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LONDON, 1884.

PUBLIC HEALTH LABORATORY WORK.

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PUBLIC HEALTH LABORATORY WORK.

PART I.—BIOLOGICAL LABORATORY.

BY

WATSON CHEYNE, M.B., F.R.C.S.

WITH

CATALOGUE OF THE EXHIBITS IN THE LABORATORY.

PUBLIC HEALTH

LABORATORY WORK.

I.

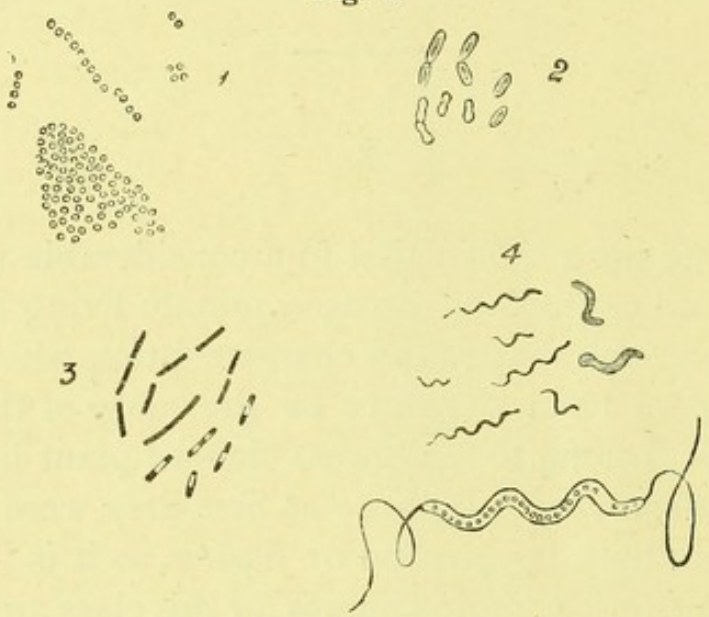
It has of late been shown that in a considerable number of infectious and contagious diseases, minute living bodies are present of various shapes and characteristics, which have in some instances been shown to be the cause of the disease. These bodies belong to the lowest class of plant life and are termed Schizomycetes, because at first they were only supposed to multiply by division or fission, as it is technically called. The most common names for this class are, however, Bacteria or Micro-organisms. There are four well-marked groups of bacteria, divided according to differences in form. These are (1) Bacteria proper, small oval or slightly elongated bodies; (2) Bacilli or rod-shaped bodies; (3) Micrococci or round bodies; and (4) Spirochætæ or spiral bodies.

Many of these micro-organisms can move actively in fluids, their progression in most instances being probably due to the presence of a lash or cilium at one or both ends. This has been demonstrated to exist in the bacteria and in some forms of Spirilla (belonging to the fourth class). Movement also is present in some of the bacilli, but the majority of those associated with disease are motionless. In most cases moving bacteria have a motionless stage. The spontaneous movements of these bodies are sometimes

difficult to distinguish from the well-known molecular motion of minute particles suspended in fluid. The former is, however, as a rule, well marked, the organisms changing their place either with a swift darting or slow undulating movement; while the latter is a dancing, or in the case of rods an oscillating motion without change of position.

The mode of growth is in most cases by division. A rod elongates and soon divides transversely to its long axis, giving the appearance of two rods joined together by their ends; these rods separate, and we have thus two individuals. In the case of the micrococci the division may take place

Fig. 1.



1. MICROCOCCI AND STREPTOCOCCI; 2. BACTERIA; 3. BACILLI; 4. SPIRILLA.

not merely transversely but longitudinally, and thus we may have pairs of micrococci or triplets, or fours, or groups; or it may take place in one direction only, giving rise to long chains. In the last case the organism is called a chain micrococcus or streptococcus. The rapidity of growth of bacteria depends greatly on the temperature and on the nature of the soil; but it has been calculated with regard to one or two forms grown on suitable soil at the ordinary summer temperature of this country, that they double their numbers at the least once in an hour, so that every individual produces 8,388,408 in 24 hours.

Not only does growth occur by fission, but it also takes place, more especially in the bacilli, by the formation of spores, (see figs. 1, 3). These appear in the rods as bright refracting, round or oval bodies, the rod after a time disappearing and liberating the spores.

These spores are of great importance, as they are extremely resistant to the action of heat and chemical agents. They may retain their vitality for years in the dry state, and grow again into the fully developed organism if placed in suitable circumstances.

Ever since the discovery of these organisms till within the last few years there have been constant and often violent discussions as to their origin, some asserting that they were always derived from a parent, others stating that they arose *de novo* in organic fluids from aggregation of organic molecules, which became vivified as the result of various physical causes—the theory of spontaneous generation Abiogenesis or Heterogenesis. The latter theory has been gradually disproved step by step till it is now no longer upheld, and there can be no doubt that whenever micro-organisms develop they have been derived from one which has come from the air, water, or surrounding objects. The fallacy arose from the fact that, when an organic fluid such as infusion of meat, was placed in a flask, boiled and the flask hermetically sealed, in a certain number of instances the fluid became turbid from the development in it of these minute bodies. As boiling a fluid for a few minutes was supposed to be destructive of all existing life, a positive result was held to be proof of the origin of these bodies *de novo*. Sometimes the fluid was boiled after the flask was sealed and the temperature was raised above 212° Fahr., and yet in a number of instances development occurred. The fallacy in these experiments is two-fold ; in the first place the upper parts of the vessel were not sterilised previous to the introduction of the fluid, and in the second place nothing was at that time known of the existence of spores. The introduction of the method of first heating the flask for some hours at a temperature of

at least 300° F. after the orifice had been plugged with cotton wool, taking care in the introduction of the impure fluid that it does not touch the upper part of the vessel, and then boiling the fluid for ten or fifteen minutes, was followed by a very great diminution in the number of instances in which development afterwards occurred in the fluid. And when it was shown by Tyndall that, if the fluid, instead of being boiled only once for a long time, were boiled on several successive days for a few minutes at a time, no instances of development occurred, the last blow was struck at the theory of spontaneous generation, and it may now be finally dismissed from consideration. Tyndall made the brilliant deduction from his observation that some of these bodies must form very resistant spores, and his object in boiling the fluids more than once was to give time for the spores not killed on the first occasion to develop into mature organisms, when they are readily killed by the second or subsequent boilings. This deduction, made before the spores in these organisms had been observed, has now been amply confirmed by microscopical observation. At the present time it is perfectly easy to maintain any organic material pure for an indefinite time if the vessel in which it is placed be sterilised at a high temperature for some hours after being plugged with cotton wool, if care is taken to introduce the fluid to the bottom of the vessel, and if the fluid be afterwards heated for an hour for two or three days in succession to a temperature even a good deal below the boiling point of water.

These organisms and their spores are found almost everywhere in nature in enormous numbers. They float in the air: in large numbers in the air of factories, towns, and inhabited rooms, in woods and forests; in smaller numbers in the air in the open country; still fewer at high altitudes; and in the air on the glaciers in Switzerland, for example, they are almost if not entirely absent. They are constantly present in water; the more stagnant the water is, the more numerous they are; they pass through the ordinary filters, and are, therefore, numerous in drinking

water. The surface of the animal body is covered with them, and in the mouth and parts of the alimentary canal they flourish in great luxuriance. All dust contains them, and the soil is the special habitat of many forms of the greatest importance in the plan of nature.

These organisms play a very important rôle in nature, and without them vegetation and with it animal life would greatly diminish if not entirely cease to exist. They are the mechanism by which dead vegetables and animals are decomposed and rendered suitable food for future generations of plants. The higher plants derive their carbon almost entirely from the carbonic acid of the air, and their nitrogen in part from the ammonia of the air and soil, and in part from nitrites and nitrates in the soil. By the combined action of the chlorophyll and the sunlight the carbon is extracted from the carbonic acid and used to form the complex organic substances of which the walls and contents of the cells of plants are composed. In the same way, also, the nitrogen must probably be in its elemental form before it can be utilised. The higher plants cannot take up complex chemical substances and utilise them as food; these must first be reduced to their simple forms. Hence, there must be some mechanism for reducing these compounds to their simple forms, otherwise the higher plants would perish for want of suitable food. Part of this destructive work is done by animals. They can take up these complex substances and utilise them as food, and, as a part of their vital action, they reduce a portion of them to carbonic acid, water, and other simple forms, in the lungs and throughout the body. But the reduction of these substances by animals is very imperfect and quite insufficient for the purpose, while, further, the dead animal body must be itself converted into these simple elements, otherwise a large amount of energy and nutritive material would be constantly lost. This gap is filled up by the lowest forms of plant life—the miscroscopic fungi, but more especially the bacteria; their existence is therefore essential for the maintenance of all life.

It must not, however, be supposed that every bacterium is capable of taking up a complex organic substance and splitting it into its elementary constituents. All take up oxygen either from the air or from the substances in which they grow, and probably all produce more or less carbonic acid, but some are only able to carry on the destructive process to a certain stage, and when their work is done other forms come to their aid and complete the change. Among these partial changes in organic substances, as the result of the growth of micro-organisms, we have the great class of fermentations which result in the production of some of the essential elements of food and many of the so-called luxuries.

There is one class of micro-organisms which gives evidence to the naked eye of the change they occasion in the material in which they grow. These are micro-organisms which produce various pigments. There are now a large number of pigment-producing organisms known. Among the torulæ there are some which produce pigments of various colours. The best known of these is one which forms a pink substance (Rosahefe). This substance only becomes pink at the surface in contact with oxygen; at the deeper parts of the growth, the material formed is colourless, but rapidly becomes red when exposed to the action of the air. In none of these cases is the micro-organism itself coloured, but it is the material produced by and surrounding it that has the property of absorbing certain portions of the spectrum. Other forms of torula produce other colours; for example a yellow torula is very common. Among the subdivision *bacterium* of the Schizomycetes there are a few which produce pigments. Chief of these is one which causes the greenish-blue colour which is sometimes seen in pus; also one which produces the so-called yellow milk, and one which gives rise to a brown colour. It is necessary to mention here that it is not only in pus or in milk that these respective colours are produced. Pigment micro-organisms always produce the same colour on whatever soil

they grow, provided that the soil possesses the necessary chemical substances. And the same pigment is always produced by the same organism. An organism cannot at one time produce a red, at another a blue, at another a yellow substance; it always produces the same colour, or where the soil is unsuitable, but where it is still capable of growth, no colour at all. There are very few *bacilli* which cause the formation of pigments, but of these the best known is the bacillus of blue milk. These bacilli can be cultivated apart from milk, and when introduced into a glass of milk which is becoming sour, but has not yet coagulated, they produce this blue change. A red pigment is also produced by a bacillus—*Bacillus ruber*. By far the largest number of these pigment-producing organisms belong, however, to the class of *micrococci*. These grow with great readiness on boiled potatoes, and also on various gelatinised organic infusions. One of the best known is *Micrococcus prodigiosus*, which gives rise to a beautiful blood-red colour. Among other colours produced are a yellow (*Micrococcus luteus*); an orange-yellow (*Micrococcus aurantiacus*); violet, green, &c. These pigment organisms are very important for experiments on the specificity of these minute bodies, and also, as will be seen later, for testing the power of various agents in destroying the vitality of these lower forms of life.

The changes produced by the other micro-organisms associated with fermentation are not so evident to the naked eye as those we have just been considering, but nevertheless it is possible to render these changes visible in some cases. For example, when most forms of bacilli grow in an organic fluid rendered solid by the addition of gelatine, this solid material becomes fluid as the result of the action of these bacilli on the gelatine. The fluidity of the gelatine is at once a test of the presence of bacilli, and an evidence of the extensive chemical alterations they produce in the soil in which they grow. I may mention a beautiful example of a chemical change rendered visible to the naked eye which occurred to me lately. A yellow torula

was being cultivated on a gelatinised meat-infusion which contained a minute quantity of blood-colouring matter. In the preparation of the material, the blood-colouring matter had been converted into methæmoglobin, a substance convertible into oxyhæmoglobin by the action of oxidising and reducing agents. On one occasion, in re-inoculating this yellow torula, a bacterium became mixed with it; the cultivation was impure. After these two organisms had grown on the gelatine for a few days, it was found that the material beneath the yellow patch, and extending far beyond the growth of organisms, had assumed a delicate pink colour, which on spectroscopic examination was found to be due to the presence of oxyhæmoglobin. Re-inoculations of this bacterium on similar soil was always followed by the same result, the bacterium evidently producing a gaseous reducing agent which passed a certain distance into the gelatine, and converted the methæmoglobin into oxyhæmoglobin. That the growth of bacteria is followed by changes in the soil in which they grow is also easily ascertainable by chemical analysis, and among the most important of these changes are the various fermentations which occur in organic substances.

The most extensive fermentation caused by micro-organisms is the conversion of glucose and maltose into alcohol, carbonic acid and other substances. This is brought about by the growth of the *Torula cerevisiæ* in solutions containing these substances. Other torulæ and also some fungi are capable of causing the conversion of sugar into alcohol, but their effect is insignificant as compared with that of the organism employed for the purpose—the *Torula cerevisiæ* or yeast plant. The torulæ are small microscopical cells, round or oval, with cell wall, granular protoplasm, and sometimes vacuoles. They grow by budding, and in some cases by the formation of spores. They grow with great rapidity in suitable sugary solutions if exposed to the air. When there is plenty of free oxygen present they do not cause much fermentation of the fluid; but if the supply of oxygen is insufficient, they grow less

luxuriantly, but produce a much greater change in the constitution of the fluid. In these circumstances they are supposed to take oxygen from some of the compounds in the material in which they grow—probably from the sugar which splits up chiefly into alcohol and carbonic acid, a small quantity of glycerine and other substances being also formed.

Many other fermentations are caused by the Schizomycetes. Thus the souring of milk is due to the growth of a small bacterium, the *Bacterium lactis* (Lister) in it. This organism can be cultivated pure in solutions other than milk, and when again inoculated into milk, the latter becomes sour and coagulates from the formation of lactic acid from the milk sugar. The butyric acid fermentation has been shown to be due to a bacillus, which only grows in the absence of oxygen, and indeed is killed by it. When cultivated in various fluids, even in Pasteur's solution, it causes the butyric fermentation. This organism is of use in the preparation, especially the ripening of Swiss cheese. It grows and causes the butyric fermentation during the first twenty-four hours, while the cheese is still under the press, and the fermentation is accompanied by the evolution of large quantities of gas. The slower development of this gas which occurs later explains the formation of cavities in the cheese. The chemical change consists in the partial transformation of the milk sugar into butyric acid. Sugar at times undergoes a viscous fermentation. This is the transformation of sugar into gum, mannite, and carbonic acid, and results in the formation of a viscid ropy fluid. This fermentation is due to micro-organisms, said to belong to the class of micrococci. Putrefaction is a fermentation accompanied by the development of a foul smell, but is a much more complex process than the other fermentations, and is probably caused by several organisms producing a succession of fermentations. This fermentation is a very important one, as during its course products may be formed which are intensely poisonous to animals, and introduced into the circulation may cause symptoms resembling those

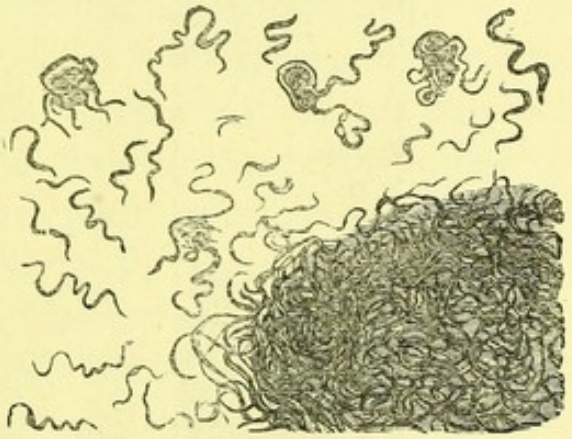
due to various alkaloids. The acetic fermentation is due to a small bacterium which converts alcohol into acetic acid. The growth of this bacterium only occurs when suitable nitrogenous and other nutritive substances are present and when the fluid does not contain more than ten per cent. of alcohol.

Other fermentations are associated with bacteria, and though not yet thoroughly worked out are undoubtedly due to them. The old idea that organic substances underwent fermentation and decomposition owing to the action of the air or other causes independent of the growth of these bodies, has been shown to be erroneous, for the most diverse organic materials may be kept for an indefinite time with suitable precautions without the occurrence of any change in them. Thus, milk may be taken from the cow with certain precautions, received into a sterilised flask, protected from the dust by a cotton wool cap, and kept for an indefinite time without undergoing any change and without the development of any organism. In the same way blood or portions of the organs from a healthy animal just killed may be placed, under similar arrangements, in vessels, and kept indefinitely, without decomposing. These experiments show not only that organic substances do not undergo fermentations if micro-organisms are absent, not only that bacteria do not originate spontaneously (i.e. without a parent) in organic substances, but also that bacteria are not present in the blood or tissues of a healthy living animal. This is a point of great importance.

Many of these micro-organisms will only grow on particular soils, while the great majority will grow on any albuminous substance. The necessary substances are water, carbonaceous and nitrogenous organic substances, and various organic salts, especially phosphates and salts of potash. One of the most important points with regard to the soil is the reaction, most bacteria requiring a neutral or slightly alkaline substance. This is, however, not invariably the case, as for the bacterium which causes the acetic fermentation, for example, an acid soil is requisite. Again, bacteria as a rule grow best in the presence of plenty of

oxygen; but there are some which will not grow unless oxygen is almost or entirely absent. Some of those which cause fermentation do so most vigorously in the presence of free oxygen, others act best when there is no oxygen, where, therefore, they must take their oxygen from the substances in which they grow. The temperature is also a point of great importance, a medium temperature of 60° to 80° F. being best for most forms. The best temperature is, however, different for different forms, some, for example the bacillus of tubercle, only growing at the average body temperature.

Fig. 2.



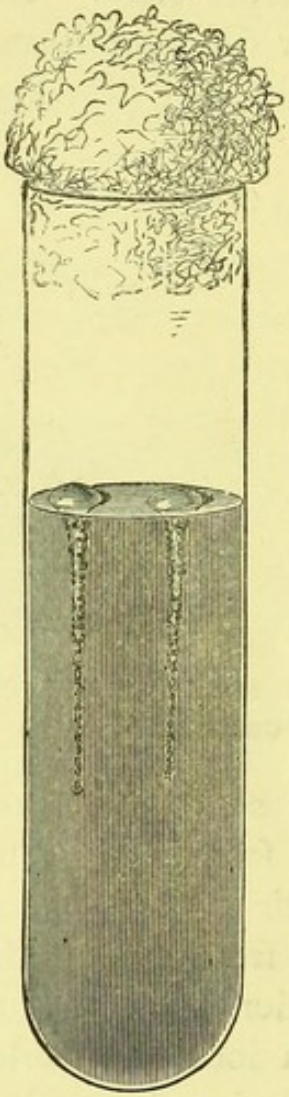
SURFACE OF COAGULATED BLOOD SERUM ON WHICH THE BACILLI ARE GROWING. × 100.

When growing on solid substances, such as gelatinised meat-infusion many forms of bacteria show distinctive characteristics in their mode of grouping, &c., and thus may be distinguished from one another, though this would be hardly possible under the microscope. Thus the *Bacillus anthracis* grows in a loose network, the rods not being closely applied to each other; the bacillus of tubercle grows in dense masses of parallel rods, which soon become more or less S shaped (see fig. 2); the bacillus of septicæmia in mice forms an extremely delicate cloud; the micrococcus of pneumonia forms pin-shaped colonies at the point of inoculation (see fig. 3), and so on. Thus by the naked eye one can pick out many organisms by their mode of growth or solid substrata.

Not only are these microscopic plants essential in nature,

by causing fermentation and decomposition of the substances in which they grow, but some forms can also prove injurious to vegetable and animal life. Those which injuriously affect plants belong almost solely to the class of fungi, while of those which are hurtful to animals only one or two are fungi, the great majority being various forms of bacteria.

Fig. 3.



APPEARANCE OF CULTIVATION OF THE MICROCOCCI OF PNEUMONIA (FRIEDLAENDER) ON GELATINE. (NATURAL SIZE.)

These bacteria may be hurtful by the production of poisonous substances which belong to the class of alkaloids, and are rapidly fatal to life in a sufficient dose. If a quantity of putrid blood be injected into a number of mice, for example, a certain number may die only after a day or two or may not die at all ; but where the quantity injected

is large the animals may die in a very short time (a few hours), as the result of the absorption of the poisonous substances resulting from the growth of the micro-organisms in the putrefying blood. Some observers state that they have been able to extract from this putrefying blood an alkaloid substance, which, injected into animals, produces the same poisonous effects as the original putrid blood. This septic intoxication is of great importance in surgery, for in wounds to which micro-organisms are freely admitted these substances are produced, and if absorbed in moderate quantities give rise to fever, or, if in larger quantities, and rapidly, to death.

This condition of septic intoxication must be carefully distinguished from the action of other forms of micro-organisms which are parasitic on the animal body, and, growing in the blood or tissues, give rise to a large number of diseases grouped together under the term "Infective Diseases." Of these there are two groups, those in which the infection occurs from a wound or open surface—Traumatic Infective Diseases—and those in which no wound is necessary and where the pathogenic organisms are supposed to be able to enter the body through uninjured surfaces. Of these the traumatic infective diseases have been most completely worked out, and have been shown in a larger number of instances to be due to the action of specific micro-organisms. Some of these pathogenic organisms are not only parasitic on the living body but can also grow outside the body on dead organic substances, being ever ready, however, to become parasitic on a living body when an opportunity offers. One of the best examples of this is the bacillus of anthrax, which in the living body does not form spores. It can, however, grow on dead vegetables such as peas, especially when lime is present, and form spores, producing the disease again when taken into a living body. Other pathogenic organisms are, however, apparently incapable of growing outside the body though they retain their vitality for a considerable time in the dry state, and can grow when they again enter

a living body. One of the best examples of this is the bacillus of tubercle, which, though it can be artificially cultivated outside the body under special conditions, can seldom if ever meet with these necessary conditions in nature.

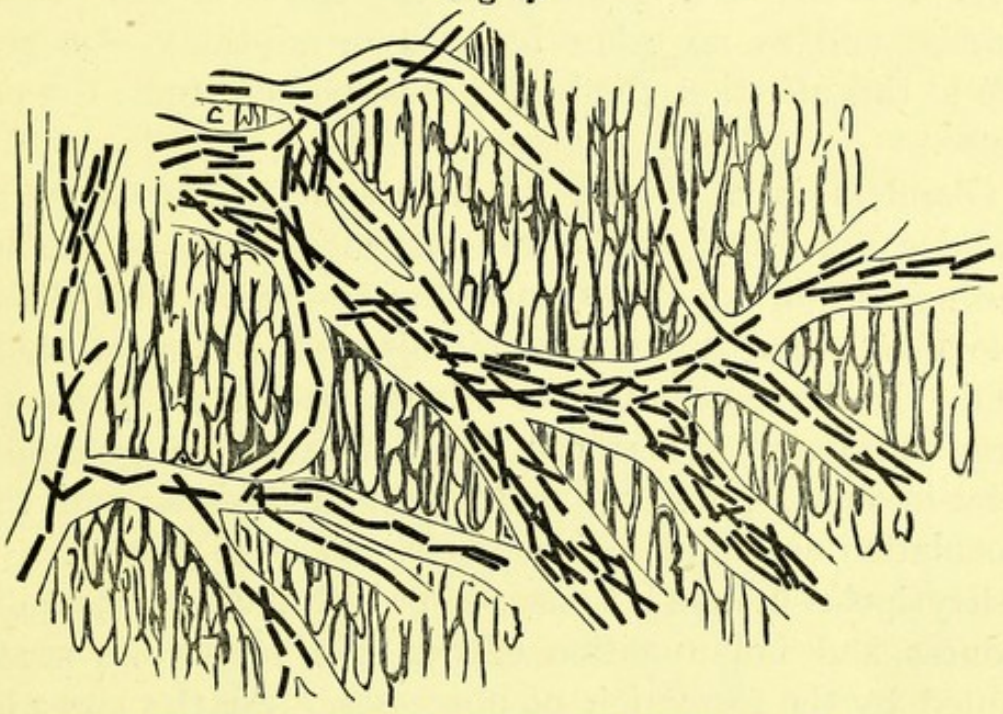
The following are the chief steps required for the proof that a given organism is the cause of a disease. Firstly, an organism of a definite form and with definite characteristics must always be found in the blood or in the affected parts of the animal body. The blood or the affected parts containing these organisms, when inoculated into another animal of the same species, must produce the same disease. Treatment of the blood or affected parts in such a manner as to destroy the micro-organisms present in them must also destroy their power of causing disease in another animal. When the diseased parts are inoculated on suitable soil outside the body the micro-organisms grow, and can be indefinitely propagated on similar soil.* When in this manner the organisms have been separated from the remains of the animal substances in which they were imbedded, their inoculation on a suitable animal must again produce the disease, the same organisms being also found in the diseased parts. This sort of proof has now been furnished for a considerable number of diseases.

The best known example of a disease due to micro-organisms in which the above proof has been furnished is that of anthrax or splenic apoplexy. This disease affects all mammalia, including man, and birds are also liable to be attacked by it. It may commence by the formation of a pustule of a carbuncular nature, but usually, especially when the disease is rapid in its course and the animal is particularly liable to it, no pustule is observed. Sometimes animals are suddenly struck down while apparently well, but generally the temperature becomes high, they stagger, bleed from the nose, mouth, &c., and rapidly die.

* The cultivation of some pathogenic organisms, for example of leprosy, and relapsing fever, has not yet been successful, but it is necessary for the absolute proof that they are the causes of these diseases.

In man the carbuncular form is not uncommon, and patients so affected may recover; when, however, the disease becomes generalised death almost always results. In the blood of animals affected with this disease one constantly finds rod-shaped organisms belonging to the class of bacilli. These bacilli are long and thick and are among the largest of the pathogenic bacteria. Not only are the bacilli present in enormous numbers in blood drawn from the body, but if after death portions of the organs are hardened in alcohol, cut into very thin sections, and stained with some of the aniline dyes, all the smallest blood-vessels

Fig. 4.



ANTHRAX BACILLI IN THE CAPILLARIES. × 700.

throughout the body will be seen to be full of these organisms (see fig. 4). The smallest quantity of blood containing these organisms rubbed into a scratch in another animal causes its death in a very short time, the same appearances being found. If this blood is exposed to a high temperature or treated with substances which destroy the vitality of these bacilli it no longer produces any effect when inoculated. If a previously heated wire is dipped into the infective blood and then introduced into a sterilised infusion, or stroked over a gelatinised nutritive material, or over a

purified potato, care being taken to prevent the entrance of extraneous organisms during and after the experiment, growth of the bacilli occurs in the fluid or on the surface of the solid substance, in the latter case forming the loose network mentioned before. From the first material a second may be inoculated, and then a third, and so on indefinitely till all trace of the original blood is lost except these bacilli. If now an animal be infected with the minutest quantity of these cultivated bacilli the same disease and fatal result follow as when the infective blood was employed. Heat or substances which kill the bacilli render the material harmless when inoculated. These facts show that the bacilli were the cause of the original disease, and as no other bacteria or anything else gives rise to this affection, the bacilli must be held to be the only cause.

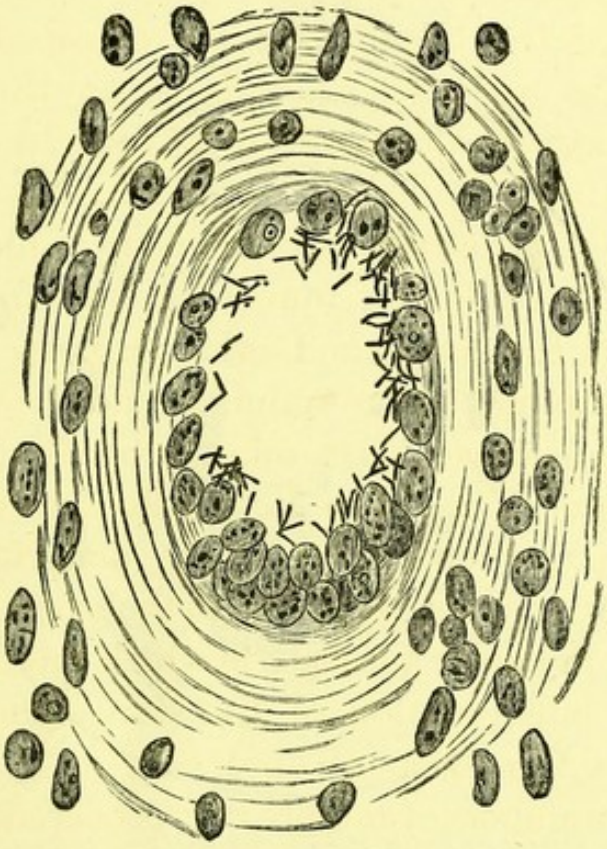
Glanders is a disease of horses in which ulcers and nodules are found in the mucous membrane of the nose and nodules in the lungs and other organs. This disease also affects man and other animals, and is almost always fatal. In the diseased parts minute bacilli are present in large numbers. They can be cultivated on gelatinised meat-infusion, potatoes and other materials, and their inoculation on animals gives rise to the same disease.

Erysipelas in man is a disease in which there is a spreading redness and inflammation of the skin, sometimes accompanied by the formation of abscesses. At the spreading margin large numbers of minute micrococci are found in the lymphatic vessels of the skin. These can be cultivated on potatoes, gelatinised meat-infusions, &c., forming whitish masses spreading over the cultivating material. The inoculation of these micrococci as also of erysipelatous pus on the ear of rabbits causes extensive redness, which generally passes off without producing any ill effects, and is followed by peeling of the skin in the same manner as occurs in man. Advanced cancerous and other diseases in man, in an unsuitable condition for operation have been benefited by an attack of erysipelas, and the use of these

cultivated micrococci has been as effectual in causing erysipelas and as beneficial to the patient as the use of erysipelatous pus.

Tubercular diseases assume a variety of forms in man of which the chief are phthisis and acute tuberculosis. In rodents we find only acute tuberculosis. In cattle and other animals there are various peculiarities, but the essential characters of the disease are the same. The inoculation of sputum from a case of phthisis, of portions of phthisical

Fig. 5.

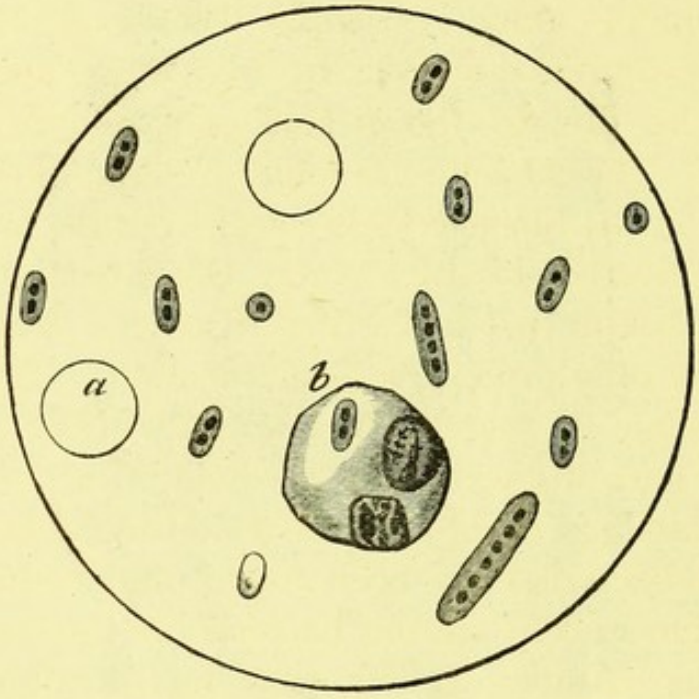


GIANT CELL FROM A TUBERCLE CONTAINING TUBERCLE BACILLI. × 700.

lungs, of tubercle of cattle, &c., into rodents gives rise to acute tuberculosis. In the same way inhalation of tubercular material gives rise to acute tuberculosis in rodents. The inoculation of other materials, provided they are not tubercular, does not cause the disease. The disease, therefore, is infective and specific. Examination of tubercular materials, sputum, phthisical lungs, acute tuberculosis, &c., shows the constant presence of a peculiar form of bacillus

(see fig. 5), differing in certain chemical characteristics from most of the other known bacteria. The destruction of these bacilli removes the infective property of tubercular substances. The bacilli can be cultivated on solidified blood serum kept at the temperature of the animal body, and forms the peculiar S-shaped growths previously mentioned. These cultivated bacilli can be indefinitely propagated in successive generations in any number of tubes containing blood serum. The inoculation of these bacilli on animals causes acute tuberculosis, identical in every respect with the disease caused by inoculation of tubercular materials. They are,

Fig. 6.



MICROCOCCUS OF PNEUMONIA. × 800.

therefore, the cause of tubercular diseases, though it is probable that a variety of conditions, such as special predisposition, are necessary before they can grow in the living body.

In the affected parts of the lung in pneumonia in man are found micrococci which have the peculiarity of being surrounded by a capsule (see fig. 6). They may be single, in pairs, or chains. They are also present in the fluid in the pleural cavity and in the sputum. They may be cultivated

on potatoes or gelatinised meat-infusion, &c. If the point of a sterilised needle be dipped into the pleural fluid, or passed into the diseased lung, and afterwards pushed into gelatinised meat-infusion, a whitish growth appears along the track of the needle, and at the surface this growth assumes the appearance, in relation to that occurring along the track, of the head of a pin. These pin-shaped growths are peculiar to the micrococci obtained from some cases of pneumonia. These micrococci can be grown through an indefinite succession of generations. Their injection into mice is followed by pneumonia, and inhalation by mice of these cultivations also causes pneumonia in a considerable proportion of the animals.

Septicæmia in mice is a rapid disease, resulting in the death of the animal in one to two days, and has been shown to be due to the growth of a minute bacillus in the blood. These bacilli are found in large numbers in the blood and in the blood-vessels throughout the body. They can be cultivated in tubes, and the inoculation of the cultivated bacilli produces the disease. Inoculated on rabbits they only produce a local affection. A peculiarity of this disease is, that while it readily affects tame mice and house mice it does not attack field mice.

For rabbits and other animals a considerable number of pathogenic bacteria have been found, and complete proof has been furnished that the bacteria are the only cause of the disease. Among others may be mentioned various septicæmic diseases in rabbits and mice, chicken cholera, pneumoenteritis in pigs, &c.

Besides the bacteria one or two fungi have been found which are capable of living in the body and causing the death of the host. Among these are two species of mucor and *Aspergillus fumigatus*. In man there is also a fatal disease termed actinomycosis, which is evidently due to a fungus living in the tissues. In man there are various skin diseases, as ring-worm, favus, &c., also due to fungi growing in the cutaneous structures.

In other diseases in man the proof is not so complete as

in the diseases of which I have been speaking above, because animals have not yet been found which are liable to the disease. Fortunately, however, for the advance of medical knowledge, so many diseases of the same type have been shown to be due to bacteria as the result of experiments on animals, that in these cases the constant presence in the diseased parts of organisms, showing definite morphological characteristics and differences from other bacteria in their mode of growth on cultivating media, leads us by analogy to assume, practically with certainty, that they are the virus of the disease. In typhoid fever minute short thick bacilli are found in the ulcers in the wall of the intestine, in the mesenteric glands, and forming plugs in the vessels of the spleen and liver, and sometimes in the lungs. These bacilli can be cultivated, and their mode of growth presents special characteristics. In cholera a bacillus is present in large numbers in the walls of the intestine, somewhat resembling in appearance the bacillus of glanders, and capable of cultivation on suitable soil outside the body. In ague, during the shivering stage, bacilli of peculiar and distinctive appearance have been found in large numbers in the blood. In diphtheria a bacillus is often found at the part where the disease progresses; it can be cultivated, and the result of experiments on animals points very strongly to the view that it is the cause of diphtheria in man; but as yet no animal has been found in which the disease can be produced with all the characteristics of the affection in man.

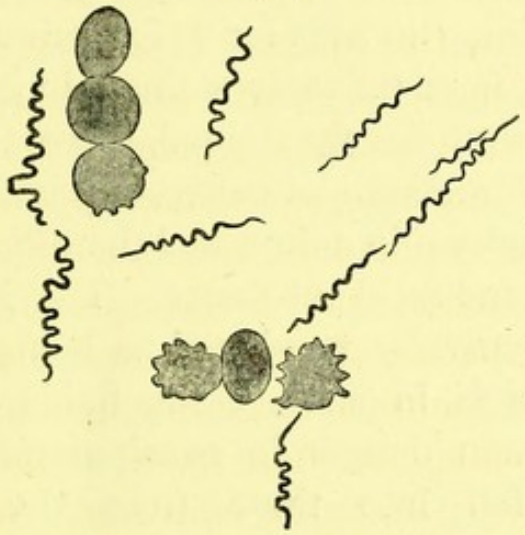
There are also some diseases in which definite organisms have been found in constant association with the morbid process, but these organisms have not yet been successfully cultivated. Thus in relapsing fever (see fig. 7), spirilla appear in the blood at the commencement and even before the commencement of the febrile attack, and increase rapidly in numbers till defervescence occurs. In leprosy enormous numbers of bacilli are found in the nodules, these bacilli being marked out from other forms

of bacteria as well by their appearance as by definite chemical characteristics.

These parasitic diseases are not confined to the higher animals, but they also affect those much lower in the scale of organisation. Thus the fungous disease of salmon and other fish is due to the growth of a fungus (*saprolegnia*) on the surface of the body; flies often die from the growth of a fungus (*Empusa muscæ*) in their bodies; and *pebrine* and *flacherie*, so destructive to the silkworm industry, are due to micrococci.

The demonstration of these bodies by means of the microscope is not always an easy matter, and when they

Fig. 7.



SPIRILLA FROM RELAPSING FEVER. × 700.

are present in tissues they can only be properly seen when they are stained. In the case of fluids one can by placing a drop under the microscope and using a sufficiently high power, generally see the bacteria and observe their movements, &c.; but when they are lying among other structures this is very difficult, and as a rule impossible. In the case of bacteria in fluids, also, it is always best to stain them. This is done by allowing the fluid to dry on the surface of a thin piece of glass (cover glass), and afterwards fixing the organisms to the glass by heating it, by passing it three or four times through a gas flame; the glass is then placed in the staining fluid for a sufficient length of

time, it is afterwards washed in water, dried and mounted in canada balsam. The materials used for this purpose are the basic aniline dyes, such as magenta, gentian violet, &c. In the case of tissues, fine sections are made, generally by means of a microtome, and stained in one of the above solutions. If now they are washed in dilute acetic acid, alcohol and oil of cloves successively, the colour disappears from the tissue, and only the bacteria and the nuclei of cells are left coloured. The processes employed are very various and not suitable for discussion in the present handbook. I may, however, mention one solution which is useful for almost all forms of bacteria. Take of a 1 to 10,000 solution of caustic potash in water—100 parts ; add of a saturated alcoholic solution of methylene blue 30 parts, shake and filter ; after staining in this for a few minutes the section may be washed, if very deeply stained, in dilute acetic acid ($\frac{1}{2}$ p. c.) ; if not very deeply stained, in water only.

There are two methods of cultivating bacteria, the one in which they are grown in a fluid and the other in which they are grown on some solid substance. The latter method is the one now generally employed as being the most free from error, though in some cases fluids are still useful. The most common danger in manipulating fluids is that bacteria may fall into them from the air, hands or instruments employed, and, growing by the side of those intentionally introduced, the two become mixed together, and the experiment is thus almost hopelessly ruined. If on the other hand a solid medium is employed, and bacteria accidentally gain access to the vessel during the manipulation, they grow at the point where they fell and do not necessarily mix with and spoil the organisms inoculated. Any impurity can thus be seen, and a fresh inoculation can be made before the organisms experimented with become contaminated by those which entered accidentally.

Fluid cultivating materials are usually infusions of animal or vegetable substances. These are in most cases neutralised, filtered, and introduced by a siphon into flasks

which have been purified by heating them at a temperature of 300° F. for two or three hours after their necks have been plugged by cotton wool. The fluid is then boiled two or three times at intervals of twenty-four hours, so that all the bacteria contained in it are destroyed, and the fluid remains pure so long as it is kept in the plugged flask. The best flasks for this purpose have a neck at the side, through which the fluid can be poured into smaller vessels. This neck is wide where it joins the bottle and narrow at the end. After fluid has been poured through it and the bottle is again placed upright a drop remains in the end so that no air enters the flask which has not been filtered through the cotton wool over the mouth of the flask. From this flask the fluid is poured into smaller flasks or tubes, which have in like manner been purified by heat, and are covered with caps of cotton wool after filling them. The fluid may be again sterilised by boiling, and then the flasks or tubes are kept for some days at the temperature of the human body. If the fluid still remains clear after a few days it may be looked upon as pure and used for experiments. The cotton wool cap being lifted momentarily, with precautions against the entrance of dust, the material to be tested, blood, pus, &c., is rapidly introduced, the cap again applied, and the flask placed in an incubator at 90° to 100° F. The material may be introduced by means of a syringe purified by heat, by platinum wire which has been heated, by sucking up a little in a capillary tube and dropping it in, &c. If growth occurs the fluid generally becomes turbid in a few days, the turbidity being due to the enormous numbers of bacteria present.

Solid-cultivating materials are boiled potatoes, coagulated blood serum, various infusions rendered solid by the addition of gelatine or agar-agar, &c. Potatoes are cleaned with a dilute solution of bichloride of mercury steamed till they are cooked, divided with a heated knife, and placed on a dish under a glass cover with wet blotting paper around to keep them from drying up. Potatoes are very good soil for a large number of bacteria, and it is much

easier to carry on pure cultivations on them than in fluids. The disadvantage is that they are opaque, and that therefore the mode of growth of the organism experimented with cannot be observed under the microscope. This difficulty is obviated by the use of infusions, rendered solid by the addition of gelatine or agar-agar. The latter is in some cases an advantage, because it remains solid at the temperature of the body, at which gelatine is fluid. These gelatinised infusions are kept in pure tubes or flasks plugged with cotton wool, or they are melted and poured out on heated glass plates which are kept in a moist chamber and protected from the dust. The best composition for a cultivating material is an infusion of meat to which is added 3 per cent. pepton, ½ per cent. common salt, and 5 to 10 per cent. gelatine, the whole being carefully neutralised. Most of the common forms of bacteria will grow on this, though modifications must be made in some instances. If this material is poured out on a glass plate and allowed to solidify, it may be inoculated with the bacteria under investigation, and their mode of growth observed. This is done by dipping the end of a fine platinum wire, which has been heated and allowed to cool, into the material containing the bacteria, and then rapidly drawing lines on the gelatine with it. Along various parts of the track of the needle bacteria remain, and if the pabulum is suitable and the temperature and other conditions correct, they grow in the form of colonies at these points. If any adventitious organism has fallen on the gelatine during the exposure it develops where it fell, and can easily be recognised as an impurity, while further cultivation may be made from the needle track before this adventitious colony has grown so large as to become mixed with those inoculated. At the same time, the gelatine being clear the growth may be observed under even comparatively high powers of the microscope, and may be photographed. As I have already stated, different organisms differ greatly in the form and mode of growth of the colonies which they form on a solid substratum, and in this way organisms, hardly

distinguishable under the microscope, may be readily separated from each other.

In other cases the pabulum employed is coagulated blood serum, and some organisms, such as the bacillus of tubercle, grow only sparingly and slowly on any other soil. The advantage of the serum is that it can be kept at the temperature of the human body without becoming fluid, and also that very few organisms liquify it while gelatine is liquified by almost all forms of bacilli.

The best cultivating material for microscopic fungi is a bread infusion, made by rubbing down bread, mixing it with water to a thick consistence, and sterilising it by heat.

I have already mentioned that when cultivations in fluids become impure, i.e. when other bacteria besides those intentionally introduced gain access to the fluid, and grow in it, the cultivation is lost, as it is a matter of great difficulty to separate the various forms from each other; at least, it was a matter of great difficulty till Koch introduced his method of cultivating on solid substrata. Before the solid method was employed the separation was made by what is termed the fractional method. Experiments were in this way successfully made by Sir Joseph Lister on the bacteria of the lactic fermentation of milk. He first estimated the number of bacteria of all kinds present in a given quantity of the fluid, for example in one drop. He then diluted this drop with boiled distilled water till every drop of the mixture thus obtained only contained one bacterium, supposing the organisms to be equally distributed throughout the liquid. To each of a large number of flasks containing sterilised milk or other cultivating material a drop of this diluted bacteric liquid was added. In a certain number of flasks nothing grew; but, in a certain number pure cultivations of the *Bacterium lactis* were obtained. This method, though very ingenious, is, however, very laborious and uncertain in its results, and is now given up in favour of the methods introduced by Dr. Koch. A sterilised, gelatinised infusion is liquefied, poured out on a sterilised plate of glass and

allowed to solidify. A fine platinum wire sterilised by heat is now dipped into the fluid containing the bacteria, and then drawn rapidly across the surface of the gelatine. In this way bacteria are sown along the track of the wire, and if a sufficiently small quantity be taken up on the point of a needle, and if the experiment be skilfully performed, it will be found that in parts of the track nothing grows, while at various points small colonies appear. It will be found on examination that some of these colonies consist of only one kind of bacteria, and pure cultivations can then be made from them. The following is another method. A minute quantity of the bacteric fluid is introduced into a tube containing the gelatinised material which has been liquefied at the body temperature. The fluid gelatine is now well shaken up so as to distribute the bacteria throughout the mass ; it is then allowed to solidify. In this way bacteria are caught at various points in the solid gelatine, and grow there to form colonies. On examination it will be found that many of these colonies are pure cultivations. It is more convenient, instead of retaining the gelatine in the tube, to pour it on a sterilised glass plate while it is still fluid, as in this way there is readier access to the colonies after their development for examination and further cultivation. These glass plates are kept in vessels to protect them from the dust, moistened blotting paper being present to prevent drying of the gelatine.

It is on this last principle that Koch's method of examining water is based. A measured quantity of the water to be examined is well mixed with a measured quantity of liquefied sterilised gelatine material, and this is poured out on a sterilised glass plate, and kept moist and protected from dust as in the previous instance. At various points in the gelatine organisms develop and their number can be counted, while, as previously mentioned, the class to which they belong may be determined by their method of growth even without having recourse to the microscope. In case of difficulty, any particular colony can be examined under the microscope, and if necessary inoculated into a

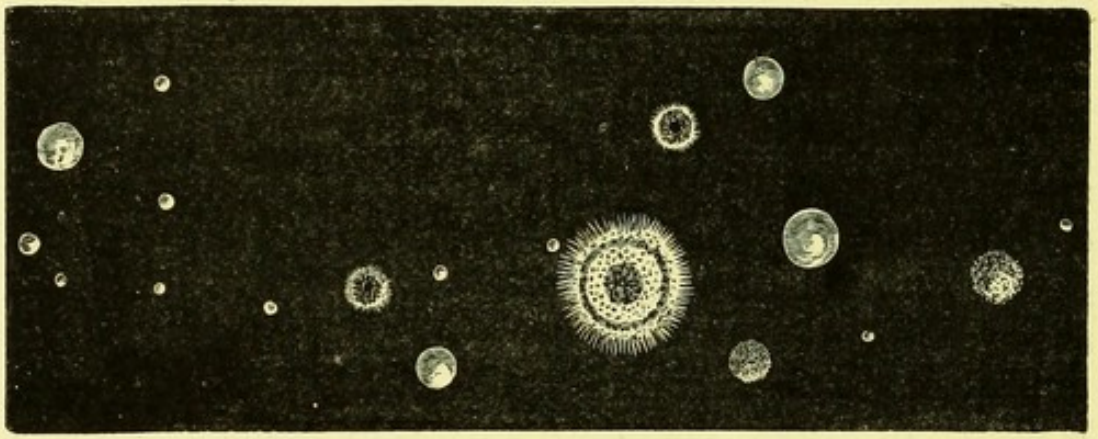
suitable animal. This method of examination has been carried out for a long time under Dr. Koch's direction in the Sanitary Institute at Berlin, and in the report of any specimen of water sent to him for examination, not only is the chemical analysis given, but also the number and kind of micro-organisms present are mentioned.

Soil is examined in the same manner. The soil to be investigated is crushed with precautions against the entrance of organisms other than those originally present and in the soil. It is then scattered over the surface of gelatine, spread on plates, as in the foregoing method, and the number and kind of the organisms which develop is in this way determined. Already valuable results have been obtained in this way. For instance, in a hospital at Amberg, an epidemic of pneumonia broke out, and a large number of patients died. Dr. Emmerich examined the soil under the floor of the ward, and found there large numbers of the peculiar micrococci, which seem to be the cause of that disease; these are not present in the same situation in healthy wards. He was in this way enabled to determine the cause of the outbreak.

By the use of the gelatine method air can also be very conveniently and accurately examined. Plates covered with a layer of sterilised gelatine may be exposed in various situations for various lengths of time, and the number and character of the organisms which fall on them may be readily determined (see fig. 8). Air may also be analysed quantitatively by the same method. Into long tubes, the walls of which, more especially the lower wall, are covered with a layer of sterilised gelatine, a known quantity of air may be aspirated and the dust allowed to settle. Development occurs at various points, and the number and kind of the organisms present in a given quantity of air may be determined. Very interesting results obtained by this method are given by Dr. Hesse in the second volume of the "Mittheilungen des Gesundheitsamtes in Berlin," and the accompanying woodcut is copied from one of his plates. Another method is employed by Dr. Miquel of the

Mont Souris observatory in Paris. He introduces a definite quantity of the air from certain localities into a large number of flasks containing sterilised infusions, and counts the number of flasks in which development occurs and the kind of organism in each flask. This method is, however, not so exact as the other, and there are many objections to it; for example: in Hesse's experiments it was found that the organisms in his tubes develop at different dates. Now, if two organisms of different rapidity of growth gain access to the same flask of meat-infusion, the one which grows first may entirely prevent the development of the second, while in many cases two organisms may

Fig. 8.



RESULT OF EXPOSURE OF A LAYER OF GELATINISED MEAT-INFUSION TO AIR. DEVELOPMENT OF VARIOUS FUNGI AND BACTERIA AT DIFFERENT PLACES ON THE GELATINE. (NATURAL SIZE.)

resemble each other very closely in microscopical appearance but differ in the appearance of their growth on a solid substratum.

One of the most important functions of these laboratories is to determine the best means of destroying the bacteria associated with disease, i.e. to determine the best methods of disinfection. Only in some of the infective diseases has the cause been as yet made out, and the bacteria already proved to be the cause of disease differ much in their resistance to various disinfecting means. The most resistant of all are, however, the spores of some bacilli, more especially of bacillus anthracis, and of a short thick bacillus

found in earth. If, therefore, substances and methods are tested as to their power of destroying these most resistant bodies, it is practically certain that they will be efficient as disinfecting means in all cases. This matter has been worked out very carefully by Dr. Koch, by the aid of his new method of cultivation. It is not merely of importance to determine what will destroy these bodies, but also what will impede or prevent their growth ; and it has been found that it is much easier to hinder the growth of bacteria than to destroy them. The method adopted by Koch was to soak sterilised threads in spore-bearing cultivations of anthrax bacilli and also in cultivations of non-spore-bearing and less resistant forms, such as micrococcus prodigiosus, and also to use dried earth, which always contains the thick bacillus with the very resistant spores. Among chemical substances able to destroy these spores with great rapidity he found that bichloride of mercury was the most potent. Mixed with the cultivating material in the proportion of 1 to 300,000, the bacillus anthracis was unable to grow. Spores of anthrax dried on threads and placed in a solution of 1—20,000 for ten minutes were incapable of development, but a weaker solution than this was uncertain. Solutions of 1 to 5,000, or stronger, destroy all spores with certainty in a few minutes ; indeed, it was found that to wet the spores with a spray of this solution and then allow them to dry sufficed for their destruction. A large number of other substances acted in the same manner, though not in such dilute solutions.

Of the various other disinfectants employed, only the following were able to *kill* the spores of the anthrax bacillus in less than 24 hours.

Chlorine water.
Bromine (1 per cent. in water).
Iodine water.
Permanganate of potash (5 per cent. in water).
Osmic-acid (5 per cent. in water).

The following acted slowly or imperfectly on the vitality of the spores.

Ether (incomplete destruction after eight days, complete destruction of life after thirty).

Aceton (incomplete after five days).

Iodine, 1 per cent. in alcohol (incomplete after one day).

Sulphuric acid, 1 per cent. in water (incomplete after ten days).

Sulphate of copper, 5 per cent. in water (incomplete after five days).

Boracic acid, saturated watery solution (incomplete after six days).

Hydrochloric acid, 2 per cent. in water (complete on the tenth day).

Arsenious acid, 1 per thousand in water (complete after ten days).

Sulphurous acid (incomplete after five days).

Sulphide of ammonium (complete after five days).

Formic acid 1·12 s. g. (complete on the fourth day).

Quinine, 2 per cent. in water ($\frac{2}{5}$) and alcohol ($\frac{3}{5}$) (incomplete after one day).

Quinine, 1 per cent. in water with hydrochloric acid (complete on the tenth day).

Turpentine oil (incomplete on the first day, complete after five days).

Chloride of lime, 5 per cent. in water (incomplete on the first and second day, complete after five days).

Chloride of iron, 5 per cent. in water (incomplete on the second day, complete after six days.

Carbolic acid in 5 per cent. watery solution killed all the spores between the first and second day. In 5 per cent. oily or alcoholic solution it produced no effect on spores, and bacilli without spores which are killed by the watery solution in a few seconds were not destroyed by the oily and alcoholic solutions till the sixth day. The question has been raised whether the evaporation of carbolic acid at the ordinary temperature would be sufficient to disinfect the air, but this must be answered in the negative. Spores of the earth bacillus placed in a vessel with carbolic acid and exposed to the vapour of carbolic acid for 45 days, developed as readily as before the experiment was commenced. On the other hand, if the vapour of carbolic acid is heated, although precautions are taken that no more is given off than at the ordinary temperature, the action becomes very rapid, so that carbolic acid vapour at a temperature of 167° F. almost completely destroys the spores of the earth bacillus in two hours, though this temperature of itself does not in the least impair the vitality of the spores.

Sulphurous acid is another disinfectant which is much used, but which turns out to be overrated. Dry micrococci are killed by a 1 per cent. vapour per volume in 20 minutes ; if moist, in two minutes. Therefore, for a disease due to micrococci it is sufficient, but it is quite different when it is tested on spores. Spores of anthrax, earth and hay bacilli, exposed for 96 hours to a vapour of sulphurous acid, at first of the strength of 6·13 vol. per cent. and after 96 hours of the strength of 3·3 per cent., were quite unaffected.

Such are examples of the results obtained by this method, and they show that though the ordinary disinfectants in use are sufficient when the virus is a bacterium which is not spore-bearing, yet where spores have to be dealt with they are insufficient. In the case of those diseases in which the cause has not yet been worked out, it is safest to treat them as if the virus possessed the resisting power of the most resistant spores, though it may turn out later that it does not do so.

In disinfecting fluids other factors come into play. Thus, one disinfectant may form compounds with substances in the fluids and lose its properties, while another which is in reality weaker may not do so, and thus be more effectual. Thus, in recent experiments on the destruction of the tubercle bacillus in phthisical sputum, Schill and Fischer found that corrosive sublimate solution (1—500 in water) added to an equal quantity of sputum failed to destroy the tubercle bacillus even after 24 hours' action, while carbolic acid (5 per cent.) added in the same proportions to sputum disinfected it thoroughly in 24 hours. And yet, acting on dry spores of bacillus anthracis the sublimate solution is much more effectual than the carbolic acid. In the case of sputum the difference probably depends on the different chemical affinities of the two substances, the sublimate either losing its antiseptic properties by entering into new combinations, or being unable to penetrate and act on the masses of secretion which contain the bacilli.

Among other methods of disinfection, the most popular are disinfection with hot air and with steam. Dr. Koch's results show that disinfection of clothing, bedding, and large

masses of material is impossible with hot air. One experiment will show where the fallacy lies. It is known that spores can resist dry high temperatures for a long time, but that two or three hours' exposure to a temperature of about 300° F. will effectually destroy them. At this temperature clothes are destroyed ; they become brown and useless. But, independently of this fact, Koch made the interesting discovery, that when a roll of clothes is put into a baking apparatus, though the clothes may become brown at the outside, the temperature in the interior of the mass is very low, and quite useless for disinfecting purposes. Thus, a piece of linen, about 40 inches long, was rolled up tightly, and 32 complete turns were in this way made, giving 64 layers from one side to the other. A maximum thermometer was placed in the middle of the roll, and between every fourth turn from within outwards. Beside each thermometer were placed spores of bacillus anthracis, of earth bacillus, and micrococcus prodigiosus, a non-spore-bearing organism very readily killed. The whole was placed in the disinfecting oven. The experiment began at 2 o'clock p.m., and lasted for four hours. The temperature of the air in the interior of the oven was taken at different times and was as follows :

At 2	p.m.	227° F.
„ 2.20	„	284° F.
„ 3	„	293° F.
„ 4	„	298° F.
„ 4.30	„	298° F.
„ 5	„	302° F.
„ 5.30	„	298° F.
„ 6	„	298° F.

When taken out at 6 p.m., the following were the readings of the maximum thermometers :

In the middle of the roll	94° F.
4 turns from the middle	109° F.
8 „ „	126° F.
12 „ „	152° F.
16 „ „	165° F.
20 „ „	175° F.
28 „ „	212° F.

If the roll was moist the result was still less favourable. A similar roll, which had been moistened, was placed in the oven at the same time, and the thermometers in it stood as follows:

In the middle	..	..	..	..	114·5° F.
4 turns from the middle		..	..	..	129° F.
8	„	„	..	..	..	131° F.
12	„	„	..	..	..	142° F.
16	„	„	..	..	..	152·5° F.
20	„	„	..	..	..	159° F.
24	„	„	..	..	..	165° F.
28	„	„	..	..	..	166° F.

Of the organisms enclosed, micrococcus prodigiosus within the central 18 turns (it was not placed further out) was unaffected, and the bacillus spores which were placed outside the 24th turn also grew. Spores of these bacilli lying free in the oven were destroyed. It is thus evident that dry heat is useless as a method of disinfecting bedding and masses of clothing.

It was also found that there was the same difficulty with steam, even though it were much superheated. The temperature in the central parts of large masses of cloth was very much below that of the steam outside, and much too low to be effectual as a disinfecting agent. If, however, the steam, instead of being shut up in a closed vessel, was allowed to flow through the vessel, there being thus a constant current of steam, at 212° F., the result was very different. In a comparatively short time even large masses were thoroughly heated throughout, and the steam at this temperature acted like boiling water, and completely destroyed the spores in the interior of the masses. Further, the steam injured the various woollen and other fabrics much less than the hot air, and it is evident that where heat is to be employed as a disinfecting agent, it must be employed in the form of a current of steam at 212° F. constantly passing over the material for about three hours.

In connection with this subject it must also be mentioned, that recent experiments have shown that it is possible to

diminish the virulence of certain of these pathogenic micro-organisms, and when this is done it is found that in some cases the inoculation of the attenuated virus protects the animal against the effects of the virulent form. Pasteur found, with regard to the organism of fowl cholera, that if it is cultivated in a thin layer of fluid for some months it loses its virulence, and may be inoculated into fowls without causing death, these fowls being now protected against attacks from the virulent organism. Toussaint found that by heating blood containing bacillus anthracis to 134·6° F., and adding carbolic acid, the organism diminished in virulence, and its inoculation protected animals more or less from the virulent form. Chauveau found that by heating for 15 minutes at 125·6° F., or for 20 minutes at 122° F., a sufficient attenuation was obtained. Pasteur cultivated these bacilli at 107·6° F., and thus gradually diminished the virulence of the organism. Koch has worked out the degrees of attenuation which are most suitable for the purpose of affording protection. It has also been found by Pasteur that a virus may be attenuated not only by cultivation in flasks, but by inoculating animals belonging to different species. In certain cases, the blood of these animals, when inoculated into animals of the species in which the disease naturally occurs, causes a mild form of the disease and protects the animal from the more virulent attack.

From this short sketch the great importance of the work done in a laboratory of this kind will be evident, and it is remarkable that in this country there is no public laboratory devoted to these researches. The functions of the bacteriological laboratory in connection with hygiene may be summarised as follows :

1. The investigation of the causes of infective diseases in man and animals, the cultivation of the micro-organisms causing them where they are due to micro-organisms, and the study of the life history of these organisms. In connection with this part of the subject we have the various methods of staining and cultivating organisms, and also of

photographing them. It is also of importance that other organisms, not specially connected with disease but nevertheless of great importance in nature, such as those associated with fermentations and food, should be studied. In this connection also the parasitic diseases of plants deserve special notice.

2. The investigation of air, water and soil, for the presence of micro-organisms, also the determination of the kinds found, and their relations to disease.

3. The discovery of the different methods of destroying these organisms, or of making them useful instead of hurtful. Here we have to do with experiments on disinfectants, and also with the valuable experiments on the attenuation of virus, and the conversion of hurtful organisms into useful vaccine materials.

CATALOGUE OF EXHIBITS

IN THE

BIOLOGICAL LABORATORY.

Microscopes and microscopical apparatus exhibited by Messrs. C. Baker, 244 High Holborn, W.C.

R. & J. Beck, 68 Cornhill, E.C.

C. Coppock, 100 New Bond Street, W.

Powell and Lealand, 170 Euston Road.

J. Swift & Son, 81 Tottenham Court Road.

Carl Zeiss, Jena.

All these makers kindly allow their instruments to be shown in use.

Staining materials exhibited by Messrs. R. & J. Beck and Dr. G. Grübler, 17 Dufour Strasse, Leipzig.

Microtomes by Messrs. Swift & Son, R. & J. Beck, and A. Frazer, 7 Lothian Road, Edinburgh.

Apparatus for bacteriological research, by Dr. Hermann Rohrbeck, 100 Friedrich Strasse.

Diagrams of parasitic diseases of plants, by Worthington Smith, Esq.

Most of the bacteria, as well as the maps of infective diseases and the vaccination statistics have been obtained from Dr. Koch's laboratory in Berlin.

The glass apparatus used in the laboratory is supplied by Mr. E. Cetti, 36 Brooke Street, Holborn, E.C.

The staining reagents used in the laboratory are supplied by Dr. Georg Grübler, 17 Dufour Strasse, Leipzig. Agent in England, Mr. C. Baker, High Holborn, W.C.

Demonstrations are given every Thursday at 4 P.M.

1. *Apparatus used in the Cultivation of Bacteria.*

Flasks of various kinds, test tubes, glass slides, glass dishes, platinum needles, glass cells, microscopic slides.

Two forms of hot stage, exhibited by T. P. Hawksley, Oxford Street.

The flasks and test-tubes are plugged with cotton-wool, placed in a hot air chamber at the temperature of 300° Fah. for three hours. In this way all micro-organisms in their interior and in the cotton-wool are destroyed and a sterilised cultivating material may be kept in them without risk of contamination.

Glass slides, &c., are placed in a beaker plugged with cotton wool and subjected to the above temperature for three hours.

Dishes, &c., may be disinfected by washing in a 1 per 1000 watery solution of bichloride of mercury. This may then be got rid of if necessary by rinsing in boiled water or washing with alcohol.

Platinum needles are simply heated to redness in the gas flame.

2. *Cultivating Materials.*

(*a*) Boiled potatoes. Old potatoes are thoroughly washed with water and then with the bichloride of mercury solution (1 per 1000), and steamed for half-an-hour. They are cut with a large previously-heated knife (the hand in which they are held being previously dipped in the bichloride of mercury solution), and placed in a glass dish with cover purified as above described. A piece of moist filter paper is placed in the glass dish to prevent drying of the potato.

(*b*) Meat infusion. One pound of meat is chopped up and infused with 37 ounces of water for two or three hours, or is placed in the water in an ice safe for 24 hours. In the latter case the meat is pressed after 24 hours to get rid of all the fluid. The fluid obtained in either of these ways is then boiled and filtered. If desirable it may be neutralized, or peptone or other ingredients may be added to it before filtration. The clear fluid is then introduced by siphon into a sterilised flask, steamed for fifteen to twenty minutes on two or three successive days, and set aside for use.

(*c*) Gelatinised meat infusion.

Constituents :—

Lean meat, 1 lb.

Gelatine (5 to 10 p. c.) 1½ to 3 ounces.

Peptone (1 to 3 p. c.) 2½ to 7½ drachms.

Common salt (·1 p. c.) 15 grains.

Water about 37 ounces.

(Instead of gelatine, Japanese isinglass (1 to 2 p. c.) may be used).

A meat infusion is obtained as described in (*b*), only half the quantity of water, however (16½ ounces), being used.

The gelatine is soaked in the other half of the water until it is thoroughly saturated; it is then added, with the water which is not absorbed, to the extract of meat. The whole is now boiled for some minutes to complete the solution. The peptone and salt are then added and dissolved. The mixture, which is acid, is neutralised by the addition of carbonate of soda or neutral phosphate of potash.

The solution, now very turbid, may be rendered clearer by beating up with it the whites and shells of two or three eggs and then boiling briskly. The egg albumen, coagulated by the heat, rises to the surface and carries with it the solid particles.

A perfectly limpid solution is now obtained by filtering the fluid in a water-bath.

The material is then introduced into the sterilised test-tubes or flasks, and steamed on three successive days for a quarter to half-an-hour on each occasion. When it cools we have a perfectly clear cultivating material, solid and remaining solid below 80° F.

(*d*) Milk. The milk (skimmed milk is best) is introduced by siphon into sterilised flasks and steamed for fifteen to thirty minutes on three successive days.

(*e*) Bread. One part of bread and two parts of water introduced into a sterilised flask and steamed for fifteen to thirty minutes on three successive days.

(*f*) Solidified blood serum.

Serum free from blood corpuscles is collected, introduced into sterilised tubes, and kept in a water-bath at 58° C. (136·4 F.) for an hour on six successive days. The tubes are then laid obliquely in a water-bath, and the temperature kept at 65° C. (149° F.) till they solidify.

The necessary apparatus is exhibited.

3. *Cultivations of Micro-organisms.*

These are growing in the various materials mentioned above. The potatoes are inoculated by dipping the heated platinum needle into a pure cultivation of the micro-organisms and stroking it over the potato. The tubes are inoculated by dipping the

heated needle into a pure cultivation and pushing it into the fresh gelatinised material, the tube being held obliquely to prevent dust falling in. The serum is inoculated by rubbing the needle carrying the bacteria over the surface.

In looking at the cultivations in gelatine, observe the production of colour, liquefaction of the gelatine, the mode of growth along the needle track and the growth on the surface.

(*a*) Pigment producing organisms. None of these are hurtful to animals.

Torula producing a black colour, cells oval, black colour only formed in contact with air, forms black colour on potato ; obtained from air.

Torula producing pink colour, cells almost round, red colour only formed in contact with air, grows on potatoes ; obtained from air.

Micrococcus Indicus.—A large micrococcus, producing scarlet colour, grows on potatoes and the gelatinised material, liquefies the gelatine. Obtained by Dr. Koch in Egypt from the air.

Micrococcus Prodigiosus.—A large micrococcus producing blood red colour, size $\frac{1}{2}$ to 1 μ in diameter. Grows on potatoes, bread, the gelatinised material, &c., liquefies the gelatine. Very common in the air in certain localities.

Bacillus producing a violet colour, liquefies gelatine, violet colour formed in contact with air ; obtained from water.

Bacillus causing fluorescence of the material in which it grows, does not liquefy gelatine.

Bacillus of green pus produces green colour and liquefies gelatine, also causes fluorescence. Obtained from wounds, and there causes the green colour sometimes seen in the discharges.

Bacillus of blue pus produces blue colour in contact with air, and liquefies the gelatine. Obtained from wounds, where it makes the discharge of a blue colour.

Sarcina producing yellow colour, growing in the gelatinised meat infusion.

Closely allied to the above, but not producing colour are

Sarcina Ventriculi found in the vomit in many cases of cancer of the stomach. Grows in whitish colonies.

(*b*) Organisms which are found in milk.

Torula cerevisiæ, the cause of the alcoholic fermentation.

Bacillus of blue milk.—A bacillus (size 2·5 to 3·5 μ in length) which is occasionally found in milk and produces a blue colour. The bacillus grows on potatoes and causes a dark blue colour

In gelatine the colour is greenish blue and the gelatine remains solid, the growth spreads out from the needle track forming a tree-like growth.

Milk inoculated with the above bacillus showing blue colour.

Bacterium lactis.—A minute bacillus (1·5 to 3 μ in length) the cause of the lactic fermentation of milk. In the gelatinised medium forms a delicate whitish growth along the needle track, grows slightly on the surface.

Milk sterilised and inoculated with the bacterium lactis showing the pure lactic fermentation.

Bacillus of butyric fermentation.—Size, 3 to 10 μ in length, below 1 μ in breadth. Grows in the gelatinised material, liquefies it and forms a scum on the surface. Produces the butyric fermentation.

Milk sterilised and inoculated with the butyric bacillus showing the pure butyric fermentation.

Micrococcus frequently found in milk. Grows in gelatine in form of delicate colonies, the gelatine remains solid. Produces no apparent change in milk.

Milk inoculated with the above micrococcus apparently unchanged.

Oidium lactis, a fungus found often in milk. Growing in bread infusion.

Milk inoculated with oidium lactis apparently unchanged.

(*c*) Organisms associated with diseases in man.

Bacillus of tubercle.—Found in all tubercular affections in man and animals; it may be cultivated on the coagulated blood serum at the temperature of the human body; it grows slowly and forms whitish irregular crusts on the surface. The specimen shown is the 21st cultivation from the lung of a patient who had died of phthisis

Bacillus of glanders.—Found in all cases of glanders; may be cultivated on blood serum or potatoes kept at the temperature of the body; on blood serum forms small round moist semi-transparent colonies. It grows very slowly on the gelatinised material at the ordinary temperature, forming a whitish mass.

Micrococcus of acute osteomyelitis.—Always found in pus from acute osteomyelitis; forms orange yellow colonies on potatoes, liquefies gelatine, and forms orange yellow deposit; produces acute osteomyelitis in rabbits when injected into the veins if bones have previously sustained any injury.

Bacillus of enteric fever.—Always found in typhoid ulcers,

mesenteric glands, frequently in spleen and liver as plugs in blood-vessels, grows slowly in the gelatinised material, forming somewhat brownish almost homogeneous growth along the track of the needle. Grows slightly on surface.

Micrococcus of pneumonia.—Found in most cases of acute lobar pneumonia, grows rapidly in the gelatinised material, forming whitish growth along the track of the needle, and a rounded mass on the surface, the whole resembling a nail.

Micrococcus of erysipelas.—Present in all cases of erysipelas in man in lymphatic vessels at spreading margin of redness; may be cultivated on gelatinised meat infusion, potatoes or blood serum; grows slowly in gelatine, forming delicate colonies along the track of the needle.

Bacillus Anthracis.—Size, 5 to 20 μ in length; 1 to 1·25 m. in breadth; may be cultivated on a variety of substances, grows in the gelatine in the form of a loose network, and soon liquefies it.

Attenuated bacilli of Anthrax.—By growing these bacilli between 42° and 43° C. (107·6° to 109·4° F.) they gradually lose their virulence, till by and by they will not kill any animal. When partially attenuated they may act like vaccine in not only not killing the animal into which they are inoculated but in protecting it from the virulent disease. The specimen exhibited will not kill any animal.

Also microscopical specimens of the bacillus of leprosy, the spirilla of relapsing fever and the *cholera* bacillus.

(*d*) Organisms fatal to lower animals but not affecting man.

Bacillus of mouse septicæmia.—Very small, ·8 to 1 μ in length; frequently present in decomposing fluids, grows in the gelatinised material, forming a delicate haziness around the needle track.

Bacterium of rabbit septicæmia.—A small oval organism (1·4 μ in length, ·7 μ in breadth) growing in the gelatinised material as a delicate brownish growth along the needle track. Probably the same as Davaine's septicæmia; very fatal to rabbits when inoculated, death occurring within 24 hours.

Fowl Cholera.—Small bacteria closely resembling in appearance and mode of growth the rabbit scepticæmia, kills fowls in 17 to 20 hours.

Micrococcus tetragenus.—A micrococcus with the cocci arranged in groups of 4; frequently found in phthisical sputum; when unstained it closely resembles sarcina; grows in the gelatinised material, forming large flattened milk-white colonies along the

needle track, and on the surface gives rise to an irregular plate. When inoculated into guinea pigs and mice, the animals die in 2 to 10 days, the organisms being present in large numbers in the blood.

Also microscopical specimens of the bacillus of malignant œdema in guinea pigs (*vibrion septique, Pasteur*), and the bacillus of foul brood in bees.

(*e*) Fungi.

Tinea or Favus Galli.—Forms crusts on the comb and wattle of fowls which may spread over the breast and back, belongs apparently to the class of torula; grows on the gelatinised material as a thin whitish growth; pure cultivations mixed with vaseline or glycerine, and rubbed on the combs of healthy fowls produce the disease.

Aspergillus flavescens.

Aspergillus fumigatus.—Growing on bread infusion. Both these organisms, when injected in sufficient quantity into the veins of rabbits, cause the death of the animals by growing in the capillary blood-vessels.

Aspergillus niger.

Aspergillus albus.—Also growing on bread infusion. Neither of these can live in the animal body.

Mucor, described by Lichtheim, kills rabbits when injected into the veins.

Mucor not pathogenic.

4. *Staining Materials and Methods.*

Bacteria are most satisfactorily examined after being stained. In the case of fluids a drop is placed between two cover-glasses, the glasses are squeezed together so as to get a thin layer, and then they are slipped apart and set up to dry. When dry they are heated to make the layer adhere to the glass, either by passing the cover-glass thrice through the gas flame, or by keeping them at from 100° to 120° C. for an hour.

Ehrlich's method is to place a lamp under one end of a brass plate and to allow the plate to stand till it has got thoroughly warm; then ascertain the part of the plate where water boils, place a cover-glass at that place, and one a little nearer to the flame and leave them an hour. They are then stained by floating them on the surface of the methylene blue solution mentioned in the text or in a methyl violet or other solution.

The methyl violet or fuchsin solution is made by adding a saturated alcoholic solution to distilled water till a sufficiently deep colour is obtained. The cover-glasses are floated on these solutions for about ten minutes, then washed in water and afterwards in a ½ to 1 p. c. solution of acetic acid, dried and mounted in Canada balsam. They may also be stained brown for photography in a saturated watery solution of vesuvin.

For tubercle bacilli a different solution is employed. Add to 100 parts of a saturated watery solution of aniline, 11 parts of a saturated alcoholic solution of fuchsin, filter and use as above. At the ordinary temperature the material must stain for 12 to 24 hours, at the body temperature for 2 to 3 hours, at a temperature near the boiling point for a few minutes. Afterwards immerse for a few seconds in diluted nitric acid (1 part of strong nitric acid to 2 parts of water). Wash in water and stain in a solution of methylene blue (100 parts of water, 20 parts of saturated alcoholic solution of methylene blue) for about an hour, wash in water, dry, and mount in Canada balsam. The tubercle bacilli remain red, all other bacteria (except leprosy bacilli) and the nuclei of the cells become blue.

For bacteria in tissues harden in alcohol for two or three weeks, then take a small piece, place in water for two or three hours, then in a strong solution of gum, freeze and make sections with microtome. Stain in the alkaline methylene blue solution or in solutions of the other stains, wash in water, dilute acetic acid, alcohol, oil of cloves or cedar, and mount in Canada balsam. For tubercle bacilli use the stain mentioned above, afterwards wash in water, alcohol, oil of bergamot or cloves, and mount in Canada balsam.

Gram's method of staining bacteria is very simple and beautiful. Take 100 parts of saturated watery solution of aniline, add 11 parts of saturated alcoholic solution of gentian violet. After cutting the sections place them in absolute alcohol, then in the above solution for two or three minutes (tubercle for some hours), then immerse in solution of iodine and iodide of potassium (1 part of iodine, 2 parts of iodide of potassium, 300 parts of water) till they are decolorized (a few minutes as a rule), then place in absolute alcohol, for a second or two in a saturated watery solution of vesuvin or Bismark brown, again in absolute alcohol, oil of cloves, and mount in Canada balsam. The bacteria appear dark blue, the tissue brown. Successful staining is only a matter of experience.

5. *Demonstration of Bacteria.*

For this good microscopes with condensers are required. More important even than powerful lenses is correct illumination of the specimen.

Various bacteria are shown under the microscope on Thursday afternoons.

A *microphotographic apparatus* is also exhibited.

Also a number of *microphotographs* taken by Dr. Koch of erysipelas, anthrax, relapsing fever, mouse septicæmia, rabbit septicæmia, pyæmia in rabbits, ulcerative endocarditis, acute osteomyelitis, &c.

6. *Examination of Air, Water and Soil for Bacteria.*

Various experiments are shown. The methods are referred to in the text.

The glass plates for the water cultivations are sterilised in an iron box shown. They are laid in glass dishes prepared as above described for potatoes, and the apparatus placed on a level plate of glass on a levelling stand. The plates of glass may be marked out in squares to facilitate the numeration of the bacteria. The cultivation is left for three or four days to develop and is then taken out, placed on a black ground, and the number of colonies of bacteria counted and their kinds ascertained under a low power of the microscope.

The tubes used in Hesse's air experiments are sterilised in the steaming apparatus after being filled with the gelatinised material. Apparatus for growing bacteria in various gases is also shown.

Also Pasteur's experiment to disprove spontaneous generation.

7. *Method of testing the Power of Disinfecting Agents in destroying Bacteria.*

Apparatus and experiments are shown.

The power of killing (1) spores and (2) mature actively growing organisms must be tested.

The organisms generally used are spores of anthrax bacilli, and for non-spore-bearing organisms, micrococcus prodigiosus.

Sterilised cotton threads are soaked in the cultivations of the organism to be tested, and are then rapidly dried in a desiccating chamber. When spores are used the threads may be kept for

weeks or months in a dry state, without the vitality of the spores being impaired. In the case of non-spore-bearing organisms, the threads must be used within two or three days after drying. The prepared threads are placed for varying periods of time in the solution to be tested or subjected to the temperature, &c. They are then removed, and in the case of immersion in chemical solutions, washed in boiled distilled water, to get rid of the antiseptic, and planted on the solid gelatinised material spread out on a glass plate and kept protected from dust. The occurrence of growth is then observed. In the case of the pigment-producing organisms, the production of the proper colour shows that the organisms have not been killed. In the case of anthrax, the method of growth is typical and easily recognised. If there is any doubt a mouse may be inoculated with the cultivation.

8. *Parasitic Diseases of Plants.*

A large number of *diagrams* are exhibited by Worthington Smith, Esq., illustrating diseases of potatoes, clover, turnips, corn, &c.

C. B. Plowright, Esq., exhibits various dried specimens of ergot, canker of apple-trees, diseases of corn, &c.

Also two plants showing the effect of parasitic fungi.

1. A plant of barberry which last year had no æcidium upon it, was on the 22nd of May last infected with germinating spores of Puccinia graminis on wheat straw. On May 30th spermogonia first began to indicate their appearance by the production of yellow spots. Three days later they became well developed, and have now (July) been succeeded by the æcidium berberidis. The straw with the Puccinia upon it used in this culture is tied up in a little bundle and placed in the same pot.

2. A well grown plant of poa trivialis infected on the 9th of May with æcidiospores from ocidium on ranunculus repens. On May 20th the infected leaves began to show sickly yellow spots. On May 22nd the perfect uredospores were developed. On June 3rd abundant development of the uredo with some teleutospores of uromyces poæ beginning to develop.

9. *Maps showing the Death-rate of Children in Germany.*

Tables showing the relative prevalence of infective diseases in various towns. These are not a complete series, but have been

lent by Dr. Struck, of the Kaiserlich. Gesundheits Amt in Berlin, to show the method of registration employed.

Dr. Struck also lends tables showing the effect of the introduction of compulsory vaccination in Germany on smallpox. There was no compulsory vaccination in Germany except in the army till 1874. The German law now compels vaccination in childhood and revaccination at 12 years of age. A third vaccination is compulsory in the army.

PUBLIC HEALTH LABORATORY WORK.

PART II.—HYGIENIC LABORATORY.

BY

W. H. CORFIELD, M.A., M.D., F.R.C.P.,

AND

CHARLES E. CASSAL, F.I.C., F.C.S.

PUBLIC HEALTH

LABORATORY WORK.

II.

THE work of a hygienic laboratory chiefly consists in the chemical and microscopical examination, and in the general study, of all those natural and artificial substances which, on account of the uses made of them, have some bearing on the Public Health.

Such work therefore includes the examination of drinking waters, of air and of soils ; of foods and drinks of every description ; of the various substances used in the construction and decoration of houses, such as wall-papers and paints ; and of the materials used for clothing, especially as regards the dyes which are applied to them. The comparison and valuation for sanitary purposes of filtering materials and disinfectants, and the examination of drugs and patent medicines also form part of the ordinary work of a model hygienic laboratory.

New methods of chemical analysis are constantly being devised, and the value of these has necessarily to be ascertained, and while there is a wide field for original work in the invention of new processes, the hygienist has also great opportunities for research in the study of the causes of pollution of water and air, the nature and degree of such pollution under different circumstances, and with different polluting agents, and the extent to which the methods at his disposal will enable him to detect and estimate these pollutions and the adulterations and impurities existing in the substances used as food.

The passing of the Public Health Act and of the Sale of Food and Drugs Acts has been attended by a very large decrease of adulteration and has greatly diminished the sale of inferior food, and of substances unfit for food, more especially in the metropolis and in the larger provincial towns. The work of the public analysts appointed under the provisions of the "Sale of Food and Drugs Act," is to a great extent hygienic, inasmuch as the samples submitted to them have to be examined not only with the view of determining whether they are of the "nature and quality demanded," but of ascertaining the presence or absence of substances injurious to health.

Many local authorities, however, have unfortunately not made full use of the powers possessed by them under these Acts, and on the other hand it has very frequently been extremely difficult to obtain a satisfactory punishment for a proved offence, even in the Metropolis. It is obviously very desirable that in all cases where an adulteration which actually is, or which may be under certain circumstances, dangerous to health, has been proved to exist in any article, a very severe punishment should be inflicted; for example, in the case of milk, an article on which infants and young children so largely depend for their nourishment.

It is not possible in a work of this kind to enter into a full account of the details of laboratory work, but a general idea can be given, and with this object it will be convenient to describe briefly some of the processes and apparatus made use of in hygienic investigations.

AIR.

The chief constituents of atmospheric air are:—oxygen, nitrogen, and carbonic acid; the first two in large quantity, the last in very small quantity. In 10,000 parts by volume of air, there are:

7,900 Nitrogen.
2,096 Oxygen.
4 Carbonic Acid.

The amount of carbonic acid varies slightly in the pure air of places differently situated.

A number of substances are continually passing into the atmosphere,—gases, vapours and solid material particles. In all close and ill-ventilated places the air has become more or less charged with the products of combustion and of respiration—carbonic acid, water vapour, and foul "organic" matter—and it becomes therefore necessary to estimate the extent to which this pollution has taken place. The quantity of carbonic acid present may be taken as the measure of the degree of pollution of air. It has been shown that the diminution of oxygen and the increase of carbonic acid in the air of inhabited places are so slight as to be of very little importance in themselves, and that the dangerous pollution of such atmospheres is due to the presence of foul organic matter. Nevertheless, as the increase of carbonic acid is proportional to the degree of such foul organic pollution, and as the amount of carbonic acid is easily and very accurately determined by the process about to be described, the quantity present is taken as a measure of the degree of pollution.

Estimation of Carbonic Acid in Air.

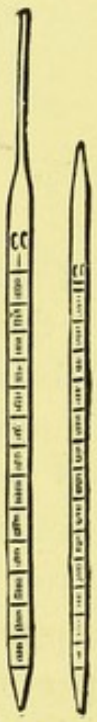
Pettenkofer's Process:—In this process, advantage is taken of the fact that carbonic acid unites with lime to form carbonate of lime, which is insoluble in water. Lime water is prepared by pouring pure distilled water over pure lime, and pouring off the clear solution from the sediment; and a rough method of estimating the quantity of carbonic acid is to place ½ oz. of this clear lime water into a 10½ oz. stoppered bottle containing the air to be tested, shaking it up and leaving it to stand. If the lime-water becomes turbid (from the formation of carbonate of lime), the air contains more than 6 parts of carbonic acid per 10,000 parts of air by volume. It has been shown that if the carbonic acid of an enclosed space exceeds that of the outer air by more than 2 parts per 10,000, the ventilation

of that space is insufficient, the fouling matter in the air being then in sufficient quantity to render the air perceptibly impure to the senses.

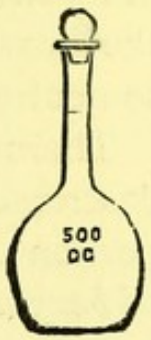
MEASURING GLASS.

The collection of a known volume of air for subsequent examination is carried out in various ways. The volume of a good-sized bottle of from two to six litres capacity and provided with a well-fitting stopper, may be taken by carefully filling it with mercury, and then measuring the volume of the mercury by pouring it into a *glass measure;* and the air of any given place may be collected by filling the bottle with distilled water or mercury, and emptying it in the place in question, or by pumping the air into the bottle with a pair of bellows, or by previously pumping the air out of the bottle and then allowing the air to be tested to enter it.

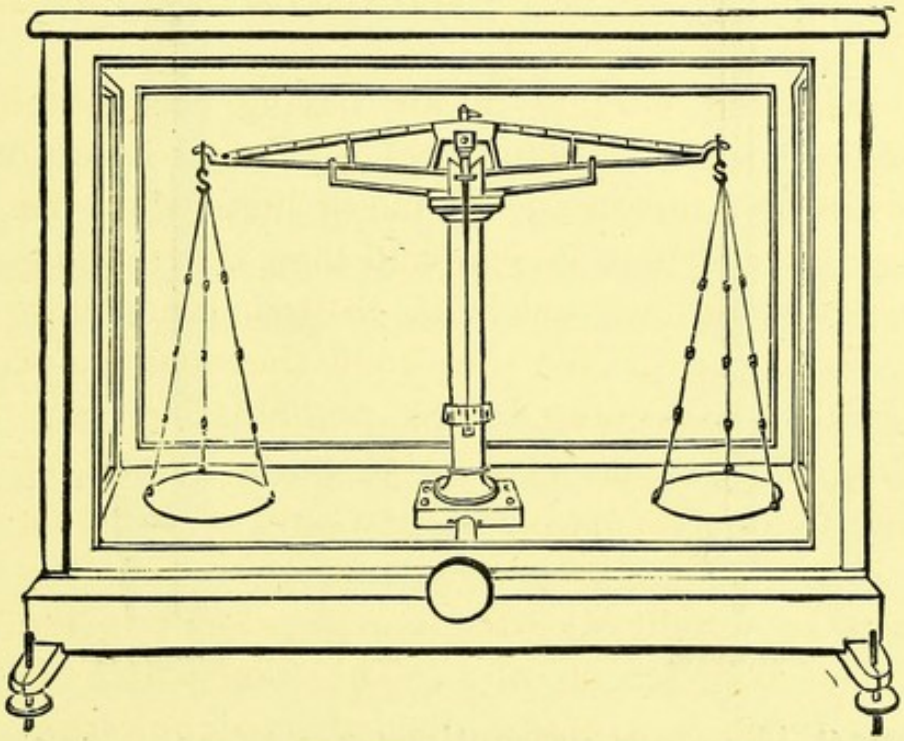
A measured volume of air having been obtained, an estimation of the carbonic acid is effected by absorbing it by means of a measured volume of lime water, the strength of which is known, and then determining the quantity of lime which has *not* combined with the carbonic acid. We thus know the quantity of lime which has entered into combination, and knowing further that exactly 56 parts by weight of lime unite with 44 parts by weight of carbonic acid, we arrive by a proportion sum at the number of parts by weight of carbonic acid present in the measured volume of air. The lime-water is measured out in a *pipette*, divided into cubic centimetres and tenths of a cubic centimetre. Its strength is determined by means of a "standard" solution of *Oxalic Acid,* which acid unites with lime to form oxalate of lime. A certain number of grammes of oxalic acid are exactly weighed out on a *Chemical Balance* dissolved in pure water to a definite volume, say 1 *litre* (1000 cubic centimetres) in a *graduated flask,* thus giving a solution, every cubic centimetre of


PIPETTES.

which contains a known weight of oxalic acid. Supposing the weight of oxalic acid in each cubic centimetre of solution to be exactly capable of uniting with one *milligramme* of lime (this being the usual strength of oxalic acid employed), it is clear that the number of cubic centimetres of oxalic acid solution required to exactly combine with or "neutralize" the lime present in a definite volume of a sample of lime water will be equal to the number of milligrammes of lime present in that definite volume. For instance, if 10 cubic centimetres of lime-water required 12 cubic centimetres of the oxalic acid solution to combine with all the lime, then the 10 cubic centimetres of lime-water will contain 12 milligrammes of lime in solution. The measured volume of lime-



GRADUATED FLASK.

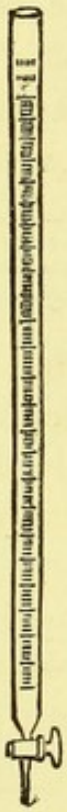


CHEMICAL BALANCE.

water is put into a convenient vessel and the oxalic acid solution added from a cubic centimetre *Burette* until all the lime is combined, a fact which is ascertained by employing an "indicator," such as the change of colour of *blue litmus paper* when brought into contact with an acid. So soon as the oxalic acid has combined with all the lime, the liquid in the test vessel turns the blue paper red. Instead of

litmus paper, litmus solution may be used in the above process; or again, paper coloured yellow with an infusion of *turmeric*, which is turned brown by alkalies such as lime, the brown colour disappearing when there is enough acid to neutralize the lime.

There are several modifications of the processes above described, by which with more or less accuracy the proportion of carbonic acid in air can be determined. The use of *baryta* water instead of lime-water is more convenient



BURETTE.

BURETTE.

and exact if proper precautions are taken. In accurate work of this kind, it is necessary to determine the temperature of the air examined and the barometric pressure at the time, in order to avoid the obvious errors due to change of volume produced by alteration of pressure and temperature.

Wanklyn's Process.—Good results may be obtained by making use of this process, which is thus carried out :—

From 2 to 3 litres of air (2000 to 3000 cubic centimetres)

are shaken up with a measured volume, say 100 cubic centimetres, of baryta water, which is rendered more or less turbid by the formation of carbonate of baryta. The solution is poured out into a cylinder made of thin glass, and the *degree of turbidity*, which is evidently proportional to the amount of carbonic acid present, is imitated in another precisely similar thin glass cylinder by mixing with another 100 cubic centimetres of the baryta water, measured volumes of a standard solution of carbonate of soda, which forms a precipitate or turbidity more or less pronounced according to the amount of carbonate added. The standard solution is measured from a burette and is made of such a strength that 1 cubic centimetre contains 1·97 milligrammes of carbonic acid (in combination with soda) which is equivalent to 1 cubic centimetre of carbonic acid.

Organic Matter.

Air always contains some organic matter, derived from animal or vegetable sources, or both. The precise nature of the organic substances is not accurately made out, but there is no doubt that those which are hurtful are chiefly nitrogenous.

Air loaded with organic matter possesses a peculiarly unpleasant odour, particularly evident in close, over-crowded rooms, and in narrow streets and courts. Prof. de Chaumont has shown that it is possible to graduate, by means of the sense of smell, the pollution of air by organic matter, with a close approach to the truth as indicated by the estimation of carbonic acid and by other chemical processes to be immediately described.

Attempts have been made to estimate the extent of organic pollution by means of permanganate of potassium, a salt which contains a large quantity of "available" oxygen, and which readily gives up some of its oxygen when placed in contact with organic matter, burning up the latter to a greater or less extent according to the nature of the organic substances present. The permanganate

dissolves in water, forming a deep purple solution. The process depends upon the extent to which a measured volume of the air will destroy the pink colour of a weak standard permanganate solution delivered from a burette. There may exist, however, in polluted air, other impurities which also decompose the permanganate, and the process is now only used as a qualitative test.

A common method for dealing with the organic matter in air consists in polluting a certain measured quantity of absolutely pure distilled water with a known volume of the air ; and then subjecting the polluted water to analysis. The water may be most conveniently examined by the *ammonia process*, shortly to be described under the head of Water. Ammonia, viz., the gaseous compound of nitrogen and hydrogen, is very easily produced by the decomposition of organic matter containing nitrogen, and may thus be made an approximate measure of nitrogenous organic matter. In order that the ammonia process may be successful in the case of air, it is necessary that a large quantity of air be washed in as small a quantity of water as possible. This may be accomplished in different ways. By means of an *aspirator*, or vessel of known capacity filled with water, which can be run out by means of a stop-cock at the bottom, air is drawn through a flask or series of flasks containing pure re-distilled water free from ammonia, the volume of air which has passed through being obviously equal to the volume of water which has run from the tap of the aspirator. Or the air may be washed by injecting it, by means of a caoutchouc ball of known capacity, into a cylindrical vessel containing a little pure water with a spray-producing apparatus, and thus washing the air by means of a water spray.

Microscopic Examination.

The solid particles suspended in the air vary of course with the locality and with other circumstances. Mineral particles, salt, soot, fungi, starch granules, pollen, and vegetable spores may be detected in air. In the air of hospital

wards, pus globules, epithelium, and various forms of bacteria have been detected. The extent to which ordinary air is loaded with solid particles may be well observed by passing a beam of sunlight or of the electric light through a darkened room.

The collection of air-dust for microscopic examination is easily effected by drawing the air through a tube plugged with purified cotton wool or glass wool, by means of an aspirator. The wool acts as an efficient filter and the dust collected upon it may be examined.

Or a glass tube has one end connected with an aspirator and is provided with a small funnel at the other passing through a cork, and terminating inside the tube in a fine point; opposite this point a thin glass disc moistened with glycerine is placed. The air being drawn in at the funnel strikes against the glass disc, which accordingly becomes coated with some of the suspended matters of the air, and may then be examined microscopically. Other plans are as follows:—

1. The air is drawn by means of an aspirator through a glass tube cooled by a freezing mixture. The moisture of the air is condensed and arrests some of the solid particles which thus remain inside the tube.

2. The air is filtered through pure *gun cotton*, the latter is then dissolved in alcohol and ether, and the dust left behind is examined. (Pasteur.)

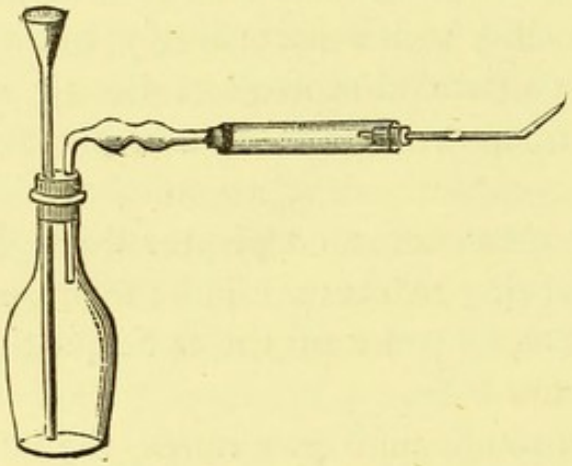
3. Fine glass threads moistened with glycerine, arranged in the tube connected with the aspirator, are sometimes used as traps to catch the suspended matters.

4. The "Montsouris" plan is also a good one. It is essentially the same as one previously described. The air passing through a small tube is made to impinge on a glass disc covered with glycerine and protected by a bell-jar.

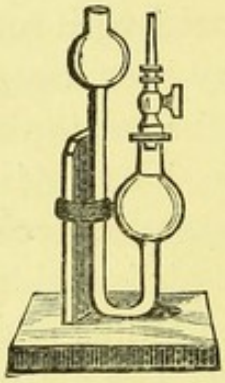
The dust obtained by any of these devices is examined under a high microscopic power—a magnifying power of from 500 to 1000 diameters being generally used.

Metallic Poisons.

Both in the gaseous form and in the form of dust some metals and their compounds are occasionally found in the air. Copper and lead have been detected in the air surrounding smelting works, arsenic in the form of arseniuretted hydrogen, of Scheele's green (arsenite of copper) and of other compounds has been found in the air of rooms papered with arsenical papers. The wall-papers of suspected rooms and the dust deposited in them have therefore frequently to be examined. If a very large quantity of air containing arsenic be drawn through a tube heated to redness by a gas flame, a "metallic mirror," or ring of metallic arsenic will be formed in the tube, which is



MARSH'S APPARATUS.



MARSH'S APPARATUS.
(*Another form.*)

recognizable by its peculiar crystalline structure and by other tests. Wall-papers may be most satisfactorily examined by *Marsh's test.* The suspected paper is treated with warm hydrochloric acid, and the solution thus obtained is introduced into an apparatus evolving hydrogen, from pure zinc and pure sulphuric or hydrochloric acid, and provided with an exit tube terminating in a fine jet. The arsenic unites with the hydrogen forming arseniuretted hydrogen, which may be lighted at the jet, and burns with a peculiar bluish flame, the flame depositing on a cold porcelain plate exposed to it, a "metallic mirror" of arsenic.

Gaseous Impurities.

In addition to those already referred to there are several other gaseous impurities which may under some circumstances be present in the air. Those which are evolved from various manufacturing operations are as a rule very easy of detection.

The principal ones are:—

Carbonic oxide, carburetted hydrogen or marsh gas, sulphurous acid, sulphuric acid, sulphuretted hydrogen, ammonium sulphide, hydrochloric acid, chlorine, ammonia, nitric acid, and organic vapours (from sewage, bone-boiling, etc.)

The suspected, air containing one or more of these substances, having been collected as before described, special tests are applied to it with a view of recognizing the more common substances. Papers impregnated with

Blue litmus	reddened by acids,
Red litmus	blued by alkalies,
Turmeric	browned by alkalies,
Acetate of lead . . .	blackened by sulphuretted hydrogen,
Iodide of potassium and starch	blued by chlorine,

are slightly moistened and exposed to the air, either in the bottle, or are exposed under a glass shade to a stream of the air, which is made to pass through it by means of an aspirator or otherwise. A proper investigation must then be made by washing the air with distilled water, and examining the watery solution obtained.

WATER.

The ordinary waters used for drinking hold in solution a large number of substances. The purest natural water is obviously rain-water collected in the open country—inasmuch as it has merely passed through the air, and has not percolated through various strata or through surface soil more or less contaminated, and thereby become charged

with some of the substances contained in the strata or soils. Taking ordinary pure spring water, we have to deal with a solution containing several salts, dissolved gases (chiefly those of the air), and a small quantity of organic matter. Carbonates, sulphates, nitrates, and chlorides of lime, magnesia, soda, and potash, and silica, are the principal mineral substances held in solution; minute quantities of the salts of iron and alumina are generally present, but the number and the proportion of the constituents is of course somewhat variable. Organic matter derived from animal or vegetable sources, or both, and salts of ammonia, may be present in drinking waters, and constitute the points of most hygienic importance.

Drinking water may be polluted in very various ways by organic matter, as from cesspools, and leaking drains in proximity to the well, vegetable filth allowed to accumulate, house refuse falling into the well, or finding its way into it by means of unsound drains, or from foul air coming up the waste-pipe into the cistern; poisonous metals, such as lead or zinc may also be present, and occasionally the water may be found polluted chiefly with gaseous substances.

The object of the analyst in the case of water is, firstly, to determine the presence or absence of impurities; secondly, the nature and quantity of such impurities, and, thirdly, to form an opinion as to the wholesomeness of the water on the data he has obtained. The most important point, and the chief difficulty, is to deal with the organic matter.

Organic matter, though at a given time harmless, may at any moment become extremely dangerous, and so long as a water is polluted to any appreciable extent with organic matter it should be condemned. The taste and the smell of a water are first noticed, and its appearance and colour when viewed in a two foot tube placed on a white slab, so that the observer can look through two feet of the water; or when looked at in a decanter or flask capable of holding about a quart. Pure water should have no sweet, or salt, or other decided taste, and should be odourless. It

should be clear and colourless, or should have only a very faint tinge of blue in a two-foot tube. In some cases, however, a water may possess a decided colour, such as the water of Loch Katrine, and yet be perfectly fit to drink —but nevertheless, yellowish and brownish, and greenish-yellow tints are always to be regarded with suspicion.

The Solid Matter.—The total amount of solid matter is measured by putting a known volume of the water into a *platinum dish* capable of containing the whole volume of water with ease, and which has previously been carefully cleaned, heated to redness over a Bunsen gas flame, and weighed. The platinum dish is placed on a *water bath* and the water is evaporated off; the dish is then weighed again, and the difference between the two weights is evidently the weight of solid matter contained in the measured volume of water. This is calculated into parts per 100,000, or into grains per gallon. If the number of grains per gallon of solid matter is to be known it is convenient to evaporate 70 c.c. of the water, inasmuch as 70 c.c. of water contain 70,000 milligrammes, and there are 70,000 grains in a gallon, so that in 70 c.c. the milligramme corresponds to the grain in the gallon; the number of milligrammes of solid matter found in 70 c.c. of water will therefore be equal to the number of grains of solid matter in a gallon of the same water. The estimation of the quantity of solid matter present in a water is a point of great importance, polluted waters as a rule yielding much higher quantities than pure waters. Further information as to the solids may be obtained by heating the solid residue over a lamp, gradually raising the dish to a red heat. Water residues containing even small quantities of organic matter will perceptibly alter in colour, and those which contain much will brown and blacken very markedly. The loss of weight on "incineration" was formerly used as an approximate indicator of the quantity of organic matter present in the water, but several other constituents are volatilised at a red heat, *e.g.*, carbonic acid from the carbonates and chloride of sodium, so that the test is not applicable in this respect.

At the same time, the determination of the volatile matter often affords very valuable information.

In certain cases it is necessary to make a complete analysis of the solid constituents of water; this is the case with mineral and medicinal waters. A large volume of the water must be evaporated to dryness for such purposes—as much as one or two litres—the residue dissolved in acid, and the various constituents, silica, lime, magnesia, potash, soda, etc., carefully and completely separated, and weighed in convenient forms.

Chlorine.—The chlorine in water is present chiefly as chloride of sodium, or common salt. Chloride of sodium usually accompanies animal matters. Urine contains large quantities of it, and waters polluted with sewage are loaded with it; hence it follows that the proportion of chlorine present in ordinary pure waters being known, an estimation of the chlorine in any given water is a matter of great importance. Should the source of the water be near the sea, or should it be water which has passed through salt-bearing strata, the chlorine determination loses in value in consequence of the excess of salt which such waters contain, and further it must be pointed out that water may be highly and dangerously polluted with organic filth of *vegetable* origin, and may contain a very small quantity of chlorine.

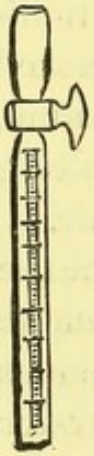
The estimation of chlorine in water is a very simple matter. Advantage is taken of the fact that silver combines with chlorine in the proportion of 108 parts by weight of silver to 35·5 parts by weight of chlorine to form the white insoluble chloride of silver. A standard solution of pure nitrate of silver is prepared by dissolving a weighed quantity of the salt in a measured volume of pure distilled water—generally 4·79 grammes of nitrate of silver are dissolved in 1,000 c.c. or one litre of water—furnishing a solution, every c.c. of which contains exactly the amount of silver necessary to unite with one milligramme of chlorine—$Ag\ NO_3 + Na\ Cl = Ag\ Cl + Na\ NO_3$. 50, 70, or 100 cubic centimetres of the water are placed in a clean

white porcelain dish or in a *beaker* standing on a white slab, and the nitrate of silver delivered from a *burette*, with constant stirring of the mixture. The exact point at which all the chlorine present has united with the silver, is observed by means of a solution of pure *chromate of potassium*, a few drops of this solution having been placed in the water. Silver nitrate reacts on chromate of potassium to form chromate of silver, a blood-red substance. This it does so soon as it has united with all the chlorine present, for which it has a greater "affinity" than for the chromium, and the appearance of the faintest red tinge is an indication that the process is ended. The number of cubic centimetres is read off on the burette and is equal to the number of milligrammes of chlorine present in the volume of water operated on. This is by some calculated into chloride of sodium, or common salt, 35·5 parts by weight of chlorine uniting with 23 parts by weight of sodium to form common salt.

Oxidised Nitrogen.—Taken along with other evidence the presence and quantity of the *nitrates* afford very valuable information concerning the pollution of water. Nitrogenous organic matters decay and become oxidised and the nitrates are yielded by the process, so that supposing that the nitrates in a water do not come in any quantity from other sources they form a measure of the extent of nitrogenous organic pollution. By itself, however, the estimation of oxidised nitrogen is not to be taken as a trustworthy guide, any more than is the estimation of any other *single* constituent, or single product yielded by the constituents, of a water. Certain pure waters contain considerable amounts of nitrates, and a water may be highly charged with organic matter and yet contain but small quantities of nitrates. The estimation of oxidised nitrogen will frequently enable the analyst to discover that a water though not actually polluted is in danger of pollution at no distant date. A well drains the soil in its neighbourhood; should a cesspool or other source of filth be situated in the drainage area, the organic matters will percolate through the soil towards the well, and in passing through the soil these matters are in great part

oxidised—nitrates being a product of the oxidation ; the nitrates find their way into the well, and if the process continues, a time will come when the soil, being saturated with filth, is no longer capable of any purifying action, and the water of the well becomes highly polluted.

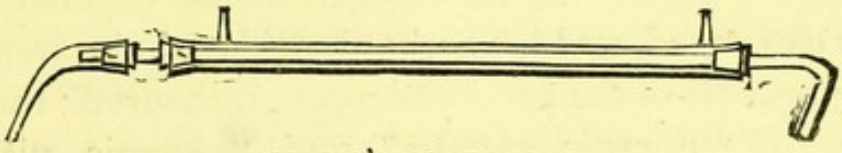
There are several methods for determining the quantity of nitrates. A measured volume of the water is treated with metallic *zinc* or *aluminium* and strong caustic potash, hydrogen is evolved and the nitrogen of the nitrate is thereby converted into ammonia. The mixture is distilled and the ammonia is determined in the manner immediately to be mentioned under saline and organic ammonia. Crum's method which is better, is essentially as follows :— A graduated tube open at both ends and provided with a stop-cock near to the top (see Figure) has the graduated part completely filled with mercury, the lower end dipping into mercury in a trough. A solution of the water-residue containing the nitrates followed by some sulphuric acid is introduced from the upper cup into the tube by means of the stop-cock ; on vigorous shaking nitric oxide is given off, and its volume measured by means of the graduations. Corrections for temperature and barometric pressure being made, the amount of nitrogen is calculated from the volume of nitric oxide, and stated as nitric acid or as nitrogen in the form of nitrates.



CRUM'S TUBE.

Organic Matters.—The "Saline" and "organic" ammonia or the "free" and "albuminoid" ammonia, that is, the ammonia present in a water as such (free or saline), and the ammonia yielded by the destruction of the nitrogenous organic matter with an oxidising agent (albuminoid or organic), are taken as measures of the degree of pollution of the water. Most nitrogenous substances can be made to yield some of their nitrogen in the form of "ammonia," for instance *Urea*, the principal constituent of urine, is converted into carbonate of ammonia by the action of ferments in water, and thus the amount of free ammonia may be an important point.

A measured volume of water—generally half a litre—is placed in a *distilling flask* or a *retort*, which is then connected with a *Liebig's condenser*, a small amount of carbonate of soda having previously been added to the water. The latter is distilled and the distillates are received into measuring glasses in volumes of 50 cubic centimetres. These distillates are "Nesslerised." The first 50 c.c., for example, is placed



LIEBIG'S CONDENSER.

in its cylinder on to a white porcelain slab, and 2 c.c. of "Nessler's" solution added to it, and the mixture stirred up. Nessler's solution, which contains iodide of potassium, bichloride of mercury and caustic potash, strikes a yellow or deep brown colour with a solution containing a very minute quantity of ammonia—with large quantities a brown solid substance is obtained as a precipitate. The colour obtained is then imitated by means of a standard solution of chloride of ammonium (NH_4 Cl) containing the $\frac{1}{100}$ part of a milligramme or the $\frac{1}{100000}$th of a gramme in every cubic centimetre. A measured volume of this solution is mixed up with 50 c.c. of distilled water *free from ammonia* and 2 c.c. of the Nessler's solution added. The water having been distilled until no more ammonia is found by the Nessler test the distillation is stopped, and 50 c.c. of a strong solution of permanganate of potash and caustic potash are added, distillation is continued, and the distillates Nesslerised as before. The ammonia now obtained (organic or albuminoid) is that whose formation is due to the decomposition of the nitrogenous matters by the action of permanganate of potash.

The number of cubic centimetres of the solution of chloride of ammonium required to give the same tint as that obtained in any given distillate is obviously equal to the number of $\frac{1}{100}$ths of a milligramme of ammonia present in that distillate. The total amount of free and albuminoid ammonia yielded by the water is thus arrived at.

Combustion Process.—This process is too long and complicated to admit of its being fully described here. A measured volume of water, not less than one litre, is evaporated to dryness with certain precautions to avoid loss and gain ; and the residue having been scraped up, is mixed with pure dry oxide of copper, introduced into a hard glass combustion tube, and heated strongly in a furnace. The copper oxide parts with its oxygen to the organic matter, which is destroyed, and the carbonic acid and nitrogen, which are the products of the combustion, are measured ; the actual quantity of "organic carbon," and "organic nitrogen" present in a given volume of water are thus obtained.

It should be clearly understood that in all these processes it is necessary to adopt certain standards for guidance. In the case of albuminoid or organic ammonia, for example, the limit 0·15 parts per million, meaning thereby 0·15 parts of ammonia (grammes, ounces, &c.), yielded on distillation by one million parts (grammes, ounces, etc.) of water, has been fixed upon as the result of experience. Pure waters known to be uncontaminated not yielding *more* than this amount, and polluted waters not yielding less. If we find less than 0·1 parts per million of albuminoid ammonia *other evidence to the contrary being absent* we conclude that the water is not polluted ; for in order that such a water may be polluted it is necessary to assume in the first place that it was originally of very exceptional purity, and secondly, that the pollution had taken place to an extent far slighter than occurs in almost every case where there has been any pollution at all.

A limit of some kind, determined in a similar way, must of course be used in any process. Other things being equal for example, the solid residue of a water should not rise much beyond 40 or 50 grains in the gallon. The limits for organic carbon and organic nitrogen are of course also based on experience.

Microscopic Examination.—This is a most important part of the hygienic examination of water and should never be omitted. The usual mode of obtaining the suspended

matter in water for microscopic examination is to allow some of the water to settle in a good sized conical glass, the sediment being taken out with a pipette. A *stop-cock* flask or *straight burette* can also be used for the purpose, and these are in some respects more advantageous. The presence of fibres of cotton, wool, silk, etc., is evidence of pollution with house refuse. Bacteria of various kinds, fungoid growths, epithelium and muscular fibres may be detected, the conclusions to be drawn from the presence of such substances being obvious.

Poisonous Metals.—The metals which may be present in drinking water and which have to be considered as regards their poisonous action, are lead, copper, zinc, and iron. The presence of an excess of lead, iron, or copper is at once detected by placing 70 c., or 100 c.c. of the water in a clean porcelain dish and adding two or three drops of sulphide of ammonium. If the water contains more than $\frac{1}{10}$ of a grain of these metals per gallon a more or less dark coloration is produced, due to the formation of the sulphides of lead, copper or iron. Zinc, which appears to be not unfrequently present in water, has to be sought for in a strong solution of the residue of the water, the sulphide being white.

Hardness.—A water is said to be "hard," when it contains a considerable amount of the salts of lime and magnesia, and to be soft when these salts are present in but slight quantity. The softest natural water is of course rain-water; among the hardest are those proceeding from springs in chalk formations. The salts of lime and magnesia decompose *soap*, and hence a hard water requires a much larger quantity of soap than a soft water to form *a lather*—a lather being only possible when undecomposed soap is present. The degree of hardness of water is determined by means of a standard solution of soap, of such a strength that 1 cubic centimetre of it contains the amount of soap that will be decomposed by one milligramme of lime. 50 or 70 c.c. of the water are placed in a stoppered bottle, the soap solution added, and the bottle vigorously shaken after each addition until the point at which a permanent lather

begins to form is reached. The number of cubic centimetres of soap is then equal to the number of milligrammes of "hard" salts in the water: one cubic centimetre is deducted as it is found that 70 c.c. of water itself consumes that amount of soap solution.

Temporary and Permanent Hardness.—By boiling a measured volume of the water for some time, the dissolved carbonic acid is driven off and the carbonate of lime and other salts held dissolved by the carbonic acid are deposited in the form of a crust. The number of c.c. of soap solution, less one, required to produce a lather in the water after it is boiled, gives the amount of permanent hardness, and the difference between this and the total hardness is equal to the temporary or removable hardness.

An approximate opinion as to the hygienic quality of a water may be arrived at without going through a series of accurate quantitative operations.

A number of tests may be applied to the water as follows:—

1. The reaction of the water is taken with litmus papers to note any acidity or alkalinity.

2. About 25 c.c., of the water are boiled with a solution of chloride of gold. In proportion to the amount of organic matter present the gold is thrown down as a blackish powder.

3. About 100 c.c. of the water are treated with a few drops of very weak permanganate of potash solution, and allowed to stand; the extent to which the permanganate is decolorised is judged of by adding the same number of drops to the same volume of pure distilled water.

4. To about 50 c.c. of the water about 2 c.c. of Nessler's solution are added, and the colour produced is compared with that obtained in the same volume of pure distilled water similarly treated.

5. Nitrate of silver solution is added, and the amount of precipitated chloride of silver observed.

6. About 50 c.c. of water are evaporated to dryness and the residue heated to redness, and the extent to which blackening or browning takes place is noted.

7. To about 25 c.c. of the water are added a few drops of a solution of brucine and a little pure sulphuric acid. If nitrates are present (even in very small amount) a pink colour of greater or less intensity is produced.

8. Oxalate of ammonia solution is added and the lime is precipitated as oxalate of lime. The amount of the precipitate is noted.

9. Barium chloride solution, with a little nitric acid, is added, and the sulphates present are precipitated as sulphate of barium.

10. Finally the water may be tested for phosphoric acid by means of a solution of molybdate of ammonia and nitric acid, and boiling after their addition. The presence of phosphates is indicated by a yellow coloration, and a yellow precipitate.

Foods and Drinks.

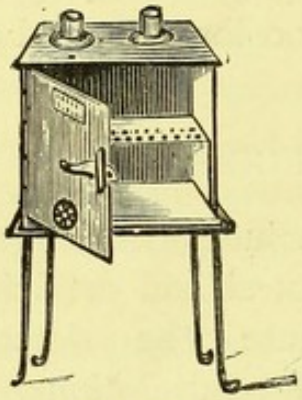
The hygienic analysis of food includes the determination of the composition of the chief natural and artificial foods in their natural and pure state, the detection of alterations, of impurities and of adulterations, and the estimation of the extent and amount of these should they exist. It is obvious that in order to form a satisfactory opinion as to the dietetic value of a given food, as to its general quality, and as to whether it has in any way been altered or falsified, a knowledge of its normal composition, and in the case of an artificial mixture, of the normal composition and properties of its constituents, should be as far as possible obtained.

The matter of greatest importance consists in separating and in estimating the quantity of the *proximate* constituents. The proximate constituents of a substance are the various definite chemical compounds entering into its composition, whereas by its *ultimate* constituents are meant the elementary bodies which build up these compounds. For example, the chief *proximate* constituents of milk are:—water, fat, caseine, milk-sugar, mineral matter (including common salt and phosphate of lime); the principal

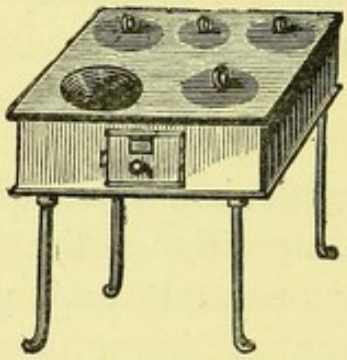
ultimate constituents being the elements oxygen, hydrogen, carbon, nitrogen, calcium, phosphorus, sodium, and chlorine.

The isolation and the determination of the respective total quantities of the ultimate constituents of such a substance as milk is a comparatively easy matter, but we do not thereby obtain very much information as to its value as a food, or as to the purity or non-purity of a particular sample of it, such information being rather obtained by a study of its proximate constituents.

In greater or lesser quantities the following substances and classes of substances enter into the composition of the great majority of foods:—Water, mineral matters



AIR OVEN.



WATER BATH.

(such as common salt and the carbonates and phosphates of lime), fats and oils, sugars, starches, cellulose or woody fibre, gums, nitrogenous compounds (such as caseine in milk, gluten in bread, gelatine, etc.), and alcohol.

In addition certain foods have of course certain specific and characteristic constituents, to which reference will be made.

Water.—The amount of water in a substance may be accurately determined by carefully weighing out a small quantity in a convenient vessel, such as a platinum, porcelain, or glass capsule, and heating it by steam on a *water-bath* or in an *air oven*, until it ceases to lose weight. The final weight deducted from the weight of substance taken gives the quantity of moisture contained in that weight. The employment of this method pre-supposes that the substance under examination is not altered or affected

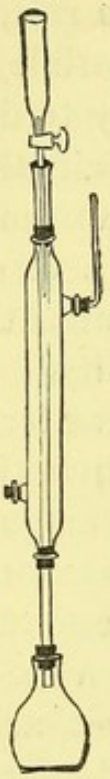
by the heat to which it is exposed, except as regards the loss of the water it contains. Means are of course taken to dry the substance in a condition which shall ensure the loss of its moisture with the greatest facility. As a rule, it is powdered and arranged in as thin a layer as possible in the evaporating vessel. It is occasionally necessary to dry in a vacuum at the ordinary temperature of the air, this being generally accomplished by placing the substance under the receiver of an air-pump together with a vessel containing strong sulphuric acid, a body which possesses the property of absorbing water-vapour with great avidity.

Mineral Matter.—The total amount of mineral matter is approximately determined by burning a weighed quantity of the substance to be examined, and weighing the amount of ash obtained. It must be borne in mind that some mineral substances, such as common salt, are volatilised at a very high temperature, and that some, such as carbonate of lime, are partially decomposed by loss of carbonic acid, but the estimation of the percentage of ash yielded affords valuable information as to the impurities and adulterations that may be present in certain foods. The amount of substance taken for examination is regulated by the quantity of ash that different foods are known to yield, and the burning off of the organic constituents is always managed at as low a temperature as possible.

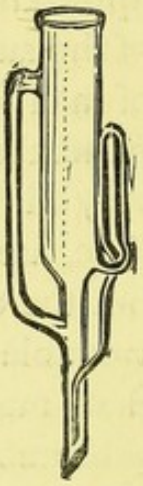
Analysis of the Ash.—An analysis of the ash is sometimes called for and, as in the case of the analysis of the solid residue of a water, is carried out by separating the different constituents from a given weight of the ash and weighing them. For example, valuable indications are afforded by determining in the ash of milk, the percentage of chlorine and of phosphates ; in the ash of beer, the percentage of common salt ; in the ash of coffee-mixtures, the percentage of silica, and in most ashes the proportion of matter soluble in pure water to matter not thus soluble.

Fat.—To estimate the quantity of fat, advantage is taken of the fact that certain organic liquids, such as *ether*, possess the property of dissolving fat, and of depositing it

when they are evaporated. A weighed quantity of the substance—which may previously require some preparation, as in the case of milk, from which the larger part of the water must first be removed—is digested with ether at the boiling temperature of that liquid, this treatment being continued until the whole of the fat has been extracted ; the etherial solution is passed through filtering paper, received in a previously weighed vessel, such as a platinum dish, and the ether evaporated off by immersing the vessel in hot water. The increase in the weight of the platinum dish is the weight of the fat. A very perfect extraction of fat, by means of ether, may be secured by placing the substance in a flask provided with an upright condenser, covering the substance with ether or other volatile extracting liquid, and boiling the latter. The vapour of the ether being condensed, falls back into the flask, and a very concentrated solution of the fat may thus be obtained. A *Soxhlet's extractor* is a convenient apparatus for such purposes—as large a quantity of fat as possible being dissolved in as small a quantity of ether as possible, a point of much importance as regards facility and rapidity of work.



FLASK, WITH UPRIGHT CONDENSER.



SOXHLET'S FAT EXTRACTOR.

Sugar.—There are a great many processes for the estimation of sugar, both chemical and physical. The amount of carbonic acid evolved during the alcoholic fermentation of a saccharine substance has been proposed and used as a measure of the quantity of sugar to which the fermentation is due ; and the specific gravity of the saccharine liquid has also been used to estimate the percentage of sugar. These two processes are not, however, now in use ; the most usual method is perhaps that of Fehling. This process depends upon the action of grape sugar (or glucose) on sulphate of copper in the presence of a

strong alkali. Certain organic substances, grape sugar among the number, possess the property of influencing several chemical reactions. If caustic potash solution be added to a solution of sulphate of copper, a bluish precipitate of hydrated oxide of copper will be produced—and if the mixture be boiled it will become black, owing to the formation of the black oxide of copper. But should the smallest portion of grape sugar be present, on boiling, the mixture will become red to reddish brown in consequence of the formation of the red sub-oxide of copper, a substance, as its name implies, less highly oxidised than the ordinary oxide. A standard solution of sulphate of copper with strong caustic potash is prepared of such a strength that each cubic centimetre contains the quantity of copper that will be exactly reduced to the sub-oxide by the action of 5 milligrammes = ·005 grammes of pure grape sugar. A known volume of the solution of copper is placed in a white porcelain dish, is heated to boiling and the saccharine liquid delivered into it from a burette, until the whole of the copper has been converted into sub-oxide, as is shown by the point of disappearance of the blue colour of the solution in the dish.

Cane-sugar has no action on the solution of copper ; but its quantity may nevertheless be determined by it, inasmuch as by boiling a solution of cane sugar with a few drops of acid, the cane sugar is converted into grape sugar. The sub-oxide of copper precipitated by a measured volume of grape sugar solution may also be separated, washed, dried and weighed, and is of course a direct measure of the quantity of grape sugar which has precipitated it.

Starch and *Woody-fibre* may also be estimated by taking advantage of the fact that by prolonged boiling with acid they are converted into a substance which is chemically identical with grape-sugar or glucose, and reacts on the copper solution in a precisely similar manner and to the same extent. It must, however, be understood that should both these substances be present in the material under examination, this method enables no distinction to be made

between them, and their separation must be effected in other ways. Where the complete separation of a number of chemically similar substances is required, the difficulties of the problem are of course greatly increased, and methods more or less complicated must be devised to cope with it.

Starch.—The chemical composition of the various starches is identical, although the forms of the granules, or corpuscles, differ greatly according to the plant from which the starch comes. It may be estimated by the process above mentioned. The specific characters of the granules of starch from different plants are very easily made out by the microscope, and fairly close approximations may be made to the true percentages of starch in certain mixtures—such, for instance, as mustard and flour, or cocoa and arrowroot, by making up mixtures of known quantities of the starch in question with pure mustard, pure cocoa, etc., as the case may be, and counting the number of starch granules in miscroscopic preparations of the same quantities of both the standard and the suspected substances.

Starches are all coloured intensely blue by iodine, and the latter substance is accordingly employed as a very delicate test for the presence of starch. Granules of starch under the microscope may thus be identified with the greatest ease.

Of non-nitrogenous bodies we have examples in the fats and the carbo-hydrates (sugar, starches, gums, etc.), all of which are compounds of carbon, hydrogen, and oxygen alone.

Nitrogenous Compounds.—Nitrogen is present in food-substances combined with other elements in a great variety of ways, forming a number of more or less complicated compounds; and several classes of nitrogenous bodies have accordingly to be distinguished. For example, of the compounds of carbon, hydrogen, oxygen and nitrogen alone we have some of the *alkaloid class* such as theine in tea and coffee, and theobromine in cocoa. These bodies act as strong poisons on the animal economy. The most

important nitrogenous bodies as regards food, however, belong to the so-called *albuminous* class, which, in addition to the elements mentioned, contain sulphur, and in some cases phosphorus. Such for instance as casein in milk, gluten in the cereals, albumen (white of egg) and fibrin in blood, flesh, etc., and vegetable albumens. Gelatine obtained from skin, tendons, and bones, belongs to another sub-class.

Our knowledge of these substances is still extremely imperfect. Many of them, formerly regarded as separate and definite compounds, are now well made out to be mixtures of several. The absolute separation and estimation of many of these compounds is therefore not always possible in the present state of our knowledge with regard to them; and it has consequently been usual to estimate the total quantity of nitrogen contained by the body under examination, that quantity being a measure of the total amount of nitrogenous matters present.

Such a determination may be carried out by mixing a carefully weighed portion of the dried substance with oxide of copper, introducing the mixture into a long tube of hard glass, and having removed the air from the latter by means of a pump, heating it to redness in a furnace. The nitrogenous substance is decomposed, and the nitrogen is evolved and measured, by allowing it to bubble up into a graduated tube filled with mercury and standing in a bath of mercury; corrections for temperature and barometric pressure being of course made.

In thus determining the total nitrogen in a substance used as food, or in any particular portion of such a substance, we are not justified in assuming that the whole of the nitrogen is present in such combinations as are valuable for feeding purposes, such, for example, as the albumens. Much of the nitrogen *may be* present in compounds which are comparatively valueless as foods, and in order to obtain further information as to the state of combination of the nitrogen, it is necessary to employ some process for the estimation of albuminous matter. Such processes depend as a rule upon the property possessed by most

albumens of *coagulating* when treated with acids or other re-agents.

Alcohol.—In the examination of beverages, the determination of the percentage of alcohol is generally the first step taken. There are several very accurate methods. Pure or absolute ethylic alcohol is a mobile, colourless, and neutral liquid of specific gravity 0·815 at 0° Centigrade and boiling at 78·3° Centigrade. The alcohol in an alcoholic liquid is extracted by distillation. One hundred cubic centimetres or more of the liquid, (beer, wine, spirits, etc.), are placed in the distilling apparatus and distilled until the matters in the retort or flask are nearly dry. The distillate having been received in a flask fitted air-tight to the end of the condenser is made up to the same volume as the volume of liquid experimented on (100 c. c. or more, as the case may be,) with distilled water. The specific gravity of this distillate at 0° C. (32° Fahrenheit) is then accurately taken in a specific gravity flask. Inasmuch as bulk for bulk alcohol is lighter than water, as ·815 is to 1, it follows that the more alcohol there is in the distillate the weight of the latter will be *pro tanto* lessened. A hydrometer is also frequently used for this purpose; it consists of a glass tube with a weighted bulb, which sinks in the liquid to a depth varying with its density, the latter being indicated by graduations on the stem. Tables have been constructed giving the specific gravities of mixtures of alcohol and water in all integral proportions, and by referring to one of these the amount of alcohol in the 100 c.c. of distillate is at once seen. Since the distillate has the same volume as the liquid experimented on, and since all the alcohol in the latter is contained by the distillate, the percentage of alcohol required is arrived at.

SPECIFIC GRAVITY FLASK.

HYDROMETER.

We now proceed to describe and explain some of the simpler processes of food-analysis, more particularly those which are of hygienic interest.

Milk, Cream, Butter, and Cheese.—The milk of different animals varies somewhat in composition, so far as the actual percentages of the typical ingredients are concerned; but the milk of any healthy animal, such as the cow, even when the methods of feeding are different, varies within well-ascertained and tolerably narrow limits, so that the analyst, knowing the average composition of the milk of healthy cows, can find whether, in a particular case, there is a departure from it. We are not for the moment concerned with departures from the normal composition, such as those which occur in milk from diseased or starved animals, or with milk which for other reasons is of abnormal character. By determining the total quantity of solid matter, of fat, of sugar, of caseine (curd), and of ash, as well as the specific gravity, the analyst is able to ascertain whether milk has been sophisticated by abstracting fat (cream), or by adding water, or by both these processes. It must be remembered that these sophistications may be of a very dangerous character. Milk is admitted to be a perfect food for the young, containing as it does a proper proportion of the ingredients of a typical food, viz., the nitrogenous, saccharine, and mineral constituents, and the lowering of its value as a food has been shown to have much to do with infantile mortality; on the other hand, the water with which a milk is adulterated may be, and very often has been polluted, and the poisons of various diseases, which find a congenial soil in such a substance as milk, are thus often carried over a wide area, and may produce their specific effects in a large number of persons.

The normal composition of healthy cow's milk, having a specific gravity of 1029 and upwards, is as follows, (Parkes):—

Water	86·8
Albuminates	4·0
Fat	3·7
Carbohydrates (sugar)	4·8
Salts	0·7
	100·0

According to Wanklyn, there are in 100 c.c. of average country milk :—

Water	90·09	grammes
Fat	3·16	„
Caseine	4·16	„
Milk sugar	4·76	„
Ash	0·73	„
	102·90	„

Specific Gravity.—Considering milk as a watery solution of lactin (milk sugar), caseine and salts (*i.e.*, compounds of magnesium, potassium, sodium, and iron, with chlorine, and with phosphoric and sulphuric acids), holding fat globules in suspension, it seems at first sight that the addition of more water should lower the weight of a given volume of milk, and hence lessen the specific gravity. The fact that fat is lighter than water must, however, not be lost sight of, and that, in consequence, increase of fat means lowering of density. The commonest instrument used in examining milk by a specific gravity test is that known as the *Lactometer.* This is simply a hydrometer applied to milk—a bulb-tube and stem weighted at the bottom. It is floated in the milk and it will sink more or less, according to the density of the liquid in which it is immersed. The lactometer may be graduated, by marking on the stem the points to which it sinks when it is placed in mixtures of normal milk with water in different proportions, or it may be graduated into specific gravities. As a matter of fact the lactometer is a very untrustworthy instrument. A more accurate method is to fill a specific gravity flask with the milk and weigh it at a particular temperature. By comparing this weight with that of the same flask filled with pure water at the same temperature the density of the milk is obtained. The specific gravity of pure milk varies from 1023 to 1035, the average being 1029. The total amount of solid matter may be determined by drying up on the steam bath, about five grammes

LACTOMETER.

of milk weighed out in a platinum capsule, and by burning this residue the percentage of ash (mineral matter) is arrived at. The amount of solids yielded by pure milk varies from 11·5 to 14 per cent.; when the percentage falls below 11, the milk is very poor, and has probably been sophisticated. The fat is obtained by extracting about 10 grammes of the milk, previously evaporated to a semi solid consistence, by means of ether; the solution of the fat in the ether, passed through a filter and collected in a weighed vessel, is evaporated in the latter until the whole of the volatile solvent has disappeared, and the weight of fat thus obtained.

After the extraction of fat there remain caseine, milk-sugar and mineral matter. By treatment with alcohol and water the milk-sugar, and part of the ash are removed; the residue consists of caseine and a little phosphate of lime, which may be dried and weighed, burnt, and the ash weighed; the latter, deducted from the first weight, gives the quantity of caseine. The milk-sugar may be determined in the weak alcoholic extract above mentioned, by means of Fehling's copper solution, as previously described under "Sugar"; or a given weight of milk may be "coagulated" with acetic acid, and the clear whey may be taken for the determination of sugar. The "solids not fat" that is, the caseine, salts, and lactin, obtained by direct experiment or by deducting the percentage of fat from the percentage of total solid matter, is a fairly constant quantity, *i.e.*, is not subject to the variation of the single constituents. This percentage is taken at 9·3, and knowing that 100 parts by weight of pure milk will yield 9·3 of "solids not fat," it is of course possible to calculate how much pure milk there is in any particular sample.

CREAMOMETER.

The percentage of cream thrown up by milk in a given time, is determined by an instrument called a *creamometer*. This is a long tube in which the milk is allowed to stand and which is graduated to give percentages.

Many ingenious adulterations of milk have been devised and

practised with a view of increasing the difficulty of detecting fraud by analysis, but they are all susceptible of detection by some modification of the processes just sketched out, and it cannot be too widely known that attempts to cheat the analyst, however clever they may be, can only in the long run result in failure. Among the other adulterations of milk the following have to be guarded against, most of them, however, being extremely rare ; starch and gum (to conceal thinness), annatto or turmeric (to give colour), glycerine, syrup, emulsions of seeds (almonds, &c.,), chalk, sodium carbonate.

Apart from the discovery of adulteration in milk, its characters when it has been altered by decomposition, or when it has been obtained from starved or diseased animals, have to be considered. The alterations in chemical composition, although in some cases very striking, are not as yet sufficiently made out to be distinctive, and the microscopic appearances are therefore chiefly to be relied upon. Milk from diseased animals soon decomposes. Blood discs, pus cells and fungoid growths can be at once detected by the microscope. The characteristic appearance of the so-called "blue milk" is due to a microscopic growth. Minute moving organisms have occasionally been detected in milk from suspicious sources. A peculiar clustering of the milk corpuscles is noticed in certain diseases.

Cream.—The composition of cream is similar to that of milk, except that it contains a much larger amount of fat. The following is the analysis of a very thick cream (Wanklyn) :—

Water	50·00
Fat	43·90
Caseine and Milk Sugar . .	5·63
Ash.	0·47
	100·00

It has been found adulterated with starch and gum, as well as with mineral matters. White of egg has also been used. The analysis is carried out in the same way as in the

case of milk, but is somewhat more difficult owing to the large amount of fat. It hardly enters into the scope of the present work to describe the necessary analytical modifications which must be applied in the case of cream.

The analysis of condensed and prepared milks and milk foods, is also beyond our present scope. In these cases the addition of large quantities of cane-sugar for the purpose of preservation, considerably complicates the methods employed.

Butter.—Butter consists principally of cohered milk fat. It contains varying quantities of salt, water, and caseine. The following is an analysis of a pure butter.

Fat	83
Curd	5
Salt (ash)	3
Water	9
	100

The average amount of water varies from 5 to 10 per cent., but may be much higher even in genuine butter. For the purpose of increasing weight, butter is beaten up with water; an excess of the latter can be detected by drying a given weight of the butter on the water bath. By simply melting the butter in a narrow tube, the fat rises to the top, the water, containing the salt in solution, and the curd, sinking to the bottom; if the amount of water is very large, this test makes the fact at once evident. Butter may also be loaded with an excessive amount of common salt. This may easily be determined by extracting with water, and estimating the quantity of salt in the solution, by means of standard nitrate of silver as in the analysis of water. The most important point, however, is the substitution of some cheaper fat. Butter fat is a mixture of the Glycerides of certain fatty acids, some of which, viz., Palmitic, Stearic and Oleic acids, are insoluble in water, and not volatile; the others Butyric, Caproic and Caprylic Acids being soluble and volatile. For detecting and estimating foreign fat,

advantage is in the first place taken of the facts, (1), that butter-fat possesses a different specific gravity to that of other fats, and (2), that it melts at a different temperature.

The fat having been separated by melting in the narrow tube and filtering it through coarse filtering paper into a dry bottle, the specific gravity may be determined by the flask as in the case of milk. The melting point, by drawing some of the melted fat into a very thin and narrow tube, allowing it to congeal therein, and placing it in a beaker of water, together with a thermometer. The beaker is nested in a second containing water, to which heat is then applied, and the temperature at which the fat *runs up the tube* is noted on the thermometer. Another valid plan is to note the temperature at which a small glass bulb will sink in the fat to be tested.

By determining the percentage of insoluble fatty acids, the analyst is able to calculate the extent to which a sample of butter has been sophisticated with foreign fats. The fatty acids insoluble in water are tolerably constant in butter fat, being about 88 per cent. of its weight, most other fats yielding about 95·5 per cent.

A given weight of the fat is "saponified" by heating with caustic potash and alcohol; the process of saponification consisting essentially in the combination of the fatty acids with potash, to form compounds called *soaps*. Some of the more volatile substances being driven off by the heat used in the process. The soap obtained is decomposed by sulphuric acid, preferably in a 'butter-flask,' a modified separating flask, and the resulting mass of fatty acids washed with hot water, the washings being allowed to escape by means of the stop cock.

The insoluble fatty acids are thus obtained in the flask in the form of a cake, which can be dissolved in ether, the solution collected in a weighed dish, the ether evaporated, and the fatty acids weighed. The soluble fatty acids may also be separately estimated.

In most samples of butter which contain foreign fat, the specific gravity, the percentages of insoluble and soluble

fatty acids, and the melting points, are all entirely different from those obtained with genuine butter. For instance, genuine butter contains about 6 per cent. of soluble, and 88 per cent. of insoluble fatty acids, whereas "butterine" contains only 0·6 per cent. of soluble, and 95·5 per cent. of insoluble fatty acids.

Cheese.—Cheese is made from milk by coagulating the caseine by the action of *rennet*, and pressing the mass obtained. It is not a much adulterated article; but it is of importance occasionally to examine the rinds for poisonous metals, not only because of the custom of using protecting foils containing lead, but also because arsenical and other metallic solutions have been applied in order to prevent the attacks of insects. The examination of cheese is carried out in the same way as that of milk and butter.

Wheat Flour and Bread.—The following is the composition of wheat flour of average quality :—

Water	16·5
Fat	1·2
Gluten, &c.	12·0
Starch, &c.	69·6
Ash	0·7
	100·0

Bread is flour made into a paste with water, treated with carbonic acid gas, which is liberated in the paste, either by the action of yeast, or by mixing it with a strong solution of the gas in water ; and subsequent baking.

The colour, smell and taste of flour and bread often yield valuable information as to quality ; indeed, these tests are valuable in the examination of all food substances. The microscope detects the presence of fungi, of which several have been found in flour, and which also occur in bread ; their presence indicating that the article is diseased or damaged. Certain animal parasites are also found in bad flour ; and a point of much importance is the detection of adulteration with other starchy substances. The characteristic

appearances of the different starches under the microscope enables this to be done with ease in the case of flour; but with bread, inasmuch as the process of manufacture alters these appearances, the starch granules swelling and bursting, the problem is much more difficult.

The chemical examination of flour resolves itself chiefly into determining the percentages of Water, Gluten, Ash, and the presence of Alum, and its quantity, if present.

The amount of water in flour is ascertained by drying one gramme in a dish, and the amount of ash by burning two or three grammes. For bread larger quantities are necessary. The more water the less is the value of the article; flour should be rejected if it contains more than 18 per cent. If the ash be more than 2 per cent. mineral adulteration or impurity is present. An easy method of detecting large quantities of mineral impurity is to shake up the flour with chloroform, when the flour floats, and the foreign mineral impurities fall to the bottom of the vessel. Gluten is estimated in flour by washing away the starch from a weighed quantity: good flour contains from 8 to 12 per cent.

Alum in Flour and Bread.—The presence of alum is most easily detected by treatment with a tincture of logwood and carbonate of ammonia. If alum is present, a blue or green colouration is obtained; the flour should previously be well mixed with water and the bread should be allowed to dry. If a blue colour be obtained a further examination is necessary, and the exact amount of alumina present must be separated out from a weighed quantity of the flour or bread and weighed. The process is a somewhat long one, and it will hardly be necessary to describe it here.

There have been rare cases of poisoning which have occurred through the presence of lead in flour, which has been introduced through the repairing of mill stones with lead. Arsenic has also been found in flour, having been introduced accidentally, and plaster of Paris (sulphate of lime) has been fraudulently sold with flour.

Tea, Coffee, Cocoa and Chocolate.—The examination of tea does not present much difficulty. The presence of foreign

leaves, of sand, and other mineral substances, and the use of exhausted leaves are the chief points to be seen to. The Chinese themselves have invented a method of adulteration which consists in steeping the leaves in gum and rolling them in sand. This they call "lie tea"; and most samples of the cheaper kinds of tea contain small quantities of it.

The tea to be examined is boiled up with water, and the leaves can then be picked out and examined. The structure of the tea leaf is very characteristic. The shape, serration, and the venation, especially the latter, being distinctive. The primary veins run out from the mid rib nearly to the border, and then turn up sharply. Foreign leaves can therefore be at once detected by their special characters. The vessel in which the tea has been boiled will contain any sand or mineral substances which may have been adhering to the leaves. The chemical examination of tea essentially consists in the determination of Water, Ash and Soluble Ash, Extract, Theine and Tannin. The extract is obtained by treating a weighed quantity of tea with hot water and evaporating a measured bulk of the solution to dryness, and weighing the residue. If exhausted leaves are present, the amounts of extract and of ash soluble in water, &c., will be obviously lower than those obtained from good tea. The amount of the total ash will be high in case of the presence of much mineral impurity.

Coffee.—It is of course in the ground state that coffee is most liable to adulteration. The chief adulterations are chicory, roasted wheat and beans, acorns and burnt sugar. By far the most common is chicory. The analysis of coffee is very similar to that of tea. Chicory may be detected by the microscope, its dotted ducts and large loose cells being very characteristic. If coffee containing much chicory be sprinkled on the surface of some water in a jar the chicory will sink, colouring the water a deep brown as it does so. Pure coffee gives a hardly perceptible colour to the water, and scarcely sinks at all. The alkaloid of coffee, viz., caffeine, is identical with theine.

Cocoa and Chocolate.—These articles, as they come before the analyst, are "prepared," and are subject, consequently, to various adulterations. The various forms of prepared cocoa are simply the ground seeds previously roasted and deprived of their covering, and mixed with starch and sugar. The microscope at once shows the nature of the mixture. There is no essential chemical difference in the composition of cocoa and chocolate. Both cocoa and chocolate are rich in fat.

Alcoholic Beverages.—This is a very wide subject, and cannot be more than alluded to here. In all cases, of course, the determination of the quantity of alcohol is the first essential point. There are several methods, the one most usually employed being as follows: A measured volume of the liquid is distilled, and the distillate made up to the same volume as the liquid taken. As previously explained, the specific gravity of the distillate at once gives the percentage of alcohol present by reference to a specific gravity table.

Special adulterations and impurities, as might be expected, have to be looked for in different beverages, *e.g.* artificial colouring matters in wine, bitter principles and salt in beer, "fusel oil" (amylic alcohol) in spirits. The addition of water to spirits is a criminal offence, but so far as health is concerned it is perhaps an advantage.

Condiments—Vinegar, Pepper, Mustard, Pickles.—Vinegar is more or less impure acetic acid. It contains from 3 per cent. to 5 per cent. of acetic acid (glacial); 3 per cent. is the minimum allowable. It is adulterated, (1), by the addition of water, (2), of sulphuric acid or other strong mineral acid, and it may contain lead, copper, or zinc, due to the action of vinegar on the vessels in which it has been kept, or on the apparatus used in its manufacture.

By taking the specific gravity the addition of water can be detected. It is preferable to distil a measured bulk of the vinegar, and to take the specific gravity of the distillate. The percentage of acetic acid is then obtained as in the case of alcohol.

The total acidity of the vinegar may be determined by means of a standard alkaline solution. The acidity is calculated as acetic acid, although other acids may be present. If the specific gravity is low and the acidity high, sulphuric acid or some other mineral acid may have been added. The processes for determining the amount of free sulphuric acid in vinegar cannot be detailed here. It may be pointed out that the ordinary methods of estimating sulphuric acid, by precipitation and weighing as sulphate of barium, do not admit of differentiating between the added sulphuric acid and natural sulphates in the vinegar. However, there is a rapid qualitative test which may be easily applied:—10 c.c. of vinegar are evaporated to dryness and burnt; the ash is then dissolved in a little water, and the reaction of the solution taken with litmus paper. If the ash is alkaline the vinegar cannot contain an excess of free sulphuric acid, if *neutral* it very likely does, the reason being, that the ash of natural vinegar consists chiefly of alkaline carbonates, but if excess of sulphuric acid is present, neutral salts will be obtained in the ash, owing to the decomposing action exerted by the sulphuric acid during evaporation.

Pickles.—The vinegar used may be examined as has just been described. The presence of such metals as copper and lead, and their quantities if present, can be determined by incinerating a given weight and using the ash for analysis. The presence of copper in pickles can be detected by inserting the bright blade of a steel knife, on which the metal is deposited as a thin red film.

Mustard.—The microscopic characters of mustard-seed are very distinctive, and the presence of adulterating ingredients can therefore be very readily made out, the more so as it contains no starch. There exist in mustard a number of very well-defined compounds, some of which contain sulphur. An estimation of the total quantity of sulphur present in a sample can be made an approximate measure of the actual quantity of pure mustard in it, as can also a determination of the percentage of *oil*, the common

adulterants of mustard containing neither of these substances in more than very small amount. The most common adulterations of mustard are wheat-flour and turmeric. The microscope at once reveals their presence and an approximation to the true percentages can be made by comparing the sample under examination with standard samples made up of pure mustard with varying quantities of the adulterants. By treating mustard thus adulterated with iodine the blue colour which the latter strikes with starch shows the presence of flour, and caustic potash solution added to mustard containing turmeric, reveals the presence of the latter by a bright red colouration of the turmeric specks. From the hygienic point of view there can be no doubt that the use of *pure* mustard with food is not desirable, and that the addition of a little starch is an advantage.

Plaster of Paris, chromate of potash (a yellow substance), clay, and gamboge, are all stated to have been found in mustard. Special tests must be made for these substances should the microscope or the percentage of ash give rise to suspicion. There is no doubt of the occasional use of gamboge, although it is rare, and it is certainly a most objectionable adulterant.

A percentage of ash higher than 5 would show mineral, and lower than 4 organic adulteration, the average ash of mustard being 5 per cent.

Pepper.—Unlike mustard, pepper contains starch, the granules being very small. It is found adulterated with ground rice, wheat-flour, linseed, and mustard-husk, and with sand. These are the only common adulterations; although some observers give a formidable list of the substances used. The determination of the ash, which varies from 1·5 to 5 or 6 per cent., and should never exceed 7 per cent., detects mineral impurities such as sand. The microscope shows the presence of ground-rice, this being indeed the commonest adulterant.

Condensed, Prepared, and Preserved Foods.—The very large number and variety of such articles renders it impossible to

do more than allude to them as substances which come under the notice of the analyst in increasing number. Some of the cheaper tinned foods are occasionally deleterious, either through imperfect preparation and consequent decomposition, with accompaniment of fungoid growths, or from the action of the foods themselves on the metal of the containing vessel s; but the extremely rare occurrence of cases of poisoning does not warrant the scare which seems to exist with regard to them.

Examination of Soils.—The complete analysis of a soil is a very long and tedious process. It will be enough to indicate that for the purposes of the hygienist the determination of the percentage of water contained, of the power of holding water, of the amount of loss and behaviour on incineration, together with a microscopic examination, is as much as is generally required for practical purposes.

Arsenical Wall-papers and Pigments.—These are now well recognised as fruitful causes of disease. There are several methods of detecting arsenic, one of the best being Marsh's, which has already been described.

Disinfectants.—The principle of the method usually employed to judge of the value of a particular disinfectant consists in comparing its preserving power in solutions of different strengths with that possessed by some standard disinfectant, such as carbolic acid. Milk or meat is perhaps the most convenient substance for such experiments. Equal volumes or weights are treated with measured volumes of the disinfectant solutions, and are examined daily by the microscope, and otherwise, for the signs of decomposition.

The foregoing sketch of hygienic analysis is necessarily very limited, but enough has been said to show the general nature of the plans employed, more particular attention having been paid to common adulteration and impurity, rather than to the processes used in the investigation of food-substances for purely scientific purposes. But it must not be supposed that the list of articles which find their way to the hygienic laboratory is by any means

exhausted. The examination of articles of clothing and decoration, of the innumerable substances used as deodorisers and disinfectants, of soils and of drugs, opens up, as will readily be seen, an extremely wide field of work.

INTERNATIONAL HEALTH EXHIBITION.

HYGIENIC LABORATORY.

(*Annexe to City and Guilds Institute.*)

Director—PROFESSOR CORFIELD, M.A., M.D. (Oxon), F.R.C.P.
Chief Assistant and Demonstrator—MR. CHARLES E. CASSAL, F.I.C., F.C.S.
Assistant—DR. W. FRASER, San. Sci. Cert., Cambridge.

THIS Laboratory is designed to show, as far as is possible in a temporary building, the arrangements suitable for the examination, from a Public Health point of view, of water, air, foods and drinks, soils, disinfectants, sanitary appliances, and other articles of Hygienic interest. In front of the Laboratory proper is an ante-room in which are arranged cases of apparatus of various kinds for exhibition and use in the Laboratory, and also a model laboratory table.

Projecting into the ante-room and entered from the Laboratory is the balance room, which should be separate from the Laboratory, but is here merely a glazed compartment, so that the operations conducted in it may be visible to the visitors; the balances, lent by Mr. Oertling, are supported on a pier with a solid foundation of masonry to prevent vibration; most of these instruments are very delicate, being capable of weighing to the one-thousandth part of a grain with comparatively heavy loads on the pans.

On each side of the balance room, in the ante-room, is a table on which are placed microscopes with various specimens for examination.

In the body of the Laboratory are placed three working-tables with bottle-racks above them, and drawers and cupboards for apparatus underneath; and around the sides, tables for microscopic work and distillations, with shelves for apparatus and bottles containing reagents, a furnace with sand bath on the top for evaporating purposes, and two glazed draught cupboards in which operations producing fumes may be conducted; these cupboards are provided with flues in which jets of gas are burning in order to produce currents of air which convey the fumes outside the building; the laboratory tables are provided with appliances for the supply of gas and water, and with sinks, the waste pipes of which are connected with a stoneware drain discharging into an open trapped gully outside the Laboratory, and having an inspection opening, with a ventilating pipe carried above the eaves, at its upper end.

The operations conducted in the Laboratory are sufficiently described in the handbook entitled "Public Health Laboratory Work," and consist chiefly in the examination by chemical, microscopical and other means, of specimens of water and air with the view of determining the nature and amount of various pollutions, and the analysis of articles of food and drinks to ascertain their quality and to detect the presence and estimate the quantity of impurities and adulterations, also the examination of filtering materials and of disinfectants, and the detection and estimation of poisonous ingredients, such as arsenic, in the colouring matters used for decorative purposes, clothing, &c.

Specimens of accurately graduated flasks, burettes, thermometers and other apparatus used in the operations conducted in the Laboratory may be seen in the cases and on the tables, and also in actual use.

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