

It is easy to ascertain whether the nerve cells of the brain are in this condition. Let us inject into the cerebral substance of a rat one-tenth of a cubic centimetre of diphtheria toxin; this dose, when introduced under the skin of another rat, does not produce even a local œdema. Nevertheless, the one which has received the toxin into the brain is soon attacked with complete paralysis. It remains paralysed for two or three days and dies.

The brain of the rat is thus sensitive to the diphtheria poison, and if this animal does not die as the result of the injection of large quantities of toxin into the subcutaneous tissue, that is because the toxin does not reach the encephalon. It is arrested in some part of the body. The natural immunity of the rat with regard to the diphtheria poison is not at all due to the resistance of its nerve cells, but to some other property of the organism.

The rabbit is supposed to be very refractory to the action of morphia; a hypodermic injection of 30 centigrammes of a salt of this alkaloid is quite well borne by an animal of 2 kilogrammes. The introduction of a single milligramme of hydrochlorate of morphia into the brain causes almost immediate effects in a rabbit of the same weight. The limbs are agitated by a trembling movement, progression is impossible; the animal remains stupefied from twenty-four to thirty hours, then it appears to improve, but it emaciates and dies in four or five days.

The nerve cells of the rabbit are thus not insensitive to morphia. When this rodent resists the injection of a large dose of the alkaloid it is because that does not reach the cerebral cells.

The facts which we have just recorded show that in acquired immunity, as in natural immunity, against certain poisons of the nervous system, the resistance is not due to an accustoming or an insensibility of the nerve cells, at least not of the nerve cells of the brain. Toxins introduced under the skin and into the blood do not attack these cells, although they have a manifest affinity for them. These poisons are without doubt retained by other cells, which play a protective rôle and probably manufacture the antitoxins. What are these cells? Perhaps the phagocytory cells which in many circumstances one sees capable of destroying the poisons contained in the bodies of bacteria. We cannot affirm that such is the case, but it seems to us that the problem of immunity against bacteria and that of immunity against the toxins will receive similar solutions.¹

THE EFFECTS OF DEAD TUBERCLE AND GLANDERS BACILLI ON ANIMALS.

By STEWART STOCKMAN, Professor of Pathology, Dick Veterinary College, Edinburgh.²

THERE are two principal methods whereby the pathogenic microbes act on the animal organism. *Firstly*, by the products of their meta-

¹ See article by Metchnikoff, "Toxine tétanique et leucocytes," *Annales de l'Institut Pasteur*, April 1898.

² Most of the work in this paper has been done in the Laboratory of the Royal College of Physicians, Edinburgh, and I wish here to acknowledge my indebtedness to that Institution.

bolism in the tissues—the toxins—they poison the system. *Secondly*, they so act on the tissue cells that a lesion is produced. These two actions are in a sense related to each other, for it cannot be denied that in the majority of cases the bacterial products are largely responsible directly or indirectly for the lesion, although the purely mechanical effects of the microbes, such as embolism, cannot be altogether disregarded. The systemic effects of the toxins are of the first importance in such fatal diseases as tetanus and anthrax, but they are secondary, although important enough in the contagious diseases that tend to run a chronic course, such as glanders and tuberculosis. Of course everyone knows that acute and rapidly fatal cases of glanders and tuberculosis do frequently supervene on chronic ones, and in these we assume from the wide distribution of the young lesion, that the poisons have been administered to the system in unusually large doses.

In the majority of cases, however, these two diseases run a chronic course, and the principal inconvenience arises from the lesion. There is a great deal of work to be done yet in the study of the tissue reaction, both as regards what actually takes place and how far the bacterial products are responsible for it. With the microbes of suppuration, tubercle, glanders, actinomycosis, and some others, the initial lesion presents many points of similarity—attraction of leucocytes, degenerative changes, etc.,—but after a time they, as it were, part company, so that in many cases, though by no means in all, it is possible to say from a simple histological examination which of these microbes is responsible for a certain lesion. That is evidence of the toxins being largely answerable for the lesion, because they are special in each case.

In most cases the appearance and seat of the lesions in tubercle, actinomycosis, and glanders, when taken with the species of animal from which they have come, give us a good idea of what disease we have to deal with. The presence of a special microbe revealed by the microscope or experimental inoculation puts the diagnosis beyond doubt. It not infrequently happens, however, that tuberculous lesions appear in an organ which is much oftener the seat of actinomycosis, and *vice versa*. Take for examples, tubercle in the lower jaw of the ox, actinomycosis in the udder or lung of the cow, and the so-called clyers, which may be due to the tubercle bacillus or the actinomyces. A microscopic examination may fail to reveal the presence of any of the micro-parasites in question, and one has to fall back on experimental inoculation, which is tedious and oftentimes impossible.

In the majority of cases of actinomycosis in animals we have to deal with what is believed to be an involution form of the parasite, and inoculation is useless. Moreover, if one examines lesions from an animal that has been treated with iodide of potassium, one may fail to find a single parasite, at least such has been my experience. As regards glanders, much of our present knowledge concerning some forms of the lesion is comparatively recent and dates from the introduction of mallein, which has established the glanderous nature of certain lesions hitherto misunderstood. A microscopic examination of the glanders lesion in the horse seldom reveals the presence of the *Bacillus mallei*, and the recent researches of Nocard seem to show that experimental inoculation and artificial cultivation may give negative results. Indeed,

it has been shown that mallein may fail to produce a reaction in the living animal although lesions of an apparently glanderous nature may be found at the autopsy. These cases Nocard regards as cured, and it is thus that he would explain the negative results of inoculation and artificial cultivation. The study of the tissue reaction to the dead and living bacteria, then, becomes all the more important. The lesions of glanders have been recently studied by M'Fadyean,¹ who first demonstrated the presence of giant cells in glanders nodules. Nocard² has studied them also from an experimental point of view. Schutz³ has recently published the result of a very exhaustive study of the histological lesions in glanders. These papers I shall have to refer to afterwards.

Knowing that dead microbes are sometimes capable of causing a lesion, it becomes all important to know how that caused by the living differs from that produced by the dead, if it differs at all.

EXPERIMENTS WITH DEAD TUBERCLE BACILLI.

Such experiments have been undertaken before. Koch showed that dead tubercle bacilli produced an abscess in guinea-pigs when injected subcutaneously.

Prudden and Hodenpyl⁴ experimented more fully on guinea-pigs and rabbits.

In their experiments they used dead tubercle bacilli from cultures on glycerine, agar, and broth. In some of their experiments they used bacilli that had been freed from their soluble products, but they found that the results were the same whether they used bacilli from agar or broth, and whether these had been washed free from their soluble products or not. They concluded that the lesions produced were due to some substance—bacterio-protein—set free by disintegration of the microbes in the tissues, or extracted in some other way. They incline more to the former view.

By subcutaneous inoculation they produced an abscess in from two to six weeks. Tubercle bacilli stainable by the ordinary methods were found in the pus.

By intraperitoneal and pleural inoculation with a milky emulsion of bacilli they produced nodules of various sizes on the serous membranes. These were made up of a central creamy looking part surrounded by fibrous tissue. The central part consisted of epithelioid cells and giant cells. Tubercle bacilli were abundant in the central part. Well marked caseation was not found.

Intravenous inoculations were made into the auricular veins of rabbits. The animals were killed and examined at intervals of from one to sixty days. A few died after the third week. In animals killed after one day the bacilli were found in the lungs, liver, and spleen, most abundantly in the first mentioned organ. The older the tubercle the fewer seemed to be the bacilli. After five days white nodules were found in the lung, some being microscopic, others quite visible to the eye. They were present up to the sixtieth day, the longest period of observation. Their structure consisted of epithelioid cells,

¹ "Journal of Comparative Pathology and Therapeutics," March 1895.

² Nocard, "Recueil Vet.," March 1896, November 1897.

³ "Journal of Comparative Pathology and Therapeutics," March 1898.

⁴ "New York Medical Journal," 20th June 1891.

giant cells, and leucocytes. Bacilli were found between the cells and inside giant cells. Later the nodule was denser and made up of epithelioid cells and loose connective tissue. After three weeks microscopic nodules, apparently having their origin inside the capillaries, were found in the liver. After five or six weeks visible nodules were found in the latter organ. Prudden and Hodenpyl conclude that the nodules originate in a proliferation of the vascular endothelium under the stimulus of dead and disintegrating tubercle bacilli. They say that "the dead bacilli seem to act as foreign bodies simply, curiously stimulating, it is true, but only foreign bodies after all." I think too much is made here of the foreign body question. Every foreign body does not produce this tissue reaction, although the power to do so may not belong exclusively to the tubercle bacillus. The action of the dead tubercle bacillus is in large part, at least, special to it, and it is the foreign body of this order that we are most likely to meet with in the tissues.

At the conclusion of their paper these authors offer some suggestions as to the influence of the products of the living germ on the degenerative changes in a true tubercle. They suggest, too, the possibility of the more fibrous tubercles being due to dead bacilli.

Curiously enough it was that last idea, and a wish to further test an opinion which I had formed as to the origin of tuberculous giant cells, that led me to undertake some experiments with dead bacilli. I had conceived the idea and started experimenting with dead bacilli before I knew of their paper. I have since read it very carefully, and wish here to acknowledge my indebtedness to the authors. I have performed most of my experiments on different animals, but many of the results confirm those of Prudden and Hodenpyl.

I must also mention that Straus and Gamaleia¹ have to some extent confirmed the results of the American authors by experimenting on guinea-pigs, rabbits, and dogs. They say little about lesions in the liver. They did not find giant cells in the nodules. That may be because in their experiments too few bacilli were arrested in one part, or because the centres of the nodules were not examined. Many of their animals wasted and died. When the clumps of bacilli were broken up and well distributed in the fluid the animals wasted and died all the same, but no lesions were found. Evidently several dead bacilli are necessary in one part to produce a lesion. If the number of bacilli was very small the animals wasted, then recovered and appeared quite healthy, but if a second small dose were administered they wasted and died. By using very small and ever-increasing doses of the dead bacillus they immunised the animal against it. With the products of the bacillus in artificial culture they could produce no lesion.

I would like first to say a few words about tuberculosis of the udder.

The tuberculous lesions in the udders of cows are extremely interesting, and suggest many possibilities.

If one examines a number of tuberculous udders from the cow, one generally finds distinct caseous nodules, but that is not the only form that the tuberculous lesion may assume. At the Edinburgh abattoir

¹ "Archives de Medecine Experimentale et d'Anatomie Pathologique," 1891.

during the last five years I have found a considerable number of very cirrhotic udders without any appearance of a caseous nodule in their substance. On examining these microscopically I have found tubercle bacilli and tuberculous giant cells, although the former were not very numerous. For that reason I think that the percentage of tuberculous udders has been slightly underestimated. These udders, however, will not very much increase the amount of tubercle-infected milk, because the affected quarters give little or no milk, and the cow is soon sent to the abattoir. The bacilli enter the udder by way of the blood vessels, and it is highly probable that at an earlier date there were distinct caseous tubercles in these udders now cirrhotic. Two possible explanations of the difference in these two lesions suggest themselves:—

(1.) The bacilli might have been overcome by the tissues to such an extent that they could no longer produce distinct caseation, but were still able to excite a proliferation of the tissues, and cause the formation of giant cells.

(2.) The bacilli might have arrived in the gland in an attenuated condition, and were thus no longer able to produce the distinct caseous lesion.

There is nothing wildly imaginative in these suggestions if we think on what we know of the tubercle bacillus. It is well known that the bacillus first acts by exciting a proliferation of the tissues. The new cells, instead of completing their development, however, tend to become caseous. Still, we know that the cells do sometimes complete their development and form fibrous tissue. This is especially the case in the old tubercles found in the ox and pig. They are often surrounded by a rim of fibrous tissue, which is invading the caseous centre. Moreover, the tuberculous lesions of the muscles—muscle is considered a bad medium for the growth of the tubercle bacillus—which I have described in the pig were distinctly fibrous.¹

I may say, too, that I have once found tubercle bacilli in fibrous nodules under the mucous membrane of the fourth stomach of the ox, and have several times found them in fibrous thickenings on the subcarpal regions of the ox. These are mainly the observations which have led me to undertake these experiments.

Experiment I.—Irish terrier bitch. I began to experiment on this animal for the purpose of trying to render her immune against tuberculosis by using the toxin and dead bacilli. I first injected her with tuberculin in doses varying from 10 cc. to 30 cc. There was no temperature reaction. I then continued with injections of the products of the tubercle bacillus in glycerine broth, from which the microbes had been separated by filtration through porcelain without previous heating. The cultures were allowed to go for from two to two-and-a-half months and longer. Beginning with 6 cc., I went to 10, 20, 40, 50, 95 cc. without disturbing the animal at all. With 95 cc. the temperature rose 8° F., but was not above the physiological limits (102·6°). I next gave her 190 cc. into the vessels and under the skin. This toxin came from a two-and-a-half months' culture. It produced a rise in temperature of 1° F. in six-and-a-half hours. This shows the feeble systemic effect of the tubercle toxin. Thinking that the toxin prepared

¹ Stockman, "Veterinarian," March 1896.

in the ordinary way was too weak to produce much effect, for there followed no local lesion whatever—it thus differs from the powerful diphtheria toxin—I thought of trying the effects of dead bacilli. Under the skin of the chest region I injected a thick emulsion of tubercle bacilli killed by three hours' steaming. The bacilli came from a glycerine broth culture that had been kept for five months. An abscess formed and burst three days afterwards. A month later a second injection of bacilli in thick emulsion was given under the skin. The latter came from a glycerine broth culture that had been in the incubator for four months, and afterwards killed by three hours' steaming. On the third day a swelling about the size of a hen's egg formed at the seat of inoculation. The skin over this abscess was aseptically, and some pus was aspirated into a sterilised syringe by passing its needle into the cavity. Cover-glass preparations made with the pus simply swarmed with tubercle bacilli; no other microbes were present. Glycerine agar tubes sown with the pus remained sterile. The abscess then was caused by the tubercle bacilli, which were beyond doubt dead. The abscess burst on the fifth day, and the wounds took a considerable time to heal up. The temperature rose to 105° F. on the morning after the second injection. It fell to 101.5° on the sixth day. On the afternoon of the sixth day 30 cc. of a filtered tubercle culture were injected. The temperature next morning had risen to 104.5° , and had fallen to 102° the day after. Three weeks after the second injection of bacilli I injected subcutaneously about 4 cc. of the dregs left in the preparation of tuberculin. This material was very rich in bacilli, which had been killed at about 110° C. Five days afterwards there was slight swelling and tenderness, but no distinct abscess formed. The temperature rose 1° on the day after, and fell to the normal on the fourth day.

One might draw the following conclusions from this experiment:—

(1.) That the soluble products of the tubercle bacillus produce little effect on the healthy organism, although they have a very decided action on animals whose bodies contain the tubercle bacillus, living or dead. (2.) That the dead bacilli are far more active than the soluble products, although this may be on account of their retaining a strong toxin in their bodies.

This bitch ran about and enjoyed perfect health for ten months afterwards. I was anxious to see if she would still react to the dead bacilli. On the 22nd March of this year the daily temperature ranged between 101.8° and 102.4° F. She received into her jugular vein the greater part of the bacilli from a two months' culture of the tubercle bacillus in 50 cc. of glycerine broth, which had been sterilised at 110° C.

23rd March. Temperature 105.5° . Animal rather dull.

24th March. Temperature 104° . Animal brighter.

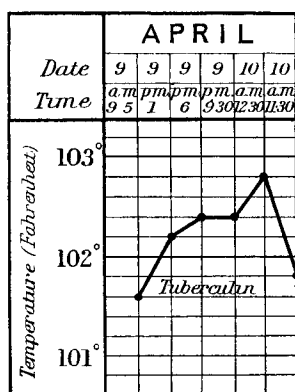
25th March. Temperature 103.8° . Animal brighter.

26th March. Temperature 103° . Animal apparently quite well.

Up to the 3rd April the temperature fluctuated between 103.2° and 103.4° F. From the 4th to the 8th April the daily temperature at 12 o'clock was 102.4° . On the 9th April (seventeen days after), at 9.15 A.M., when the temperature was 101.6° F., 20 mm. of the dilute tuberculin were injected. My reason for starting at this hour was, of course, to get the observation over in one day.

The annexed chart will show the temperature reaction.

CHART I.



The variation was 1.2° , but it did not go much beyond the physiological limits. The results of tuberculin in the dog, however, have not been satisfactory—so far at least. Cadiot records the result of the test on fifteen tuberculous dogs. The rise in temperature amounted to from $.5^{\circ}$ to 1.7°C . In eight cases the temperature rose no more than one degree. In three cases there was no rise, but a diminution of temperature followed by death.

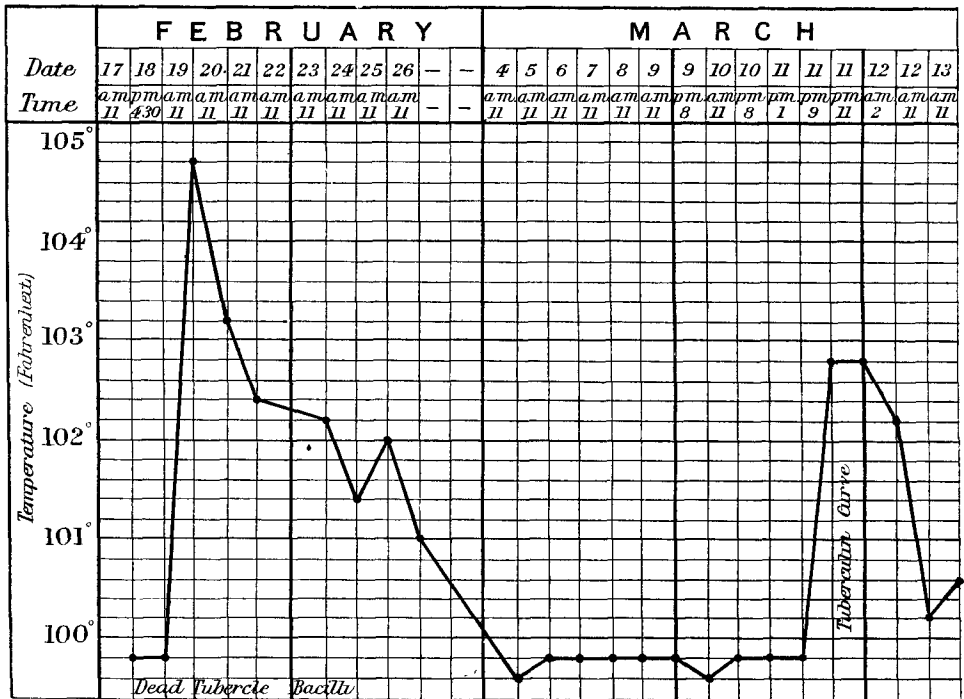
The animal is still alive and has always enjoyed perfect health. I am observing her to see whether she will develop a fibroid condition of the lung.

Experiment Ia.—Spaniel bitch. With a view to raising the temperature for a certain experiment of his own, Dr Noel Paton injected some dead tubercle bacilli into the external saphena vein of this animal's hind leg. He killed her twenty days afterwards, and was good enough to let me have the lungs and portions of the liver. The lungs contained many small, firm, greyish nodules, about the size of a pin's head. I have not yet examined these microscopically. The liver showed no alteration to the naked eye, but the microscope revealed small cellular collections identical with those found at the start of an ordinary tubercle caused by the living bacillus entering by way of the blood-vessels. The temperature of this animal did not rise, but I think too few bacilli were used.

Experiment II.—An aged pony was tested with tuberculin on the 17th February 1898. It did not give any reaction. The normal temperature was 99.8°F . On the 18th February two cultures of the B. Tuberculosis in 55 cc. of glycerine broth, which had been incubated for two months, were killed by submitting them to the boiling temperature for one hour. The fluid was pipetted off until the bacilli were left as a thick emulsion. As one can understand, the microbes were very numerous. At 4.30 P.M., the bacilli from the two cultures were injected into the left jugular vein of the pony with all antiseptic precautions.

The annexed chart will show the temperature reaction after the inoculation, and also the result of the tuberculin test applied three weeks afterwards.

CHART II.



The reaction was 3° F. It will be seen that the temperature after the injection of dead bacilli fell to the normal just about the time that it should have been rising had the animal been inoculated with living bacilli. The animal was afterwards killed for the dissecting room, twenty-five days after the bacilli had been injected.

Autopsy.—The lungs were simply studded with small white nodules, varying in size from a pin point to a pea. They to some extent resembled miliary tubercles, but they differed from them in being denser and less yellow. One or two small areas about the size of a bean were very solid and of an amber colour. The latter much resembled the larger nodules that one finds in the lungs of sheep affected with parasitic pseudo-tuberculosis. There was also slight broncho-pneumonia. The jugular vein at the seat of inoculation showed a nodule in its wall. This nodule was about the size of a pea nut. It was comparatively firm, but showed one small softened centre. Cover-glass preparations, made with the softened material and stained by carbol fuchsin, showed numerous bacilli. None of the other organs showed lesions visible to the naked eye. With several nodules from the lungs guinea-pigs were inoculated into the peri-

toneal cavity. They did not become tuberculous. The bacilli then were dead.

Microscopic.—The lung nodules are made up chiefly of epithelioid cells and loose connective tissue, containing a good many spindle cells. There are a few giant cells in some of the nodules. These giant cells, however, are fewer in number and not quite so distinct as those found in natural cases of tuberculosis. I shall afterwards state my ideas as to the significance of the tuberculous giant cell. Numerous bacilli are present between the cells of the nodule, either as single rods or in clumps. At some places they have assumed a somewhat circular arrangement, such as one often finds at the periphery of the giant cells in an ordinary tubercle. The bacilli are easily stainable by the ordinary methods used for the tubercle bacillus. There is no distinct caseation, but there are some very small homogenous patches which stain diffusely.

A few abortive giant cells and a few better formed ones are also present. The best method of showing the giant cell is to stain with picro-carmin a section cut on the freezing microtome. It is difficult to find them in thin sections cut in paraffin, although distinct giant cells are well enough seen in sections of ordinary tuberculous nodules cut in the latter way. Contrary to what one finds in the ordinary tubercle, those caused by the dead bacilli are vascular. The lumen of the smaller artery, however, is often narrowed by a periarteritis.

Vein.—The nodule in the wall of the vein has a similar appearance to those found in the lung. The cellular centre, however, is more extensive, and there is a tendency for it to drop out of unembedded sections.

It seems to me that the giant cells are most numerous in the larger nodules, and these contain the greatest number of bacilli.

I attempted to repeat this experiment on another horse, but the animal died of embolism, in great part I think due to air bubbles in the fluid.

I have now another animal under experiment. I will inject him with tuberculin at several intervals in order to see if he will cease to react.

Experiment III.—This experiment was undertaken to see whether a very large number of dead bacilli in one part would not produce distinct caseation or a more plentiful formation of giant cells. For the experiment I used tubercle bacilli that had been incubated in glycerine broth for two months and then submitted to a temperature of about 110° C. for half-an-hour. The dead culture had been kept for nine months and it was sterile. On the 20th April a dense emulsion of these bacilli was injected directly into the lung through the chest wall, using a very fine needle. From the 20th April to the 2nd May the temperature ranged between 100·8° and 101·3° F. The animal was well and lively all the time. This cat was killed by chloroform on the 23rd May, about one month from the time of inoculation. A large nodule about the size of a pea nut was found in the lung. The nodule was not caseous. The pleura was quite normal. The lung nodule was fixed in corrosive sublimate for microscopic examination.

Microscopic.—The nodule is made up of epithelioid cells and spindle cells. There are very few giant cells, not one distinct. The bacilli

are very numerous. There seems to be less fibrous formation than in the horse. The lung of the cat is, I think, not so well suited as that of the horse for the study of giant cell formation. Pulmonary tubercle of the cat is often in the form of broncho-pneumonia without distinct nodules, but of this I do not wish at present to make too much, as I have not had the opportunity of studying many cases.

Experiment IV.—The object of this experiment was to see whether the dead bacilli would act through the alimentary tract or not. So far as I know this has never yet been tried. The importance of such experiments is great, because once we have boiled suspected milk or flesh we consider that the danger is past. For the experiment I used a very young kitten, as they are considered easy to infect with tuberculosis by the ingestion method. On the 25th of April a kitten, six weeks old, received about 10 cc. of the emulsion used in Experiment III. This kitten must have swallowed millions of dead bacilli. She lived in perfect health until the 23rd of May, when she was killed by chloroform. All the organs appeared healthy. The mesenteric glands and portions of the ileum were fixed in corrosive sublimate for microscopic examination. They were found perfectly normal. Sections stained by carbol-fuchsin showed not a single bacillus. At present I have another cat under observation. This animal is receiving periodical doses of the dead bacilli *per os*. I also intend making some observations on the effect of the gastric juice on the living and dead microbes.

SIGNIFICANCE OF THE GIANT CELL.

It is usually accepted that the tuberculous giant cell is the result of a multiple division of the cell nucleus without a corresponding division of the cell protoplasm. By tuberculous giant cells I mean the apparently multinucleated bodies with a yellowish centre. I do not think that the multiple division theory applies to the giant cells found in animals. I cannot see how degenerating cells, for such they seem to be, could be capable of performing a function which is usually regarded as a proof of vitality, viz., reproduction.

Moreover, I have never observed mitosis in well formed tuberculous giant cells. This, however, is not an insuperable objection, as they might divide by another method.

I have seen several cells together apparently taken with degeneration in the act of nuclear division. The nuclei of these, however, were very indistinct, and their degenerate bodies had not yet completely fused. I do not think that the so-called tuberculous giant cell is really a cell, but that it is simply the commencement of caseation. I would explain its formation somewhat thus:

When the tubercle bacillus arrives in an organ it begins to multiply and stimulates the cells to proliferate; a nodule is the result. The bacilli will be most numerous in the part where they are first arrested. Their products will there be more concentrated, and one may reasonably expect to get the most marked initial changes at that place. Now, the local action of the living tubercle bacilli on the tissues is to produce caseation as well as to cause proliferation, and the former will begin where the microbes are most numerous. The cells in the neighbourhood of the bacilli, then, will be the first to undergo caseation; their bodies stain yellow with picro-carmin.

The nuclei resist longer, and they, along with broken up chromatin substance, can be seen for a considerable time in the degenerated patch.

It is known that the nucleins are not acted on by the gastric juice digestion, and can thus be separated from tissue cells. Possibly they are less easily acted upon by the toxins than the cell bodies, and hence their persistence. In further support of this view of the giant cell I would point out that colonies of bacilli are frequently, though not always, found around the margins of the so-called giant cells, and in the tubercles produced by dead bacilli intravenously injected in the horse the giant cells seem to be most numerous where the bacilli have been arrested in clumps. The apparent absence of bacilli from the neighbourhood of certain giant cells might be explained by supposing that the damage was due to a very small number of active ones, too few to be easily found in sections. One cannot say but what a few living bacilli may be as powerful as a large number of dead ones. Again, it might be that the tissues have overcome many of the bacilli in certain nodules and prevented the changes from going further. The dead bacilli experimentally injected certainly do in time disappear from the nodules, and we may expect those killed *in situ* to do the same. In the centre of the so-called giant cell one may find a single stray nucleus; usually one sees only chromatin substance, but even that may have disappeared. Many of the nuclei at the margin are breaking up. It is at the margin of the giant cell that they are mainly found, and they may be to the number of twelve or more. If these nuclei be carefully looked at, one often sees that they are in reality surrounded by the cell bodies in different stages of degeneration. This is best seen in the tubercles provoked by the dead microbes, possibly because the caseation is neither so rapid nor so marked as in the case of active living bacilli.

In some of the former one can see contiguous cells with caseous bodies not yet fused. Their nuclei are still present, but show up less distinctly. Some of the latter are in capillary vessels and seem to arise from the endothelial cells. As caseation extends, other cells at the periphery are included. The nuclei of the cells first struck with degeneration become disintegrated, while fresh ones are taken up at the margin. By and by the degenerating patch may increase to such an extent that the cell-like form is lost and it takes the appearance of a caseous area. Some of the cells included at the margin resemble certain of the white blood corpuscles. These may be phagocytes that have come out to surround the dead patch and have perished in an attempt to digest the bacilli.

These experiments seem to show so far that one may with impunity drink milk containing dead bacilli. That, of course, does not mean that cows with tuberculous udders should be tolerated in byres. They also suggest the possibility that some of the very chronic lesions in animals contain only dead bacilli. To decide this, of course, one would have to inoculate products obtained from a large number of these lesions in question. The production of a lesion in the abdominal organs of guinea-pigs would not in all cases be enough to prove the vitality of the bacillus, because one might possibly inject a sufficient number of dead ones to act. Artificial culture on glycerine agar, however, would decide the question.

I hope in a future paper to give the results of certain experiments on the above lines, and I think they may have a not unimportant bearing on the subject of meat inspection. The fact that I obtained a reaction with tuberculin in animals inoculated with dead bacilli says all the more for tuberculin, but I am inclined to think that the majority of animals giving a reaction have living bacilli in their bodies.

In a future paper I shall give the results of further experiments on this subject, as it is important to know about what quantity of dead bacilli must be injected before a reaction can be obtained, and how long the animals will continue to react. I am also doing some experiments with attenuated bacilli, but of these I wish to say nothing at present.

EXPERIMENTS WITH THE DEAD *BACILLUS MALLEI*.

My experiments with dead and attenuated glanders bacilli have not as yet been extensive. I mean at present only to describe one experiment on the horse. One of my reasons for entering into this subject at such an early stage of the investigation is, that I have found the parasitic nodules of Schütz in the lungs of two experimental ponies selected haphazard. The works of Nocard¹ and Schütz² are too recent to require to be quoted at any length. Nocard has produced glanders tubercles in the lungs of horses by causing them to ingest living cultures of the *Bacillus mallei*. Some of these tubercles are perfectly translucent "*les tubercules translucides*," and Nocard asserts that in many of them the bacilli have been overcome or are in process of being overcome by the phagocytes. Some of the guinea-pigs inoculated with an emulsion made from these tubercles do not become glandered, nor can one always obtain cultures of the *Bacillus mallei* from them. Schütz, who has also done feeding experiments with glanders bacilli, found nodules due to a parasite in the lungs of many of his experimental animals and others. He hints that Nocard has mistaken these parasitic nodules for translucent glanders tubercles. What Nocard describes as "*les tubercules translucides*," however, are dense collections of leucocytes; one might almost compare them to small areas of white pneumonia, if I may use the expression. These, I think, are different from the parasitic nodules described by Schütz. However, it is useless to speculate on the identity or difference of certain lesions, when the matter could be decided by an actual comparison of specimens, and I think that this might form an interesting subject for discussion at a future congress.

Nobody can doubt that Schütz has frequently met with these parasitic lesions, but one is inclined to think that he must be in a badly infected area, for no other observer has so frequently noted them. Still, they are often easily passed over, unless one examines the lungs after slicing, and I must confess to having found them in the lungs of the first two horses that I examined carefully.

In view of Nocard's results, the possibility suggested itself to me, that the bacilli might be so modified by their contact with the digestive juices that they arrived in the lungs in an attenuated condition, and

¹ Loc. cit.

² Loc. cit.

thus fell an easier prey to the phagocytes. I do not mean to assert this, I merely put it forward as a preliminary suggestion. It might also be that fewer bacilli reach the lung when they are given by the mouth.

Experiment I.—Dead glanders bacilli. An aged pony was used for this experiment. The object was to see if the dead *Bacillus mallei* produced a lesion at all comparable with that caused by the living, and also whether the animal would react to mallein afterwards. On the 15th April at 12.15 A.M. the pony underwent a preliminary test with mallein. One might almost dispense with this precaution in Edinburgh, where glanders is a disease now rarely met with. This animal did not react. The variations of temperature will be found on the subjoined chart of the whole experiment. The mallein did not increase the agglutinating action of the serum on dead cultures of the glanders bacillus. On the 22nd April at 10 o'clock the pony received the bacilli from nine potato cultures of the *Bacillus mallei*, that had been incubated for twenty days and then killed by exposing to chloroform vapour for thirty hours.¹

The temperature rose during the first eight hours (*see* chart), and the animal was rather seedy, but he soon recovered as the fever passed off. The agglutinating power of the serum on dead glanders bacilli was slightly increased. On the 28th April the animal was tested with mallein.² The temperature rose no more than 1.6° F. (*see* chart), but it remained slightly above the 99.8° F.³ for two or three days. There was no swelling nor any systemic disturbance. The test was again applied on the 6th May at 11 P.M. with a dose of 1½ cc. of mallein. The temperature rose only 1° F. this time. There was a small local swelling about the tenth hour. It was tense and painful, but never increased beyond 2 inches in breadth, and had completely disappeared on the 10th May. The pony was killed on the 13th May.

Autopsy.—The greater part of both lungs showed very slight signs of broncho-pneumonia, and the organs were, as a whole, slightly more solid than normal.

Two lobules on the surface were the seat of hæmorrhagic infarction. The anterior lobe of the left lung was more solid than any other part, but it floated in water. On section it was of a dirty greyish and red colour. The posterior lobe of the same lung was decidedly congested, but this may have resulted from embolism. Under the pleura and in the substance of the lung were a few well-defined nodules. These were rather difficult to find in the substance of the organs, but I isolated about a dozen for microscopic examination.

They were very hard, about the size of a swan shot, and well defined from the lung tissue.⁴

When the nodules were cut across the central part resembled very dense caseous material. It was perfectly dry, and contained lime salts. At first sight I thought I had produced the translucent tubercles, but a closer examination convinced me that the nodules must have been there long before the bacilli were injected. Moreover, they were not at all like the white translucent patches described by Nocard. The

¹ The bacilli were certainly dead, as two platinum loopsful injected into the peritoneal cavity of a guinea-pig produced no effect.

² This mallein was kindly sent me by Professor M'Fadyean. The dose was 1 cc.

³ This was the normal temperature of the pony.

⁴ The nodules, along with portions of the lung, were fixed in 4 per cent. formol.

liver of this pony also showed similar hard nodules on its borders. The latter were yellower in colour than those of the lung. All were calcified. I think the parasite is the agamous form of the *Sclerostoma armatum*. In the livers of other horses I have found the same nodules, along with parasites that were beyond doubt the *Sclerostomata*. Again, in the lungs of the experimental horse that died from air embolism, I found a single nodule of the same kind after slicing the organs.

Microscopic.—The small nodules were embedded in paraffin and cut with the rocking microtome. They were found to consist of a fibrous periphery and a cellular centre. The extent of the cellular area varied in different nodules, and one may assume that the nearer the fully developed fibrous tissue is to the centre, the older is the tubercle. The most central part was invaded by lime salts. The majority of the cells were of the connective tissue type and lay in a loose fibrous matrix. A variable number of leucocytes with coarse eosine staining granules were also present. Indeed, many of the granules had been set free from the cells and could be distinctly seen outside. These coarse eosinophilous cells are a perfectly normal constituent of the horse's blood, and the shedding of the granules seems also to be a normal process. I have met with these granules often when using the haemocytometer, and my colleague, Professor Mettam¹ has lately drawn attention to their presence in the normal tissues of the horse. The granules are especially abundant in the liver nodules. Since these cells are normal constituents of the blood their presence need not be wondered at in any tissue formation. Schütz² says that they are absent from true glanders nodules, and nobody has yet demonstrated their presence there. It might be that this cell is not attracted by microbes. Its presence, however, can hardly be considered pathognomonic of any lesion, since it is a normal constituent of the blood.

The vessels at the periphery of the nodules are well formed, those towards the centre are embryonic. The same description may be applied to the liver nodules, except that around the periphery there is a great deal of some black pigment. All those that I have examined have had their start in a vessel of considerable size, much too large to arrest the glanders bacillus, and I do not think any pathologist would be likely to take the above formations for glanders tubercles. I have not been able to find any object that could without doubt be put down as a parasite in any of the lung nodules examined, but that is probably because I have examined only very old ones. The dead bacilli, then, had nothing to do with these formations, which I think must be identical with those described by Schütz.

The capillaries over large areas of the lung were very much distended. In some places several adjoining ones had come into contact and completely obliterated several air cells. These areas at first sight looked like commencing glanders lesions, especially as the neighbouring alveolar wall were similarly thickened. A closer examination, however, showed most of the cells to be red blood corpuscles inside capillaries, and the number of leucocytes to be about normal in proportion. No bacilli could be seen. There

¹ "Veterinarian," May 1898.

² Loc. cit.

was a very small amount of exudate in many of the air cells. The latter changes were, I think, due to the bacilli injected, but nothing like a true glanders tubercle was found.

I had intended to illustrate this paper with micro-photographs, but regret that the latter arrived too late for publication. I may, however, be able to insert them in a future paper.

QUARTER-EVIL, OR BLACK-QUARTER.

By J. M'FADYEAN, Royal Veterinary College, London.

Definition.—The disease which is commonly called quarter-evil or black-quarter in England, and black-leg in Scotland, is a bacterial affection, caused by a specific micro-organism—the bacillus of quarter-evil. In France the disease is generally known by the term symptomatic anthrax (*charbon symptomatique*), originally suggested by Chabert (1782) for those cases of disease in which after a period of fever and systemic disturbance an inflammatory tumour makes its appearance in some part of the body. This author believed that quarter-evil and anthrax were merely symptomatic varieties of the same disease, and he proposed to apply the term *fièvre charbonneuse* to those instances in which the disease ran its course without the development of any tumour. Chabert is generally credited by French authors with having effected a great improvement in nomenclature when he introduced these terms, and with having thereby differentiated from charbon various other affections, such as “putrid and gangrenous fever.” It is, however, difficult to understand what were the precise diseases other than anthrax and quarter-evil to which the term charbon was applied prior to the date of Chabert’s monograph, and it is obvious that Chabert’s definitions left it possible to confound with anthrax several other different diseases, and tended to confirm the opinion, then widely held, that anthrax and quarter-evil were not etiologically distinct.

In this country, long before the discovery of their respective organisms, anthrax and quarter-evil were recognised as things so different as to merit different names, and such confusion as exists at the present time is almost entirely ascribable to the adoption of the French nomenclature by some modern authors. The retention in France of the names proposed by Chabert may be defended on the ground that they are established by long custom, but nothing whatever can be said in favour of displacing the name quarter-evil by symptomatic anthrax. As will be shown in the course of this article, the diseases anthrax and quarter-evil have scarcely a feature in common, and in face of this fact one cannot help being struck with the curious perversity of reasoning which led the older veterinary authors to regard them as manifestations of the same morbid condition.

Discovery of the Bacillus of Quarter-evil.—Feser and Böllinger (in 1876) appear to have been the first to note the presence of rod-like motile bacteria in the lesions of quarter-evil. Both of these authors regarded these organisms as the cause of the disease, and both claimed