhibiting microbes, and this formed the starting point of intestinal bacteriotherapy.

Bienstock attributed the resistance which milk possesses naturally towards putrefaction to the microbes which it contains, and not, as the old idea maintained, to the presence in it of casein or milk sugar ; he found that the *B. lactis aerogenes*, an acid producer, and even the *B. coli*, prevent the development of the *B. putrificus*, the agent of putrefaction, whether in an experimental flask or in the intestine. Inoculated along with the *B. coli*, the growth of *B. putrificus* is inhibited, because it can only develop in an alkaline medium, and the *B. coli* produces from the sugars of the food materials an abundance of acid.

The idea of an inhibiting action exerted by an acid-producing bacterium on the putrefying organisms is correct, but the above example was not the best ; in the first place because the *B. putrificus* is rare in the human intestine, and secondly because the *B. coli*, while playing a useful part in certain cases,

is in others itself a putrefying organism. Kolbrügge's opinion can no longer be maintained that the *B. coli* is the beneficent bacterium *par excellence*, growing in the appendix as in a sort of hot-house put specially there by Providence!

The antagonism is chiefly between the *B. perfringens* of Welch and the *B. bifidus* discovered by Tissier in the normal flora of the healthy breast-fed infant.

There is a general antagonism between the simple saccharolytic bacteria which produce acid from starches and sugars and the simple *proteolytic* bacteria which carry the digestion of meat to the stage of indol, skatol, phenol, and ammoniacal salts. When the bacteria which oppose each other are both proteolytic and saccharolytic (hence called mixed ferments), those which produce the most acid inhibit those which produce less.

For example, the *enterococcus* is inhibited by an acidity of 2·45 per 1000; the *B. perfringens* at 1·60 per 1000; the *B. acidi paralactici* at 5·39; the *B. bifidus* at 4·90. The two last are evidently successful antagonists of the *B. perfringens*.

The facts established by Bienstock and by Tissier and Martelly may be expressed as the following law: in media containing protein along with more than 10 per 1000 of sugar, a "mixed ferment" can arrest the development and the action of a simple ferment, whereas a strong mixed ferment (*i.e.*, a strong acid producer) can inhibit a weaker. These inhibiting actions are entirely due to the quantity of acid which the bacteria produce in the course of their consumption of the carbohydrates.

Principles of Intestinal Bacteriotherapy.—Metchnikoff's experiments on microbial associations in cholera contain the germ of a method of treatment which consists in using inhibiting bacteria. The experiments of Bienstock and Tissier indicate that the saccharolytic bacteria form a natural obstacle to the action of the putrefying microbes. The idea of a bacteriotherapy had already been formulated by the well-known children's physician, Escherich, practically in the following terms: "It is necessary to employ the 'acid

fermentations' as antagonists of the 'alkaline fermentations, either by adding to the diet carbohydrates such as lactose (milk sugar), or by giving cultures of acid-producing bacteria."

It had long been the custom to employ as remedies for diarrhœa, intoxications, and intestinal putrefactions, first purgatives, then antiseptics, of which the most popular was β-naphthol. This treatment has been given up.

Several days of β-naphthol treatment fail to diminish the number of bacteria in the alimentary canal (Stern).

"Since intestinal antisepsis is based on such a weak foundation," said Quincke in a treatise on intestinal auto-intoxications, "it was natural to search for another method of combating the decomposition caused by bacteria in the intestine, and the idea came to me to try to supplant the injurious bacteria by beneficent ones, just as weeds are suppressed by the growth of useful crops, the starting point being the antagonism of different microbes observed in artificial culture media." Quincke tried the yeast of beer. Later there was employed in infantile diarrhœa the *Bacterium lactis aerogenes*, the antagonist of *B. proteus*.

Intestinal bacteriotherapy has now become part of practical medicine and is applied in two principal forms. The first is to administer those foods which are produced by natural fermentations and which contain great numbers of bacteria; the second is to give along with a suitable diet artificial cultures of bacteria possessing antiputrefactive properties. In both cases it is the antagonistic action of microbes towards the bacteria of decomposition of which one takes advantage.

Fermented foods, rich in bacteria, were employed long before modern researches on microbial antagonism came into being.

Humanity has known from time immemorial the whey of milk and the various forms of cheese. It was long before the days of science that there were invented koumiss, kvass, kephir, lebenraib and yoghourt. In the Bible we read that Abraham offered to his guests sour milk as well as sweet. Moses says that Jehovah has given his people to eat of the sour

milk of cows and the milk of goats (at the same time, it is true, the fat of lambs and of sheep and of kidney). There can be no doubt that sour milk is more ancient even than the Bible itself.

The artificial cultures chosen for their chemical properties are chiefly *B. bifidus*, *B. acidi paralactici* and the lactic ferment which has become popular under the name of the Bulgarian bacillus; they are given either singly or in association. They may be taken either in the form of clotted mik or in bouillon. The characters of the Bulgarian bacillus are briefly the following: it produces 25 grams of lactic acid per litre of milk; not more than 0·50 gram of succinic and acetic acid, traces of formic acid, no alcohol, and no acetone. It hardly attacks the proteins at all, and has no pathogenic power (G. Bertrand and Weisweiler). Although it does not inhibit the development of the *B. coli* in a culture containing peptone, it at least prevents it from producing phenol and greatly reduces its indol production.

The mechanism of the action of the lactic ferments in general is not quite settled. They appear to diminish the number of anaerobes and of the *B. coli*. A diet of sour milk reduces the ethereal sulphates of the urine more than does a diet of sweet milk.

The Large Intestine is a Useless Organ.—Since the large intestine performs no digestive action and absorbs poison, it follows that it is not only a useless but an injurious organ. Medicine may possibly perfect methods of treatment and diets to prevent it from doing harm; surgery perhaps will reach this end more quickly. However that may be, certainly no one has ever proposed that the large intestine should be removed from every human being; but it is not the less true that from the zoological point of view it is a useless inheritance left to us by our animal ancestors.[1]

[1] We must consider it as a useless inheritance from our ancestors during evolution, though they no doubt derived some benefit from it. Comparative anatomy teaches us that, among all the vertebrates, it is only the mammals which are provided with a large intestine properly speaking. Birds, reptiles, and other lower vertebrates do not possess one. . . . .

Examples of human beings who have lived after the partial or total removal of the large intestine or after the suppression of its function are far from being rare. A patient of Körte survived eight operations on his intestine; another of Wiesinger remained in a satisfactory condition after losing both his transverse and descending colon. Another of Ciechomski lived for thirty years with a fistula which had put the large intestine out of use; he married and had children. Tavel's patient (studied by MacFadyean, Nencki, and Sieber), aged sixty-two, had to digest her food for six months without the large intestine, yet she remained well. A patient of Mauclaire has been living for years in fair health with her large intestine out of use. The histories of these patients are to be found in the most respectable surgical journals.

An English surgeon, Lane, recently published an account of thirty-nine removals of the large intestine, partial or complete; he lost nine out of the number, *i.e.*, 23 per cent. Many of the survivors, formerly subject to enteritis and severe neurasthenia, had never been able to enjoy life till it had become thus "simple." The mortality is still too great for this operation to be anything but an exceptional procedure. But surgical technique is progressing every day. What surgeon would

It is probable that the large intestine acted as a reservoir for the waste products of digestion. The mammals having to run fast to escape from their enemies or to capture their prey, were inconvenienced by the necessity of stopping to empty their bowels. A large intestine under these conditions would be of the greatest use. Thus we see that those mammals which run fastest, such as the horse and the hare, possess the most highly-developed large intestine and cæcum. It is remarkable that, among birds, the runners such as the ostriches and cassowaries have similarly acquired a large intestine and possess cæca more developed than all the other feathered creatures. It is thus the demand made by the struggle for life which has led to the formation of the large intestine among the vertebrates. The high development of this organ as a reservoir for the solid excreta has produced in its turn the development of a very rich bacterial flora. . . . . Man having no necessity for the large intestine and its flora either for the digestion of cellulose or for prolonged retention of the residues of digestion derives no advantage whatever from its possession. On the contrary he suffers very numerous inconveniences. . . . . It is easy to conceive how an organ thus become useless contributes to the shortening of life." Metchnikoff, Wilde Lecture, Manchester Memoirs, 1901.

have prophesied fifty years ago that the removal of the appendix would become as simple an operation as the extraction of a tooth?

From the scientific point of view it is quite legitimate to conclude that in a mammal so advanced in evolution as is civilised man, both life and digestion are possible in the absence of a large intestine.

Is the Intestine Permeable for Microbes?—There exist in the massive flora of the intestine, bacteria both actually and potentially pathogenic. After a wound which penetrates the intestinal wall, the bacteria, which were harmless while in the alimentary canal, invade the general peritoneum and cause a fatal peritonitis. Immediately after death the intestinal bacteria invade all the tissues and begin the work of putrefaction. To prevent this happening during the lifetime of the animal it is obviously necessary for the intestine to be impermeable, and this impermeability must be due to the living healthy mucous membrane.

This opposing force is sometimes thrown out of action. There are certain diseases which can only be explained by supposing that the pathogenic bacterium has passed from the intestine into the blood and into the organs. In hog-cholera, which is caused by a virus still unknown, the lesions of the lung contain a microbe which develops under cover of the true virus and forms a regular complication in this disease. This microbe is a normal inhabitant of the intestine of healthy pigs. In all infectious diseases in which infection occurs by ingestion, and which are not exclusively confined to the alimentary canal, it is necessary to suppose that the microbe passes from the intestine into the blood either directly by the capillary vessels of the mucous membrane or by the more roundabout way of the mesenteric lymphatic glands. Typhoid fever, for example, is mainly a disease of the intestine, an enteritis, but during the whole course of the fever the bacillus circulates in the blood, and occasionally forms colonies in distant organs such as the lungs and the bones.

A very simple explanation suggests itself: the intestinal

barrier has been pierced by a little wound permitting the passage of the bacteria. This happened in the case of Pasteur's sheep, which took anthrax when he fed them with anthrax spores mixed with minute splinters. But this explanation does not hold for all cases. Numerous facts exist which establish the permeability of the healthy mucous membrane.

The importance of the problem was perceived when the conditions of infection with tubercle began to be discussed. Behring thought, in agreement with Chauveau, that the bacillus penetrates into the body of man and cattle, not only by the respiratory passages, but also through the digestive tract. This idea was suggested to him by the predominance of mesenteric lesions in infantile tuberculosis, compared with the predominance of pulmonary lesions in the adult; he thought that tuberculosis always began in childhood in the intestine and did not invade the lung until maturity. The intestine of an adult, he maintained, does not permit the passage of the bacteria as does that of a child, the reason being that in the infant, as in general among new born animals, the intestinal mucosa is not coated with the continuous and perfect covering which develops later; the covering is discontinuous, interrupted and open to the passage of bacteria. No intestinal lesion can be found because none exists: there has been penetration without violence.

Behring's idea has been developed to such an extent that some observers declare that invasion by the intestine is more frequent than invasion by the lung; and during the search for proofs and comparisons they have studied closely the penetration of the intestine not only by the bacillus of tuberculosis, but by various other bacteria, pathogenic and non-pathogenic, and even by inert dust particles. Pulmonary anthracosis, the disease or rather the histological condition which consists in the impregnation of the lungs by carbon particles from the air of mines and factories, has formed the field of battle for the partisans and opponents of Behring's idea, and a multitude of experiments have resulted, not without instructive consequences.

Dust particles can reach the lung by passing through the

intestine, but that is not the rule and can only be produced by administering the dust in large quantities in rapid succession: these are artificial and exceptional, not natural, conditions. It is the same in the case of bacteria. In an animal in perfect health they do not penetrate the intestinal wall, but they can be made to do so under the influence of fasting and fatigue (experiments of Ficker). One may say that their passage is the first and a very early sign of an agonal phenomenon, an anticipation of that exodus of bacteria which occurs immediately after death.

We must not forget, however, that, according to the statements of Porcher and Desoubry, bacteria pass from the intestine into the blood each time the animal digests, and that to get sterile sera it is necessary to bleed anti-diphtheria and anti-tetanus horses only during the intervals between digestion.

The question is less simple than it looks, because the mucous membrane is quite as active as, if not more so than, the bacteria. Not only is it built up of active living cells, but there are continually traversing it other living motile cells, the leucocytes, which play an active part in the phenomena of digestion and absorption, of defence and of resistance.

The cellular mechanism of defence does not invalidate the conclusion of this chapter. Being useless for digestion, and a constant cause of putrefaction, the intestinal flora is a permanent source of danger for the body. The intestinal flora represents in our interior the external world with its unregulated fermentations only limited by their mutual competition. It represents in the interior of our bodies the innumerable legions of bacteria in nature, which find their pabulum and their prey in fermentable material of every kind, and which are as eager to attack the proteins and carbohydrates of our living tissues as to attack the sugar and the casein of fermenting milk. The power of resistance of the body is counterbalanced by its sensitivencss towards the poisons which are being continually secreted, and which are capable at least of accelerating the approach of old age if not of directly causing death.

Researches on the intestinal flora are bound to take a

greater and greater place in experimental medicine. With the help of their results microbiology will then attack the problem of those chronic diseases, diseases of nutrition, such as gout, rheumatism, and diabetes. Finally the discovery of the so-called invisible viruses proves that the field of action of bacteria is much wider than had been suspected and that there may well exist legions of active bacteria in situations where none have yet been seen.

CHAPTER III

FORM AND STRUCTURE OF MICROBES

Animal and vegetable microbes—Protozoa—Moulds and bacteria.
Morphology of bacteria—Membrane; vibratile cilia, capsules, Multiplication, Spores.
Pleomorphism of bacteria—The place of bacteria in a general classification.
The nucleus in bacteria: the diffuse nucleus.
Reproduction and the possession of sex among microbes.
Chemical composition.

"MICROBES" is the name given to those living beings which can only be seen by the aid of the microscope. They are the simplest organisms found in nature. Some belong to the animal kingdom, others to the vegetable. The bacillus of tuberculosis and the typhoid bacillus belong to the class of bacteria, and the bacteria are plants. The trypanosoma of sleeping sickness is an infusorian, *i.e.*, an animal.

Whether animal or vegetable, almost all microbes consist of a single cell. When there are several cells, they are practically identical individual cells in juxtaposition.

The tissues and organs are systems of cells; microbes possess neither tissues nor organs.

Like every cell a microbe possesses a nucleus and protoplasm.

The vegetable cell is enveloped by a membrane whose composition is not well known, and even the motile ones are more rigid than the animal microbes.

Microbes are of all living creatures the most widespread. They exist wherever there is organic matter.

Protozoa.—In a hay infusion—a handful of hay in a

vessel of water—one can see under the microscope motile creatures, whose surface is furnished with cilia, whiplets or flagella in vibration; these are the infusoria, some with flagella, others with cilia. Sleeping sickness is caused by a *trypanosoma*, a flagellate protozoon found at certain times in the blood, in the lymphatic glands of the neck and in the cerebro-spinal fluid. These protozoa resemble minute flattened worms in the interior of which there are two nuclei, one large, one small; on one side there is an undulating membrane bordered by the flagellum, which is continued beyond the anterior end.

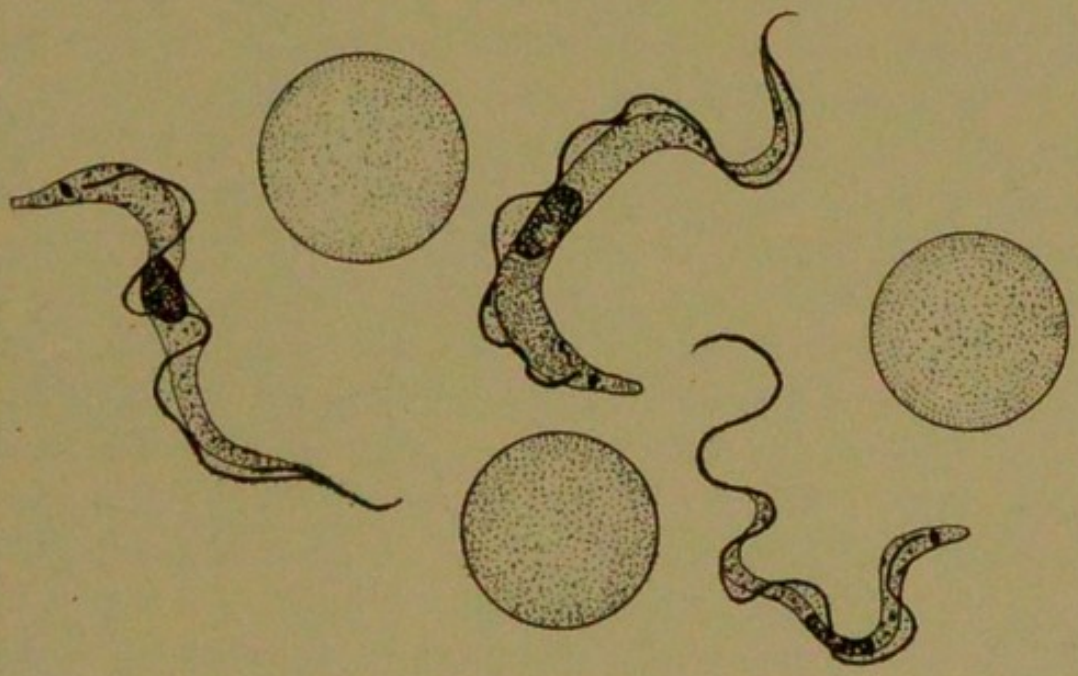


FIG. 15.—Trypanosomes and blood corpuscles.

The trypanosomes multiply by longitudinal division beginning with the nucleolus and continuing through the flagellum, the large nucleus, and the protoplasm. There is a possibility that sexual reproduction exists.

There is often found in the intestine of rabbits an animal parasite which passes through a complicated series of different forms; it possesses a *cycle of evolution*, one portion in the rabbit, one portion outside. This parasite is a coccidium belonging to the class of sporozoa. The microbe of malaria is a parasite of the blood corpuscles, a hæmatozoon, which has for long been regarded as related to the coccidia, but which

possesses certain characters, inclining one rather to derive it from the flagellates. It too passes through a cycle of development, one stage being in the blood of man, the other in the body of a mosquito of the genus *Anopheles*. According to the stage of development, the hæmatozoa of Laveran have both asexual and sexual reproduction, the latter occurring in the body of the mosquito.

It cannot be too often insisted on that no protozoon is properly known until all its cycle of development has been investigated and settled.

Moulds.—A slice of bread or a piece of lemon left to itself under suitable conditions of temperature and moisture is soon covered with a fine growth of a whitish or greenish tint; this is formed by the moulds, lower fungi belonging to the vegetable kingdom. Under the microscope a particle of this growth, teased in a drop of water, shows a felt-work of tubes or filaments, sometimes segmented at intervals, sometimes not; this felt-work is the mycelium. From the mycelium little stalks stand up vertically bearing a head which itself carries little granules resembling fruit. In the *Mucors* the head is a spherical sac stuffed full of these granules. In *Penicillium* the head consists of ramifications or digitations dividing in their turn, the last sections pinching off little pieces, the *conidia*; the whole resembles a little brush. In *Aspergillus* the fructification resembles the flower of an onion.

A fragment of mycelium put into a suitable medium reproduces the mycelium. The conidia can do the same. In the case of the mucorines two cells can join, fuse, and produce an "egg"; often these two cells are different, and the reproduction may be regarded as sexual. If mucor is grown at the bottom of a sugary fluid it no longer forms an abundant mycelium; on the contrary there can be found nothing but short pieces, round or ovoid, which reproduce themselves by budding like yeasts. But any of these pieces put in a sugar-containing medium in contact with the air, forms a mycelium on the surface. This transformation of a

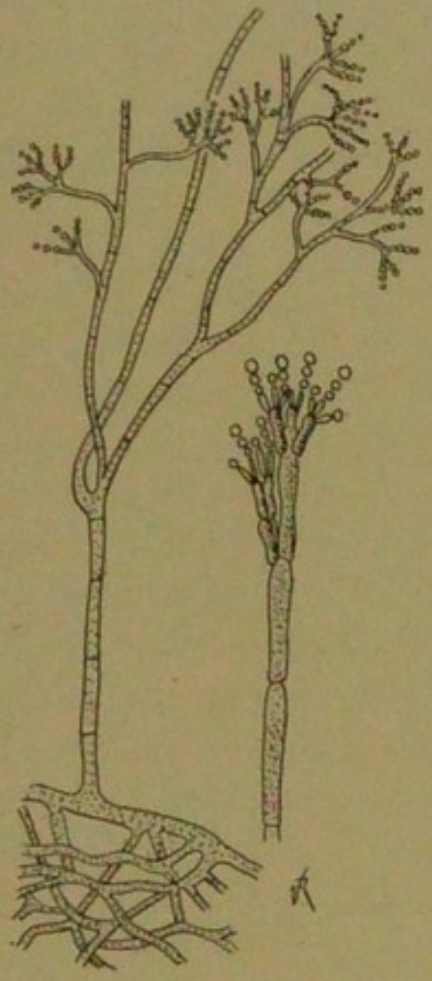


FIG. 16.—*Penicillium.*

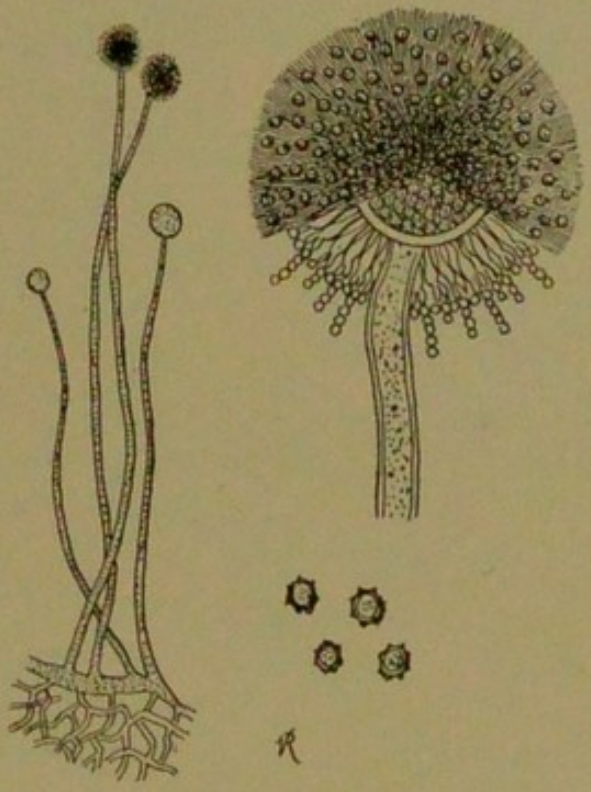


FIG. 17.—*Aspergillus.*

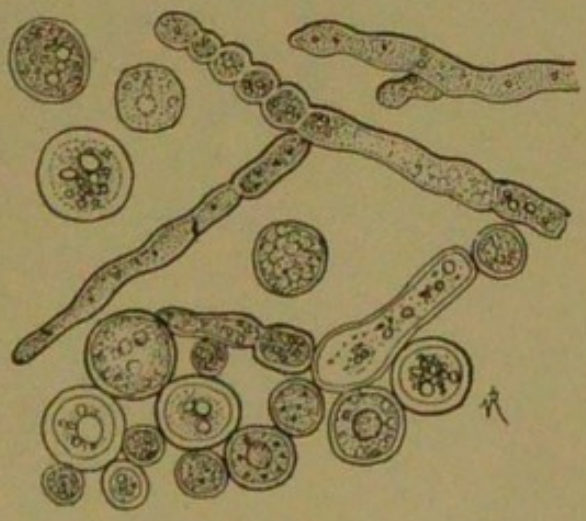


FIG. 18.—*Mucor* taking on the yeast form when grown at the bottom of a sugar solution.

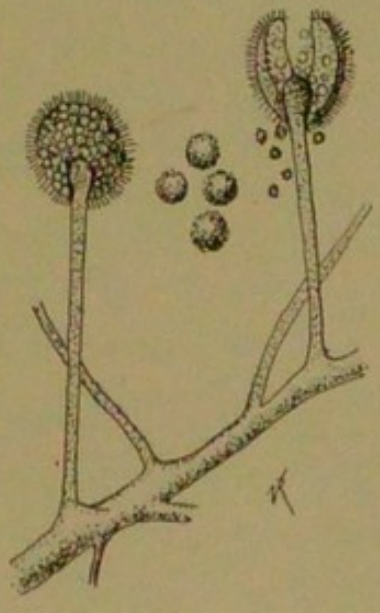


FIG. 19.—*Mucor.*

mycelium into a yeast-like form, which also produces fermentation like a true yeast, was discovered by Pasteur, who thought that in the course of ages the yeasts had been evolved from moulds which had become adapted to the fermentative life.

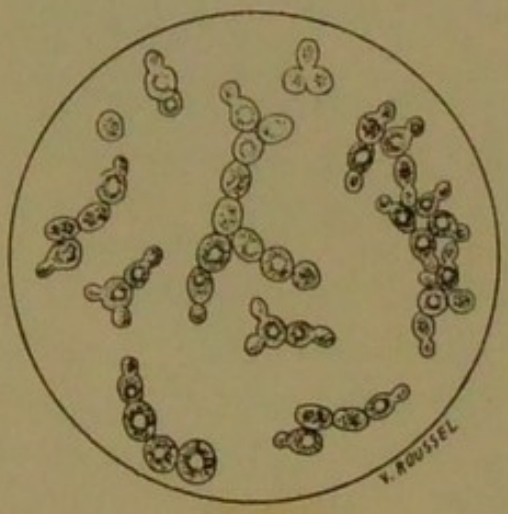


FIG. 20.—The yeast of beer: high or tumbling yeast.

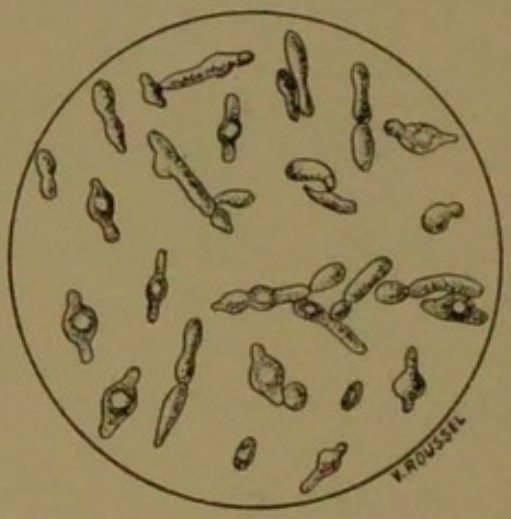


FIG. 21.—Spindle-shaped yeast.

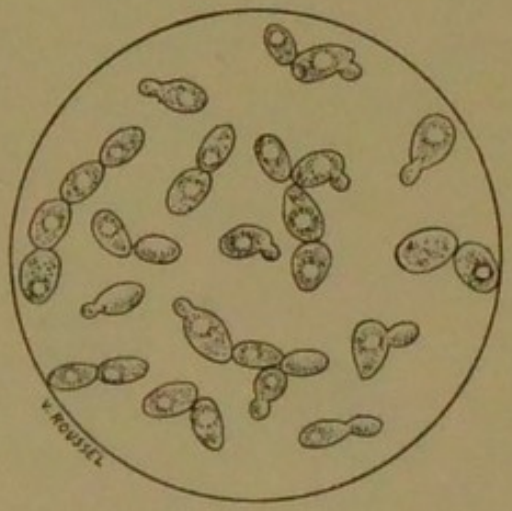


FIG. 22.—The yeast of wine.

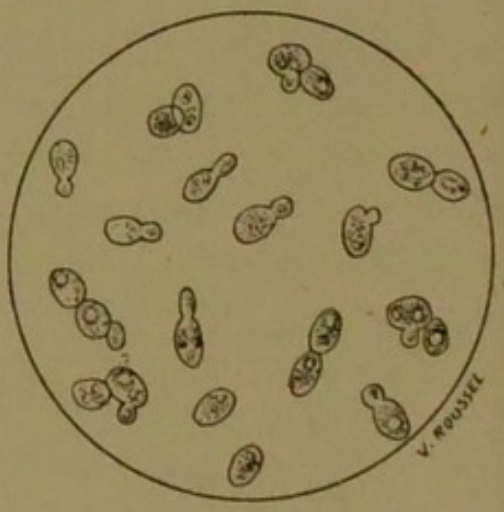


FIG. 23.—The yeast of beer: low or heavy yeast.

Yeasts.—Yeasts are fungi of the Blastomycete group. They consist of round or elliptical cells which multiply as a rule by budding; some multiply by equal division into two parts. Yeasts may furnish spores, which number 2 to 10, and may remain enclosed in the mother cell until by the bursting of this latter their germination becomes possible.

Bacteria.—The term "microbes" in current language most frequently signifies bacteria. The bacteria belong to the lower plants, are unicellular, contain no chlorophyll, and are almost all incapable of taking up carbon from the carbonic acid of the air. They are incapable of life except in the presence of ready made organic matter, and are in consequence confined either to a saprophytic existence (moulds on a fruit) or to a parasitic (as in the case of the typhoid bacillus in the intestine). Scattered as they are throughout nature the bacteria are the chief agents in the decompositions and recombinations of organic and living matter.

The usual classification of bacteria depends on their external form and is merely provisional. The round bacteria are called micrococci or *cocci*. The streptococci are, as Pasteur described them, like rosaries or necklaces; the staphylococci are like bunches of grapes. According as the micrococci in their multiplication divide in two or three planes in space, they appear arranged in the mulberry form or in the form of a *sarcina* or woolpack.

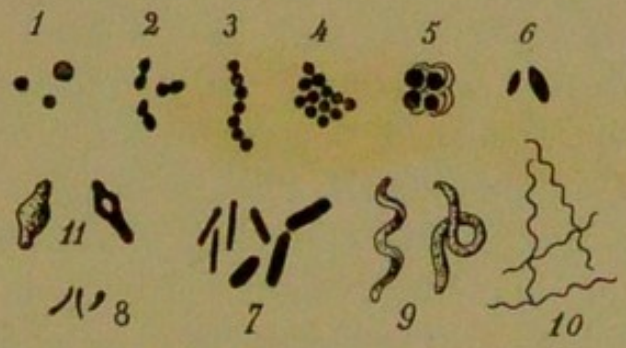


Fig. 24.—Principal bacterial forms. 1. Cocci.—2. Diplococci.—3. Streptococci.—4. Staphylococci.—5. Sarcinæ.—6. Bacteria.—7. Bacilli.—8. Vibrios.—9. Spirilla.—10. Spirochætes.—11. Clostridium.

The bacilli are long bacteria, the ends of which are sharply cut; the *bacterium*, properly speaking, has the ends rounded like a spindle or shuttle. The curved bacteria are known as vibrios, spirilla, and spirochætes.

The bacterial cell is clothed by a membrane and is not naked like an amœba. It is this sheath, resistant and elastic up to a certain point, which permits a corkscrew spirillum to maintain its form in spite of its movements, and the motile and flexible bacteria to recover their shape after distortion. The membrane takes on colours different from the protoplasm. It is not of the same composition in all the bacteria. Some-

times it is of cellulose, sometimes it resembles chitin, sometimes it is impregnated with fats or waxes like the bacillus tuberculosis. In the membrane of certain bacteria iron and silicon have been found. Sometimes in old cultures one can see membranes without contents which resemble empty sheaths.

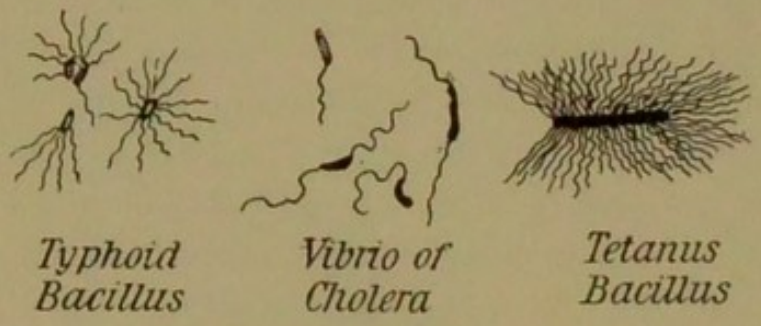


FIG. 25.—Flagella of bacteria.

Certain bacteria possess besides Brownian movement an independent motility; they can be seen performing long journeys, pirouetting on their own axis. It has been calculated that the cholera vibrio can travel 18 centimetres in the hour, which is equivalent to ten or fifteen times its length in a second. This motility is due to cilia or flagella analogous to those of the vibratile epithelial cells or the ciliated infusorians. They are often longer than the bacterium which carries them. In old cultures they become detached and scattered through the medium. It is doubtful whether they are fine expansions of cellular protoplasm which have passed through pores in the enveloping membrane or whether they are expansions of this membrane itself; whether they are not mere propelling organs but rather tentacles of some sort which increase the surface of the bacterium and thus play a rôle in nutrition, especially in the young bacteria. No definite reply can be given to these questions. According to G. de Rossi's experiments on agglutination and immunisation the cilia behave like protoplasm. Their action ceases at very low temperatures and above 50° C. When a bacterium provided with cilia only at one end (certain vibrios) is attracted towards a definite substance (positive chemiotaxis), the cilium or cilia take the front position and mark the head-end of the bacterium—just as the flagellum does in the case of the trypanosomes.

Certain bacteria such as the pneumococcus and the pneumobacillus are surrounded by a sort of case or capsule. One capsule may enclose several bacteria: when a large mass of bacteria is included in one enormous capsule what is called a *zooglæa* is formed. Bacteria which appear encapsulated in the albuminous fluids of the infected body do not always possess them in artificial culture. The capsule appears to be a defensive secretion. The anthrax bacillus defends itself against the toxic action of the serum of the rat by surrounding itself with a thick sheath made up of a sort of mucus which fixes and renders harmless the toxin. In the blood of lizards, which are very resistant to anthrax, the bacillus surrounds itself with a thick mucous envelope. The streptococci occasionally acquire in the body of resistant guinea-pigs a

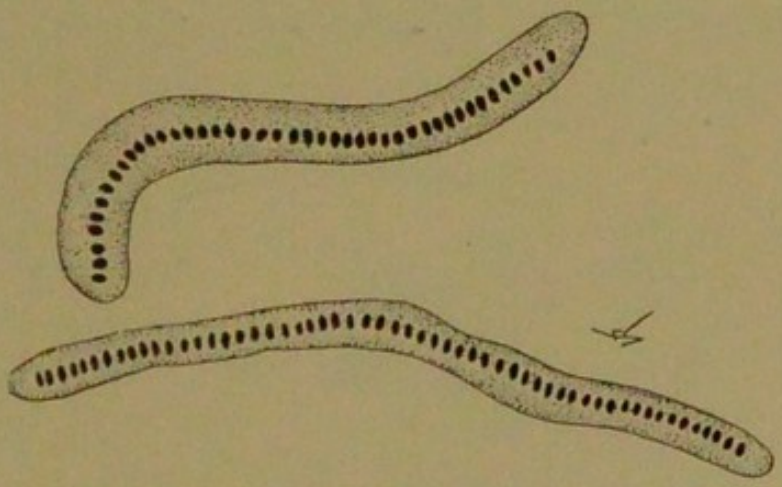


FIG. 26.—Streptococci surrounded by a sheath of mucus.

similar defensive covering. The streptococci of chronic infections (otitis and nasal infections) are frequently encapsulated. The sarcinæ are not usually pathogenic but encapsulated sarcinæ have been described which were pathogenic for laboratory animals.

Bacteria multiply by transverse divisions, *Pasteuria ramosa*, described by Metchnikoff, being an exception. One bacterium put on a suitable medium produces after a certain time two, these two producing four, and so on; it is easy to imagine to what enormous numbers this geometrical progression may lead. In nature and in artificial culture media, multiplication is

limited by the quantity of available nourishment and by the accumulation of the products of excretions ; the cultures end by poisoning themselves. But even in a drop of culture medium, the energy of multiplications is enormous. One filament of the *Bacillus ramosus* studied by Marshall Ward doubled its length in thirty-five minutes ; in twelve hours a single bacillus produced four millions, and one piece of one-hundredth of a millimetre in length produced in twelve hours the equivalent of a thread of 40 metres. Pasteur followed under the microscope the growth of the yeast of wine in grape juice at 13° C. One globule produced 10 millions in twenty-four hours when nothing intervened to limit its development. It is easy to understand now how the infinitely minute grows to form a mass and brings into play enormous forces.

Spores.—Certain bacteria produce spores, the spore appearing as a shining point in the filament ; the protoplasm of the cell diminishes as the spore increases, as if sporulations were a condensation of the living matter. Later the bacillus disappears and the spore is free. Being enclosed in a resisting sheath the spore resembles a seed, capable of prolonged preservation and of sprouting into a new bacillus when the conditions become favourable. It is by means of their spores that the bacilli of tetanus and of anthrax persist so tenaciously in nature. The anthrax bacillus is killed by heating to 60° C., the spore not till after three minutes' boiling at 100° C.

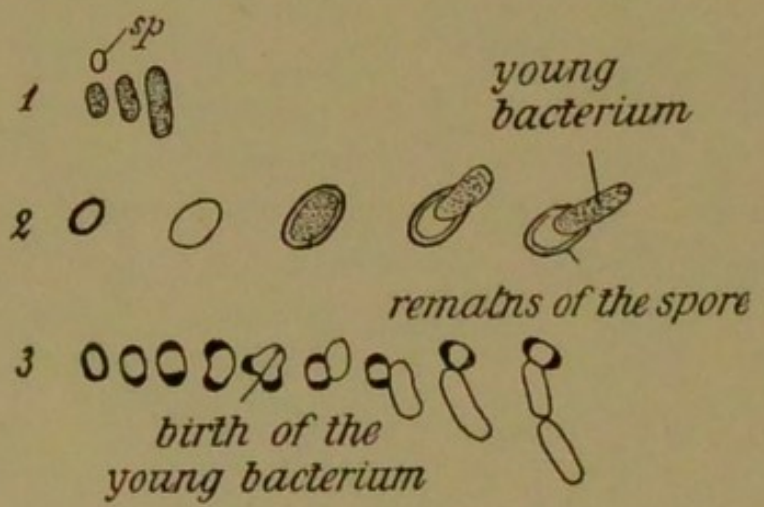


FIG. 27.—Various types of germination of spores. 1. The spore germinates by growth in all dimensions. 2. Germination by a sort of terminal budding. 3. The spore germinating by a sort of lateral budding. (After De Bary and Prazmowski.)

The spores which develop in the interior of bacteria are *endospores.* The name of *arthrospores* has been given to those rounded granules which certain bacteria produce by segmentation, by a sort of pinching off, or perhaps by a shrinking of their substance ; for example the cholera vibrio, which does not possess endospores.

The properties of an anthrax bacillus are fixed, and transmitted to the new generation, by a sort of heredity, by means of the spore.

It is not impossible that some sexual differentiation exists in the sporulating bacteria.

Pleomorphism of Bacteria.—The bacterial forms have not the immutability of crystals, nor even the relative stability of species among the higher plants and animals. They are variable to an extent that absolutely confounds the bacteriologist. In a pure culture such a diversity of forms may appear, forms long and short, rounded and thread-like, that the culture

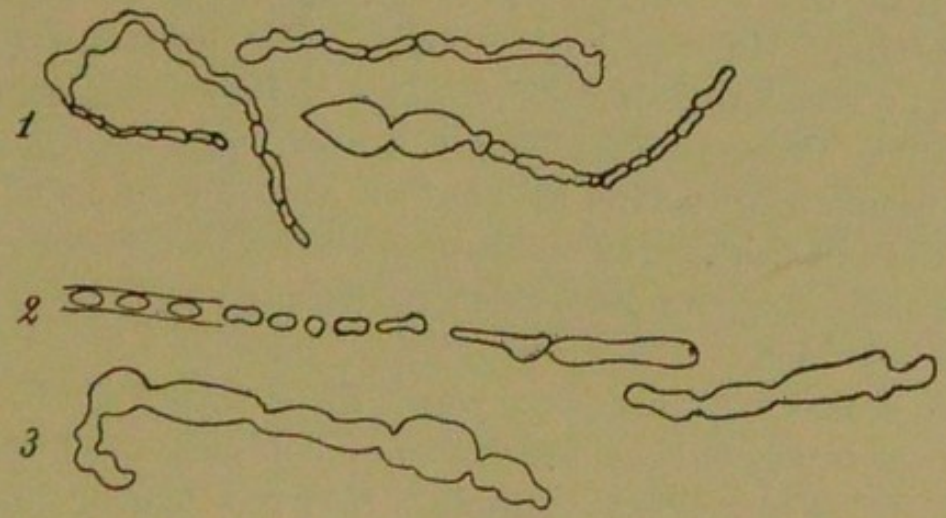


FIG. 28.—Involution forms : 1. Lactic bacilli.—2. Bacillus subtilis.—3 Anthrax bacilli.

might be thought impure. The bacillus of diphtheria transferred from a natural to an artificial medium, or from one artificial medium to another, appears short or long according to circumstances and gives in old cultures forms which resemble staphylococci. One bacterium exists so variable that it has been called *Proteus*, the protean bacillus. The *Bacillus prodigiosus*, the bacterium which gives red colonies

and thus produces the miracle of the bleeding host, multiplies very quickly in alkaline media, producing coccal forms ; these cocci, reinoculated on a faintly acid medium, multiply less rapidly and produce bacillary forms. Bacteria are then multiform or *pleomorphic* organisms.

In old cultures or in cultures on unfavourable media, forms are seen diverging from the typical : a micrococcus may give relatively enormous globules ; a bacillus may give forms racket-shaped, club-shaped, pear-shaped, &c. A bacillus which ordinarily never branches may show branching forms. These abnormal shapes are called involution forms, again examples of pleomorphism.

In the early days of bacteriology this pleomorphism was copiously discussed. Nägeli held that there were no bacterial species, but that the bacteria formed an immense group in which neither species nor families nor genera were to be distinguished. A coccus might give, under suitable conditions, a rod form or a spirillum and the function might be as variable as the form. He would not admit that a given fermentation always corresponded to a definite bacterial form, but held that the same microbe might be in its turn an acetic ferment, a lactic ferment, or a butyric ferment. For him bacterial specificity did not exist. Hence arose the belief that it was easy to render pathogenic an inoffensive bacterium like the *B. subtilis* of hay infusions.

The botanist Cohn exaggerated the stability of bacterial species as much as Nägeli exaggerated their pleomorphism, and bacteriologist-physicians, in their desire to discover for each infectious disease a specific agent, followed Cohn's idea. It was obviously impossible to hold precise opinions about the pathogenic bacteria if there existed no stability in their forms. R. Koch accused the "pleomorphists" of making their observations on impure cultures or on infusions containing already a very numerous flora ; it was not pleomorphism that was present, but confusion.

Another observer, Kurth, however, demonstrated the pleomorphism of the *Bacterium Zopfii* in pure cultures ; there-

upon the partisans of Cohn and Koch declared that though the saprophytic species might lack stability, the pathogenic species were stable. In their eyes the bacillus of anthrax was always a bacillus. But it had to be recognised later that pleomorphism existed even among pathogenic species, for example the cholera vibrio and the bacterium of fowl-cholera.

Finally the stability school maintained the necessity of distinguishing between the constancy of the mere shape and that of the species—a cholera vibrio may modify its form and yet remain always the vibrio of cholera—the tadpole of the green frog does not resemble an adult green frog, yet it belongs without any doubt to the species green frog. But it is certain that it is not only the form, but also the species *qua* species which is variable amongst the bacteria, and this variability is even more characteristic of their physiological properties than of their shape. The bacillus of tuberculosis, amongst others, is susceptible of very divergent adaptation.

Bacterial species exist, but they are all of the kind called in the language of the science of classification "*ill-defined*." "That the methodical classifier should be frequently embarrassed in his attempts to set up his artificial barriers, is very far indeed from being surprising. The world was not created for the special pleasure of descriptive botanists, and it is equally interesting to science to possess the demonstration that a classification is impossible as to have established a classification, had such been possible" (E. Duclaux).

In any case their faculty of adaptation and their power of variation sufficiently explain the history of bacteria. The pathogenic species must have come from saprophytic species by adapting themselves to certain animal bodies. The same virus, adapting itself in the course of ages to the human species and to the cow, has produced the two diseases small-pox and cow-pox, and this adaptation has become the principle of a wonderful prophylactic procedure. It was also by causing variations in the virus by physical and chemical action that Pasteur first prepared vaccines.

More recently search has been made among bacteria for

examples of mutation resembling those studied by the botanist Hugo de Vries. In a pure culture of bacillus coli Massini found that certain individuals suddenly acquired the power of fermenting lactose, and that this property was transmitted to their descendants. The attenuation of the anthrax vaccines of Pasteur is an example of a property acquired and transmitted hereditarily by the spore.

Precisely because of this pleomorphism and plasticity of bacteria, one must take care not to imagine mutations too frequently. Occasionally modifications may be got which are difficult to fix and which do not constitute true races. The bacillus prodigiosus has been cultivated at 37·5° C., without producing its red colour, for a series of generations; but after the thirty-fifth passage, when put again at 22° C., it reproduced its pigment. Besides, mutation is not to be defined simply as a sudden variation. There must be transmission by sexual reproduction in addition. One could not speak of mutation except in cases such as those of the anthrax bacillus, where there has been suspected a sort of sexuality in the spore forms. It is none the less true that the life of bacteria presents numerous facts in accordance with the Darwinian laws. "Bacteriology, like all branches of biology, has gained by the application of the theory of evolution, and has made a fair return by supplying the Darwinian theory with a striking confirmation" (Metchnikoff).

The Place of Bacteria in Classification.—Leeuwenhoek, describing in 1683 in his *Arcana naturae detecta* the micro-organisms of the mouth, which he observed through lenses polished by himself, represented them as animalculæ. In 1838 Ehrenberg assigned them a place in his work on the Infusoria, classing them with the Vibrios, which he regarded as animals. But their evolution and their activities indicate that the bacteria are lower plants.

Are they to be classed among the fungi (moulds and yeasts) or among the algæ? In the absence of chlorophyll, bacteria resemble fungi, and they have long been called *Schizomycetes*, *i.e.*, fungi multiplying by transverse division. There are

bacteria, the Streptothriceæ, which, by their filamentous and branching appearance, form a link between the bacteria and the simplest fungi. On the other hand, there are yeasts which resemble bacteria in producing endospores and multiplying by division instead of by budding.

It is, however, with certain algæ, the Cyanophyceæ or blue algæ that the relationship is most marked. The Cyanophyceæ reproduce by division, presenting different shapes, long, round, and curved, resembling cocci, coccobacilli and bacilli. They are pleomorphic like the bacteria. They possess chlorophyll in contrast to the bacteria, but the pigment which the Cyanophyceæ possess in addition to their chlorophyll, the phycochrome (most often greenish-blue, soluble in water and diffused throughout the cell) is not without some analogy to the pigment of certain bacteria. Certain algæ clump together in mucilaginous sheaths resembling the zoogloœa of certain bacteria. The Cyanophyceæ do not form endospores but have arthrospores like some bacteria. There are species like *Beggiatoa*, in which it is far from certain whether they should be classed with the bacteria or with the Cyanophyceæ. These analogies are so striking that a family, the *Bacteriaceæ*, has been made for the algæ which border on the *Cyanophyceæ*.

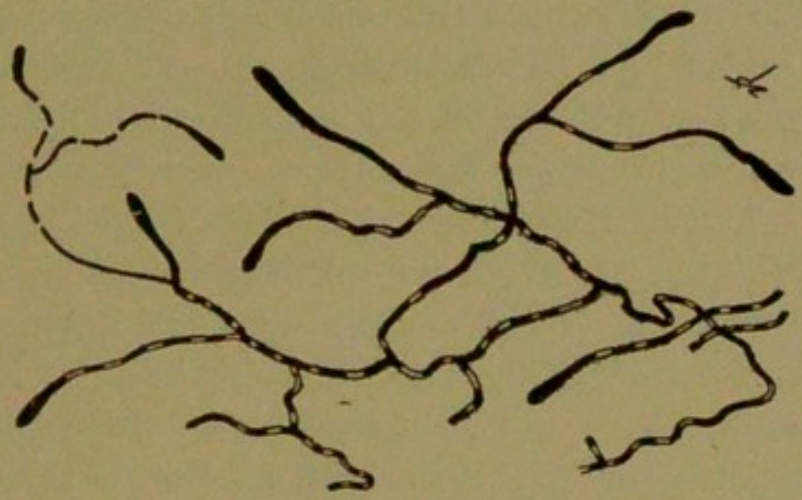


FIG. 29.—Streptothrix. Branching bacteria.

But in view of the differences which exist between these two families and the similarities which link up the bacteria and the fungi (moulds and yeasts), certain authorities make of the bacteria a heteroclitous group where there are brought together representatives more or less altered or degenerated of various lower plants. There exist no doubt bacterio-algæ; there are

also bacterial moulds and bacterial yeasts; there are even protozoal bacteria, *i.e.*, animal bacteria, the Spirochætes.

The conclusion then is that bacteria are not the original forms from which the others were derived but that on the contrary the bacteria are derived from the more definitely characterised fungi and algæ, the specific characters having become obliterated by the parasitic habit. The branching forms rarely observed in the bacillus tuberculosis, the endospores of the anthrax bacillus, the arthrospores of the Streptothrices are all atavistic stigmata.

The Nucleus of Bacteria.—The usual nucleus, consisting of a distinct mass in the body of the protoplasm, does not exist in the bacterial cell.

A cell without a nucleus ! Several observers (A. Fischer, Migula, Massart) do not shrink from such a paradoxical statement. They can see no trace of a nucleus; what others have taken for it, large or small, is only, in their opinion, an empty space or a vacuole, taking part in the cell-division and capable of dispersion into smaller vacuoles. The scattered granulations in the protoplasm are not grains of chromatin, for they have none of its reactions. They are either products of metabolism or reserve materials. They have been called *metachromatic* bodies, because when stained with blue or violet stains they take on a different, reddish tint.

Such observations are best made on certain large bacteria found in nature. It is remarkable that the *bacillus asterosporus*, which was the one selected by Migula to demonstrate the absence of the nucleus, has been employed by others to demonstrate the presence of one or even several well-defined nuclei. Able cytologists complain that the supporters of the nucleus have been deceived by appearances; that they have taken for chromatic masses the transverse segmentations which appear in certain large bacteria at the moment of division, and that they have even chosen for their demonstration bacteria which are not true bacteria; the *Bacterium gammari* of Vejdowsky is said to be a fungus approaching the yeasts and multiplying by division, while another bacterium studied by

the same author in the digestive tube of an annelid (Bryodrilus) ought rather to be considered as a mould.

To-day one can agree neither with those who affirm that there is no nucleus nor with those who describe a well-defined nucleus. In reality, the bacteria possess a *diffuse nucleus*. Instead of forming a mass with sharp contours, the chromatin is scattered through the protoplasm in the form of granules quite distinct from the metachromatic granules.

Long ago Weigert maintained that the nucleus is, as it were, melted or dissolved in the cytoplasm, for it is precisely the nuclear stains which stain the bacteria best; Bütschli considered that the cytoplasm of bacteria is reduced to a thin layer lying in apposition with the membrane, and that the bacterium, far from lacking a nucleus, is in reality almost all nucleus. Further, what purpose would a large quantity of cytoplasm fulfil, cytoplasm whose special function is nutritive? Bacteria are parasitic, and absorb their nourishment ready prepared. Their chief business is to multiply, to increase in numbers; it is quite natural for them, therefore, to possess an enormous nucleus like the spermatozoa and the embryonic cells, so much so that one might even say that bacteria are "free nuclei."

Schaudinn has furnished a brilliant support to these ideas by means of his observations on the largest known bacillus, the *Bacillus Bütschlii*, found by him in the intestine of cockroaches. The chromatin granules are scattered through the protoplasm; to produce the two spores they come together, and the chromatin condenses at the two ends. The condition of a diffuse nucleus is no less evident in the *B. maximus buccalis* of Swellengrebel.

The diffuse nucleus is not confined to bacteria. There are several protozoa which normally possess a well-defined nucleus, but at certain moments in development or in certain conditions of nutrition this may be seen changing into a diffuse nucleus. The diffused nuclei have been called by R. Hertwig *chromidia*. Several varieties exist, and the nucleus of *B. Bütschlii*, like the nuclei of bacteria, in general should be considered as consisting

of chromidia. The nuclei of the Cyanophyceæ have been the subject of similar disputes; in these inferior algæ also the existence of a chromidial nucleus is admitted, quite distinct from the metachromatic bodies.

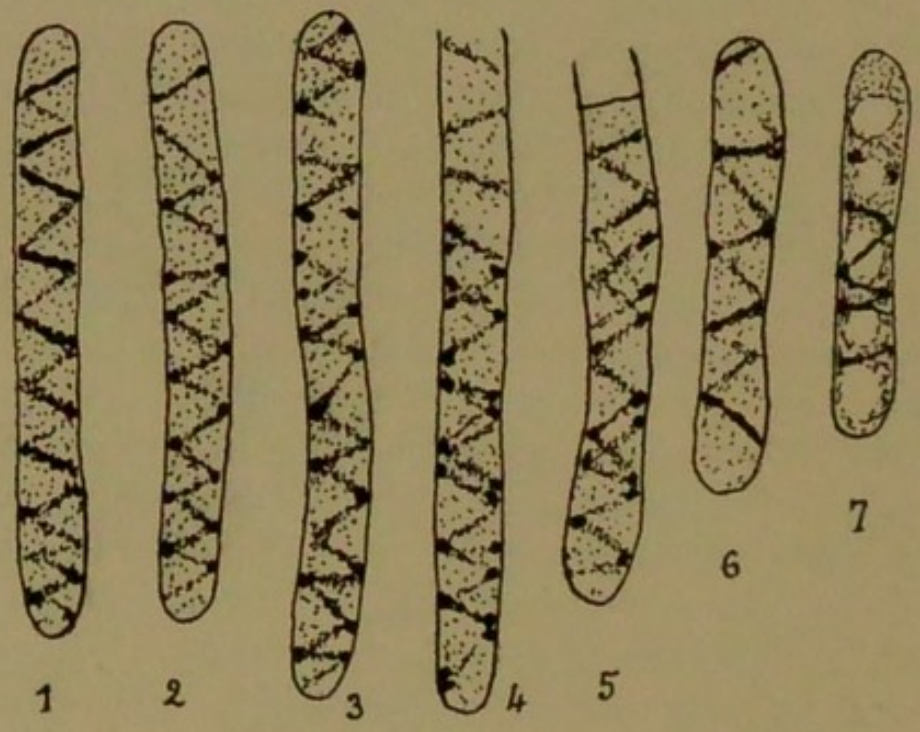


FIG. 30.—*Bacillus maximus buccalis.* (After Swellengrebel.)

The spiral filament represents a system of chromidia in process of division (4 and 5).

Reproduction and Sex.—A species is said to possess sex when fertilisation occurs by the fusion of two nuclei differentiated into male and female.

In the hæmatozoa of malaria the sexual act takes place in the body of the mosquito by the conjunction of the flagellum and the female cell, and at this point the sexual cycle begins.

Since Schaudinn's discovery of the trypanosome forms in the cycle of certain hæmatozoa closely related to those of malaria, one must admit the existence of male and female forms in the case of trypanosomes. Besides the asexual multiplication by longitudinal division there would thus exist among trypanosomes true sexual reproduction.

Among the fungi the ovum, the complete reproductive form,

results from the fusion of protoplasm as well as nucleus of two differentiated cells, the gametes, one male, the other female. Heterogamy exists when the male gamete is manifestly

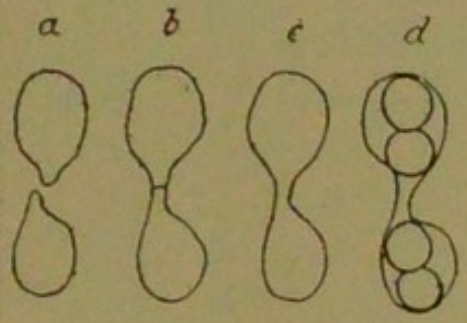


FIG. 31.—Successive stages of conjugation in a *Zygosaccharomycete*. (After Barker.)

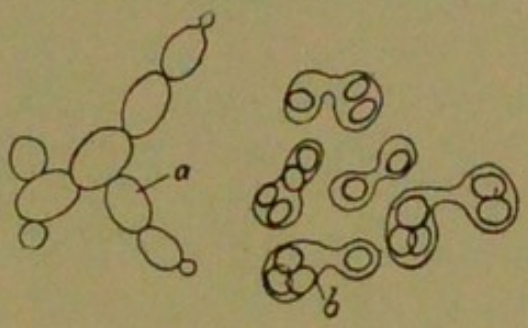


FIG. 32.—*Zygosaccharomyces* yeast. (*a*) Vegetative cells : (*b*) Asci.

different from the female—isogamy when they seem identical. The ascus and the basidium are modes of sexual reproduction.

In the yeasts, examples of conjugation are not lacking. In *Zygosaccharomyces* before the intracellular formation of the spores, two neighbouring cells put out each a little bud ; the two buds join and along the little canal thus formed fusion of the nuclei takes place (according to Barker). The two cells

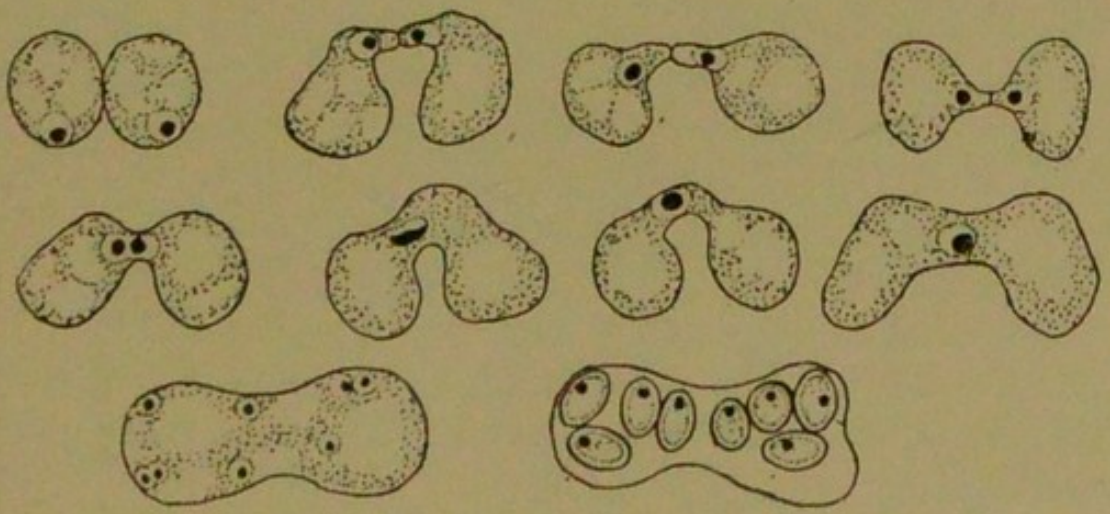


FIG. 33.—Nuclear fusions and spore formation in *Schizosaccharomyces octosporus*. (After Guilliermond.)

remain united forming a dumb-bell-shaped ascus. The same process occurs in certain schizosaccharomycetes, where the shape of the ascus also recalls its origin from two cells. Since

the cells which join belong to the same strain, and to the same generation or to two generations very near each other, this conjugation in the yeasts is an example of isogamy.

In the bacteria it has long been observed that multiplication by transverse division, *i.e.*, asexual reproduction, appeared to be an absolute rule; but Förster has described a species of conjugation among the sulphur-forming bacteria; they come together in twos or threes, put out little processes, unite and finally separate; this is a conjugation between individuals which are doubtless differentiated, and resembles somewhat the well-known conjugation of infusoria.

The observations of Schaudinn on *B. Bütschlii* have demonstrated the existence of conjugation also among the bacteria—at least among those which produce endospores. The ordinary multiplication by transverse division exists in *B. Bütschlii*, but in the individual about to form spores, other peculiar phenomena are seen: the individual begins by dividing into two, but the septum disappears almost as soon as it is formed and is dissolved, the two cells which had just been divided by the septum melting again into a single cell; an exchange of chromatin takes place between them, and finally almost all the chromatic granules collect at the two poles to form two spores. Short as is the time that the septum remains, there have, nevertheless, existed, during that moment, two cells which have conjugated: and this is, according to Schaudinn, a rudimentary sexual act. An analogous conjugation occurs with *B. Sporenema*.

It is really an *autogamy* or fusion between two elements of the same cell, or to be more precise it is a conjugation between two daughter-cells of the same mother-cell, and is hence called paedogamy.

Numerous cases of autogamy have been described among the protozoa. Autogamy is the opposite of the amphigamy which occurs when there is conjugation of two individals of well-differentiated sex.

What relations are to be established between these two methods of fertilisation? Is autogamy the simple primitive

type from which the other is derived, or is it simply a degenerated degraded form? As far as bacteria are concerned,

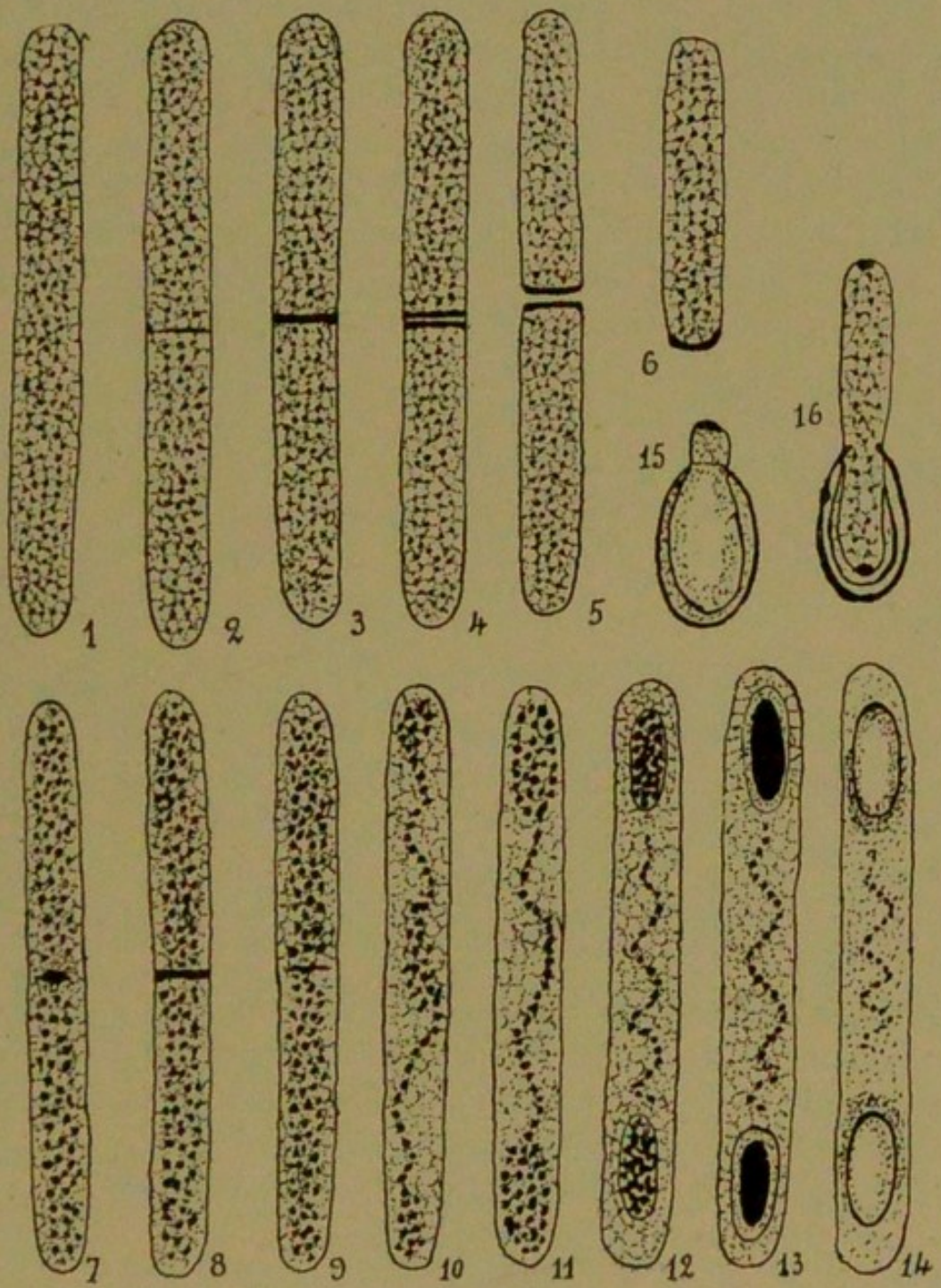


FIG. 34.—*Bacillus Bütschlii.* (After Schaudinn.) 1 to 6. stages of transverse division without sporulation.—7 to 9. Rudimentary sexual process.—10 to 14. Spore formation.—15 to 16. Spore germination.

Schaudinn regarded the conjugation which exists in *B Bütschlii* as an evidence of sex, though rudimentary, degraded and residual. Without absolutely excluding the possibility of

primitive autogamy the Schaudinn school considers autogamy in general as a regressive type.

The truth is that sexual differentiation of the gametes is universal in nature even in the lower orders. Researches on the Protista permit the recognition of sexual dimorphism in every nucleus, *i.e.*, there is in every nucleus a double substance and a double function, one specially nutritive, the female, the other specially reproductive, the male. Every protozoon cell is, to some extent, hermaphrodite, but with one or other element predominating. Even in autogamy, the elements which fuse are differentiated.

Sex is thus universal in nature, and it is from this point of view that Schaudinn had to consider the rudimentary conjugation of *B. Bütschlii* as a degenerate type. As with the structure of the nucleus, the fact of autogamy indicates again that bacteria belong to an order degraded by the parasitic habit.

Chemical Composition.—The chemical composition varies with the species studied, and in the same species with the age and the nutriment. Duclaux observed in the cells of a fifteen-year-old yeast that the proportion of fatty material, instead of being 5 per cent. as in young yeasts, had risen to 20–30 or even 52 per cent., the proportion of nitrogen varying from 1 to 4.

Bacterial substances contain proteids, fats, and sugars, but as there are so many proteids and so many sugars, the figures can only give a general indication. The cell is a "well stocked laboratory," and one which is always in activity.

The organic materials isolated from bacterial bodies of very diverse species are the following:—

Coagulable albumins in the expressed juices; globulins; a protamine (from B. tuberculosis); proteoses (by peptic digestion).
Glycoproteids. Phosphoproteids and their derivatives: nucleins, nucleic acids, xanthin bases, and pyridin bases.
A substance resembling chitin or keratin (B. tuberculosis).
Products of hydrolysis of proteins: amino-acids and hexone bases.
Carbohydrates: Cellulose, hemicellulose, and sugars.
Fats and waxes, neutral fats (capsulated bacteria, B. diphtheria, B. tuberculosis). Free fatty acids (B. tuberculosis); waxes (B. tub.); lecithin

(B. tub., 16 per cent., according to Kressling); phosphorized fats; in cultures of B. tuberculosis of a certain age the content of fat and wax varies between 20 and 39 per cent.
Ash: B. tuberculosis 8 per cent., B. coli 8·5 per cent.

Ruppel gives the composition of the Bacillus tuberculosis as follows:

Tuberculinic acid	8·5 per cent.
Nucleoprotamine	24·5 ,,
Nucleoproteid	23 ,,
Fats and waxes	26·5 ,,
Ash	9·2 ,,
Proteids (keratin)	8·3 ,,

Most of these substances pass into the extract, which is known as tuberculin.

For comparison: the human body, taken as a whole, contains 65 to 70 per cent. of water, the plants used for food 60 to 80 per cent., algæ 90 per cent.

Bacteria are specially rich in albuminoids. The moulds contain more carbohydrate than nitrogenous material, their outer covering containing cellulose.

Yeasts contain much nuclein; as they grow older the fat content increases; their outer covering also is cellulose. The mineral matter consists of phosphoric acid, potassium, magnesium, sodium, silicon, lime, sulphur, and oxide of iron. Yeast cells are richer in glycogen than the liver cells of the rabbit (31 and 10 per cent. of the weight of dried yeast).

CHAPTER IV

PHYSIOLOGY OF THE MICROBES

Nutrition—The definition of food material—Nutrition of the Mucedineæ—Raulin's experiments—Bearing of these experiments—Nutrition of yeasts and bacteria—Importance of the chemical constitution of the medium—The bacterium of sorbose—The idea of the 'soil'—Respiration—Aerobic and anaerobic life.
The purple bacteria.
Secretion of diastases or enzymes.
Products of the cultures—Products of excretion.
Auto-intoxication of the cultures.
Production of heat—Production of light—Production of pigment.
Action of heat on microbes; thermophil microbes.
Action of light; ultra-violet rays.
Physiology of Protozoa: cells possessing a great abundance of diverse functions—much differentiated—Nutrition; digestion, respiration Irritability—Reproduction—Parasitism—Adaptations and specificities—Life cycle and secondary host—Cultures.

THE microbes act in nature as transformers of energy. The energy which they get from oxygen and from various food materials they transform into products of excretion, heat, light, and work, retaining a portion used up in the building of their own substance. Like the higher creatures they display anabolism and catabolism. Each one is like a little vortex, from which the organic matter emerges different from its state on entry. It is characteristic of living beings to transform the molecules of their food materials into more complicated molecules. For example, from sugar they produce cellulose, from carbohydrates along with the nitrogen of ammonia they prepare the nitrogenous compounds.

To construct these new arrangements of the molecule, energy

or heat is necessary, and the microbe provides this by burning up a portion of its food material. Thus, while part of the food is raised to a level of higher organisation, another portion, on the other hand, is reduced to a simpler condition. For example, part becomes protein, part carbonic acid and water, the latter not being incorporated in the living substance.

Similarly, a microbe, like the *Aspergillus niger*, burns up with the help of the oxygen of the air the sugar which is furnished by the food, and this combustion puts at its disposal a certain number of calories ; one molecule of sugar weighing 160 grams furnishes 673 calories during its transformation into CO_2 and H_2O. The yeast of beer, which decomposes sugar less completely, *i.e.*, into alcohol and carbonic acid, only yields 33 calories. If the yeast had the nutritive requirements of the aspergillus it would have to use up nearly twenty times more food. Nutrition of this kind is characteristic of a ferment, and the fermentative power is greater the more of the food-stuff the cell is obliged to break down.

Lacking chlorophyll as they do almost universally, the microbes cannot take up carbon directly from the air as do the green plants. They demand their food ready made so that they can destroy it, turning it into carbonic acid and water. The heat derived from this destruction takes for them the place of the energy furnished by sunlight to the plants which contain chlorophyll.

The food of a microbe may then be defined as "every material from which a given microbe, under the conditions of the experiment, can take the material necessary for its development and the heat necessary to render it independent of solar energy." In calculating the total energy coming into play in the protoplasmic activity a certain amount has to be accounted for as external heat, and it is even the rule for an excess to appear as heat, so that there is a rise of temperature in the medium. Thus a vat at the surface of which acetic fermentation is going on gets notably hotter, and the same is the case in grapes undergoing vinous fermentation. But under certain limited conditions the protoplasm can perfectly

well employ, as part of its food, substances already oxidised and incapable in any way of furnishing heat, the conditions being that these are made to enter into a nutritive compound in the manufacture of which heat-furnishing transformations occur in sufficient amount. For example, the nitric bacteria, as Winogradsky has shown, can take their carbon from carbonic acid on condition that at the same time they transform nitrous acid into nitric. Similarly, the ferments which fix nitrogen can take up this gas from the air on condition that they destroy at the same time by oxidation sugar or some other hydrocarbon capable of furnishing heat during oxidation." (E. Duclaux).

Cellular protoplasm elaborates food by means of the diastases which it contains, and has peculiar wants and preferences according to the species. It must therefore be difficult for a microbe to find the particular nourishment which suits it best. The artificial cultures of microbes which are so useful in scientific research and in medicine demand practically for each microbe those food-stuffs which nourish it in nature.

Nutrition of the Mucedineæ (Raulin's Experiments).—Raulin succeeded in composing with perfectly pure sugar and mineral salts an artificial medium more favourable to the growth of *Aspergillus niger* than any occurring in nature. It is thus a *cultivation* in the fullest sense of the word.

Raulin's Medium.

Water	1·500	grams.
Candy-sugar	70	,,
Tartaric acid	4	,,
Ammonium nitrate	4	,,
,, phosphate	0·60	gram.
Potassium carbonate	0·60	,,
Magnesium ,,	0·40	,,
Ammonium sulphate	0·25	,,
Zinc ,,	0·07	,,
Ferrous ,,	0·07	,,
Potassium silicate	0·07	,,

This medium was prepared by a series of trials and demanded a most admirable patience, for he had to make

modifications in the number and quantity of the constituents, to compare the weights of the growth obtained, to determine the temperature and the hygrometric condition of the atmosphere, and even to take account of the shape of the culture flasks, which affected the oxygen supply.

If potassium is cut out from the above formula, the crop of aspergillus is 25 times less, all the other conditions remaining the same; if the figure 25 is taken as representing the measure of utility of potassium, the utility of the other foodstuff may be represented as follows: nitrogen, 153; phosphorus, 182; sulphur, 25; silicon, 1·4; magnesium, 91; zinc, 10; iron, 2·7.

If you take the ratio between the weight of one of these food-constituents and the weight of the crop due to its presence, you get a number which expresses its specific utility, *e.g.*, for zinc $\frac{22{\cdot}5}{0{\cdot}04} = 560$.

This ratio was in several experiments found to be 953. For nitrogen it is 17; for phosphorus 157; sulphur, 346; potassium, 64; magnesium, 200; iron, 857. It is to be noted that aspergillus takes up zinc from a medium in which the zinc is diluted 1 in 50,000.

If 1 in 1,600,000 of silver nitrate is added to the fluid, aspergillus spores no longer sprout in it: even when simply poured into a silver vessel the fluid dissolves sufficient silver to prevent growth. The plant is thus an indicator so sensitive as to mark, by its refusal to sprout, such small quantities as 1/240th of sulphate of copper, 1/8,000th of platinum bichloride, 1/50,000th of perchloride of mercury.

Zinc has a definite food value but iron acts differently, namely, by neutralizing a substance which is produced by the growth and becomes injurious to it, perhaps sulphocyanic acid.

Tartaric acid maintains the acidity of the medium and prevents bacteria from contaminating the culture, for almost all bacteria refuse to grow except in an alkaline medium. Hence a culture of aspergillus in Raulin's fluid produces its own asepsis. The sugar is only assimilated after inversion by

a diastase of the fungus ; three parts by weight of sugar furnish about one of growth. When glucose is supplied instead of saccharose, the culture starts off more quickly ; lactose is not a good food-stuff. Alcohol (in quantity equivalent to the weight of sugar) interferes with the germination of the spores, but in the adult form the fungus gets on quite well with it ; alcohol is thus a poison to the embryo, but good food for the adult. Starch paste (boiled) can supply the necessary carbohydrate nourishment, but raw starch alone prevents germination ; the adult would, however, attack it as a sort of last resource.

The Bearing of Raulin's Experiments.—These are not mere laboratory fantasies but are in reality almost the first exact experiments on the conditions of plant cultivation and growth. For every plant in the fields, for every microbe in the laboratory, a Raulin's fluid is demanded, a fluid which would represent ideal conditions for development : all the improvements in the technique of cultivation are attempts in this direction. For example, the first cultures of the tubercle bacillus were made with great difficulty on coagulated blood serum : good growth was only obtained by adding glycerine to the nutritive medium (Nocard and Roux). Almost all our culture media for bacteria are empirical : blood, ascitic fluid, and serum are supplied to them because we know that they live well in the animal body in these fluids. If our knowledge of bacteria was as far advanced as is that of *Aspergillus* it would be possible to make media of known and constant composition with measured quantities of the constituents, and these would undoubtedly be of the greatest value in the preparation of vaccines and toxins. When we consider that *Aspergillus* is sensitive to zinc in the dilution of 1 in 50,000 and to silver nitrate in the proportion of 1 in 1,600,000, it is possible to foresee to some extent the solution of many technical difficulties and to appreciate the extent of what remains to be discovered in connection with the action of manures, food-stuffs and drugs. Raulin's experiments show to what degree bacteriology and medicine depend on the progress of chemistry.

We know how to prepare a *clean* vaccine fluid by culture on the flanks of the calf, but we cannot prepare *in vitro* a vaccine lymph bacteriologically *pure*. This would be possible if we knew the nutritive demands of the vaccine virus. This virus is a microbe still unknown, but probably living in the interior of the epidermal cells; it finds itself there in a medium in which reducing actions predominate. Répin succeeded in preparing a reducing medium by means of a living reducing agent put into a culture flask (the tyrosinase extracted from the mushrooms of the genus Russula); with this he got a commencement of growth in the vaccine under artificial conditions. It is obvious what problems lie in wait for those who try to grow bacteria in the laboratory.

The Nutrition of Yeasts.—The yeasts are of such great industrial value that every detail of their nutrition has had to be studied. They demand phosphoric acid and potassium, magnesium, lime, and sulphur; their nitrogenous food they take from ammoniacal salts and they can use those albuminoid substances which are soluble in water, dialysable, and more or less insoluble in alcohol, and which exist in serum; also they can use urea and allantoïn. The carbohydrates they employ are in the first place sugars, then various alcohols, acids and organic salts. From its food the yeast accumulates reserve material, *i.e.*, glycogen. Yeast attacks the food-stuffs supplied to it by means of its diastases; and in doing so, while toiling for its own purposes, it toils for ours—exactly like a hive of bees.

Alimentation of Bacteria.—The minerals employed in nutrition are rather varied; sulphur, phosphorus, calcium, magnesium, potassium, sodium, traces of iron, traces of chlorine. As carbohydrate food, sugars and glycerine; as nitrogenous, ammoniacal salts and peptones, natural proteins like blood serum and asparagine. The food-stuffs are supplied by meat infusions, *bouillons* with peptone and salt, by animal fluids such as serum, urine, ascitic fluid, milk, and by fruit juices. The preferences which bacteria show for certain foods are employed for diagnosis, because bacteria are characterised not less by their food preferences than by their shape.

Sulphur is indispensable in the culture fluid of the sulphurous or sulpho-bacteria (*Beggiatoa*, *Lamprocystis*, and other species described by Winogradsky); these can do almost entirely without organic food material and grow well in water which contains only 4·8 milligrams of this per litre, but which contains two milligrams of sulphuretted hydrogen. This they decompose, fixing the sulphur and accumulating it in the cell in the same way as yeasts with glycogen. Certain *Beggiatoa* contain 80–95 per 100 of their weight of sulphur. When put for two or three days in non-sulphurous water they oxidize their sulphur turning it into sulphates. If the dearth of sulphur continues they die.

The ferro-bacteria (*e.g.*, *Crenothrix polyspora*, *Cladothrix dichotoma*, *Leptothrix ochracea*) oxidize the carbonate of iron protoxide, $FeH_2(CO_3)_2$, and accumulate the hydrate of ron oxide. Instead of iron oxide a deposit of oxide of manganese has been observed in certain cases.

The Importance of the Chemical Constitution of the Medium.—Pasteur observed the relations between the chemical structure of the food material and the physiological action on it of the microbe. A *Penicillium* uses up dextro-tartaric acid, leaving intact the lævo form until the former is completely exhausted. *Penicillium glaucum* and certain yeasts can decompose optically-inactive sugars, burning up the dextrorotatory form while sparing the lævo.

Following in the track of Emil Fischer, there have been observed relations between the molecular constitution of a sugar and its value as food or fermentable material to a yeast or in general to any definite ferment. Among the numerous sugars with the general formula $C_nH_{2n}O_n$ (n being a whole number 1, 2, 3, 4, etc.), the ordinary yeasts only ferment those with the carbon atoms numbering 3 or a multiple of 3. Sugars which are isomeric, but whose molecule does not possess the same stereochemical configuration, do not behave exactly the same towards a given yeast. In a mixture of glucose and levulose one or other is the first to be decomposed, this varying with the strain of yeast.

There exist also, it is true, "fermentations by force of example," where a fermentation started on a certain sugar may extend its attack to another sugar which at first was not fermentable: galactose, for example, can be fermented when it has been "baited" by glucose. Similar affinities, capable of modification by custom, come into play without doubt in the sensitiveness or resistance, natural and acquired, of an animal body towards a pathogenic ferment. Beneath the biological specificity there lies a chemical specificity.

One of the best examples that can be quoted is that of the bacterium of sorbose studied by G. Bertrand.

This makes a selection among the polyatomic alcohols, attacking only in their molecule a link of the formula CH·OH, transforming it into CO and consequently producing always a ketone body. Further, for this link to be attacked its hydroxyl OH must not be on the side of the H atom of the neighbouring link CHOH. Finally the secondary group attacked is always next to one of the primary group CH_2OH, which terminates the chain, at least in the formulæ below C_7. The stereochemical structure of the sugars thus plays an important part in the matter: it is on it and on it alone that the possibility of attack by the bacterium depends. Its action is so narrowly specific that it only transforms certain chemical groups, taking no interest in the rest of the molecule, whatever may be its mass and structure. A fermentation of this kind has all the value of a chemical reaction.

Food-stuffs, culture media, infected organisms, represent for the bacteria their *soil*. "If we consider," says Bertrand, "on the one hand the differences in chemical composition, even qualitative, which may exist between two closely related species, and on the other hand the extraordinary variety of proteins which it is possible to conceive of nowadays, it will hardly appear unreasonable to compare animal species, or physiological variations of the same species, to culture media varied like those which I used in the study of the sorbose bacterium, nor to account for their immunity or susceptibility towards a given microbe by a chemical or even

merely stereochemical difference in their composition." In two culture media, identical except that one contains sorbite, the other dulcite, a different sugar, the former supports the sorbose bacterium, the latter is refractory. To the bacillus tuberculosis distributed everywhere, all the "soils" are not the same. The chemical study of the soil ought to go hand in hand with the study of the microbe.

Respiration : Anaerobic Life.—Oxygen is the primary food of creatures which have respiration. Lavoisier showed that oxygen is indispensable to life.

During his study of the fermentative change of calcium lactate into butyrate, Pasteur discovered the *vibrio butyricus*, and made the fundamental observation that this organism lives without free oxygen and even dies on contact with the air :

"Pure carbonic acid passed for however long a period through the fluid in which they are growing has no effect whatever on their life and multiplication. If atmospheric air is passed through instead for one or two hours under precisely parallel conditions they all die, and the butyric fermentation which depends on their presence ceases immediately." (Pasteur.)

Those micro-organisms for which free oxygen seemed to be a poison were called by Pasteur *anaerobes*. There exist "strict anaerobes," "strict aerobes," which cannot exist without free oxygen, and "facultative" bacteria capable of living in either condition.

In broth the aerobes form at the surface a little collar, a ring, or a pellicle ; in a drop of fluid under the microscope they can be seen to make their way towards the periphery, where there is the best provision of oxygen.

If a filament from a green alga, a plant containing chlorophyll, is put into a suspension of motile aerobes, and a small spectrum of sunlight is allowed to fall upon it, the bacteria can be seen collecting at the points where the chlorophyll assimilation and the production of oxygen are most intense, *i.e.*, at the red and violet regions of the spectrum, the *B* and *C* lines and the *F* line of Frauenhofer (Engelmann's experiment).

On the contrary, the anaerobes avoid the surface of the

drop of water and the neighbourhood of air bubbles. They can be grown well under shelter of a pellicle of aerobic bacteria which prevent the passage of air, this being the best method of cultivating the tetanus bacillus. There is no necessity to suppose, as does Kedrowsky, that the aerobes

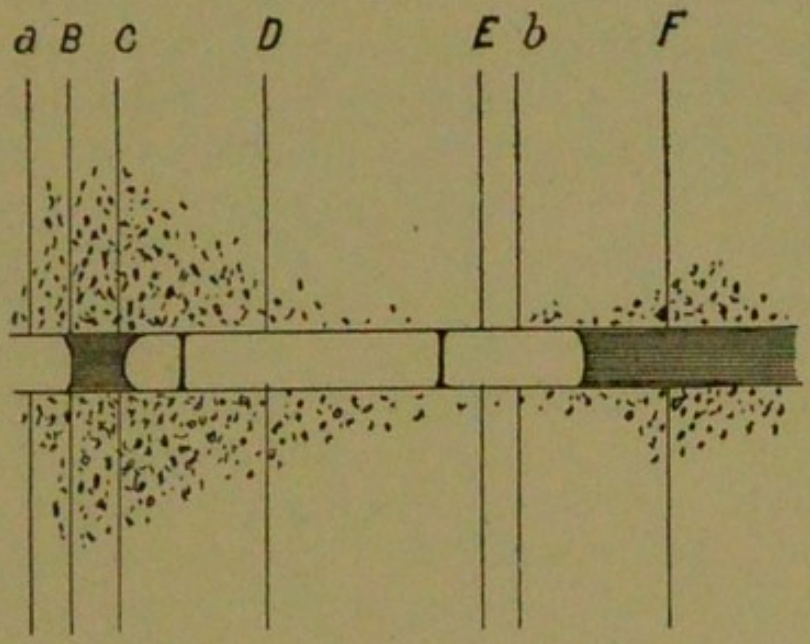


FIG. 35.—Engelmann's spectrum. Bacteria seeking oxygen swarming round an algal filament lying on a spectrum. The grains of chlorophyll are not represented. The lines of the spectrum mark out the regions on which the bacteria collect, *i.e.*, the points where most oxygen is being liberated.

secrete a special ferment which permits anaerobic growth; it is sufficient that the anaerobes are cut off from free oxygen.

The addition to a tube of ordinary broth, aerated and hence unsuitable for the culture of anaerobes, of sterile animal or vegetable tissue, *e.g.*, a fragment of flesh or a piece of banana, allows the anaerobes to grow, the tissue acting as a reducing agent. It is not at all correct to say, however, that the anaerobes live without oxygen. They only live, as Pasteur said, without *free oxygen gas*. They use up the oxygen which is present in combination in the nutrient fluid and decompose the food-stuffs to procure oxygen from them.[1]

[1] Pasteur (1861): "There exist, besides the living beings already known which without exception, at least in the general opinion, live and breathe only on condition of being able to assimilate free oxygen gas, others whose respiration is so powerful that they can live cut off from the air by

The decomposition is generally only partial and to procure the quantity of oxygen and energy necessary the anaerobes have to attack large quantities of the food-stuffs. Such behaviour is typical of ferments and accordingly the anaerobes usually produce powerful fermentation. "Fermentation is life without air," was Pasteur's dictum. It is in particular the study of alcoholic fermentation which supports this statement. When a yeast grows in a shallow mass of fluid with an extensive surface its cells multiply abundantly: there is a great increase in yeast protoplasm but little or no alcohol. When inoculated on the contrary at the bottom of the fluid without access of air, the growth is feeble but produces alcohol in quantity, varying in proportion to the completeness of the anaerobic conditions. The differences in the form and functions of *mucor* when aerated or deeply immersed have already been mentioned. Several microscopic plants, mucedineæ and yeasts, exhibit a whole series of transitional forms between aerobic and anaerobic growths. Anaerobic life appears to be an asphyxial condition against which the microbe contends or adapts itself by changing its manner of nutrition. Not only the mucedineæ and the yeasts but all living cells, animal and vegetable, act like ferments and produce alcohol when forced to live cut off from the air in presence of sugar. Such is the case in the experiment of Pasteur and J. B. Dumas with the plums kept under a bell-jar: they use up the air and fill the jar with carbonic acid; their sugar diminishes and they become charged with alcohol. A similar case is that of ripe fruits left to themselves in an atmosphere of limited volume, as, for example, with the apples and pears kept in a closed vessel in the experiments of Lechartier and Bellamy. Another is that of seeds starting to germinate cut off from oxygen; they produce alcohol, using up their reserve material (Maze's

seizing upon the oxygen of certain compounds, in which there is in consequence a slow progressive decomposition. This group of living organisms is composed of ferments precisely similar to those of the first group, living like them, assimilating like them carbon, nitrogen, and phosphates, and like them requiring oxygen, but differing from them in their power of doing without free oxygen gas and carrying on their respiration with the oxygen derived from unstable compounds."

experiment). There is even alcohol in animal tissues. There is, therefore, nothing surprising in the presence of alcohol throughout nature, in the soil, in water, in air, and in the sea; if it is true that the latter contains one millionth of its weight (one gram per cubic metre) there must be an enormous supply.

Since the discovery by H. Buchner of zymase—the diastase by which the yeast decomposes sugar—we know that it is on the zymase rather than on the anaerobic conditions that the alcoholic fermentation depends. But since the zymase only appears when the yeast is shut off from the air, it too is "an asphyxial function" and we return to Pasteur's formula.

Duclaux has re-established the continuity between the two methods of respiration by his idea of the constant operation of the zymase in aerobic as much as in anaerobic life and by maintaining that alcohol is produced by living tissues, not pathologically but normally. "Alcohol is a normal and necessary product in the digestion of the hydrocarbons of the seed. When oxygen is present, this alcohol is burnt up and escapes observation. To demonstrate it the plant must be submitted to a degree of asphyxia which just lets it live, or rather, which permits the action of the zymase which it contains. It is not the asphyxia which produces the alcohol, it only renders it perceptible."

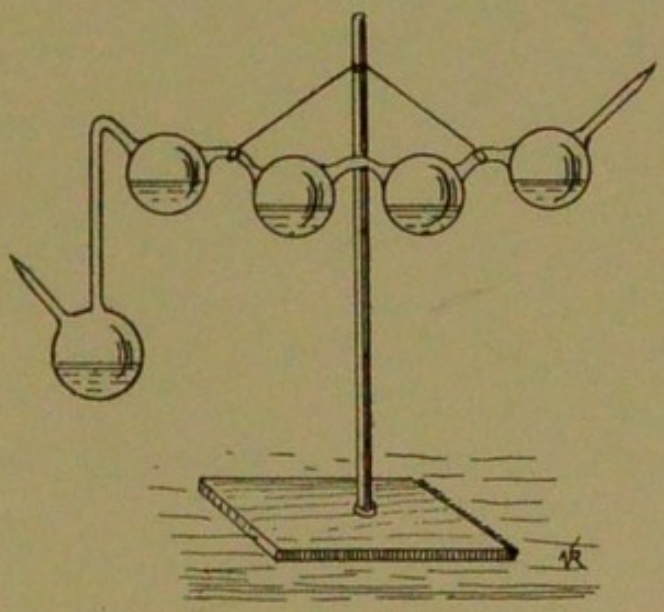


FIG. 36.—Cochin's experiment.

Further, absolute anaerobiosis does not exist either in nature or in our artificial cultures. The pretty experiment of Denys Cochin shows that yeast even under anaerobic conditions the most complete possible cannot do without oxygen

indefinitely, *i.e.*, in traces. Yeast cells are made to grow shut off from air in a series of communicating flasks all carefully sealed. The flasks are inoculated in series, the second with the yeasts of the first and so on. To isolate each flask from the preceding the little communicating tube is sealed in the flame. Towards the tenth generation, fermentation is seen to stop and only revives when a little oxygen is admitted to the confined atmosphere.

Pasteur had already observed that an air bubble about the size of a pin-head was sufficient to reawaken a slackening fermentation.

All microbes require oxygen but their requirements are very unequal. Betweeen the aerobes and the strict anaerobes there exists every intermediate condition. Each species requires an oxygen pressure suitable for itself just as do the higher animals, which are of course aerobic organisms.

By exhausting the air under a bell-jar, Khoudiakow observed that the *B. butyricus* could still multiply at the pressure of five millimetres, *Clostridium butyricum* at ten millimetres, the *vibrion septique* and the tetanus bacillus at twenty millimetres ; the bacillus of systematic anthrax at forty millimetres, at which pressure the latter microbe behaves like an aerobe, using up the oxygen in oxidations.

An anaerobe like the *B. butyricus* can be trained to live under an oxygen pressure greater and greater up to fifty millimetres, a pressure ten times greater than that which it will bear normally. This acclimatisation of anaerobes to contact with air can be carried out within certain limits in the laboratory, so much so that it has even been thought useful to create the barbarous phrase ' aerobisation ' of anaerobes.

Khoudiakow has made a complementary experiment by modifying the pressure on aerobes. *B. subtilis*, grown on gelatin, lives fairly well under three atmospheres, but begins to suffer at four. At the other end of the scale it still grows well at ten millimetres of pressure, but not at five. *Aspergillus niger* has for minimum and maximum five millimetres and three atmospheres. Spores are more resistant to the action of air

than bacilli. The spores of *Bact. butyricum* are scarcely affected by the action of air for 265 days, whereas the growth of the bacillus is inhibited by the action of air for fifteen hours.

Oxygen is a food which bacteria take from compounds liberating it more or less readily; anaerobes take it from compounds which retain it and resist decompositions. Although it is not absolutely true that fermentation is life without air (there are fermentations which go on in presence of oxygen), it is true that anaerobiosis favours the majority of fermentations and is the usual condition for these.

In nature, anaerobes occur wherever there is little penetration of air, or where the air is diluted or replaced by other gases, as, for example, in the earth, in mud and slime, in sewage, in the ooze of the sea, in dunghills, and in the intestines and excrements of animals; and it is in these surroundings that the most important fermentations and putrefactions of organic matter take place.

Respiration of the Pigmented Bacteria.—The purple bacteria (a certain number of which are also sulphobacteria) contain a pigment, *bacterio-purpurine*, quite distinct from the pigment of *Bacillus prodigiosus*. According to Engelmann, these pigmented bacteria absorb the infra-red rays of the spectrum (of wave-length 0·8 to 0·9 μ) and employ them, as also the red rays, in the decomposition of carbonic acid from the air and the liberation of oxygen, just as plants do with chlorophyll; they have a "chromophyll" function analogous to the chlorophyll function of green plants. This opinion is not shared by all observers: according to Molisch, the purple bacteria are not capable of decomposing CO_2 nor of assimilating directly inorganic compounds. They certainly differ from other bacteria in their power of using light in their nutritive process, but their foods are still organic food-stuffs ready made and they cannot do without these: they are not capable of the synthetic function of the green plants. They can assimilate organic food-material in the dark like other bacteria, but they have advanced a step by adapting themselves to light and by using it to increase their nutritive resources. But

they have remained at that stage: they have not cast off the necessity for ready-formed organic food, *i.e.*, the parasitic habit, nor can they break up the carbonic acid of the air, liberating oxygen and absorbing carbon. The purple bacteria then occupy a position intermediate between the saprophytic or parasitic habit of bacteria in general and the chlorophyll property of the higher plants. They carry on, like the latter, a sort of photosynthesis, but what they synthesize with the aid of light is still organic material like the ordinary bacteria.

Secretion of Diastases or Enzymes.—Microbes act through their diastases; fermentations are thus diastasic reactions. The diastases carry on the transformations of matter both by breaking down and building up, and it is through them that the bacteria transform energy.

The discovery of diastases and the possibility of extracting them, of isolating them (not completely pure), and of making them act without the presence of living cells, represents a great acquisition to the dominion of chemistry in the field of the study of life and fermentation.

A further step was made when Bertrand demonstrated the prominent rôle taken by the mineral elements associated with the enzymes; the activity of *laccase* depends on the proportion of manganese present, and the whole reaction behaves as if laccase were a salt of manganese with a weak acid. Besides, all the diastasic reactions can be performed by chemical agents and the part played by diastases seems to be that of amplifying and stopping the action of the latter.

The nature of diastases is still unknown, and we shall not dwell here upon the manner of preparing them, on the causes of error which may creep into this technique, or on the theory of diastasic actions in general.

One microbe is capable of secreting several diastases. With the proteolytic enzymes have been grouped the *lysins* including the hæmolysins of bacteria. The solution of the cell attacked may indeed be only the sequel to an action on the cell membrane or protoplasm, injurious but not actually dissolving. The best known hæmolysins are those of the

tetanus bacillus, tetanolysin; of the staphylococci, staphylolysin; of the cholera and pseudo-cholera vibrios, vibriolysin; of the streptococci, streptocolysin; of bacillus pyocyaneus, pyocyanolysin; lysins also exist in the cultures of *B. typhosus*, of fowl-cholera, of the anthrax bacillus and in the bacillus of diphtheria (in this case doubtless in the body of the bacillus, not excreted).

The majority of the bacterial hæmolysins are destroyed by heating to 56° C.; that of the bacillus of fowl-cholera, however, is only destroyed at 70° C.; while pyocyanolysin stands boiling for a long time and is only destroyed in half an hour at 120° C.

Along with these enzymes should be classed the lysins which attack the leucocytes (leucocidine of Vandevelde) and other bacteria (pyocyanase of Emmerich).

Products of Cultures. Microbic Excretions.—A medium in which a bacterial culture has grown contains bodies which were not present before the inoculation. These are products of the activity of the microbes in relation to their food-stuffs; they do not come exclusively from the microbe itself, but they are products of these and bear their mark. It is often difficult to draw the line between true secretory products, the diastases and toxins—and the residual substances remaining after enzymatic action, the study of which ought to be included with that of the fermentations and putrefactions—and the excreta, the catabolic products, properly speaking.

The distinctions which are currently drawn between these products depend often on the purpose we have in view. We stop a fermentation when it has reached the stage of the products which are useful, as, for example, in the manufacture of beer, wine, and cheese; if left to continue, the organic matter breaks down finally to the simplest substances, water, carbonic acid, and ammonia. In nature a ferment only ceases when it has furnished the materials for a new fermentative process.

"A bacterial product is a substance incapable of being attacked under the conditions of the experiment by the

bacterium which has produced it, but which can in its turn become a food material if the conditions are altered, if the bacterium takes on new properties, or if other microbes step in" (Duclaux). Every living thing lives on the products of others.

As the richness of a culture increases, its growth slackens: the medium becomes less and less favourable, the food material becomes exhausted and the bacterium, by no means always capable of living on its own residues, ends by being embarrassed by the substances it has produced. Acid-producing bacteria cease to grow when the acidity reaches the point where vegetation is no longer possible. Alcohol acts like an antiseptic towards the yeast that produced it and acetic acid does the same for the ferment of vinegar. The bacterium however can often fall back from the food of its real choice to a sort of famine ration: when the acetic ferment has used up all the alcohol it burns up the acetic acid. When a yeast has no longer any sugar it consumes the glycerine which it has produced at its expense.

Products of excretion exist which stop the growth of cultures by a sort of auto-intoxication. The foulest waters are those which are least easy to infect, because, according to Miquel, they contain substances of bacterial origin injurious to bacterial growth. If such foul waters are concentrated at a low temperature and the filtered result is added to pure water this latter becomes incapable of supporting life. Boiling destroys these inhibitory substances, which indicates perhaps that they are of the nature of diastases. There is said to be in fæcal matter an inhibitory substance which checks the extraordinary multiplication of bacteria in the intestine, and this also is to be regarded as a diastase; Conradi and Kurpjuweit compare its energy to that of carbolic acid; without having been able to isolate it they were able by dialysis to make it act without the bacteria themselves. They call it an "autotoxin." Others, however, question this, not having been able to find it either in tube cultures or in the human intestine, and explain the inhibition by the exhaustion of the medium, in the same way

that Pasteur explained the immunity of the body by the exhaustion of the food material which it supplies to the microbe.

Exhaustion of the food-stuffs and action of the excreta may exist together. For example, when gelatine is inoculated with 6 millions of bacillus coli per milligram of medium no growth takes place; the bacteria die and disintegrate although the medium is not exhausted.

Further, an "exhausted" medium can be regenerated by filtering it through porcelain and heating it without adding any new food material (Eijkman). In cultures of B. coli of five days at 37° C., there is of all the bacteria which can be seen and counted under the microscope, only one living in fifteen, and after a week only one in forty (Hehewerth). The antitoxin of one bacterial species can act on other species.

Heat Production.—The combustion of the food liberates a quantity of energy which is not entirely used up in the construction and support of the bacterial cells; there remains an excess of heat which raises the temperature of the medium. The yeast of beer undergoing anærobic fermentation heats up the whole mass by 3·9° C. (Eriksson). In heaps of manure or hay, the temperature may rise to 50 or 70° C., and in hay even to 96° C. Cohn found in the masses of moist cotton a micrococcus which discharges carbonic acid and raises the temperature to 67° C., when care is taken to avoid loss of heat by radiation.

But it is not very certain that the heating of hay is really due to microbes. It is only the spores which can resist temperatures bordering on 100° C., and spores exert no activity. Further, no bacteria are to be found in the places where the heating begins, and finally, hay sterilized at 120° C. can heat like normal hay.

According to Bœkhout and Ott de Vriés, the spontaneous heating of hay is a chemical phenomenon, the cause of which is still unknown.

Production of Light.—Rotting wood and the corpses

of sea-beasts frequently emit light. Butcher-meat left to itself in a cool place for two or three days half-immersed in 3 per cent. salt solution very often becomes luminous. Dead leaves fallen in the forests occasionally give out a dim, steady light. Now it is not the animal or vegetable tissue which shines; it must be microbes, moulds, or bacteria.

Luminous microbes have been discovered in the Baltic Sea, the North Sea and the Indian Ocean, and phosphorescent bacteria in the Elbe. At present about fifteen moulds and about thirty bacteria are known to be "photogenic" or "luminous."

A simple recipe may be given: take a fresh herring, sprinkle it with 3 per cent. salt solution, leave it at a temperature of about 10°, add a little sugar, glycerine, and peptone; in two days the flesh and the juice become luminous.

The production of light depends on the temperature and the food supply. Sometimes a temperature from 20 to 30° C. suits best; most often lower temperatures are more favourable. The phenomenon has been seen to occur at +45° C., and at −20° C. The luminous bacteria seem to like salt; some only require a nitrogenous medium, others require in addition carbon. But the indispensable substance is oxygen, and when this is exhausted the luminosity ceases. If a bubble of air is made to pass, by turning upside down, through a long tube in which there is a culture of a luminous bacterium, which has just become extinguished for lack of oxygen, a wave of light can be seen passing along the tube. There is no luminosity in a vacuum.

Strains have been produced by natural selection so luminous that their light can be seen in full daylight. If a flask coated on the inside with gelatine is inoculated with the *Bacterium phosphoreum* or the *Pseudomonas lucifera*, one gets a microbial lamp which with an eye a little accustomed to darkness allows one to read the time from a watch or to read moderately large print. It might be possible even, it seems, to employ such lamps in powder-magazines or mines, for they do not emit heat.

Certain fishermen employ as bait luminous fragments of dead fish, a luminous bait provided by bacteria.

With the aid of the spectroscope, Molisch distinguished in the light of *Pseudomonas lucifera* the colours green, blue and violet. R. Dubois photographed colonies of these bacteria with their own light alone. Although feeble this vegetable luminosity exerts, like sunlight, heliotropism ; the shoots of young plants such as vetches, peas and lentils turn towards it on germinating ; but it is incapable of exciting the chlorophyll function.

Substances which kill the microbe abolish its luminosity. Dubois stated that he had isolated a substance which shone on contact with the oxygen of the air, *luciferine*, but others failed to repeat this experiment. It is possible that the microbe secretes a substance which as soon as it is produced is destroyed by oxidation giving out in the process luminous rays. Many organic substances, aldehydes, ethereal oils, carbides of hydrogen, fats, and alcohols, when they combine with free oxygen in an alkaline medium, can emit light closely resembling that of the above bacteria ; perhaps the bacteria give out light thanks to the oxidation of substances in the cell, such as lecithin, cholesterin, and ethereal fats ; but there is one difficulty : the living cell does not contain free oxygen.

To prove that the luminosity of bacteria is a chemical phenomenon independent of the life of the cell which produces it, it would be necessary to repeat with these bacteria the experiments which have been made with the secretions of certain animal cells. With the photogenic substance of *Luciola italica* one can write, and the writing becomes luminous whenever it is moistened. If the luminous organs of *Lampyris noctiluca*, which have been dried and preserved *in vacuo*, are moistened with a drop of distilled water, the luminosity reappears. Paper soaked in the secretion of certain *Myriapods* can shine when moistened, even after two months. In these cases the luminosity cannot be attributed to a living cell.

Bacteria which have never been exposed to light shine quite as well as those grown in daylight. Their luminous property

is therefore not like that of the salts of strontium and barium, a case of re-emission of light formerly absorbed. It is the discharge in the form of light of energy absorbed in another form.

Production of Pigments.—Numerous bacteria exist, whose cultures possess colour, green, violet, red, blue, black, and fluorescent; these colours have nothing in common with the green colour of chlorophyll plants, for they are diffused throughout the cells, whereas chlorophyll is agglomerated in distinct masses. The bacteria which produce coloured cultures without their cells themselves containing the pigment are the more numerous; the pigment is therefore an excretory product which diffuses into the medium or collects in little masses which can be seen under the microscope at the side of the bacteria. All the coloured bacteria might be put in this category if one admits that the bacteria which contain a diffuse pigment ought to be classed as algæ.

Staphylococcus aureus and various sarcinæ produce colonies of a golden yellow: the pigment is a fatty substance (*lipochrome*) insoluble in water but soluble in alcohol, benzine, chloroform, ether and carbon bi-sulphide and capable of saponification; it turns to blue or bluish-green on the addition of sulphuric acid and to orange or red on the addition of alkalies.

Everyone has heard of the miracle of the "*bleeding host*," the sacred bread which becomes covered, more by accident than miracle, with red spots having a reddish-brown, somewhat metallic lustre. It only means that it has become invaded by one of the commonest bacteria, one which is present abundantly in air, milk and dust, especially at the end of summer and in autumn, the *B. prodigiosus*. The pigment is insoluble in water, soluble in alcohol; sulphuric acid turns it into reddish-brown, alkalies into yellow. Reducing agents decolorise it as does light, though only after some time.

There appears occasionally on the surface of milk a bluish colour, sometimes as a uniform film, sometimes in rings or marbling; this is due to the *bacillus cyanogenes*. The colour is soluble in water, insoluble in alcohol, ether, and chloroform.

Grown in pure culture in sterile milk, the colour is merely grey; to get the typical blue the collaboration of an acid-producing bacterium is necessary. In nature this is provided for by the lactic bacilli. The B. cyanogenes produces at the same time a green fluorescent pigment.

The *Bacillus pyocyaneus* (Gessard) is the colour-producing bacterium which has been most studied. It used to be thought that it was the cause of blue pus; but it confined itself really—in the days before antiseptics—to diffusing its blue pigment through the linen of the dressings. This blue colour, "*pyocyanin*," is soluble in water and chloroform, insoluble in alcohol; it becomes pink in acid solution, yellowish in alkaline, and is a base closely approaching the ptomaines.

The B. pyocyaneus produces in addition a fluorescent pigment and a green pigment not fluorescent; and, finally, old cultures take on a smoky brown tint. By heating, by inoculating on special media, and by animal passages it is possible to dissociate or to associate these different colours in the same microbe and to create different strains or even a non-pigmented variety; the green fluorescent pigment is particularly associated with phosphatic food, but the strains thus obtained depend on the medium and on the technique employed; they are not fixed, and are rather transitory varieties than true strains. The chromogenic function lending itself thus to modification it is obvious that it is not one of the essential properties of the bacterium.

With the microbe, as with higher creatures, habits are more easily changed than nature.

The majority of the chromogenic bacteria produce their pigment at moderate temperatures, 20 to 25° C.; at 37° C., the *B. prodigiosus* and the sarcina grow excellently, but produce no pigment. They prefer a slightly acid medium, but fluorescence requires the medium to be alkaline. The starches are excellent food materials, which explains why the *B. prodigiosus* grows so well on the Sacred Host. The essential nutritive material is oxygen, and with certain exceptions none of them produce pigment when shut off from air.

Action of Heat on Microbes.—Just as on ordinary thermometers the temperatures are marked for taking a bath or for keeping silk-worms, so it would be possible to mark the points at which microbes develop best. Each species has an optimum temperature; below this, it grows feebly; above it, it begins to suffer and dies; heat, indeed, is the sovereign disinfectant. Adapted as they are to the surroundings which shelter and nourish them, the bacteria are parasites, not only in regard to their food supply, but also for their heat surroundings.

Although the names of different species might thus be written on almost every degree of the thermometer, three types may be distinguished, with intermediate individuals. The majority of the bacteria of water and of soil and the phosphorescent bacteria of fish grow well at 15—20° C.

The majority of pathogenic microbes demand in cultures the same temperature as that of the body in which they lived as parasites. The tubercle bacillus of the mammals develops best at 38° C.; that of birds at 41–42°, and that of fishes at 15–20° C., *i.e.*, practically like a water-bacterium.

The third group is that of the *thermophilic* bacteria. They demand and support temperatures so high that other bacteria would rapidly be killed. They have been found in rivers, in sewage, in cheese and in the human intestine. The majority are motile and possess spores. In the hot springs of Ischia, and in the fumaroles of Naples there are bacteria which live at 60° C. Miquel found in the Seine a species living best at 67° to 70° C. In a spring at Luchon, Certes and Garrigou found a bacterium developing at 64° C., the temperature of the water. In the upper layers of the soil, Globig discovered species which grow well at 65–70°. Mlle. Tsiklinsky has studied the thermophilic bacteria of the human intestine; they are all aerobes.

It is remarkable that thermophilic species from the surface of the soil have been found in the most varied latitudes, from the tropics to the Hebrides and Norway Perhaps those of the cold countries (rare) can live shut off

from air at 35–40° C., and can thus remain alive in the intestine of animals.

The majority of non-sporulating bacteria are killed in a few minutes at a temperature in the vicinity of 60° C. Further, the nature of the medium in which they are heated must be reckoned with; they perish more quickly in acid than in alkaline fluids, and dry heat kills them much less quickly than moist. The spores, being resistant forms, are only killed at much higher temperatures: 100° C., during 2–4 minutes for the anthrax spore. In one single species, there are spores which stand the same temperature twice as long as their companions. At higher temperatures resistance is much shorter, for example for certain sporulating bacteria of the soil and of hay (in saturated steam):

100°	resistance	16 hours.
115°	,, ...	½ hour.
130°	,, ...	5 minutes.
140°	,, ...	scarcely 1 minute.

The spores of moulds, studied for the first time by Spallanzani, stand thirty minutes of dry heat at 127–132° C., but in moist surroundings they die below 100° C. The spores of *Ustilago carbo* in the presence of saturated water vapour perish at about 60° C.; dry, they stand 120° C. Spores are more resistant than the bacilli, because they contain less water, *e.g.*, 38 per cent. instead of 62 per cent. Tyndall's method—discontinuous heating, at intervals, about one hour per day for three days in succession—succeeds at a relatively low temperature because the protoplasm in taking up water becomes more vulnerable.

Heating coagulates the protoplasm, and this coagulation is the more rapid and easy the more water the protoplasm contains. Albumin dried *in vacuo* over sulphuric acid can be heated beyond 100° without losing its solubility in water (Chevreul). Since coagulation is not an instantaneous but a progressive phenomenon, instead of talking of the "temperature of coagulation" and the "lethal temperature" it would

be better to speak of the lethal zone and of the zone of coagulation.

It was from the effects of heat on the bacilli that Pasteur discovered the anthrax vaccines.

Microbes stand low temperatures very well. Long ago Cagniard de la Tour observed that yeast kept at −90° C. in a mixture of carbonic acid and ether does not lose its power of fermentation. After twenty hours at −130° C., 108 hours at −70° C., the spores of *B. subtilis* still germinate, and the spores of anthrax are still virulent. According to MacFadyen's experiments, bacteria kept for six months at the temperature of liquid air (about −190° C.) or ten hours at the temperature of liquid hydrogen (−252° C.) remain living and virulent.

Action of Light.—Light is injurious to bacteria and is thus a disinfectant.

The active rays are the chemical rays of the spectrum which act by oxidizing the protoplasm: the bacteria do not die when the sunlight strikes them in a vacuum.

The anthrax spores stand sunlight for about thirty hours in contact with air and eighty hours when shut off from air (Roux). Even *in vacuo* in pure hydrogen the bacteria do not resist indefinitely. There is therefore something else than simple oxidation taking place. The action of the air is associated with an action belonging more particularly to the light, and the oxidation affects not only the bacterium but the medium in which it is.

The bactericidal rays are *par excellence* the ultra-violet rays, as can be proved by cutting off certain parts of the spectrum by means of various sorts of screens. Glass of a thickness of 1·35 millimetres completely abolishes the action. A solution of oxalic acid of 10 per cent., which limits the spectrum up to 300$\mu\mu$, acts in the same way, whereas bacteria are destroyed through a screen of sulphocyanide of potassium of 10 per cent. which cuts down the spectrum to 265$\mu\mu$ (experiments with an electric arc); the active portion of the rays of such an arc must lie between these limits. A blue

specimen of rock salt cuts off all visible light without cutting off the active ultra-violet portion, and rays which traverse it destroy bacteria.

The action of ultra-violet rays is practically equally rapid in the presence as in the absence of oxygen. They produce a little peroxide of hydrogen in the medium of suspension, but in quantities 400 times too weak to be active; hence the action is not due to the peroxide. By putting in the path of the ultra-violet rays from a mercury lamp a plate of white glass of one millimetre thickness, all the ultra-violet spectrum is cut off beyond the rays 3027–3022; the latter only penetrate the glass very much weakened, and in this case the bactericidal action is much diminished. By far the most powerfully bactericidal rays are those which have a wave-length below 2·800 units. "Protoplasm (albumin, gelatine, and serum) absorbs the ultra-violet rays below 2·900 units: it is therefore the rays absorbed by the cells which exert the destructive action."

The ultra-violet rays have been studied with a view to the destruction of cancer cells. Exposed to the ultra-violet rays the tubercle bacillus loses its property of taking on a stain which is acid-fast. An exposure of ten minutes kills them. An exposure of one minute attenuates them, and, inoculated in guinea-pigs, they now produce a slow lingering infection; the animal lives for months, whereas the controls die within forty days at most. After an exposure of three minutes the bacilli no longer grow on potatoes. The toxin of tubercle, tuberculin, which stands heating at 134° C. for half an hour, is destroyed by five hours' exposure to ultra-violet rays. The solutions should be exposed n a layer of two or three millimetres and kept shaken. Tuberculin exposed to the rays *in vacuo* is destroyed much more slowly than tuberculin exposed in air (M. and Mme. Henri and V. Baroni).

Certain coloured and fluorescent substances such as eosin erythrosin, and bengal-rose, are injurious to bacteria; and still more so to infusoria, in presence of light, but are quite or almost harmless in the dark. These have been called *photodynamic substances*. Several have been employed in photography

to sensitise plates towards rays which alone are chemically inactive. They exert the same action on ferments and on the toxins and anti-toxins of tetanus and diphtheria. This action is entirely due to oxidation and only takes place when oxygen is dissolved in the fluids of the experiment. Thus in a solution of iodide of potassium, with eosin added and exposed to light, iodine is set free; this does not occur if the solution is freed from oxygen. It has been thought that this oxidizing action is due not to oxygen but to ozone (O^3).

Physiology of Protozoa.—It must not be thought that all the protozoa because they are unicellular are primitive creatures and rudimentary ancestors of higher animals; their cell is adapted to all the requirements of life and possesses, at least in some degree, all the properties of higher animals; it may be more independent, and richer than certain cells of vertebrates. Both by structure and by function the protozoa are complex and highly differentiated creatures.

Ehrenberg, an old scientist, who studied them very carefully, held this belief, but in a naïve and inaccurate form. Protozoa to him were animals possessing in brief all the organs of higher animals; he saw in them a digestive tube, a brain, eyes, kidneys, a heart, an ovary and vessels. But nothing of that really exists; the protozoa have simply nuclei, vacuoles which digest the food, others which expel the waste products and a protoplasm full of varied movements and currents. But although they do not possess the miniature organs which roused Ehrenberg's admiration, they are none the less capable of taking up food, digesting it, and expelling the waste, of moving, and of reproducing. They possess all the functions of animal life, but more simply and more purely than among the higher animals; what one might call the chemical and physical model of life is in them more visible, more exposed to the eye. That is why their study is so attractive and so fertile; it is to it we owe our best knowledge and our best ideas on life in general, so there is no necessity for the surprise expressed by those who have only read their family "Buffon" (Buffon's Natural History), that scientists should be passionately

studying infusoria and amœbæ rather than sharks and elephants.

Life means always the combustion of protoplasm, according to Lavoisier's dictum, and this protoplasm replaces its losses by assimilating food. Three methods of assimilation we know: the chlorophyllous plants with the help of sunlight decompose the carbonic acid of the air, manufacture hydrocarbons, and finally turn the starch into more complex substances which go to build up their protoplasm; the green plant thus starts with non-organised substances for all its food-supply; animals feed on plants or on other animals which have already fed on plants; parasites absorb food which has already been prepared ready-made for them by their hosts. All three methods exist in the protozoa.

There are some which, possessing chlorophyll, manufacture their food exactly like green plants: these are the plant-animals which form the link between the two kingdoms. They prepare by synthesis a starch or a para-starch (Bütschli) and can satisfy their life conditions in water containing mineral salts, provided they receive the light of the sun. Certain species lose their chromatophore granules when ready-made food is supplied to them, permitting them thus to dispense with the labour of "photo-synthesis." Certain flagellates live in symbiosis with green algæ which supply them with starch: in such a case the protozoon may be said to be attacked by a useful infection: further, this infection may be conveyed from one to another.

The other protozoa capture their prey, frequently in the form of living creatures. Whether they have a mouth or not, whether the food penetrates their bodies with the help of a current produced in the water by cilia or flagella, or whether it is the protoplasm of the protozoon itself which issues from its envelope, introduces itself into the body of the prey, and thus devours it from the inside, as it were reversing the rôles, in all cases the important point in the nutrition is *intracellular digestion*: the food is enclosed in a little spherule inside the protoplasm where little by little it is

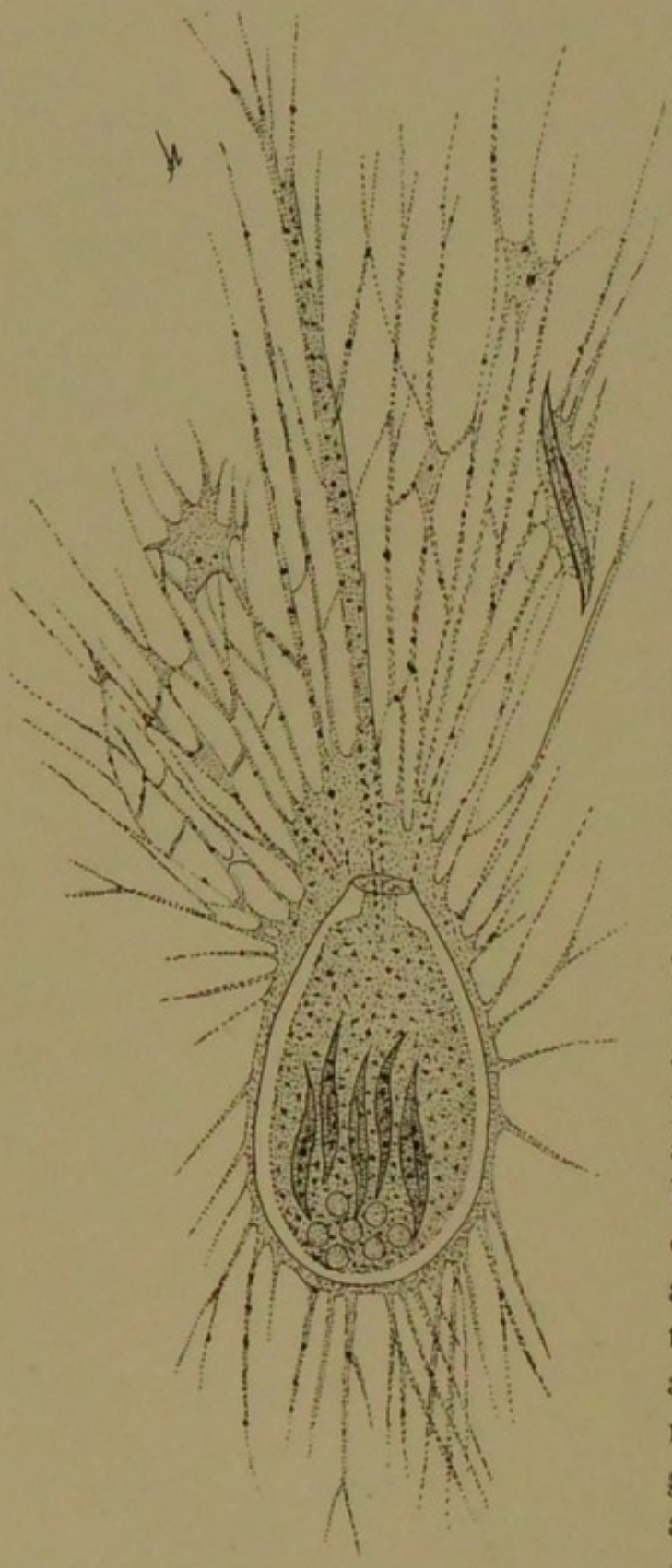


FIG. 37. — *Gromia oviformis* in the act of capturing in its pseudopodia a diatom, which being too large for ingestion is digested outside the body in this way. (After Max Schultze)

dissolved by means of the digestive juices. A leucocyte which seizes and digests a bacterium in a higher animal acts in precisely the same way. The amœba is the prototype of that phagocytic digestion which occupies so large a place in both natural and acquired immunity.

Of what nature is this digestion from the point of view of chemistry? In the digestive vacuoles the reaction is acid and from certain myxomycetes (*Fuligo varians*) and rhizopods (*Pelomyxa palustris*) a ferment resembling pepsine and acting in an acid medium has been isolated. On the other hand, Mouton and Mesnil have extracted from amœbæ and paramecia a ferment which digests gelatine and fibrin in an alkaline medium just like trypsin.

According to other investigators the digestive medium is first

acid, then alkaline, just as in the stomach and small intestine of mammals.

The solid refuse of digestion is evacuated by an anus and the liquid residue collects inside protoplasm in a little spherical sac which from time to time expels its contents externally: this latter is the contractile vesicle which Ehrenberg took for the pulsating heart.

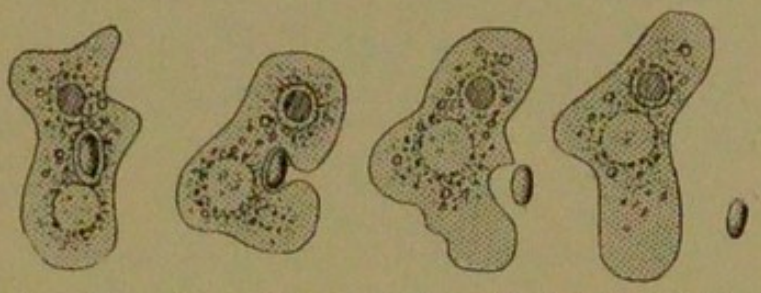


FIG. 38.—An amœba expelling the residue of its food: various stages. (After Verworn.)

Oxygen is a primary necessity to protozoa as to bacteria: the digestive vacuoles contain oxygen, the contractile vesicles discharge carbonic acid, *i.e.*, aerobic respiration. The protozoa which live in surroundings deprived of free oxygen have, it is certain, a method of respiration analogous to that of anaerobic bacteria; they draw their oxygen from reserve materials which they have stored within themselves, *e.g.*, glycogen. It is believed, without being absolutely certain, that certain infusoria can, like the intestinal worms (ascarides), break up glycogen with the formation of valerianic and carbonic acids. Among the products of excretion have been found uric acid and phosphate of lime (Schewiakoff). Excretion is a fairly active process since the vacuole contracts often (every four to eighteen seconds according to temperature in *Stylonychia pustulata*). According to Maupas the infusoria discharge during a space of time which varies from two to forty-six minutes a volume of liquid equivalent to the whole volume of the animal. Stimulation from the exterior is always accompanied in the protozoa by a manifestation of energy: they possess irritability. The excitant may be a touch, a ray of light, heat, electricity or a chemical substance, and the protozoon in its reactions to the stimulus acquires habits such that its physiology is full of as many problems as that of the higher animals, not excepting problems in psychology. Although possessing neither nervous

system nor sense organs, even to such a degree as the sea-anemone, the protozoon is capable of choice and of determination, these phenomena of course remaining more or less mechanical. Superposition and propagation of impressions exist among protozoa, and after long and minute observations it has been maintained that no essential difference exists between them and the most complicated metazoa: activity is neither more nor less mechanical in the one set than in the other.

The chief business of all living creatures is reproduction. Among protozoa there exist several principal methods for this, each presenting numerous variations. They may divide by nuclear division: they may divide by budding, and in this the greatest diversity occurs in the number, size, and arrangement of the buds. They may sporulate, *i.e.*, their protoplasm may break up, and each fragment consisting of a bit of protoplasm and a bit of nuclear material can reproduce a creature similar to the mother-cell which sporulated. When life-conditions are difficult, certain protozoa encyst, *i.e.*, they contract inside a resistant shell, and under shelter of this various modes of reproduction may take place. Between reproduction by division and that by sporulation intermediate forms exist (*Tillina* and *Colpidium*), and the continuity in nature can always be detected by the imagination.

Parasitic protozoa, those which to subsist require to emigrate from one host to another, most often reproduce themselves by sporulation, and the reproduction is the more lavish the greater the difficulties encountered by the species in propagation.

Parasitism tends to modify the species in a retrograde direction but the losses may be compensated for by new acquisitions. Locomotory organs, protective envelopes and the apparatus designed for capturing and digesting food become simplified or disappear altogether. But parasites are in general more prolific and they acquire other organs, hooks or suckers, by which they can better cling to their host. As their life conditions or their habits become narrowed down,

they present those phenomena of strict adaptation which are equivalent as between the soil or the host and the parasite—to

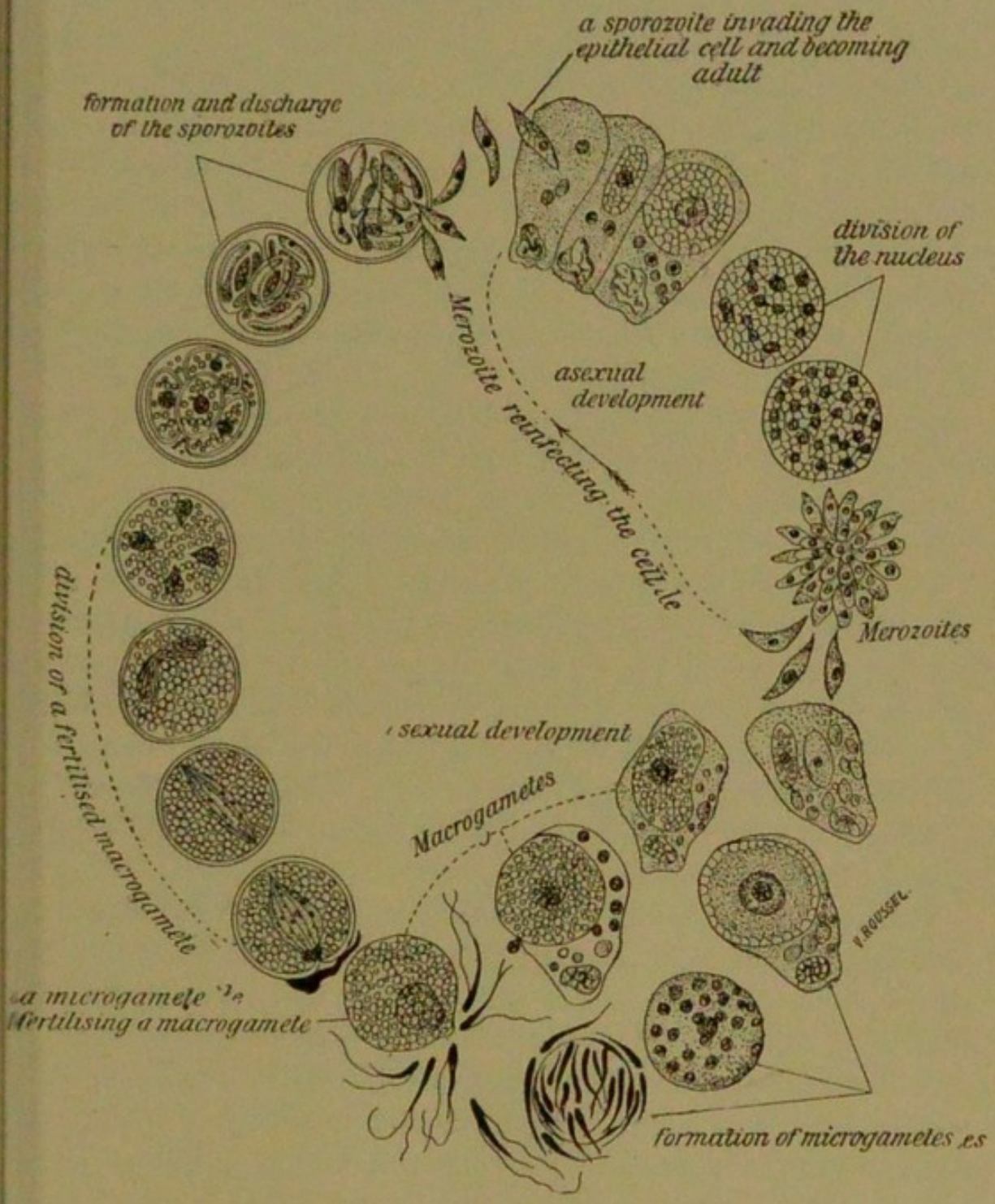


FIG. 39.—Life cycle of a Coccidium (*Coccidium Schubergi*). (After Schaudinn.)

true specificities: they cannot endure any other habitat, food, or host.

For example, *Costia necatrix* which lives attached to the

surface of the bodies of fishes can no longer live even in the same water when it becomes detached and floats free. The infusoria from the paunch of ruminants or the caecum of horses cannot live outside the bodies of these animals except at body temperature, 37°C.: they are thus examples of semi-parasitism. The parasites of mammalian blood are habituated or even confined to life at definite temperatures; hence the effects of climate and season.

The same parasites attach themselves to one host only and their presence becomes a specific character of the latter. The parasite of malaria is peculiar to man and among the mosquitoes it can only inhabit those of the genus *Anopheles*. But one trypanosome can live in several species of host, one species often serving as a sort of reservoir for others (it is probable, for example, that cattle form a reservoir for the *Trypanosoma gambiense*, which causes sleeping sickness in man).

Lamblia intestinalis is a parasite of the small intestine: gregarines are only found in the large intestine, in the peritoneum, and in the genital organs of their hosts (invertebrates): coccidia inhabit the epithelial cells: the hæmosporidia of malaria only the red corpuscles of the blood: while the sarcosporidia only occupy the muscle cells. But parasites exist which infect all the organs of the host, *e.g.*, *Myxobolus pfeifferi* in the barbel disease.

Chemiotactic phenomena, positive or negative, are observed among protozoa as among bacteria, and it is by an action of this sort or by a choice of soil (which closely resembles it) that the affinity of the sporozoites of the malarial hæmosporidia is explained for the salivary glands of the mosquito which inoculates man. The hæmosporidia sucked from the blood of the patient gain the stomach of the mosquito and there enter upon the sexual cycle, a cycle which cannot go on in any other surroundings: this specific action is no doubt due to certain physical and chemical conditions which are only realised there and of which the following fact may give some idea: the appearance of sexual forms in human blood is favoured by adding a little distilled water to the blood of

a preparation (Manson). The ideas of specificity "of soil" and of virulence must resolve themselves among the protozoa as among the bacteria into physical and chemical factors.

The parasitic protozoa of the intestine of one host pass into another host through the external world in the state of spores or cysts. The parasites of the blood cannot enter the blood of a new individual (in nature) except through the intermediation of a blood-sucking insect, and a portion of their life-cycle takes place in this intermediary. Thus the parasite of Laveran is inoculated from man to man by the *Anopheles* mosquito. In these cases the principal host is the one in which the sexual phase of the life-cycle takes place; the host in which occurs the non-sexual reproduction is only the secondary or "intermediate host." With regard to the parasite of malaria man is the intermediate or secondary host, and the principal host is the mosquito. The tsetse fly is the principal host of the *Trypanosoma gambiense* of sleeping sickness.

Though the protozoa are frequently parasites, they are often themselves attacked by parasites, by bacteria, by chytridiaceæ, by saprolegnaceæ, and by algæ. The algæ, however, may be useful commensals, furnishing a food-stuff—starch; but other parasites may kill the protozoa which they infect, provided the latter does not defend itself and overcome its parasite by devouring and digesting it—again by intracellular digestion.

To study the bacteria pure cultures can be made in which they find their food material in solution. The protozoa have, doubtless, methods of nutrition much more complicated, for their culture is more difficult. Several trypanosomes, parasitic in the blood, or, more precisely, in the blood plasma, have, been grown in pure culture by Novy and MacNeal on media to which blood was added. The parasites of malaria (parasites of the red corpuscles) have not been cultivated. The amœbæ can be grown on condition that suitable prey is supplied: if in a culture there are only amœbæ and as prey a bacterium

for example the B. coli in pure culture, the culture is said to be "pure-mixed." The prey may consist of a dead bacterium.

In the absence of cultures experiment is difficult; the study of protozoa is still necessarily attached to the study of their forms, and physiological study takes of necessity the second place to morphological. The method *par excellence* consists in following the life-cycle of protozoa in their natural surroundings or in their hosts. The study of bacteria has been capable of greater advances, thanks to pure cultures in well-defined media which permit of chemical analysis. The application of similar methods to protozoa is infinitely desirable, not only for the sake of medicine, but also to extend our knowledge of the phenomena of life in general.

CHAPTER V

PATHOGENIC MICROBES—INFECTION

ORIGIN.—*Specificity*—*Virulence.*—How virulence may have been acquired—Evolution of microbes—The 'para' and the 'pseudo' forms—Diminution and augmentation of virulence—Pasteur and attenuation of the virus.

INFECTION.—The conflict between the microbe and the body—Methods—of transmission—Latent microbism—Germ-carriers—The number of microbes sufficient to produce infection—Microbial associations—Paths of penetration and inoculation—The rôle of the intestine—Seats of election and susceptible cells—Incubation.

ORIGIN : SPECIFICITY : VIRULENCE.

THE idea of pathogenic microbes arose as a result of Pasteur's labours on fermentation.

For years the bacteridium had been seen in the blood of anthrax animals without giving rise to the thought that these microscopical rods were the cause of the illness. After the discovery of the bacillus of butyric fermentation, Davaine considered that the bacteridia were the cause of anthrax as a sort of peculiar fermentation having for its subject the body of an animal.

We do not yet know the pathogenic microbes though they certainly exist in small-pox, in vaccinia, in measles, in scarlatina, in mumps, and in hydrophobia. That of syphilis remained unknown up to 1905. Pasteur was the first to handle invisible microbes with sufficient confidence to discover a method of vaccination. His discoveries in hydrophobia aroused researches on the so-called invisible microbes. A nervous

disease which resembles hydrophobia, the acute poliomyelitis of children, has recently been studied by the same experimental method.

The microbial doctrine has still opponents, more or less masked, who accuse microbiologists of being able to see nothing but the microbe and of imagining that this is the whole malady. The body takes some part, there is no doubt; the malady is a sort of fermentation, but one taking place in a medium capable of resisting the ferment. Pasteur recreated medicine by introducing into it the spirit and method of the exact sciences, but he knew as well as any that diseases do not rage in an inert material. This, however, does not prevent the various incidents of the disease from being at bottom physico-chemical phenomena.

The Origin of Pathogenic Microbes.—The pathogenic microbes are not instruments of a perfidious Providence, and created to chastise man, animals, and plants. The pathogenic species, are species the result of selection and adaptation. They grew first of all as saprophytes on individuals who suffered no damage, as is the case to-day with many bacteria growing on animal bodies. They multiplied upon ill-nourished and fatigued individuals and found on a definite animal species nutritive materials and a chemical "soil" which suited them. Certain bacteria have become strict parasites, incapable of living even temporarily in the external world, *e.g.*, the bacillus of leprosy. These views of Pasteur are quite in conformity with the spirit of Darwinism.

From the beginning there has been happening what occurs every day, *i.e.*, there has been a struggle between the parasite and the body. Not only does the body defend itself against the bacteria, but the bacteria defend themselves against the body. Each is capable of gathering strength or immunising itself against the other, and these are simply different aspects of adaptation and of natural selection. "The science of bacteria, as with all the branches of biology, has profited by the theory of evolution and, making a just return, it has supplied the Darwinian theory with a striking confirmation.

The great discovery of Pasteur of the attenuation of viruses proves the plasticity of microbial species and the facility with which they modify their primitive characters. The history of bacterial diseases shows also the great rôle which these infinitesimal creatures have played in natural selection, for is it not they which have caused to disappear in the course of ages certain vegetable and animal species insufficiently armed to resist them? . . . Experimental medicine has studied the adaptation of certain pathogenic microbes which permit them to attack the body in spite of the defences opposed to them. Here it is probably a question of a selection of individuals endowed with particularly stable characters." [1]

Starting with a bacterium almost non-virulent, Pasteur succeeded in infecting with anthrax in succession, by the method of passages, the new-born mouse, the adult mouse, the young guinea-pig, the adult guinea-pig, the rabbit, and the sheep. Vincent by introducing into the peritoneal cavity little collodion sacs containing bacteria which are nourished by the body fluids, while being protected from the cellular defences, rendered pathogenic for the guinea-pig and the rabbit such saprophytic microbes as *B. megatherium* and *B. mesentericus vulgatus*, but the virulence thus acquired disappeared as soon as the artifice which produced it was suspended. These experiments do not permit of the conclusion that pathogenic species can be created at will in the laboratory; only more or less stable variations are got. It is not with such ease that we are likely to reproduce what Nature has taken centuries to accomplish. It is very probable that small-pox and vaccinia are two modifications of the same virus. Yet the production of vaccinia with small-pox has not yet been successfully performed. The experiments said to have proved variolo-vaccination are still disputed. Nothing has been able to produce from B. coli a Typhoid bacillus. There exist various families of the Tubercle bacillus, which may be secular adaptations from the same strain, adaptations to the human species

[1] Metchnikoff, address read to the Cambridge festival in honour of the Darwin centenary, June, 1909.

and to the ox, to birds, reptiles, frogs, and fishes, but no one has ever succeeded in producing a tubercle bacillus of the human type from the tubercle bacilli (or acid fast bacilli) of the frog. Nocard's experiments in which he transformed the human bacillus into the bird bacillus by repeated passages have not been confirmed. The researches of recent years confirm the idea of Th. Smith and R. Koch, that the human bacillus and the bovine bacillus cannot be transformed one into the other, at least under the time conditions of our experiments; the fixity of these acquired characters has even raised the hope that it might be possible to vaccinate cattle against bovine tuberculosis by means of bacilli of the human type and *vice-versâ*, perhaps, men with the bovine bacillus or products derived from it.

The Darwinian conception of evolution in pathogenic microbes is nevertheless true, although direct proofs are lacking. Similarly the simian origin of man would be quite as certain, although some of the proofs were lacking, and even although we might not be able to demonstrate forms intermediate between man and monkey.

Specificity.—It is necessary to distinguish between the specificity of the microbe and that of the disease.

Typhoid fever is caused by the bacillus of Eberth and by it alone—specificity of the disease. The typhoid bacillus remains the typhoid bacillus in cultures and in the intestine; it does not tend towards either the dysentery bacillus or the coli bacillus—specificity of the microbe. These two ideas hang together; the same causes must produce the same effects if there is to be any science at all, but to produce the same effect it is absolutely necessary that the cause remain the same.

A disease appears with certain symptoms and anatomical lesions due to a definite microbe, but these symptoms and these lesions may be produced by others; for example, the tubercle is the lesion *par excellence* produced by the tubercle bacillus of Koch, but tubercles are also produced by the bacillus of glanders. There are not so many possibilities of reaction in the body as there are bacteria; fever, effusions,

congestion, false membranes, tubercles, are all properties of the body rather than of the microbes. The specificity of the disease consists in a definite combination of symptoms along with the invariable presence of a definite bacterium. To render possible the study of disease it was a primary necessity for a certain disease to be the result of a certain micro-organism, and further for this latter to be of fairly stable natural characters. The typhoid bacillus causes typhoid fever, but if it were capable of changing its characters the hygiene and prophylaxis of this disease would be without any sure foundation.

Microbiology, medicine, and hygiene therefore can no more do without this idea of specificity than science in general could exist without the idea of causality.

Medicine has always been pursuing this conception, but has only finally seized it by the help of microbiology and chemistry. Even the beliefs in "miasmata" and "epidemic causes" were already attempts in this direction. Common sense has always been a believer in the specificity of transmissible diseases. It was for that reason, as we read in the Old Testament, that the Israelites isolated lepers. Herodotus knew that leprosy passes from man to man; Galen believed in the specificity of hydrophobia, of scabies, of granular conjunctivitis. The idea of a specific disease was bound to suggest the idea of a specific agent.

The fact that those who have had small-pox scarcely ever take it a second time, but are still quite virgin soil for measles and scarlatina and *vice versâ*, spoke again in favour of the specificity of diseases. Jenner's discovery even furnished a general principle of diagnosis between specific viruses.

In favus, in pityriasis versicolor, in the ring-worms, and in thrush, there had already been observed before the days of Pasteur the constant presence of the same microscopical fungi, and it had even been concluded that these diseases were due to parasites. But the opponents of this idea maintained (there are perhaps still existing some who believe this) that these organisms were not the cause, but in a way constant

"*witnesses*" of the diseases, and simply the inhabitants of lesions which they had not produced—just as the same moulds are usually found in pots of the same jam left open to the air.

The specificity of infectious diseases has been demonstrated by Pasteur and Koch.

Since there is no "spontaneous generation," at least in the world of the present day, it is impossible for the micro-organisms to originate in the diseased tissues, and the science of fermentation has proved that a given cell, inoculated in a sterile fluid of known composition, produces in it definite and constant phenomena. These ideas were taken up by medicine when Davaine maintained that the bacteridium was the cause of anthrax, and when Obermeier held that recurrent fever was caused by the spirochæte found in the blood of the patients during the fever.

To prove the specific activity of a ferment it is necessary to isolate and make pure cultures of it and to re-inoculate it. It was this that we learnt from Pasteur and Koch. Koch's memorandum on tuberculosis remains the complete model for the discovery of *the* microbe of a disease and of the demonstration of its specificity.

Specificity of function is the point of capital importance. The fixity of form in bacteria is of great use in the search for and identification of them; but on this latter point science has been obliged to become less exacting; the form of the bacteria is not always a sufficient distinguishing character. They are really defined by their chemical and physiological actions. Thus the tubercle bacillus is better defined by its staining peculiarities (a physico-chemical reaction) than by its shape; still better by the appearance of pure cultures than by staining; and better still than by the cultures, by its excretion of tuberculin. Finally, the study of the reactions of the body, *i.e.*, of immunity, has shown that the cells of the patient respond in a specific manner to the attack of the bacterial cells, and both medicine and hygiene daily employ the property of specificity in the *antibodies*.

The "Para" and "Pseudo" Bacteria.—We may now

go on to cite a series of facts which have modified the idea of rigorous specificity among microbes, a specificity which in the early days of medical bacteriology was believed to be absolute; or at least these facts have restricted this idea (by compelling us to create new varieties), although we may still regard it as sufficient for the purposes of medicine and hygiene.

It was long the custom to talk simply of *the* bacillus of diphtheria, *the* typhoid bacillus, *the* B. of dysentery, *the* meningococcus, *the* cholera vibrio, etc., but little by little there have been discovered bacteria, close relatives of each of these typical microbes, but not possessing all their characters.

From the time of the first bacteriological discoveries in diphtheria, bacilli were isolated from the mouth exactly similar to the pathogenic bacillus, but non-toxic; the best known is that described under the name of Hoffmann's bacillus. They have been called *pseudo-diphtheria* bacilli, and have been found in diphtheritic sore throat, in scarlatinous sore throat, and in the normal conjunctiva, even sometimes in vaccine lymphs. Some of them are pathogenic for the guinea pig, and produce in it a septicæmia bearing no resemblance to diphtheria. They are not affected by antidiphtheritic serum and are incapable themselves of being used to produce such a serum. Roux's opinion was that it was a question of degenerated diphtheria bacilli, or of bacilli not yet adapted, not having yet found the conditions capable of exalting their virulence. It must be added that the name of pseudo-diphtheria has been incorrectly applied to bacilli which do not deserve the name, even by their form.

It is no use playing with words. It is on the clinical facts the problem ought to depend. There are found in the most typical cases of diphtheritic sore throat diphtheria bacilli possessing every degree of toxicity, and also bacilli which are not pathogenic (*i.e.*, for the guinea-pig, since they cannot be inoculated in man). All these bacilli are called diphtheria bacilli. On the other hand, there exist non-diphtheritic affections both of the throat and of the nose in healthy individuals where bacilli resembling diphtheria but non-toxic

are to be found; the name of pseudo-diphtheria has been agreed upon for them. Are they capable of becoming toxic? Under the conditions in which we can experiment it is scarcely possible to give an answer. There is a possibility, and even a probability. But once the natural selection has occurred, it is undoubtedly the toxic bacilli which get all the chances of passing from mouth to mouth and of maintaining their hereditary privileges.

The specificity of the meningococcus has had to be defended against a group of bacteria resembling it in form and cultural characters—the *pseudo-meningococci.* The true meningococcus may be distinguished from these by various biological reactions, but it is certain that the principal character from our point of view is the property of causing meningitis, and it is difficult to say whether or not, and under what conditions, the "pseudo" forms may acquire this power.

Not only have there been distinguished three pathogenic types of the dysentery bacillus (Shiga, Flexner, and Strong), but a whole group of pseudo-dysentery bacilli has been admitted, and side by side with the true bacillary dysentery there have been decribed dysenteriform affections caused by these "pseudo-bacilli." In reality the biological reactions have proved that there is only one fundamental type of pathogenic dysentery bacillus, but that there exist none the less satellites of this which possess biological importance, although of less importance from the point of view of medicine.

Hygiene cannot afford to neglect the non-pathogenic cholera vibrios, often very difficult to distinguish from the vibrios isolated from genuine fatal cases of cholera. Since the prophylaxis is based on the discovery of the microbe, and since no laboratory animal exists which readily takes cholera, it has been necessary to employ refined diagnostic procedures, and these do not solve the scientific question of the relations in nature between these different vibrios. In certain maladies closely resembling typhoid fever (but almost always benign, rarely fatal), bacilli have been found which only differ from the typhoid bacillus in certain cultural peculiarities, differing also

from the B. coli which is so abundant in the normal intestine. Two principal types can be distinguished, A and B : the latter is nearer to the B. coli, the former to the B. typhosus. They are called para-typhoid or para-coli bacilli, but do not on that account call in question the specificity of the Typhoid bacillus ; the latter rather appears as a chosen specimen out of a numerous family containing other well-defined pathogenic bacteria, among which are not only these para-A and B, but various bacilli producing meat-poisonings, diarrhœa among animals, and the pneumo-enteritis of pigs. This family is even the one in which the biological reactions have been found most suitable for establishing fairly definite degrees of relationship. We class them by their relations to the human species, putting at the head of the column the typhoid bacillus. It is evident that if we were calves or pigs our point of view might be somewhat modified.

One of the properties characterising the tubercle bacillus of Koch is its acid-fastness (it takes on stains with difficulty, but once stained by the suitable colour, it resists the decolorising action of acids). There exist numerous acid-fast bacilli and even numerous bacilli capable of producing tubercles in the tissues. Between the human and bovine bacilli and the bacilli found in grass, in manure, and even in smegma, there is a long series of intermediate types. Are we to believe that among the acid-fast and para-tubercle bacilli the ancestor of the tubercle bacillus of men and the ox is to be found? It is rather a philosophical question and escapes experimental examination. But the general truth of the Darwinian ideas compels us to this belief. According as one is physician or veterinary surgeon, according as one is engaged in diagnosis or in treatment, according as one is accustomed to think as a naturalist and to class all living beings in groups, so one tends to insist on differences on the one hand, on resemblances on the other. The production of tuberculin and the re-inoculation of tuberculous lesions in series in a given species are methods of differentiation which do not invalidate the existence of a great natural family.

Small-pox and vaccination are without doubt of common origin; Jennerian vaccination is based as much on their relationships as on their differences. In the same way the distinction between the human bacillus and the bovine bacillus is not inconsistent with a common origin. The numerous experiments which have been made to vaccinate cattle with the human bacillus prove that it is a question of two adaptations from the same type, and unfortunately they are not sufficiently differentiated for one to be sure that the bovine bacillus is incapable of producing in man a tuberculosis, not merely local and benign, but general and fatal.

The specificity of bacteria is only relative, but it suffices for the carefully performed bacteriological diagnosis which is so useful in medicine. Instead of the solitary types which once seemed to constitute the whole species, we know now varieties and families of which one member only may be the constant cause of a definite disease; and this is of advantage to both medicine and hygiene. In theory it is evident that the only point of view to be accepted is that of the plasticity of microbial species, *i.e.*, the Darwinian theory.

Virulence.—Virulence is in the first place the capacity in a microbe to settle and develop in the bodies of animals; secondly, its capacity of secreting its toxic substances. Even in the strictly toxic diseases, such as tetanus, the intoxication is not the whole malady; there is a preliminary, the penetration of the microbe, which may or may not find suitable conditions for its growth.

Virulence is a variable property, and it was in connection with modifications in virulence that Pasteur had the intuition of the possibility of attenuating a virus.

Diminution of Virulence.—In cultures on artificial media in the laboratory the virulence diminishes spontaneously; the media may be improved by adding animal fluids (serum, blood, ascitic fluid). A little too much or too little acidity or alkalinity, too much or too little peptone or salt, may cause our nutrient broth to lower the virulence of the strain from the change in its reaction.

The diphtheria bacillus and the streptococcus are not suited

by acids; the cholera vibrio finally suffers from the alkalinity which it itself produces; the presence of fatty materials is injurious to anthrax bacillus. There exist processes for diminishing virulence: 1. The action of a high temperature (Toussaint, Pasteur), *e.g.*, a temperature of 41° to 43°C., instead of the body temperature of 36–37°C. 2. Temperature + aeration (Pasteur's experiments on fowl-cholera and swine-erysipelas). 3. Desiccation (Pasteur: preparation of the spinal-cord of rabbits in the treatment of hydrophobia). 4. Light, pressure, oxygen under pressure. 5. Antiseptics (Roux: carbolic acid, potassium bichromate, etc.).

Increase of Virulence—Passages.—For this purpose one provides a microbe with the food-conditions which suit it best; oxygen of the air for the B. diphtheria; extracts of putrefying meat for the B. of tetanus (Brieger and Cohn). The virulence is augmented by accustoming the bacteria to the body against which they are being prepared; the feebler individuals are destroyed by the natural defences, and a selection of the strongest members takes place.

A bacterium may be habituated to the guinea-pig by compelling it to live in the peritoneum of this animal enclosed in a collodion sac, which permits the penetration of the nutrient juices while keeping off the leucocytes. It is a culture in the living body. Habituation is chiefly produced by the method of passage, *i.e.*, by inoculating the bacterium in an animal and from this animal into another (generally of the same species). In certain cases a degree of virulence is reached which cannot be exceeded in the species of animal employed; thus the virus of hydrophobia becomes *virus fixe* after a certain number of passages through the rabbit. Passages do not perceptibly affect the tubercle bacillus, but have a pronounced influence on the streptococcus. According to Marmorek the streptococcus, which required at first a dose of 1 c.c. to kill, could be brought by passage to kill with a dose of 0·000000000001 c.c. Pasteur raised the virulence of the anthrax bacillus by passing it through new-born animals, then through older ones, then adults, and finally through different species.

Passage does not give the same results in all cases, and

qualitative variations occur which render it necessary to take into account the species of animal employed. Pasteur, after exalting the virulence of the virus of rabies for the dog by passage through rabbits, found that passage through monkeys weakened it. The bacillus of swine-erysipelas becomes more virulent (for the pig) after passage through pigeons, less virulent by passage through rabbits. Passage through a foreign species has been used to prepare vaccines; thus the virus of vaccine lymph—undoubtedly of the same origin as that of smallpox—has become a "vaccine" by passage through the cow; the pox of pigeons becomes a "vaccine" for pigeons by passage through the fowl. In passing from fowl to fowl the spirillum of Marchoux and Salimbeni gets weaker. And while it is true that a feebly virulent anthrax strain can "recover" its virulence by passages beginning on new-born mice, in the spirillosis of fowls, on the contrary, according to Marchoux, it is precisely the young of the species concerned which is the animal of choice for weakening the virus and preparing an efficient vaccine for the adult.

Further, the age of the "young" individual must be reckoned precisely: it is known that the new-born child is less susceptible to Jennerian vaccination than the child of three or four months.

The modification of virulence may be expressed by a difference in dose in relation to a "soil" agreed upon. The example of Marmorek's streptococcus gives a numerical measure of the increase in virulence. The spirochæte of Schaudinn was inoculated from man into anthropoid apes, from these into the lower monkeys, thence into the rabbit, and thence into the guinea-pig; but this has chiefly been a case of progress in the technique of inoculation, for nowadays it is possible to inoculate directly from man into the rabbit.

Attenuation of Viruses.—There was in Pasteur's laboratory a culture of the bacterium of fowl cholera which was being reinoculated daily and was of constant virulence. It happened that a culture was taken for inoculation which had remained untouched for several weeks in the incubator;

the fowls became ill but did not die, and further, they resisted a second inoculation of a very virulent strain which killed the controls. This was the first demonstration of an attenuation produced by keeping in contact with air at incubator temperature. By taking cultures of different ages, a scale of virulence could be produced—a series of "vaccines."

If on re-inoculating an enfeebled culture, one obtained a virulent culture, it would not be correct to speak of attenuation, but merely of enfeeblement—transitory lowering of the virulence.

Attenuation is only applied to a permanent enfeeblement, one which passes from one generation to the next. One cannot say hereditary because, strictly speaking, heredity only occurs when there is sexual reproduction. In the experiments on fowl cholera the new cultures showed themselves to be weakened in series; it was thus a true attenuation.

"If you take each one of the cultures whose virulence has been attenuated as the starting point of successive cultures and without a perceptible interval in the starting of the cultures, the whole series will reproduce the attenuated virulence of the one serving as starting point. Similarly, a culture of zero virulence reproduces another of the same" (Pasteur).

A similar process of attenuation appeared at first inapplicable to the anthrax bacillus; as the culture grows older the bacterium sporulates and the spores are not affected by the conditions which act upon the bacillary form. One could only therefore expect attenuation from an anthrax bacillus which did not produce spores.

Now at 42°·5 the anthrax bacillus does not sporulate. Pasteur cultivated it therefore at 42°·5 in order to diminish its virulence by the action of the heat and the air.

It was then found that sporulation, instead of being an obstacle to attenuation, was a condition entirely favourable; reinoculated at 35°, a bacillus attenuated at 42°·5 produced sporulating bacilli, but the bacilli germinating from these spores possessed the same degree of virulence as the bacilli

from which the spore was derived. Pasteur had obtained an attenuation fixed by the resistant form, the spore; "vaccine viruses fixed in their germs, with all their peculiar qualities without any possible alteration."

It was by these modifications of virulence that Pasteur explained the behaviour of the great epidemic diseases:

"There exist virulent diseases which appear *spontaneously* in every country: such is, for example, the typhus fever of armies in the field. Without doubt the germs of the microbes responsible for these maladies are to be found everywhere. Man may carry them on his body or in his alimentary canal without suffering great harm, but they are, nevertheless, ready to become dangerous when, under conditions of overcrowding and successive development on the surface of wounds, in weakened bodies or otherwise, their virulence becomes progressively reinforced.

"Virulence thus appears under a new light to us and one which is distinctly disquieting for mankind, unless nature during the past centuries has already met with all the opportunities possible of developing virulent or contagious diseases, which is highly improbable.

"A microscopical organism, harmless for man or for a given animal species, is simply a creature which cannot develop in our bodies or in the body of the given animal, but there is nothing to prove that if this microscopical creature succeeded in penetrating another of the many thousand species in creation, it might not invade it and produce in it disease. Its virulence, reinforced then by successive passages through individuals of this species, might become powerful enough to attack such and such an animal of higher position, man, or the domestic animals. In this way it is possible to create new virulences and new contagions. I am much inclined to think it is thus that there have appeared throughout the ages small-pox, syphilis, plague, yellow fever, etc. . . . and further that it is by phenomena of this kind that there from time to time appear certain great epidemics. . . ."

Infection.

Infection may be defined thus: the attack on one living being by another which penetrates it and lives parasitically at its expense.

It is simply one case of the universal struggle and competition among the species.

The conflict between the invader and the invaded resolves itself into a question of nourishment and digestion. "The parasite attacks by secreting toxic or dissolving substances, and defends itself by paralysing the digestive and expulsive powers of its host. This latter exerts a noxious effect on its aggressor by digesting it or eliminating it from its body, and it too defends itself by means of secretions."[1]

Infection exists among the amœbæ. An amœba invaded by the parasites described by Metchnikoff under the name of *Microsphæra* finally succumbs. Certain infusoria are infected by Acinetians, which pierce their cuticle and invade them. The green euglena is subject to infection by lower fungi of the chytridian group; they lose their green chromatophores and become literally anæmic. Infectious diseases are not the peculiar privilege of man and the higher vertebrates.

On the great problem of the origin of microbes, their mode of transmission, and the way in which they penetrate the body, bacteriology and hygienic science have accumulated many facts.

The microbes inhabit the air, water, the soil, animals, and plants, and gain access to the patient either directly by simple contact with another patient or thanks to more or less numerous and various intermediate agents. Contact is sufficient for the transmission of measles, small-pox, and scarlatina; these are the contagious diseases properly speaking. The air may carry the germ from one individual to another. In the air the B. tuberculosis may float, attached to particles of dried dust or to moist droplets, projected into the air by the patient

[1] Metchnikoff, *Pathologie comparée de l'Inflammation*.

during speech or coughing (Flügge). Water-supplies convey the cholera vibrio and the typhoid bacillus. Along with garden

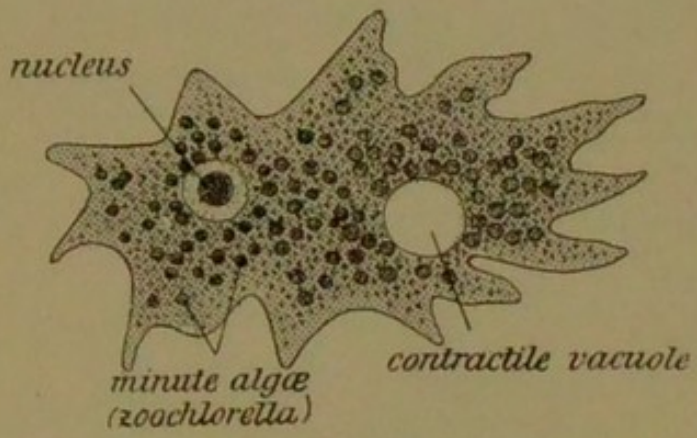


Fig. 40.—Amœba (*Amœba viriais*). (After Gruber.)

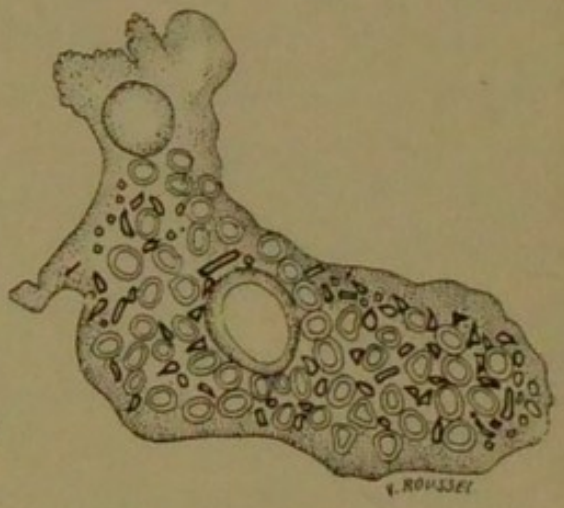


FIG. 41.—An amœba dying full of parasites.

FIG. 42.—Green euglena enclosing a chytridium (lower fungus). (Metchnikoff.)

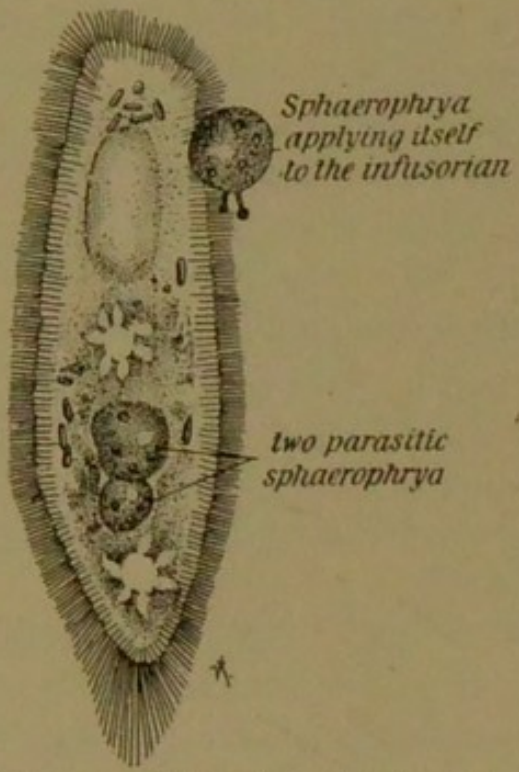


FIG. 43.—Infusorian attacked by acinetian parasites (*Sphærophrya*). (Metchnikoff.)

earth the tetanus spore may gain access to a wound ; the soil may spread anthrax spores over sheep pastures. The foods which are eaten raw (milk, meat, vegetables) convey what they have gathered from the soil or from the bodies of animals.

The vehicle of transmission may be a living creature instead of an inert object; fleas carry plague from rat to rat and from man to man. The intermediate inoculating agent may constitute a storehouse and even a culture chamber for the virus, *e.g.*, the tick in spirillum fever. This intermediate agent becomes properly speaking a *host* when the germ undergoes in it, and can only undergo in it, a cycle of changes by which it attains the stage at which it can infect us; for example, the mosquito for the parasite of malaria and the tse-tse fly for the trypanosome of sleeping-sickness. The transmission of bovine piroplasmosis from ox to ox is conducted by two individuals: a tick becomes infected, produces larvæ, and these larvæ inoculate another ox.

Latent microbism is said to exist when the organs and tissues contain germs which remain for a longer or shorter time unsuspected.

Pasteur thought that our organs and tissues were normally aseptic: "The human body is completely closed to the introduction of the germs of fermentation"; and he was still more right in adding, "Except the alimentary canal and again except in certain pathological conditions." The body defends itself well against the microbes which enter it; "latent" microbes can only be those which escape phagocytosis at least temporarily. The best example is furnished by cases of spontaneous tetanus, appearing under the influence of a heatstroke; this tetanus is due, according to Vincent, to the germination of spores which have penetrated the body by some unknown path, and have remained there for several days or even several weeks, till the day when the excessive heat accompanied by fatigue interrupted the phagocytic defence.[1] The experiments of Porcher and Desoubry teach us that the blood is rarely aseptic during digestion.

Auto-infection is said to exist when an individual is infected

[1] Tetanus spores inoculated in the blood or under the skin of rabbits are only eliminated after three or four weeks (subcutaneous inoculation), or even after three months (intravenous inoculation). They 'wake up' and germinate, should favourable conditions supervene, among others necrosis in the tissues (Tarozzi).

by bacteria of which he is himself a carrier. The term of auto-infection gained a precise signification only when germ-carriers became known.

Germ-carriers are those individuals who harbour the microbe of the disease yet present no symptom of this. The fact is not entirely novel (it has been known for long that the pneumococcus exists in individuals recovered from pneumonia, and Pasteur discovered it in the saliva of a child dead of rabies), but it is only during recent years that all its importance has been recognised.

There exist carriers of typhoid and paratyphoid bacilli, of diphtheria, of cholera, of dysentery and of meningococcus There are individuals who have acquired the microbe without *yet* contracting the disease—precocious carriers : others recently cured and not yet freed from their bacteria—convalescent carriers ; individuals cured weeks, months, or even years before—chronic carriers : finally there are occasionally individuals who have never had the disease—healthy carriers or "paradoxical carriers." It is of great importance to recognise germ carriers especially in communities where the general life is intimate and confined, as in schools and barracks.

In typhoid fever in particular the germ-carriers are most often women. According to Frosch's statistics, there is one woman for every five cases of typhoid, but of every five chronic typhoid-carriers four are women. The typhoid bacilli in chronic typhoid-carriers select as their favourite habitat the bile-ducts.

Conditions of Infection.—These are very variable, varying with the virulence, the resistance of the body, and the method of inoculation.

The number of attacking bacteria can be calculated fairly closely in experiments, but in the natural disease it is impossible to tell how many bacilli are necessary to determine a given infection.

The rabbit is extremely sensitive to the bacilli of fowl-cholera. Now, according to Watson Cheyne, if 10,000 to 30,000 bacteria are injected there is only a local abcess ; above

this figure a general infection is practically certain ; but on the other hand the same observer states that one bacillus is sufficient to produce fatal anthrax in the mouse. There are certain experimental facts as regards the smallest quantity of bacilli capable of producing tuberculosis but these have not an absolute value. H. Buchner found that to spray 100 c.c. of a dilution of 1 per 100,000 of tuberculous sputum was sufficient to give miliary tubercle of the lung to guinea-pigs. The sputum employed contained approximately 80,000 bacilli per c.c. and he calculated that each guinea-pig had been exposed to the attack of 100 bacilli (these figures are of course very approximate). In the experiments of Preisz, $\frac{1}{1000}$ milligram of sputum, containing about forty bacilli, was found sufficient. In the experiments of Findel, guinea-pigs inhaling about sixty bacilli regularly took tuberculosis.[1]

Microbial Associations.—The microbes which cause diseases are never derived from pure cultures. Their virulence is modified not only by the chemical properties of the medium and the "soil" on which they fall, but by the presence of other species favourable or the reverse. There are streptococci which aggravate diphtheritic sore throats. The coliform bacillus, the torula, and the sarcina described by Metchnikoff favour the production of intestinal cholera. In tetanus the associated bacteria are helped also by other favouring factors, such as splinters, bruising of the tissues, and blood clots. It was formerly thought that the streptococcus of erysipelas was antagonistic to the anthrax bacillus. Pasteur observed an antagonism between the B. pyocyaneus and the anthrax bacillus ; if these two bacteria are inoculated on a medium in lines which cross each other, the anthrax bacillus grows very feebly at the points of intersection. The proteolytic enzyme of the B. pyocyaneus, the pyocyanase, is injurious to many bacteria, and occasionally plays the part of a disinfectant (Emmerich).

[1] Tuberculous sputum (caseous material) frequently contains 50,000 bacilli per mg. In cultures half-dried on paper there are about 35 to 40 millions per mg.—with a possible error of one million (Chaussé).

In the infections due to a well characterised microbe, capable of itself of causing the disease, the associated bacteria most often act by turning upon themselves the phagocytic attack ; their rôle is thus secondary.

The study has hardly commenced of those bacterial associations which have no specific pathogenic power, but which act nevertheless favourably or the reverse by the products of their metabolism. Their sphere is chiefly the alimentary canal, and, according as the dominant flora of the intestine is acid producing or produces indols and phenols, the general health escapes or is subject to the action of the sclerosing toxins. These microbial associations thus constitute a certain condition or disposition rather than a true disease. There can be distinguished in it a fundamental flora and an accidental flora, and these can be modified by fortifying one species at the expense of the others ; it is in this that bacteriotherapy consists.

In the mouth, microbial associations produce a disposition, more or less marked, towards the development of inflammations of the throat.

The body presents a field capable of infinite variations ; we have to reckon with species, age, and physiological conditions : hunger, cold, and fatigue. Experiment alone could teach us that a mammal, such as the rabbit, is more sensitive to avian tuberculosis than to the tuberculosis of mammals, or that the rabbit is extremely sensitive to the bacillus of fowl-cholera and the pigeon to that of swine-erysipelas. The Algerian sheep is more resistant to sheep-pox than the sheep of Camargue. In general very young animals are more sensitive than adults, yet the young pig hardly ever contracts swine-erysipelas under three months.

Hunger, heat, or cold, and fatigue act by depressing the phagocytic defences.

Paths of Penetration into the Body.—The mosquito inoculates the virus which it carries either under the skin or directly into a blood-vessel. The spirochæte produces syphilis only when it is inoculated strictly in the subcutaneous

cellular tissue. The virus of hydrophobia spreads from the region of the bite to the nerve centres along the nerve trunks. Tuberculosis appears different according as it is inoculated along one path or another ; in natural disease different methods of propagation are associated. The two following ideas are of great importance.

1. *The rôle of the intestine in those diseases in which the infection is not purely intestinal.*—The question was long ago put by Chauveau in relation to tuberculosis. Behring has taken it up again, and maintains that every case of pulmonary tuberculosis in the adult is the extension of an intestinal tuberculosis acquired from milk in the earliest infancy, but remaining latent for years. Tuberculosis in general, according to him, does not attack the lung until it has pierced the barrier of the intestine. A similar origin has been claimed for other infections which finally settle in the lung, such as the pneumococcal inflammation. A comparative case was found in the anthracosis of coal-miners (the impregnation of the lung with coal-dust), and since it is easier to experiment with inert dust particles than with virulent bacteria, anthracosis has become the field of study and discussion in the question for and against intestinal infection.

It has been settled by experiment that living bacteria can pass through the intestinal mucosa like dust particles without leaving any lesion as a mark of their passage. But such passage only occurs as a rule when massive, repeated doses are ingested, and when there are in the intestine such injuries as favour penetration. It is therefore chiefly by inhalation and by penetration of the lymphatics and blood-vessels in the neighbourhood of the pharynx that the tubercle bacillus reaches the lungs.

2. *Seats of election and receptive cells.*—The hydrophobia virus fixes itself on the nerve-tissue, the parasite of malaria on the red corpuscle of the blood, other protozoa on the white corpuscles. The dysentery bacillus, inoculated under the skin, proceeds to make a home for itself in the large intestine. The bacillus of swine-erysipelas inoculated in the pigeon

is chiefly found infecting the large endothelial cells, the "Kuppfer cells," of the capillaries of the liver.

If the virus of small-pox or of sheep-pox is injected intravenously, it is simply carried by the blood and settles and multiplies in the skin. In those diseases which Borrel has grouped together under the name of *Epithelioses*, there exists a fairly strict cellular specificity: the virus only develops vigorously in the interior of epidermal cells, and these cells from the moment of infection take on a special character; they are therefore justly called the *receptive cells*. The reason why we have failed to inoculate certain diseases is that the proper site of inoculation has not yet been discovered, *i.e.*, the receptive cell, or that this receptive cell requires to undergo, before becoming truly receptive, certain modifications which are still unknown and which we cannot reproduce.

"In cancer our methods of inoculation in a normal individual fail to strike the receptive cells and to transform the normal into cancer cells: it is this which constitutes the whole etiological problem of cancer" (Borrel).

Between the date of penetration of the virus and that of the appearance of the disease there is a period of *incubation*, during which the virus propagates itself, multiplies and affects the cells on which depend the symptoms. The duration of the incubation varies primarily with the virus, secondarily with its quantity and the path by which it has gained access. For example, the incubation is quite short when experimental septicæmia is produced with a virulent streptococcus, whereas in human leprosy it may last for years. In rabies the period of incubation is shorter, and the disease more violent when the bite is on the face than when it is on the leg. In tetanus (a toxin disease), the longer the incubation the less serious the disease.

The spirillum of recurrent fever causes a disease of the septicæmic type, *i.e.*, the microbe inhabits the blood. The tubercles characteristic of tuberculosis and glanders represent a reaction of the cells of the mesoderm, while the pustular diseases like small-pox are typically reactions of ectodermic

cells. But the same disease may be in different phases septicæmic or localised in a tissue, and in any case the blood itself is simply a tissue whose cells are motile. It was long thought that typhoid fever was an infection localized to the small intestine and to the Peyer's patches there, but in reality it is septicæmic during the whole of the febrile period. There are also septicæmic phases in pneumonia and tuberculosis.

Microbes are not inert particles but living cells acting through their secretions and toxins. Cholera is an intestinal infection, but it is fatal to its host from a general intoxication. Every infection is to some extent an intoxication. Even the macroscopic parasites, to which formerly only a physical activity was ascribed, the bothriocephalus, the ankylostoma, the trichina, secrete poisons which have been studied.

Microbes, ferments, and toxins are inseparable terms, and for this reason the discovery of the diphtheria toxin by Roux and Yersin in 1888 began a new era in bacteriology.

CHAPTER VI

INFLAMMATION AND PHAGOCYTOSIS

The comparative pathology point of view—Inflammation throughout the series of natural species—defined by phagocytosis—Inflammation among invertebrates without nerves or blood-vessels—The phagocytes and intracellular digestion — Chemiotaxis — Phagocytes in man—Phagocytosis in the chronic infections—The examples of the squirrel-rat, spermophilus, and of the jerboa.

THE essential fact of inflammation is the reaction of the phagocytes towards the injurious material (Metchnikoff). Phagocytosis is not a theory but a doctrine, a collection of accumulated facts. It has put the finishing touch to the work accomplished in medicine by Darwin, Virchow and Pasteur. From Darwin it has derived its fundamentally evolutional character : it is founded primarily on comparative pathology and demonstrates the persistence of the same phenomenon throughout all the animal species. It has derived from Virchow its foundation on cellular pathology, *i.e.*, on the essential rôle of the body cells in disease. From Pasteur it derives the fundamental idea of the rôle of microbes in the production of infections.

It required a zoologist applying the method of comparative study to demonstrate that the only constant phenomenon in the different forms of inflammation is the active incorporation of injurious elements by fixed or, more often, migratory cells which are capable of digesting these. *Inflammation is essentially phagocytosis and phagocytosis is summed up by intracellular digestion.*

The four cardinal symptoms redness, heat, pain and swelling represent only an external definition of inflammation. The sum total of the facts is so complex that many observers in former days refused to give a simple definition, proposed to abandon the vague term of *inflammation*, and limited themselves to a description of the variety of facts.

The tissues, the vessels, and the nerves of the injured part participate in the state of inflammation: to which is the primary rôle to be ascribed? Virchow maintained that it was the tissues, and that these were in a state of supernutrition at the expense of the *fluid* parts of the blood; the cells multiply at the injured point and it is from the tissues of this same region that the numerous cells of the inflammatory exudate are derived. Inflammation taken as a whole represents a danger to the body.

But when Cohnheim, in his observations on the frog's mesentery exposed to the air, discovered diapedesis or the escape of the white corpuscles through the walls of the vessels, and when it was established that the pus cells instead of developing on the spot by the proliferation of the cells of the connective-tissue came from the motile cells of the blood, the primary fact of inflammation seemed to be the vascular irritation, the other appearances being secondary. Cohnheim thought he had proved this by his well-known experiment; a frog's tongue is ligatured at the base so as to stop the circulation. On untying at the end of forty-eight hours the circulation is re-established, but is now of the inflammatory type with diapedesis.

But if the vascular phenomena are of primary importance it is difficult to account for the fact that microbial or other foreign substances introduced under the skin produce an inflammatory reaction, but fail to do so when injected into the vessels themselves.

Light was first thrown on the problem by the comparative study of lower organisms.

Inflammation in the Lower Organisms.—The jelly-like plasmodium of a mycetozoon pricked or burnt responds

to the injury by movements of attraction or repulsion (varying according to circumstances) of its protoplasm. A foreign body introduced into it is engulfed and then rejected.

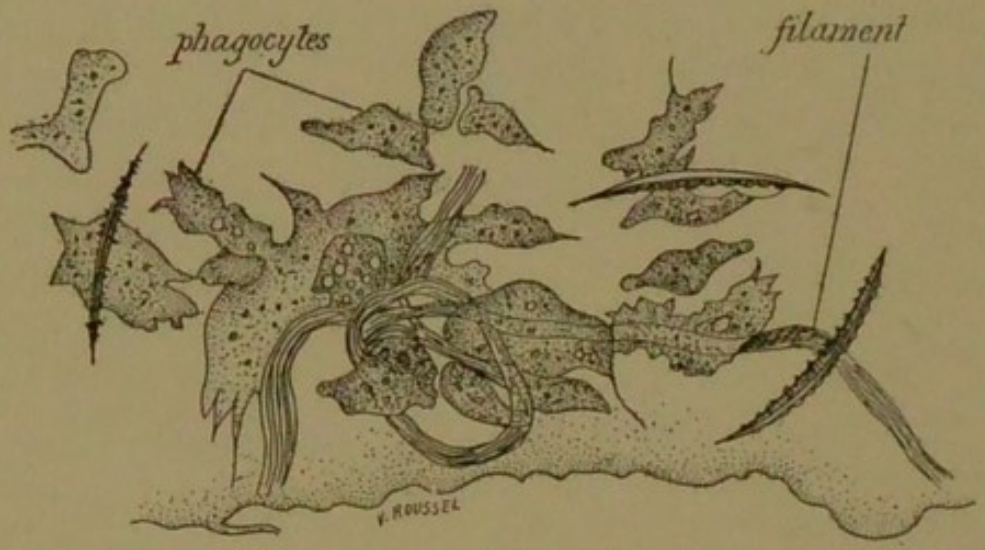


FIG. 44.—Plant filament surrounded by the phagocytes of Spongilla. (Metchnikoff.)

If into the body of a sponge a little glass tube or an asbestos fibre or any sharp foreign substance is introduced, motile amœboid cells from the mesoderm soon come and surround it, the same cells which are capable of surrounding and digesting both inert granules and living prey. This engulfing power of

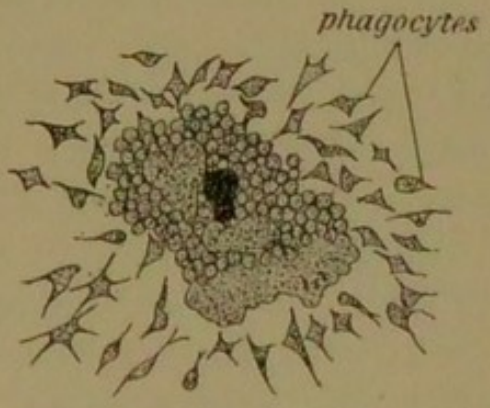


FIG. 45.—Mass of phagocytes round a spike in *Bipinnaria asterigeria*. (Metchnikoff.)

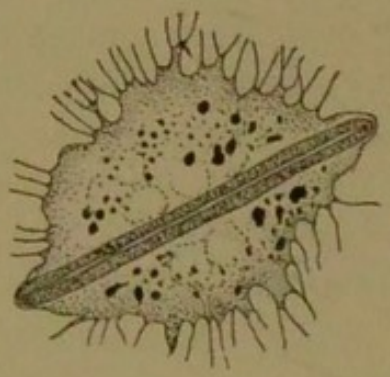


FIG. 46.—Phagocytes of the worm collected round a foreign body. (Metchnikoff.)

the mesodermic cells (and of certain endodermic cells also) is aided by the sensitive contractile ectodermic elements.

The larvæ of a certain sea-anemone (*Astropecten pentacanthus*),

transparent and thus easily observed, have neither nervous system nor vessels nor muscles: they react towards a penetrating foreign body by an accumulation of amœboid mesodermic cells. In a larger larva, *Bipinnaria asterigeria*, the cells of the mesoderm can be seen engulfing particles of carmine or of indigo and surrounding a splinter or a drop of blood with masses of cells equivalent to a plasmodium. They also engulf bacteria introduced under their outer skin.

In all these cases inflammation occurs without blood or blood vessels. Among the Annelids which possess a closed

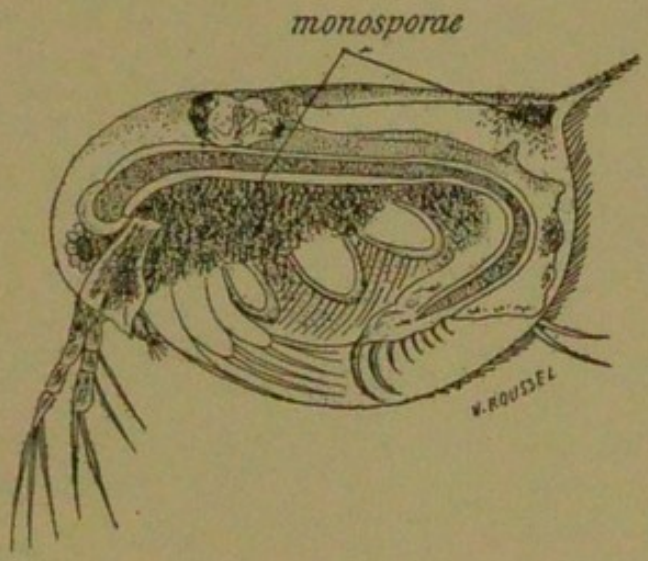


FIG. 47.—Daphnia infected by *Monospora*. (Metchnikoff.)

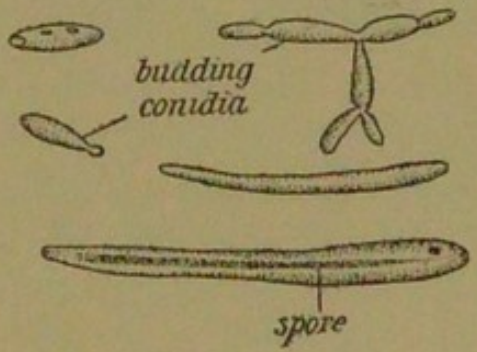


FIG. 48.—Different stages of *Monospora*. (Metchnikoff.)

vascular system, the reaction against foreign bodies takes place in the same way without any intervention of the blood vessels.

In *Lumbricus*, whose male sex-glands are infected by gregarines, there is a struggle between the two organisms, the gregarine encysting itself and becoming surrounded by an outer covering of protective chitin, while the amœboid cells surrounding it join together and form a sort of armour-plating which stifles it. The blood-vessels remain inactive.

For an example of parasitism exactly parallel to an infectious disease there is nothing better than to observe *Daphnia magna* being invaded by a microscopic fungus, the *Monospora bicuspidata*. The motile cells engulf the spores of the mould and

attempt to destroy them by digestion: a struggle takes place, sometimes the Daphnia is victorious, sometimes it succumbs.

In the young of a vertebrate, the axolotl, if the non-vascular rudiment of the fin is pricked with a needle charged with a little carmine or indigo powder, the migratory cells can be seen

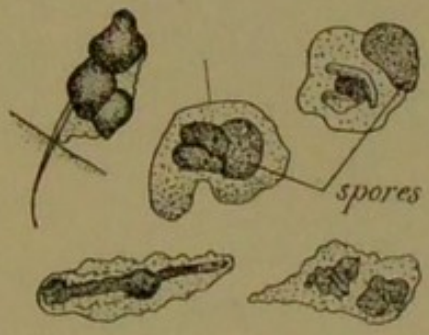


FIG. 49.—Spores of *Monospora*, surrounded by leucocytes of Daphnia. (Metchnikoff.) The spore is transformed into granules.

FIG. 50.—Two leucocytes of Daphnia surrounding a conidium of *Monospora*. (Metchnikoff.)

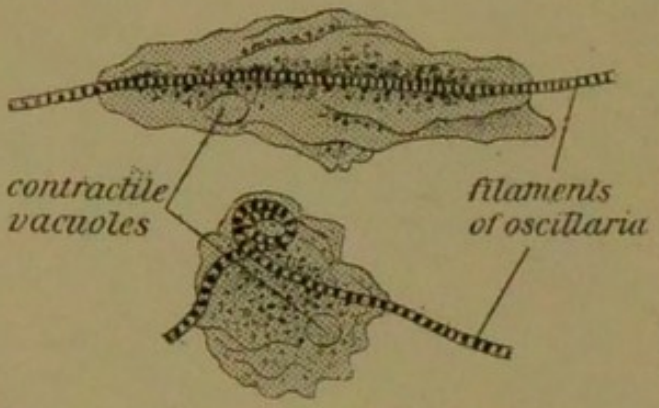


FIG. 51. - An amœba (*Amœba verrucosa*) incorporating a filament of Oscillaria. (After Rhumbler.)

hastening to the injured point and engulfing the particles. In the older stages of the axolotl and in the tail of tadpoles, where a well-developed vascular system exists, inflammation is accompanied by a dilatation of the vessels and by diapedesis: the reaction is more violent, but the essential process is the same as in the non-vascular invertebrates.

"It is quite evident that inflammation in the vertebrate, in which the protective phagocytes emerge from the vascular system to attack the aggressor, differs from the analogous phenomena in the invertebrate only from the purely quantitative standpoint. . . . The morbid phenomena, properly speaking, such as the lesion or the primary necrosis, equally with the processes of repair which succeed the inflammation, do not belong to it and must not be confused with it" (Metchnikoff).

The phenomena of vascular dilatation and hyperæmia are no

more to be regarded as inflammation, says Cantacuzène with justice, than the congestive phenomena which accompany ovulation or precede coitus can be called fertilisation.

Phagocytes, phagocytosis and digestion.—Metchnikoff has given the name of *phagocytes* to those cells which are capable from their own activity of seizing and incorporating solid particles. (There is no question here of the faculty of absorbing substances in solution.)

Certain phagocytes are migratory cells : for example the white corpuscles of the blood. Others are fixed cells—for example many of the endothelial cells of the blood-vessels and lymphatics, the endothelial cells of the omentum and the neuroglia cells. Others originally motile may become fixed at a certain period in their existence.

The fundamental property of phagocytes is intracellular digestion.

Metchnikoff's first observations were on the property of intracellular digestion in the intestinal epithelium of a great many Turbellarians. In the Cœlenterata and the Sponges digestion is intracellular, *i.e.*, the nutritive particles instead of being digested in a cavity with the help of juices poured out by digestive cells undergo digestion in the interior of the cells themselves.

The Metazoa have inherited this property from the Protozoa. Originally all the cells of the inferior Metazoa are capable of phagocyting ; there are both ectodermic and endodermic phagocytes ; later the function devolves entirely on specialized cells belonging to the mesoderm.

The phagocytic cells of man are descendants of cells whose normal function was to digest intracellularly, and through them we still possess this method of digestion, side by side with the extracellular digestion which occurs in our stomach and intestine.

Phagocytosis is thus a function very wide-spread among living beings, and the struggle against infection is only a particular case of it.

" The phagocytes are those cells which have best preserved

the primitive amœboid type. They are in general the least differentiated elements in the body, but they are also the most independent and possess the greatest vitality. They assist in building up the young animal during the embryonic period, and when the tissues begin to wear out, when old age is coming on, it is the phagocytes which consume the senile cells incapable of recovery and take their place. The renewal of cells and tissues which goes on slowly and continuously in a great many animals is, like the abrupt transformation which occurs in metamorphosis, the work of phagocytes."

The importance of pathological phagocytosis from the medical point of view should not make us forget that a *normal phagocytosis* exists. The histolysis in the larvæ of insects, the destruction of the tail in the tadpole forms of the Tunicata, the degeneration of the tail muscles in the tadpole of the Frog and Toad, the destruction of the myelinated nerve fibres in Wallerian degeneration, the shrinking of the ovarian follicles, the fixation of the ovum on the mucous membrane of the uterus, the daily destruction of the red corpuscles of the blood which goes on in the spleen, all are examples of normal phagocytosis.

The phagocytes are guided or directed in their choice and perception of the bodies which they ingest, by a peculiar sense whose manifestations are known by the name of *chemiotaxis.*

It has been known since the time of Pfeffer and Stahl that cellular organisms and plasmodia are attracted by certain substances (positive chemiotaxis), and repelled by others (negative chemiotaxis). They become accustomed to substances which at first repelled them and finally are attracted by these. Massart and Ch. Bordet have systematically studied chemiotactic actions, by introducing under the skin of the frog capillary tubes containing chemical substances, microbes and their products. Lactic acid, glycerine, bile, and guanin repel the leucocytes; sterilised cultures of both saprophytic and pathogenic microbes attract them. Positive chemiotaxis may be considered as the *appetite*, which prepares for intracellular digestion. Everything is not yet explained in this distant action. Chemiotaxis is analogous to the sensations of higher

animals, and the sensations of a plasmodium obey like ours the law of Weber.

Chemiotaxis is a sort of chemical sense.

The phagocytes have also a sort of tactile sensibility. The leucocytes in their reaction apply themselves to the exciting body over the largest surface possible.

In defensive phagocytosis, the struggle of the body against the parasitic invaders, it is not necessary to suppose any purposeful cause but simply a function developed by evolution and selection. "Those lower animals in which the motile cells directed themselves towards the enemy, engulfing and destroying them, survived, whereas others in which the phagocytes did not act, were condemned to perish. All the useful characters and among them those which are concerned in the inflammatory reaction have become fixed and transmitted without the intervention of any preconceived purpose whatever" (Metchnikoff). Thus in the invertebrates with soft skins in which bacterial invasion occurs easily there has been a selective process at work in the phagocytic apparatus and the defensive measures have become perfected. Among the invertebrates possessing a natural protection, such as a chitinous covering, infection is rarer, but the means of defence have not found suitable conditions for their employment and development, so that the infected organism succumbs. The phagocytic arrangements are much reduced in the Insects, and in these the parasitic fungi have great difficulty in penetrating the cuticle, but if they are successful the insect is destroyed (for example in the beetle *Cleonus punctiventris* invaded by *Isaria destructor*). The nematode worms which are protected by a thick skin do not even possess cells capable of movement.

The phagocytes of man are both fixed and motile. Among the fixed are the large mononuclears, the Kuppfer cells of the liver, certain endothelial cells of the lung (the dust-cells) and the myeloplaxes of the bone-marrow. The motile phagocytes are the white corpuscles or leucocytes in general (except the small lymphocytes), the polymorphs, the eosinophils, the large mononuclear cells of the blood and of the lymphatic organs.

Metchnikoff has divided the phagocytes into *macrophages* and *microphages*; the former are chiefly concerned in the absorption of cells and cellular debris, and include the large mononuclears, the fixed phagocytes of the spleen, of the peritoneum and of the lymphatic glands. They digest the blood corpuscles and other phagocytes. The microphages are the polymorphs; their principal function is to digest bacteria. There are exceptions. In certain cases the microphages take up cells (red cells among others), while in certain cases the macrophages take up bacteria; the large mononuclears surround the tubercle bacillus producing the giant cell, and take up also the spirochætes of recurrent fever and of syphilis.

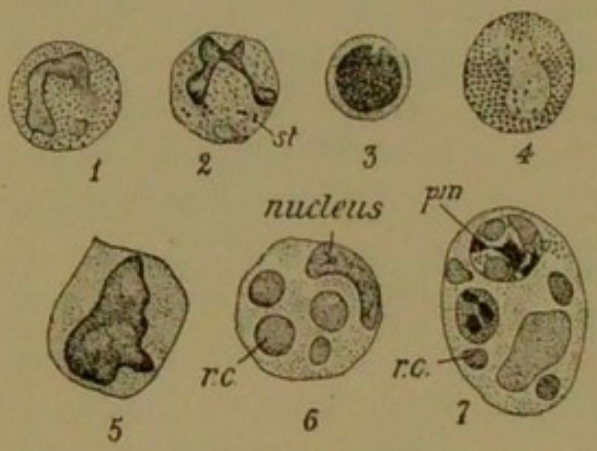


FIG. 52. — Different leucocytes. — 1. Polymorph. — 2. Microphage (polymorph) taking up staphylococci, *st.* — 3. Small lymphocyte. — 4. Eosinophil. — 5. Large mononuclear (macrophage). — 6. Macrophage from the peritoneum of a guinea-pig taking up red corpuscles. — 7. Macrophage from the peritoneum taking up polymorphs (microphages) and blood corpuscles.

It has sometimes been maintained that the phagocytes only take up dead bacteria and not living virulent ones; this is a mistake to which it will be necessary to refer again in connection with immunity. Under the microscope there can be seen inside the phagocytes living and even motile bacilli, and cultures can be obtained by inoculating into broth phagocytes full of microbes: the leucocytes are destroyed and the liberated bacteria multiply. They were still quite alive therefore, although already seized by the phagocytes.

The phagocytes secrete digestive ferments. Rossbach has demonstrated the existence of a starch-splitting ferment in the leucocytes of the tonsils. The cells of pus can digest fibrin and gelatine, and must thus secrete proteolytic ferments. In cases of acute muscular atrophy the progressive digestion of

the muscle fibres can be observed in the interior of the phagocytes. The bacilli or cells taken up by the phagocytes become distorted, and before disappearing lose their affinity for stains. In the various cases of immunity the phagocytes digest the bacteria by means of *endo-enzymes*.

The surface of the skin, and in particular of the mucous membrane, is being continually besieged by bacteria, and never a moment passes but some point in the body is in a state of subinflammation. The phagocytes are in continual operation on the surface of the tonsils, of the mucous membrane of the intestine, and of the alveoli of the lungs.

Phagocytosis plays a pre-eminent part in chronic infections, especially in tubercle, and the tubercle itself is a phagocytic formation. In contrast to Baumgarten's contention that the tubercle is built up by epithelial cells, *fixed* cells from the diseased tissue itself, lung, liver, or kidney, Metchnikoff and his pupils have proved that it is really composed of migratory mesodermic cells which have come from elsewhere to the infected point. Borrel followed the formation of the tubercle from the time of the first contact between the white corpuscles and the bacilli, and found that injected bacilli were engulfed by the polymorphs while still in the circulation. The polymorphs perish and degenerate (in two or three days) and are followed by the macrophages which fuse together into a sort of little plasmodium with several nuclei, which is characteristic of the tuberculous lesion, and is called by the anatomists the *giant cell*. Later, the tubercle may soften, and there may be a new afflux of polymorphs attracted chiefly by the bacteria of a secondary infection.

In the squirrel-rat spermophilus, a rodent rather resistant to tubercle, the phagocyted bacilli lose their staining properties, degenerate, swell up and finally appear as yellowish bodies, such as *are never observed either in cultures or outside the cells*, and can only be residues of phagocytic digestion. In another rodent, the jerboa, there is found, especially in tubercle of the spleen, instead of bacilli amorphous bodies built up of concentric layers, which are encrusted with

phosphate of lime and may be dissolved by an acid. Observation of these tubercles at different stages shows that the concentric layers correspond to secretions of the bacillus which has been defending itself against the phagocytes. Analogous formations are known in actinomycosis (the club-forms of the granules). There is no essential difference between the struggle of the tubercle bacillus against the giant cell and the

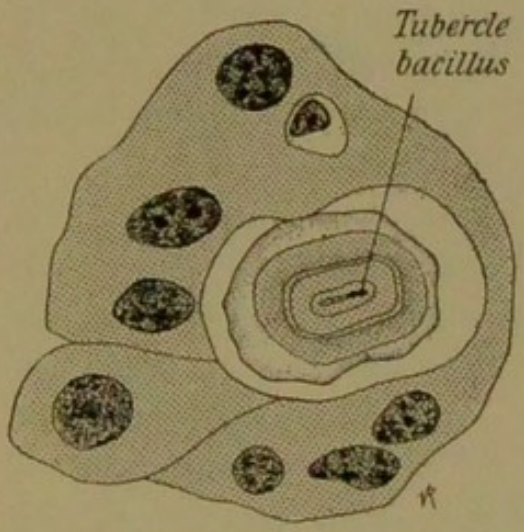


FIG. 53.—Giant cell from the spleen of the jerboa: it contains a tubercle bacillus surrounded by concentric layers. (Metchnikoff.)

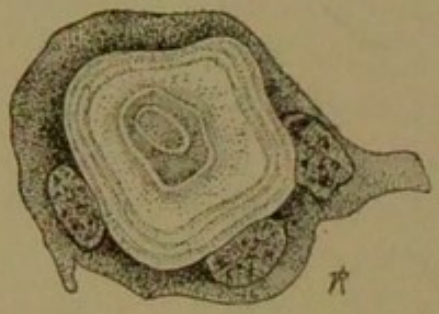


FIG. 54.—Giant cell enclosing the final stage of a calcareous particle.

struggle of the gregarines and the nematodes (larvæ of Gordius or Rhabditis) against the phagocytes of the worm.

Inflammation is thus defined by phagocytosis: the vessels and nerves have their importance, but are merely accessory. Infection, inflammation, and immunity can all be seen in miniature in the examples of the Bipinnaria with its splinter surrounded by motile cells, and of the Daphnia with its globules in the act of devouring the spores of Monospora.

CHAPTER VII

THE PATHOGENIC PROTOZOA : FILTER-PASSING VIRUSES

Protozoal diseases—Laveran's discovery—Importance of the morphology and the life-cycle—Intracellular protozoa—Heredity in bacterial and in protozoal diseases—Diseases due to the so-called invisible microbes. —The ultramicroscope—Filtration—Various types of virus capable of passing filters—Microbes of extreme minuteness described in the pustular diseases of the epithelium—Lesions of the infected cells.

The Pathogenic Protozoa

The name of Pasteur must be inscribed at the head of this chapter. It was by his study of *pébrine*, a protozoal disease of silk-worms, that our ideas on the microbial diseases were so much advanced.

The studies on anthrax, the labours of Koch and the great discovery of the attenuation of viruses led the new science in the direction rather of bacteriology ; the protozoa had even been somewhat forgotten, when in 1880 Laveran discovered among them the cause of malaria. Since that date their importance in pathology has never ceased to grow.

The methods of research cannot be quite the same as in bacteriology ; they have not the same simplicity as the bacteria. In the case of the tubercle bacillus, the cholera vibrio, and the streptococcus, we practically know only one single constant fixed form for each, and there is no sign of a life-cycle. The majority of the pathogenic protozoa on the contrary go through a cycle in their existence whose successive forms may be very diverse and this cycle may take place, not in a single host, but often in two different ones. The discoveries of Ross on the

plasmodium of malaria present the best example of the labour necessary to reconstruct the biology of a parasite common to man and the mosquito.

PROTOZOAL DISEASES

Rhizopods

Amœbæ.—Amœbic dysentery and liver abscess. A ciliated infusorian, *Balantidium coli*, may produce the same disorders.

Hæmatozoa [1]

Trypanosomes.—Sleeping sickness (human Trypanosomiasis).—Trypanosomiasis of animals: nagana, surra, dourine, mal-de-Cadéras.
Leishmania.—"Leishmanioses": Kala-azar (the oriental sore, Biskra button, Aleppo button, etc., being particular cases).
Piroplasmoses.—Bovine Piroplasmoses (due to *Piroplasma bigeminum*, *P. parvum*, *P. mutans*; canine, ovine, and equine Piroplasmoses.
Plasmodia.—Malaria with its varieties: tertian, quartan, and æstivo-autumnal or tropical tertian.
Spirochætes.—Relapsing fever (European, African, Asiatic, American).
The spirillosis of fowls and geese.
Human spirillosis = syphilis.

The more the study of these cycles was advanced, the more one was compelled to acknowledge relationships between forms which did not seem in the least related. Established classifications have several times got into difficulties from trying to express genealogical relationships between very diverse forms. Schaudinn saw in the life-cycle of the same parasite trypanosomes, spirochætes, and amœboid forms. It has been necessary to recognise a relationship between the sporozoa (hæmo-sporidia) and the flagellates. The affinities of the spirilla and spiro-

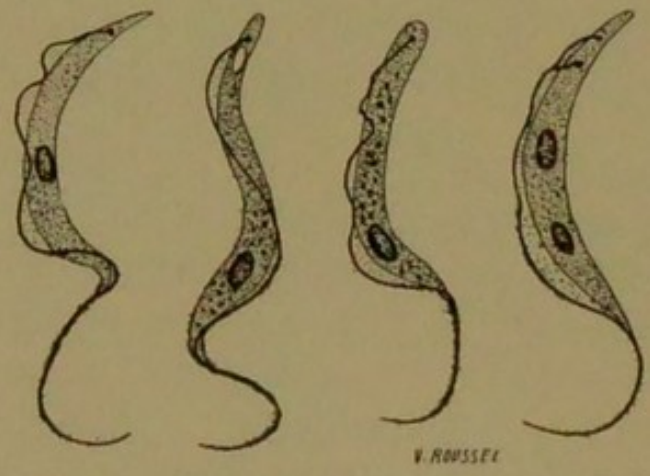


FIG. 55.—Trypanosomes of sleeping sickness (*Trypanosoma gambiense*): the form on the extreme right is in process of division.

[1] According to the recent views of Hartmann, originating in Schaudinn's ideas, all the hæmatozoa mentioned here may properly be arranged in one natural group.

chætes are not yet clearly determined. These questions, which seemed of zoological and philosophical interest rather than medical, have nevertheless come into the domain of medicine since the discovery of the microbe of syphilis and its treatment. From the study of the pathogenic protozoa there have arisen many new ideas.

In the study of bacteria the methods are their isolation and pure culture, the study of their biochemical reactions, and of experimental inoculations. Their physiology, *i.e.*, the study of their functions, takes precedence of the study of their forms, *i.e.*, their morphology. In the case of the protozoa, morphology has the first place. It is no longer a question of describing one cell, but a cycle of very dissimilar cellular forms with reproductive phases, sometimes sexual, sometimes asexual. And it is only at this price that certainty can be attained on the methods of transmission of these microbes and on the basic ideas for medicine and hygiene. Cultivation has been successfully accomplished only in the case of certain species, the trypanosomes of the rat, amœbæ in mixed culture, and piroplasma, and it does not give the same help as do bacterial cultivations.

No intracellular protozoon has yet been cultivated.

It is the knowledge of the life-cycle which gives the key to the transmission. The most complicated modes of transmission among the bacteria are very simple in comparison with those of malaria and sleeping sickness. The living carriers of certain bacteria, *e.g.*, the rat-flea in the case of plague, appear to be carriers pure and simple, *i.e.*, possess practically the same importance as the needle of a syringe or a lancet. The mosquito is more than a mere carrier of the hæmatozoon of Laveran ; it is a second host and in it, and it alone, the parasite accomplishes the sexual phase of its life-cycle.

It was originally thought that the tsetse fly (*Glossina palpalis*) whose bite produces sleeping sickness was a simple carrier of the virus and only remained infective for a few hours after sucking it from the patient's body. But the recent experiments of Kleine, confirmed by Bruce, have proved that the tsetse is

really a second host. It remains infective for about 24 to 48 hours after the moment of drawing infected blood, then for a period of about 17 days it is non-infective, again becoming infective for a period of about 60 days' duration. The trypanosome undergoes in it an evolution with sexual reproduction.

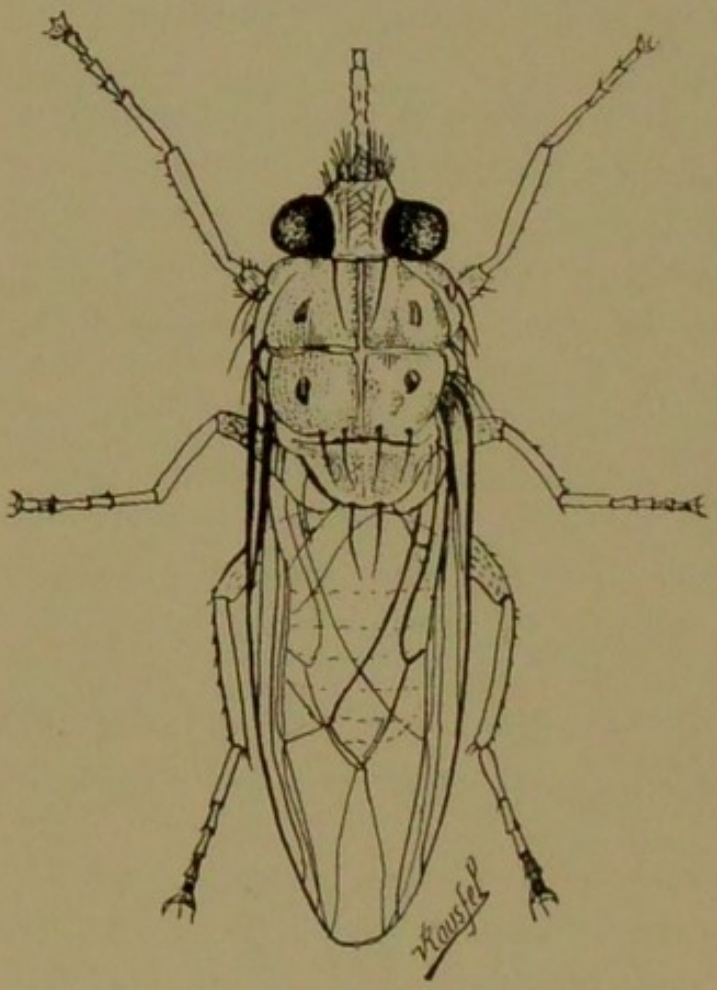


FIG. 56.—*Glossina palpalis*, the tsetse fly which carries sleeping-sickness.

The protoplasm of protozoa appears to possess faculties of adaptation and variation much more extensive than that of bacteria. Biologically speaking it is a long journey for a parasite of the stomach of the mosquito to reach the blood of man. In the successive phases and habitats there are forms and structures so different that it requires strict proofs to convince one that it is really the same species. The *Leishmania donovani* of Kala-azar (a disease of India and the Mediterranean) is in the intestine of a bug a flagellate form: in man it is an intracellular form deprived of all locomotory organs. The degradation resulting from parasitism has abolished in many forms the characteristic structures and in many cases permits of only provisional classification.

These degradation phenomena are more striking among the protozoa because we know on the other hand their complete cycle. The bacteria, as we have seen, have reached in their apparent simplicity perhaps the final stage of degradation, and

they conceal their origins, of which we can with difficulty discover a few vestiges.

The protozoa may injure their host both by mechanical and by chemical means. The *Entamœba histolytica* destroys and strips the epithelial cells from the intestine, abolishing at certain points the impermeability of the healthy mucosa. *Myxobolus pfeifferi* produces atrophy of the muscle fibres. *Lentospora* destroys the bones and cartilages of the trout.

The toxins of the protozoa are little known. If they exist they are difficult to isolate and demonstrate. The *Sarcocystine* of Laveran and Mesnil, which kills the rabbit and only the rabbit, is a well-characterised toxin, but no similar substance has been isolated from the cultures of trypanosomes, or from the blood of animals with a trypanosome infection. The blood of a malarial patient, filtered at the moment of the paroxysm, is not quite harmless to a healthy individual. The blood of an animal infected with *trypanosoma gambiense* produces an appearance of somnolence in experimental animals, but it is difficult to say how much of this is due to products of the parasite and how much to the host. These are researches which will have to be continued; there is no reason to believe in advance that toxins and endotoxins do not exist in the pathogenic protozoa.

Heredity in Protozoal Diseases.—The protozoa are frequently intracellular parasites. Bacteria also may inhabit cells, for example the bacillus of leprosy, the bacillus of swine-erysipelas, and the tubercle bacillus. But in these cases it is the cell which has taken up the bacterium, the cell being mesodermic and naturally phagocytic; the microbe has been captured; no bacterium ever penetrates by its own activity into a living cell. On the contrary, many protozoa have during their life-cycle a motile form, amœboid or flagellate, thanks to which they can penetrate spontaneously the cells of their host.

This fact is of capital importance from the point of view of heredity in disease. When one sees the young of an anthrax-infected mother born with an anthrax pustule, one might think

that the disease was hereditary, but in reality it is a case of contagion or of transmission at short radius; the placental filter has been injured (Chamberland's experiment). Nowadays heredity in tuberculosis is no longer believed in; what is inherited is at most a physiological predisposition of the soil (and even that is a vague and uncertain idea), or, alternatively, conditions of life in which the bacillus, everywhere to be found, can flourish. There is no hereditary infection in the strict sense unless the fertilised ovum is infected by the

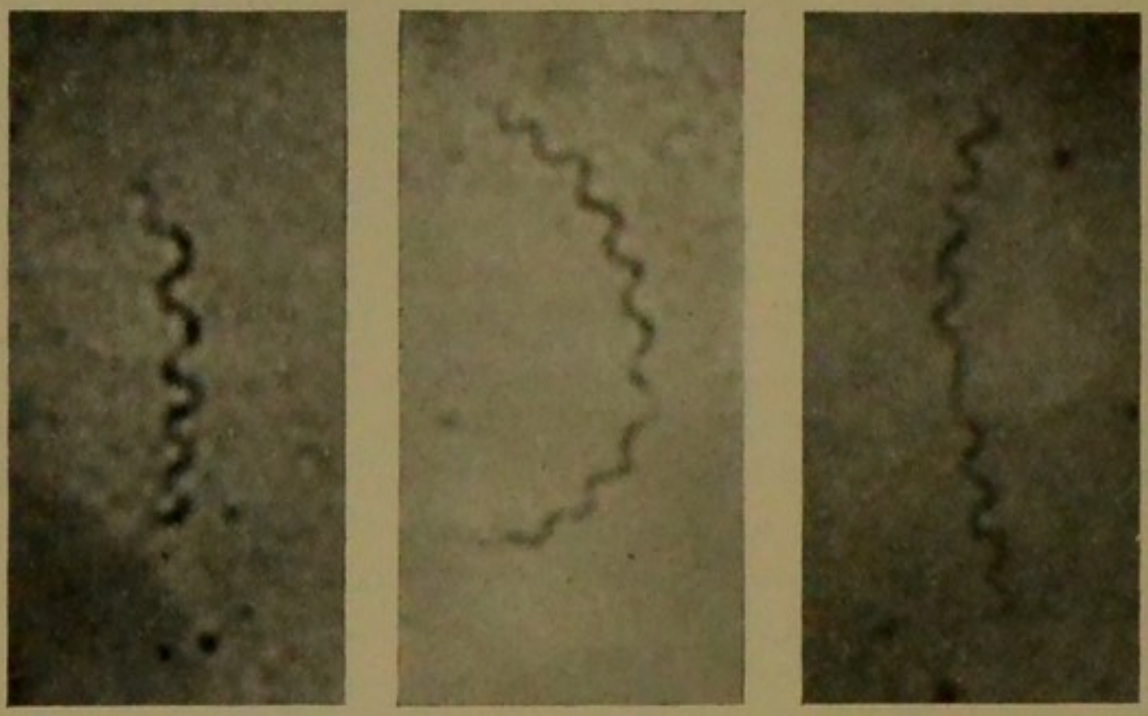


Fig. 57.—The spirochæte of Schaudinn in the liver of a congenital syphilitic. (From a microphotograph. Magnified 3,000 times.)

parasite (among the vertebrates either from infection of the female cell or of the spermatozoon, or of both); in such a case the disease is truly congenital. There is no certain example of such a fact among the bacterial diseases, and this is the reason why the idea of heredity among them has lost so much ground.

From their power of penetrating cells the protozoa frequently infect parasitically the ovum, thus producing hereditary infections. The first thoroughly demonstrated example of hereditary infection was that of the *pébrine* of the silk-worm,

rendered so famous by Pasteur. Under the microscope the presence of the germ was demonstrated in the egg, and it was recognized that the infected eggs produced caterpillars which formed the point of departure for the infection of the following year. In general, in this example the heredity is only of one generation, for the silk-worm infected in the egg rarely survives to become an adult; it is the other silk-worms, infected late in their larval stage, which succeed in reaching the adult condition after more or less great vicissitudes, which produce the infected eggs.

Eckhardt has found coccidia (*Coccidium tenellum*) in the white of hen's eggs, and, according to him, these parasites produce an early infection of the chicks which in consequence very soon die.

The higher vertebrates have an interest in the hereditary transmission of protozoal diseases from two points of view; either it occurs in themselves or it is a condition of the infection of an invertebrate host which transmits to them the disease. Thus the piroplasmosis due to *Piroplasma bigeminum* is transmitted from ox to ox by a tick, *Rhipicephalus annulatus*, but it is not the same individual tick which carries the piroplasma from one ox to another. One tick becomes infected from an infected ox, and it is its progeny, a daughter tick, which infects the healthy animal. On the inheritance of the parasite in the insect-carrier depends the propagation of the disease in the vertebrate.

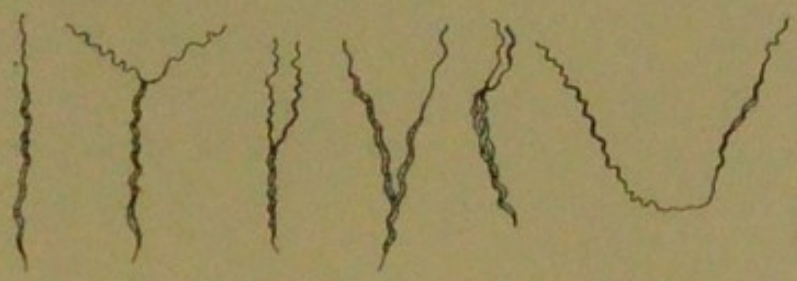


FIG. 58.—The spirochæte of syphilis: forms in longitudinal division.

An inheritance is probable also in other diseases of vertebrates which are transmitted by ticks, *e.g.*, the spirillosis of fowls, African tick-fever and recurrent fever. R. Koch saw the spirochæte of African recurrent fever in the egg of the tick which transmits it, *Ornithodorus moubata*. In fowls

infected with the spirochæte of Marchoux and Salimbeni the parasite may penetrate the egg, particularly the yolk, and in this case inheritance may occur from vertebrate to vertebrate. Hygiene has to take account of these facts: to abolish a parasite it is insufficient to destroy it in the vertebrate; the invertebrate host also has to be abolished since it is capable of transmitting the parasite to its descendants, the egg of the infected insect preserving the disease in nature somewhat as the anthrax spore keeps alive anthrax.

Schaudinn considered the spirochæte which he discovered in syphilis to be a protozoon. Now the clinicians regard syphilis as a disease which can be inherited. The case of new-born infants with syphilis does not alone prove an hereditary infection, the spirochæte being very motile might be transmitted from the mother to the fœtus through some lesion of the placenta. *Congenital* syphilis is by no means necessarily a syphilis by *conception*. But from certain observed facts such true inheritance is very probable. The spirochæte has the power of spontaneously penetrating cells: it has even a predilection for epithelial cells: further, although there are no certain observations of its presence in a spermatozoon, it has been seen in the spermatic tubules in close relation to the epithelial cells (in congenitally syphilitic boys), and it has been seen (Levaditi and Sauvage) in the protoplasm of the ovarian follicles of female children. Are infected ova in the woman capable of fertilisation, and, if fertilised, capable of normal development? It seems possible in view of the clinical facts and by analogy with the case of the tick *Ornithodorus*, whose eggs infected with spirilla give rise to larvæ which as adults are capable of conveying the infection.

To sum up, we know from Finger and Landsteiner's experiments that the semen of an adult syphilitic is, as a substance, capable of producing syphilis, and we know that the congenital syphilitic of the male sex shows the parasite developing in the seminal gland in contact with the epithelial cells, but no one has ever seen a spirochæte in an adult spermatozoon. In the female subject there can be no doubt of the possibility of

transmission by the general circulation through the blood, *i.e.*, through the placenta; and further, spirochætes have been seen in the interior of the ova in female congenital syphilitics; it is not known with absolute certainty whether, and how, a spirochæte passes from the general circulation of the mother, or from the spermatic fluid of the father, into the ovum which after fertilisation is to produce an embryo infected from the start, whose life in consequence will be more or less soon cut short. But taking all the facts we know we have almost a complete demonstration of true inheritance.

Protozoal disease and hereditary disease are two terms so closely associated in our minds to-day that the protozoal nature of the spirochæte is invoked to support the hereditary character of syphilis, and this latter is brought forward as an argument in favour of the protozoal nature of the spirochæte: there are at the base of this somewhat easy-going argument facts which are sufficiently certain.

The striking analogies between syphilis, a spirochæte infection, and sleeping sickness, a trypanosome infection permit of the belief that the spirochæte of Schaudinn is a protozoon. Among the more or less late complications of syphilis are locomotor ataxy and general paralysis. Now there is also known an ataxic condition in dogs infected with trypanosomes (Spielmeyer's experiments), and there exists a general paralysis with all the mental stigmata of that disease in men attacked by sleeping sickness (G. Martin and Ringenbach).

There are doubtless more protozoal diseases than we think to-day, and it may quite well be that protozoa are the cause of those infections whose nature is still unknown—*e.g.*, yellow fever, cattle-plague, and the horse-sickness of the Transvaal. Yellow fever in particular is transmitted by a mosquito (*Stegomyia fasciata*), which does not infect until after the 12th day from the time at which it was itself infected. The individual bitten passes through a period of prostration which lasts three to five days, and at this moment his blood becomes infective for the mosquito, but only for a period of three days; these facts indicate a life-cycle in the mosquito and in man,

a series of different forms (cf. malaria) which appear and disappear in the blood. These forms are unknown and must be extremely minute.

From the immunity point of view also the protozoal diseases present characters quite different from the bacterial infections.

The Viruses Capable of Passing Filters.

The bacteria which we study under the microscope are unequal in size. The *Bacillus Bütschlii* we mentioned in connection with the nucleus of bacteria is a colossus in comparison with the bacilli of fowl-cholera, with the *Micrococcus parvulus* of Veillon, or even with the little bacillus found in influenza by Pfeiffer. There probably exist bacteria still smaller. Our best microscopes do not allow us to distinguish a particle whose thickness is less than 0·1 μ. The bacteria smaller than 0·1 μ are therefore invisible under the microscope ; they are *ultra-microscopic*. Since there are many diseases in which the microbe remains unknown we are tempted to ascribe to them ultra-microscopic microbic agents. Already in 1884 Pasteur said that the virus of rabies was too small for us to be able to see it.

The study of these extremely small microbes only commenced in 1898 with an experiment by Löffler and Frosch on the virus of foot-and-mouth disease, which no one has yet made visible. The serous fluid from an ulcer (in which no microbe can be seen) is diluted with water and filtered through a porcelain bougie (similar to those of the Chamberland filters) ; there results a perfectly clear fluid free from visible microbes which is capable of transmitting the disease to a fresh animal ; this is the first example of a virus passing through filters, or, as it is commonly called, a filtrable virus.

Since 1898, the existence of filtrable viruses has been proved by experiments in about twenty diseases, the chief of which are foot-and-mouth disease, pleuropneumonia of cattle (rinderpest), yellow fever, swine-plague, cattle-plague, small-pox, and rabies.

The study of these viruses is far from being advanced ; one only, that of cattle pleuropneumonia, has been seen (and even its form is subject to discussion), obtained in pure cultures and treated like an ordinary bacterium. In vaccinia, small-pox, sheep-pox, hydrophobia, trachoma and molluscum contagiosum, microbes have been described but they are still hypothetical ; the proof is still to seek.

The expression, "invisible microbes," originally employed to describe these, has been abandoned as inexact, and they have been called the "so-called invisible," and later the "filtrable viruses." Invisible microbes are simply microbes which have not yet been seen ; for example, the syphilitic virus was classed among them till the day when Schaudinn discovered the Spirochæte. The classic example of pleuropneumonia shows that a microbe may traverse a porcelain filter without being invisible. Little vibrios and even little protozoa (*Micromonas Mesnili*, of Borrel) have been found in water ; these pass through a filter and can yet be quite easily seen. A filtrable microbe is not necessarily visible.

The name of ultra-microscopic microbes is the most correct, because many of these micro-organisms, too small to be seen under the microscope, can be studied with the ultra-microscope. Everyone has heard of this improvement, consisting in examining the object not as lighted from below and seen by transmitted light, but lighted from the side so as to appear as a bright point or line on a dark field. In the observation of microbes which are perfectly visible the ultra-microscope is not the instrument of choice for studying the structure ; a well-stained preparation is still the best. The ultra-microscope furnishes to medical bacteriology above all an economy of time and trouble ; it makes the finding of the microbes a more rapid process, for example, when there are very few trypanosomes in the blood or spirochætes in the fluid from a lesion suspected of being syphilitic ; but these are quite visible microbes.

Are the ultra-microscopic viruses always microbes ? May it not be a question, at least in certain cases (as Beijerinck has

suggested for the mosaic disease of the tobacco plant), of a fluid, living contagium which is literally invisible? This hypothesis of "soluble viruses" has been put forward, but hitherto no positive proof has been given.

The essential procedure in the definition of the ultra-microscopic viruses is filtration; it is the current method of isolation. The liquid containing the virus is passed through a filter—for example, vaccinal pulp rubbed up in water; the filtrate collected is virulent, and with it attempts at cultivation can be made. The filters employed are of the well known forms; the majority are hollow bougies, like those which are used for filtering drinking water; they are made of porcelain (Chamberland filter), of infusorian earth (Berkfeld filter), of asbestos, charcoal, plaster, etc.

These filters do not act towards microbes as does a sieve used to sift seeds of unequal size, or as the metallic grids used for separating sand of different coarseness. It would not be right to conceive the large microbes as being kept back because they are bigger than the meshes, whereas the little ones pass easily through, just as the smaller fishes pass through the meshes of a net. Even the bacteria of average size, such as the vibrio of cholera, are smaller than the pores of our filters, and their size would permit them, to use the simile of Duclaux, to pass through, as a train passes through a tunnel, without rubbing against the walls; what keeps them back is that they are held against the walls by the capillary pressure.

Filtration is not a simple mechanical operation, various factors act in it: the quantity of the virus, the motility of the microbe, the pressure, the degree of dilution, the nature of the liquid more or less albuminous, the temperature, the duration of the filtration, and the texture of the filter. All these factors have to be taken into account in these experiments. As a rule, several filtrations are performed one after another, the first, rougher and more rapid, prepare for the final one by freeing the liquid from particles which block up the pores. One must especially avoid having thick albuminoid substances present; they soon cover over the surface of the filters. By prolonging

the time it would be possible to make microbes pass which are not ordinarily "filtrable," but this would not be really a filtration but a culture propagating itself by extension from one side of the filter to the other. This is what happens in the filters of water supplies which are badly kept. Bacteriologically speaking, they are no longer filters at all ; instead they are continually infecting the drinking water with the microbes which they are supposed to be keeping out.

Filtration can show that some quite minute microbe exists in a given infection : it gives no information as to its nature. Fortunately, the labours of Jenner and Pasteur have proved that it is possible to study a virus without seeing it. It can be purified (precisely by filters as it happens), inoculated, and its resistance to physical and chemical agents (heat, antiseptics, etc.) determined, as well as the conditions of preservation and attenuation. All the viruses enumerated at the head of the chapter have been treated in this way and processes of immunization have been discovered against certain of these viruses which are still unknown, a paradox which has become familiar to us through Jennerian vaccination and the antirabic treatment of Pasteur.

To say that a virus is filtrable is to give it an external rough definition ; there are undoubtedly in this group very different microbes ; some may be bacteria, others protozoa. Borrel has described a protozoon which passes the rough filters. In the life cycle of the *Hæmamœba Ziemanni* of the little owl, Schaudinn has described motile forms smaller than the microbe of peripneumonia ; it is admitted that even the most visible protozoon may have ultra-microscopic stages.

Several groups may henceforth be distinguished among the diseases due to filtrable viruses :

1. Pleuropneumonia of cattle : in this the microbe has been filtered, cultivated, and finally seen. It would seem that it ought to be easy to describe it. At first it was said to be a bacterium, extremely fine cocci namely, which it was possible to see under the ordinary microscope, not singly, but in amorphous masses. Recently, Bordet has described by means

of culture in a special medium, forms resembling spirochætes. Borrel, using a different technique, has criticised the forms seen by Bordet and concluded that it is not a spirochæte but a new type lying perhaps between the protozoa and the bacteria, and still incapable of precise definition.

2. Cases of blood infection or septicæmias such as the horse-sickness and the catarrhal malarial fever of sheep (studied in particular in the Transvaal), yellow fever, cattle-plague, avian-plague, and hog-cholera appear to be of the same

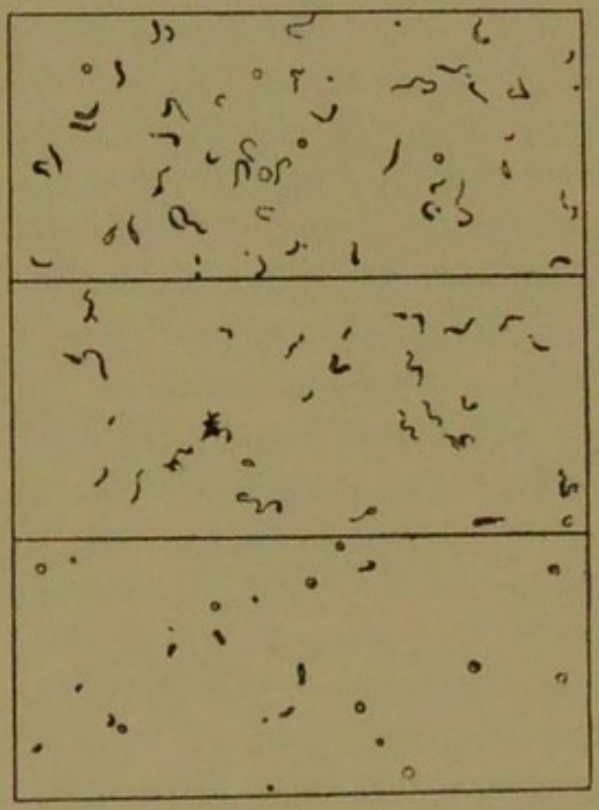


FIG. 59.—Various forms of the microbe of bovine pleuro-pneumonia according to Bordet.

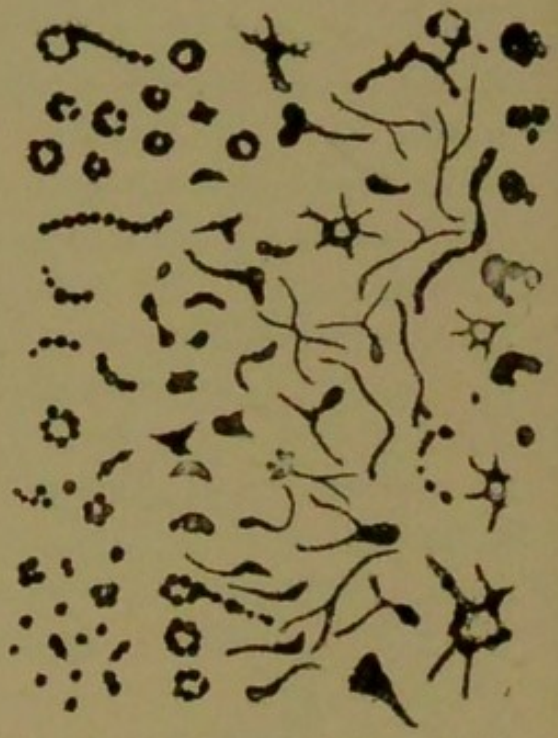


FIG. 60.—Various forms of the microbe of bovine pleuro-pneumonia according to Borrel (higher magnification than in Fig. 59).

nature. Horse-sickness and the sheep disease which resembles malaria only exist in localities where there are certain definite mosquitos ; like, malaria, too they were formerly called miasmatic disease. Horses do not take horse-sickness even when they are exposed to conditions of climate and altitude reputed to be dangerous, provided they are protected from mosquitos by means of wire-screens. The "heart-water" of ruminants is transmitted by a tick (the bont-tick—*Amblyomma hebraeum*).

Yellow fever is inoculated by a mosquito, *Stegomyia fasciata*. These diseases have all the behaviour of protozoal infections. By reason of certain procedures of immunization which are common to them all, horse-sickness, cattle-plague, and hog-cholera present some points of similarity.

3. The diseases characterized by localization to, and lesions in, the epithelium, for example, small-pox and vaccinia, foot-and-mouth disease, molluscum contagiosum of birds and man, scarlatina, the jaundice of silk-worms, and trachoma or granular conjunctivitis are perhaps to be classed in this group.

Small-pox or vaccinia is the type-specimen of these infections; the characteristic lesion is the pustule and the pustule is a collection of epithelial cells containing the virus and forming a focus of culture *in vivo*. The cells which build up the little tumour have no longer the normal structure of the epidermic or Malpighian cells; they have become globular, voluminous and "dropsical"; the nucleus is altered, being swollen and frequently out of position; beside the nucleus there appears a mass of abnormal material called the "cellular inclusion."

This mass was for long regarded as a parasite, as the visible stage of a protozoon which possessed other stages more minute or ultra-microscopic. Nowadays it is known that it is merely a deformation of the nucleus appearing after the invasion of the cell by the virus. It is, as it were, the stigma of the presence of a virus within the cell, or even within the nucleus.

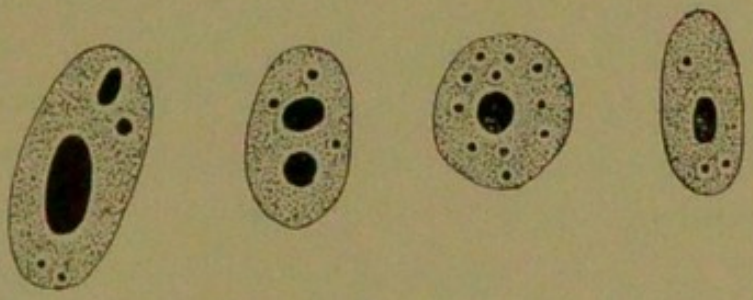


FIG. 61.—Negri bodies in rabies; the hypothetical microbe of rabies at different stages. (After Calkins.)

These stigmata have been described under different aspects and names in small-pox, vaccinia (Guarnieri bodies), rabies (Negri bodies), *molluscum contagiosum* (described by Virchow), trachoma, and the jaundice of the silk-worm, and the similarity of these infections can no longer be doubted.

Borrel has discovered in the cells of molluscum contagiosum minute corpuscles very equal in size and distinct from the nucleus, from the chromatin and from the protoplasm: they are small enough to pass through filters, and sufficiently abundant and resistant to physical influences, as temperature and drying, to explain the powerful nature of the contagium in these diseases.

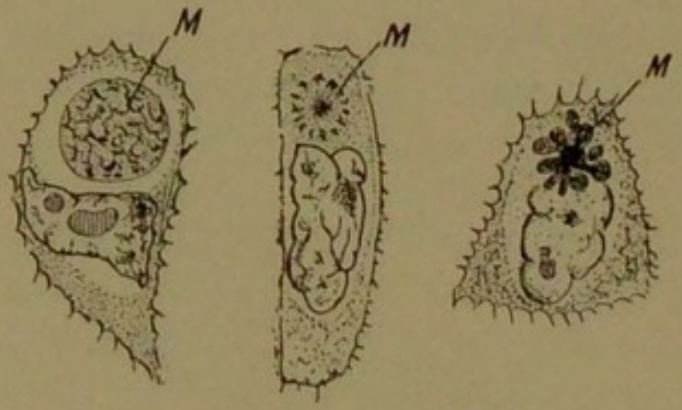


FIG. 62.—Mallory bodies; the hypothetical microbe of scarlatina under different aspects. (After Calkins.)

This may perhaps be the type of microbe so long sought for in small-pox and granular conjunctivitis, but it is necessary to speak with reserve as cultivation has not yet been successful. What is certain is that there exist ultra-microscopic bacteria and protozoa sufficiently small to traverse the pores of filters made of asbestos, porcelain, plaster, or infusorian earth. Great discoveries are still to be made in this domain—a domain opened up twelve years ago by the study of foot-and-mouth disease and pleuro-pneumonia.

The curiosity of investigators ought not to be monopolised by the diseases occurring in man and animals. There is no reason why there should not be invisible microbes elsewhere in those fermentations which go on everywhere in nature. They may also quite well play a part in the life-cycle of plants. Just as insects produce injuries and mutilations in plants, so the ultra-microscopic microbes may be responsible for the variations and mutations which occur in the vegetable world. Microbiology may hope here again to bring its support to the Darwinian doctrine.

CHAPTER VIII

THE TOXINS

Microbial and Vegetable Toxins—Endotoxins

Microbial and vegetable toxins—Definition—Soluble toxins—Characters—Toxins and diastases: resemblances and differences—Incubation Penetration of the body—Elective fixation—Wassermann's experiment—Vegetable toxins: ricin, abrin, crotin—Production of antitoxins—Endotoxins—Definition—Toxity of microbial bodies—Toxin and endotoxin of the cholera vibrio—Do anti-endotoxins exist?—Importance of intravenous inoculations.

Microbial and Vegetable Toxins

We know toxins as properties, not as substances, properties of certain broth-cultures, or properties of the bodies and extracts of the bodies of bacteria. Their nature and their exact chemical constitution are unknown, for they are bound up with albuminoid substances the chemistry of which is still in its infancy.

The science of toxins is therefore more physiological than chemical, and the chief method of experimentation is on the living body. The quantitative element is introduced by measuring the incubation times, the temperature, or the magnitude of the local phenomena, such as œdema and the duration of the symptoms of intoxication.

In certain cases it has been possible to replace the animal experiment by experiments *in vitro*, and to measure, with exactitude, certain phenomena, easy to observe, such as the lysis of the red corpuscles of the blood (hæmolysis).

Useful discoveries are much oftener reached by instinct than

by reason. If progress went on logical lines the idea of antibodies in general ought to have been the first, and from it the existence of antitoxins ought to have been deduced. But, on the contrary, it was the discovery of a particular antitoxin which led to the study of antibodies in general.

In experimental medicine, the chief business is not to build up systems of ideas, *i.e.*, to philosophize, but simply to search patiently with many trials and many a re-beginning. The inquisitive, prying, intuitive people have the advantage over those who reason.

At the very beginning of the researches on toxins we find an experiment of Pasteur: the filtrate of a culture of fowl cholera produced in a fowl the symptoms of the disease in the absence of microbes. At first it was thought that the bacterial toxins belonged to the group of alkaloid substances, the ptomaines, found by Selmi in dead bodies, in certain molluscs, and in bacterial cultures (*e.g.*, muscarine, neurine, &c.). It is true that bacteria can produce poisons of this type (Brieger), but these poisons do not produce a specific intoxication like that observed in such a well-defined disease as tetanus. Later, when it had been observed that microbes killed by heat are not harmless, but when inoculated produce a local suppuration, attempts were made to isolate the "poison" by making protein extracts of the bacterial bodies; but the bacterial *proteins* of H. Buchner are not specific poisons; from very diverse bacteria one can extract almost the same poisons. They consist of excretions or residues of nutrition of the bacteria, and are found in particular in old cultures.

Excluding these alkaloids and proteins, the following are the substances studied as toxins:—

1. *The Soluble Toxins.*—The type-specimen is the diphtheria toxin or the tetanus toxin. These are secretions of bacterial cells, just as the pancreatic juice is a secretion of the gland cells.

2. *The Endotoxins.*—Examples: typhoid endotoxin, plague endotoxin. These are poisons which remain attached to the cellular protoplasm, and do not diffuse at all or very little in

broth-cultures. It is necessary to destroy the cell to set free the poison. The process may be exemplified by the zymase production of Ed. Buchner, in which the yeast cell is ground up and expressed.

3. A special group has to be made for the poison of the tubercle bacillus (tuberculin), and that of the bacillus of glanders (mallein). These diffuse into the broth, but there remains some in the substance of the bacterium ; hence the bodies of tubercle bacilli form an active tuberculin.

Besides the specific toxins, cultures may contain non-specific poisons, the proteins of Buchner.

Soluble toxins.—The toxins of diphtheria, of tetanus, and of botulismus are known (the latter being produced by an anaerobic bacillus growing in meat). They can be obtained by cultivating the bacteria with an abundant supply of air (in the case of the bacillus of diphtheria), or in broth deprived of oxygen (in the case of the tetanus bacillus). The bacteria are removed from the fluid by filtering through porcelain bougies. Those strains of bacilli are picked out which furnish the best toxins, for good toxins are necessary for the production of good antitoxins.

The Characters of the Soluble Toxins.—The soluble toxins possess very definite characters ; in the first place they are strictly specific ; the symptoms produced by the diphtheria toxin are quite distinct from the symptoms of tetanus. Their action is specific in another sense, as it varies in the different animal species. The fowl does not react to the tetanus toxin as does the mouse or the human subject. Secondly, they are extremely potent: a good culture of tetanus can kill the mouse in a dose of $\frac{1}{100000}$ c.m. The diphtheria toxin kills a guinea-pig of 250 grams in a dose of $\frac{4}{1000}$ c.c.[1] The

[1] " 1 c.c. of the active fluid toxin produces on evaporation *in vacuo* 0·01 gram of dry residue. Deducting the weight of ash and the portion insoluble in alcohol (which has no toxic activity) there remains 0·0004 gram of organic matter. It is certain that the greater portion of this weight consists of substances other than the diphtheria toxin. Yet this minute quantity is sufficient to kill at least eight guinea-pigs of 600 grams each, or two rabbits of 3 kil." (Roux and Yersin.)—1 c.c. of a good tetanus filtrate dried *in vacuo* leaves 0·04 gram of dried residue of which 0·025

botulismus toxin is fatal in the dose of $\frac{1}{1000}$ c.c. (by subcutaneous inoculation in each of these cases). Toxins are soluble in water and in glycerine, and can be precipitated from solution in virtue of the fact that they adhere to precipitates and coagula; precipitation is a method of purification. They are unstable or "labile" substances, heat, light, and oxygen destroying them fairly quickly. Exposed for an hour and a half to the temperature of 55° C., the tetanus toxin loses its toxic properties; at 60° C. it is destroyed in thirty minutes; at 68° C., in five minutes. Toxins bound up with dried precipitates are more resistant; the dry tetanus toxin is still slightly active after an hour at 80° C., and even after fifteen minutes at 120° C. Sunlight "inactivates" a solution of tetanus toxin in fifteen to eighteen hours. When a photo-dynamic substance (*e.g.*, 1 per cent. eosin), is added, the toxin is "inactivated" after six hours' exposure to light; with 2·5 to 5 per cent. eosin it is "inactivated" even in the dark.

Comparison between Toxins and Diastases.—The chemical constitution of diastases is not much better known than that of the toxins. In comparing toxins with soluble ferments or diastases, although it is possible to note analogies, it is for obvious reasons impossible to give a chemical definition. Like diastases, toxins act in a very small dose, are soluble in water and in glycerine, and are weakened by filtration, and are sensitive to the action of oxygen, of heat, and of light; also to changes in their reactions and to various chemical substances which "poison" or destroy them. Roux and Yersin, who pointed out these analogies at the time of their work on toxins in 1889, did not see in them more than a suggestion: "It seems to us that the diphtheria poison has many analogies with the diastases. Its activity is quite comparable to these and to the activity of venoms. We do not mean, however, that it produces phenomena of hydrolysis such as the diastases produce. It neither inverts sugar nor digests

gram is organic matter. Supposing that the whole of this is toxin (which is a great exaggeration), the lethal dose for a mouse would be 0·000,000,25 gram.

fibrin. If we compare it to the diastases, we do so without forming any rash opinion as to its chemical action and simply in order to sum up some of its properties." This reservation still holds good.

The disappearance of the toxic property by heating to 60° does not necessarily mean the destruction of a diastase. Heating modifies the reaction of the fluids, especially of organic fluids, and coagulates the proteins: this coagulation may inhibit the action of certain substances or properties without destroying them. The toxins have a character possessed neither by chemical poisons, *e.g.*, strychnine or potassium cyanide, nor by the diastases: the action of these chemical poisons is almost instantaneous, and a zymase put into a solution of sugar begins to ferment it at once. The toxins, on the other hand, when injected into the body, only manifest themselves after a silent period of apparent inactivity, the period of incubation. By changing the path of introduction, *e.g.*, by injecting intravenously instead of subcutaneously, and by increasing the dose, the period of incubation may be rendered shorter: it is never reduced to zero. Further the *minimum* incubation period varies with the species of animal inoculated.

It is admitted that the tetanus poison, to reach the nerve-centres, has to travel along the peripheral nerves from the site of its production, and within certain limits the period of incubation varies in proportion to the distance of this: but even when this delay is cut out the incubation period does not reach zero. Meyer and Ransom inoculated the nerve-centres of cats directly and still found a *minimum* incubation of three to five hours.

This inevitable incubation period suggests that the toxin of the culture is not the poison which kills the animal, but that the toxin inoculated undergoes in the body certain transformations (fermentations?) which produce the true poison, the action of which is direct and immediate. This secondary poison was said to have been demonstrated by inoculating mice with extracts of the organs of animals actually in tetanus, but these experiments have not given constant results as the symptoms

produced differed from the pure tetanic symptoms. It is not yet possible to believe in the existence of pro-toxins analogous to the pro-diastases such as the pro-fibrin-ferment.

The toxins act in extremely minute doses, like the diastases, of which a very small quantity can determine a chemical change in a very large mass of material. But there are alkaloid poisons which also act in a very small dose: a man dies after absorbing the five-millionth part of his weight of aconitine or digitalin. The mere fact of possessing an action produced by very small doses is not equivalent to being a diastase; it may be an ordinary chemical phenomenon; an artificial catalyst such as colloidal platinum is "inactivated" by one thousand-millionth part of hydrocyanic acid; Graham's solution of ferric hydrate under certain conditions is sensitive to the presence of 1/5,000,000th of ferrocyanide of potassium.[1] Finally, the phenomena produced by toxins are phenomena taking place in living creatures, which makes it all the more difficult to determine whether they are diastasic in nature or simply due to ordinary chemical reactions. It is true that the toxins of diphtheria and tetanus can "kill" 20 to 100 million times their weight of living animal, but these figures must not be allowed to produce this illusion: the quantitative relationship is complicated by a qualitative element whose importance cannot be exaggerated. When a horse is killed by 1/80,000th of its weight of tetanus toxin, the toxin is not acting on the whole mass of the horse: to produce death it is sufficient for it to act on the medullary nucleus of the vagus nerve, a group of cells scarcely weighing two grams. The diphtheria toxin similarly acts *in an elective fashion* on a group of cells in the medulla or in the cardiac ganglia. To take facts of another order, the fixation of carbon monoxide on the hæmoglobin of the blood is not a diastasic phenomenon (on the contrary it arrests a vital diastasic function of the first importance), yet the toxic power of carbon monoxide in proportion to the weight of the body is certainly more than 100,000.

[1] J. Duclaux—*La chimie de la matière vivante*, Chapitre X.

Penetration of the Body.—To reach the sensitive cells the toxins do not always follow the same path. Injected subcutaneously they pass into the lymph, then into the blood. Injected into the blood they save time, since the passage through the lymph is avoided. Introduced into the alimentary canal the botulismus toxin retains its potency, but the diphtheria toxin and the tetanus toxin are inactive even after being swallowed in much more than a lethal dose. This destruction or neutralization cannot be attributed to an action peculiar to the intestinal epithelium nor to any extent to the influence of the bacteria and their fermentations in the digestive tube, but chiefly to the action of the digestive secretions, the pepsin, and above all the pancreatic juice.

According to the experiments of Meyer and Ransom and of Marie and Morax, the tetanus toxin does not pass directly from the site of inoculation to the nerve centres ; it penetrates the peripheral nerves at their motor terminations, and follows these nerves to reach the centres. All three species of nerve fibre, motor, sensory, and sympathetic, can carry it ; but the carrying power of the nerve depends absolutely on the integrity of the axis cylinder. By employing antitoxin it is possible to localize the action of the toxin to certain definite territories and paths. Dissociation experiments have shown that the antitoxin acts by neutralising the toxin still in circulation, but is no longer capable of neutralising toxin absorbed by the nerve trunks (these do not absorb antitoxin). The fact that in certain animals, man and the horse, tetanus always begins by a contraction of the muscles of the jaw ("lockjaw") only means that even after a stab or a wound at the end of a limb sufficient toxin passes sufficiently quickly into the circulation to affect the centres on which the innervation of these muscles depends.

The cells of the central nervous system are sensitive to many poisons, whether these reach them by the nerve filaments or through the blood. Directly introduced into the centres the poisons act in a smaller dose, and often produce different symptoms from poisons injected subcutaneously or into the

blood stream. Cerebral tetanus is more like a mental disease, a sort of delirium, than the systematic tetanic contractions which follow the wound of a limb. In the rat which has received tetanus toxin intracerebrally, the incubation is from forty-eight hours to three days, and if the observer did not know with certainty that he had injected tetanus toxin he would never recognize tetanus in the disease which he observes. Psychical manifestations predominate; the rat is anxious and vigilant; without apparent cause it is seized with sudden terrors, and runs madly round its cage. . . . During the crisis it seems to obey an internal impulse . . . . and on careful observation the question forces itself whether many psychical phenomena in man may not also be produced by the fixation on certain nerve cells of bacterial toxins elaborated in the intestine or in some other part of the body at some particular moment (Roux and Borrel).[1]

Selective Fixation of Toxins.—For a toxin to kill in the minimum dose it must possess a selective affinity for cells whose function is important, and must proceed to fix itself on these cells and not on others, when introduced into the circulation. Thus it is necessary for a medullary nucleus to attract to itself the few thousandths of a milligram of tetanus toxin introduced into a human body. The intoxication depends entirely on this *fixation* of the toxin, and it has long been compared to a dyeing process.

Even in the inorganic world, and among dead substances of animal or vegetable origin, numerous examples exist of the fixation of a substance in solution more dilute even than are the toxins in the blood. The examples which follow are taken from the book of J. Duclaux already quoted. Pre-

[1] In the rat $\frac{1}{10}$ c.c. of diphtheria toxin subcutaneously does not produce even local œdema, but a rat receiving this dose intracerebrally is soon completely paralysed, and after two or three days of inertia it succumbs. A rabbit of less than 1,500 grams supports perfectly 30 centigrams of a morphine salt injected subcutaneously, whereas 1 milligram of morphine hydrochloride injected into the brain produces almost immediate effects in a rabbit of the same weight. A tuberculous guinea-pig succumbs when injected intracerebrally with a dose of tuberculin 200 times smaller than when injected subcutaneously.

cipitated sesquioxide of iron absorbs arsenious acid (and probably also phosphoric acid) till there remains in the fluid less than one-thousand-millionth part (A. Gautier). A skein of white silk dipped in a solution of eosin so dilute that the eye can perceive no colour, *i.e.*, to about one in a million, is dyed pink in the course of a few hours. Silk can take up 1·3 per cent. of picric acid from a solution containing only 0·006 per cent. The absorptive power increases the greater the dilution. Passing to living cells, we find that a culture of *Aspergillus niger* can take up from a solution measuring 250 c.c. and containing one-half milligram of zinc, *i.e.*, 1/500,000, practically the whole of this metal (Javillier); the proportion of zinc increases to about 1 in 10,000 in the aspergillus cells and falls below 1 in 10,000,000 in the fluid which remains. Certain plants can absorb copper from solutions containing only 1 in 100,000,000. Certain marine plants, such as the ordinary sea-wrack, fix abundantly the iodine and silver which exist in traces only in sea-water.

The whole industry of dyeing is founded on similar fixations: fabrics are sensitive to and fix selectively the colouring materials. Picric acid, which stains the skin, does not dye cotton. The microscopical preparations of histologists and biologists depend on this principle of the selective fixation of the stains by different anatomical elements: magenta is fixed by the nucleus, picric acid by the protoplasm, indigo-carmine by the connective-tissue fibres.

Ehrlich has pointed out that in jaundice the kidneys and the liver become charged with bile-pigments, whereas the brain remains free. When certain derivatives of paraphenylene-diamine are administered to mice, the central part of the diaphragm is found stained brown much more intensely than the periphery, and the muscles of the eyes, of the larynx, and of the tongue are much more deeply stained than other muscles: this may be because these muscles are in continual activity, receive more blood, and are the seat of more intense oxidations. Methylene blue in the living animal is fixed by the sensory fibres, by the nerve-endings for taste and smell,

by the nerves of the plain muscles and the cardiac muscle, and by certain fibres in the nerve centres: it does not stain the motor-endings of striped muscle with the exception of those of the eyes, of the larynx, and of the diaphragm (Ehrlich).

The majority of the staining substances fixed by the cerebral cortex are also taken up by fatty substances: now the cortical cells are rich in *lipoids* such as cholesterin, lecithin, and cerebrin. The substances which stain "*in vivo*" are soluble in these lipoids, the *non-vital* stains are not. Narcotics, cerebral poisons, have an activity proportional to their co-efficient of absorption by the lipoids, and both this activity and this co-efficient vary with the temperature. Antipyretics also have, without doubt, a selective action on certain cells.

The famous experiment of Wassermann and Takaki showed that a similar selective absorption takes place between the cerebral grey matter of mammals and tetanus toxin when these are mixed together in a test-tube; after a certain time the tissue fixes the toxin and the liquid is no longer toxic.

The brain of cold-blooded animals, *e.g.*, the lizard and the tortoise, does not fix toxin at all, or very little. The brain of the frog does not fix toxin *in vitro* (and yet the frog kept in a fairly hot room is sensitive to tetanus toxin). The fixing power of the brain seems to be proportional to its lipoid content, and there is less of these substances in the brain of cold-blooded animals. Brain material treated with ether, which dissolves the fats, loses a great part of its power of fixation: brain which has been boiled no longer fixes at all. Filtration of a suspension of brain material (removing the cell elements) also destroys the fixing property. Cholesterin, lecithin, and cochineal, a fatty material extracted from the cochineal insect, all fix toxin, but when heated to 60° in presence of moisture, or after a previous maceration in an alkaline fluid, the latter is no longer a fixative.

The fixation of toxin by sensitive cells is a phenomenon of the order of dyeing or *in vivo* staining—a phenomenon of molecular adhesion.

There is not in Wassermann's experiment, as was thought at

first, a destruction or neutralisation of the toxins by an anti-toxin elaborated by the brain cells. The fixation can be modified by altering the physical conditions: brain matter emulsified in physiological saline solution (0·8 per cent.) is a stronger fixative than the same emulsified in distilled water, but ten times weaker than the same emulsified in salt solution of 10 per cent. The brain gives up the toxin more or less quickly and restores the toxicity to water when it is allowed to macerate, and also after drying or digestion with papaïne: the toxin liberated has all the biological properties which it had before its intimate contact with the brain matter. This observation negatives the hypothesis of a secondary toxin elaborated by the cells from the toxin received and capable of acting immediately without incubation.

It is because of this fixation property of the cells of the body that the toxin injected disappears more or less rapidly from the blood and cannot be recovered, or only in very small degree, from the excretions. In the rabbit seventeen hours after injection no free toxin is to be found either in the blood or in the organs, and there is never any in the blood at the moment when the tetanic symptoms commence (about forty-eight hours after intravenous inoculation.—A. Marie).

Since the tetanus toxin may be fixed on cells other than the nerve-cells, it is evident that the former keep back at least a portion of the toxin, acting thus as a sort of screen to the nerve-cells. For example, the rabbit is less sensitive to tetanus than the mouse and the guinea-pig because its spleen fixes the toxin and saves its brain. Hence it is not the power of fixation in general which explains the sensitiveness of a particular animal, but the selective fixation on certain definite cells whose activity is indispensable to life, for example, the cells of the medullary nuclei or of the sympathetic ganglia.

Scorpions can stand very large doses of tetanus toxin without symptoms; the toxin rapidly disappears from the blood and accumulates in the liver. The alligator, which is refractory to tetanus, retains in its blood for more than a month toxin which has been injected into it. The carp, the axolotl,

and the frog, kept at a low temperature, do not take tetanus and retain the toxin intact in their blood for months. The tortoise, which does not take tetanus either at high or low temperatures, retains in its blood for months enough toxin to give tetanus to mice on injecting it. The fowl also, very little sensitive to tetanus, retains toxin in its blood for long periods.

The frog, which is refractory to tetanus in winter or when kept at a low temperature, takes tetanus in summer or when it is kept warm in an incubator at about 30° C. At this temperature the poison disappears from the blood and the organs much more quickly than in the cold. The course of the tetanic symptoms can be interrupted at will in the frog kept in the incubator by putting it again at a low temperature. In this way the phenomena may be suspended as long as the chilling continues; if it is again put in the incubator the symptoms recommence at the stage at which they were interrupted. In the frog, fixation and response are, therefore, to a certain extent dissociated, for in the cold the toxin is fixed by the cells yet the disease does not appear.

Vegetable Toxins.—The bacterial toxins are vegetable toxins since bacteria are microscopic plants. They have their analogies in the higher plants, for example, *ricin* extracted from the seeds of the common castor oil plant; *abrin* from the *Abrus precatorius* or jequirity; *crotin* from the plant *croton tiglium*.

Ricin inoculated subcutaneously can kill a rabbit in a dose of 0·1 mg. per kilo. body-weight; it agglutinates into masses and dissolves the red corpuscles of the blood. The agglutination is so rapid and powerful that it is necessary to keep shaking the tube in order to perceive the hæmolysis. The chemical nature of ricin is not exactly known; it is not absolutely certain that it is an albuminoid substance (Jacoby). Abrin agglutinates the red corpuscles but is not a powerful lysin. Crotin requires a dose of several centigrams per kilo. to kill a rabbit.

Toxins and Antitoxins.—A fundamental difference exists between the poisons of known chemical composition,

such as the alkaloids and glucosides, and the toxins properly speaking. The toxins alone produce antitoxins in the animal treated by graduated doses, *i.e*, the antitoxins employed in serotherapy. Antitoxins exist against ricin, abrin, and crotin, but there are none against solanin and saponin, which are glucosides. The glucosides although capable of being fixed by sensitive cells, do not produce anti-glucosides. Only those poisons which are capable of giving rise to antitoxins can be regarded as true toxins.

Endotoxins.

In contrast to the soluble toxins, the endotoxins are defined as the poisons contained in the bodies of bacteria and not spontaneously set free in cultures. Whereas the toxins are secretions of living bacteria, the endotoxins, according to the strict definition of R. Pfeiffer, are only set free by the destruction of the bacterium. It is the protoplasm of the bacterium itself which acts as a poison on absorption by the body.

To bring the endotoxins into line with a well-known example, one may compare them to the zymase of Buchner, the ferment of yeast not excreted, or scarcely excreted, but set free by grinding up with sand and expression of the juice under a pressure of several hundred atmospheres. But this comparison is not to be taken to mean that the endotoxins are diastases.

Bacterial extracts have been produced by Buchner's process, but nowadays endotoxins are obtained by simpler procedures. MacFadyen grinds up the microbes at the temperature of liquid air; others subject the cultures to combined maceration and shaking. Besredka takes young cultures, dries them, and re-suspends them in saline solution.

The endotoxins are distinct from the proteins; the latter are practically the same in the different species of bacteria. To deserve their name they ought to be specific and to give rise to antitoxins.

It is in the cholera vibrio, the plague bacillus, the typhoid bacillus, and the bacillus of dysentery that they have been chiefly sought for and thought to have been found. It is necessary to employ this qualified method of expression, because their definition and even their existence is still disputed. This chapter in the physiology of bacteria contains many uncertainties, and the facts observed cannot always be made to agree.

The endotoxins obtained by different workers from the same bacterium do not seem to possess the same properties, especially from the point of view of the toxic dose and the resistance to temperature; but it is also well known that with tetanus toxin different workers do not obtain equal specimens either.

Between the endotoxins described as having the same general characters there exist differences which are not to be found between the diphtheria and tetanus toxins; thus the plague endotoxin is destroyed by heat from about 75° C. onwards, whereas the typhoid endotoxin resists 127° C. (according to Besredka). But physical differences are known to exist between the diphtheria and tetanus toxins also, which, though less striking, are none the less real.

The dysentery endotoxin is easily obtained, the typhoid endotoxin less easily, the cholera endotoxin with great difficulty.

The following are the principal points disputed.

1. The fundamental phenomenon on which depends the existence of specific endotoxin is the toxicity of the bacterial bodies themselves. The nature of the bacterial bodies must first be agreed upon, and no agreement will be reached unless young bacteria are taken, avoiding as much as possible the alterations caused by manipulations, however carefully or cautiously conducted. Pfeiffer is inclined to regard with some suspicion the endotoxins which accumulate spontaneously in old cultures, considering them as products of a destruction of the bacteria, which is accompanied by chemical alterations (fermentative), of the details of which we are ignorant. These

errors may be avoided by employing young bacteria from cultures of twelve to eighteen hours on solid media.

2. But in many cases difficulties are met with when one attempts to determine the relations between this endotoxin and the soluble poisons secreted by the same bacteria and apparently true toxins. For example, the cholera vibrio certainly cont ins an endotoxin in Pfeiffer's signification, but it is none the less true that the cultures contain an excreted poison, the poison studied by Roux, Metchnikoff, and Salimbeni, on which the hope of a serotherapy in cholera has been founded. It is this poison which is absorbed into the body and produces the cramps, the chilling, and death, whereas the vibrios, however numerous they may be, remain in the intestine and only very rarely invade the blood and the tissues. "The free poison," says Pfeiffer, "does not exist in cultures except when these are already old and contain many vibrios already destroyed and autolysed." The cholera toxin of Roux and Metchnikoff is, however, chiefly secreted during the first days of a culture kept under the requisite conditions (a virulent vibrio and a well-aerated culture). This example is not in favour of Pfeiffer's opinion, at least in the case of cholera, for although he admits that the endotoxic bacteria can also secrete other soluble poisons, he maintains that the latter are quite different from endotoxins and do not possess their specificity.

3. Do the endotoxins give rise to antitoxins in the body of an immunised animal, as do the soluble toxins? This is the most disputed question of all. Without denying the existence of anti-endotoxins in principle, Pfeiffer's school considers that hitherto none have been obtained which have passed satisfactory tests, and that the sera prepared against plague and typhoid fever have not hitherto been successful precisely because they do not contain anti-endotoxin.

To prepare an anti-endotoxin, as in the preparation of an antitoxin, it is necessary to inject several times into an animal, for example, the horse, the toxic substance, in this case the bacterial bodies, entire or broken up. Good results are not obtained when endotoxins are injected subcutaneously; intra-

venous injection is essential. By intravenous injection of young microbes, Besredka obtained anti-endotoxins which, both *in vitro* and in the animal, neutralise the toxic action of the bacterial bodies. Since the production of antitoxin is the essential character of toxins, these experiments would prove the reality of endotoxins as specific poisons.

It is difficult to say to what degree the sera hitherto prepared against typhoid, plague, and dysentery are anti-microbic or antitoxic, *i.e.*, active against infection or against intoxication.

Although inferior to antidiphtheritic or antitetanic sera, the antidysenteric and antiplague sera have already given results sufficiently good to encourage us to bring to perfection the endotoxins and their anti-endotoxins.

CHAPTER IX

Tuberculin and Mallein—Animal Toxins—Venoms

Tuberculin and mallein—Koch's phenomen—Action of tuberculin—Local and general reactions—Resistance of tuberculin towards physical agents which destroy other toxins—Specificity—No antituberculin—Habituation to tuberculin—Cutaneous reaction of v. Pirquet—Tuberculin and anaphylaxis.

Animal toxins—venoms—The venoms in the animal kingdom—Snake poisons—Physiological action of venoms—Digestive properties—Hæmolysis by venoms—Rôle of lecithin—Lecithids—Natural immunity of certain animals towards venoms.

Tuberculin and Mallein

These poisons are found in old broth cultures of the bacillus of tubercle and the bacillus of glanders. They are prepared by combined maceration and heat from glycerine extracts of the cultures. They exist also in the bodies of the bacteria and are thus in a sense endotoxins. But hitherto no antitoxins to them are known.

Tuberculin and mallein from their physiological properties occupy a place apart. Tuberculin may be taken as the type.

Koch's Phenomenon.—The discovery of tuberculin originated in the "Koch's phenomenon": when tubercle bacilli are inoculated subcutaneously in a guinea-pig nothing is seen at the point of inoculation for from ten to fourteen days, then a nodule appears which later produces an open sore, which refuses to heal; the corresponding lymphatic glands are swollen. If, however, a guinea-pig already tuberculous is reinoculated after four to six weeks, there appears on the third

day, without any nodule developing, a necrosis of the skin over a zone of half to one centimetre; the necrotic patch becomes detached, and the ulcer heals up and closes without any swelling of the corresponding glands. The same process takes place when, instead of living bacilli, bacilli killed by boiling are injected the second time.

The tubercle bacilli therefore act in a different fashion in the healthy organism from the organism already tuberculous. Koch observed that a large dose of bacilli killed the tuberculous guinea-pig, whereas a very small dose produced an improvement in their condition and healed up the initial ulceration. He saw in this a principle of treatment. Since the bacilli are not readily absorbed, he replaced them by an extract from cultures; this was the original tuberculin.

This substance is practically harmless to non-tuberculous animals, but is fatal to tuberculous animals in a very small dose. In absolutely minimal doses, repeatedly administered, it exerts a curative effect on certain tuberculous lesions. According to the size of the dose, it can act as a poison or as a remedy. These are its fundamental properties.

Local and General Reactions.—Koch ascribed the curative effect to the necrotic action which he had noticed after reinoculation of bacilli in tuberculous guinea-pigs. Tuberculin he said in 1890, does not kill the bacilli, it kills the living tuberculous tissues; it does not even act on tissues already dead, such as the caseous masses. It acts on cells in the same way as the bacillus tuberculosis itself, but the soluble product has a much more extensive radius of activity than the bacillus.

In what way does this necrotic action become therapeutic? Because in a necrotic tissue the bacillus is badly nourished and grows feebly; the dead tissue becomes a sort of slough or sequestrum which the body strives to get rid of. The action of tuberculin is, in a sense, surgical. It might be hoped that every part affected by it might be thrown off, and this is sometimes possible in tuberculosis of the skin and of the lungs. In many cases, however, it is impossible, and it

has always been feared that tuberculin might cause a necrosis of the tuberculous tissue without completely killing the bacilli, and might thus set them free and inoculate them on a tissue till then unaffected.

When the tuberculin action does not quite reach the degree of necrosis, i. merely produces around the tuberculous focus an active inflammatory reaction with an afflux of leucocytes, which may build up a fibrous, cicatricial tissue in place of the tuberculous ulceration.

Tuberculin does not produce only local reactions; it provokes a general reaction of the body, the most obvious sign of which is fever. In large doses the reaction occurs even in a healthy individual according to R. Koch, who observed this in himself; but it is very probable that he had at that time some tuberculosis, and it has been maintained that tuberculin is entirely inactive in subjects who have never been attacked by the bacillus.

Three or four hours after the inoculation of $\frac{1}{4}$ c.c., Koch observed "twinges of pain in the limbs, a feeling of fatigue, and a tendency to cough. The symptoms became more pronounced, and about the fifth hour he had a violent rigor which lasted a whole hour with general uneasiness, vomiting, and fever (103°·3 F.). The symptoms began to settle about the twelfth hour, and on the following day the temperature was normal; a heaviness of the limbs and stiffness were perceptible for several days after. The point of inoculation remained red and painful for a considerable time."

In the treatment of a tuberculous patient with tuberculin, doses are employed which do not produce these violent symptoms. As far as possible no symptom, not even fever, ought to occur. A well-conducted treatment produces an improvement in many patients; this fact is certain, but the mechanism of these cures is not yet well understood. The indications and contra-indications for treatment are complex, and cannot be settled except by a physician with a large experience of tuberculosis and tuberculin treatment.

The febrile reaction which follows the inoculation of a dose

too small to be dangerous is employed in the diagnosis of tuberculosis, both among animals and in man.

Tuberculin is very different from other toxins. It bears much less resemblance than they to the ferments. In the liquid condition it stands a temperature of 120° to 150° C. In the solid state, heated dry in sealed tubes, it stands 250° C. "If it is a substance derived from albumins," said Koch, "it cannot be a toxalbumin in view of this resistance to heat and of its dialysing properties." It may be exposed to sunlight for months without losing its activity. Heating with acids (for example, $\frac{1}{50}$th hydrochloric) and with alkalies simply weakens it without destroying it. Perhaps it is not a simple poison: in the condition in which we get it, it has no more claim to be pure than our diphtheria and tetanus toxins. Maragliano thinks that it contains, besides the poison which causes the fever and is not a toxalbumin, a poison which, on the contrary, lowers the temperature of the body, is destroyed by heating to 100° C., and is possibly a true toxalbumin.

Tuberculin without tubercle bacilli does not reproduce tuberculosis. To provoke suppuration, caseation, and the typical lesion, the tubercle, living or dead tubercle bacilli are necessary: fluid tuberculin does not even produce the lesions which dead bacilli can give rise to: it has nothing in common with them but its destructive action on the cells and its power of raising temperature.

It is eminently specific, not producing any definite effects except in tuberculous individuals. In this it differs entirely from the other bacterial poisons. It only acts on a prepared soil, a soil prepared by the bacillus tuberculosis itself, *i.e.*, by an agent to which it is closely related both by origin and constitution. This specificity, though very marked, is not absolute. Tuberculin acts similarly, less, it is true, than in tuberculous individuals, but more than in healthy subjects, on patients affected by lesions resembling anatomically the tubercle, *e.g.*, glanders and actinomycotic nodules. This extension of its field of action is perhaps due to the close biological relationship between the tubercle bacillus and the

micro-organisms of these diseases, perhaps to the existence of the same anatomical type of lesion, the tubercle, or perhaps to similar inflammatory and phagocytic reactions.

Antituberculin?—Does tuberculin in animals, tuberculous or not, treated and habituated to its effects, give rise to the production of an antituberculin comparable to antitoxins or even anti-endotoxins? No; an antituberculous serum comparable to antidiphtheria or even antiplague sera does not exist, in spite of all efforts to discover it. Tuberculin in tuberculous subjects excites the production of certain reaction- or anti-bodies, but no true antitoxin. No treated individual has ever furnished a serum capable of neutralizing tuberculin either *in vitro* or *in vivo*, but serum of this kind can produce precipitates and clumps in a suspension of bacilli; this action is, however, inconstant and of little value in medicine.

Wasserman and Bruck have given the name of *antituberculin* to the reaction products which fix themselves on tuberculin as anti-bodies do on antigens (*v.* Chap. X.). The interpretation of these experiments is a question of some delicacy, and we shall see how doubtful are the relations between the presence of antibodies and the existence of an immunity, *e.g.*, a resistance in the case of tuberculous individuals.

Habituation to Tuberculin.—By careful, repeated injections the tuberculous subject may be made to protect himself against the fatal action of tuberculin, and to enjoy a general improvement in his condition, without stopping the progress of his tuberculosis. Guinea-pigs may be made to support fifty lethal doses yet their lesions progress in the ordinary way—or even more quickly than usual. It is not yet well known what are the relations between the physiological action of tuberculin and the progress of a chronic tuberculosis.

This habituation has been called immunity to tuberculin. It is not, however, an immunity or even a resistance to tuberculosis. It does not appear on repeated injections of *equal* small doses of tuberculin; in this case, the febrile reaction, which was absent at first, finally appears and becomes severe. The fever remains absent when one proceeds by *increasing* doses (always

to be done with caution). With small equal repeated doses, tuberculin behaves like a poison towards which the tuberculous patient becomes more and more sensitive.

Cutaneous Reactions.—A drop of very dilute tuberculin, applied by means a prick or very superficial scarification on the non-tuberculous skin of a tuberculous subject, excites at the point a reaction which may extend to the vessels and lympathic glands of the neighbourhood—V. Pirquet's experiment; this represents a new diagnostic procedure, the *cuti-reaction*. It has been modified and perfected by dropping the tuberculin between the eyelids (*conjunctivo* or *oculo-reaction* of Wolff-Eisner and of Calmette) or by inoculating it with a very fine needle in the depths of the skin itself (the *intra-dermo-reaction* of Mantoux).

It is a reaction of extreme interest, for it occurs at a non-tuberculous point, *i.e.*, a point containing no bacilli, in a tuberculous individual; and can only be explained by supposing that the whole body has become impregnated with substances formed under the influence of the tubercle bacillus.

Animal Toxins—The Venoms[1]

In the struggle for existence, certain animal species have acquired, as their means of attack and defence, organs which secrete and inoculate toxic substances. These animal toxins are the venoms, and such venoms are known at almost every level in the animal scale.

Venoms in the Animal World.—Among the Cœlenterata the Actinians produce certain poisons which can be extracted from their tentacles, *thalassine* and *congestine*, well known as having formed the subject of Ch. Richet's experiments on anaphylaxis. These poisons are perhaps the cause of the disease of sponge-fishers, who dive quite naked without a diving-suit; the disease consists in burning of the skin and swelling, with gangrene and violent fever.

The pedicles of the Sea-Urchins (Echinoderms) contain a

[1] *Vide* papers of Noguchi and Calmette.

poison which stands boiling. This poison, in nature, is dangerous to crabs and fishes; in the laboratory it is found to kill the rabbit.

Among the Arthropoda, the Spiders and Scorpions (Arachnidæ) secrete active poisons. The excretory tubes of the poison glands of venomous spiders open at the point of the two appendices which are furnished with claws at the end and situated on each side of the mouth. The poison kills the small animals on which the spiders feed, and causes in man pain and contracture at the bitten point—a sort of miniature tetanus. The poison of certain spiders contains a *haemolysin*, *i.e.*, it lakes blood, making the hæmoglobin of the corpuscles diffuse into the surrounding liquid (arachnolysin). The bite of the Tarantula (*Lycosa tarentula*) is only dangerous for the small animals on which they feed, and is quite harmless to man. According to Brehm, all the stories of the effects on man of the Tarantula bite are nothing but fables and fantasies.

The venom of the Scorpion (*Scorpio occitanus*) of the South of France can kill a guinea-pig in a dose of half a milligram of dry extract; for a rabbit one milligram. The scorpion is the subject of a legend which says that when it is enclosed by a circle of fire it commits suicide with its own poison. Now the scorpion is in reality immune to scorpion venom, towards which its serum acts like an antitoxin (Metchnikoff).

Among the Myriapoda the Centipedes, and among insects the Hymenoptera, secrete venoms. The poison extracted from two bees (by grinding up the terminal part of their bodies in 1 c.c. of water) is sufficient to kill by asphyxia a mouse or a sparrow. It also is a hæmolytic poison.

There are many poisonous fishes. As a rule their poison glands are found at the base of the dorsal or caudal fins, or under the spine of the gill-flap. These venoms all resemble more or less that of the weever-fish, which has been most studied. Locally it causes pain and swelling, with fever and vomiting. At the time of spawning the poison is more abundant and more active. The tropical tetrodons are most

venomous at the time of greatest activity of their reproductive glands.

The Toad (Batrachia) manufactures poison in its parotid gland and the glands of the skin, but it has no other way of secreting it than by contracting its skin and covering itself with a viscous, nauseating slime, which is rather poisonous when injected into small animals such as mice. Phisalix and Bertrand have extracted two poisons from the toad, '*bufotaline*' and '*bufotenine*,' a poison of the nervous system. At spawning time the cutaneous glands of the male toad are full of venom, whereas those of the female are empty; but the poison accumulates in her eggs, from which it may be extracted by means of chloroform.

The Salamanders possess on their sides and tail poison glands, and it is to the fluid which these secrete that they owe their fame as animals capable of living in fire and even of extinguishing it—pure legend, of course. Their secretion permits, at most, of their surviving a few seconds.

One venomous animal exists among the mammals, the Ornithorhynchus. Its poison gland is situated on its thigh, and the secretion escapes by a spur or claw on the hind feet. The poison resembles that of the snake *Lachesis*, but is much more feeble.

Medical men have been especially attracted to the study of snake-venoms. The vipers of our own country have few victims, but in India the cobra kills as many as an epidemic disease. In 1889, in India, 22,480 human beings and 3,793 domestic animals died of snake-bite. Of those bitten 25 to 30 per cent. die within ten or twelve hours. The importance is obvious of the researches which led to the antivenom serotherapy (Calmette).

As they issue from the glands the venoms resemble a thick, oily saliva more or less yellow. Their physical properties vary a good deal with the genus. The venoms of the *Viperidae* do not dialyse through a membrane and are destroyed entirely at 75° to 80° C. (*Lachesis* even at 65°). Those of the *Colubridae* pass slowly through vegetable membranes, with

greater difficulty through animal parchment: they resist heating at 100° C. and are only completely destroyed at 115–120°.

From the chemical point of view both consist of proto- and deutero-albumoses: the albumins they contain are not toxic. S. Faus. has extracted from cobra venom an "ophio-toxin" which contains neither nitrogen nor sulphur nor phosphorus.

By using dried venom, which can be accurately dosed by redissolving in a known volume, it has been possible, as with the vegetable toxins, to determine the minimal lethal dose per kilogram in different species of animal. As with tetanus toxin the size of the animal bears no relation to its sensitiveness. With one gram of dry cobra-venom it is possible to kill 1,250 kilos. weight of dog, 2,000 k. of rabbit, 2,500 k. of guinea-pig, 1,430 k. of rat, 8,333 k. of mouse, 20,000 k. of horse, and 10,000 of man, *i.e.*, 165 adults of average weight. The horse is thus the most sensitive of all these animals.

The toxicity of the venom is very variable: it is more active (and doubtless less abundant) after the casting of the skin and after a prolonged fast.

Physiological Action of Snake-venoms. — The physiological action of snake-venoms is complex. They act on the cells of the organs, on the liver, the kidney, and the spleen: on the endothelial cells which line the interior of the blood vessels (especially the rattlesnake poisons): on the nervous system (according to Rogers the venoms of the *Viperidae* paralyse the vasomotor centres, those of the *Colubridae* the respiratory centres): on the blood, one of the oldest known effects and one much studied recently because the solution of the red corpuscles or *haemolysis* is a phenomenon very obvious and easy to study *in vitro*. The condition of the blood at autopsy varies according to the dose of the poison and the time; this is why the same poison is called coagulant or anticoagulant by different workers.

Snake-venoms have the properties of digestive ferments. They can dissolve coagulated blood and can destroy the cells of the vessel coats and even of the muscles. Cobra-venom

digests albumins but without reaching the peptone stage. The digestive properties are destroyed by heating to 70° C.

Pancreatic juice, as is well known, when perfectly pure cannot alone digest albumin; it has to be "activated" by a "kinase" ferment, secreted either by the intestinal mucosa or by the leucocytes. Now snake-venoms can take the place of this kinase and activate a pure inactive pancreatic juice. This is all the more curious since the pancreatic juice when activated digests and destroys venoms, which, as a rule, have in consequence no action when taken by the mouth. The venom secretion is thus for the snake itself a normal physiological secretion of great use in the digestion of the huge meals for which snakes are famous. There is further nothing surprising in the fact that non-venomous snakes, *i.e.*, those not provided with poison-fangs, still possess glands capable of secreting venom; it is simply used in the digestion of their food.

Venoms thus, like toxins, resemble ferments—with the same reservations. They are very closely allied to toxins. Their action, like that of toxins, is not simple; just as in tetanus toxin several different substances or functions can be distinguished, a nerve-cell poison and a poison of the red blood corpuscles, so several physiological activities can be distinguished in the same venom. But venoms act without any incubation period, or at any rate with a very short one. It is only the time elapsing between inoculation and death that varies with the dose, and that within rather narrow limits.

Like the toxins, the venoms are destroyed at relatively low temperatures (resistance up to 100° to 110° C. does not interfere with the analogy). They act in minute dose and deteriorate or are destroyed by light, by photodynamic substances, by iodine perchloride, and alkaline hypochlorites.

Finally, and most important, the venoms give rise to *antivenoms*, as toxins do to antitoxins. It is practically only with the help of the antivenoms indeed that the specificity of the venoms can be definitely demonstrated.

Venom Hæmolysis.—The venoms are hæmolytic

poisons, and since hæmolysis is a phenomenon much more convenient to follow experimentally than paralyses or nervous symptoms, they have been much studied, and from certain points of view are better known than the microbial toxins. The study of the toxins has profited by that of the venoms. Thanks to some beautiful experimental results, there is reason to believe that, with the help of the venoms, the study of toxins in general may make the advance so much desired by science, and from a physiological subject, studied only in the living animal, become a part of chemistry with its definite reactions.

Some definitions and examples must first be given.

When a rabbit is repeatedly inoculated with, for example, defibrinated sheep's blood, the rabbit's serum acquires the property of dissolving the red corpuscles of the sheep: these latter suspended in physiological saline solution in a test-tube with a little of this rabbit's serum, instead of settling intact and leaving a colourless supernatant fluid, break up, liberate their hæmoglobin, and colour the fluid red. The rabbit's serum has become *haemolytic* for the red corpuscles of the sheep.

Bordet has shown that hæmolysis depends on the operation of two substances, or rather of two functions, of which we shall have much to say in connection with immunity: one is the *alexine* or *complement* of normal serum (destroyed by heating to 56° C. for one hour); the other is the *sensibilisatrice* or *immune-body* of the serum of immunised animals, such as the rabbit above mentioned (it stands heating to 68° or 70° C.).

The latter owes its name to the fact that it prepares or renders *sensitive* the red corpuscles towards the action of the complement. The complement completes the action of the "sensibilisatrice," hence its name. If we take the blood corpuscles of a goat and add a little cobra-venom, hæmolysis occurs. But if the blood corpuscles are first carefully washed with physiological saline so as to be entirely freed from traces of blood serum which might adhere to them, no hæmolysis

occurs when the venom is added. But if now to this mixture of carefully washed goats' corpuscles and venom a little normal blood serum is added, hæmolysis proceeds at once. It would seem from these facts that the venom acts like a sensibilisatrice or immune-body, the normal serum providing the alexine or complement.

But there are other facts which forbid this interpretation. Normal serum activates the venom, it is true, but even when heated to 65° C. or higher it still activates: there exist even normal sera which cannot activate venom hæmolysis until they have been heated at 100° C. It is inconceivable that it is the complement which is the active agent since complement is destroyed at 56° C.

Further, washed corpuscles of certain animals are laked by the venom without any addition of fresh serum (corpuscles of dog, rat, guinea-pig, and man). Again, in the case of the washed goats' corpuscles, normal serum is not the only substance which can activate: laked red corpuscles, *e.g.*, of the guinea-pig, can take its place quite well. Finally, in this last example it is not the fluid which acts, but the stromata or bodies of the corpuscles which have shed their hæmoglobin, and these still possess their activity after heating to 100° C. It is thus impossible to attribute the activating action to the complement or even to the serum as a fluid. The active substance is not the complement nor is it an albumin, for albumin coagulates below 100° C. It is not a ferment, for a ferment heated in solution above 100° C. is no longer active. It is a definite chemical substance present in the serum and in the stroma of the blood corpuscles, namely *lecithin*. Lecithin is a well-defined chemical body, unlike albumin, for which we are unable to write a formula, still more unlike the complement and the immune body which, like the ferments, are known as activities or properties, not as substances. In venom hæmolysis, therefore, our knowledge is more complete and clear than in the hæmolysis of hæmolytic sera.

Lecithids.—When blood of any species is easily laked by venom without addition of serum (*e.g.*, in man, the rat,

and the guinea-pig) it means that the lecithin of the corpuscles readily detaches itself and unites with the venom. When the blood is laked only when serum is furnished in addition, it means that the lecithin of the globules themselves is firmly bound and difficult to set free. In certain cases heating to 65° C. or even higher is necessary to liberate lecithin from its combination with hæmoglobin. The experiments of this kind have many complications of detail, since the three factors coming into play—the blood corpuscles, the lecithin, and the venom—are subject to many variations. A step in advance has been made in what may be called the mechanical or purely chemical explanation of hæmolysis by the discovery that lecithin forms with venom a combination of a chemical character in which neither lecithin nor venom can be recognized. Kyes has named this combination or "couple" *lecithid*, or since his experiments were on cobra-venom, *cobra-lecithid*.

In its physical properties (solubility in water, alcohol, ether, chloroform, and acetone) the lecithid differs from lecithin as much as from venom. It can be isolated in a crystalline form and redissolved in water. It acts on the blood of all animal species, and that without any incubation period. The delay observed in the action of venoms is not a period of incubation, but merely represents the time necessary for the formation of the lecithid compound. If the ready-made lecithin is added to the blood the hæmolysis is more rapid than on the addition of the two elements separately.

Strictly speaking, however, the venoms do not act without incubation: the time taken by lecithid formation represents the minimum incubation period. It is quite possible that in the action of microbial toxins there may occur a slow formation (*i.e.*, with a longer incubation period) of compounds analogous to lecithids.

Cholesterin behaves as an antagonist to lecithin; it has no effect on complement, but prevents the combined action of the lecithid by affecting the lecithin; it thus forms a sort of *antihæmolysin* or *antitoxin the composition of which is definitely*

known. It is one of the dreams of biological chemistry to discover an equivalent to cholesterin for the other toxins.

Still more interesting is the fact that the peculiar anæmia produced by injecting cobra-lecithid into an animal can be prevented by giving cholesterin. It would seem that cholesterin acts towards lecithin and lecithid, not only as an antagonist, but as a remedy.

There are, of course, facts which prevent us from regarding as entirely similar the cobra-hæmolysin, on the one hand, and the solvent properties or hæmolysins of normal and immune sera (sera derived from animals prepared by the injection of blood corpuscles) on the other. These, however, do not invalidate Kyes's conclusions in his experiments on the lecithids. The study of snake-venoms has shown a participation of chemically-defined substances in phenomena hitherto only known from the biological point of view. It would be of immense interest, both theoretical and practical, to discover an analogous mechanism in the effects of toxins.

Certain animals enjoy a natural immunity towards snake-venom, which, however, is never absolute. It is possible that these animals have received from generation to generation small doses of the venom as the result of being bitten, and that they have in consequence elaborated an antivenom. This forms the starting-point of artificial immunisation and antivenom serum-therapy. It is known that the blood of certain animals possesses normally a weak antitoxic action against diphtheria and tetanus toxins; these animals similarly must have harboured tetanus and diphtheria bacilli.

The animals possessing the most remarkable resistance towards snake-venoms are the hedgehog and the mongoose. To see the dramatic combats which take place when the mongoose tackles the cobra, one has only to read Kipling's marvellous tale of the war between "Rikki-Tikki" and "Nag" in the *Jungle Book*.

CHAPTER X

IMMUNITY

Phagocytosis and Humoral Immunity

Early ideas of Pasteur on immunity—Opposition to the phagocytic doctrine—Cellular and humoral immunity.
Antigens and Antibodies—Complement—The two substances: Bordet's experiments.
Phagocytosis a fact capable of direct observation—Ferments of the leucocytes—Analogies with the digestive ferments.
Pfeiffer's phenomenon—Opsonins and bacteriotropins—Antibodies not an exact measure of the immunity.

It is not necessary to have studied medicine or science in order to ask oneself what is this immunity which appears in infectious disease. Certain bacteria are pathogenic for certain animal species and not for others; the guinea-pig, for example, does not take fowl-cholera and the fowl does not take anthrax. Among people living in one family under the same conditions and among soldiers in the same barracks living under the same rules we see disease attacking some while others remain free. Finally it is a popular conviction that anthrax does not occur twice and that, as a rule, once an individual has had measles or small-pox he never takes it again: these are everyday examples of acquired immunity.

"Immunity against infectious diseases ought to be understood to mean the sum total of all the phenomena to which is due the resistance of an animal body to the microbes which produce these diseases" (Metchnikoff). Immunity may be innate or acquired. Natural acquired

immunity appears when there is spontaneous recovery from an infectious disease. Immunity the result of human interference (vaccinations, serotherapy) is artificial acquired immunity.

After his work in collaboration with Chamberland and Roux which established the attenuation of viruses and the value of preventive inoculations, Pasteur, being a chemist, conceived immunity as a chemical process. He considered that the reason why the bacillus of fowl-cholera fails to grow in the fowl vaccinated against this disease was that the body of such a fowl no longer contained the necessary food-stuffs for the development of the microbe. The muscle which has been severely affected by disease has become, even after complete recovery, in some way incapable of supporting the life of the microbe, as if this latter during its previous growth had made to disappear from the muscle some principle which life is incapable of renewing and the absence of which prevents the development of the micro-organism.[1]

He filtered a culture of the fowl-cholera bacillus and found that a re-inoculation of the bacterium in the fluid thus freed from the first germs always failed: when fresh nutritive substances were added to the filtrate, growth took place. It was not, therefore, the presence of some excretion, but the absence of some nutritive substance which explained "the immunity of a culture filtrate, or of the fowl considered as a natural culture medium."

In natural innate immunity also he refused to recognise the presence of an inhibitory substance, basing his faith on the celebrated experiment of the fowl refractory to anthrax but rendered susceptible by chilling, and appealing to the "constitution" or to a "vital resistance," by which he conceived a struggle between the parasites and the body-cells for the oxygen and food materials available. But when it was found that bacteria could grow perfectly in the blood of animals possessing a complete immunity, Pasteur's early conception could no longer be maintained in its primitive simple form.

Even to-day immunity has still to be defined as a complex

[1] *C. R. Acad. des Sciences*, 1880, p. 247.

of biological phenomena, for in spite of the hope of men of science some day to get beyond "vitalistic" explanations, no explanation in chemical terms can yet be given. Immunity is a function of the cells. Immunity means phagocytosis. Further research may fathom the nature of this activity and give a chemical explanation as in the case of peptic or pancreatic digestion, but the cellular activity is indisputable and is not a theory but a collection of facts, a doctrine in the true sense.

The principles of this doctrine of phagocytic immunity have already been indicated in the chapter on inflammation. It is necessary to read in Metchnikoff's book his "historical review of our knowledge on immunity" (Chap. XVI) to comprehend how much his doctrine has developed.

From the historical point of view it had to oppose the ruling conceptions, not only in medicine, but in pathological anatomy and in physiology. The few observers who had seen microbes inside the white corpuscles had never deduced from this a protective function: quite the contrary, for authorities of the rank of Waldeyer and Robert Koch firmly believed that the microbes found in the leucocytes only a field of growth and a means of dispersion throughout the body. Haeckel, also, had no idea that the presence of foreign particles in the amœboid cells was the result of an active engulfing process. The development of the phagocytic doctrine brought it into opposition to the humoral theory, which was sustained under the most varied forms by the most celebrated supporters. As with many other doctrines which have eventually been admitted as scientific truths, the doctrine of phagocytosis was revolutionary in conception and had to conquer by main force.

It originated in zoology and is a result of the comparative method. From the study of the biology of organisms low in the scale of life, stage by stage it gained the field of medicine. These stages we have indicated in the observations and experiments already described in connection with the digestive activity of the mesodermic cells, intracellular digestion in general, the reaction of Bipinnaria to the introduction of splinters, the diseases of such lower animals as are transparent

and suitable for observation in the living condition, *e.g.*, Daphnia, and finally the infectious diseases of animals and man. "I have sought to develop the conception that the intracellular digestion found in unicellular organisms and in many invertebrates has been transmitted by heredity to the higher animals and in them has become fixed and preserved in the amœboid cells of mesodermic origin." Phagocytosis is in harmony with the Darwinian principles of evolution among living beings.

The essential fact of immunity is the intracellular absorption and digestion of microbes and probably of toxins under precisely the same conditions as in the absorption and digestion of cellular elements and albuminoid fluids in general when introduced into the body.

The general laws are the same whether it is a question of the absorption of extravasated blood after a wound or an internal hæmorrhage, or of blood corpuscles injected into the peritoneal cavity of a guinea-pig ; whether one is dealing with cells so diverse as spermatozoa or epithelial cells, injected into the peritoneum of a foreign species, or with complex albuminoid fluids such as blood-serum, milk, egg-albumin, or finally with the bodies or toxins of bacteria. Laying aside the historical development let us now attack the mass of facts accumulated on the subject of immunity.

Taking a general view of the observations and interpretations which are multiplying every day but are far from being universally clear or certain, two points of view are continually being opposed to each other, *the activity of the cells* and *the activity of the body-fluids* ; the cellular theory and the humoral theory of immunity.

The supporters of the *cell theory* do not deny the participation of the body-fluids separated, more or less artificially, from the cells, *i.e.*, the phagocytes ; but they maintain that the cells are the primary and principal agents, the humoral properties being secretions or excretions of the phagocytes, and the final stage in the destruction of the microbes being digestion in the interior of the phagocytes.

The supporters of the *humoral theory* consider that in immunity the body-fluids (serum, exudates, &c.) possess or acquire destructive properties independent of the cells; that there is a non-phagocytic destruction of bacteria (and poisons), and that even when this destruction appears to be completed in the interior of the leucocytes, the rôle of the latter is limited to seizing and absorbing bacteria already killed.

Of course the antagonism between these two standpoints is not irreconcilable, and intermediate theories exist. The priority of the cells, however, as compared with the fluids

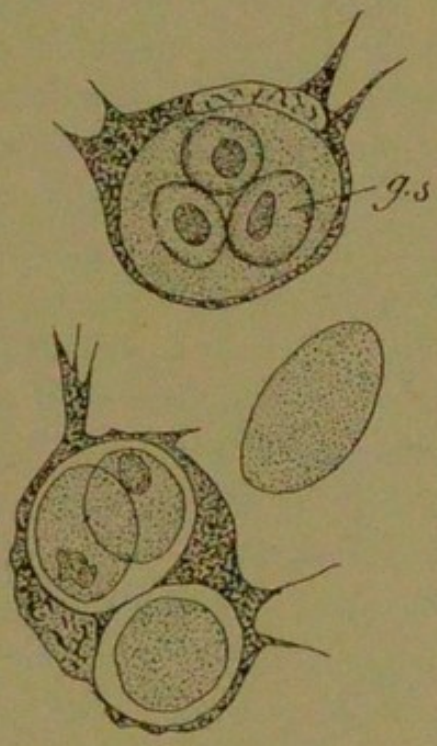


FIG. 63. — Phagocytosis of the red corpuscles of the goose by the phagocytes of the snail. (Metchnikoff.)

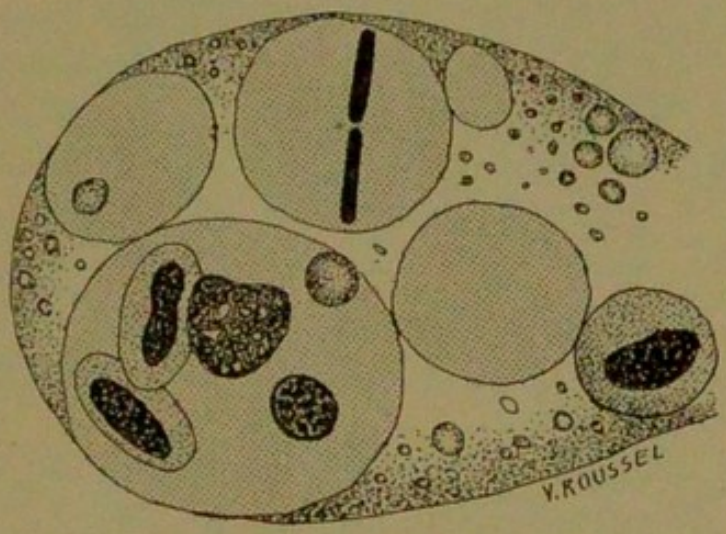


FIG. 64.—Intracellular (phagocytic) digestion in an intestinal cell of *Planaria*. (Metchnikoff.)

independent of the cells, is still the subject of dispute; no theory succeeds in explaining the facts of immunity without acknowledging the activity of the phagocytes and the importance of intracellular digestion.

The humoral theory first took the field with claims or aspirations to be a "chemical" theory, when some at least of the phenomena of immunity were successfully reproduced out-

side the body in the test-tube. It must be carefully kept in view that all the most plausible arguments in its favour are supplied by "*in vitro*" experiments. It is a laudable tendency to attempt to reduce biological phenomena to a mechanism reproducible at will, but it must not be allowed to distort the facts of nature. The study of immunity is, above all, the study of an infected body defending itself. We know neither the nature nor the composition of the substances concerned, albuminoid or otherwise: we are not even always sure that the substances postulated really exist. Too often we yield to the tendency to describe as substances what we only observe as functions; these functions have been symbolised by names and by signs, and some have even come to see in them actual things and things with an actual shape. It cannot be denied that at present, while we are still awaiting the advances so generally longed for, vitalism (in the sense in which certain critics of the phagocytic theory employ the word) represents the most realistic conception.

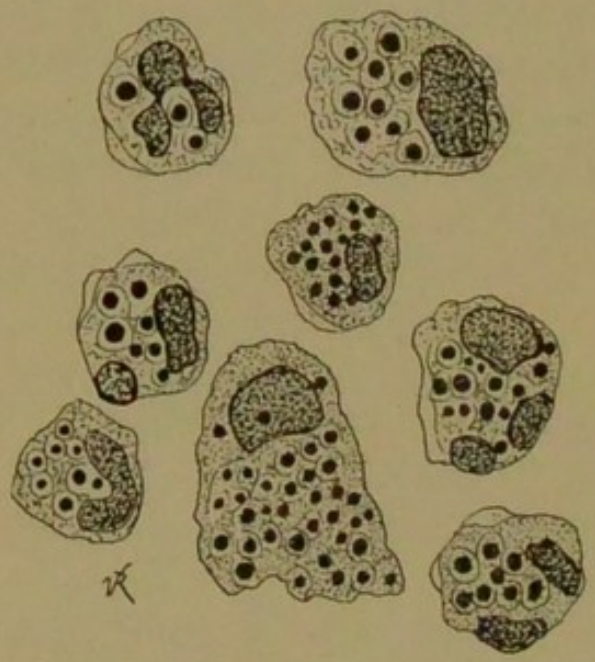


FIG. 65.—Phagocytes taking up spores of the tetanus bacillus (heated).

Metchnikoff has therefore never ceased to recall and emphasize the differences which separate the corresponding (one cannot say "the same") phenomena "*in vivo*" and "*in vitro*": not that he disputes the importance of the latter, but to emphasize the necessity of associating them always with the phenomena in the living animal. *Experiment ought always to deal as much as possible with the living creature itself.* The cells and the body-fluids are hardly to be treated as substances capable of preservation in bottles, and this fact we will have occasion to recall more than once.

Antigens and Antibodies.—The cells which play a part in immunity are known: they are the phagocytes, the micro- and macro-phages. The humoral properties correspond to what are known as antibodies.

The antibodies are the products (substances or properties) of a reaction of the body towards a natural or artificial introduction into it of certain foreign substances, bacteria and their poisons, vegetable poisons of other kinds, and various albuminoids all known by the name of *antigens*. The exact definition of an *antigen* is its capacity of exciting in the injected (or infected) body the production of an antibody.

The discovery of the antibodies was so much more a splendid biological acquisition in that its practical importance was at least equal to its theoretical. The first antibodies studied were the antitoxins of diphtheria and tetanus. The discovery of the diphtheria toxin by Roux led to the discovery of its antitoxin in the hands of Behring. The neutralisation in an ordinary test-tube of a toxin by an antitoxin was one of the first and most brilliant "*in vitro*" experiments in immunity. It might certainly seem that this neutralisation could take place equally simply in the living animal with no intervention of the cells, but like a chemical combination.

When an animal of species A is injected with the red blood corpuscles of an animal of species B, the serum of the former acquires the property of dissolving the globules of the other species: it becomes hæmolytic and the prepared animal is said to have developed a *hæmolysin*. When the body is vaccinated against the typhoid bacillus, the serum acquires the property of agglutinating a homogeneous suspension of typhoid bacilli: it is said to have produced an *agglutinin*. The serum of an animal A which has been injected with the blood or the serum of an animal B of a different species forms a precipitate when to it there is added a little of the serum B; there is said to have been developed a *precipitin* to B.

Hæmolysins, agglutinins, and precipitins are the antibodies of which the blood corpuscles, the bacteria, and the serum-proteins are the antigens.

Complement or Alexine.—Before the discovery of the antibodies, at the time when attempts were being made to transfuse human patients with the blood of other mammals, in particular of the sheep, it had been noticed that the normal blood-serum of certain animals destroys the red corpuscles of other species: Buchner attributed this property to a defensive substance which he called *alexine*. Its chemical composition is unknown; it is thought to be an albuminoid substance: it is known to disappear from serum on dialysis and to act after a period of inactivity or incubation; it is destroyed by temperatures about 56° C., and only acts in presence of salts. Buchner classes it along with the digestive ferments.

The serum of many animal species, expressed from the blood after coagulation, possesses the property of killing *in vitro* many infective bacteria without any apparent assistance from the body-cells: this destructive action is similarly attributed to the alexine or complement, and it is this bactericidal property which represents the simplest and crudest fact on which the whole structure of the humoral theory of immunity has been reared.

The Two Substances.—Bordet showed in 1895 that the serum of an immunized animal contains two substances, or rather two functions. Take a guinea-pig which has received intraperitoneally spaced injections of cholera vibrios: its serum now destroys these *in vitro*, and is said to be bacteriolytic. Heated to 56° C., it loses this property, but on the addition of a little fresh serum, recovers it. The bacteriolytic action thus demands primarily a substance present in the fresh normal serum of any animal species (non-immunized), a substance which heating to 56° C. destroys: it is the alexine or complement. But it demands also another substance present in the serum of treated animals and absent in normal serum, which is not destroyed by heating under 65° C.

Bacteriolysis (the destruction of bacteria by serum) is thus due to the collaboration of two functions, by custom regarded as two substances. One, thermolabile, is the alexine or complement and exists in all freshly prepared normal sera.

The other, thermostable, only exists (with rare exceptions) in treated immunized animals: in the example given above it makes the cholera vibrio susceptible to the action of the alexine or complement, or it may be said to fix this to the bacteria, or, finally, it may be regarded as forming the connecting link between the alexine or complement and the vibrios. It has been called by Bordet the '*sensibilisatrice*.'[1]

The idea of two substances is not a theory, as Bordet remarks with great justice; there is nothing hypothetical in it. It is simply putting in words the facts observed, in particular those of re-activation.

Hæmolysis, agglutination, precipitation, in a word, all the reactions in which antibodies play a part, proceed in the same fashion and Bordet's discoveries have thus a general application.

A fundamental experiment performed by Ehrlich and Morgenroth completes Bordet's observations. The immune body becomes fixed on the antigens (bacteria or blood corpuscles) without producing in them any visible modification, so that eventually the fluid containing it is completely robbed of immune body; this fixation prepares the way for the destruction of the corpuscles or the bacteria, but this latter does not occur till the complement is added. On the other hand, the complement does not fix itself to the antigen when alone, but only by the intermediation of the immune body.

This collaboration of two substances or ferments in one complex physiological process is not the sole example in biology. The digestive ferments of the pancreas, amylase, saponase, and especially trypsin, fail to exert their full activity without the collaboration of the enterokinase, a ferment secreted in the juice of the small intestine not by the cells and glands of the mucous membrane itself, but by the lymphoid tissue, which is composed of white corpuscles: the importance of this fact will be seen (Pawlow; Chepowalnikoff; Delezenne).

[1] The alexine is called by Ehrlich the *complement* or complementary substance; the sensibilisatrice is often called the immunizing substance; the intermediate substance, *immune-body* or amboceptor.

The antibodies quoted may themselves give rise to antibodies : anticomplements and anti-immune bodies may be prepared.

We know now all the factors which come into play in the phenomena of immunity : on the one hand cells, the phagocytes ; on the other the body fluids, containing ferments the actions of which supplement each other, the immune body and the complements ; we know also that the experiments of bacteriolysis and hæmolysis *in vitro* appear to indicate that the chief phenomena of immunity are independent of the phagocytic cells. Let us now examine the problem more closely and see if phagocytosis stands the test brought against it by the humoral theories.

Phagocytic Immunity

In every case in which the body possesses immunity the bacteria against which immunity exists are devoured by the phagocytes, which collect in crowds, incorporate, and digest them. " Looked at from this standpoint immunity becomes a phenomenon much more general than a mere resistance of the body to infectious disease." On ultimate examination it reduces itself to a phenomenon of cellular susceptibility, of chemiotactic influences, and of intracellular digestion, *Immunity is a phenomenon of digestion.*

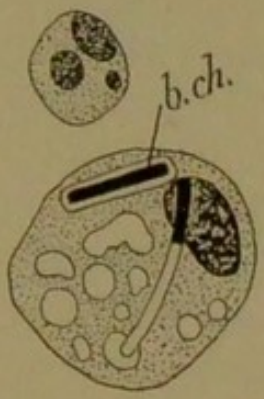


Fig. 66. — Phagocytosis of anthrax bacilli by the macrophages of the rat's liver. (Metchnikoff.)

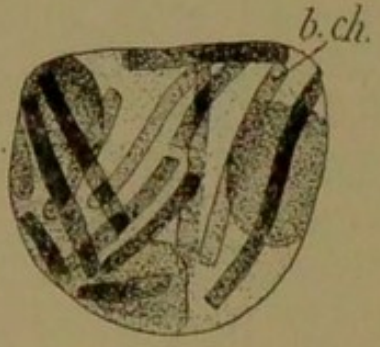


Fig. 67. — Microphage of the rat full of anthrax bacilli. (Metchnikoff)

Phagocytosis can be directly observed in many cases of natural immunity: the disease of Daphnia presents one of the simplest and most typical examples, and similar ones have been observed among other invertebrates. Among the vertebrates the frog owes

its resistance towards the anthrax bacillus to phagocytosis, the same bacillus growing excellently in the body-fluids deprived of cells.

Similarly, the anthrax bacillus grows very well in the body of a fowl, although the fowl is very resistant to inoculation. The effect of cold in rendering it susceptible (the famous experiment of Pasteur) is to be ascribed to a benumbing of the phagocytes. In the case of the dog resistant to anthrax, or of the guinea-pig to the spirillum of relapsing fever, or to the cholera vibrio injected in small dose in the peritoneum, the engulfment and digestion of the microbes by the phagocytes are visible facts and the figures appended are better than any description.

On the other hand, the bacteria cannot be said to be expelled from the body through the various excretory organs. They are never found in the urine, provided the kidney filtering action is intact. They are never found in the sweat unless by faulty technique a little infected blood gets mixed with it.

There is no digestion, even intracellular, without digestive ferments. Under the microscope the digestion of the ingested microbes can be seen going on in the digestive vacuoles of the phagocytes, and by means of the dye, neutral red, the acidity of the part in which digestion is proceeding is equally easy to demonstrate, as in the case of the digestive vacuoles of a myxomycete or an amœba, or as in the intestinal cells of Planarians or Actinians. Metchnikoff considers that there are two varieties of leucocytic digestive ferments corresponding to the two great groups of phagocytes, the macrophages, which digest chiefly the cellular elements and the bacteria of chronic infections such as the tubercle bacillus, and the microphages which digest chiefly bacteria. They can be obtained by making extracts of those organs which are rich in phagocytes, the lymphatic glands, the spleen, and the bone-marrow. In natural immunity the digestive ferment of the leucocytes is simply the *complement*.

There have been, however, many disputes regarding this point and regarding the origin of complement. Certain observers have recently maintained that the complement has

nothing to do with the white corpuscles. They have made extracts of leucocytic exudates withdrawn from the body, and shown that these extracts were either without bactericidal power or that the bactericidal substance they contained possessed properties quite different from those of complement.

It is true that in extracts of white corpuscles prepared by maceration or freezing no complement can be detected capable of destroying bacteria, but such experiments do not prove that the production of complement by these is impossible. The complement may easily be lost in the course of the maceration and freezing, rather brutal processes in any case for living cells. It is also possible that the complement may be neutralised by some antagonistic substance contained in the leucocyte, some sort of anti-complement: we are certainly far from knowing all the substances contained by leucocytes. It is conceivable that they may respond to a slight injury, received in the course of the manipulation of the blood, by discharging into the surrounding fluid complement alone, whereas when more seriously injured they may discharge the neutralizing substance. Later it will be seen that Pfeiffer's phenomenon when correctly interpreted supports this view: in the body thoroughly immunized against the cholera vibrio but with the white corpuscles uninjured, the vibrios are not destroyed by the body-fluids and are altered only in the interior of the cells.

According to Metchnikoff the complement is *secreted* by the phagocytes, never *excreted*, *i.e.*, poured out into the serum or the body-fluids, so long as the phagocytic process remains normal; it is only discharged when the phagocyte has been injured or *phagolysed*, as this semi-destruction has been called. It resembles the zymase of the yeast cells of beer, which are only liberated by processes which break up the cell. The fact is one of great importance, as will be found again in the discussion of Pfeiffer's phenomenon: the complement action never takes place outside the bodies of the phagocytes except when there has been phagolysis.

In acquired immunity, *i.e.*, in an animal which has

recovered from an infection or which has been treated in the laboratory, other ferments develop which did not exist, or hardly existed, before the appearance of immunity: these are the sensibilisatrices, or immune-bodies or amboceptors of Ehrlich. They are secreted by the macrophages to some extent, but chiefly by the microphages, and are found in the spleen, in the lymphatic gland, and the bone marrrow at a stage in the immunizing process when they are still absent from the blood. They resist a higher temperature than the complement, and have properties resembling the ferment *enterokinase* of the small intestine. Just as the enterokinase prepares fibrin for the action of trypsin, so the immune-bodies prepare the bacteria or other cell-elements for the action of the complement: this is an analogy between extracellular and intracellular digestion which ought to be emphasized.

There is not in any given animal a series of different complements: the complement from the same animal performs indifferently hæmolysis and bacteriolysis, and dissolves equally well the typhoid bacillus and the cholera vibrio. The immune-bodies, on the contrary, are specific, being developed during the immunization against the invading cells (by inoculation or natural infection).

Complement is discharged into the fluids bathing the phagocytes only when phagolysis has occurred: the immune-bodies, on the other hand, are readily excreted by the phagocytes; they resemble, not zymase, which is firmly bound to the protoplasm of the yeast cell, but sucrase, which is easily discharged. Recovery or inoculation does not increase the quantity of complement, but greatly develops the quantity of immune-body. In natural immunity the presence of immune-body is difficult to demonstrate, probably because there is little of it in existence, and what there is is contained in the phagocytes; but in acquired immunity immune-body is abundant and is found, not only in the plasma and serum of the blood, but in exudates and œdematous fluids. Examples of acquired immunity exist in which the body remains poor in immune-body and in which the body-fluids entirely lack it; in these it is necessary to

assume that the action goes on in the interior of the phagocytes.

To refute the old opinion that the leucocytes form a good culture medium for bacteria and serve as convenient vehicles for them, it has been necessary to show that in the phagocytes the bacteria die and are digested. To refute the opinion that the phagocytes simply incorporate bacteria already damaged or killed by other (humoral) actions, it has been necessary to show that bacteria are ingested in a living and virulent condition: as a matter of fact living motile bacilli (*B. pyocyaneus*) can be seen in the interior of a frog's leucocyte. Fatal anthrax can be produced in a guinea-pig by inoculating anthrax bacilli already engulfed by the phagocytes of a frog: it is only necessary not to wait too long, or digestion in the leucocyte may be completed. Pasteur noted that it was perfectly easy to kill the fowl and the rabbit by inoculating them with bacilli of fowl cholera already incorporated by the leucocytes of the refractory guinea-pig. The bacteria attacked by the phagocytes are therefore thoroughly alive and virulent.

If we take an animal immunised against vibrios and inoculate it with the same microbes against which it is immune; if we withdraw now a drop of the exudate provoked by the inoculation and make of it a hanging drop in a sealed chamber at incubator temperature, we find that the phagocytes thus withdrawn from the body promptly die and the bacteria grow in their interior as if in culture. But from the phagocytes withdrawn from the animal a little later no such culture can be obtained; the phagocytes have had time to digest the bacteria. No better conception can be furnished than this of the life and death of bacteria in the phagocytes.

It has been said that the body-fluids have already attenuated the virulence of the microbe before its capture by the phagocyte. If such previous attenuation exists their remains still to be settled whether it is due to a cellular or to a humoral action: in any case it is far from being the rule. In Charrin and Roger's experiments the streptococcus, the pneumococcus, and the pyocyaneus bacillus grown in the serum of an immunized

animal no longer killed fresh animals : but this was due to the fact that they were saturated with the immune serum which contained immune-bodies : deprived of these by thorough washing, they regained their original virulence.

It has further been said that the microbes act through their toxins and that the body-fluids of an immunized animal begin by neutralizing this toxicity, after which the bacteria fall easy victims to the phagocytes. But if this were the case why should there be such profound differences between the immunity towards the microbes and that towards their toxins? Why should there be in animals immunized against the bacillus pyocyaneus or the cholera vibrio a complete resistance to infection with these microbes along with a susceptibility to the toxins equal to that of a fresh animal?

In all these objections to phagocytic immunity it is always the idea of a direct primitive action of the body-fluids which appears, the idea of the humoral theory. Since it is admitted that the immune-bodies circulate in the plasma whereas according to Metchnikoff the complement remains in the phagocytes, since the phagocytic theory maintains that no excretion of complement occurs without phagolysis, it ought certainly to have been on this point that the humoral theory should have made its attacks. This is the central point on which turns the whole question : if without phagolysis there is no extracellular destruction of microbes in an immunised animal, when destruction takes place outside the phagocytes it means there has been an abnormal lesion, a phenomenon unlikely to occur spontaneously in nature and probably only an experimental accident ; it was the celebrated experiment of Pfeiffer which threw the question into prominence.

Pfeiffer's Phenomenon and the Humoral Theory

It was the following experiment of Behring and Nissen which, after the primary investigations of Flügge, Nuttall and Buchner, seemed best to explain immunity by the bactericidal power of the body-fluids ; the serum of guinea-pigs well vaccinated against

the vibrio Metchnikowii, a cholera vibrio, becomes much more powerfully bactericidal than the serum of fresh guinea-pigs.[1]

It is easy to immunize guinea-pigs against lethal doses of the cholera vibrio injected intraperitoneally. Pfeiffer took a guinea-pig thus prepared, injected into it a certain quantity of vibrios, and then abstracted from its peritoneum a little of the exudate. He found that after a few minutes the vibrios had almost entirely disappeared from the peritoneum; they had been transformed into granules, the first stage of destruction, the "commas" turning into "dots." Later these granules dissolved in the peritoneal fluid like a piece of sugar in water. The same phenomena were observed when the vibrios were injected along with immune guinea-pig serum into the peritoneum of a fresh guinea-pig.

Pfeiffer's interpretation was that in the immunized body the bacteria are destroyed directly by the body-fluids without the intervention of the leucocytes.

Such then is Pfeiffer's phenomenon, so long discussed and for long a sort of touchstone in the two immunity doctrines. Metchnikoff and his pupils have subjected it to merciless criticism. First of all they showed that the granule formation takes place also outside the body when the vibrios are mixed with a little fresh serum from an immunised guinea-pig, or even when to the same serum, which from age or heating has lost its complement, a little fresh peritoneal fluid is added. (It was, in fact, while repeating Pfeiffer's experiment that Bordet discovered the two substances in the serum of immune guinea-pigs). In the test-tube, as in the peritoneum, the vibrios fall victim to the action of the complement through the intermediation of the immune-body.

Since the granule formation is due to the combined action of the two substances, and since we know that the leucocytes do not readily shed their complement, which is therefore

[1] It must be said in this connection that the experiment was insufficient to permit a general conclusion on the nature of acquired immunity; a similar experiment with other bacteria gives a different result and even the vibrio itself when injected into an immunised animal remains alive in its body for several days.

rarely found in the normal body-fluids, this destructive action ought not to take place in these nor in any position except the peritoneum. As a matter of fact, if the immune guinea-pig is injected under the skin, in the anterior chamber of the eye, or in the fluid of a passive œdema, the phenomenon does not take place: the immune-body is present, but not the comple-

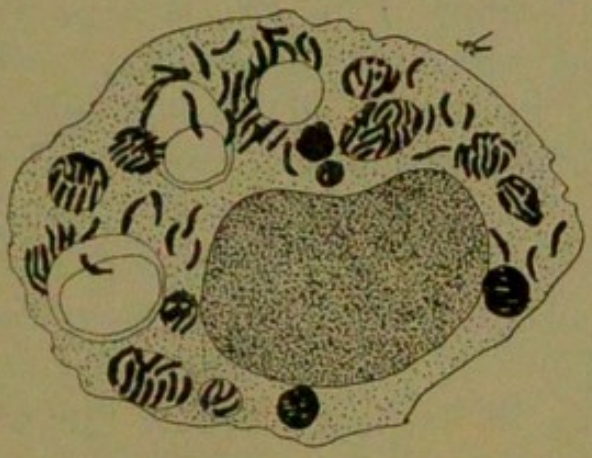


FIG. 68.—Cholera vibrios phagocyted by a macrophage of the guinea-pig and not yet transformed into granules. (Metchnikoff.)

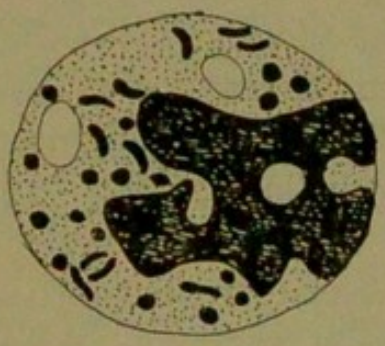


FIG. 69.—Cholera vibrios phagocyted by a microphage of the guinea-pig and turned into granules. (Metchnikoff.)

ment. When this latter is added in the form of a little fresh serum, the transformation into granules occurs.

Why then does the phenomenon occur in the peritoneum if the complement remains inside the leucocytes? It is because the mere act of intraperitoneal injection produces phagolysis. The injection of any liquid into the peritoneum, water or nutrient broth, for example, destroys some at least of the leucocytes which are found in it: they discharge, as is known, one ferment under these conditions, that which produces coagulation of the blood; in the same way, they discharge this other ferment, the complement, which acts upon the sensitized vibrio.

If then this initial phagolysis could be prevented, the phenomenon of Pfeiffer would also fail. Experiment has proved this: by injecting into the peritoneum sterile broth, freshly prepared and tepid, the leucocytes are rendered much less sensitive to a succeeding injection, and in the peritoneum thus habituated, thus "prepared," Pfeiffer's phenomenon does

not occur. When phagolysis is thus prevented, the vibrios in all the variations of the experiment possible fail to be transformed into granules but disappear by digestion in the interior of the phagocytes. If we take a guinea-pig strongly immunized against the vibrio of cholera and inject these bacteria directly into its circulation by the jugular vein, we find half-an-hour after no granule transformation in the circulating blood; the vibrios retain their shape and are to be seen inside the leucocytes. No phagolysis has occurred, accordingly no Pfeiffer's phenomenon, and no extra-cellular destruction by the body-fluids.

The resistance of the immunized guinea-pig depends so much upon phagocytosis, that if the leucocytic activity is paralysed by means of a dose of opium, the animal succumbs to a smaller dose of vibrios than is necessary to kill the non-narcotized animal.

The destruction after phagolysis, Pfeiffer's phenomenon, is not even a general fact. It is true of the cholera vibrio, a fragile bacterium, but even with the typhoid bacillus, also comparatively fragile, it is only a modified Pfeiffer's phenomenon which occurs. With the bacillus pyocyaneus there is still greater resistance, and greater still with the bacilli of swine-erysipelas and anthrax. *In those cases in which the humoral action is imperceptible, phagocytosis is active and constant.*

Opsonins and Bacteriotropins.—There exist more recent theories which, while recognizing the action of the phagocytes, attribute to the body-fluids an important part in immunity: they are said to prepare the bacteria for phagocytic digestion. These preparatory substances or actions are the opsonins of Wright and the bacteriotropins of Neufeld.[1]

[1] In their experiments these workers have pursued the same general method; they have studied the phagocytosis occurring with leucocytes withdrawn from the body and suspended in glass tubes, *i.e.*, the phagocytosis *in vitro* already studied in the old experiments of Denys and Leclef. Metchnikoff himself did not fail to compare phagocytosis *in vivo* and *in vitro*, having observed the incorporation of the anthrax bacillus by leucocytes suspended in urine and in aqueous humour which had been boiled and thus deprived of all antibodies.

According to Wright the opsonins are the principal and determining cause of phagocytosis, the act of incorporation by the leucocytes being only the final concluding operation. Opsonins, being the essential factor in immunity, ought not to be present in the serum of fresh animals, *i.e.*, there ought not to be any phagocytosis without opsonins, no spontaneous phagocytosis. But spontaneous phagocytosis is incontestable, as has been established by the experiments of Metchnikoff and Bordet : it is only necessary in *in vitro* experiments to allow enough time for it to take place : and from the moment that the existence of spontaneous phagocytosis is granted opsonins can no longer play the primary part in the phagocytic process.

It is certain that the presence of normal serum favours phagocytosis *in vitro* (Wright and Douglas, etc.) : the serum acts on the bacteria, which are capable of fixing certain of its elements. Are the opsonins substances or properties new and unknown before Wright's researches? Numerous experiments ascribe to the opsonins of normal serum the same properties as characterize the complement. They are products of the leucocytes.

In the serum of immunized animals, which favours phagocytosis much more actively than normal serum, the opsonins are not to be distinguished from the immune-body : they can be used for the same re-activation experiments (Levaditi) and they also are products of the phagocytes.

The *bacteriotropins* of Neufeld are considered by the majority of workers as being equivalent to the opsonins of immune serum and to the immune-body.

There is no reason to deny these actions which favour phagocytosis. The work on opsonins and bacteriotropins is simply, to use Ehrlich's expression, a new flowering of the phagocytic doctrine. In Wright's and Neufeld's experiments it is chiefly the experimental method which Metchnikoff criticises. Leucocytes taken from the body, washed and in a different surrounding medium, can no longer accurately represent the phenomena occurring in the living body. The conditions are

abnormal and yet one knows that it is only in abnormal conditions that the leucocytes discharge complement. "The least change in the salt content of the surrounding fluid is sufficient to modify notably the phagocytosis. The leucocytes, of patients suffering from various diseases present a marked diminution in their vital activities. The destruction of bacteria is the work of phagocytes which are living and vigorous" (Metchnikoff, Nobel Lecture). Washing, chilling, maceration are quite sufficient to destroy the complement of the leucocytes; how is it possible to conclude after such procedures that the leucocytes do not contain the complement?

The opponents of phagocytosis declare that it is the humoral properties which undergo the most marked increase during immunization. There is no doubt of such a development of bacteriotropins, opsonins and immune-bodies—which in any case are phagocytic products. But it can be shown experimentally that the phagocytes are modified in immunity and modified sooner than the body-fluids. Leucocytes taken from an animal vaccinated against some microbe and injected into a fresh animal protect the latter from several lethal doses of the microbe, whereas the leucocytes of a normal animal fail. (Pettersson's experiment). The white corpuscles of the immunized animal supply protective substances at a time when the blood-fluids are not yet affected: and it is owing to the leucocytes that the body remains refractory after the body fluids have already lost their protective properties (Salimbeni).

Serum is a fluid into which have been poured the ferments of the lencocytes, the fibrin-ferment and the complement. Injury to the leucocytes is necessary before blood will coagulate. By very delicate operations and with great trouble it has been possible to separate the blood corpuscles and obtain a plasma which remains—for a certain time—incoagulable. Now the properties of such plasma are very different from those of serum: the leucocytic excretions are absent. It is, however, so difficult to obtain a true plasma identical with that of the circulating blood that such experiments have to be very carefully analysed. When quickly prepared immediately after

bleeding the plasma contains no complement, but every minute afterwards injured leucocytes pour into it little by little the active substance.

Antibodies and Immunity.—There are innumerable facts preventing us from regarding as a law any correspondence between the quantity of antibodies in the serum and the degree of immunity of the animal: this is a definite proof that there is something else besides the humoral properties and that the preponderating part is played by the cell elements.

The serum of guinea-pigs inoculated against anthrax was found by Behring and Wernicke to be incapable of protecting fresh guinea-pigs from a fatal infection. Pfeiffer immunised guinea-pigs against the bacterium which he regards as the cause of influenza in man, but these immunized animals did not produce a protective serum.

In protozoal diseases such as malaria, there seems to be immunity in certain cases, but no one has ever demonstrated a protective property in the serum. To take an example among the invertebrates, the larvæ of the rhinoceros beetle (*Oryctes nasicornis*) are immune to anthrax and in them the phagocytic incorporation of injected anthrax bacilli can be very well seen; yet the blood fluid of these larvæ forms a culture medium equally favourable to the anthrax bacillus, to which they are immune, as to the cholera vibrio which produces in them a fatal infection.

Again, the dog is extremely resistant to anthrax, yet the anthrax bacillus grows very well in dog-serum; all these are examples of immunity in which the bactericidal power does not play a part. It has been known since the experiments of Behring that rat's serum possesses a remarkable destructive power towards the anthrax bacillus; now the rat is not immune to anthrax, and the degree of immunity which it does possess is due to the phagocytes. The bactericidal substance exists in the leucocytes, but not in the circulating plasma, nor in plasma carefully prepared by Gengou's method. It exists in the serum, but only because it has been discharged into this by the leucocytes. The rat is extremely susceptible to

anthrax if it is inoculated with a very fine needle so as not to provoke a hæmorrhage at the point of inoculation.[1]

Immunity towards toxins could be made to furnish analogous examples in abundance; it will be discussed in the next chapter.

In many cases Pfeiffer has seen his guinea-pigs, which had been thoroughly immunized against the cholera vibrio, succumb to the injection of a moderate dose of vibrios. Yet the serum of these guinea-pigs was capable of producing Pfeiffer's phenomenon.[2]

Tuberculin carefully employed exerts a favourable effect in many tuberculous patients, and leads to the production of antibodies in their serum. Jochmann has quite recently made several observations of this kind under the direction of R. Koch, and has sought for a correspondence between the appearance and quantity of the antibodies and the resistance of the patient. He found it was impossible to maintain that the presence of antibodies meant recovery. Certain patients manifested great clinical improvement simultaneously with the appearance of antibodies; in others, the improvement was quite as great without the antibodies, while in other cases the appearance of the antibodies coincided with marked and fatal aggravation of the malady. It is obviously impossible to draw any conclusion as to the immunity of these patients from such *in vitro* experiments.

[1] In this example of the rat there is no question of an antibody produced by immunization, but rather of a want of agreement between the *natural* immunity of the animal and the *natural* bactericidal power of the serum.

[2] Quite recently Citron has shown that the serum of rabbits actively immunised against the so-called bacillus of hog-cholera possesses protective properties for guinea-pigs, whereas it has none of this for fresh rabbits. Rabbits prepared with extracts of the bacilli and without active immunity of their own (they succumbed to the test inoculation of living bacilli), nevertheless furnished a protective serum for guinea pigs. Choukewitch taking up this question again, prepared rabbits by large intravenous inoculations of the same bacilli, but in the killed condition. One rabbit of the lot acquired immunity towards the living microbes, but in every one of the series, not only the immune individuals but also the non-immune, the blood contained an abundance of antibodies, immune-bodies, opsonins, etc. . . . . It even contained much more than the serum of rabbits rendered truly immune by subcutaneous inoculations of virulent bacilli.

Immunity is entirely a cellular function and the inherent tincture of "vitalism" in the phagocytosis doctrine is unavoidable.

"The final phagocytic reaction is represented by the physical or physico-chemical processes in the digestion of microbes, conducted with the help of cytases, and favoured by the presence of immune bodies; in the resistance to poisons the phagocytes must also exert chemical influences. But before these phenomena occur, the phagocytes present activities which are purely biological; such are the chemiotactic perceptions and movements directed towards the threatened spot, the engulfment of bacteria, and the absorption of toxins, and finally the secretion of substances utilized in cellular digestion" (Metchnikoff, *L'Immunité*, p. 590).

CHAPTER XI

IMMUNITY

Toxins and antitoxins—Chemical and physical conceptions of immunity.
Side-chain theory—Origin of the antibodies—Theory of chemical equilibrium.
The physical point of view : Bordet—Phenomena of absorption or molecular adhesion—Explanation of specificity—Analogies between the reactions of antibodies and the reactions of colloids – Lipoid actions.
Phagocytosis and toxins—The body plays an essential part—Origin of antibodies and Wassermann's experiments—The phagocytes in their connection with mineral poisons and microbial poisons, toxins, and endotoxins.

It was the discovery of antitoxins which inaugurated the study of antibodies. It was the necessity of explaining the action of antitoxins on toxins which gave rise to the theories on this peculiar problem and on antibodies and immunity in general.

It was thought at first that in the immunized animal which manufactures the antitoxin, as well as in the animal immunized by the injection of the antitoxic serum, the cells play a part. Buchner enunciated the hypothesis that the body produces antitoxin by transforming the toxin ; he quoted, as a distant analogy, the transformation of one compound into another by polymerization. But it is difficult to understand how there could be such a disproportion between the toxin injected and the antitoxin produced ; the horse produces, according to Knorr, for one unit of toxin injected 100,000 units of antitoxin.

Buchner performed a pretty experiment, which has lost none of its interest, by showing that after accounting for the differences in weight and in natural susceptibility, a mixture of

tetanus toxin and antitoxin which is neutral for the guinea-pig is fatal to the mouse; it is impossible to avoid the conviction that the body, that of the guinea-pig or of the mouse in this example, counts for something in the phenomena. The same ideas were maintained by Roux at the time of the discovery of serotherapy.

But, on the other hand, the action of antitoxin on toxin seemed to be a neutralization, and to behave both *in vitro* and *in vivo* like a chemical reaction; and in practice it was found necessary for medical purposes to titrate the sera in this way to measure their activity; the ideas of Buchner and of Roux were then laid aside, only to be rediscovered later; the biological phenomena were subordinated as much as possible to quantitative studies, and the endeavour was made to represent the action of antitoxins on toxins as a chemical reaction.

The Side-chain Theory.—The best known chemical theory for the action of antitoxins and antibodies in general is that of Ehrlich, which is currently known under the name of the "side-chain theory." The primary idea of its author was to find in the facts mutual relations as much as possible fixed and independent of the body, and to eliminate all "vitalism" in favour of exact quantitative work. To begin with, he adopted the method of *in vitro* experiment. The nature of the tetanus and diphtheria toxins had been rendered much clearer by the study of other toxins more easy to work with, such as hæmolysins, agglutinins, ferments, and anti-ferments (ricin, abrin, rennin, and antirennin, etc.). Preliminary experiments *in vitro* showed the general applicability of the same laws. Originally Ehrlich believed that the curative and preventive action *in vivo* was equivalent to the neutralising action towards the diphtheria toxin *in vitro*, a belief which later experiments were to disturb.

The second step was to demonstrate that antitoxin does not destroy toxin, but that the two bodies combine to form a compound (neutral from the physiological point of view), just as an acid and a base combine to form a salt.

Antitoxin does not destroy toxin because the two reacting bodies can be recovered. For example, the neurotoxin of cobra venom resists heating to 68° C. ; when the neutral mixture of venom and anti-venom is heated, the toxin can be recovered if this is done during the ten minutes which follow the preparations of the mixture (Calmette's experiment). From a neutral mixture of cobra-hæmolysin and anti-venom, it is possible to recover the hæmolysin by the action of hydrochloric acid ; the recovered hæmolysin manifests its action on the addition of the necessary lecithin (Morgenroth's experiment). A mixture of diphtheria toxin and antitoxin (twenty-four hours' contact), harmless to the rabbit, recovers its toxicity when treated with hydrochloric acid, the toxin being set free (Morgenroth and Willanen's experiment). Heat, filtration, the action of a digestive diastase, are other methods of dissociating the toxin-antitoxin combination provided the intervention takes place without too long delay.

How then are the toxin and anti-toxin to be represented? Probably as albuminoid substances of large molecules capable of being represented by stereochemical models and possessing a nucleus on which are grafted lateral chains. To conceive of the action of the toxin on a cell, it is only necessary to imagine the molecules of the cell protoplasm as containing figures of the same kind. The toxin molecules enter into relation or combination with the cell molecules by means of these atom groups or side-chains. Ehrlich calls them "receptors" or "haptophorous groups." The term "side-chains" was borrowed directly from the chemistry of benzene. Such stereochemical symbols were introduced into science by Emil Fischer to represent the specific action of the ferments : one body acts specifically on another, because an atom group of the one is adapted to an atom group of the other, as with a key and the corresponding lock. Thus the haptophorous group of a toxin fixes itself on the receptor of a cell, both haptophore and receptor being "side-chains."

This in fact is the central idea of the theory, the same mode of chemical action, the same relations between receptors and

haptophorous groups, conceived after the manner of the reactions of organic chemistry; and this molecular stereochemistry explains all vital phenomena: the action of a toxin on the cell, the action of antitoxin on toxin, the production of antitoxins, and immunity in general.

Finally, since the same combinations and linkages go on in the metabolic changes of all living matter, the conception of side-chains becomes a general theory of nutrition; so much so that, with all his chemical language and mechanical attitude of thought, Ehrlich arrives at the same formula as Metchnikoff with his "vitalistic," or rather, biological explanation: immunity is a function of nutrition.

This idea has been Ehrlich's guiding principle in all his scientific work, and it is from it that we have to start in order to arrive at the principal laws of immunity.

Let us represent the protoplasm molecule as possessing numerous and varied functions, the agents or the bases of which are distinct atomic groups. This molecule consists of a central nucleus (analogous to the nucleus of the aromatic compounds), which maintains its continuous individuality, and of numerous side-chains or receptors which act towards the nucleus as organs of communication or nutrition. The primary food-stuffs and the toxins which circulate in the blood and body-fluids have haptophore groups which are fixed by the cell receptors; and it is thus that all the modifications of the protoplasm are carried on.

Take a poison like the tetanus toxin introduced into the body. We know by definite experiments that it is "fixed" by various cells and in particular by the nerve-cells. The toxin molecule is treated by certain definite receptors of the nerve-cell as a food material; it possesses a haptophore group which hooks on to the receptor, but it possesses also a toxophore group, an atom group which exerts the toxic action; it is through the haptophore group that the toxophore group is fixed by the cell and acts as a poison.

If the toxin has been injected in sufficient quantity, numerous cell receptors are occupied, monopolized by the

toxic molecules; the cell, deprived of the use of these receptors, has its functional activity diminished and its nutrition threatened. But all injured protoplasm possesses a reparative or regenerative power; the cell reproduces receptors; it even manufactures a great many more than is necessary. To follow Weigert's dictum the living matter overstimulated by the lesion regenerates itself much beyond its needs; these receptors regenerated in excess are cast off by the cell and pass into the body-fluids; and it is these which fix and neutralize a fresh dose of toxin injected into the body.

Antitoxin is nothing but these Free Receptors.—It acts like a lightning-conductor. Withdrawn from the body which produces it and introduced into another animal, it retains the same fixing property and is the active agent in therapeutic sera.

Thus there is no essential difference between the receptors which produce the antitoxin and the normal "nutrition

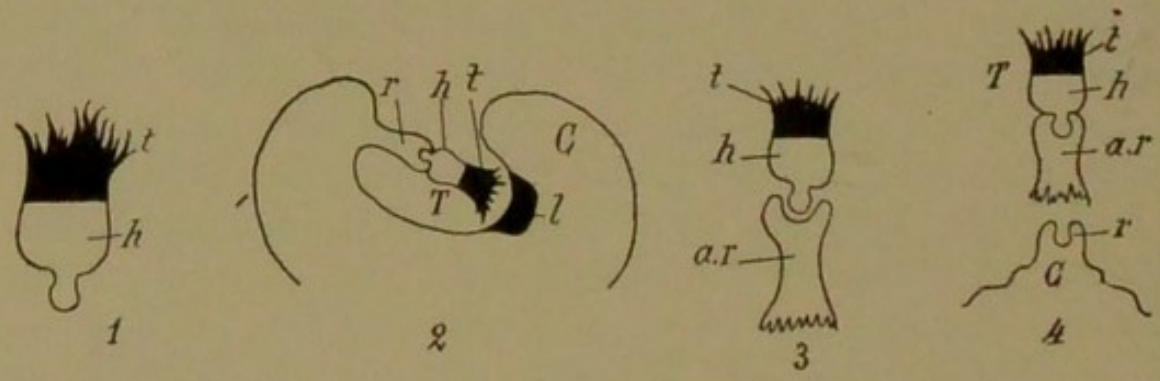


FIG. 70.—Diagrams to represent Ehrlich's theory.

1. Haptophore group *h* and toxophore group *t*.
2. Cell *C* injured at *l*: its receptor *r*. The toxin is represented by *T* with its haptophore group *h* and its toxophore group *t*.
3. A toxin molecule: haptophore group *h*: toxophore group *t*: antibody or free receptor *a. r.*
4. A cell *C* with a fixed receptor *r*: a detached free receptor forming the antibody *a. r.*: the toxin *T* with its haptophore and toxophore groups *h* and *t*.

receptors" of the cell. A cell susceptible to the poison produces an antidote, but the antidote may also be produced by cells which are insusceptible, *i.e.*, not only by the "noble" cells, but also by the connective-tissue cells, and it is necessary

to add, in the light of the experiments of the Metchnikoff school, by the leucocytes.

In bacteriolysis and hæmolysis as defined by Bordet's experiments, also immunity phenomena, the complement and the amboceptor (or immune-body) come into play. Complement is an atom group already present in the body, whereas amboceptor is analogous to the antitoxin: it possesses, however, two haptophore groups, one uniting with the cells (blood corpuscles or bacteria), the cytophil group, the other linking up the complement, the complementophil group. The antibodies are receptors or amboceptors set free and detached from the cells which form them.

It is impossible to follow here all the developments of the side-chain theory. Ehrlich has complicated it almost to excess in order to include in it the infinite complexity of facts observed in experiments with the various antibodies, antitoxins, hæmolysins, bacteriolysins, and the other cytolysins, precipitins, and agglutinins. The dominant idea is always to give a chemical interpretation of nutrition and, as a particular case of this, of immunity.

The conception was first intended to explain the physiology of toxin and antitoxin action, and it is to this that it is best adapted; but it has had to undergo complication, not only to include the largest number possible of the ascertained facts, but to act up to its original intention as an explanation in terms of chemistry.

It is to explain all these facts[1] that the distinction between

[1] For example these, that the broth culture of the diphtheria bacillus which constitutes crude toxin is not a simple substance: nor is there any reason to believe that the broth cultures of the tetanus bacillus is any more so, since it contains at least two poisons, tetanolysin and tetanospasmin. The products of cell-life are frequently very complex: Ehrlich, for example, quotes with justice cinchona bark with its twenty odd alkaloids and the liver cells with their round dozen ferments. Toxin left to itself, even protected from light and heat, rapidly becomes modified: it feels the effect of age and not only deteriorates in activity but undergoes qualitative changes. Toxin acts after a period of incubation. The neutralization by antitoxin no longer proceeds in the same way when the appropriate dose of antitoxin is added in separate fractions instead of all at once (Danysz-Dungern phenomenon).

the haptophorous and toxophorous groups has been conceived, and that in toxin the toxones and toxoids have been postulated. But the complexity is also due to the necessity of making the facts conform to the idea of the chemical nature of these actions, and especially to the law of *multipla*, which demands that the same quantity of toxin should always be neutralized by the same quantity of antitoxin. The theory flatters itself also on its capacity to explain the specificity of the antibodies ; for the complexity of the groups is supposed to be sufficiently great to permit a hæmolysin against goat corpuscles to differ from a hæmolysin against rabbit corpuscles. Even for the fairly numerous cases in which specificity is not rigorous an explanation is forthcoming: different receptors possess certain elements in common, and it is possible that a serum which precipitates horse serum may also precipitate the serum of the ass.

The side-chain theory has been of great service ; it has, its supporters say, a great " heuristic " value, *i.e.*, it has been the means of discovering many interesting phenomena, and has led Ehrlich on to his chemotherapeutic studies, in which he has gained such magnificent successes.

Nevertheless, it cannot be said that these fortunate results prove the truth of the theory: the discovery of " 606," for example, proves neither the existence of the side-chains nor the truth of the chemical theory of immunity.

Theory of Chemical Equilibria.—Again to explain the action of toxin and antitoxin, Arrhenius, Madsen and Walbum have proposed another chemical theory. They criticize the complexity of the theory of Ehrlich, they do not admit the complex nature of the diphtheria toxin, and they attach great importance to the experiment of Danysz-Dungern on partial saturation. The facts can be explained by conceiving the toxin + antitoxin reaction as a chemical interaction, not between a strong acid and a strong base, but between a weak acid and a weak base, for example, ammonia and boric acid. The reaction toxin and antitoxin is comparable with those reactions known as reversible and is governed by the law of

mass-action of Guldberg and Waage, if one supposes a state of unstable equilibrium to exist among the combinations.[1]

The combination toxin + antitoxin must therefore be dissociable ; Arrhenius and Madsen are of the opinion that they have demonstrated this by their experiments on the diffusion of the mixture in a column of solidified gelatine and by their distribution experiments (*e.g.*, distribution of agglutinin between bacteria and the immersing fluid). Many facts which led Ehrlich to his hypothesis on the toxins are explained by Arrhenius in terms of these dissociations, and in general by the fact that antibodies and antigens have only a feeble affinity for each other.

To the diffusion experiments it has been urged in reply that the dissociation only takes place because the mixture poured on the gelatin has not had time to form the final combination ; while to the hypothesis that a quantity of *free* toxin is present in the mixture, it has been replied that if this were the case antitoxin would never act in the body ; the body would fix the free toxin, the toxin-antitoxin equilibrium would be disturbed, a new quantity of toxin would be set free, and so on.

Physical chemists regard the dominant idea of Arrhenius's theory with great reserve ; they doubt the justice of employing the laws of chemical equilibrium and rates of reaction in speculations as to the reactions which go on between bodies of which nothing is known from the chemical point of view. Nernst has verified the use made of the laws of reversible actions, and he denies the possibility of applying to colloidal substances laws established only for homogeneous liquids.

This does not mean that there is anything odd in attempting

[1] When a substance in solution of a molecular concentration m reacts with another substance in solution in concentration n, the mass of the substance formed by their combination is in a given time proportional to the product mn.

For example, when in a given volume 3 molecules of alcohol react with 2 molecules of acetic acid, the quantity of acetic ether formed in a given time is $3 \times 2 = 6$; if 5 molecules of alcohol react with 3 molecules of acetic acid the quantity of acetic ether formed in the same time is expressed by $5 \times 3 = 15$.

to apply physico-chemical laws to biological phenomena in spite of the variability of living creatures and of their products. It is impossible for biologists to refrain from seeking quantitative laws and from applying physico-chemical laws to immunity phenomena. Quantitative results have been obtained in the study of diastases, and Ehrlich has discovered facts of the greatest interest by means of his experiments *in vitro* on titrations and measuring. It is only necessary to agree upon the conventions necessary (in physics itself these cannot be dispensed with) and not to employ such unjustifiable expressions as "*guérison in vitro*" (*in vitro* cure).

It is always possible to return to the biological point of view when this becomes necessary, as Ehrlich himself did, when considering Weigert's ideas on the regeneration of protoplasm.

The Physical Point of View.

Bordet rejected Ehrlich's system, and compared the "antigens + anti-bodies" reactions to phenomena of absorption and molecular adhesion, even before the closer comparison with colloidal reactions had been arrived at.

He does not only complain that the side-chain theory is too complicated; he criticises its whole disposition.

Immunity is a problem not yet ripe; and the solution will probably come from a quarter quite unexpected. There are enormous gaps in our knowledge. Why, then, make adventurous generalizations when the biological facts are far from permitting this? Every theory that can be constructed must base itself for the moment on facts not yet demonstrated. Let us keep close to the experiments, and be content to advance step by step. Ehrlich's theory is dangerous, in that it offers too readily conceptions which have the appearance of explanations, and which, therefore, are apt to dull the appetite for research. "For my part," adds Bordet, "I have been unwilling to construct a theory; I do not adduce any general conceptions; the hypotheses which I have proposed are

scarcely worthy of the name, they differ so little from mere critical statements of experimental results. Even at the risk of being regarded as incapable of generalizing, I prefer to stick to the facts without moulding them into a system."

In immunity phenomena, we observe certain activities ; but why materialize them and picture each by an atomic group? In the side-chain theory we are told that the antibody is nothing but a cell-receptor affected by the antigen. This identity is not proved. Why should not the cell with its power of adaptation and reaction produce some new and original substance?

When the "substance" which we call agglutinin clumps cells or bacteria, does it really bring into action an atomic group, or side-chain, which attaches itself and another group which agglutinates? The explanation is artificial. In reality it is not the agglutinin which agglutinates ; it is a salt. The antigen (bacteria) and the antibody (agglutinin) form a combination which produces floccules, or, as it is expressed nowadays, is "flocculable" by electrolytes. It is this couple or "complex" which agglutinates.[1]

An analogous coupling must be regarded as taking place in all the reactions of antigens and antibodies, and Ehrlich's theory is wrong in attributing everything to the antibodies and nothing to the antigen. There are no "amboceptors" in reality, there only exist "uniceptors" capable of being absorbed.

It was therefore not with the purpose of disputing details that Bordet accumulated his experiments on the mode of fixation of the complement on the immune body ; in this field he has discovered the principal facts which render the side-chain theory impossible as a dogma, if not altogether impossible as a conception of certain phenomena. The important fact is that there is never absorption of complement by an immune body without the presence of an antigen, so

[1] The salt acts on bacteria saturated with agglutinin but it acts also on bacteria which have absorbed various chemical substances, iron, uranium, or aluminium.

true is it that it is the antigen-antibody compound which absorbs the complement. Although the complement of one animal species may differ from that of another species, yet in a given serum, in opposition to Ehrlich's theory, there exists but one complement or rather one complementing property (exp. of Bordet, Gay, Muir and Browning, etc.). It is due to Bordet's correct attitude on these points that the Bordet-Gengou reaction (complement fixation) has been capable of such successful application in various bacteriological diagnostic methods, and recently by Wassermann in the diagnosis of syphilis.

Finally, since the complement attaches itself, not to the immune body, but to the antigen-antibody combination, there is no reason to suppose the existence of a haptophore group of the "complementophile" kind, indispensable to the immune body if complement is to be fixed. This question has for some time been a sort of *test* for the side-chain theory, and it seems to have resulted in favour of Bordet's ideas.[1]

From the beginning of his researches in 1896, Bordet has imagined immunity reactions, not as chemical combinations, but as physical phenomena of absorption or molecular adhesion. He considered that in agglutination (where the bacteria are passive, since dead bacteria also agglutinate) serum acts by modifying the relations of molecular attraction between the bacteria and the fluid bathing them, and that, in the first phase of the phenomenon at least, the bacteria behave like particles in general. Under the influence of Duclaux's ideas, he observed the resemblances between agglutination and coagulation. From the point of view of their coagulating and dissolving properties, he compared the active sera to the digestive juices, and, like Metchnikoff and after him Ehrlich, he also saw in immunity, though from a different point of view, a particular case of the physiology of digestion.

At that time the results of the study of colloids had

[1] Experiments of Ehrlich and Sachs, of Sachs and Bauer; of Bordet and Gay and Bordet and Streng on hæmolysis by ox-serum.

scarcely begun to be applied to the study of immunity, and the body-fluids, the toxins, and the antitoxins had not yet been studied from the point of view of their colloidal constitution. The phenomenon of adsorption (*ad* in preference to *ab* as expressing the idea of attraction or adhesion) is a very general one, and does not depend absolutely on the colloidal state. Bordet therefore, in explaining his conception, preferred to employ the comparison with dyeing processes. The action of antitoxin on toxin appeared to him to resemble, for example, the action of iodine on starch: the immune body which prepares bacteria or cells for the action of complement he regarded as acting after the manner of *mordants* in dyeing, intermediary substances necessary for the fixing of certain dyes on certain cloths. Thus in hæmolysis the union of the immune-body with the blood corpuscle (anti-body + antigen) forms a combination possessing a greater adsorptive affinity than the normal corpuscle: the complement tends to become precipitated on the sensitized corpuscle, and the attraction which the latter exerts is more powerful the more heavily it is sensitized.[1]

Inorganic substances present similar phenomena. Water runs off a watch glass coated with paraffin without sticking to it, but if the water contains barium sulphate in suspension it wets the paraffin and spreads over it; this depends on the fact that the surface of the paraffin becomes coated by molecular adhesion with a thin white film of barium sulphate, which water can wet; this film is not removed by rinsing in water, and can only be removed by rubbing. There are even substances which inhibit this fixation of the barium sulphate on the paraffin, just as there are substances which inhibit the fixation of complement by sensitized corpuscles.

On all the most important points of the question of the toxin and antitoxin combination, the physical theory is the

[1] Those sera which possess the power of inhibiting hæmolysis act by keeping the complement in a condition of greater suspension or dissemination in the fluid; they thus render it more stable, unlike saline solution, which produces a condition of instability in which the complement condenses itself or is precipitated on the attracting sensitized cells.

reverse of the chemical theory of Ehrlich; the one simplifies where the other complicates.

In the chemical theory the same quantity of antitoxin ought to combine with the same quantity of toxin, so, to account for the irregularities in the actual facts, there have been introduced the hypotheses of the haptophore group separate from the toxophore, of the toxones, toxoids, &c. On the contrary, Bordet supposes that the antitoxin really unites with the toxin in varying proportions: the toxin can fix, can, so to speak, dye itself with toxin in greater or less amount just as starch can take up varying quantities of iodine and become thereby stained a more or less dark blue. In the same way, in hæmolysis the corpuscles can absorb variable amounts of the active substance according to the concentration of the solutions and the duration of the contact. The distance of this idea from the theory of chemical equivalents is apparent. When a given quantity of toxin is mixed with a quantity of antitoxin insufficient for complete neutralization, what occurs is not a monopolization of the antitoxin by a portion of the toxin molecules, forming a complete combination with it while the rest of the toxin remains free (*i.e.*, the chemical conception). On the contrary, the antitoxin is equally distributed over all the toxin present, so that the latter is attenuated throughout and presents a diminished activity. To return to the same analogy, it is faintly dyed. It can produce toxic effects qualitatively different from those due to an intact toxin or a toxin completely neutralized without necessitating the hypothesis of a special chemical condition (toxones).[1]

The phenomenon of Danysz-Dungern (the antitoxin has a different action on toxin when the mixture of the same quantities is made at one instead of several additions) does not compel the hypothesis that in toxin there are several chemically

[1] The fact first observed by Ehrlich, namely, the difficulty of preparing exactly neutral mixtures of toxin and antitoxin, can thus be easily explained. The effects produced by a hæmolysin more or less neutralized and the experiments of Grossberger and Schattenfroth on the toxin and antitoxin of the bacillus of quarter-evil have confirmed Bordet's views of the nature of the toxin + antitoxin reaction.

distinct components. If a large piece of filter-paper is dipped in a rather dilute solution of a dye it is faintly stained; if the piece is cut into small pieces and these are immersed in turn for a certain time, we find that the first pieces take up the colour and leave almost none for the last. Substitute for the dye the antitoxin and for the paper the toxin; if the mixture is made at one blow the toxin is attenuated in its whole bulk: if, however, the mixture is made by several additions, the first portions of the toxin take up the antitoxin and the later portions, not being neutralized, remain much more toxic.

Further, the fact that toxin and antitoxin mixtures (and in general antigens and antibodies) become in time less dissolvable and more stable is also explained by adsorption phenomena.

When a piece of cloth is placed in a dyeing vat the dye loses its attachment to the dissolving fluid and adheres more and more intimately to the cloth until it can no longer be redissolved. A similar example may be quoted in connection with the precipitates produced by alcohol in certain albuminous fluids, precipitates which are fairly easily redissolved in water immediately after their precipitation, but which are no longer soluble when a certain time has been allowed to elapse for their aggregation. When three substances exist together, two of them may compete with each other in the fixation of the third; it is thus that the protective action of certain substances is to be explained, as, for example, the albuminous substances of the blood which protect blood corpuscles against the action of soap (Meyer) or of eel-serum (Frouin).[1]

One thing that the physical theory has not yet explained is the specificity of the reactions of immunity, but it is not incapable of explaining even this.

It is not difficult to imagine that slight physical modifications may change the affinities on which depend the molecular attraction; that is certainly no more difficult to imagine than

[1] Citrate of soda protects blood corpuscles against the agglutinating and hæmolytic action of sulphate of barium. The lecithin of ox-serum is held in check as regards its action on guinea-pigs' corpuscles by some albuminoid material.

the innumerable molecular groups postulated by the side-chain theory. For example, the antiserum prepared by an animal against a protein has not the same properties as the serum obtained against the same substance previously subjected to heat (exp. of Obermayer and Pick). Hens' serum agglutinates the lipoids extracted from the red corpuscles of the rabbit much more vigorously than those from the ox. It is easy to found a theory for specificity on the absorption phenomena, and such theories already exist ; hitherto they have been too philosophical, but it is satisfactory to know that experiment has already furnished the germ of a scientific explanation.

The Colloids.—After Bordet's explanations of agglutination and hæmolysis in terms of molecular attractions and cohesions, and in the light of his comparison of these phenomena to dyeing processes, Zangger, Landsteiner, and Jagic established experimentally the first analogies between immunity phenomena and the physics of colloids.

Reactions between colloids or between colloids and true solutions can be reduced to phenomena of molecular attraction, of absorption, and *adsorption*. The bodies participate in the reactions in *variable proportions*, influenced by temperature and pressure. Colloids of opposite electrical charge exert on each other a "flocking" or precipitating action, which may be masked when one or other is in excess in the mixture ; one colloid may inhibit the precipitation of another colloid by a salt. In many cases, the law of the opposite electrical charge may be masked by the fact that the proteins are amphoteric colloids, and may neutralize acids and alkalies equally well, behaving in acid solution as bases, in basic solutions as acids.

Are the antigens and antibodies of immunity colloids? The only ones whose chemical composition is known, namely, the lipoids (fatty bodies typified by lecithin or cholesterin), behave, in watery suspension, like the colloids ; the others, which probably belong to the proteins, behave like colloids from the point of view of diffusion in dialysis, heat, and instability, and their principal reactions are closely analogous

to those of colloids. In any case, there might exist between the antibodies and antigens on the one hand, and the colloids on the other, considerable differences without preventing the general laws of attraction and adsorption from being equally applicable to the former as to the latter.

Agglutination and precipitation closely resemble the flocking of colloids. Bacteria behave towards a precipitating serum in the same way as they behave to certain substances quite foreign to the body, such as gelatine or gum-arabic.

Bacteria, in presence of a solution of ferric chloride, are protected by the colloidal ferric hydrate from agglutination by their specific agglutinin.

The phenomena of specific hæmolysis (*i.e.*, hæmolysis by the serum of immunized animals) have been imitated by attacking the corpuscles by means of such systems as silicic acid + lecithin, colloidal ferric hydrate + dog-serum, or saponin + taurocholate of sodium: the two kinds of phenomena may be expressed by the same curves (Zangger, Mlle. Cernovodeanu, and V. Henri). Complement can be fixed by the most diverse substances, by Witte peptone, yeast cells, the cells of organs, and various precipitates.

The toxin + antitoxin reaction has been "imitated" by the interaction of arsenious acid and colloidal ferric hydrate. The phenomenon of Danysz-Dungern can also be reproduced with colloids; the final result in the precipitation of a colloidal suspension is different according as the precipitant is added in one large or in fractional doses.

The reactions between cholesterin and various poisons and between cholesterin and lecithin have the appearance of colloidal reactions. One may even observe affinities of the character of the specific affinities. For example, cholesterin neutralizes saponin and tetanolysin, but has no effect on ricin or staphylolysin. Complement is absorbed, as has been seen, by a great variety of substances; agglutinin, however, possesses affinity chiefly for the colloidal protein, while tetanus toxin is chiefly fixed by the lipoids. It is legitimate to conceive of

these affinities as depending upon physical conditions, including that of the electrical charges.

The *lipoid substances* which are found in such abundance in nerve-tissue and which constitute a constant component of protoplasm, according to Overton furnish the cell with a sort of envelope through which the food materials have to pass and which behaves as a sort of colloidal atmosphere; the principal members of this group are lecithin and cholesterin. It is inconceivable that they do not play some part in immunity phenomena which are phenomena of nutrition. Hæmolysin, for example, undoubtedly induces changes in the lipoid coating of the red blood corpuscles.

The lipoid extracts of red corpuscles readily fix normal hæmolysins, whereas the lipoid extracts of bacteria fix certain immune-bodies. The lipoids also are capable of fixing the complements.

We have already mentioned two series of experiments in which lipoids play a most definite part. In the first place we have the activation of cobra venom by lecithin (Kyes), in which the latter appears to play the part of complement. According to Noguchi, triolein, oleic acid, exerts the same effect and loses its activity quite like complement when heated. Oleic acid is said to be capable of activating specific hæmolysins, and silicic acid, which alone possesses a very feeble hæmolytic power, is said to form with lecithin a complex or "lecithid" which is much more powerful.

In the second place lecithin plays the part of antitoxin towards certain toxins. The bile or the soluble elements of bile neutralize snake venom in appropriate dose; the cholesterin is the active constituent. Cholesterin and lecithin neutralize the botulismus toxin. Cholesterin neutralizes saponin, solanin, agaricin, vibriolysin, lecithids of cobra venom and of the poison of bees: again it is its cholesterin content which makes serum neutralize saponin. Finally in the celebrated experiment of the fixation and neutralization of tetanus toxin by the cerebral cortex (Wassermann and Takaki's experiment) the lipoids of the grey matter are the active

agents; the brain extracted with ether loses much of its neutralizing power, and the dye carmine neutralizes because it contains lipoids derived from the cochineal insect (Metchnikoff).

It is facts such as these which encourage investigators to pursue the line opened up by Bordet's experiments, although there is no absolute promise that they will find in this the key to immunity.

If we add now that phagocytosis is not incompatible either with Bordet's theory or with Ehrlich's, it is true enough, but it is not all the truth. It is not a question of reconciling theories. There are only two "theories," that of Ehrlich and that of Bordet, which, with their conjectures, their uncertainty, their attempts at explanation, and their continual state of incompleteness, are striving to round off the positive *doctrine*, the expression of undoubted facts, namely phagocytosis. When a physiologist is studying digestion he founds his study on facts which are essential and certain, such as the action of trypsin and enterokinase; this is no theory; it is only when he proceeds to interpret this action in terms of physics or chemistry by fixations or combinations that he enters the domain of theory. Similarly, it is no way of recognizing the capital importance of phagocytosis to admit that antibodies and other humoral properties are produced by the phagocytes. The essential fact is the destruction of the microbes by incorporation and digestion. Extraphagocytic destruction is so much an exceptional case that it cannot even be brought in as opposition.

There would be no temptation to forget this fact if, instead of limiting our attention to human pathology, we kept in view the universality of intracellular digestion throughout the series of living beings. Phagocytosis is quite different from a medical theory. It is a doctrine as fundamental in general biology as is the existence of the cell or the variation of species.

Toxins and Phagocytosis.—The immunity of an animal towards a toxin is not to be ascribed simply to the activity of its body-fluid. We must take account of the body also in the reaction. There are many facts indicating a similar state of

affairs, as in the experiment of Heymans and Masoin on the neutralization *in vivo* of hydrocyanic acid by hyposulphite of soda.

In vivo the hyposulphite of soda acts as an antidote or chemical antitoxin to hydrocyanic acid. Now no one has ever succeeded in reproducing this experiment in the test-tube, whereas in the body it is perfectly easy. "In consequence it is legitimate to appeal to certain peculiar conditions in the living animal, which, however, does not exclude the possibility that the transformation of the toxic substance into a harmless material may be due to a chemical reaction."

Fresh nutrient broth possesses an antitoxic action towards abrin intoxication (Calmette). The serum of an animal immunized against certain toxins or venoms protects other animals to a greater or less extent against the action of *other* toxins or *other* venoms; here there can be no question of a specific direct antitoxic action.

The fresh blood of the crayfish is capable of preventing the fatal intoxication of mice by scorpion venom; yet the crayfish is killed by a dose of scorpion venom three or four times smaller than that necessary to kill a mouse, and the blood of the crayfish has no protective action for another crayfish.

Roux and Vaillard observed long ago that animals might die of tetanus although possessing an abundance of antitoxin in their blood. There are certain horses originally good furnishers of diphtheria or tetanus antitoxin which suddenly cease to produce this in their serum although they remain immune.

Rabbits may be immunized against tetanus by inoculating them under the skin of the tail several times in succession with tetanus spores along with a little lactic acid; the animal becomes resistant to the toxin and yet 100 volumes of its serum fail to neutralize a single minimum lethal dose of toxin (Vaillard). The antitoxic power of the body-fluids is thus not sufficient to explain acquired immunity, since it is not an invariable fact in animals rendered immune.

The actions of the body itself have again to be reckoned with in attacking the question of the origins of antitoxins

We have already mentioned the old opinions of Buchner and Roux. Buchner considered that the antitoxin was derived from the toxin, and Metchnikoff advanced the opinion that certain body-cells might produce this transformation. But, it has been protested, how could a horse react to a single unit of toxin by producing 100,000 units of antitoxin? The toxin may, however, be seized by certain organs which retain it for a long period and transform it slowly. The toxin may induce in the cells which produce antitoxin the very stimulus which Ehrlich was among the first to appeal to. The experiment of Roux and Vaillard on rabbits immunized against tetanus would thus be explained: after repeated bleedings the antitoxin power of the blood rapidly regained its former titre. But why should the serum of healthy animals sometimes have a certain antitoxic power? Because without having actually suffered from diphtheria or tetanus they may have harboured diphtheria or tetanus bacilli in their bodies: in the intestine of the horse for example the tetanus bacillus abounds.

Whether or not the toxin is transformed into antitoxin, it is certain that the antitoxin is a product of the body: no other way of producing it is known. Ehrlich formerly thought that the cells sensitive to the toxin were its chief producers; but if this were true, antitoxin ought to be present in these cells and be capable of neutralizing the toxin. Wassermann and Takaki's experiment seemed to prove this: the brain tissue of mammals ground up with tetanus toxin, neutralizes it, furnishing a mixture which no longer gives tetanus to animals. But Wassermann's experiment has in reality a quite different signification. The brain does not act as an antitoxin, for, if injected into a guinea-pig along with a dose of toxin, but at separate points in the body, it has no protective action whatever, and does not act in the least like a dose of antitoxin. Further, if the so-called neutral mixture is injected into the thigh of a guinea-pig, the animal becomes tetanic, whereas it remains protected if the injection is made into the peritoneum. The neutralizing property is in reality a property peculiar to

the cerebral cortex (and the grey matter of the spinal cord) of *mammals* only ; in fowls immunised against tetanus the brain has much less neutralising power than the blood, the liver, or the kidney. The brain of the frog does not neutralise the toxin, although under certain conditions the frog is susceptible to tetanus.

Cholesterin, lecithin, and even olive oil and carmine (a substance derived from the fatty body of the cochineal insect) are capable of neutralizing a certain quantity of toxin ; now the brain substance contains both cholesterin and lecithin. It is, however, another lipoid in the brain, *protagon*, which chiefly fixes the toxin and perhaps permits of its transport along the nerves (Landsteiner and Botteri.) A. Marie and Tiffeneau have recently insisted that in Wassermann's experiment it is not a destruction which takes place, but a combination from which the toxin may be recovered. Anyhow, the neutralization by the cerebral tissue is a phenomenon of molecular adhesion, analogous to a dyeing process.

By injecting the tetanus toxin directly into the brain it has been shown that this very brain substance which, ground up in a glass, neutralizes the toxin, does not neutralize in the living animal the most minute dose. It is therefore impossible to suppose that the brain is a source of antitoxin (Roux and Borrel).

An immunized rabbit, rendered resistant to toxin by subcutaneous inoculation, succumbs when the toxin is injected into the brain : the antitoxic action is therefore due to cells lying between the periphery and the centre ; the poison is neutralized *en route*.

What are these cells? We find that sublethal doses of tetanus toxin produce in the fowl a great afflux of leucocytes into the blood ; further, in a fowl injected with tetanus toxin, far less toxin is to be found in the blood than in (aseptic) exudates rich in leucocytes. Metchnikoff therefore considers that the protection of the body against toxins also depends on the leucocytes.

The rabbit can stand large doses of atropin injected subcu-

taneously or into the circulation, but it is very susceptible to intracerebral injection; is it the leucocytes which dispose of the poison when injected into the veins? This is possible, for the poison can be recovered from the leucocytes after it has disappeared or is only to be found in traces in the blood plasma (Calmette).

When guinea-pigs are injected intraperitoneally with arsenic trisulphide, a salt which is only slightly soluble, the particles are taken up by the macrophages. When a soluble salt like potassium arsenite is injected, when the animal recovers it is in the leucocytes that the arsenic is found on chemical analysis (Besredka). The phagocytes also absorb lead salts (Carles). The white corpuscles of the blood are thus equally capable of resisting mineral as microbial poisons.

When a guinea-pig is inoculated with the mixture of mammalian brain ground up with tetanus toxin the solid particles attract the phagocytes which seize them and with them the attached toxin. If the toxin is injected along with particles to which it does not attach itself (*e.g.* the mixture of toxin + frog's brain) it can diffuse, and the leucocytes no longer protect the animal.

After the discovery of the antitoxins one was apt to think that in every infection there was an intoxication and that the neutralization of the poisons ought to precede phagocytosis which is only a secondary phenomenon. But experience has shown that the phagocytes can also digest the microbial toxins.

Certain bacteria secrete substances which weaken and destroy the phagocytes; among these antiphagocytic microbial poisons may be classed the *agressins* of Bail. Now the phagocytes are capable of absorbing and digesting these substances without any outside assistance.

Certain bacterial extracts prepared outside the body and injected in sufficient quantity are prejudicial to phagocytosis. Yet the same bacteria which furnish these extracts are absorbed by the leucocytes when these latter have their activity reinforced.

When dead typhoid bacilli are injected into the peritoneum

of a guinea-pig they are of course incapable of producing an infection, but as they contain the typhoid endotoxin the animal dies nevertheless from the intoxication. But if before the injection the peritoneum is "prepared" so that the bacteria at once meet with a great number of vigorous leucocytes the poison is absorbed by these and the animal is saved.

A staphylococcus habituated to the rabbit by the method of passages, secretes a poison which is very injurious to the leucocytes of this animal; but if along with the staphylococci vigorous living leucocytes are injected into the pleura, the rabbit is protected against the intoxication (Bail and Weil).

Immunity against toxin therefore can be reduced, like the immunity against bacteria, to the simple fact of phagocytic digestion. Metchnikoff finds himself justified in the light of all these experiments, in thinking that it is the phagocytes which take up poisons and which perhaps themselves elaborate the antitoxins employed in serotherapy.

CHAPTER XII

ANAPHYLAXIS

Definition of anaphylaxis—Experiments of Richet and Portier—Anaphylaxis to various poisons—Anaphylaxis to normal serum—Arthus' phenomenon—Serum sickness: observations of V. Pirquet and Schick—Serum anaphylaxis in guinea-pigs; phenomenon of Th. Smith—Anaphylaxis to cells and organ extracts—Passive anaphylaxis.

Study of Richet's poisons and serum anaphylaxis—Anti-anaphylaxis (Besredka): not a vaccination—Application to serotherapy—Heating of sera—Theories of anaphylaxis—General theory of antibodies.

ANAPHYLAXIS is the opposite of vaccination. An animal is vaccinated when the first attack by a virus (microbe or toxin) produces in it changes through which it is protected against another more serious attack. If you suppose the animal to become, after the first attack, not more resistant, but more susceptible, you have in a word the root idea of anaphylaxis.

The paradox is even greater than this definition shows, for anaphylaxis exists not only towards viruses against which immunity also occurs, but also it appears towards substances which in the normal individual are apparently quite innocuous, for example, egg-white, milk, and serum. In this way the first injection with these substances, instead of producing immunity, seems to destroy the natural immunity of the normal animal.

The supersensitiveness produced by a first inoculation and brought to light by a second is not due to a cumulative action, such as may be observed after several successive doses of certain drugs; in anaphylaxis the effect is out of all proportion to the quantity of material ingested.

This chapter of experimental medicine was opened up by the experiments of Richet and Portier on the poison of Actinians (1902).

Anaphylaxis to Poisons—Richet's Experiments.—From the tentacles of actinians a poison may be extracted which has been called *congestin*, because it produces in the animals injected an intense congestion of the viscera, of the stomach, liver, kidney, and, above all, the intestine. The fatal phenomena do not appear until after several hours of incubation. If a dog which has recovered from a small dose is inoculated after a certain interval of time with $\frac{1}{20}$ of the quantity of the first dose, violent symptoms suddenly appear after a few seconds, severe vomiting, difficult respiration, paralysis, profuse and blood-stained diarrhœa. On comparing the size of the dose with its effect, it works out that the dog has become, between the first injection and the second, eighty times more susceptible.

Anaphylaxis to Normal Serum—Arthus' Phenomenon.—If a rabbit is injected subcutaneously every six days with 5 c.c. of horse serum, it is found that after the fifth injection the serum is absorbed with difficulty; succeeding injections produce local lesions which continually increase in severity, from simple inflammation to actual necrosis of the tissue. The same phenomenon may be produced with milk instead of serum.

"Serum-sickness": Observations of Von Pirquet and Schick.—Therapeutic sera (antidiphtheria, antitetanus) are almost always got from immunized *horses*; they are not invariably devoid of toxicity for man. In a small proportion of cases, about 14 per 100, the injection is followed by various symptoms, not very severe it is true, which only appear after an incubation period of five to fifteen days, and consist of urticaria, erythemas, and pains in the joints. But should the patient fall ill again months or years later, long after the serum has disappeared from his body, and should he be reinjected with it, the symptoms are reproduced and are more frequent (86 per 100, according to Weil-Hallé and Lemaire), more

intense, and more rapid; they appear within an hour, or even within a quarter of an hour, after the injection.

In the light of these observations V. Pirquet observed later that the unknown germs of vaccine lymph produce a premature reaction in the skin of those individuals formerly vaccinated and now supersensitive. The same observation in its turn suggested to him the idea of applying a droplet of tuberculin to the skin of a tuberculous patient to test his susceptibility, and this was the origin of the cutaneous reaction of tuberculosis.

Through these observations the study of anaphylaxis entered the sphere of human medicine, and the important question arose, How are we to render harmless our therapeutic sera?

Serum Anaphylaxis in the Guinea-pig. The Phenomenon of Th. Smith.—The question entered the laboratories when attempts were made to elucidate the fact observed in the American serotherapeutic institutes, that guinea-pigs which had already been employed in the titration of antidiphtheritic serum (injected with a mixture of toxin and serum) became eventually supersensitive to horse serum. If three to twelve weeks after the titration injection 5 c.c. of horse serum is injected under the skin or especially into the peritoneum, the animals immediately manifest anxiety and discomfort; respiration is rapid and laboured, the heart becomes weaker, the temperature falls below normal and after one hour 50 per cent. of the animals die, whereas normal guinea-pigs support doses of the same serum five times as great without any disturbance. The phenomenon is specific, for guinea-pigs receiving horse serum in the first dose behave on re-injection quite like normal guinea-pigs towards rabbit, goat or ox serum. In the toxin + antitoxin titration mixture the antitoxin, *i.e.*, the horse serum, is responsible for the supersensitiveness induced. A small dose sensitizes more certainly than a large dose; even one millionth of a c.c. may suffice! The symptoms resemble serum-sickness in man; there is an incubation period of twelve days at least after the first injection. The supersensitive condition persists for

months (experiments of Otto, Rosenau and Anderson, Besredka).

Anaphylaxis to Cells.—Animals injected with red corpuscles, washed (to prevent the action of the serum) or unwashed, resist the first injection well but support a second badly.

Similarly with bacterial cells, typhoid, paratyphoid bacilli, etc., they present more or less severe symptoms after the second injection. The specificity of this reaction does not appear to be very strict. This variety of anaphylaxis is of great practical importance for in the preparation of anti-plague sera it is necessary to inject the cultures into the jugular vein of horses, and severe symptoms are quite common.

Organ Extracts.—The phenomena induced by injections of extracts of spleen, lymphatic glands, bone marrow, or spermatozoa are analogous to the preceding. The supersensitive state can be induced towards extracts of the crystalline lens of the eye and an animal thus prepared reacts only towards the lens tissue, no matter from what species of animal, even when it presents no reaction to the serum of that animal.

A tuberculous patient becomes supersensitive to the product of the tubercle bacillus, to tuberculin. But in this case it appears that only the disease itself can induce this excessive susceptibility permanently: it is very doubtful if it can be produced by the injection of the bacterial bodies or of tuberculin. Physiologically speaking it is an anaphylactic phenomenon.

Passive Anaphylaxis.—M. Nicolle has shown that if a fresh rabbit is given a large dose of the serum of a rabbit rendered anaphylactic to horse serum, the fresh rabbit takes on an anaphylaxis which is therefore called *passive*. It may in its turn present the typical symptoms on injection 24 hours after the preparatory injection. This brief delay shows that it is a true passive anaphylaxis, and not an active anaphylaxis induced by small quantities of horse serum which might have remained in the serum of the sensitive rabbit. This experiment demon-

strates the existence of an *antibody* in the serum of the sensitive animal; this antibody has been transferred to the other animal just as the diphtheria antitoxin of the horse can transfer passive immunity to man.

Passive anaphylaxis can be produced particularly well by injecting the serum of an anaphylactic rabbit into a normal guinea-pig. Passive transference has also been produced in the case of Richet's poisons. It is therefore a general fact, although experiments of transference between animals of the same species do not always succeed.

From these fundamental experiments there have proceeded several sets of researches; especially with the poisons and with serum considerable advances have been made. It is not at all certain in spite of their manifest similarities that the laws of anaphylaxis against poisons and serum (or other non-toxic protein) are really entirely the same. There is always this difference, that in the one case it is a question of substances manifestly toxic to the normal body, whereas in the other the substances are such that the healthy animal shows no visible reaction.

For the practical point of view, these researches, both on the poisons and on the sera (because of serotherapy), are of obvious value; there is an actual disease to prevent or cure.

It does not seem as if the anaphylaxis to egg-white should interest us to the same extent. We do not take albumen or milk by subcutaneous, intraperitoneal, or intravenous injections. If, as we know already, our body tends to resist the introduction of foreign albumens, we have a digestive tube which transforms these into our own specific protein; that is perhaps why so few examples exist hitherto of anaphylaxis acquired by taking food. Further, it is probable that we are much more exposed to the cumulative action of toxic bodies, *e.g.*, the phenols of the digestive tube, than to an anaphylaxis to the proteins of the ox or of the fowl. But the digestive tube may occasionally be defective as a defensive agent.

Poisons also may be absorbed by other routes, such as the

pulmonary alveoli or the skin. We are here in a region as yet little explored; there are many mysteries in the action of drugs, there are still greater mysteries in the experimental study of diseases of nutrition. Once again anaphylaxis raises this general problem of nutrition, towards which already the whole study of immunity is directed, and it is probable that, did we know more of this, we would be less ignorant of the nature of life, old age, and death.

Let us consider then separately what knowledge has been gained on the subject of anaphylaxis to those substances which have been most studied, the poisons and serum.

Researches on the Poisons.—There have been studied by Richet the congestin of Actinians, the congestin extracted from mussels, and a vegetable toxin, analogous to abrin and ricin (well-known from Ehrlich's experiments), called crepitin, and extracted from the plant *Hura crepitans* of the Euphorbiaceæ, known in Brazil under the name of *Assaku*. The mytilocongestin (from mussels) produces vomiting, a symptom very definite and easy to observe, a great convenience in experimental work. "The symptoms of anaphylaxis to crepitin are exactly the same as with actino- or mytilo-congestin, and even the most acute observer, I am sure, could not distinguish them. . . . There is the same profound abolition of all innervation, both motor and sensory, and above all, vaso-constrictory: there is the same intense hæmorrhagic congestion of the intestine with an enormous fall in the arterial pressure" (Richet).

It is to be observed that these belong to the group of slow poisons—resembling thus the bacterial toxins--which differ from the crystalloid group such as strychnine, and it is probable that on this depends their anaphylactic effect.

An animal is rendered anaphylactic because the first injection has induced in it, after a period of incubation, the formation of a new substance, an antibody, the product of a reaction of the body. This antibody is not itself poisonous, but it liberates a poison when it comes in contact with the congestin or crepitin of the second injection.

The antibody is named by Richet *toxogénine*, and the new poison, *apotoxine*. He therefore imagines anaphylaxis after the formula: *Toxogénine + Congestine = Apotoxine.*

He considers that the apotoxin results from the action of the two substances on each other, the one, congestin, introduced from without and non-toxic in the dose employed for the test injection, the other, toxogenin, non-toxic, prepared by the body itself as a result of the first small injection of congestin. In a similar way we have emulsin and amygdalin acting together to produce hydrocyanic acid.

At first the existence of this toxogenin was hypothetical, but experiment has in two ways proved its real existence—1. When a normal animal is injected with the blood of a sensitized animal, it becomes anaphylactic without any period of incubation, *i.e.*, there is passive anaphylaxis. 2. Richet has several times succeeded in demonstrating the reaction with crepitin *in vitro*. The mixture in a test-tube of anaphylactic serum and poison, when injected after incubation, produces immediate anaphylactic effects: the test-tube contains both the antibody and the antigen. 3. The toxogenin does not exist only in the blood; it is also to be found in the brain tissue. By mixing crepitin with brain substance (freed from blood) or even with the alcoholic precipitate from the brain substance of an anaphylactic animal, immediate anaphylactic symptoms can be produced in a fresh animal: there is thus "cerebral anaphylaxis" *in vitro*. There is thus antibody present in the nerve-cell and capable of reacting with the antigen at the moment of the anaphylactic shock. It is this very reaction which Besredka regards as the essence of the anaphylactic shock, which appears to him to be eminently a cerebral phemonenon.

According to Richet those animals which have received an anaphylactising toxin become from this sole fact more sensitive to other poisons. The injection of an antigen, for example crepitin, renders an animal more sensitive to toxic action of other kinds, for example to that of apomorphine, although the increase of sensitiveness is particularly directed towards the antigen itself. There exists therefore, it would

seem, a sort of general anaphylaxis in addition to the specific form of anaphylaxis. Richet is inclined to consider that in the phenomena of anaphylaxis there is in reality only a relative specificity, and that the apotoxin is a poison without any specificity which attacks and paralyses the central nervous system, affecting in this particularly the vasomotor centres. "It seems to me very probable that in the study of the whole field of the different forms of anaphylaxis produced by different substances a great analogy if not identity would be discovered in the symptoms of all forms of anaphylaxis, so that we may be permitted to believe in the general analogy if not in the identity of all the different apotoxins, the various anaphylactic poisons. There would thus be a very simple conclusion, namely, that there is one poison and one only, the apotoxin produced in all the forms of anaphylaxis."

Richet's poisons produce in the body, besides anaphylaxis, immunity. With mytilocongestin the anaphylaxis disappears after about six weeks and the immunity persists. Anaphylaxis thus gives place to its opposite "prophylaxis" and toxogenin to antitoxin. The two conditions "develop side by side from the moment of the first injection. Hence it is necessary to distinguish closely between the immediate and the late effects. During the anaphylactic period there is a striking supersensitiveness as regards the immediate symptoms, but there is already some immunity towards the late effects of the poison. If the animal survives the immediate effects of the second dose, it presents no further symptoms during the days following.[1] . . . *Anaphylaxis is the first stage in prophylaxis.*" These views recall those of Behring. "However paradoxical it may seem, there can be no doubt that horses which have become strongly immune as the result of treatment with tetanus toxin yet possess a histogenic supersensitiveness of their tissues towards the tetanus toxin." These properties are

[1] Richet applies the same idea to serum-anaphylaxis and to anaphylaxis in general. "The anaphylactic reaction is a defensive function and is destined to maintain intact the chemical constitution and homology of each animal species by preventing foreign albumins from entering the protoplasm of the cells so as to modify their specific chemical structure."

probably common to many toxins, but in serum anaphylaxis the same characteristics do not necessarily exist.

Anaphylaxis to poisons thus means a hastening of the reaction of the body towards microbial toxins: it is a process adapted for rapid defence and in particular for defence against small doses: it is a sort of alarm signal sent to the body-cells calling its attention to minute quantities of poison which without this would have been insufficient to induce immunity: immunity is established thanks to the preceding anaphylaxis. Perhaps anaphylaxis is equivalent to Ehrlich's superexcitation of the cells required for the production of antitoxins.

Serum Anaphylaxis.—A preparatory injection of serum in very small dose (1/250 to 1/1,000,000 c.c.), a period of incubation, a test injection larger than the first and producing violent symptoms, these are the conditions of serum anaphylaxis in the guinea-pig.

On observing the symptoms it is impossible to avoid the idea that the guinea-pig has fallen victim to some cerebral lesion which was latent during the period of incubation but has been revived by the test injection. The symptoms of this lesion ought to be still more definite if the injection were made into the brain itself. The nerve-centres can be more directly attacked by intracerebral or intradural injections or through the circulation by the intravenous route (Besredka).

One quarter of a c.c. injected intracerebrally produces the same phenomena as 5 c.c. intraperitoneally and produces them with much greater regularity, for instead of about 25 per cent., practically all the guinea-pigs die. The experiments are thus on a surer footing. Should the still unknown reaction take place in the brain cell itself, *i.e.*, the reaction between the poison and the antibody manufactured by the body as a result of the first injection, one might hope to modify the anaphylactic phenomena by acting on the nerve-cells of the supersensitive animal. And this is really the case, as is shown by a very pretty experiment of Roux and Besredka. The sensitive guinea-pig is put to sleep with ether or put under the

influence of a suitable dose of alcohol; an intracerebral injection which kills the sensitive control leaves the narcotised guinea-pig unharmed.[1]

In practice intracerebral injections have supplied Besredka with a useful means of measuring the toxicity of a serum, for example, anti-diphtheria serum, from the point of view of the symptoms of serum sickness. Should a method be discovered of diminishing or destroying the toxicity of sera, the improvement acquired could thus be estimated. In particular, the method will tell us whether a serum may be safely injected by the route by which it manifests its greatest toxicity, but also its greatest efficacy, *i.e.*, intravenously. Experience has shown that a serum, very toxic the day of the bleeding (minimum lethal dose $\frac{1}{32}$ c.c.), rapidly loses this toxicity during the ten days following (lethal dose $\frac{1}{16}$ c.c.). It continues to diminish slowly, for a month and a half, but after about two months it remains almost indefinitely at the same level (lethal dose $\frac{1}{8}$ c.c.), and never becomes non-toxic. For example, a sample of serum kept in Roux's laboratory for thirteen years still kills sensitive guinea-pigs in a dose of $\frac{1}{4}$ c.c. intracerebrally (Besredka).

Antianaphylaxis.—Anaphylaxis is a morbid state predisposing to the occurrence of accidents. But these may be prevented. Antianaphylaxis is the name given by Besredka to the condition of insensibility to which the sensitive animal can rapidly be brought. But though in current language one talks

[1] There exist other physiological shocks which are deadened by narcosis, and it is impossible to avoid a comparison between the above experiment and one of Jellinek's (the director of the laboratory of electrical pathology in Vienna) reported in a recent lecture by D'Arsonval.

"A rabbit is fairly easily killed when the opposite poles of an alternating current of 1,500 volts are placed in its mouth and rectum, whereas a rabbit of the same breed but so deeply chloroformed that all its vital phenomena have ceased is at once reawakened and saved from death by the same current.

"At the time that this experiment was published an English engineer, Aspinall, observed that two electrical engineers who had during sleep come into accidental contact with an alternating current of 3,000 volts were simply awakened by burning sensations in the back without other injury." (Jellinek).

vaguely of "vaccinating" against anaphylaxis by Besredka's method, *antianaphylaxis is in reality not a vaccination.*

Originally it was conceived as a true vaccination, and to produce it Rosenau and Anderson made a series of injections of 5 c.c. of serum intraperitoneally, starting before the period of incubation for the anaphylactic state was up: they proceeded as one does for antitoxins.

But this idea had to be given up when it was seen that it was sufficient to protect the guinea-pig to give it, not a series of injections, but a single injection, not a large injection, but a minimal one, $\frac{1}{150}$ to $\frac{1}{100}$ c.c., *i.e.*, much less than the toxic dose; finally, and above all, that the resistance of the guinea-pig develops the day after, or even some hours or minutes after, the injection, according to the route employed. After subcutaneous injection the resistance is present in four or five hours; after intradural injection in about two hours; after intraperitoneal in an hour and a half; ten to fifteen minutes after intravenous injection. The shortness of this latter incubation period may be employed to render the resistance more and more complete: it is simply a question of repeating these minute doses intravenously every ten or fifteen minutes: each injection reinforces the effect of the preceding: this is the method of "continuous vaccination" (*vaccinations subintrantes*). It is not even necessary to inject by the veins: by taking a little more time one may proceed by other routes and even changing routes in successive doses. In this way a guinea-pig may be protected from as many lethal doses of serum as is desired (Besredka).

In this there is the germ of a method which may be applied to man. Certain sera, in particular the anti-plague serum, have to be injected in large quantities, and as far as possible by the veins. The anti-diphtheria serum is 500 times more active, other things being equal, if introduced directly into the circulation instead of under the skin (Berghaus). Besredka's process promises to relieve such intensive serumtherapy from all danger of death by anaphylaxis.

What sort of immunity then is this which develops with such

small doses and so rapidly ?[1] A vaccinated animal departs from the normal to acquire a new condition ; in sensitized animals under these circumstances there is not the departure from a normal state but there is an appearance of returning towards it. Antianaphylactic vaccination proceeds like a disintoxication and *antianaphylatic immunity seems to be a return to the natural immunity*.

The sensitizing injection induces the formation of an antibody ; at the moment of the test injection it is probable that the toxic serum combines with or rather fixes itself on this antibody and this abrupt reaction produces a shock which the nerve-cell resents profoundly. It is "disintoxicated" by it, but with such brutal suddenness as to kill the animal. When by narcosis with anæsthetics or alcohol the sensitiveness of the nerve-cell is diminished, as in the experiment of Besredka and Roux, it becomes disintoxicated during sleep, just as in a surgical operation under chloroform, and wakes up free. If instead of injecting as in the test injection a massive toxic dose, a minute dose is introduced ($_2\frac{1}{000}$ c.c. intravenously, for example), the disintoxication of the body, and in particular of the nerve-cells, is carried out gradually, little by little ; in the continuous vaccination method the disintoxication leads back the animal by degrees to its normal condition.

To the normal condition, but not quite, for the injection leaves as a rule a trace of serum in the body with which it re-sensitizes itself in time ; the guinea-pig has become a fresh animal, and like a fresh animal may be sensitized. It may lose its virginity, regain it, and again lose it. Certain experimenters do not believe that the body ever becomes again normal.

The digestive tube is, physiologically speaking, as much a barrier to the invasion of the body by foreign proteins as it is

[1] "The immunity to a toxin does not appear till after eight days at least and is more pronounced the more numerous and prolonged the successive doses : it is accompanied by the appearance of the antibody in the serum, and finally it never extends to the nerve-centres. On the contrary anaphylactic immunity is established after a single injection, is practically instantaneous, is never accompanied by the appearance of antibodies and finally always extends to the nerve-centres, the brain and spinal cord" (Besredka).

towards bacteria: by its very power of digesting and assimilating them it renders inoffensive those substances which, if injected under the skin or into the blood stream, would perhaps be toxic: it maintains the natural immunity.

But for the present it must be pointed out that Besredka's conception only applies to substances like serum which are harmless in general to the normal body even when injected direct into the veins. It is not necessarily true of the tox-albumins, which are toxic from the first.

We have briefly indicated two procedures for rendering a sensitive animal resistant, narcosis during the test injection and the method of continuous vaccination with small doses. The same result may be obtained by heating the toxic serum to certain definite temperatures. Heating produces an effect unrealized hitherto by any chemical means. Serum heated to 100° C. (diluted to prevent coagulation) becomes practically harmless for both intracerebral and intravenous injections: But this heated serum, no longer lethal, can still protect against the anaphylactic shock by gradually disintoxicating the sensitive cells as in the method of small doses or narcosis; no doubt the mechanism is the same.

Heating is thus a good means of lowering the toxicity of a serum. But sera heated to 100° C. lose all preventive and curative power, and it is impossible therefore to exceed 59–60° C. Experiment shows that heating for several hours at this temperature diminishes considerably the anaphylactic toxicity. The reason for the low toxicity of the Pasteur Institute sera, duly recorded by various laboratories, is simply that their sterilization is effected by means of several heatings at a low temperature (without antiseptics).

Man is not alone in profiting by laboratory research on anaphylaxis. In certain countries the Pasteur vaccine against anthrax is injected along with a few c.c. of an anti-anthrax serum: the method is named sero-vaccination. Many cattle are inoculated over again every two years, so that severe anaphylactic effects are not uncommon. Recently sero-vaccination was repeated on 180 cattle in Roumania. The

animals were divided into two lots, the first receiving 1 c.c., as an anti-anaphylactic injection, five hours before the sero-vaccination. In the result it was found that during the twenty-four hours following the sero-vaccination, not one of these showed any anaphylactic symptoms, whereas ten of the other ninety showed symptoms, such as œdema of the muzzle with salivation, œdema of the vulvar and anal mucous membranes, and colic (Alexandresco and Ciuca).

Theories.—No final theory for anaphylaxis yet exists, but there are various hypotheses which keep experimental work active. There is one point, however, which is certain, that it is impossible to explain the phenomena without the presence of an antibody formed by the animal as a consequence of the first injection. This has been demonstrated by M. Nicolle in connection with Arthus's phenomenon.

It is probable that the anaphylactic shock is due to the union of antibody and antigen, that this union is abrupt and affects especially the nerve-cells, which does not mean that the nerve-cells produce the antibody. On the contrary, everything points to their not creating the supersensitiveness, but being passively affected in it.

According to Vaughan and Wheeler, who studied in particular the anaphylaxis to egg-white, the sensitizing injection, as an antibody, provokes the formation of a *proferment* which can only act after the reinjection by splitting the protein molecule into two components, one toxic, the other not. As a matter of fact it is possible in the laboratory to produce from albumin two such components, but the toxic one is also toxic for normal animals quite as much as for sensitive ones: this therefore is not an explanation of anaphylaxis, though it shows that there exist toxic elements in substances normally non-toxic.

It was natural to attempt to identify the antibody of anaphylaxis with some antibody already known in the reactions of immunity. This was Friedberger's idea: in serum anaphylaxis the antibody is nothing but the precipitin, in anaphylaxis to blood corpuscles a hæmolysin. The facts, however, do not confirm Friedberger's theory.

Besredka's theory approximates more closely. In anaphylaxis three activities come into play, the *sensibilisinogen*, the property of serum by virtue of which it sensitizes, the *sensibilisin*, the property due to the body and corresponding to the antibody generally admitted, and the *antisensibilisin*, by which is meant the property of normal serum in virtue of which it combines with the sensibilisin and determines the anaphylactic shock.

Thus serum is toxic, not because it contains a poison ready made, but because two substances, non-toxic themselves, "enter into abrupt combination within the nerve-cells and thus disturb their equilibrium."

Why should small doses sensitize so well, whereas massive doses only do so after a long delay? The answer is that a minute dose induces the formation of sensibilisin without furnishing it with anything on which to fix: in consequence it remains avid, in the nerve-cells among others. After a large dose the sensibilisin manufactured is neutralized as it forms by the "anti-sensibilisin" of the serum and anaphylaxis is therefore delayed. It seemed probable at first that by heating the serum at suitable temperatures it might be possible to dissociate the two activities which do not depend on the body, *i.e.*, the sensitizing and toxic properties. But on closer examination it was found that heating acted equally on both; the two in reality follow the same curve. It had also to be recognized that the toxic function, the sensitizing function, and the vaccinating or anti-anaphlyactic function, are all fundamentally identical and occur in the same serum, but that they *correspond to different physical conditions of that serum.*

To sensitize, a serum must be injected highly diluted; to produce the anaphylactic shock it must possess its normal concentration; to "vaccinate" or protect against the shock it must unite very slowly with the sensibilisin of the body or must unite with it in minute quantities at a time (*e.g.*, in the method of "continuous vaccination").

Heated at 100° C. it is no longer toxic or very slightly

so, because being partly coagulated its fixation is delayed. The action of serum, sensitizing, vaccinating, or toxic, depends thus on its physical condition.

There are therefore really only two activities of serum in question, that of the antigen and that of the antibody. But according to its physical condition, according to the manner and time of its injection, the antigen plays the part of sensitizer, of vaccinator or of toxin. These explanations, it must be mentioned, are confined to the field of serum-anaphylaxis as it is known to-day.

We always return thus to the antigen and the antibody, *i.e.*, to the activities which we found to exist in all the phenomena of immunity.

General Theory of the Antibodies.—M. Nicolle has proposed a general explanation embracing anaphylaxis as a particular case of the general physiology of the antibodies. Every antigen induces in the body the simultaneous formation of antibodies of two classes, the coagulins and the lysins. The coagulins condense albuminoids and toxins (to speak only of these two varieties of antigen); the lysins, on the contrary, break them up and liberate from them their real toxic components:[1] in intoxication by proteid poisons it is not these themselves which injure the body, but secondary poisóns elaborated by it itself. The fate of the animal depends on its species, on the nature and quantity of the antigen, on the channel of inoculation and on the route by which the assaulting dose is introduced.

Thus, in the phenomenon of Th. Smith, the supersensitiveness is explained by the development of a lysin and the absence of all coagulin makes of it the type example of pure supersensitiveness. In bacterial anaphylaxis, the super-

[1] When the coagulins predominate they rapidly condense the antigens, giving the body time to attack them bit by bit without liberating enough poison at a given moment to cause toxic or, at least, fatal symptoms. The lysins on the contrary, when they predominate, make their appearance as the agents of an inevitable and often fulminating intoxication, for the body has only limited protection against the true endotoxins and the true toxins, no more than it has against alkaloids, for example" (Nicolle).

sensitiveness is due to a lysin which sets free from the bacteria a true endotoxin.

Infection and intoxication arouse in the body a many-sided conflict between these coagulins and lysins, which are in general the *good and evil antibodies.* A lytic action may, however, be salutary when it occurs slowly, whereas it is lethal when it takes place abruptly: under these formulæ may be arranged all the facts mentioned in the course of this chapter. "Although diametrically opposed to each other, as inevitable results from their definition, immunity and supersensitiveness may co-exist in the same individual, as well as succeed each other, often again and again."

Nicolle's theory is frankly inclined to the physical theory of immunity, without overlooking the intimate relations which exist between the physical properties of bodies, and their chemical constitution. It also sees in immunity phenomena of nutrition; for the body digests the antigens, and the theory supposes simply that every digestive act is due to the successive application of a coagulin and a lysin.

CHAPTER XIII

APPLICATIONS OF BACTERIOLOGY

Diagnostic Methods.

Direct diagnostic methods—Direct diagnosis of the microbe—Cultures from the blood—Examination of fæces—Indirect diagnostic methods—Cytodiagnoses.

Biological diagnostic methods—*Agglutination*: specificity and group agglutinations: variations in bacteria from the agglutination point of view—*Precipitation*: employment in forensic medicine and in the adulteration of foods—Applications to anthropology: confirmation of the simian origin of man—*Complement-fixation*: first experiment of Bordet: clinical application—Wassermann's reaction and the sero-diagnosis of syphilis—The nature of the substances coming into play in this reaction—*Supersensitive reactions*: tuberculin.

The simplest and surest way to diagnose an infectious disease is to demonstrate the presence of the specific microbe, *i.e.*, direct bacteriological diagnosis. When this is impossible, indirect diagnosis is resorted to, *i.e.*, the lesions of the tissues which are constant accompaniments of a virus which is invisible are sought for. The presence in exudates of certain cell elements is noted, or the body-fluids and bacteria are made to react together specifically (antibodies and antigens): in the latter case it is more properly a case of biological diagnosis.

Direct Diagnostic Methods.

The microbe is sought for wherever there is a possibility of finding it; blood, exudates and transudates, pus, mucous discharges, false membranes, ulcers and chancres, sputum,

cerebro-spinal fluid, urine, fæces, all may be examined. Such observations are completed by cultivating and experimental inoculations.

Direct Diagnosis of the Microbe.—This has to suffice when it is a microbe which cannot be cultivated. It is sufficient when the microbe has characteristics which cannot be mistaken. The sight of the malarial parasite of Laveran, of a filaria embryo, of a trypanosome (in man) is a certain diagnosis. Medicine has profited by every step in advance in technique: the ultra-microscope now permits us to see trypanosomes and spirochætes living and motile much more easily than with the best microscope.

Pasteur's method of "seeding" silk-worms was based on direct diagnosis. Direct diagnosis is currently employed in connection with sputum and false membranes; it is completed by culture and inoculation. Inoculation is the rule when the pneumococcus is observed in sputum: subcutaneously inoculated it kills a mouse within twenty-four hours: the mouse is *the* pneumococcus reagent.

Blood Culture.—Blood, taken aseptically from an animal not in a state of active digestion, and kept free from external germs, may be kept indefinitely without putrefying. If microbes develop in the blood itself or in nutrient broth into which it has been put, it means that these microbes existed in the blood during life. Pasteur's observations on the sterility of normal blood form the foundation of the diagnosis of infectious diseases by blood culture. The streptococcus was isolated by him for the first time from the blood of women suffering from puerperal fever.

It has been proved by blood culture that gonorrhœa, which is usually quite a local disease, may infect the blood with gonococci and cause arthritis and endocarditis. In a frankly acute pneumonia it is the rule to find pneumococci in the blood.

In typhoid fever blood cultivations have given results which have upset our conceptions of this disease. It used to be thought an exclusively local disease of the intestine and

the lymphoid organs: even when the bacillus had been found in the urine and in the red eruption spots on the skin it was still thought that it only exceptionally entered the blood. But when cultures were regularly made from the blood of typhoid patients it was perceived that the typhoid bacillus is present there during the whole febrile period of the disease and again during relapses: in typhoid fever, therefore, the acute enteritis is complicated by a blood infection, a septicæmia.

The examination of the faeces is regularly performed in the diagnosis and prophylaxis of cholera, typhoid fever, and dysentery, particularly in order to detect *germ-carriers*. Also in the stools are sought the eggs of worm parasites and of ankylostoma and amœbæ. The bacteriological examination of fæces is becoming more and more common in proportion as our knowledge of the intestinal flora is increasing; the composition of this flora is a guide to the state of digestion and nutrition, and aids the physician in his choice of a diet, especially in infants, who are so often threatened by infections of the alimentary canal.

Indirect Diagnostic Methods.

Even when no microbe is found in it, the blood may furnish diagnostic proofs from its leucocytic formula.

Cytodiagnosis (Widal) depends on the examination of the cells floating in pleural effusions or cerebro-spinal fluids. Different cells are found in a pleurisy due to the bacillus tuberculosis and in one due to heart disease. Cytodiagnosis of the cerebro-spinal fluid is an indirect diagnostic method in certain tuberculous and syphilitic affections.

Biological Methods: Agglutination.

The first clinical sero-diagnostic method was discovered by Widal.

When a drop of a broth culture of the typhoid bacillus is looked at under the microscope, the bacteria are seen actively motile, isolated from each other and dispersed regularly

throughout the liquid; the suspension is "homogeneous." If a trace of serum is added from an animal prepared by injections of typhoid bacilli or from a patient suffering from typhoid fever, the bacilli lose their motility and collect into masses: they are said to become agglutinated by the serum. If the serum is added to a broth culture floccules can be seen with the naked eye forming and sinking to the bottom of the tube; agglutination is also a sedimentation. Normal serum never possesses this property, certainly never to the same degree.

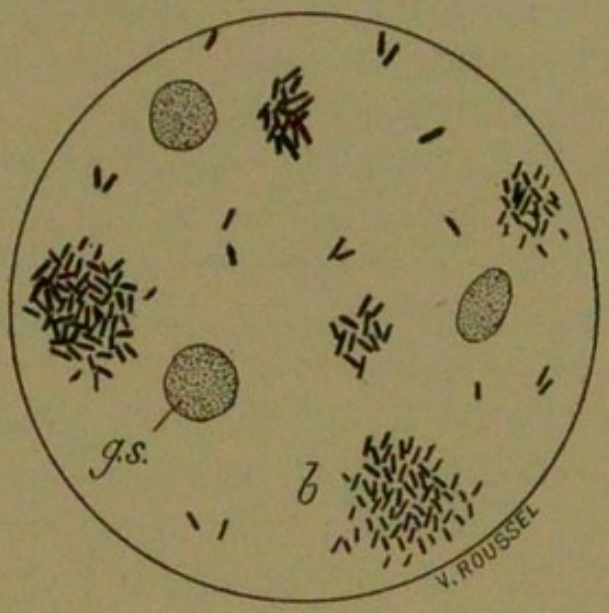


FIG. 71.—Agglutination of the typhoid bacillus by the serum of a typhoid patient: *b*, clump of bacilli: *g.s.*, blood corpuscles left in the serum.

The agglutinating power may be measured by trying the effect on a suspension of the bacilli of various dilutions of the serum: one may say that such and such a serum agglutinates at 1 in 50, 1 in 100, 1 in 1000. . . .

The reaction is capable of two applications. With a bacillus definitely known as a genuine *B. typhosus*, one can say that the serum which agglutinates it is an antityphoid serum. If, on the other hand, with such a definitely known serum we find a bacillus agglutinated, we may say that it is a true *B. typhosus*. Agglutination may be used to diagnose now the bacillus, now the disease. The agglutinating power depends on an antibody named the agglutinin. This substance keeps much longer than the time required for its carriage to long distances for examination, when this is necessary. Dead bacteria also agglutinate, so that the method may be employed even without living cultures. Seroagglutination is therefore the simplest and most convenient of the biological methods.

In performing the test it is necessary to avoid certain sources of error, among others the existence of bacterial strains

which agglutinate spontaneously or do not agglutinate at all. Strains can be artificially produced which refuse to agglutinate: this resistance of the microbe to the action of the serum is an example of the immunity of the microbe towards the animal, the converse case of the immunity of the animal towards the microbe.

Agglutination is applied to as a test for suspected bacteria found during cholera scares in suspected water or choleraic diarrhœa; the serum employed is obtained from an animal prepared by several immunizing injections of a definitely known cholera vibrio.

Agglutination has also been applied to the diagnosis and prophylaxis of bacillary dysentery and epidemic cerebro-spinal meningitis. In tuberculosis it has only been applied as a means of controlling the treatment by tuberculin. As a diagnostic agent the tuberculin test is much more convenient and certain (R. Koch). But agglutinin is no better than the other antibodies (vide p. 208) for the estimation of the resistance of the body towards tubercle.[1]

Precipitation.

If we take a culture of *B. typhosus* and filter it, we obtain a clear fluid free from bacteria. If a little of a very active

[1] Agglutination is the touchstone in the study of bacterial strains and their variations. Recently Bordet and Sleeswyk, working with the bacillus of whooping-cough discovered by Bordet, have created in the laboratory varieties of this analogous to the varieties of the dysentery bacillus.

What fixity do these varieties or strains possess, created as they are by growth on different media and separated by their different reaction to such-and-such a serum? What is of interest here is the conclusion of these workers. They maintain, at least as far as the agglutinating action of serum on bacteria is concerned, that sera "do not act on the fundamental bacterial substance, which is inherent to its life and whose presence is indissolubly bound with the nature and constitution of the species, but that they act on substances to some degree accessory, of possible but facultative presence, whose production is in no way one of the bundle of hereditary, immutable characters which give to a living creature its own physiognomy and autonomy."

It is obvious that this interpretation of the facts is not in favour of a chemical theory of immunity: the more one considers the agglutination as a surface reaction, the more probable becomes the physical explanation, *i.e.*, the explanation of Bordet.

antityphoid serum is added, the mixture becomes turbid and a precipitate forms which settles at the bottom of the tube. Instead of agglutination we have precipitation (R. Kraus).

If we inject a rabbit repeatedly with proteins of animal origin, horse serum, eel's serum, defibrinated fowl's blood, its serum thus immunized forms a precipitate when mixed with the serum of the eel, of the horse, or of the fowl (Tchistovitch and Bordet).

The precipitating property of sera thus prepared is ascribed to an antibody, *precipitin*. There are bacterial precipitins and protein precipitins, the former being simply a species of the latter : all proteins, animal or vegetable, in clear solution, are precipitated by the corresponding antisera : the action is quite specific.

This reaction is nearly related to the agglutinating reaction. It is scarcely ever used in the diagnosis of infections, but for the differentiation of protein substances it has furnished a very sensitive test which is employed in forensic medicine : nowadays serodiagnosis is applied to the detection of bloodstains and to the adulteration of meat and milk.

A man is accused of murder and in his house is found a garment stained with blood : the accused (he may be, for example, a butcher) declares that it is ox-blood and justice demands that serodiagnosis be applied to the stains. Some of the stains are therefore cut out of the cloth and extracted with physiological saline and with this liquid the precipitation test is applied : if the spot was due to human blood, the liquid produces a precipitate with the serum of an animal previously treated with human blood.

A merchant is accused of selling for pork-sausages sausages made from horseflesh. An extract is therefore made with water from the suspected meat. If the sausage contained horseflesh, the extract gives a precipitate with the serum of an animal previously injected with horseflesh extract. The method can still be applied when the meat has been smoked and dried. By the same method may be detected cow's, goat's milk, &c. The reaction is sensitive to 1 in 100,000, *i.e.*,

it gives a result with an extract containing only $\frac{1}{100000}$ of its weight of the protein to be determined.

The precipitation test has given positive results with the organs of forty-year-old mummies. It is even said to have been successful with mummies of 3,000 to 5,000 years, but in this case there is some doubt. It is probable that these latter may contain albumoses, but probably they no longer contain coagulable albumins.

The same biological reaction has furnished an additional proof of evolution. Just as there are group agglutinations, so there are group precipitations. The antiserum which precipitates rabbit serum precipitates also hare serum. Anti-dog serum precipitates with both dog and fox serum. The serum against the horse precipitates also the serum of the ass and of the tapir. The relationship, the *blood relationship* literally, between the goat and the sheep, between the fowl and the pigeon, and between the domestic and wild pig can thus be demonstrated.

What interests us more particularly is that the serum of a rabbit prepared with human serum precipitates with the serum of monkeys, a definite proof that we are their near relations. This relationship extends even to the lemurs, which are only half-monkeys, and is closer in the case of the monkeys of the Old World than with those of America. Grünbaum, who experimented with forty-six species of monkeys, maintains that from the point of view of the quality and quantity of the precipitates furnished by their sera, he has been able to detect no difference between man, the gorilla, the orang-outang, and the chimpanzee: this new proof of the correctness of man's simian origin may be welcomed.

Complement Fixation.

This new sero-diagnostic took origin from an experiment of Bordet: he was trying to show that there is in a serum, not several, but a single complement and that it is the same complement which acts in bacteriolysis as in hæmolysis.

We know that complement (*i.e.*, fresh normal serum) is fixed by the combination antigen-antibody. Let us prepare two such combinations:

(1) Cholera vibrios and anticholera serum (heated to 56° C. to destroy its complement).

(2) Blood corpuscles and hæmolytic immune body (hæmolytic immune serum heated to 56° C.).

Now on the first combination fix complement (*i.e.*, add fresh serum to the mixture and put in the incubator: the complement exerts its bacteriolytic action).

Now add the second combination ready for hæmolysis and return to the incubator. If the serum contains hæmolytic complement over and above the bacteriolytic, its presence will be shown by the laking of the corpuscles and the red tint spreading throughout the mixture. If, on the other hand, no hæmolysis occurs, it means that the first fixation has used up all the complement of the fresh serum: there is no hæmolytic complement therefore distinct from the bacteriolytic.

Given a mixture complement + immune-body (antibody) + antigen, we have always a means of knowing if the complement has been fixed, namely, to add a mixture of blood corpuscles and hæmolytic immune-body ready for hæmolysis. Laking of the blood indicates the absence of a previous fixation of the complement. For example, a serum is sent to us of a man who has been suffering for some time from fever; typhoid fever is suspected; if this is the case his blood ought to contain an *antibody* (antityphoid immune-body) and in the mixture of complement + serum of the patient + typhoid bacilli, the complement will be fixed on the bacilli through the intermediation of the serum. If after a suitable lapse of time one adds to the same tube a mixture of hæmolytic antibody and red corpuscles, the complement being no longer free, the corpuscles are not dissolved.

Bordet's reaction furnishes therefore a means of recognising by the presence or absence of hæmolysis the presence or absence of another immune-body (antibody), and in conse-

quence of discovering the effect remaining in the animal body from a previous infection.

It has been used like agglutination to diagnose both a past infection and the nature of a bacterial strain. There is no reason to use it in the diagnosis of typhoid fever, Widal's reaction being much more convenient. It is very useful in those cases where agglutination cannot be employed.

The Sero-diagnosis of Syphilis: Wassermann Reaction.—Wassermann's reaction is simply the application of the Bordet-Gengou reaction to discover the syphilitic antibody in the serum of patients affected by this malady. In practice it is rather delicate, and demands an experienced worker. In theory nowadays it is regarded as a precipitating reaction between certain colloid substances of the serum to be examined and the colloids of an organ extract which is employed as "antigen."

The reaction is not strictly specific; in leprosy, in trypanosoma infections in animals, perhaps in scarlatina, modifications of the serum often occur which give a positive reaction. The method is nevertheless capable of clinical application, since the physician has only to decide between a trypanosoma infection, such as sleeping sickness, which scarcely exists in Europe, leprosy, which produces characteristic lesions, scarlet fever, which is easy to diagnose, and syphilis, which is extremely common; the selection is therefore easy. A laboratory reaction which, taken by itself, does not give an absolute diagnosis, becomes nevertheless an absolutely certain sign when taken along with the sum-total of clinical and biological indications.

As a matter of fact, the Wassermann test, if carried out with the best technique, is positive in about 90 per cent. of the cases of primary syphilis, in 100 per cent. in secondary syphilis, and in 50–60 per cent. of late tertiary cases (Citron's figures); the proportions are rather lower in the statistics hitherto collected by the Pasteur Institute. The reaction becomes more definite the more acute the infection and the more active the virus.

There is some doubt as to whether it indicates syphilis in

full activity or rather only that the individual has been affected at a more or less distant period, and that the infection is now latent. According to certain observers, it is always the sign of active infection, although in a part of the body where the lesions are not perceptible, the proof being that specific treatment makes the reaction disappear, whereas it reappears during relapses or recurrences of the disease. The experience hitherto with "606" does not apparently authorize such a simple interpretation, however.

In many cases the diagnosis of syphilis is only too easy ; the lesions visible and active are sufficient proof. But humanity suffers from many ills which are more or less distant consequences of an attack of syphilis, neglected, forgotten, or even unsuspected. Since syphilis is a curable disease the value of Wassermann's reaction in the diagnosis of these obscure cases is obvious : lesions of the liver, of the heart, of the aorta, and failure of the sight may all by its means be assigned to their true cause and yield to the specific treatment. An old clinical law holds that the mother of a syphilitic infant, without being herself infected, is immune to infection from her child, and thus may nurse it without danger ; in reality, it turns out, she is infected : the serum reaction proves it. A Dresden physician, Rietschel, has thus diagnosed syphilis in 10 per cent. of wet nurses apparently healthy, and the confirmation was obtained when it was found that the children of these women eventually showed signs of syphilis.

Syphilis unsuspected or without symptoms is a disease less rare than is generally thought, and Wassermann's reaction furnishes us with an additional weapon against this formidable enemy.

Clinical medicine has long maintained the syphilitic origin of general paralysis of the insane, and of locomotor ataxy. The sero-diagnosis brings a striking proof of this. Not only the serum but the cerebro-spinal fluid of general paralytics furnishes a positive reaction in 88 per cent. of all cases, and in 95 per cent. of advanced cases. In locomotor ataxy the percentage is not so high.

The reaction is also of value in the distinction between general paralysis and other mental affections which bear an external resemblance to it, among others from *dementia praecox*. Bordet's reaction has also been applied in the diagnosis of hydatid cysts due to the echinococcus.

Also it has been employed in the detection of blood and foreign proteins for which it is an even more sensitive test than precipitation: it requires, however, such delicate manipulation, and is so extremely sensitive, that it can hardly be trusted in certain cases; according to Friedberger it is capable of detecting the minute trace of protein present in human perspiration, so that the hands of the experimenter himself may introduce an error.

It has been employed in the differentiation of different races of man and of monkeys: not that the distinction between man and monkeys is so absolutely necessary as to show the sort of hierarchy which exists. Man is as far removed from the orang as the orang is from the macacus; however, man is more nearly related to the orang than the latter is to certain species of macacus. It is even possible, it is said, to distinguish by serodiagnosis an individual of Mongolian race from a Malay.

The biological reactions thus are of value, not only in the physiology of immunity and of nutrition, but even in zoology and anthropology.

Tuberculin Tests.

A dose of tuberculin which produces no effect in a healthy subject may determine in a tuberculous individual a definite reaction, consisting of general and local phenomena, fever, inflammation, and œdema round the tubercles and at the site of injection.

It was long thought that the reaction only took place in the neighbourhood of the tuberculous lesions themselves, and consisted in the inflammation and destruction of a certain number of cells, the fever being due to the absorption of the cellular debris. Von Pirquet, however, discovered that the skin of a tuberculous patient reacts to tuberculin at every point,

and it has therefore been necessary to suppose that the body is impregnated throughout by some substance produced by the chronic infection.

The body responds to the attack of the tubercle bacillus by a reaction product, an antibody, and the tuberculin reaction appears to be produced by the coming together or combination (not necessarily in the chemical sense) of this antibody and tuberculin ; that is roughly how the facts are being interpreted nowadays.

The body by producing this antibody is said to become "sensitive" to tuberculin. The tuberculous individual behaves differently from the healthy subject, being more sensitive, and his excessive susceptibility acting as a warning to him of his danger. It is in this way that the supersensitiveness which is a phase of immunity (*v.* Chap. XII., page 240) has occasionally been interpreted. There is some resemblance between the tuberculin reaction and the anaphylactic shock ; the diagnosis of tuberculosis by tuberculin is based on the "supersensitiveness" of the individual.

The reaction represents in a sense a resistance to the action of tuberculin ; we have seen that the prototype of this reaction, Koch's phenomenon, consists in the expulsion of the re-inoculated virus by the infected subject ; it is therefore also an immunity reaction.

Tuberculin was at first employed as a remedy, but later only as a diagnostic agent. It was even more studied in veterinary science than in medicine, because it was easier to make experiments and control them by autopsies on animals. Even to-day the tuberculin test is quite as important in veterinary as in human practice.

In the diagnosis of tubercles, nowadays, smaller doses are used for subcutaneous injections than at the time of the first trials of tuberculin.

The temperature reaches its greatest height about eight hours after injection. A positive reaction indicates merely "tuberculous lesions" and nothing more. It gives no information as to the site or the age of the lesions, nor

as to the probable fate of the patient. The reaction may be negative in exhausted, cachectic tuberculous patients.

The number of individuals who give a positive reaction much exceeds the number of those who show clinical signs of tubercle: latent dormant tuberculosis, or even healing tubercle, gives a reaction because the body is saturated with the products of its reaction to the tuberculous infection.[1]

In veterinary practice, it happens that animals once subjected to tuberculin injection fail to react to a second test performed twenty-five to thirty days after the first; this fact has been employed in fraudulent dealing to throw out the diagnosis, but the second test will still act if a double dose is given, and if the temperature is taken at the fourth hour (instead of the tenth); the fever appears prematurely (Vallée).

Cutaneous Reactions.—In individuals affected by tuberculosis, if the skin is subjected to a quite superficial scarification on which is put a drop of tuberculin, a red spot is seen to appear after a few hours. This swells, and comes to resemble a little vaccine pustule. It was this phenomenon which was discovered by Von Pirquet. Several variations have been tried. It is possible to do without scarification by dropping into the inner corner of the eye a drop of a dilute solution of tuberculin; the conjunctiva reacts with more or less intense inflammation and more or less exudate; this is the ophthalmo-reaction of Wolff-Eisner and of Calmette.

It was observed during subcutaneous injections of tuberculin, that the needle-track of the syringe, being wetted with a trace of the tuberculin, became red and swelled up; from this

[1] The statistics of Franz dealing with certain regiments of the Austro-Hungarian army are worth reproducing, since the author had the opportunity of observing the same men during several years.

In a regiment of four hundred apparently healthy men recruited from a district where tuberculosis was rife, 61 per cent. gave during their first year of service a positive reaction; of 323 similar men in their second year of service 68 per cent. were positive. Of 279 healthy men recruited from a district where tuberculosis was scarce, there were 30 per cent. of positive reaction. These 1002 individuals furnished in the course of the seven following years sixty-four cases of manifest tubercle and twenty died of it.

subordinate phenomenon an independent diagnostic test has been established by making a simple prick in the skin (Moro).

Further, it is possible to inject into the thickness of the skin itself a drop of the solution containing only $\frac{1}{100}$ of a milligram of tuberculin; there is thus obtained a very definite diagnostic reaction: the intradermo-reaction (Mantoux).

These procedures are all very convenient, especially in the examination of children; the methods of pricking and of intradermo-reaction are less severe than the intra-ocular instillation.

The cutaneous reaction of V. Pirquet gives about 85–90 per cent. of positive results in children presenting signs of tuberculosis, 20 per cent. in children with no clinical symptoms, and 48 per cent. in doubtful cases. The percentage of positive reactions *in children not clinically tuberculous* varies with the age. During the first 6 months of life it is practically 0; from 6 to 24 months 2 per cent.; from 2 to 4 years 13 per cent.; from 4 to six years 17 per cent.; from 6 to 10 years 35 per cent.; from 10 to 14 years 55 per cent.; in adults there are at least 77 per cent. of positive reactions.

The great value of V. Pirquet's cutaneous reaction is that with a little prick, which is itself quite harmless and produces no trace of fever, and with a minimal quantity of tuberculin (down to $\frac{1}{1000}$ of a milligram), it is possible to tell if a child is affected with tubercle bacillus.

The younger the child the more definitely does the reaction signify a true active infection, since tuberculosis is less frequent—and more recent—the younger the infant. In the adult, it indicates for the most part a tuberculous impregnation, great or small, recent or remote.

The cutaneous tuberculin reaction, performed as it has been on thousands and thousands of human beings, has taught us that man becomes infected with tubercle in childhood from his first to his fourteenth year; so that tuberculosis, although it may only become serious and fatal at a more advanced age, is really a disease of children and not of adult life. It is then during childhood that protection is necessary and since we

know that the child is infected by the individuals surrounding it, it is in the family itself and in the home that contagion must be prevented. The disease is kept up and spread by infected members of the family and by servants and also by unhealthy houses, in particular by dark, airless rooms. The cutaneous reaction tells us that of a hundred adults harbouring the tubercle bacillus, only twelve are destined to die of tubercle. It is therefore very evident that many human beings recover from the attack of the bacillus and that man is an animal naturally resistant to tuberculosis ; in most of us a sort of spontaneous vaccination takes place. These facts ought not to be overlooked in the search for a method of treatment or prevention of tubercle.

CHAPTER XIV

VACCINES AND SERA

Vaccination with unknown viruses—Small-pox: inoculation and Jennerian vaccination—Sheep-pox—Rabies: Pasteur's treatment—Vaccination with microbes of attenuated virulence—Anthrax, Swine-erysipelas, Pleuropneumonia of cattle—Vaccines against cholera, plague, typhoid fever—Attempts at anti-tuberculous vaccination.

Bacteriotheraphy: intestinal, buccal, nasal—Applications to surgery. Serotherapy, Diphtheria, Tetanus, Venoms, Cholera—Serum against plague, bacillary dysentery, cerebrospinal meningitis—Anti-streptococcic sera—Anti-tuberculous sera.

Sero-vaccinations: Sheep-pox—Bovine plague—Swine-plague.

Virus-vaccines sensitized—Experiments of Ehrlich and Morgenroth. Besredka's vaccines: Typhoid fever, Plague—Virus-serum in the treatment of rabies.

Phagocytic therapy.

It is fairly easy to immunise laboratory animals against bacteria by repeated doses of the living microbe: a preliminary experiment determines the minimum lethal dose, and the first injections are to be kept well below this. Immunization is possible also with viruses which are incapable of killing in any dose.

With extremely virulent bacteria, immunization is much more difficult. It is impossible to immunize a guinea-pig against anthrax, for example. In man, where one is not entitled to run the slightest risk, it is impossible to begin even with very small doses of living virulent microbes. Hence arises the great importance of Pasteur's discovery in the history of active immunization, the discovery of the attenuation of a virus. Only *vaccines* may be used in human immunization.

In general, a non-virulent microbe neither kills nor im-

munises, whereas a virulent organism kills before immunity has time to develop. It is often necessary to find intermediate stages of virulence. This is what Pasteur perceived, and hence arose his two vaccines against anthrax.

It is possible to vaccinate with viruses which one handles without actually knowing their nature, with cultures of bacteria virulent or attenuated, and with bacterial products freed from the living microbes.

Vaccines

I. *Vaccinations with Unknown Viruses.*

It is not necessary actually to know the microbe of a disease in order to immunize against it. The vaccinations with unknown viruses are, as a matter of fact, among the most perfect known to medicine.

Small-pox.—The microbe of small-pox is unknown, yet preventive inoculations have long been carried on. There exist two methods, both empirical in origin; the older of the two has been entirely given up in favour of Jenner's method of vaccination. *Inoculation* or *variolisation* consisted in inoculating the virus of small-pox itself, so as to produce one or more cutaneous pustules, the development of which saved the body from a general attack. Small-pox acquired in the natural manner invades the whole body: the virus may enter perhaps by the tonsils or the respiratory passages, gaining the blood stream from these and thence reaching the skin and mucous membranes. Once the eruption has appeared it is too late to intervene. When the natural virus is inoculated artificially on the skin, it may give rise to a general infection later on, but in general the first local pustules produce rapidly sufficient immunity to prevent such a general invasion; but not always; occasionally inoculation results in a fatal attack of small-pox. The virus inoculated was by no means constant, and it was always a case of working in the dark. A correct and witty account of the history of inoculation is to be found in the eleventh of Voltaire's *Lettres Philosophiques*.

There exists among cattle a disease resembling small-pox, and also due to an unknown microbe, the *cow-pox*, which attacks also those who milk infected cows. Those individuals who have suffered from the pustules of cow-pox are immune to small-pox.

This idea, popular in origin, had long been current among farmers and cattle-breeders in certain districts of England, France, Holland, and Germany when Jenner took it up and consecrated his life and his genius to publishing it abroad.

He made of the subject a definite experimental study: he inoculated in turn the virus from animal to animal, from the animal to man, from man to man, and finally submitted his "vaccinated" subjects to experimental inoculation with small-pox.

In all countries where public health is well organized vaccination has been made compulsory; in consequence smallpox has almost disappeared from Germany: the rare cases recorded there are cases of immigrants or inhabitants on the frontiers. It has very much diminished in France and is being actively combated in the French colonies. It may one day become extinct.

Three improvements have been introduced in Jennerian vaccination since Jenner's time. In the first place, it was recognized that in many cases vaccination does not protect for the whole of life, and periodical revaccinations have become customary and even in certain countries compulsory. In the second place, instead of vaccinating from man to man the vaccine employed is derived from animals. Jenner practised "arm to arm" vaccination, *i.e.*, transmitted from man to man a virus whose distant origin was cowpox. This practice had the inconvenience of occasionally transmitting human diseases such as syphilis. From 1860 onwards vaccination in Europe has been performed with "animal lymph" taken from vaccine calves (in certain colonies from buffalos, rabbits, zebras, &c.).

Finally we have learned to preserve and purify the supply of

virus by mixing it with glycerine, so that it is no longer necessary to vaccinate directly from the calf. Inflamed arms are nowadays quite the exception.

According to certain observers there are really only two types of smallpox, the pox of sheep and that of man. All the others are said to be mere modifications of these two types; for smallpox is very apt to become modified in adapting itself to different animal species.

Vaccinia, in this theory, would simply be an adaptation of human smallpox to cattle, and, starting with this idea, attempts have been made to transform smallpox into cowpox experimentally by inoculating from man to the calf. This question of variolo-vaccinia has been very much studied and is being taken up again to-day. The results obtained have been much discussed, and it is not yet absolutely proved that it is possible to produce in the laboratory in a short time a change which nature has probably required centuries to accomplish.

Rabies.—Pasteur's treatment takes advantage of the period of incubation, which is on the average six weeks. The patient ought to be treated as soon as possible after the bite and before the appearance of symptoms.

In the process there is inoculated the *virus fixe*, which is kept going in the nervous system of the rabbit, is contained in the spinal cord, and may be modified by drying the latter in the dark at the temperature of 23°. To begin with, a cord dried for fourteen days is injected, and at the end of the treatment a cord dried for three days. The rate of treatment varies according to the site and gravity of the bites, there being three formulæ, the fifteen-day, eighteen-day, and twenty-one day treatments. The mortality in rabies has been reduced to 0·3 per cent. instead of 15 to 20 per cent.

The microbe of rabies is unknown, and Pasteur treatment is one of the crowning glories of experimental medicine. Consider the facts which it has been necessary to determine before arriving at its application: in the first place, the virus of rabies exists in the brain of rabid animals; further, it is possible to make a pure inoculation of it; the virus of any

rabid dog taken at random inoculated under the *dura mater* of the brain of a rabbit after trephining the skull produces rabies in the rabbit after a variable period of incubation; but after about twenty passages from rabbit to rabbit the incubation period falls to six to seven days; the virus has then become the *virus fixe*, and the passages from rabbit to rabbit are really cultures in the living body. The culture exposed to dry air, but sheltered from light, gradually loses some of its virulence. The dried cords, inoculated in the form of emulsions under the skin of animals, produce in them a certain and stable immunity against the strongest virus inoculated under the *dura mater*. The cultivation *in vivo* of the *virus fixe* was for Pasteur the key to vaccination against rabies after the bite of the rabid animal.

There is some doubt whether the virus of the dried cords really undergoes attenuation. Even in 1885 Pasteur said that it was rather a rarefaction which occurred. "The delays observed in the duration of incubation of the rabies communicated day by day to rabbits, to estimate the virulence of our cords dried in contact with air, result from a *diminution in quantity* of the virus contained, and not from a diminution in virulence." A variation of Pasteur's treatment, the treatment of Högyes, consists in replacing the graduated desiccation by a graduated dilution.[1]

[1] The nature of this *virus fixe* cultivated in the rabbit is rather a question. Nitsch and Marx, who inoculated themselves with fresh *virus fixe*, consider that after several hundred passages in rabbits the virus becomes harmless to man; they consider it as a true Jennerian vaccine for man, resulting by adaptation to a different species. But it is not right to maintain the absolute innocuousness of *virus fixe*. It may well be harmless when injected into the subcutaneous tissue, but would it continue to be so if injected in a region rich in nerve filaments such as the face or the end of finger?

It is probable that the virus of dried cords is destroyed by the body, that the microbe is absorbed, and that protective substances are produced in consequence and are found in the blood of treated individuals. But these substances play only a secondary part, for there is no constant relation between the properties of the serum of an animal and its resistance to rabies. There are certain animals refractory to rabies, such as a species of tortoise whose blood nevertheless possesses no antirabic power. It is impossible to avoid thinking of the phagocytes in such a condition.

ii. *Vaccination with Attenuated Cultures.*

Anthrax.—The anthrax vaccines are cultures attenuated by contact with the air at a temperature of 42·5° C. Two graduated vaccines are employed: the weaker kills mice and small guinea-pigs, the stronger kills adult guinea-pigs and even a certain percentage of rabbits. The animals treated with these preserve their immunity for about a year, and it has been calculated that during the twenty years which followed their introduction at least twelve million animals were inoculated. The method of anthrax vaccination has never required alteration since its discovery by Pasteur, Chamberland, and Roux, and the celebrated experiment of Pouilly-le-Fort.

Swine-Erysipelas.—In this the same principle is employed, *i.e.*, a virus attenuated in the laboratory. But the bacillus of swine-erysipelas does not produce spores, and a method of sero-vaccination has been invented instead (*v. infra*).

iii. *Pleuropneumonia of Cattle* (*Rinderpest*).

The discovery of the microbe dates only from 1898, but vaccination had been practised against the disease a considerable time before. Willems employed as virus the serous fluid from the diseased lungs: he had observed that inoculation of this in a healthy animal produced variable effects according to the site of injection chosen: injected on the trunk or neck the infection was fatal; at the tip of the tail or of the ears it produced only a limited inflammation which left the animal immune towards the naturally occurring disease.

The first improvement was introduced by Pasteur, who showed that the pure virus could be got by taking the abundant exudate produced in the subcutaneous tissue of a calf after inoculation there. Later, pure cultures were substituted.

The tip of the tail is a good site for vaccination, since the tissue is dense and the absorption slow. A few animals lose their tails as a result of the vaccination.

iv. *Cholera, Typhoid Fever, Plague.*

Cholera.—Human cholera is a toxic disease, the toxin being secreted by the vibrios invading the intestine. The true remedy for cholera, therefore, should be an antitoxic serum. It was before our present conceptions as to the toxicity of the cholera vibrio that Ferran, in 1884, tried to vaccinate men against cholera by injecting the living microbe subcutaneously. This method has been taken up and modified by Haffkine, who has tried it in India, where cholera is always present. In vaccinated individuals he found the mortality seven times less than in the non-vaccinated, but although the death-rate may be lower, the severity of the symptoms is quite as great among the former as among the latter, which is rather disconcerting, as in other kinds of preventive vaccination this does not occur. The solution of the cholera problem lies elsewhere, but it is worth while recalling the attempts of Ferran and Haffkine, because their method has proved of more value in other diseases.

Typhoid Fever.—Typhoid fever is a toxic enteritis, but it is at the same time a blood-infection; blood-cultures show that there is septicæmia. The bacillus inhabits chiefly the small intestine, but it also extends to the blood, the spleen, and the bone-marrow. Hence there is much more hope of success than with cholera for preventive injections of the bacteria under the skin. Bacilli killed by heat are employed. The injection produces some swelling and pain with fever and stiffness the discomfort lasting about two days. Two, or even three, injections ought to be made. The treatment is succeeded by what is called a "negative phase," during which the individual may be in a condition of lowered resistance.

Anti-typhoid vaccination has been chiefly employed hitherto in the English army in India, in the Transvaal, in Egypt, and in Cyprus. A certain number of the inoculated individuals have nevertheless taken the disease (in many of these the vaccination had been insufficient), but in a form milder and

with less frequent relapses. The mortality is about four times less among the inoculated than among the non-inoculated. As far as can be judged at present, the effect of the vaccination lasts for about four years.

There is no necessity to submit a whole family to the discomforts of inoculation when one member has taken typhoid; it is sufficient to isolate the patient and to disinfect strictly the excreta, linen, etc. Vaccination is adapted only for the members of an ambulance and hospital corps during an epidemic, or for troops campaigning in a country where typhoid is raging, and where it is difficult to adopt hygienic measures.

Plague.—Haffkine's vaccine consists of bacteria killed by heat. The inoculations, especially the first, produce inflammation and pain with a high temperature, the illness lasting for about three days. Two, or even three, inoculations ought to be made. Such vaccinations are suitable in the case of plague, as this disease is a general infection, spreading throughout the blood and organs. In 1902–1903 about half a million persons were inoculated in the Punjab, and fairly accurate information could be gathered regarding a portion of them. On comparison we find :—

186,797	inoculated,	with 3339	cases = 1·8 per cent.
		,, 814	deaths = 0·4 per cent.
639,630	non-inoculated,	with 49,433	cases = 7·7 per cent.
		,, 29,733	deaths = 4·7 per cent.

It appears that the inoculation continues to be of benefit for several years after.

Anti-tuberculous Vaccination.—As far as is known, vaccination against tubercle is possible with living bacilli only, dead bacilli being ineffective, but there is no vaccine as yet which is entirely safe.

Behring has sought "Jennerian" vaccines, *i.e.*, he has tried to vaccinate one species, cattle, with the virus derived from another species, man. Innumerable experiments have already been made on bovo-vaccination, and they prove at least that a certain degree of immunity may be created towards tuber-

culosis. But even among cattle the method is not yet suited for practical application.

In man there exists an auto-vaccination, since practically all adults have been slightly affected by this disease, and only a small fraction succumb. Both in medical and surgical practice it is common to find tuberculosis of the skin, of the joints, and of the bones healing up and even apparently protecting the patients against contracting pulmonary phthisis. It is on these lines that is to be sought a method of vaccination against tuberculosis. Up to now none exists.

Bacteriotherapy.

Vaccines and sera are not the only remedies furnished to medicine by bacteriology. Bacteriotherapy consists in modifying a given flora by adding to it other bacteria which are antagonistic or favourable to certain microbial species. In discussing the intestinal flora we have indicated the principles of the bacteriotherapy of intestinal infections. In the future this will be developed, and health-giving bacteria will form part of our diets.

Bacteriotherapy has also been attempted in the case of cancer and lupus, where injections of streptococci have been given, and in the case of chronic boils, where injections of yeast have been tried. These attempts are still semi-empirical, but this is no reason for despising them.

The lactic bacilli have not been utilized in intestinal bacteriotherapy only. Surgery has employed them to combat puerperal infections, injuries occurring during labour, inflammations of the cervix, and even peritonitis. They evidently act by modifying the "soil." It is obvious that they find their chief success in combating infections of closed cavities, such as we have in rhinitis, sinusitis, ethmoiditis, and otitis.

Bacteriotherapy in the mouth is not less rational than in the intestine. The lactic flora has succeeded in curing chronic inflammations of the gums and obstinate pyorrhæa alveolaris. All the infectious diseases which enter by the

mouth, the tonsils, and the nasopharynx may be combated by this bacteriotherapy, and already very encouraging observations exist. For example, epidemic cerebro-spinal meningitis starts from the nasopharynx, and there are meningococcus carriers just as there are diphtheria carriers. It is the more important to employ the lactic bacilli against the meningococcus from the fact that *in vitro* the former antagonize the latter (A. Berthelot). The bacterium most to be recommended for the purpose is the *Bacillus bulgaricus* administered in powders and derived only from young fresh cultures in liquid media. It is not necessary to insist upon the inconvenience of the use of antiseptic washes and to contrast with these the biological disinfection produced by modifying the flora.

Sera.

Serotherapy means treatment by means of the sera of immunized animals. It may be preventive or curative, or both. The animal furnishing the serum has produced its own immunity, *i.e.*, it possesses active immunity. The animal which receives such a serum acquires a passive immunity. Serotheraphy is equivalent to the transfusion of antibodies.

The antitoxins were discovered by Behring in 1890.

Antitoxic sera are not the only therapeutic sera: by immunizing an animal with the bodies of bacteria antibacterial serum can be prepared: with such sera there may be transfused bacteriolysins, agglutinins, bacteriotropins, in fact all the antibodies they contain, but above all there is transferred their property of exciting the reaction of the phagocytes.

Certain antibacterial sera are also anti-endotoxic in the sense that they are capable of neutralizing endotoxins. They are best prepared as we have seen by *intravenous* injections of the antigen. Finally, sera may be prepared against viruses only slightly known or not known at all; the preventive action of serum in general may be disregarded.

Antidiphtheria Serum.—This is a serum of horses immunised against the diphtheria toxin. The treatment of the

horse begins not with pure toxin but with a mixture of toxin + Gram's iodine or of toxin + antitoxin. Every horse has its own susceptibility, and the treatment requires a good deal of skill. It is impossible to foretell the supply of antitoxin from any particular horse.

The serum treated aseptically is put into little bottles and "tyndallised," *i.e.*, sterilised by heating to a low temperature on several successive days. It is stored in a dark, cool place, and under these conditions it only loses about one-tenth of its activity, so long as it remains clear, even after several years.

Sera possessing great activity per unit volume have been sought for by selecting those horses which are active producers, or by concentrating or "refining" the serum by various procedures, freezing, precipitation, &c. The best method is still to take an active serum, to keep it pure and preserve it carefully without further treatment.

The titration of the serum is indispensable: only serum of known activity is issued for serum therapy. Hitherto, titration has only been possible by resorting to experimental animals, guinea-pigs. The laboratories of all countries have agreed to adopt the method proposed by Ehrlich. The serum is standardized by mixing with a toxin which has itself been estimated against a standard antitoxin. The activity of the serum is expressed in antitoxic units which, like all units of measure, are conventional. The standard antitoxin is preserved with precautions similar to those with which are guarded our standard weights and measures.

But in spite of all the precision introduced, we are still dealing here with a biological, not a chemical process. Experience has made clear three points on which too much stress cannot be laid. (1) The serum ought to be inoculated as soon as possible after the appearance of the disease. A little serum given early is worth more than a great deal of serum given too late. Similar guinea-pigs receiving the same dose of toxin may be cured by a small dose at the sixth hour, whereas at the eighth hour even a large dose fails. In hospital, unfortunately, the children are usually brought too

late, and it is *on this account* that the mortality in diphtheria is still 10 per cent. (or 14 per cent. in epidemics; L. Martin).

(2). There should be no fear of giving large doses: in fact in bad throats large doses ought to be resorted to at once.

(3). Intravenous injection is the most efficacious: it is indicated in all serious cases. This holds good for all sera.

There is said to exist still in certain minds a certain scepticism as to the value of antidiphtheritic serum but it is scarcely credible. The figures speak for themselves. The death rate from diphtheria in the Sick Children's Hospital in Paris before serotherapy was about 50 per cent.; it has fallen to 6—10 per cent., and would be much less if the children were treated at the very beginning of the disease.

The serum is used as a prophylactic against diphtheria in schools, barracks, crêches and hospitals: a dose of 5—10 c.c. protects for about three weeks.

Anti-tetanic Serum is an antitoxic prophylactic serum. The serum furnished by the Pasteur Institute possesses an activity such that $\frac{1}{100000}$ c.c. suffices to neutralize *in vitro* 100 lethal doses for a mouse.[1]

In human tetanus when the incubation is long and the progress slow, the serum may check the extension, *i.e.*, may have a limiting effect. According to the figures collected by Vallas the serum has lowered the death rate from about 75 per cent. to 45 per cent. The mortality remains about 70 per cent. in those cases where the incubation period is less than eight days.

[1] When tetanus toxin and a sufficient dose of antitoxin are injected at different points of the body a minute quantity of toxin escapes neutralization and induces a slight local tetanus which is always recovered from.

It is possible to cure animals inoculated in the paw if the serum is injected at the first appearance of symptoms: when the poison has reached the nerve-centres the antitoxin is of no avail. Roux and Borrel had the idea of bringing the antitoxin in direct contact with the brain so as to prevent the poison reaching the centres. By intracerebral inoculation they succeeded in curing a number of guinea-pigs which had manifested tetanus and were therefore doomed to die. But they do not extend their conclusions to other animals. In man the first symptoms of tetanus affect always the medullary centres so that cerebral treatment has even less hope of success than in the guinea-pig.

The employment of this serum in veterinary practice is due to the efforts of Nocard. He collected, now a long time ago, 2,708 cases of horses injected immediately after one of those operations frequently followed by tetanus (castration, amputation of the tail, inguinal or umbilical hernia) ; not a single case of tetanus occurred. A second group comprising 600 animals, treated one to four or even more days after an accidental wound (nail-prick, harrow-tooth wound, kicks, wounds soiled with earth or manure, etc.), presented one single case of mild tetanus (a horse treated five days after the accident, a nail-prick in shoeing). The veterinary surgeons who supplied these 3,308 cases observed in their practice at the same time 314 cases of tetanus (of which 220 were horses) in animals not treated with serum. In 705 equidæ, wounded or operated on, Labat observed three cases of tetanus among the untreated, not a single case among the treated animals. According to statistics supplied by eight veterinary surgeons to Vallée of Alfort, from 1898 to 1906, 13,126 animals were inoculated as a preventive measure, and not one took tetanus. During the same period, two of these surgeons alone had 139 cases of tetanus among animals not treated. At present the Pasteur Institute supplies to veterinary surgeons more than 100,000 doses per annum.

There is no reason known to science why man should not derive a similar advantage. There exists, however, with regard to the prophylactic serotherapy of tetanus in man a distrust or scepticism which finds vent from time to time in the discussions of surgical societies or congresses. It has been maintained that the employment of preventive injections has not affected the death rate from tetanus in Paris ; that tetanus occurs fairly often in spite of prophylactic injections ; that the conditions which permit of the prophylactic employment in veterinary practice are unrealizable in human medicine, and finally that in man the serum is less active than in the horse. When examined none of these objections hold good. The numerous unsuccessful cases depend on the conditions of tetanus intoxication and its neutralization ; there is not one of

the objectors who, if he received a tetanus-suspected wound, would not call for a prophylactic injection of serum for himself.

It is necessary to avoid giving too small doses and, should the wound fail to heal, to repeat the injection several times; of course, the surgical cleansing of the wound should never be omitted.

Antivenom Sera.—These are prepared by immunizing horses, beginning with venom + calcium hypochlorite and ending with pure venom. Eventually they can be made to support 80 lethal doses (2 grams of dried venom) at a single injection. The immunization is a difficult operation to bring to a satisfactory end; frequently it is interrupted by serious accidents, endocarditis, nephritis, and abscesses. It takes 16 months of treatment to get a good serum from a horse (Calmette). Antivenom serum is *polyvalent*, *i.e.*, is prepared with several species of venom.

The quantity of serum required to save life increases with the susceptibility of the animal and the delay in the application of the remedy. As a rule 10–20 c.c. suffice to save a bitten human being.

Anticholera Serum.—Cholera is an acute intoxication due to a poison elaborated by the vibrios in the intestine. This toxin has been sought for and an antitoxic serum attempted. The serum prepared against the bacteria is not active against the poison; the principle of serotherapy is thus quite different from the Ferran-Haffkine method of immunization.

The cholera toxin prepared in 1896 by Metchnikoff, Roux, and Salimbeni presented this peculiarity, that it resisted heating to 100° C. for a quarter of an hour. Horses are prepared by intravenous injections.

Anticholera serum is still at the trial stage. Its only trial outside the laboratory was in 1909, during the epidemic of St. Petersburg. The doses ought to be large, 150–350 c.c., given 100 c.c. at a time intravenously. There are cases so fulminant as to defy almost any remedy. In the above trial cases, not

very numerous, the mortality was reduced by about a half. In the laboratories work is still going on towards the improvement and perfection of an anticholera serum.

Antiplague Serum.—It is derived from horses immunized against the plague bacillus. The treatment is long and difficult.

The statistics are in need of discussion. Whereas Mayer, for example, records numerous cases of cure, other physicians declare that there are more deaths among the treated than the non-treated: they allege that the serum is too weak and the Hindu too susceptible to plague. When these unfavourable statistics are more closely examined, it becomes apparent that the serum has been given too late, in too small dose, by subcutaneous injection, and not long enough continued: the patients, abandoned after the fall of the fever, died in many cases fifteen to thirty days after the last serum injection. In man, plague is all over in five to six days, and if treatment is only carried on during the third or fourth day cure is well nigh impossible.

The injections ought to be massive and repeated. It is no use trying to treat plague with the doses suitable to antitetanus serum.[1]

It is evident that against pneumonic plague the serum which we possess at present is not to be relied on, but that against bubonic plague it has already been of service. It may be of great value in a population where the hygiene is good, to complete the isolation of a case of plague by giving prophylactic injections to the contact individuals, and thus to limit the epidemic focus.

Antidysenteric serum is given in doses of 20, 80 to 100 c.c.—the latter doses in very serious cases where there are more than 100 motions of the bowel per day. Between 1905

[1] Duprat, using doses of 300 c.c., succeeded in curing thirty-eight out of forty-five plague patients treated, a mortality of only 15 per cent.

Employing intravenous injections (100 c.c. repeated after twenty-four hours), Penna had a mortality of 14·2 per cent. in 200 cases. Ferrari at Sao Sabastiao had only 7·2 per cent. of fatal cases among forty-four. Ferrari's statistics throw light on the different modes of injection: by the skin, 38 per cent. death-rate, intraperitoneally, 18 per cent, intravenously 7·2 per cent.

and 1907 Vaillard and Dopter applied the serum in 512 acute cases of all ages, of whom 32 had certainly fatal dysentery and 170 a very severe attack. The average death rate was only 1·3 per cent., whereas in different epidemics it has varied from 10 to 25 or 50 per cent. The serum relieves the patients of their terrible abdominal pains, of the vomiting, and of the hiccough; the stools become less numerous and less painful, the temperature falls, and a feeling of comfort succeeds that of absolute prostration. These good results occur as a rule in the course of one day.

The **antimeningococcus serum** of Flexner is employed in epidemic cerebro-spinal meningitis. It has hardly any action when injected subcutaneously. It must be injected at the site of the disease—*i.e.*, into the cerebro-spinal canal by lumbar puncture. The treatment should be begun as soon as possible and be repeated. It has lowered the mortality from 60 to 80 per cent. to 25 to 10 per cent.

Antistreptococcic serum is a polyvalent serum—*i.e.*, it is prepared with several strains of streptococci. It is difficult to get an accurate idea of its efficacy in man. The infections in which streptococci are responsible are multiple, and the serum is employed in the most diverse diseases, from puerperal fever to scarlatina and acute rheumatism. Large quantities of this serum are supplied from the Pasteur Institute both for medical and veterinary use, but unfortunately it is rare to get any information as to the results obtained.

Antituberculous Sera.—Maragliano's serum is supplied by horses treated with the soluble constituents of the tubercle bacillus.

Marmorek's serum is furnished by horses immunized with "primitive bacilli," *i.e.*, young bacilli which scarcely possess any of the acid-fast property due to the wax and fat constituents. The same horses are at the same time immunized against streptococci, since the streptococci are frequently associated with Koch's bacillus in tuberculous lesions. As regards this serum opinions differ. Certain observers report a favourable action on cases of tuberculosis of

the bones and joints and on early cases of pulmonary tubercle (checking the fever and improving the general condition).

Sero-Vaccinations.

When a vaccination *per se* involves a certain risk it may be associated with a specific serum: the combined treatment is equivalent to an active immunization under cover of a passive protection.

Sheep-pox.[1]—In France the law enjoins the vaccination against sheep-pox of all the flocks in the affected regions, whereas it forbids it in the regions free from the scourge. In Algeria sheep-pox is endemic, and the Algerian sheep are much more resistant than the French sheep. In consequence, Algerian sheep, which are imported in great numbers, may be landed apparently healthy, yet may induce a malignant epidemic in a French flock. Nowadays only vaccinated sheep may be imported.

Borrel, who perfected the method of vaccination by showing how to prepare cheaply large quantities of pure virus, has prepared an active anti-serum by "charging" with virus sheep which had recovered from the disease. When injected into a sheep twenty-four hours before the virus this serum prevents the development of the disease (in a dose of 20–30 c.c.), and even inhibits the inoculation pustule. By graduating the dose of serum it is possible by inoculating the virus simultaneously to produce simply an immunizing pustule without any risk of a general infection.

The serum employed alone is sufficiently active to render certain a prophylactic treatment of the disease by curative and preventive injections in a flock. It permits of the importation of Algerian sheep without danger of infecting French territory. The sheep to be imported receive the serum three weeks before shipment. Inspection eliminates those which were incubating the disease at the time of inoculation, and which might therefore be shipped while still ill. This serotherapy is

[1] Non-existent in Great Britain.—*Translators' note.*

of advantage to the Algerian breeders, and helps in the suppression of the disease.

Cattle plague or *rinderpest* is an epizootic disease which has caused incalculable losses throughout Europe, especially by attacking the herds of pure blood. The mortality always exceeds 50 per cent. The microbe is unknown, but it is known that the virus can pass through filter bougies, even the finest: it is therefore an ultramicroscopic organism. An animal which survives possesses an immunity which seems quite invincible: as soon as the illness is over any quantity of virulent blood may be injected into it without killing it. But it is not possible to immunize by a method similar to that in sheep-pox, for inoculation produces an attack as severe as the naturally acquired disease.

The animals may be made to acquire an immunity lasting for four to six months by injecting the bile of animals dead of the plague, but the method is not practical.

A good serum can be obtained by "charging" with virus an animal which has recovered. The sero-vaccination of Kolle and Turner consists in injecting simultaneously this serum and virulent blood: they must be injected at different spots.

It is always risky to employ a virus in therapeutics: there is always the danger of spreading it or introducing it into a country where it does not hitherto exist. The serum alone suffices for prophylaxis, for treatment, and for the prophylaxis of the healthy animals. The prophylactic dose is one of 50 c.c. Capable veterinary surgeons have succeeded by using the serum alone in reducing the mortality to 12 per cent. (Nicolle). The same principles are at work in the treatment of the horse-sickness of the Transvaal and the plague of pigs, hog-cholera, which are also septicæmic diseases due to an ultra-microscopic virus.

Sensitized Vaccines.

The vaccines employed against typhoid fever, cholera and plague may be rendered less painful, more prompt, more powerful and more durable by impregnating the bacteria of

which they consist with the corresponding anti-sera (Besredka). After soaking in the serum the bacteria are washed free from all trace of it: they must only retain what they have been able to fix: there must be no free serum.

These qualities of such vaccines are due to the immune body which they have fixed.[1]

Phagocytic Therapy.

When the leucocytes are counted to determine the diagnosis and prognosis in certain infectious diseases it is really a case of measuring to some extent the reactions and natural means of defence of the body: the laws of phagocytosis are being applied. It was natural not to stop at recording these phenomena, but to proceed to active intervention by attempting to modify, fortify or direct the phagocytic apparatus.

The great danger in surgical operations, especially on the abdomen, is an infection of the field of operation: formerly it was the custom to flood this with antiseptics; the bacteria were destroyed but the tissues were injured. Nowadays not only has antisepsis given place to pure asepsis but the attempt is made to summon to the field of operation, particularly to the peritoneum, legions of the cells capable of taking up bacteria and healing wounds. For this purpose it is customary to inject into the abdominal cavity either blood serum (warmed to body temperature), or substances which

[1] The association of an antirabic serum with the injection of the emulsions of spinal cord may be regarded as a sero-vaccination or as treatment with a sensitized vaccine.

Such a serum cannot be relied upon to prevent rabies in animals: still less can it furnish the basis of treatment for man. But it has been employed with success in conjunction with the *virus fixe* of the Pasteur treatment to induce the greatest possible absorption by the body. In such virus-serum mixtures it is *only the virus which immunizes:* the serum merely favours absorption. An excess of serum would be actually injurious and must be avoided.

In man the virus-serum treatment is employed in those cases where the treatment has to be rapid, *i.e.*., when the patient presents himself long after the bite or where the injuries have been very serious (A. Marie).

exert a positive chemiotaxis on the leucocytes, *e.g.*, nucleic acid.

Again, in the method of Bier, which consists in cupping and surrounding with elastic bands to retain a large volume of blood around an abscess, a boil, or other acute infection, it is also the phagocytes which are called upon, for the artificial œdema which is produced is accompanied by an afflux of leucocytes (Schimodaira's experiment).

Wright's method of vaccino-therapy consists in treating infections by means of inoculations of bacterial bodies (the bacteria of the infection itself), in order to increase the opsonic power of the serum (*v.* Chap. X.); the treatment would not be applicable without the quantitative and qualitative control in which the phagocytes are employed.

For some years the study has been going on of those chemical actions capable of stimulating phagocytosis and reinforcing the phagocytes. Weak solutions of quinine (0·002 per cent.) increase the phagocytic power, whereas solutions fifty times stronger produce the opposite effect. The peptones have been recognized to be powerful stimulants. According to quite recent experiments, it will be necessary to add to these iodoform and various chemical substances which are soluble in fats, and which doubtless act in virtue of their consequent power of penetrating protoplasm in solution in the lipoids. These substances are true chemical *stimulins*.

It is possible that a new and most interesting chapter is about to open in therapeutics with the discovery of Hamburger among others: the action of certain mineral springs is said to be due to their power of exciting phagocytosis (experiment with the water of the Virchow spring in Wiesbaden).

Thus, chemistry and physics are far from taking possession of medicine and relegating phagocytosis to the region of pure speculation. On the contrary, the doctrine of phagocytosis, which has already bloomed a second time in the purely biological researches on opsonins and bacteriotropins, plays the premier rôle still as inspiration and explanation in the study of the chemical therapeutic agents.

CHAPTER XV

CHEMICAL REMEDIES

CHEMIOTHERAPY

Ancient origin of chemical therapeutic agents—"Sterilization" of the infected animal—Protozoal diseases: example of quinine—Association of chemical research with animal experiment.
The arsenical bodies and "606" or *Salvarsan*.
Progress in the treatment of sleeping-sickness and syphilis—Observations on immunity in Protozoal diseases.
Strains resistant to drugs—Strains resistant to sera—The future of chemiotherapy.

CHEMICAL therapeutics is old as the world. It fills our pharmacopœias, still further enriched by organic chemistry. But all over it furnishes only symptomatic remedies: one induces sleep, another stimulates the heart, others deaden pain. The drugs which really cure are easily counted, few exist besides quinine and mercury.

Chemical therapeutics of to-day is engaged in searching for other examples of the order of quinine and mercury, drugs capable of destroying pathogenic microbes without injuring the cells of the body. In theory and practice it is chemistry applied to therapeutics.

Such remedies should resemble more or less the antiseptics and should have as function sterilization. When the antiseptic properties of corrosive sublimate became known, R. Koch tried to inject it into anthrax-infected guinea-pigs to destroy the bacteria, but the animals died. This simple experiment puts the problem well before us with all its difficulties. Corrosive sublimate cannot be employed to "sterilize" a

living creature because it kills the cells of the organs at the same time as the bacterial cells. Quinine is a good therapeutic agent because it destroys the parasites of the red corpuscles without acting—at least in general—on the corpuscles themselves.

There are cases where the sterilization of a tissue may be performed without the least inconvenience. Mercury is the traditional remedy in syphilis: its internal application is limited by its toxicity; but after a contact possibly dangerous, it may be applied as a prophylactic to the skin. Metchnikoff's experiments on the chimpanzee and on man have proved that simple rubbing with calomel ointment sterilises the skin where the microbe of syphilis has just been inoculated and prevents infection. This preventive treatment, which is as efficacious as it is simple, is already practised.

Origins.—The renaissance of chemiotherapy has had several causes. The first is the revolution due to Pasteur's teaching. When a disease is found to be due to a germ, to a germ which is known and which can be made to transmit the disease by inoculation into a laboratory animal, then there is room for experimental therapeutics. Animal experiment permits us to go much further than clinical observation. Nevertheless, the progress of microbiology at first seemed about to throw drug therapy into the background. Marvellous biological remedies were discovered, both preventive and curative, surpassing in specificity and efficacy all the drugs of the Pharmacopœia, *e.g.* the Pasteur vaccine against anthrax, vaccination against rabies, and all the serotherapies. The idea at once suggested itself that all infectious diseases might be treated in similar ways.

But that has not proved to be the case; there is a group of infectious diseases against which vaccination and serotherapy have achieved nothing or almost nothing, the protozoal diseases. In no case has the serum of an animal susceptible to the trypanosome of sleeping sickness, and immunized against this microbe, been capable of exerting a curative effect upon the experimental disease in other animals. In malaria, all the

biological methods have failed, and quinine remains the sovereign remedy. Hence, instead of searching for a vaccine or a serum in protozoal diseases, the search has been directed to finding an equivalent of quinine for each.

When fortune favours, the history of cinchona bark and quinine may be recapitulated. Chance or an empirical discovery or a tradition of unknown origin, then enriches humanity with a remedy on which chemists and experimenters exercise their talents. This is the history of the recent arsenical remedies.

The discovery of the microbes and the necessity of discovering for protozoal diseases other remedies than vaccines and serum, have been aided by the development of chemistry itself, and especially of industrial chemistry. The great aniline-dye factories have been the chief furnishers of the laboratories of experimental medicine. The remedies tried have been varied as the dyes are varied, and in many cases *chemiotherapy* has been really a *chromotherapy*.

The method of research consists in associating experiments on the living animal with the reactions of organic chemistry. A certain body endowed with a certain property is known ; the molecule of this body possesses a principal nucleus on which are grafted secondary atomic groups ; it is found that the property desired depends upon one of these groups ; this group is then shifted about, varied or substituted by another group ; experiment then informs us as to the properties of the new compound and as to the relations between molecular structure and therapeutic effect.

Ehrlich has rendered great services to medicine by combining more closely than had ever been done, the technique of the chemist with the *in vivo* experiments of the biologist. His guiding principle, which dominates his whole career and is to be found in his earliest works, is the attempt to explain vital actions by a similar mechanism as in the reactions of organic chemistry. He has represented by stereochemical formulæ the reciprocal reactions of bodies of the chemical composition of which we are almost entirely ignorant, the toxins and antitoxins.

He has disarticulated protoplasm into atomic groups, both structural and functional, which act like the organs of the cells; he has extended the anatomy and physiology of the tissues and organs by imagining a sort of molecular anatomy and physiology for the cell.

The cell is a microcosm in which multiple functions take place by continual exchanges between the protoplasm and the external world. Each of these functions is represented by a group or "side-chain," capable of entering into (chemical) combinations with corresponding groups in foreign substances, food-stuffs, poisons, and also drugs. All the vital phenomena can be reduced to a series of such exchanges, which are nutrition phenomena and retain this character, whether it is a protein which is being assimilated, or a serum producing immunity, or quinine killing the malaria parasite.

For a substance to act on a cell, whether an organ cell or a microbe, it must fix itself on the protoplasm. Did not a philosopher in the middle ages declare that drugs ought to have points or hooks to enable them to seize upon the organs? There is scarcely any phenomenon which haunts the mind of the biologist more than this fixation, which, according to some, is a physical phenomenon, one of molecular adhesion, according to others the chemical interplay of the side-chains.

The antitoxin injected into a patient has affinities only for the corresponding toxin. But the arsenic which we inject into an individual suffering from sleeping-sickness possesses affinities both for the parasites and for the cells of certain organs. It is a double-edged weapon. We must suppress, or at least attenuate as much as possible, the dangerous affinity in favour of the useful one, a problem in chemical substitution. The task is chiefly met with in connection with protozoal diseases, but it is not impossible to aim in a similar fashion at the bacterial infections.

We already know examples of these, we may call them, *elective* affinities. Ehrlich himself showed long ago, that methylene-blue possesses a special affinity for the living nerve-fibres; there has even been derived from this *in vivo* staining, a

valuable method for anatomical analysis as follows: the animal is injected with methylene-blue and is then killed, and one finds in sections the nerve-fibres as fine blue lines. By injecting the same dye into a frog, the nervous system of the parasites of its body-cavity can be stained in the most delicate way. There are in cells, granules which are stained electively by neutral-red, others by pyrrol-blue. One colour is taken up by the nerve-cell, another by the fatty material, and these stains are more or less specific. Drug treatment ought to be imagined in the same way. To cure syphilis, it is necessary to find a chemical compound which will "stain" electively the parasite of Schaudinn without staining the cells.

It is curious that we find here again this analogy of staining, so often brought forward by both Ehrlich and Bordet, but with different significations. It is still too soon to ask which is true, the chemical or the physical theory.

The great merit of a theory is its usefulness, and Ehrlich's theory has certainly stimulated much work. It is not necessary to retain all the scaffolding once the house is built.

The new impulse of chemiotherapy is scarcely six years old. Research has followed different tracks and attached itself to different chemical substances: hence there arose several methods in which various remedies were soon combined and alternated.

Here is a list of the principal remedies tried against the protozoal diseases.

1. *Trypan-red and the benzidine colours.*

2. *The Triphenylmethane series:* malachite-green, brilliant-green, crystal-violet, victoria-blue, parafuchsin, tryparosane. . . .

3. *Antimony, tartar emetic, etc.* In historical and logical order this series was studied after arsenic and atoxyl, the chemical relationship of antimony and arsenic suggesting this trial. Tartar emetic acts on the parasite of sleeping sickness: it has been employed in man. Salmon has observed that it acts—slightly—in syphilis.

4. *The arsenical bodies and atoxyl.* Arsenic is one of the most ancient remedies. Even before Dioscorides and Pliny,

the Chinese had employed it. As soon as it was found that sleeping sickness had as its cause a trypanosome, the arsenical treatment was applied to it. But arsenious acid was too toxic to be easily employed, and the discovery of trypan-red and the first successes reported with the *chromotherapy* might have led to its abandonment, had not W. Thomas brought again into prominence the substance *atoxyl* in 1905. Atoxyl, discovered by Béchamp in 1863, hardly entered the sphere of human medicine until 1902, when it was employed in dermatology, both by intravenous and subcutaneous inoculation. Thomas has the credit of employing it against sleeping sickness.

It is not, as was first thought, metarsenical aniline, which contains 37·7 per cent. of arsenic. According to Ehrlich and Bertheim, it is the monosodic salt of paraminophenylarsenic acid, but it is still the compound discovered by Béchamp.

It contains 24 per cent. of arsenic. It is a white, crystalline powder soluble in water and easily sterilized. It may also be applied as an ointment. Although almost thirty times less toxic than arsenious acid excessive doses produce nephritic symptoms and above all disturbance of vision reaching even to complete blindness.

Atoxyl has been employed in the trypanosome infections and in several spirillum diseases, such as recurrent fever and syphilis: it exerts both a prophylactic and a curative action. It has been said that it no longer acts in the trypanosomes in sleeping sickness once these have penetrated into the cerebro-spinal fluid: but according to several observers the meninges are in general quite permeable (L. Martin). *In vitro* it acts neither on trypanosomes nor on spirilla, so that the body itself must play an active part in the treatment.

Atoxyl would be a perfect remedy were it non-toxic. By ringing the changes on the chemistry of this arsenical subject a series of compounds have been discovered of which the last is 1,500 times less toxic than the first.

Three compounds superior to atoxyl have been discovered in this series: in the first place *arsacetin*, which is four times less toxic than atoxyl for the mouse and distinctly less toxic

for the monkey; secondly, *arsenophenylglycin* (arsenophenyl glycocollate of sodium), two to four times less toxic than atoxyl and more active in killing the parasites and in prophylactic power. Quite recently Strong and Teague in the Philippines have been treating surra in horses with arsenophenylglycin on the scale of veterinary practice and not of laboratory experiment, and have cured more than one-third of the animals, besides checking the epidemic. In the series of bodies studied by Ehrlich arsenophenylglycin had the number 418. Finally we reach the body numbered 606, the *dioxydiamidoarsenobenzol* ($C_{12}H_{12}O_2N_2As_2$), of which the hydrochloride is employed as the celebrated remedy termed "606" or *Salvarsan.*

5. The toxicity of these chemical remedies, the necessity of treating relapses and the fear of rendering habituated to the drug both the patient and the parasite, have suggested the method of *combined* or more correctly *alternated medication* (Laveran). The drugs associated may be of the same series or of a different chemical series from the principal drug. In spite of the good qualities of atoxyl arsenious acid still retains the first place in the alternated treatment.

Treatment of Sleeping-Sickness.—From 1906 onwards atoxyl was the remedy most employed in the treatment of sleeping-sickness. R. Koch during his sojourn in the Sese Islands on the Victoria Nyanza, gave half a gram two days in succession: in about eight hours the parasites disappeared from the blood and from the swollen glands. They remained absent for about ten days, when the injections were repeated; without inconvenience this double injection was continued every six days for from four to six months.

Koch observed further that on ceasing the injections the parasites reappeared in the glands after a minimum period of eleven days; from the twenty-fifth day they could be found in about 25 per cent. of the patients treated as above; they then disappeared and after the sixtieth day were not to be found again. But the disappearance from the glands was not a final cure: there were relapses. The prolonged treatment, how-

ever, permitted the campaign of prophylaxis; the patients thus treated had no longer parasites in their blood and could not therefore furnish the glossina carriers with new supplies of virus.

Mild cases were cured after a treatment of from four to six months. At the end of 1907 Koch calculated that the death-rate among the treated individuals was between a tenth and a twentieth of what it had been among the non-treated.

Relapses occur even among the patients treated from the earliest period, and even 14 months after the treatment ceases. The more intense the treatment the later the relapse.

Soamin (atoxyl with one molecule of water in addition) was apparently rather less toxic than atoxyl; arsenophenylglycine produces pain at the point of injection, but it seems to act in patients in whom atoxyl fails.

Of the remedies supplied by other chemical groups the best hitherto is tartar-emetic injected intravenously. It ought to be tried when atoxyl fails and the association of atoxyl with tartar-emetic has often seemed better than either of these drugs alone. With one injection per week it is possible to keep the blood and glands free from trypanosomes. The combinations atoxyl + mercury and atoxyl + sulphide of mercury (orpiment) have been tried; atoxyl remains the basis of all the remedies.

The Treatment of Syphilis.—Experimental study of syphilis is quite recent and dates from the announcement of Metchnikoff and Roux that the disease can be inoculated with certainty in anthropoid apes, presenting not only the primary symptoms, but secondary symptoms as in man. By his discovery of the specific microbe, Schaudinn furnished us (1905) with the best means of controlling under the microscope both inoculation and treatment. For centuries the idea has prevailed that syphilis is exclusively a human ailment; since 1903 it has been transferred from man to the higher apes and from these to the lower, and now it has been inoculated in rabbits and guinea-pigs. Man however remains the chief.

Six years ago the medical world would have been astonished at a prediction that syphilis and the trypanosome diseases

would one day be treated practically by the same remedies. Yet analogies were already in existence to act as a guiding principle, for the name of horse-syphilis had long been given to a trypanosome disease, dourine, which possesses clinical resemblances. It was Schaudinn, again, who maintained from his study of the facts the relationship between trypanosomes and spirochætes; he was convinced that a protozoon cannot be properly known until its biological cycle has been followed completely, and he therefore studied the hæmatozoa as a zoologist and found in the development of the same species trypanosome forms and spirochætes. When the spirochæte of syphilis came under his eye, he was not only prepared to see it but to believe it and maintain it. There was great discussion in the scientific world as to the relationships of this new microbe: was it a protozoon or a bacterium? These researches had the happy result of drawing attention to a problem which concerns medical practice much more closely than was guessed, and they prepared the way for the revolution in the treatment of syphilis.

This sprang from an old idea. In an article dated 1867 in the *Dictionnaire des Sciences médicales*, one may read that arsenic occasionally succeeds in completing a cure after mercury and iodine: "it is especially the symptoms which have resisted the former treatment which yield to the action of arsenic."

The arsenical treatment of syphilis is an adaptation of the arsenical treatment of the trypanosome infections, inspired by the ideas of Schaudinn and the labours of Ehrlich.

Salmon was the first to undertake in Metchnikoff's laboratory methodical studies with atoxyl. The well-known toxicity of this substance confined him to small doses. Arsenophenylglycine, employed by Alt in general paralysis, was found to produce temporary improvements. It was in December, 1909, that Ehrlich mentioned for the first time a new substance containing the same arsenobenzol group as arsenophenylglycine, namely, the dioxydiamidoarsenobenzol, famous to-day under the name of "606" or *Salvarsan*.

All that has been published hitherto on Ehrlich's remedy con-

firms the great progress it has realized. It is to be hoped that the future will confirm these promises and make of this drug one of the great victories of therapeutical science.

Observations on immunity in Protozoal Diseases. Strains resistant to Sera. The future of Chemiotherapy.—To chemiotherapy, also, we owe much new knowledge on the immunity to the protozoal diseases, and on immunity in general.

The question of the dose is here of capital importance; the object is to begin and to finish the treatment at one blow, but it must be a sledge-hammer stroke. Small doses cure for a time without producing immunity, and relapses therefore occur. In the treatment of a relapse the trypanosome is found to be different. Sometimes it becomes more sensitive to the action of the drug. Sometimes it gives the impression "that as the result of the absorption of those parasites killed by the atoxyl, the body acquires a degree of immunity which then interferes with their normal development" (Ehrlich). As a rule, however, such immunity is ephemeral.

But what is most commonly observed in following the course of a first attack on to a relapse, and again from one relapse to another, is a diminution in the efficacy of the remedy. The question is, is it the body or the parasite which has changed? We find it is the parasite, for when inoculated now into a fresh animal it still resists the same drug. The injection of a drug in small doses is in fact the best way to render a trypanosome resistant to it, and further to create a *strain* of trypanosome which resists the remedy and preserves this resistance even after hundreds of passages, transmitting it as an acquired character.

Trypanosome strains have been created in the laboratories resistant to trypanred, to benzidine dyes, atoxyl, and tartar-emetic. The resistance is a group resistance, *i.e.*, it applies to all drugs of the same series or chemical family: a trypanosome resistant to trypanred is still sensitive to the arsenic bodies; it resists, however, also the benzidine dyes, which are a good deal different from trypanred. It can be seen by testing various drug-groups against the same resistant strain that biology agrees

with chemistry in putting tartar-emetic, *i.e.*, antimony, in the same group as arsenic.

The resistance acquired towards the bodies of one group presents, however, different degrees; thus, in the arsenical group a strain resistant to atoxyl is still sensitive to arsenophenylglycine; if it is now rendered resistant to this latter substance it still remains sensitive to tartar-emetic and arsenious acid. Again subjected to arsenious acid it becomes resistant to tartar-emetic; no strain, however, has yet been created resistant to arsenious acid.

These observations have important consequences for practice; they indicate that it may be necessary to attack the same trypanosome by different remedies and form the reason for the method of combined or alternated drug treatment. The drugs superpose their actions on the parasite, but not necessarily on the body, because their toxicity does not bear entirely on the same organ cells. Further, the different degrees of resistance which exist towards drugs of the same group show that it is proper to associate atoxyl and arsenious acid, although they are both arsenical bodies. Attacking the same parasite with several remedies is doing the same, says Ehrlich, as the entomologist who pins out a butterfly with several pins.

Although the difficulty may be overcome by using these combined treatments the appearance of resistant strains is always a danger. In sleeping-sickness, for example, there is only one good drug, atoxyl, to destroy the trypanosome, and in presence of strains resistant to this, one is rather helpless, the other remedies being either too weak or too toxic. The danger would be aggravated should the trypanosomes preserve hereditarily their acquired resistance through their passages in the tsetse-fly which conveys them. It would represent the creation of a more virulent and less curable disease, not only in an individual, but throughout a whole country.

Things do not go on in the living body exactly as in a test-tube. This fact, so often referred to already, must be insisted upon again.

There are certain chemical substances which act on the

trypanosome neither *in vitro* nor *in vivo*; others are active in both, for example, tartar-emetic.

Methylene-blue kills certain spirochætes in the test-tube in a dilution of 1 in 6,000,000, but has no action in the body of a mouse even when in 500 times greater concentration.

Atoxyl does not act *in vitro* but acts in the living body. There are even substances which in the body stimulate the multiplication of certain parasites; one must here reckon with the body as a factor as well as the parasite, and the problem of the best drug to use both in the first attack and in relapses is a new one for each species of trypanosomes, and for each species of animal: chemical therapeutics is therefore not likely to become more simple.

When an animal acquires immunity towards a bacterial infection, there develop, as we have seen, *antibodies*, as manifested by new properties of the serum, bactericidal, preventive, and curative. Is it the same in protozoal infections? Do the chemical remedies induce the formation of antibodies?

The serum of animals infected with trypanosomes possesses microbicide and preventive properties, weak it is true, but definite; they appear in the course of chronic or subacute infections. There is reason to believe in the presence of an immune body analogous to those known in antibacterial immunity; it acts by inducing a phagocytosis of the parasite.

Strains of protozoa may arise resistant to the serum of animals, infected or provisionally cured, just as with the chemical remedies; in many cases it is a true variety formation, for the acquired character can be transmitted through several generations (counted by mouse passage).

The antibodies in animals infected by trypanosomes originate doubtless from trypanosomes destroyed and absorbed. At the same time, the rapid destruction of a great number of the parasites may flood the body of the host with substances which act as poisons—a sort of endotoxins. In a man suffering from sleeping sickness, a disappearance of the trypanosomes under the influence of atoxyl is often followed by an attack of fever (L. Martin).

A dose of a chemical remedy, itself non-toxic, sometimes kills the mice while destroying their parasites. Ehrlich warns against treating with "606" infants suffering from the septicæmic form of congenital syphilis; he fears an intoxication with substances derived from the suddenly dissolved spirochætes.

The paradox that a patient may give a negative Wassermann reaction which after treatment with "606" becomes positive, is to be explained, according to Ehrlich, by the production of antibodies as a consequence of a non-curative dose. And it is a local production of endotoxin, under the influence of arsenic or mercury, which produces the sudden revival of cutaneous eruptions in syphilis, known as the phenomenon of Herxheimer.

These observations on strains resistant to drugs and to sera have thrown light on the question of the virulence of protozoa. Bacteria also acquire resistance to the defensive powers of the higher animals; the streptococci and the anthrax bacilli clothe themselves with a mucous capsule; the typhoid bacilli become insensitive to the agglutinins of the patient whom they are infecting; other bacteria secrete *agressins*; all these are manifestations of resistance. This "immunization of microbes" raises the thought that the laws of virulence may be fundamentally the same for both bacteria and protozoa.

There is nothing to exclude the possibility of a drug-therapy in bacterial infections also, and it is always possible that our vaccines of to-day, and in particular our sera, marvellous discoveries as they are, and originating in the most scientific empiricism, may give place to physico-chemical remedies better defined and more direct. It is from chemistry that we have to expect advances in the healing art. The phrase of Duclaux is very apt in this connection.

"*With Pasteur chemistry invaded the field of medicine probably never to leave it.*"

GLOSSARY

Actinomycosis—an infectious disease affecting cattle and sometimes man, characterized by tumour growths of the jaw, the lungs, the tongue, &c., and due to a *streptothrix*, q.v. : known popularly as woody-tongue.

Algæ—cellular cryptogamaceous plants including many seaweeds.

Amylase—a ferment capable of decomposing starch.

Ankylostoma—the "hook-worm," a parasite of the small intestine, common in the tropics and among miners, and producing severe anæmia, the "miners' anæmia."

Annelids—a class of Vermes, or worms, including the common earthworm.

Ascitic fluid—the fluid producing abdominal dropsy.

Ascus—an enlarged cell of a fungus in which the spores are developed, usually the terminal end of a hypha or thread.

Auto-intoxication—the poisoning of the body by materials developed within itself: gout, arterio-sclerosis, &c., are supposed to be auto-intoxications.

Basidium—the spore-bearing hypha (or thread) of a fungus.

Bothriocephalus—a broad tape-worm occasionally infecting man and derived from the ingestion of certain fish in which its cystic stage occurs.

Botulismus—the poisoning produced by the consumption of meat, particularly in the form of sausages, in which an anaerobic bacillus, the B. Botulinus of Van Ermenghem, has grown and produced a toxin, the botulismus toxin.

Brownian movement—dancing vibratile movements seen among minute particles, even of inert substances such as charcoal, when suspended in a fluid and examined under the microscope : it is not to be confused with true motility.

Calories—units of heat : large and small calories distinguished by capital and small letters ; small calory is the amount of heat necessary to raise 1 gram of water 1° centigrade : large calory the amount necessary to raise 1 kilogram 1° centigrade.

Carbohydrates—compounds of the three elements, carbon, oxygen, and hydrogen : the chief representatives are the sugars and starches.

Catalysts—substances which modify the rapidity of a reaction without forming part of its final products : such a reaction is termed "catalysis" (Berzelius, 1835) : for example peroxide of hydrogen, H_2O_2, is decomposed into water, and oxygen on contact with spongy platinum, or platinum in a state of fine division. The platinum remains unaltered : it has acted merely by its presence.

Chemiotaxis—a sort of "chemical sense" by means of which living cells seem to seek the substances favourable to them, and avoid those which are injurious. Positive and negative chemiotaxis are indicated by movements of attraction and repulsion respectively, *i.e.*, by approach or flight.

Chromatin—the substance composing the greater part of the nucleus of cells, so called because it stains very deeply with certain dyes.

Chromidia—masses or networks of chromatin distinct from the nucleus itself.

Colloids—Graham gave the name of "colloids" to those substances which in watery solution do not dialyse (*i.e.*, do not pass through a parchment membrane dipping in pure water), or dialyse extremely slowly in contrast to the "crystalloid" substances which rapidly dialyse. Types of these two classes are gum and salt. Nowadays it is more usual to speak of "substances in the colloidal state" than of "colloidal substances." The "colloidal state" is a state of *suspension* of one substance in another as contrasted with true solution. For example gutta-percha forms a true solution in alcohol, a colloidal solution in water. In the animal body the cells, the cell-membranes, and the body-fluids all consist of colloidal solutions, the condition and activities of which are regulated by physico-chemical laws : the colloids are thus of extreme interest to biologists.

Conidium—a spore of a fungus produced asexually and borne on a special branch.

Cytoplasm—the protoplasm of the cell-body as distinguished from the nucleus, the protoplasm of which is known as nucleoplasm.

Daphnia—the common water-flea.

Dialysis—*vide* Colloids.

Diapedesis—a phenomenon discovered by Cohnheim : the white corpuscles of the blood (leucocytes) emigrate from the interior of the blood-vessels by a process of active movement not passive expulsion. They insinuate themselves between the cells forming the walls of the capillaries, and drag themselves through as narrow threads to recover their rounded shape outside.

Diastases—also known as *ferments* or *enzymes*: they are substances which can produce fermentations in the absence of living cells: their chemical nature is unknown and they are defined simply by their activities: they occur in the form of organic substances of indefinite composition which are soluble in water. In the embryo of grain (barley, &c.) there exists a diastase which transforms the starch of the grain into sugar in the presence of warmth and moisture, the process of "malting." (*Vide* Fermentation.)

Electrolytes—bodies which in solution break up into "ions" carrying electrical charges which are equal and opposite. For example, common salt in solution in water breaks up into a sodium ion carrying a charge of negative electricity, the cation and a chlorine ion carrying a charge of positive electricity the anion. The presence of electrolytes in water renders it a good conductor of electricity, hence the name.

Enzymes—*vide* Diastases.

Epizootic—infectious disease occurring widespread among animals—contrasted with "epidemic" in which the disease affects man.

Fats—compounds of carbon, oxygen, and hydrogen in the form of "esters" of glycerine, *i.e.*, compounds of glycerine with a fatty acid. Palmitin, stearin, and olein are fats in which glycerine is combined with palmitic, stearic, and oleic acids. The natural fats are mixtures in varying proportions of palmitin, stearin, and olein. The "saponification" of fats is their decomposition into the two elements glycerine and fatty acid, and the combination of the latter with sodium or potassium ("salting out" in the language of soap manufacture). Soap is thus a salt of sodium or potassium with a fatty acid, *e.g.*, stearate of sodium. By saponification 100 grams of fat can furnish 90 grams of fatty acid.

Fermentation—a chemical transformation produced by the action of living cells, *e.g.*, the cells of the yeast of beer, or by the action of the secretions of these cells (diastases), *e.g.*, zymase extracted from yeast cells. A typical fermentation is that of sugar by yeast in which the sugar is broken up into carbonic acid, water, and alcohol.

Fibrin—a protein (more exactly, a globulin) which forms the clot in coagulated blood. It does not exist in circulating blood in the living animal, but is derived from a different substance which exists in this and is termed *fibrinogen*. "Defibrinated" blood is blood from which the fibrin is removed as it forms by whipping with a bunch of twigs immediately after the blood is drawn. Such blood is no longer capable of coagulation.

Flora—the aggregate of plants growing without cultivation in a given district or indigenous to a particular geological formation: hence applied to the aggregate of bacterial species inhabiting the intestine, the mouth, &c.

Gametes—sexually differentiated cells which unite to form the fertilized cell, the *zygote*.

Glucosides—substances which are capable of decomposition into a sugar (glucose), and various other organic substances, alcohol, phenol, aldehyde, &c. For example, amygdalin the active principle of bitter almonds breaks up into glucose benzaldehyde (the odorous constituent) and hydrocyanic (prussic) acid. Many poisonous plants contain glucosides as their active principle.

Glycogen—a carbohydrate analogous to the starches, stored by the body in the cells of the liver and muscles, and drawn upon by the body for the supply of sugar consumed by the cells during muscular work and other activities.

Humours—body-fluids, for example the aqueous and vitreous humours of the eyeball.

Incubation—the time elapsing between the moment of penetration of the body by a virus (microbe or toxin) and the moment when the first symptoms appear.

Indol—Among the products of the digestion and putrefaction of proteins (q.v.) there appears a substance, *tryptophane*, from which the indol bodies are derived, *indol* and *skatol* (indicated by various colour reactions depending upon their relationship to indigo) : also a substance, *tyrosin*, from which are derived *paracresol* and *phenol* (carbolic acid).

Inflammation—the reaction of living tissues to injuries and infections. The essential fact in inflammation is the activity of cells, the phagocytes which are capable of taking up substances and digesting them.

Isomeric—with different chemical or physical properties but containing the same quantity of chemical elements in the molecule : the difference is due to the different arrangement in space of the atoms in the molecule.

Lipoids—a group of substances such as *lecithin*, *cerebrin*, *protagon* *cholesterin*, possessing certain of the properties of fats (λιπον—fat). The name was introduced by Overton to indicate the possession by these bodies of powers of solution similar to fats, in particular for anæsthetics. According to Overton the outermost layer of protoplasm in a cell consists of lipoid substances and the cell absorbs only those materials which are soluble in the lipoids.

Medulla—the *medulla oblongata* or bulb is the continuation of the spinal cord within the skull before its junction with the brain : it contains the nuclei of certain cranial nerves regulating the respiration and the heart-beat.

Molluscum contagiosum—an infectious skin disease characterized by small teat-like protuberances.

Mucedineæ—fungi of the group to which the ordinary white mould *mucor mucedo* belongs.

Mutations—sudden variations appearing in individuals of one species and capable of transmission to their descendants.

Mycetozoa or Myxomycetes—protozoa with naked protoplasm occurring on damp surfaces in the form of jelly-like masses, living on organic debris, amœbiform without mycelium but later plant-like.

Myeloplax—a multinucleated cell occurring in the marrow of bones.

Nematodes—a class of worms with thread-like body, mouth and intestinal canal, including the parasitic thread-worms, &c.

Neuroglia—cells in the nervous system which correspond to the connective-tissue cells in other organs.

Nitrification—the transformation of salts of ammonia (chiefly the sulphate and the carbonate) into nitrites and nitrates under the influence of the nitrifying bacteria.

Plasma—the fluid portion of the blood freed from the red and white corpuscles which float in it : it contains fibrinogen but, not having been allowed to coagulate, no fibrin (*vide* Fibrin).

Proteins—substances resembling egg-white and containing nitrogen, carbon, oxygen, hydrogen, and sulphur.

Ptomaines—products of putrefaction, some of them poisonous, isolated from putrefying organic matter (first discovered in dead bodies).

Putrefaction—the decomposition of protein substances by microbes and their ferments with the production of gas, foul smells, and sometimes poisonous substances : as a result of putrefaction organic matter is restored to the state of inorganic elements.

Septic—derived from σεπσις—corruption and applied to material which can produce or undergo bacterial infection, *e.g.*, a septic wound, a septic instrument, a septic dressing. Hence the words "antiseptic," a substance acting against this action, and "aseptic," the absence of sepsis. In modern surgery antisepsis has given place to asepsis.

Serum—the clear fluid expressed from the clot of coagulated blood : it represents the fluid portion of the blood minus the cells and minus the fibrin which forms the clot (*vide* Fibrin).

Stereochemical—the chemistry of matter may be treated as depending on arrangements of the atom in space in the molecule (*vide* isomeric).

Sucrase—sugar-splitting ferment.

Symbiosis—the living together of dissimilar organisms each dependent on the other : the best examples are the lichens where a fungus and an algæ live together.

Urease—urea-splitting ferment : producing ammonia from the urea, the main nitrogenous constituent of urine.

Vagus—the nerve supplying the heart, lungs, and stomach.

Vitalism—the modern application is to a theory which postulates, at least provisionally, some other activity in the life processes

than mechanical, physical, and chemical forces. The theory of immunity as being due to phagocytosis calls in the activity of living cells the mechanism of which is not known. It is certain, however, that in vital activities it is not necessary to assume anything beyond physico-chemical phenomena which will some day become clear. Biologists no longer talk of a "vital principle."

Zymase—*vide* Fermentation.

INDEX

INDEX

Abrin, 168
Acidity, inhibiting power, 10, 42
Acidophilus, 35
Acinetians, 121
Actinians, venom of, 178, 234, 238
Actino-congestin : *see* Congestin.
Adulteration, detection of, 255
Aerobes, 80
  in fermentation, 5
  in putrefaction, 10
Agglutination, Bordet's explanation, 221
  diagnosis by, 252
Agglutinin, development of, 193
  power of, 253
Agressins, 231
Albuminous materials, putrefaction of, 9
Alcohol production, 82
Alexine : *see* Complement.
Algæ, 299
  bacteria relationship, 63
  coals, 7
  nitrogen fixation, 20
Algal coals : *see* Bogheads.
Amboceptor : *see* Immune-body
Amœbæ, culture, 105
  digestion, 99
  excretion, 101
  infection among, 121
  inflammation in, 134
  pathogenic, 142
Amylase, 299
Anærobes, 80
  in fermentation, 5
  in putrefaction, 10
Anaphylaxis, 233
Ankylostoma, 299
Annelids, 299
"Antagonistic force," 10
Anthracosis, 47, 127
Anthrax, immunity, 207
  infection, 122
  vaccination, 270
  bacillus, antagonistic microbes, 125
  encapsulation, 57
  involution forms, 59
  light effect on, 96
  lysin, 87
  phagocytosis of, 196
  respiration, 84
  virulence, 117
  virus attenuation, 119
Antianaphylaxis, 242
Antibodies, 193, 248
  immunity, 207
Antiendotoxins, 171
Antigens, 193
  Bordet's researches, 218
Antinomycosis, 299
Antisensibilism, 247
Antitoxins, 168, 210, 227
Apotoxine, 239
Arachnolysin, 179
Arsacetin, 290
Arsenic chemiotherapy, 289
Arsenophenylglycin, 291
Arrhenius immunity theory, 216
Arthraspores, 59
Arthus' phenomenon, 234
Ascarides, respiration, 101
Ascitic fluid, 299
Ascus, 229
Aseptic breeding, 30
Aspergillus, 52
  Niger, fermentation, 5
  nutrition, 73, 75
  respiration, 84
  zinc absorption, 165

Atoxyl, 290
Auto-infection, 123, 88
Auto-intoxication, 299
Avian plague, 154
Axolotl, infection, 134
Azotobacter, 19

BACILLUS, 55: for bacilli of different diseases *see under* disease.
acidi paralactici, 42, 44
acidophilus, 35
amylobacter, 6, 7
asterosporus, 64
bifidus, 35
chemical properties, 44
inhibiting power, 42
botulinus, 299, 37
toxin, 159
Bütschlii, nucleus, 65
reproduction, 68
size, 150
coli, culture of, 89
in intestine, 34, 36, 37
mutation of, 62
para-, 115
putrefaction inhibited, 41
cyanogenes, pigment of, 92
exilis, 35
lactis ærogenes, 34
putrefaction inhibited, 41
maximus buccalis, 65, 66
megatherium, 109
mesentericus, 35, 109
perfringens, inhibiting action, 42
in intestine, 34, 36
prodigiosus, pigment, 92
pleomorphism, 59
putrificus, Bienstock, 11
inhibiting action, 41
in intestine, 36
pyocyaneus, inhibiting action, 125
Pfeiffer's phenomenon, 204
pigment, 93
radicicola, 21
ramosus, 58
III. of Rodella, 34
sporogenes, 36
sporonema, reproduction, 68
cold effect, 96
subtilis, involution forms, 59
respiration, 84

Bacteria, 55
alimentation, 77
chemical composition, 70
diseases, 107
heat effect, 94
light effect, 96
luminous, 89
nitrogen fixation, 19
organic cycles, 41
pleomorphism, 59
respiration, 80
toxins, 168
Bacteriaceæ, 63
Bacterial beds, 17
Bacteriolysis, 194
Bacterio-purpurine, 85
Bacterio-therapy, 273
intestinal, 42
Bacteriotropins,
Bacterium aceti, 5
butyricum, respiration, 80, 84, 85
gammari, 64
phosphoreum, 90
sorbose, 79
zopfii, 60
Basidium, 299
Beggiatoa, 63
nutrition, 78
Behring, immunity experiment, 201
on intestinal permeability, 47
Bellamy, fermentation experiment, 82
Bengal rose, bactericidal action, 97
Bertrand, G., experiments on medium, 79
Besredka, anaphylaxis theory, 241, 247
endotoxins, 169
Bienstock, putrefaction experiments, 10, 41
Bier, phagocytic therapy, 284
Bitumen, bacterial origin, 8
Bleeding, post, 92
Blood, pathogenic microbes in, 251
Bloodstains, detection of, 255
Blue algæ: *see* Cyanophyceæ.
Bogdanoff, aseptic breeding, 31
Bogheads, formation, 8
Bordet, Ch., agglutination, 254
chemiotaxis, 136
complement fixation, 256
hæmolysis, 183
immunity theory, 218
pleuropneumonia, 154

Bordet-Gengou reaction, 220
Borrel, anti-tetanic serum, 276
pleuropneumonia, 154
Bothriocephalus, 299
Botulismus, 299
Brownian movement, 299
Bruck, antituberculin of, 177
Buchner, H., antitoxin formation, 210
tubercle infection, 125
Bufotaline, 180
Bufotenine, 180
Bulgarian bacillus, 44
Bütschlii, on bacterial nucleus, 65

CAGNIARD DE LA TOUR, cold effect on bacteria, 96
Calcicolæ, 22
Calcifugæ, 22
Calmette, antitoxin experiments, 212, 228
ophthalmo-reaction, 262
Carbohydrates, 300
fermentation, 5
synthesis, 3
Cattle plague : *see* Rinderpest.
Celluloses, fermentation, 5, 6
Centipedes, venom, 179
Cerebrin, 302
Charrin, immunity experiments, 200
Chemiotaxis, 300, 136
Chemiotherapy, 285
Cheyne, Watson, infection experiments, 124
Chlorella, nitrogen fixation, 20
Chlorophyll, 3, 99
Cholera, anticholera serum, 278
infection, 122
vaccination, 271
vibrio, 40
agglutination test, 254
endotoxin, 170
flagella, 56
microbes favourable to, 125
phagocytosis, 202
pseudo-, 114
toxin, 171
virulence diminution, 117
Cholesterin, 302
lecithin reaction, 185
poisons reaction, 225, 226
Chonkewitch, immunity experiments, 208
Chromatin, 300
Chromidia, 65, 300
Citron, immunity experiments, 208
Cladothrix dichotoma,
Clostridium, 55
pasteurianum, nitrogen fixation, 19
Coagulins, 248
Coal, bacterial formation, 7
Cobra venom, 181
Cocci, 55
Coccidia, life cycle, 103
parasitism, 104
Cochin, Denys, fermentation, 83
Cohen, microbial heat production, 89
Cohendry, aseptic breeding, 32
Cohn, stability of species, 60
Cohnheim, inflammation, 131
Colloids, 300
immunity analogies, 224
Comma bacillus, 40
Complement, 183
bactericidal properties, 194
Ehrlich's theory, 215
non-specificity, 256
fixation, 256
Complementophil group, 215
Congestion, 178, 234
Richet's researches, 238
Conidium, 300, 52
Conjunctis-reaction, 178
Conradi : inhibition, 88
Contagious diseases, 121
Costia necatrix, 103
Cow-pox, 267
Crenothrix polyspora, 78
Crepitin, 238
Crotin, 168
Cuti-reaction, 178
Cyanophyceæ, bacteria relationship, 63
nitrogen fixation, 20
Cystococcus, nitrogen fixation, 20
Cytodiagnosis, 252
Cytophil group, 215
Cytoplasm, 300

DANYSZ-DUNGERN PHENOMENON, 215, 222
Daphnia, 300
infection by monospora, 133

Denitrification, 12
Diagnosis, microbial, 250
Diapedesis, 300
Diarrhœa, infantile, 38, 43
Diastases, 301
  secretion, 86
  toxins compared, 160
Diplococci, 55
Diphtheria, antidiphtheric serum, 274
  bacillus, lysin, 87
    pleomorphism, 59
    pseudo-, 113
    toxin, 158, 159
    virulence, increase and diminution of, 116, 117
Dubois, B., bacterial luminosity, 91
Duclaux, fermentation, 83
Dumas, J. B., fermentation, 82
Dysentery, antidysenteric serum, 279
  bacillus, endotoxin, 170
    pseudo-, 114
    receptive cell, 127

Ehrlich, chemiotherapy research, 287
  immunity theory, 211, 213
  serum titration, 275
  side-chain theory, 211
Endospores, 59
Endotoxins, 158, 168
Engelmann, respiration experiments, 80, 85
Entamoeba histolytica, 145
Enterococci, 35
  inhibiting power, 42
Enterokinase, 199
Enzymes : *see* Diastases.
Eosin, bactericidal action, 97
Epidemics, 120
Epitheliosis virus, 128
Erythrosin, bactericidal action, 97
Evolution :
  bacterial, 108
  serum action, evidence of, 256

Facultative bacteria, 80
Falloise, intestinal putrefaction, 38
Fats, 301
  fermentation, 5
  resistance to decomposition, 9
Fermentation, 5, 301
  anaerobic production, 82
  chemical constitution of medium, 78, 79
  heat production, 89
  phagocytosis analogy, 195
Ferments : *see* Diastases.
Ferrau, cholera vaccination, 271
Ferro-bacteria, 78
Fibrin, 301
Fibrinogen, 301
Ficker, intestinal permeability, 48
Filtration, 152
Findel, infection experiments, 125
Finkelstein, on diarrhoea, 38
Fishes, venoms of, 178
Foot-and-mouth disease virus, 155
Fowl-cholera, infection, 124
  bacillus, lysin, 87
    size, 150
    virus attenuation, 118
Friedberger, anaphylaxis theory, 246
Fungi, bacteria relationship, 62
  coals, 7
  sex, 66

Galactose, fermentation, 79
Germ-carriers, 124
Giant cell, 139
Glossina palpalis : *see* Tsetse fly.
Glucose, fermentation, 78
Glucosides, 302
  fermentation, 5, 6
Glycogen, 302
Granular conjunctivitis, *see* Trachoma.
Granulobacter, 7
Gregarines, 104
Gromia oviformis, digestion, 100
Guarnieri bodies, 155

Harmatozoa, pathogenic, 142
Haemolysin, 179
  secretion of, 86, 193
Haemolysis, Bordet's theory, 221
  specific, 225
  of venoms, 182
Haffkine, cholera vaccination, 271
  plague vaccination, 273
Haptophorous groups, 212
Heat-production, microbial, 89
Heredity, 145

Herxheimer, phenomenon of, 297
Hippuric acid, fermentation, 10
Hoffmann's bacillus, 113
Hog-cholera, 46, 154
Högyes, rabies treatment, 269
Horse-sickness, 154
Humoral immunity, 191
Hymenoptera venom, 179

Immune body, 183, 195
  Ehrlich's theory, 215
  specificity, 199
Immunity, 187 *et seq.*
  anaphylaxis, 240
Incubation of disease, 128, 302
Indol, 302
  toxicity, 37
Infection, 121
  intestinal, 127
Inflammation, 130, 302
Infusoria, 51
  excretion, 101
  infection, 121
Inoculation, 266
Intermediate substance: *see* Immune body.
Intestine, flora, 11, 27, 33
Intra-dermo reaction, 178

Jochmann, tuberculin experiments, 208

Kala-azar, 144
Khondiakon, 84
Koch, R., sleeping sickness treatment, 291
  specificity of disease, 112
  tubercle phenomenon, 173
Kurpjuweit, on inhibition, 88
Kyes, lecithids, 185

Laccase, 86
Lactic bacilli, bacteriotherapy, 273
  fermentation, 43
  involution forms, 59
Laîné, artificial nitre beds, 16
Lamblia intestinalis, 104
Lamprocystis, 78
Lampyris noctiluca, 91
Latent microbism, 123
Lechartier, fermentation experiment, 82
Lecithids, 184, 226
Lecithin, 184, 226, 302
Leguminosae bacteria, 20 *et seq.*
Leishmania: pathogenic, 142
  donovani, varying forms, 144
Lentospora, 145
Leprosy, incubation period, 128
  bacillus, 108
Leptothrix ochracea, 78
Leucocidine, 87
Leucogytes, 48, 136, 138: *see also* Phagocytes.
Levulose, fermentation, 78
Lignite, bacterial formation, 7
Lipochrome, 92
Light production, microbial, 89
Lipoids, 166, 302
  colloids, analogy with, 224, 226
Lithocolletis caterpillars, aseptic condition, 31
Luciferine, 91
Luciola italica, 91
Lumbricus, inflammation in, 133
Lysius, 86, 248

MacFayden, bacterial extract, 169
  cold, effect on microbes, 96
Macrophages, 138, 197
Madsen, immunity theory, 216
Malaria, infection, 123
  mosquito, 143
  parasite, 51, 104
  path of penetration, 126
  receptive cell, 127
  reproduction, 66
Mallein, 173
Manure, denitrification, 14
  fermentation, 7
Marmorek's serum, 280
Martelly, putrefaction experiments 10
Marx, virus fixe, 269
Massart, chemiotaxis, 136
Massini, bacterial mutation, 62
Maze, fermentation experiment, 82
Meat, adulteration detection, 255
  putrefaction, 10
Meningitis, antimeningococcus serum, 280
  bacteriotherapy, 274
Meningococcus, 27
  pseudo, 114
Mercaptan, poisoning, 37
Metachromatic bodies, 64

Metchnikoff, aseptic breeding, 32
cholera bacillus, 40
non-bacterial life, 30
phagocytic immunity, 197, 198
Micrococci, 55
Micrococcus parvulus, 150
Microphage, 138, 197
Microsphaera, amoebae infection, 121
Miguel, inhibition, 88
Milk, fermentation, 5
putrefaction, 11, 41
Molisch, microbial luminosity, 91
respiration, 85
Molluscum contagiosum, 302
virus, 155
Monospora tricuspidata, Daphnia infection, 133
Morgenroth, antitoxin experiments, 212
Moro, aseptic breeding experiments, 32
Moulds, 52
heat effect, 95
nitrogen fixation, 20
Mouth, microbes in, 27
Mucedinae, 302
nutrition, 74
Mucor, 52
Mucous membranes, microbes in, 26, 46
Müntz, nitrification experiments 13, 16
Mycelium, 52
Mycetozoa, 303
inflammation, 131
Mycoderma vini, 5
Myeloplax, 303
Myriapods, 9
Mytilocongestin, 238
Myxobolus pfeifferi, 104, 145
Myxomycetes: *see* Mycetozoa.

NÄGELI, pleomorphism, 60
Negri bodies, 155
Nepticula, aseptic condition, 31
Neufeld, bacteriotropins, 264
Nicolle, M., antibodies theory, 248
Nictric ferment, 13, 74
Nitrification, 12, 303
Nitrobacter, 13
Nitro-bacterium: *see* Nitric ferment.
Nitrogen fixation, 18
Nitrosococcus, 13
Nitrosamonas, 13
Nitrous ferment, 13
Nitsch, virus fixe experiments, 269
Nobécourt, intestinal putrefaction, 38
Nocard, anti-tetanic serum, 277
Nose, microbes in, 26
Nucleus, bacterial, 64
Nutrition, microbial, 72
Nuttall, aseptic breeding experiments, 31

OCULO-REACTION: *see* Conjunctivo-reaction.
Oïdium lactis, 11
Omeliansky, cellulose fermentation, 6
Opsonins, 204
Ornithodorous monbata, 147
Ornithorhynchus venom, 180

PANCREATIC JUICE, venom reaction, 182
Para, bacteria, 112
Para, cresol, 302
Parasitism, 102
Passages, method of, 117
Pasteur, anaerobic life, 80
fermentation experiment, 82
immunity, 188
microbial association, 125
non-bacterial life, 28
nutrition, 78
pathogenic microbes, 108
pleuro-pneumonia vaccination, 270
putrefaction, 10
rabies treatment, 268
virulence, 117
Pasteuria ramosa, 57
Pathogenic microbes, 107
Peat, bacterial formation, 7
Pébrine, 146
Pectose, fermentation, 7
Penicillium, 52
nitrogen fixation, 20
nutrition, 78
Peptolytic microbes, 10
Petroleum, bacterial origin, 9
Pettersson, immunity experiment, 206
Pfeiffer's phenomenon, 201

Phagocytes, 135, 138
Phagocytosis, 130, 191 (figs.), 100
chemical stimulation, 284
immunity, 187
toxins, 227
Phagolysis, 198
Pharynx, microbes in, 26
Phenol, 302, 37
Phosphorescence (animal), 90
Photodynamic substances, 97
Phycochrome, 63
Pigments, microbial, 92
Piroplasma, bigeminum, 147
Piroplasmosis, 142
infection, 123, 147
Pirquet, von, serum-sickness, 234
tuberculin, 178, 262
Plasmodia, 142
Plague, anti-plague serum, 279
vaccination for, 272
Plants, microbes in, 28
Plasma, 303
Pleomorphism, 59
Pleuropneumonia, microbe, 153
vaccination for, 270
Pneumococcus, germ carriers, 124
in throat, 27
Pollen coals, 18
Portier, anaphylaxis experiments, 234
Precipitation diagnosis, 254
Precipitin, 193, 255
Preisz, tubercle infection experiment, 125
Prophylaxis, 240
Protagon, 230, 302
Proteins, 303
putrefaction, 9
Proteolytic microbes, 10
Proteus, intestinal, 12, 37
pleomorphism, 59
putrefaction, 10
Protozoa, 50
diseases due to, 141, 286
physiology of, 98
Pseudo-bacteria, 112
Pseudomonas lucifera, 90
Ptomaines, 303
Purple bacteria, 85
Putrefaction, 3, 303
intestinal, 37
Pyocyanase, 87
Pyocyanin, 93
Pyocyanolysin, 87
Pyorrhea alveolaris, bacteriotherapy, 273

Rabies, incubation period, 128
vaccination, 268
-virus, path of penetration, 127
receptive cell, 127
virulence increase, 117, 118
Raulin, nutrition experiments, 74
Raulin's medium, 74
Receptive cells, 127
Receptors (Ehrlich), 212
Recurrent fever, 147
Reproduction, microbial, 67, 102
Respiration, 80
Rhipicephalus annulatus, 147
Rhizopods, pathogenic, 142
Rhizopus nigricans, 11
Richet, anaphylaxis experiments, 234
Ricin, 168
Rinderpest, 154
sero-vaccination, 282
Rivet, intestinal putrefaction, 38
Roger, immunity experiments, 200
Roux, immunity experiments, 228, 276
pseudo-bacteria, 113
serum anaphylaxis, 241
toxins, 160

Saccharolytic microbes, 10
Sacred host : *see* Bleeding host.
Salamander venom, 180
Saltpetre, bacterial formation, 15
Salvarsan, 291, 293
Sarcinæ, 34
pigment production, 93
Sarcocystine, 145
Sarcosparidia, 104
Scarlatina, 155
Schaudinn, nucleus, bacterial, 65
reproduction, 68
syphilis, 118, 292
Schick, serum-sickness, 234
Schizomycetes, 62
Schizosaccharomycetes, 67
Schloesing, nitrification experiment, 13
Schottelius : *see* Aseptic breeding
Scorpion venom, 179

Sea-urchin venom, 178
Sensibilisatrice : *see* Immune body.
Sensibilism, 247
Sensibilisinogen, 247
Septic tank (sewage), 16
Sero-agglutination, 252
Sero-diagnosis, 255
Serotherapy, 274
Serovaccination, 245
Serum, 274, 303
Sewage purification, 16
Sex, microbial, 67
Sheep-pox, sero-vaccination for, 281
Side-chain theory, 211
Skatol, 302
Skin, bacteria in, 26
Sleeping sickness : atoxyl treatment, 290
infection, 123
treatment, 291
trypanosome of : *see* Trypanosoma gambiense.
tsetse-fly, 143
Sleeswyk, agglutination experiments, 254
Small-pox, vaccines, 266
virus, 128, 155
Smith, Th., anaphylaxis phenomenon, 235, 248
Snake venom, 180
Soamin, 292
Sorbose, bacterium of, 79
Spallanzani : heat effect on moulds, 95
Spider venom, 179
Spirilla, 55
Spirillosis, infection, 123, 148
virus, 118
Spirochætes, 55
diseases due to, 142
Spore coals, formation, 8
Spores, 58, 102
heat resistance, 95
Staphylococci, 55
in skin, 26
Staphylococcus aurens, 92
pyogenes, 37
Staphylolysin, 87
Starch fermentation, 5
Stegomyia fasciata, 149
Sterigmatocystes, 20
Stichococcus, 20
Stomach, microbes in, 27
Streptococci, 34, 55
anti-streptococcic serum, 280
encapsulated, 57
in skin, 26
virulence, 116, 117
Streptocolysin, 87
Streptothriceæ, 63
Stylonychia pustulata, 101
Sucrase, 303
Sugar, fermentation, 5, 78
soil-enrichment, 20
Sulpho-bacteria, alimentation, 78
respiration, 85
Sulphuretted hydrogen poisoning, 37
Sulphurous bacteria : *see* Sulpho-bacteria.
Surgery, phagocytic therapy in, 283
Swine-erysipelas : vaccination, 270
-bacillus, 127
virulence increase, 118
Symbiosis, 303
Syphilis, chemio-therapeutical treatment, 292
hereditary, 146
sero-diagnosis, 258
transmission, 148
spirochæte, path of penetration, 126

Takaki, toxicity experiments, 166 226
Tannase fermentation, 6
Tarantula venom, 179
Tartar-emetic, in sleeping sickness, 292
Tetanolysin, 87, 215
Tetanospasmin, 215
Tetanus, anti-tetanic serum, 276
favouring factors, 125
incubation period, 128
infection, 122
latent, 123
bacillus, flagella, 56
path of penetration, 163
respiration, 84
toxin, 159
virulence increase, 117
Tetrodon venom, 180
Thalassine, 178

Thermophilic bacteria, 94
Thierfelder, aseptic breeding, 31
Thomas, W., atoxyl use, 290
Tissier, putrification experiments, 10
Toad venom, 180
Toxins, 157, 210
  phagocytosis, 227
Toxogenin, 239
Toxophore group [Ehrlich], 213
Trachoma virus, 155
Trypanosoma, 51
    culture, 105
    diseases due to, 142, 290
    parasitism, 104
    sexual reproduction, 66
  gambiense, 51, 105
    toxin, 145
Trytophane, 302
Tsetse fly, 143
Tubercle bacillus, chemical composition, 71
    culture media, 76. 94
    detection, 112
    light, effect of, 97
    in lung, 27
    para-, 115
    path of penetration, 127
    phagocytosis, 138
    virulence increase, 117
Tuberculin, 173
    chemical composition, 71
    diagnosis by, 260
    immunity, 208
    light, effect on, 97
Tuberculosis, antituberculous serum, 280
    antituberculous vaccination, 272
    cutaneous reactions, 262
    heredity, 146
    infection, 47, 121, 125
    tuberculin treatment, 174
    tuberculin diagnosis, 260
  bacillus : *see* Tubercle-bacillus.
Typhoid bacillus, agglutination test, 252
    blood infection, 251, 46
    in decayed food, 37
    endotoxin, 170
    flagella, 56
    infection, 122
    lysin, 87
    para-, 115
Typhoid bacillus, Pfeiffer's phenomenon, 204
  -fever, germ carriers, 124
    vaccination, 271
Typhus fever bacillus, 120
Tyrosin, 302

ULTRA-MICROSCOPE, 151
Urea fermentation, 9
Urease, 303
Urine fermentation, 9
    toxicity, 38
Urobacteria, 9
Urtilago carbo, 95

VACCINATION, 265
    anaphylaxis, 234, 242
    continuous, 243
    sero-, 281
Vaccines, 265
    passage of, 118
    sensitized, 282
Vaccinia virus, 155
Vaillard, immunity experiments, 228
Variolisation, *see* Inoculation
Vaughan, anaphylaxis theory, 246
Venoms, 178
    anti-venom sera, 278
Vibrio, 55
Vibriolysin, 87
Vibrion septique, putrefaction, 10
    respiration, 84
Villes, Georges, leguminosæ experiments, 20
Vincent, pathogenic microbes, 109
Virulence, 116
Virus, attenuation of, 118
    filtrable, 150
    fixe, 268
Vitalism, 303

WALBRUM, immunity theory, 216
Wassermann, antituberculin, 177
    toxin experiments, 166, 226
  reaction, 258
Weever fish venom, 179
Weigert, 65
Welch's bacillus : *see* Bacillus perfringens.

Wheeler, anaphylactic theory, 246
Widal, biological diagnosis, 252
Willanen, toxin experiment, 212
Wollmann, aseptic breeding, 31
Wolff-Eisner, ophthalmo-reaction, 262
Wright, opsonins experiment, 204

YEASTS, 54
  chemical composition, 70
  cold, effect on, 96
Yeasts, fermentation, 5
  milk putrefaction, 11
  nutrition, 77, 73
  reproduction, 67
Yellow fever, 149, 154
Yersin, toxins, 159

ZOOGLÆA, 57
Zischeria, aseptic condition of, 31
Zygosaccharomycetes, 67
Zymase, 83, 169

R. CLAY AND SONS, LTD., BRUNSWICK ST., STAMFORD ST., S.E., AND BUNGAY, SUFFOLK.