A brief review of our knowledge of the relation of micro-organisms to diseases in man / by William Robert Smith.

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BY

WILLIAM ROBERT SMITH, M.D., D.Sc., F.R.S.Ed.,

BARRISTER AT LAW;
FELLOW AND MEMBER OF COUNCIL OF THE INSTITUTE OF CHEMISTRY;
PROFESSOR OF FORENSIC MEDICINE IN KING'S COLLEGE, LONDON;
MEDICAL OFFICER OF HEALTH FOR WOOLWICH,
ETC.

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A BRIEF REVIEW OF OUR KNOWLEDGE OF THE RELATION OF MICRO-ORGANISMS TO DISEASES IN MAN.

When you did me the honour, Sir, to ask me to read a paper before the members of this Society upon the relationship of micro-organisms to disease, so far as our knowledge has extended to enable us to speak with some certainty as to such organisms being the direct cause of certain well-known specific infective disorders, I was thoroughly alive to the importance of the subject, and regretted that for such a review you had not chosen one better able to deal with it.

Such a relationship is now, I think, on all hands, admitted to be very intimate; and although it may not be of such a universal nature as some perhaps would have us believe, yet, so far as many diseases are concerned, it has been abundantly shown to be of a most direct and positive nature.

A knowledge of what is known regarding these minute bodies is not only necessary for pathological study, but for the rational treatment also of infectious diseases; in that department of medicine known as preventive, the special value of such knowledge is very evident. So long as the cause of a disease is unknown, the views held as to its origin must be vague, and the measures adopted against it frequently ineffectual; but when the cause is known, and especially when it can be isolated and its life-history studied, together with its behaviour outside the body, its mode of entrance into the system, and the best methods for destroying it, then indeed, and only then, can measures be adopted both precise and effectual.

A medical officer of health should, however, not alone be conversant with the literature on this subject, but should have a practical acquaintance with the best methods of research. It is to be regretted that opportunities have not been afforded in this country such as those which exist in Berlin for health-officials to obtain this practical knowledge;

and it is a subject for congratulation that measures have now been taken to remove, in some measure, this defect.

The bacteria were for a long time included in the animal kingdom; but, from a more precise study of their morphological and physiological characters, their modes of reproduction, and the obvious relation which they bear to the algæ on the one hand, and to the fungi on the other, have led observers to regard them as the lowest stage of plant life. They are divisible into three main groups: the first, where the cells are round, when they are termed cocci; secondly, where the cells are more or less elongated, varying from short rods, which are twice as long as they are broad, to long threads—these are the bacilli; thirdly, when the organisms are elongated and twisted, like a corkscrew—these are called by Cohn spiro-bacteria, and by him are further divided into the spirochætæ—where the rod is flexile and long, and the turns of the screw close together—and the spirilla proper —where the rods are stiff, shorter, and the turns of the screw wider apart.

In all investigations of the relation of micro-organisms to disease, it is necessary to bear in mind that in no case can it be said to have been proved that a particular infectious disease is due to a particular micro-organism, if any one of the following conditions laid down by Dr. Koch remains unfulfilled.

1. The micro-organism must be found in the blood, lymph, or diseased tissues of man or animal suffering from, or dead of, the disease.

2. The micro-organisms must be isolated from their nidus, from the blood or tissues, as the case may be, and cultivated in suitable media, *i.e.*, outside the body, and by such means as will effectually exclude the accidental introduction of other micro-organisms. These pure cultivations must then be carried on from one cultivation to another through several successive generations, in order to obtain them free of every kind of matter derived from the animal body from which they were taken in the first instance.

3. A pure cultivation thus obtained must, when introduced into the body of a healthy animal susceptible to the disease, produce the disease in question; and

4. It is necessary that in this inoculated animal the same micro-organism should again be found.

These points naturally suggest the sequence in which the various processes must be adopted in the practical study of micro-organisms associated with disease; but, in reference to the first, it is well we should note that great differences

exist as to the presence of the organism in the blood and in the tissues; in some cases the micro-organism, although present in the blood, cannot be detected in the tissues; whilst in others, although present in the tissues, cannot be found in the blood. Whether these differences depend upon defects in our methods of examination, as is very probable, remains yet to be satisfactorily settled.

We will proceed now to the consideration of the diseases in question.

Tuberculosis.—The etiology of this disease is of the greatest moment and highest possible interest, and, as we all know, it is to the brilliant researches of Professor Koch of Berlin that we are indebted for the exact knowledge we now have of this disease.

It was some time since that Buhl pointed out the connection which existed between miliary tuberculosis and primary cheesy foci, and suggested that general tuberculosis was to be regarded as a disease due to the absorption of a virus originally present in a primary cheesy nodule, and causing self-infection; the way in which the virus was distributed through the body being explained, in great measure, by Ponfick's discoveries relative to tuberculosis of the thoracic duct, and Weigert's detection of tubercle in the walls of the veins. Such facts, however, whilst bearing upon the question of the diffusion of the tubercular virus throughout the body, did nothing to show that the virus was communicable from one person to another, rendering the disease an infectious one.

Many attempts were made, towards the end of the last century, to artificially produce tuberculosis, without result. The first successful experiments were made by Klencke, in 1843, who induced extensive tuberculosis of the lungs and liver in rabbits by inoculation into the veins of the neck of portions of miliary and infiltrating tubercles from man. These experiments were, however, not continued, and were consequently soon forgotten. Later, Villemin undertook a series of investigations on the subject, working throughout with great care and thoroughness, inoculating not only with tubercular matter obtained from human beings, but also from cases of bovine tuberculosis. These researches, not only from the methodical character and great number of the experiments, but also from the employment of suitable control experiments, appear to have conclusively proved the question in favour of the infective theory. These experiments were repeated by other observers; the adherents of the infective theory seeking to improve the details of the

experimental methods, and its opponents, on the other hand, striving to prove that tubercular material contains no specific virus, and that true tuberculosis could be induced by inoculation with non-tubercular matter. To the decision of this question Cohnheim and Salomonsen contributed greatly, by selecting for inoculation the anterior chamber of a rabbit's eye, by which means a separation of the cases is made into those in which successful inoculation with tubercular material has been accomplished, from those in which some other infective material has been introduced with the tubercular virus; for with this latter material subcutaneous inoculation often causes a more or less widely diffused cheesy infiltration, not unlike that of tubercle, whilst in the eye a general inflammation of short duration is produced, which cannot be mistaken for the slow characteristic development of tuberculosis following inoculation. On the other hand, the course of a successful tubercular inoculation is very characteristic. After a somewhat long incubation period, single grey, hardly perceptible nodules appear in the iris, starting from the site of inoculation; these gradually increase in size, and become yellowish in the centre; they caseate, and generally exhibit all the characteristics of the true tubercular nodule; later on, the whole organism becomes affected—the neighbouring lymphatic glands, the lungs, spleen, liver, and kidneys. In no case did tuberculosis of the iris follow an inoculation with non-tubercular material, and in no case has spontaneous tuberculosis of the iris ever been observed in rabbits. Such a method of infection, therefore, presented obvious advantages over others, establishing, as it did, the fact that tubercular materials apparently differing widely from each other were characterised by one and the same specific contagium; but no decision could be obtained as to the nature of the contagium, whether it consisted of specific organisms gifted with constant properties, or whether, on the other hand, it was composed of particles of an organised or unorganised nature arising only under certain abnormal conditions within the body.

Following the conditions laid down as necessary to establish the parasitic nature of a disease, the efforts of Professor Koch were directed in the first place to show the presence of a pathogenic organism, and subsequently, by isolation and inoculation experiments, to demonstrate its causal connection with the disease.

He began his efforts by the examination of recently developed grey tubercle from the lungs of animals killed three or four weeks after inoculation, in which material the virus

might confidently be expected to be present; sections of such organs, hardened in absolute alcohol, were examined, together with cover glass specimens of the crushed grey tubercle, but all such attempts to discover the presence of micro-organisms failed. At this stage advantage was taken by Dr. Koch of experience previously gained, which was to the effect that in certain cases the deepest staining and clearest differentiation of bacilli from the surrounding tissues were obtained by using dyes of alkaline reaction; and of all the ordinary aniline dyes, methylene blue, bearing the freest addition of alkali, was chosen (to a watery solution of it caustic potash was added so long as the dye remained clear, and no precipitate was produced). When cover glass specimens were exposed to this staining fluid for twenty-four hours, very fine, rod-like bodies were detected for the first time in the tubercular material, which further observations showed to possess the power of multiplication and spore formation. The method subsequently adopted for demonstrating the presence of these organisms was to make cover glass preparations dried in a very thin layer, and heated two or three times in the flame of a Bunsen burner, or to take very thin sections of organs thoroughly hardened in alcohol, and stain them with a solution consisting of aniline water 100 c.c., alcoholic solution of methyl violet or fuchsin 11 c.c., and 10 c.c. of absolute alcohol.

The preparation should remain in the staining fluid twelve hours, the staining being facilitated by slightly warming the solution; subsequently the preparation should be immersed in dilute nitric acid (1 to 3) for some few seconds, then rinsed for some minutes in 60 per cent. alcohol, and placed in a dilute solution of vesuvin or methylene blue, again washed in 60 per cent. alcohol, dehydrated in absolute alcohol, cleared with cedar oil, and mounted in Canada balsam.

This method of staining is of importance, so far as the tubercle bacilli are concerned, for no other bacillus, with the exception of the leprosy bacillus, gives the same colour reaction. The bacilli of tuberculosis and leprosy resemble each other in various ways. Still, a distinction can be observed, even in this process of staining; for whereas the bacillus of leprosy can be stained by the same method as the tubercle bacillus, the converse does not hold good; the former stains by Weigert's plan for colouring nuclei, the latter does not.

The tubercle bacilli appear as small rods of the length of a quarter to half the diameter of a red blood cell. They are not quite straight, but show slight breaks or bends, and often have a tendency to curve.

In tubercular organs the bacilli are variously distributed; they are generally found where the tubercular process is just beginning, or where it is rapidly spreading; they are within the cells near to the nucleus. A cell may contain two, or three, or more bacilli; sometimes they are much more numerous, being arranged parallel to each other in closely packed heaps. At this stage the relationship which previously existed between the bacilli and cells cannot be made out, because the cells, having undergone considerable change, are on the point of death, whilst the nuclei are also undergoing disintegration and transformation into irregularly shaped particles. These become less numerous, until at last all that is left is a homogeneous cheesy mass, which will not stain, and in which all the cells originally present are dead. Few bacilli are present, for they likewise perish, or pass on to the spore forming stage; and in so doing lose their capability of staining, the presence of such spores being revealed by the infective properties of the cheesy substance which contains them. It is important to note the order of these changes. The tubercle bacilli are the primary phenomena; these are surrounded by cells, which, undergoing decay and death, form the caseous masses. The relationship of the bacilli to the giant cells is also noteworthy, although it is now known that giant cells are present in other pathological processes, and are not a specific product of tuberculosis.

As soon as giant cells appear in a tubercular mass they are found to contain the tubercle bacilli. In slow progressing tubercular processes, when the bacilli are present only in small numbers, they are found almost exclusively in the giant cells; whilst in the more rapid processes, the bacilli are present in much greater numbers, the giant cells containing as many as fifty or sixty bacilli in one cell; and it is also interesting to note that a kind of antagonism exists between the nuclei and the organism, the latter being found near the periphery of the cell, or as far as possible from the nuclei. When the bacilli multiply rapidly, they encroach upon the nuclei and rupture their walls, with the result that they fall to pieces and cause destruction of the cell.

Nineteen cases of miliary tuberculosis were examined by Dr. Koch, and in no case were bacilli absent from the nodule; the smaller and more recent the nodule, the larger the number of bacilli present; and in the larger nodules, which showed caseation in their centre, the number of bacilli were found to be decreased. In the liver and spleen of these cases the bacilli were found almost exclusively in the giant-cells; in the meninges of the brain, the tubercles

were found to be rich in tubercle bacilli, frequently in the neighbourhood of the smaller arteries, and sometimes in the interior of the vessels themselves.

Of pulmonary phthisis he examined twenty-nine cases, and detected the tubercle bacilli in all; and in no case in which he examined the sputum from tubercular lungs did he fail to detect the organism; so, as pointed out by him, their demonstration is a matter of great diagnostic importance. Frequently such bacilli are found to contain spores. The organism was likewise detected in the tubercles connected with ulceration of the intestines; and in the adjacent mesenteric glands the bacilli were found in dense masses, chiefly at the periphery of the caseous portions. Lichtheim, Gaffky, and others, have also shown that in the evacuations of phthisical patients, with tubercular ulceration of the intestine, they are also present in large quantities, their peculiar staining properties rendering their detection easy.

In addition to the foregoing, Koch has found the bacillus in cases of tuberculosis of various organs, such as the tongue, kidney, etc., in scrofulous glands, in the granulation tissue in the neighbourhood of tuberculous joints and bones, in the tuberculosis of animals, bovine, equine, in swine, goats, sheep, fowls, monkeys, guinea-pigs, and rabbits.

From these facts, therefore—that the tubercle bacilli are always present in tuberculosis, and are found under no other morbid conditions; that they precede, both as to place and time, all the special pathological changes of that disease; and that their number, their appearance and disappearance, are in direct relation with its course—the conclusion was to be drawn that the tubercle bacillus stood to the disease as its direct cause, and not merely as a chance accompaniment.

To render this conclusion a certainty, it was necessary to isolate the organism from the diseased organs; to cultivate it outside the body, and study its cultural peculiarities; and, lastly, by inoculations with the bacilli freed from admixture with the products of disease, to artificially produce tuberculosis in animals.

Koch obtained pure cultivations by inoculation of blood-serum with material from the interior of lymphatic glands taken from a recently killed tubercular guinea-pig; or from small superficial pulmonary cavities in the human subject, the outer wall of which, after treatment with corrosive sublimate, was removed with hot instruments; or from bovine tubercles. From whatever source the material is obtained, the inoculated tubes must be kept at a constant tempera-

ture of about 37° C. The growth takes place slowly, no growth being visible before the tenth or fifteenth day, when small whitish points or spots appear on the surface; these, if sufficiently numerous, soon coalesce and form a thin, greyish white, lustreless covering on the serum. Other noteworthy points are, that the growth does not cause liquefaction of the serum, nor does it penetrate its substance, but always remains loosely attached to the surface; the growth also takes place in agar-agar peptone mixture, and on solidified hydrocele fluid. The cultures attain their maximum development in about four weeks, and remain unchanged after that time.

It was proved by numerous experiments that, at a temperature of 42° C., no growth occurred in three weeks; at 30° there was but little growth; and between 28° and 29° no growth at all; the best temperature being from 37° to 38°. Owing to this fact, it is evident that the tubercle bacilli do not thrive in the outside world in temperate climates; and, considering also the narrow scope tubercle bacilli have as regards soil, the organism can only be considered as a true parasite, finding the conditions necessary for existence alone in the animal or human organism.

Inoculation experiments with tissues containing tubercle bacilli were carried out, either by inserting in a small pouch like wound of the abdominal wall of guinea-pigs, or in the anterior chamber of the eye in rabbits, small fragments of tissue from various organs in cases of human miliary tuberculosis from phthisical lungs, strumous joints, glands, etc. In this way Koch infected 79 guinea-pigs, 35 rabbits, and 4 cats, and in all, without a single exception, tuberculosis resulted, and in all the characteristic structure of tubercle and the presence of the tubercle bacilli were demonstrated.

Further infection experiments were carried on under the strictest precautions, and with the greatest attention to detail, by the inoculation of bacilli in a pure state, quite isolated from all elements of the body.

In all, some 217 animals were thus infected, made up of 94 guinea-pigs, 70 rabbits, 9 cats, and 44 field mice; and these, without exception, became tubercular. The infection was accomplished by subcutaneous injection, injections into the anterior chamber of the eye, into the peritoneal cavity, into a large vein, and by inhalation. In these experiments, therefore, made with pure cultures, tubercle bacilli, and tubercle bacilli only, could have been the cause of tuberculosis; and this proves tuberculosis to be an infective disease, depending on the presence of tubercle bacilli. Further, in

all cases of tuberculosis tubercle bacilli are present; and the manner of their appearing further proves that they stand to the disease as cause.

These observations of Koch have been generally confirmed by numerous observers—notably, by Weichselbaum, who discovered the bacilli in the blood of a man suffering from acute miliary tuberculosis, and by Mr. Watson Cheyne in this country.

Leprosy.—The connection of a bacillus with this disease was first pointed out by Hansen, in a report made by him to the Medical Society of Christiania in 1874; these observations were subsequently confirmed by Neisser, who described the effect of various staining agents upon them. The organisms are found in the mucous membrane of the mouth, pharynx, and larynx, in the neoplasms of the skin, in the interstitial deposits in the peripheral nerves, etc., and in the lymphatic glands, spleen, and liver. In the skin they are present in the circumscribed nodular formations, as well as in the more diffuse infiltrations. They are found almost entirely in the large round cells described by Virchow as lepra cells. These cells exceed in size an ordinary white blood-cell, and they contain several nuclei. The bacilli are either equally diffused throughout the cells, or they are collected in bundles consisting of six or seven bacilli lying parallel to each other. They are not often found in the connective-tissue spaces between the cells; but their presence has been demonstrated in the blood-vessels.

The bacilli can be stained with fuchsin or with Ehrlich's acid solution of eosin-hæmatoxylin, and they are found to be very delicate rods, sometimes pointed at both ends, 0.004 to 0.006 mm. long, and less than 0.001 mm. thick; in other words, one-half to three-quarters the length of a red blood-cell, the breadth being about one-quarter the length. Some are motile, others not; many possess bright spores, others show a more or less beaded arrangement, consequent upon local collections of protoplasm within the sheath. They can be cultivated in blood serum and solutions of meat-extract.

Inoculation experiments, so far, upon monkeys and domestic animals have failed; but that the bacilli and spores are the cause of leprous growths is shown by the absolute constancy with which the bacilli are found, and the immense numbers which are nearly always present—a fact verified by the examination of material from Norway, Spain, East Indies, Roumania, Palestine, China, and Australia.

It may also be shown that the presence of the bacillus

will cause the typical development of a migratory cell into that of the so-called lepra-cell as described by Virchow. They are introduced into the body either as bacilli or as spores, and remain for a time quiescent; these are probably deposited in the lymphatic glands, the invasion of the system subsequently occurring, the skin, the peripheral nerves, the testicles, spleen, cornea, cartilage, and liver being infected.

Erysipelas.—The contagious character of this disease was for some time overlooked; and, after its recognition in this country, this character was still denied by observers in Germany and France. Numerous observers have discovered bacteria and micrococci in connection with this disease; but, in order to establish the parasitic nature of a disease, it is not enough to demonstrate the presence of bacteria in the organism, nor even to find them in every case of the disease, but there must be proved a specific and morphologically distinct micro-organism. It is to the researches of Fehleisen that we are indebted for our exact knowledge on the subject of the etiology of this disease. He examined some thirteen cases: from eleven of these he excised small pieces of skin, and found the lymph-vessels—both of the skin, especially the superficial layers of the corium, and of the subcutaneous cellular tissue—to be filled with micrococci, particularly in the form of streptococci. They were also found in the lymph-spaces and channels of the skin, but not in the blood vessels. In this latter respect the observations of Fehleisen are opposed to those of other observers, such as Lukomsky, Billroth, etc. In the zone near the sharp edge of the erysipelatous patch the inflammatory changes appear; the tissue of the cutis is swollen; the lymph-vessels are filled with micrococci, and along their course is found a more or less extensive small celled infiltration. In the older parts of the affected skin the micrococci are wanting, the small celled infiltration being alone present.

Cultivations were made from small pieces of skin cut out with heated scissors from a surface which had been thoroughly well cleansed. These were placed on nutrient jellies of various kinds and on blood-serum; the best results were, however, obtained by placing the bits of skin in the jelly which was liquefied at 40° C. In this way they were brought in better contact with the nourishing medium. After being kept for some little time at this temperature, the gelatine was allowed to solidify, and a temperature of 20° C. subsequently maintained. In two days a number of

small white points appear, which grow slowly until the sixth or seventh day, when they come to a standstill, forming a delicate white layer. The organism grows better at the body temperature on coagulated blood-serum, and continues for a longer time.

Inoculation experiments were made with rabbits. Nine of these animals were taken. One of them did not take, the neighbourhood at the site of inoculation being only slightly reddened; the other eight animals developed typical erysipelas. Six of these were inoculated at the tip of the left ear in four different places. Within forty-eight hours the temperature rose, and a sharply defined redness spread from the point of inoculation, mainly in the direction of the veins to the root of the ear. On holding the ear to the sunlight, the affected zone was seen to be of a brilliant red colour, the vessels being distinctly visible owing to dilatation. In two or three days the process reached the root of the ear, and as the ear resumed its ordinary character, the redness spread to the head and neck.

Another rabbit was inoculated on both ears; on each side erysipelas set in, spreading to the back of the neck, and there meeting.

In the eighth case, after inoculation, and the spread of the erysipelas to the middle of the ear, the ear was amputated, and in twelve hours the temperature became normal, and the animal remained perfectly well. On examination of the amputated ear, the lymph-vessels were found filled with micrococci, and the appearances generally coincided exactly with those found in man.

The duration of the erysipelas, with the exception of the last case, was in all the rabbits from six to ten days, and in no case did the animal die.

Human beings were subsequently inoculated with the organism, the inoculations being undertaken with the view of obtaining certain therapeutical advantages—lupus, epithelioma, keloid, carcinoma of the mamma, and lymphatic gland enlargements of various kinds having been partially or entirely absorbed as a result of an attack of erysipelas.

Fehleisen selected cases of nodules in the gluteal region, cancer of the breast, sarcoma of the orbit, and lupus, in all seven cases, and in six erysipelas was induced, in some cases with benefit; in all there was initial shivering, the characteristic redness spreading on the surface, fever, and lastly, resolution without any suppuration.

It is interesting to note that the spread of these micrococci takes place exclusively along the lymphatics; in no case in

true erysipelas has Fehleisen found them in the blood-vessels; the development of the micrococci also takes place primarily in the lymph-vessels, and spreads by active growth in all directions. It is to be distinguished from the organism present in suppuration processes by the clinical fact that suppuration never supervenes upon the inoculation of the true erysipelas micrococcus; whilst, on the other hand, phlegmon can with certainty be produced by inoculation with the other.

In the seventh patient who was inoculated, erysipelas did not supervene, and it was found he had suffered from frequent attacks of erysipelas previously, his last attack being one of the face, only two or three months before; it was proved by experiments on the other cases that an attack of erysipelas will confer a short period of immunity. The same observer found that a 3 per cent. solution of carbolic acid, or a 1 per cent. solution of corrosive sublimate, destroys the vitality of the micrococcus.

Suppuration and Septic Diseases.—Professor Koch has described in a paper, translated by Mr. Cheyne, in the New Sydenham Society's *Transactions*, a number of diseases in animals, which have, in some respects, a great likeness to the surgical infective diseases of man; these diseases were shown to run a definite course, and their specific nature was demonstrated by the reproduction of their prominent symptoms by the inoculation of a specific microbe found in connection with the disease. In this way we have become acquainted with a septicæmia in mice, a progressive abscess formation in rabbits, a pyæmia in rabbits, etc. From such investigations the inference was correctly drawn that it could only be a question of time before the surgical infective diseases of man were more thoroughly studied and their etiology more perfectly known.

Professor Ogston of Aberdeen was the first to demonstrate the constant presence of micrococci in acute abscesses; he pointed out that in some cases these organisms occurred as streptococci, in others as staphylococci, and these he described as distinct organisms. Rosenbach also recognised these two forms, but came to no definite conclusion regarding them; he made a number of cultivations from the pus of thirty unopened acute abscesses, and isolated five different kinds of microbes; one of these, a bacterium, which was twice as long as it was broad, and which quickly liquefied gelatine, he has never since found in unopened abscesses. A little of this cultivation injected into the eye of the rabbit was quickly followed by sub-acute suppuration without any marked constitutional affection.

The organism most frequently found by him was a micrococcus, occurring in large groups, according to Ogston, in masses resembling a fish-roe or bunch of grapes; these groups appear, however, to consist of two kinds, distinguishable by producing different colours when cultivated, the one producing golden yellow opaque colonies, the other white opaque masses; this difference in colour is always present, whether cultivation be made on agar-agar, potatoes, blood-serum, egg albumen, etc.; they agree, however, in their mode of growth, their microscopic character, and their effect on animals, and have been designated staphylococcus aureus and staphylococcus albus.

The other two organisms are described by him as micrococcus pyogenes tenuis and streptococcus pyogenes.

It will be, perhaps, well to say a few words in reference to each of these.

1. Staphylococcus pyogenes aureus. Cultivations of this organism show in twenty-four hours distinct evidence of growth, first of a whitish-yellow, and then of an orange-yellow colour. The growth increases in width, forming roundish masses. It grows more slowly in the cold, and rapidly liquefies gelatine, the growth then falling to the bottom, and becoming of a dark orange colour. It also grows well on blood-serum and potatoes. When exposed to the air, the growth shrivels up and loses colour, and becomes more difficult to inoculate. Excluded from the air, it retains its vitality for a long time. Microscopically, it is a very small coccus. Injection of this organism into the knee or pleural cavity of rabbits or dogs is most deleterious. Rabbits so treated are generally found dead in the morning, and, if they survive, a severe inflammation follows. When injected into the knees of dogs, the animal survives, but an abscess quickly follows.

2. Staphylococcus pyogenes albus. This grows in luxuriant opaque white masses. Microscopically, and in its effects on animals, this organism cannot be distinguished from the preceding one.

3. Micrococcus pyogenes tenuis. This microbe is only occasionally found; the colonies produced by it are so delicate as to be scarcely visible. On agar-agar around the growths these deposits are formed almost as transparent as glass. Microscopically, the cocci are irregular in size.

4. Streptococcus pyogenes. There are two forms of streptococci in connection with traumatic infective diseases: one I have already indicated as being the cause of erysipelas; the other is the one under consideration. Microscopically,

no characteristic distinction exists between these two kinds of cocci, or between these and another, which I shall presently refer to as being connected with puerperal fever; but culturally, and in their effects upon animals, marked differences are observable.

Cultivations on gelatine of the streptococcus pyogenes forms at first slightly whitish or somewhat translucent round dots, which grow sparingly at 20° C. At a higher temperature, on agar-agar, the growth is more rapid, but still the dotted character is preserved. As the culture becomes older, the growth goes on more in the centre, and assumes a slight brownish colour; the outer border has often a wavy, dotted character, produced by the formation of heaps of cocci. It also grows well on blood-serum, and does not liquefy gelatine.

The streptococcus erysipelatosus has often the tendency to form flat rings, the borders of which are thicker and more irregular; the colonies are also whiter and more opaque than in the case of streptococcus pyogenes; there is also a marked formation of processes, giving the culture the character of a fern-leaf. In the depth of gelatine the growth is somewhat more marked also. The most striking difference, however, is in the effects produced upon animals.

Injections of cultivations cause the formation of abscesses or a local inflammatory nodule; but rabbits are not seriously affected by the streptococcus pyogenes. Mice appear to be more sensitive. Rosenbach noted that two out of six mice inoculated died of progressive suppuration on the third or fourth day after inoculation.

These organisms were either found alone or together by Rosenbach; *e.g.*, staphylococcus alone, 16 times; streptococcus alone, 15 times; staphylococcus and streptococcus together, 5 times; micrococcus pyogenes tenuis, 3 times.

The staphylococcus pyogenes aureus was found alone in a case of empyema of a child, and in a case of perinephritic abscess; whilst a pure cultivation of staphylococcus albus was obtained from a case of spontaneous suppuration of the knee joint. They were found together in a case of large abscess of the abdomen.

Rosenbach had the opportunity of examining six cases of pyæmia. The streptococcus was found five times, partly in the blood, and partly in the metastatic deposits during life; twice combined with staphylococcus pyogenes aureus, but in larger numbers. All these cases were fatal; in one, which recovered, staphylococcus aureus was alone found.

Pyæmia may be divided into two classes or groups; the

first consisting of those cases which occur in connection with larger or smaller local affections, but always of great size, such as a suppurating knee-joint, a large, suppurating, contused wound of the soft parts, a suppurating compound fracture, and the like. In these cases the general symptoms are maintained by local deposits, which continuously supply the body with morbid materials, giving rise to the condition described by Ogston as micrococcus poisoning.

The second group consists of cases in which nothing worthy of the name of a local centre exists. The infective material enters through a small puncture or scratch, the general pyæmic affection following a single, often transitory, infection. Rosenbach believes the streptococcus pyogenes to be the cause of pyæmia.

There is an affection resembling erysipelas which is apt to attack butchers, leather-dressers, etc. From a small wound, generally on the finger, a reddish infiltration extends, spreading with a sharp margin, and coming to an end in one or two weeks, over the metacarpus. There is no fever associated with it. A micrococcus was obtained from the part in the same manner as Fehleisen obtained his micrococcus, which grows on agar-agar as beautiful delicate colonies. Inoculations made with them cause a little brown circle, with itching and burning, which slightly increases in size, and then fades away.

With a view of accurately determining the etiology of such acute purulent inflammations as osteomyelitis, whitlow, etc., Garré made a series of inoculations upon himself, into a small wound reaching as deep as the corium made on the outer border of the root of the nail of the left little finger; he placed, by means of a platinum needle, a small quantity of a pure cultivation of staphylococcus pyogenes aureus, obtained from the blood of a patient suffering from acute osteomyelitis; a slight inflammatory redness appeared for a few hours and then disappeared. He then inoculated three small wounds in a corresponding part on the ring finger of the left hand, with the result that pain, inflammation, and suppuration supervened, and from the pus cultivations of the staphylococcus aureus were obtained. He further rubbed over the skin of the left forearm some of the same cultivation; pain, heat, redness, and swelling followed, with numerous pustules, and in four days an enormous carbuncle appeared, which reached its height on the sixth or seventh day; on the eighth day, pus pressed out of the depth of the wound was inoculated on agar-agar and gelatine, and the resulting cultivation showed microscopically and macroscopically the

staphylococcus aureus. From these experiments the deduction is made that carbuncle and whitlow are infective diseases, caused by the same coccus as is found in osteomyelitis, viz., the staphylococcus pyogenes aureus.

Gonorrhœa.—The organism which is connected with this disease, the micrococcus gonorrhœæ (gonococcus), was discovered by Neisser in 1879; it is constantly present in the pus, and very frequently in the epithelial cells; the cocci generally occur in pairs or groups, and their opposing surfaces are flattened; they are found at the very commencement of the disease, and diminish in nnmber as the disease becomes chronic. This organism is difficult of cultivation, not growing on gelatine, but slowly growing on blood-serum, forming a thin, greyish-yellow coating. Bunner inoculated a cultivation into the urethra of a healthy woman, with the result of producing acute urethritis.

Puerperal Fever.—An inquiry into the etiology of this disease I undertook at the request of the committee of the Brown Institute, and in a paper read last November at the Royal Medico-Chirurgical Society, I have recorded the result of my observations. I obtained from the heart's blood of a patient, who had died a few hours previously from well-marked puerperal fever, a micrococcus, which was present in large numbers; from another patient, who was an inmate of St. Bartholomew's Hospital, I obtained the same organism from the blood taken from the finger.

This organism, in its microscopical and cultural peculiarities, closely resembled those already described as belonging to the organism described by Fehleisen as the cause of erysipelas and the streptococcus pyogenes; its effect, however, upon animals is most distinctive; mice inoculated with it invariably die in the course of two or three days, and the organism can be recovered from their heart's blood; and the ears of rabbits inoculated with it show a well-marked diffused redness, which shows no disposition to spread,and very shortly disappears.

Glanders.—This disease in man is very rare, and it is only within the last sixty or seventy years that its occurrence has been clearly recognised; there is no evidence to suggest that the disease ever originates in man, but is generally communicated by direct inoculation of virus from a diseased animal.

Researches into the etiology of this disease were undertaken by Dr. Löffler and Prof. Schütz. They first sought for definite forms of bacteria among the specific products of glanders contained in the glanders nodules; sections were

also obtained from a horse which had suffered from glanders of the lungs, liver, spleen, and septum nasi; after treatment by various staining agents these were examined microscopically, and fine rods, about the size of a tubercle-bacillus, were occasionally found.

Cultivations were made from a number of small particles taken from the nodules present in the lungs and spleen of a horse which had suffered from glanders, on the blood-serum of a horse, and in three days numerous transparent droplets were found on the surface, which, upon microscopic examination, were found to consist of bacilli, presenting the same characters as those previously found in the nodules; they are very minute, being about the size of a tubercle bacillus; they are brought out by staining with a concentrated watery solution of methylene blue, and then washing with very dilute acetic acid, the water subsequently being extracted by alcohol and the preparations cleared in cedar-oil.

These cultivations were carried through four generations, and then a small quantity was inoculated on the mucous membrane of the nose, and on both shoulders of an old, but otherwise healthy, horse; in two days the animal exhibited signs of high fever, and in eight days showed all the well-known clinical appearances of glanders. The animal was killed some five or six weeks after inoculation, when, upon examining the body, evidence was forthcoming that the animal had suffered from old glanders, so that the experiment could not be considered as absolutely conclusive; fresh cultivations were, however, made from the glanders material upon sterilised blood-serum, with the result that the same transparent droplets in the course of three days appeared, which were found to contain the same bacilli. The same organism was obtained by cultivation from several other glandered horses.

Rabbits, mice, and guinea-pigs were inoculated with the cultivation. In the rabbits, in some cases, local ulcerations and swelling of the neighbouring glands were found; in others ulcers on the septum nasi and glanders nodules in the lungs.

Inoculation of white-mice gave negative results; but of field-mice the results were satisfactory; at the *post-mortem* examination of these the spleen and liver were found infiltrated with small yellowish-grey nodules, from which the bacilli could be obtained.

In guinea-pigs the results were well marked at the site of the inoculation. An ulcer appears with a very indurated base, which speedily enlarges; other ulcers follow, the neigh-

bouring glands enlarge, and general infection follows; nodules and ulcers appear on the nasal septum, and nodules in the internal organs, followed by nodular growth in the testes, ovaries, and vulva; cultivations from all these organs always produced the same pure cultivations, identical with those previously obtained from the horses.

Two horses were now inoculated, the one with a cultivation which had been carried through eight generations, the other with a cultivation carried through five generations. Both of these animals developed the well-known signs of glanders, and the usual *post-mortem* appearances of the disease were found; one of the animals died from the disease, the other was killed; the organism was recovered in the ordinary way by inoculation of blood-serum with particles of the nodules.

Relapsing Fever.—In the blood of persons suffering from this disease a spirillum—the spirillum Obermeyeri—is found; they disappear from the blood during the non-febrile stages; they form long, wavy, flexible threads of varying length; they are motile, and stain well with methyl violet or Bismarck brown. Dr. Carter succeeded in producing relapsing fever in monkeys by injecting the human blood containing the spirillum; the monkey's blood was found to contain the organism in large numbers. Koch has cultivated it artificially, and found it growing as long spiral threads.

Actinomycosis.—The actinomyces, or ray-fungus, which is the cause of this disease, grow in roundish masses, varying in size from small grains to those as large as a hempseed; they are of a yellowish colour, and have a granular surface; microscopically the lumps are seen to consist of a number of radiating threads, with swollen club-shaped ends, forming a sort of rosette; frequently narrow, thread-like hyphæ are seen forming coils in the central parts, or growing peripherally out of the mass. In cattle the disease most commonly begins in the mouth, by the formation of firm tumours in the jaw, the alveoli of the teeth, and particularly by an enlargement and great induration of the tongue, giving rise to the so called wooden tongue; occasionally similar tumours are found in the skin and lungs.

Dr. Israel was the first to point out a disease in man characterised by metastatic abscesses in various internal organs, due to the presence of a fungus, which was proved to be "actinomyces"; since then a large number of cases have been observed, and one was reported recently at a meeting of the Royal Medico-Chirurgical Society by Dr. Douglas Powell and Professor Crookshank. Dr. Israel states that he

has cultivated the actinomyces on solid ox-serum; in fluid media the growth does not succeed, owing to. the swelling up and death of the corpuscles.

Anthrax.—This disease in its various forms has been known for many hundreds of years as affecting both man and animals; but until comparatively recent years the internal form of the disease received but little attention. In 1878 Dr. Russell recorded an outbreak amongst horse-hair workers in Glasgow; and in 1880 Mr. Spear, in his report to the Medical Officer of the Local Government Board, pointed out that the so called woolsorters' disease, so prevalent in Bradford, was nothing more than anthrax. There are two principal varieties of the disease, one presenting a characteristic malignant pustule, the other having no external lesion; these two forms are, however, etiologically identical. A third class is sometimes made, viz., that of malignant œdema; and a fourth variety consists in a small pustule, or slightly inflamed tumour or boil, which does not possess the striking characters of the malignant pustule.

From the report before mentioned, it is gathered that the disease has been known in Bradford since the importation and working of alpaca, but that the cases have been more numerous since the introduction in 1863 of the Van mohair, the fleece of a goat inhabiting the Van district of Asia Minor, as a textile fabric.

From a careful examination of a large number of cases, Mr. Spear shows very clearly that the symptoms of the disease, as it affected the Bradford workers, were those of anthrax; and this view was confirmed by the microscopical examinations and pathological experiments made by Dr. Greenfield, for it was shown that the blood, the pericardial fluid, and various tissues of the body contained an organism having all the characters of the bacillus anthracis; further, that if animals, such as guinea-pigs, be inoculated with these fluids, effects would be produced identical with those produced by inoculation with the blood of animals dying of splenic fever, such effects being death in a brief space of time, and the presence in the blood of the anthrax organism; and, further, that the organs of such inoculated animals, when examined microscopically, showed lesions similar to those seen in animals dying from anthrax inoculation.

Let me quote one or two examples. A man named Greenwood, aged 48, was attacked with the disease. Blood was taken from his arm, which was found to contain a few bacilli, and inoculations were made of guinea-pigs on the inner side of the thigh. These animals died in twenty-nine

hours. An examination of their bodies showed the typical characters of anthrax, and bacilli were recovered from the spleen and heart's blood. Upon the death of this patient, guinea-pigs were inoculated with blood obtained from the heart, with fluid expressed from a bronchial gland, and with serum obtained from the pleura; and in every case the animals died within forty-eight hours. The blood, on examination, was found to contain large quantities of the anthrax organism, and the spleen, which was enlarged, also swarmed with bacilli. Cultivations in aqueous humour, made from the splenic blood of two of these animals, produced typical growths of the bacillus.

Another case was that of a man named Heaton, who died after exhibiting well pronounced symptoms of this disease. Inoculations of animals were made with blood obtained from the spleen, and with fluid obtained from the lungs. In both cases the animals died within forty-eight hours; bacilli were found generally in the body; the spleen was much swollen, and contained numerous masses of bacilli.

An observation upon which Dr. Greenfield laid great stress, as affording evidence of the mode of infection in woolsorters' disease—viz., by the inhalation of the spores adhering to the wool of the fleeces of animals dead of anthrax—was the co-existence of mediastinal cellulitis, an engorged or hæmorrhagic condition of the bronchial glands, great serous effusion into the pleuræ, causing collapse of the lungs, and, lastly, the presence of a number of multiple pustules in the bronchi.

From recent observation by Dr. Koch and others, it appears certain that infection by the alimentary canal can be readily produced by spores.

The single bacilli, as they appear in the blood, measure between 0.005 and 0.02 mm. in length, and 0.001 to 0.0012 in thickness, and are truncated. The spores, produced by growing the bacilli with free access of air, are about 0.001 mm. thick, and about 0.002 to 0.003 mm. long. They are not stained by the ordinary dyes. They grow well in any fluid containing proteid material between the temperature of 15° and 43° C., but best between the temperatures of 25° and 40° C. They elongate and divide rapidly, and grow out into long, curved, and twisted filaments, forming bundles. Microscopically they are seen to be composed of a thin hyaline sheath, which contains a row of cubical or rod-shaped masses of protoplasm, which stains readily with aniline dyes. According to the length of the bacilli, the number of these masses of protoplasm varies. Some of the elements appear

constricted in the middle, preparatory to division; the elements are connected by a fine septum. When grown on a solid medium, such as gelatine or agar-agar, some of the elements assume a torula-like form, and, as such, multiply by gemmation and division, and form clusters, or arrange themselves in chains.

The anthrax-bacillus liquefies gelatine. When grown in the depth of a fluid, the bacillus does not form spores; but, grown on the surface, with free access of air, the bacilli having developed into filaments, proceed to form spores. Spore formation occurs at all temperatures, from 18° to 45° C., and this consists in the appearance of bright, glistening, spherical bodies in the protoplasm of an elementary cell. This body enlarges, and ultimately becomes of an oval shape. Under the most favourable conditions, each cubical or rod-shaped mass of protoplasm includes one spore, in which case the filament would contain an almost unbroken row of spores. In a dry state these spores stand heating to 100° C. for over an hour without being killed; but in the moist state, at exposure to steam at 100° C., they are killed in fifteen minutes. Many interesting and important experiments have been made with this organism by Pasteur, Koch, Klein, and others; but these are beyond the scope and intention of this sketch.

There are other diseases with which micro-organisms have been found connected, such as cholera, scarlet fever, diphtheria, pneumonia, syphilis, enteric fever, etc.; but these I refrain from considering, partly because the connection has not been generally admitted, and partly because, in the case of one of them—scarlet fever—it has but very recently been brought under the notice of this Society by my friend, Professor Klein.

I am indebted to the writings of Dr. R. Koch, Dr. Fehleisen, Mr. Watson Cheyne, and others for valuable information in the preparation of the foregoing paper.—W. R. S.