A report on the destruction of micro-organisms during the progress of inflammation / by M. Armand Ruffer.

A REPORT

ON THE

DESTRUCTION OF MICRO-ORGANISMS

DURING THE

PROGRESS OF INFLAMMATION.

BY

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Reprinted for the Author from the British Medical Journal, May 24, 1890.

PRINTED AT THE OFFICE OF

THE BRITISH MEDICAL ASSOCIATION, 429, STRAND W.C.

THE DESTRUCTION OF MICRO-ORGANISMS DURING THE PROCESS OF INFLAMMATION.

THE attention of pathologists has often been arrested by the fact that the introduction of a living contagium into animals gives rise to lesions differing in intensity, naked-eye and microscopical appearances, according to the strength and quantity of the inoculated virus, and also according to the species, age, etc., of the animal into which the virus has been, accidentally or artificially, introduced.

Among the lesions consecutive to the subcutaneous inoculation of living virus, none present a greater interest than those met with at the spot where living micro-organisms have been introduced under the skin. In some infectious diseases, diphtheria, for example, the bacilli which are the primary cause of the disease are found in the diphtheritic patch, that is, in the primary lesion and there only. In other words, diphtheria is the type of a local infectious disease, the general symptoms being caused, secondarily, by the absorption of the poisons secreted by the specific bacilli of diphtheria. In other infectious diseases—tubercle, glanders, for example—the primary lesion is slight; the bacilli invade the system by stages, and produce well-marked local pathological changes in the glands, spleen, liver, lungs, etc.; in fact, in every organ in which, from mechanical or other reasons, the specific micro-organisms are temporarily arrested. Other micro-organisms, such as those of fowl-cholera when inoculated into non-resistant animals, give rise to no local symptoms whatever. The spot where the virus is introduced shows no signs of inflammation other than those produced by the slight wound; but if the spleen, liver, kidneys of the inoculated animal be examined shortly afterwards, the characteristic bacilli are found in enormous numbers in every one of these organs. The infective process has then become general.

Among the micro-organisms which, when inoculated into animals, produce well-marked naked-eye and microscopical lesions at the point of inoculation must be reckoned the characteristic bacilli met with in the disease generally called quarter-evil in this country (*charbon emphysémateux*, *Rauschbrand*). This disease, as Arloing, Cornevin, and Thomas have proved, is caused by a specific bacillus, to which these observers have given the name of *bacterium chauvoei*.[1] The bacilli are anaërobic organisms, and may be cultivated in beef-broth or gelatine, provided these media have been freed from air by a stream of some inert gas such as hydrogen or ordinary coal gas. The bacilli closely resemble in appearance those of anthrax, but differ from the latter in being very motile, somewhat shorter and thicker. Lastly, the bacilli often contain well-defined spores at each end.

[1] The term bacillus chauvoei is a far better name for it, for the micro-organism presents all the characteristics of a bacillus, and I therefore adopt it.

When inoculated subcutaneously into non-resistant animals, that is, in animals such as guinea-pigs, in which the disease ends fatally, the bacilli produce a highly characteristic lesion at the point of inoculation. The lesion consists of a tumour containing a large amount of more or less clear fluid, in which float a small number of amœboid cells. The neighbouring muscles are highly œdematous, owing to the presence of a large quantity of clear serous fluid and migrating cells. In guinea-pigs the whole process seems to end there, for, although the animal invariably dies when a sufficient number of bacilli are introduced under the skin, the characteristic pathogenic micro-organisms are found at the point of inoculation only and nowhere else.

The inoculation into guinea-pigs affords, on account of the constant strength of the virus, excellent opportunities for studying the processes going on at the seat of inoculation. Arloing, Cornevin, and Thomas have shown that the muscles of animals which have died of quarter-evil dried at a temperature of 100° to 104° contain an attenuated virus. This virus, when inoculated into large animals, such as bullocks, produces a very mild form of the disease, from which these animals recover. By drying the virus at varying temperatures the same observers have obtained two kinds of virus, which I shall designate as A and B. The virus A is the stronger of the two, and, provided sufficient quantities of it are used, its subcutaneous introduction into a guinea-pig is followed by death of the animal after a period varying from forty to forty-eight hours. The virus B, on the other hand, when inoculated in small quantities, is followed by a passing temporary and localised lesion. When enormous doses of B are introduced subcutaneously, death follows in four, five, or six days. These two kinds of virus, when contained in a well-stoppered bottle and in a dry place, keep their virulence for any length of time. The dose of virus to be used can be accurately weighed, and by varying the strength and dose a mild or malignant, acute or chronic, form of the disease is produced at will.

By carefully studying the naked-eye and microscopical appearances of the point of inoculation, it is possible to answer the following question: "Is the inflammatory process going on at the seat of inoculation a protective process or not?" The answer to that question is, as we shall see, in the affirmative.

The next query then is: "By what process or processes is the animal body protected against the invasion of the pathogenic micro-organisms?" Theoretically speaking, there are four ways in which the body may be protected:

1. "The bacilli may not meet in the surrounding media with the physical conditions and chemical materials necessary to their existence." It is clear that if micro-organisms do not find the necessary heat, moisture, and food, they must remain in the same latent condition as the dried virus. This is not the case, however. On the contrary, the bacilli are called into active life again as soon as they are introduced under the guinea-pig's skin.

2. "The tissues of the animal may mechanically impede the passing of bacilli into the system." It is a well-known fact that certain tissues oppose a mechanical barrier to the entrance of pathogenic micro-organisms. If not abraded, the surface of a healthy skin, with its thick layer of epidermis, is not easily penetrated by microbes, and the same may be said of the dorsal surface of the tongue. On the other hand, virulent bacilli, such as anthrax bacilli, which are endowed with slight power of movement only, when introduced under the skin, soon force their way through the loose meshes of the surrounding connective tissue into the neighbouring lymphatic or blood vessels. The bacillus of

quarter-evil is endowed with considerable motility, and there is no reason to suppose that it could not penetrate into the blood or lymphatic vessels. The mechanical resistance of the surrounding tissues as a cause of immunity in this disease must, therefore, be excluded.

3. "The liquids which during the process of inflammation pass out of the vessels may have a toxic influence on the pathogenic bacilli," or

4. "The amœboid cells present in the primary lesion may take a share in destroying micro-organisms and arresting their progress."

I propose to show that the liquids of the guinea-pig's organism, far from having a deleterious action on the micro-organisms of quarter-evil are, on the contrary, an excellent cultivating medium for the latter. Further, I hope to prove that the destruction and arrest of micro-organisms at the point of inoculation are due chiefly, if not solely, to the action of the amœboid cells present in the inflammatory effusion.

I have to thank here the staff of the Laboratoire de Pathologie Générale in Paris for providing me with the necessary material and animals, although I alone am responsible for the views expressed in this paper.

The animals used in my experiments were always adult guinea-pigs. The mode of inoculation was the following: The animal being under the influence of ether or chloroform the hair was cut with scissors, the abdomen or back shaved and thoroughly cleansed with soap and water and a strong (1 to 500) solution of corrosive sublimate. A small incision, about $\frac{1}{8}$ of an inch, was made with an aseptic knife, cutting through the skin and subcutaneous tissue until the muscular layer was exposed. The muscles were then separated from the subcutaneous tissue with the handle of the knife. A small but sufficent quantity of the powder, containing the strong virus (A), was introduced with the point of the knife between the muscles and subcutaneous connective tissue, the opening closed with a silk suture, cleaned with sublimate solution and covered with collodion. Needless to say, the small wound so made never showed the slightest trace of inflammation, and was always firmly healed by the next day.

At the end of twelve to eighteen hours a small hard swelling is formed at the seat of inoculation. This tumour is evidently fluid, and crepitates on pressure as if its contents consisted of gas and fluid. The swelling increases in size, and soon becomes harder and more resistant. The parts surrounding it are then considerably thickened, the walls of the abdomen, for instance, feeling twice as thick as natural. After twenty-four hours or so, the animal shows evident signs of a general illness; it refuses food, does not run away when touched, gets more and more drowsy and generally dies just within a period of forty-eight hours after the inoculation. The *post-mortem* examination was always made within ten minutes of the death of the animal.

On examining the tissues at the spot where the inoculation had been made, the remains of the powder containing the virus are found lying in a kind of cavity which is full of almost clear fluid. The walls of this cavity are lined by a thin, greyish and fibrinous layer. The muscles surrounding the cavity are highly œdematous, so that on cutting through them, a large quantity (10 ccs. to 20 ccs. or more) of clear transparent fluid may be collected. This fluid is, occasionally, strongly tinged with hæmoglobin. The muscles and neighbouring parts present no other naked-eye changes.

The nearest lymphatic glands are sometimes slightly enlarged, but the other internal organs appear quite normal as a rule. Occasionally, there is a very slight enlargement of the spleen, together with congestion of the liver and kidneys. Sometimes, on the other hand, the latter organs appear to be somewhat paler than natural.

The microscopical appearances are, however, of far greater interest. To study them fully, the development of the bacilli must be watched and the processes going on in the walls of the cavity containing the virus, in the surrounding muscles and other organs must be followed.

It has already been stated that the virus used is carefully dried, and, as bacilli require moisture in order to show any signs of life, we must suppose that their activity before inoculation is latent. If, twelve hours after the inoculation, a sharp capillary pipette is introduced into the centre of the tumour already formed, a little of the clear transparent fluid carefully drawn off and examined under the microscope, an enormous number of bacilli are found moving about in the liquid. Here and there a leucocyte is seen. As a rule the wandering cells met with at this stage are empty, but occasionally one holds two, three, or more bacilli in its interior (see Fig. A, Nos. 1, 2, 3).

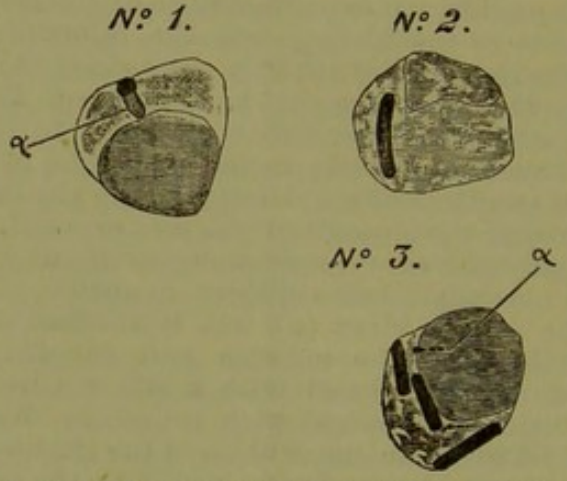

Fig. A. Serous exudation twenty-four hours after inoculation with virus A. Vesuvin and gentian-violet stain. Vérick microscope ; oc. 3, × $\frac{1}{12}$; oil immersion ; Abbé's condenser. 1. Microphage containing (a) partially digested bacillus. 2. Microphage containing two bacilli joined end to end. 3. Microphage containing bacilli ; a represents a bacillus in the last stage of digestion.

In cover-glass preparations stained in an alkaline solution of methyl-blue, or better gentian-violet, the bacilli floating in the liquid are, to all appearances, perfectly normal and, according to the reagent used, stain of a very lovely dark-blue or purple colour. Most of the bacilli contained in the cells stain normally, but here and there an intracellular bacillus shows signs of degeneration. These signs of degeneration need not be insisted on here, as they will be fully described when the destruction of the bacilli taking place in the abscess wall is spoken of.

I must call attention here to a frequent source of error, when the object in view is to study in cover-glass preparations the relation of the cells to the micro-organisms they contain. It is of great importance that the cover-glass should not be passed through the flame, as the cells are frequently damaged, burst, and their contents scattered by heat. Hence, instead of passing the cover-glass through or holding it over a flame, it is better to place it in an incubator for some little time and let it dry more slowly. I feel convinced that some of the contradictory results

obtained by observers who have studied the question of phagocytes are chiefly due to this error in manipulation.

A drop of the liquid in the tumour withdrawn and examined at the end of forty-eight hours, or immediately after the animal's death, contains an enormous number of free bacilli. The fluid, as a rule, is somewhat turbid, owing to the presence of wandering cells in fairly large numbers. These amœboid cells belong to the small variety (microphages), and are either uni- or multi-nucleated. The nuclei possess hardly any intranuclear network, and are surrounded by a small amount of protoplasm only. Many of these cells contain bacilli, some in enormous numbers, as many as ten micro-organisms being sometimes enclosed in one cell. The micro-organisms in the liquid show no signs of degeneration, whilst some of the bacilli contained in the amœboid cells have clearly undergone a process of digestion. Floating in the liquid are seen remnants of the dried blood used for the purpose of inoculation.

It would appear, therefore, that the fluid constituents of the inflammatory effusion, far from having any harmful or toxic effect on the bacilli, have, on the contrary, the power of stimulating the latter into active life. The bacilli seemingly find in the inflammatory fluid poured out around them all the conditions of heat, moisture, and food necessary to their existence. On the other hand, the amœboid cells of the inflammatory effusion have the property of taking into their interior and destroying bacilli—a property of which we shall have more evidence presently.

In order to get a clear idea of the processes taking place in the wall of the cavity—which, for brevity's sake, I shall call the abscess wall—this part and a few millimètres of the surrounding tissues must be removed immediately after death with sharp scissors and plunged at once into absolute alcohol. After remaining in this fluid for forty-eight hours at least, the alcohol is extracted with chloroform or ether, and the piece of tissue soaked in paraffin at 42° C. for twenty-four hours or longer. The sections must be fixed on the glass slide by means of glycerine albumen and double stained with alum-carmine and gentian-violet, or logwood and gentian-violet. Both modes of staining give very good results; but whereas the carmine-violet sections are, perhaps, prettier and clearer, the relation of the bacilli to the cells is more easily studied in the logwood-gentian-violet preparations.

On microscopical examination, the free aspect of the abscess wall is covered everywhere by an enormous number of bacilli, lying free in the coagulated exudation-fluid, staining in a perfectly normal manner, and showing, to all appearances, no traces of degeneration.

The abscess wall itself consists of an innumerable number of small migrating cells. Some of these amœboid cells have but one single nucleus, others contain two, three, or four nuclei. In many, the nucleus has the triradiate aspect found in other inflammatory conditions—for example, pneumonia. The amount of protoplasm round the nucleus is very small as a rule. Close to the free surface of the abscess wall these cells are pressed together in enormous numbers, and so closely packed that the contours of each are scarcely to be recognised.

In the deeper regions of the abscess wall the cells are massed together less closely. The muscular tissue around contains, within its meshes and between its fibres, a large number of small, round migrating cells. The latter are met with even in places where not a single bacillus is to be seen. Here and there, especially

in the deeper strata of the abscess wall, a few cells occur, which are larger, have a single clear vesicular nucleus, with a well-marked intranuclear network, surrounded by a large amount of coarse protoplasm, and not infrequently contain in their interior remnants of degenerated bacilli and leucocytes. These cells probably represent the first stage in the development of macrophages. They do not attain their full size owing to the early death of the animal. On the whole, macrophages play quite a secondary part in this disease.

Many leucocytes found close to the free surface of the abscess wall contain in their interior a large number of characteristic bacilli. Nevertheless, many micro-organisms remain quite free between the cells in the surrounding medium, which has been coagulated by the action of the hardening reagent (see fig. B, *f. b.*); but whereas the latter micro-organisms do not show signs of degeneration, many of those contained in the migrating cells have undoubtedly undergone a process of disintegration. The number of micro-organisms in the deeper layers of the abscess wall at some distance from the free surface gradually becomes smaller and smaller, and whereas in the upper strata some micro-organisms are free between the cells, the bacilli in the lower strata of the abscess wall are almost always contained in the interior of microphages. Lastly, a few millimètres away from the free surface, although the number of migrating cells is still very large, no bacilli are to be seen, except, occasionally, the remnant of a dead micro-organism contained in an amœboid cell.

The presence of the bacilli of quarter-evil in the tissues of the guinea-pigs, therefore, gives rise to a most intense inflammatory process characterised by the exudation of large quantities of liquid, and the emigration of an enormous number of amœboid cells. I have been unable to satisfy myself that the fixed cells of the connective tissue take any part whatsoever in the process of inflammation or in the destruction of micro-organisms.

The muscular fibres, even at some distance from the point of inoculation, are separated by a large amount of coagulated liquid, in which float a few leucocytes. In sections stained with carmine and gentian-violet no bacilli are met with between the muscular bundles, even where the œdema is most intense. The serous exudation and the emigration of white corpuscles in that situation are not, therefore, due to the presence of the bacilli as such, but to some other cause, most probably to the irritation produced by the absorption of the products of the life of micro-organisms.

The histological changes in the vessels and muscles need not detain us here, as they are immaterial to the object of this paper. The lymphatic glands, spleen, liver, heart, etc., if examined immediately after death, contain no bacilli; at least, none could be seen on microscopic examination. From the many difficulties attending the making of cultures of the *bacillus chauvoei*, it would not be right to conclude that the various organs of the inoculated animal contain no bacilli at all, but we may conclude that, at any rate, the number of such bacilli is small.

The changes in other organs are so slight that it would serve no useful purpose to take up space in describing them. The spleen, however, presents some microscopical appearances, which, as far as I know, have never been described before. Kölliker was the first to demonstrate the fact that certain cells of the spleen have the power of taking into their interior and destroying red blood corpuscles. Lately, I have been able to satisfy myself that these cells are true amœboid cells, for not only do they destroy red blood corpuscles and leucocytes, but they absorb dead

substances also, such as vermillion.[2] They are, therefore, true phagocytes.

In the spleen of guinea-pigs which have died of quarter-evil, the number of phagocytes holding partially digested red blood corpuscles in their interior is enormously increased, and I have, in one field of the microscope (oil immersion), and in a thin section, observed ten or more of these cells full of red blood corpuscles in various stages of degeneration. It appears as if, in the course of this disease, some poison is excreted by the bacilli which, entering the blood, weakens the resistance of red blood discs, and allows them to be more easily destroyed by the phagocytes of the spleen.

At the beginning of this paper, it was stated that it is possible to produce in guinea-pigs a mild form of the same disease, by using a weaker form of the virus, which I have called virus B. A small quantity of this virus—say 5 centigrammes—inoculated into an animal produces such an insignificant lesion that it is not easy to follow the struggle between amœboid cells and micro-organisms at the spot where the virus has been introduced. This difficulty is easily overcome by enclosing the virus in small glass cases, made by joining together with shellac two thin cover glasses; three sides being closed and the fourth left open, to enable leucocytes and inflammatory fluids to penetrate as far as the powder containing the bacilli. The introduction of a foreign body, like a glass case, acts as an irritant, and a few hours afterwards it is surrounded by an enormous number of migrating cells. The fluid constituents of the inflammatory exudation are drawn by capillary force into the glass case, while some of the migrating cells also wander into it.

One of these glass cases filled to one-eighth of its height with the dried, weak virus, and carefully introduced under the skin of a guinea-pig's back, is at the end of twelve hours filled with a somewhat thick, turbid, greyish liquid. A drop of this fluid is drawn off with a fine capillary pipette, and fresh preparations made, whilst others are stained in the usual manner. The slides contain an enormous number of the characteristic bacilli exhibiting, in unstained specimens, the most lively movements, and to all appearances active and quite healthy. Occasionally, the micro-organisms form long chains of ten, fifteen, or more bacilli joined together end to end. In fresh preparations many leucocytes are also seen moving about in the liquid, of which many leucocytes are empty, but a few contain a varying number of micro-organisms, some of which are already degenerated.

The outer aspect of the glass-case is covered with an enormous number of characteristic micro-organisms, which have found their way from the inner aspect of the glass case to the outer. A number of leucocytes are also seen in the same situation. Most of these are empty, but some are filled with micro-organisms presenting most varied signs of degeneration.

The tissue immediately surrounding the glass-case, removed with a sharp pair of scissors, plunged into absolute alcohol and stained in the manner previously described, is found to consist of an enormous number of cells which have migrated out of the vessels owing to the irritation produced by the foreign body. No micro-organisms are to be detected in the lower strata of the wall of leucocytes surrounding the foreign body. In the part immediately touching the glass-case some leucocytes contain numbers of micro-organisms, of which some are normal and some evidently degenerated.

If instead of placing the modified or weak virus B in a glass

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[2] Unpublished observations.

case it is simply inoculated under the skin and the animal killed forty-eight hours after the operation, the tumour formed at the point of inoculation is small, and consists mainly of a little gelatinous material. This tumour excised *en bloc*, plunged into absolute alcohol and examined in the same way as the other tissues, is found to consist of inflammatory fluid coagulated by the action of the alcohol, the remnants of the powder used for inoculation, and an enormous number of characteristic bacilli and migrating cells.

The bacilli present in the coagulated fluid of the inflammatory exudation are perfectly normal in size and appearance. Each particle of the powder containing the virus is surrounded as if by a cloak, by a thick layer of micro-organisms. The leucocytes in the neighbourhood are full, almost to bursting, with an extraordinary number of micro-organisms showing the most varied and typical forms of degeneration. In some sections it is impossible to find a single leucocyte which does not contain in its interior five, six, and sometimes as many as eighteen bacilli. The process of destruction when the weak virus is used exactly resembles the process of destruction when the strong virus is inoculated, but the destructive process in the case of the former is far more intense and far more localised than in the case of the latter.

It will be convenient to speak now of the forms of degeneration as seen in the micro-organisms of quarter-evil when they have been taken into the interior of lymphocytes. I will describe these changes as seen in preparations examined with a Vérick microscope, oil immersion, 1-12th or 1-13th, ocular 1 or 3, and Abbé's condenser. The bacilli not contained in cells I shall for brevity's sake call extracellular micro-organisms, whilst the bacilli in the interior of leucocytes must go by the name of intracellular micro-organisms.

The extracellular micro-organisms present in the coagulated exuded fluid, when carefully examined, consist of rods somewhat shorter and thicker than anthrax bacilli. Spores are not often present when the virus is inoculated into guinea-pigs. The bacilli stain of a deep blue colour with methyl-blue and intensely red with fuchsin. The best method to stain sections, however, is to place them in alum carmine for ten minutes, wash in water and absolute alcohol, and then to pass them through gentian violet, a solution of iodine in iodide of potassium, water, absolute alcohol, anilin-xylol, pure xylol, and finally pure terebene. The bacilli are of a deep purple colour, and stand out very sharply from the carmine-stained cells.

The extracellular bacilli, though staining uniformly, are not all of the same length, and not of the same thickness (see Fig. E, *a*, *b*, *c*.) These differences in size are due partly to an actual difference in size, partly to two or more being joined together (see Fig. E, *d*), and possibly to the fact that some are placed more or less obliquely in the preparation. None show any signs of degeneration whatsoever.

The appearance of the intracellular bacilli is very different. A large number are, to all appearances, quite healthy (see Fig. B, *a*), stain uniformly, and are of normal size and shape. Here and there, however, an intracellular bacillus is found, which, instead of staining as deeply as the others, assumes a light purple tinge (see Fig. B, Microph. 1, 3, 4). This tint may be perfectly uniform, but at other times part of the bacillus only is stained of a dark purple colour, whilst other parts of the same micro-organism assume a lighter tinge (see Fig. C), and the most varied forms of degeneration can be observed.

Sometimes the centre of the bacillus takes up the colouring

matter badly, whilst the periphery stains deeply still (see Fig. C, *a*, and Fig. D, No. 5, *c* and No. 2, *b*). The micro-organism then consists of a central, badly-staining core and a dark, deeply-staining sheath (see Fig. D, No. 5, *c*, No. 2, *d*, and Fig. C, *a*, *b*, *c*). Sometimes one edge only of the micro-organism retains the colouring matter deeply, whilst the remainder assumes a light purple colour. In another stage the whole bacillus is of a uniform pale-purple hue, owing to the gradual breaking up and disappearance of the colouring matter in the sheath (see Fig. D, No. 4, *a*, *b*, Fig. C, *g*).

In other bacilli the degeneration, marked by the loss of power of fixing the colouring substance, begins at the periphery of the micro-organism, the centre still retaining the colouring matter, while the edges remain unstained (see Fig. C, *e*, *f*). The central coloured part becomes less and less in amount, so that a small streak of it only is left (see Fig. C, *g*, *h*). Later on the central part itself breaks up, and forms a very thin, interrupted, irregular streak lying in the interior of a bacillus (see Fig. C, *i*).

The process of degeneration in other bacilli affects the whole breadth of the bacillus uniformly. One end of the bacillus stains normally, whilst the other end is of a pale, homogeneous colour (see Figs. B, C, Fig. D, No. 3, *a*). Sometimes an intracellular bacillus consists of a row of dots staining darkly, and embedded in a less deeply-stained material (see Fig. C, *k*, *l*), or of parts dark and light alternately (see Fig. D, No. 3*a*).

At the same time that these changes take place in the coloration of the bacilli, the latter undergo alterations in shape and size. Whereas, the bacilli present in the inflammatory fluid are usually straight and very rarely bent (see Fig. E, *e*), many of the intracellular bacilli are curved on themselves (see Fig. D, No. 3 *a*, *e*, No. 4 *a*, No. 5 *a*). This is better seen in sections made at the point of inoculation of animals inoculated with the weak virus (B), in which, as we have seen, the bacilli have a tendency to join together end to end. These intracellular rods are nearly always curved, and present a highly characteristic appearance (see Fig. C, *m*, *f*).

The intracellular bacilli in later stages of degeneration show a diminution in thickness. Many are much thinner than normal (see Fig. D, No. 1, *a*, *b*, and Fig. C, *k*, *f*, etc.); and whereas the diminution in thickness of some bacilli is uniform, in others it begins at one extremity, the bacillus looking as if one end of it were being eaten away (see Fig. D, No. 3, *c*, No. 2, *d*.) The contours of the intracellular micro-organisms also, instead of being sharply defined as in the extracellular bacilli (see Fig. B, *f*, *b*), are irregular, and their exact outline is by no means easily made out (see Fig. D, No. 3, *a*, see Fig. C, *n*). In a later stage, the bacilli become thinner and thinner, more and more irregular (Fig. C, *f*), and lose whatever power they still possessed of retaining colouring matter, so that, finally, nothing is left in the cells but small dots (Fig. C, *p*, *q*, *r*, *s*, *t*, etc., also Fig. D, No. 2 *a*, *c*), some of which still retain more or less colouring matter, whilst others appear as light highly refracting granules, or very pale rods looking like the remnants of the sheaths of dead bacilli.

Occasionally, intracellular bacilli are met with which, instead of taking up aniline dyes, stain with carmine or logwood. Others stain partly with carmine and partly with the aniline dyes used, looking like little dots stained purple and red alternately. These variations in staining prove that intracellular micro-organisms do not react like extracellular bacilli towards chemical reagents.

When a small dose of the weak virus is inoculated subcutaneously, the struggle between leucocytes and bacilli is evident on the fifth day or even later, but the number of micro-organisms gra-

dually diminishes, so that after the fourth day hardly any bacilli are found at the seat of inoculation. On the fifth day, the few bacilli which can still be seen are contained in migrating cells, and show evident signs of the most advanced degeneration.

The study of the initial lesion of animals in which a chronic form of the disease has been induced by the inoculation of large quantities of a weak virus (B) (0.40 centigramme or more) is not less interesting.

The animals inoculated with a strong virus perish so soon after the introduction that the struggle between the cells and micro-organisms is more or less one-sided, for, although the animal dies, most of the cells present at the point of inoculation are normal, and show no signs of degeneration. When, however, a chronic form of the disease is produced, many of the lymphocytes perish as the result of their struggle with the invading bacilli.

If the animal dies on the fourth or fifth day, sections through the exact spot where the virus has been inoculated show that the bacilli have infiltrated the neighbouring muscles to a far greater extent than in the acute form of the disease. Many bacilli are extracellular, lie in the coagulated inflammatory effusion, and are perfectly normal and healthy (Fig. E, *a*, *b*, *c*, *d*, *e*), staining well with aniline dyes, and retaining colouring matters in normal fashion. A few micro-organisms are contained within amœboid cells, and often present various forms of degeneration (Fig. E, *d.l.*^{3} *d.l.*^{4}); but whereas in the acute form of the disease most of the wandering cells are healthy, many of the cells met with in the more chronic affection are markedly degenerated. In other words, they become true pus cells—that is, dead cells.

The nucleus of such a cell, instead of staining deeply with carmine or logwood (Fig. E, *h.l.*), possessing a coarse intranuclear network, and being marked off sharply from the surrounding protoplasm, stains rather more diffusely (Fig. E, *d.l.*^{1}). The nucleus often shows signs of breaking up (Fig. E, *d.l.*^{2}), and in many cases is not marked off sharply from the surrounding protoplasm (Fig. E, *d.l.*^{1}). In later stages the nucleus undergoes distinct fragmentation, three or four fragments of nuclei lying in the protoplasm of the cell. In the last stage the nucleus disappears, nothing remaining but a pale round mass of protoplasm, which no longer takes up colouring matter. Not infrequently one of these cells is contained in a larger cell, possessing a clear vesicular nucleus, surrounded by a large amount of somewhat coarse protoplasm—in other words, it is taken into the interior of a typical macrophage.

Some of the dead lymphocytes contain bacilli which either appear healthy or, not infrequently, are more or less degenerated (Fig. E, *d.l.*^{3} *d.l.*^{4}). It might be supposed that these cells died owing to the entrance of bacilli into their protoplasm. Many degenerated cells, however, are found in which not a single living or dead bacillus is to be seen. These cells probably never contained bacilli, but perished as the result of the toxic influence of the poisons secreted by the micro-organisms surrounding them (Fig. E, *d.l.*^{1}, *d.l.*^{2}, etc.). In the chronic form of the disease, therefore, it is possible to follow the struggle of the bacilli and lymphocytes—a struggle in which the former and sometimes the latter perish.

I do not intend to discuss here what part phagocytes play in preventing the entrance of micro-organisms into the system in other diseases, or in destroying the vegetable parasites which have already invaded the animal body. Since Metschnikoff's first paper the literature on that subject has become so great that the space at my disposal would not suffice to give even a short summary of the facts contained in the various papers. I there-

fore intend to state here in a very few words the deductions which in my opinion are to be drawn from the experiments just described. I must, however, mention here that Rogowitch, in his paper on Quarter-Ill published in *Beiträge zur allgemeinen Pathologie* (V. iv, 4, p. 291) has stated that the leucocytes present in the inflammatory exudations contain no bacilli in their interior. I am quite unable to conceive how this author has arrived at this conclusion, for in not a single one of my experiments have I failed to find an enormous number of micro-organisms in the interior of leucocytes. Moreover, I have demonstrated this fact to several competent pathologists.

In the first place, it is clear that the fluids present in the inflammatory exudation have no toxic influence whatsoever on the pathogenetic bacilli which give rise to the disease called "quarter-evil." Not only is there no evidence to show that the fluids have the power of destroying micro-organisms, but they seem to have a stimulating effect on their growth, for the latent bacilli contained in the strong virus (A) are called into activity as soon as the dried virus is bathed in the inflammatory fluids present at the spot where the virus is introduced. On microscopic examination, the bacilli are found to be motile and present the characteristic staining reactions of healthy micro-organisms. Theoretical considerations suggest that if inflammatory fluids have any toxic action at all on the bacilli of quarter-evil, this action ought to attain its maximum when the weak virus (B) is inoculated. We see, however, that this supposed toxic action, if it exists at all, is not to be demonstrated by the means at our disposal, for the bacilli of the weak virus show signs of active life as soon as they are in contact with the *living* fluids of the inflammatory exudations. I have purposely called these fluids "*living fluids*," for I do not wish to imply that the same liquids, if taken out of the animal body, and after undergoing the peculiar changes which all protoplasmic fluids undergo outside the body, do not have a toxic action on pathogenic bacilli. The properties of *dead* or coagulated blood, for instance, are utterly different from those of *living* blood, and the physiological actions of the two fluids are also vastly different. Similarly, the *living* serum of the guinea-pig has no toxic influence on the bacilli of quarter-evil; but it would be unwise to argue from this that the *dead* serum of the same animals has no harmful influence on the same bacilli.

In the second place, it is evident that the amœboid cells which migrate to the place where the virus is inoculated arrest the progress of the bacilli and have the power of destoying them after taking them into their interior. The migrating cells, packed closely together, form a kind of living wall, shrovgh which micro-organisms can only penetrate with the greatest difficulty. Further, many lymphocytes contain bacilli in various stages of degeneration, and whereas enormous numbers of bacilli are present in the fluids of the inflammatory exudation, very few or none are found in the deeper parts of the tissue. The wall formed by the migrating cells has proved an almost impassable barrier to the micro-organisms. The localisation of the bacilli at the spot where they are introduced is, therefore, due to the action of the amœboid cells which have migrated to that point. In other words, inflammation in this disease serves a useful purpose. Clinically, the local lesion produced is a sign that the animal organism is fighting against the entrance of micro-organisms into the system.

In some diseases in which the inflammation at the point of inoculation (that is, soft chancre, diphtheria, quarter-evil) is well marked the whole process, as far as the penetration of the micro-organisms is concerned, ends there, though, in spite of this locali-

sation, animals or man frequently die. For some diseases, the pyocyanic disease in rabbits and diphtheria, the death of the animal has been proved to be due to the formation and absorption into the system of poisons secreted by the specific organisms. The evidence that this is the case in quarter-evil also is not conclusive, but we may assume that this supposition is true here also, more especially if we remember that Roux and Nocard have proved that the pathogenic micro-organisms of quarter-evil secrete, at the point of inoculation, a certain amount of chemical substances, which, when injected into animals, confer immunity against quarter-evil. It may therefore be assumed that the same micro-organisms produce not only chemical *vaccinating* substances but also chemical *toxic* substances.

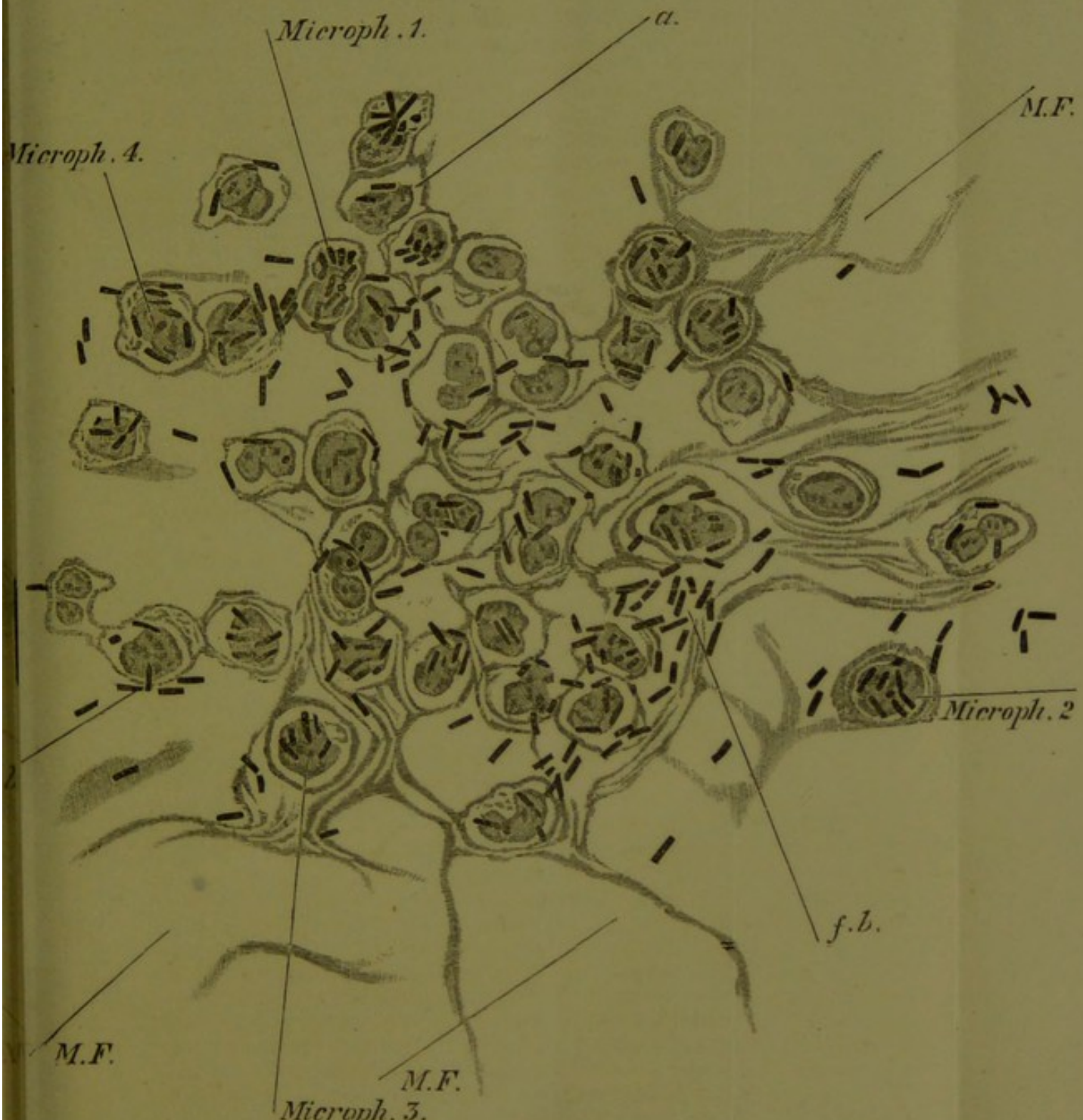
The bacilli contained in the weak virus are, as we have seen, called into life and activity as soon as they are introduced under the guinea-pig's skin though the animal, nevertheless, does not perish. It is difficult to explain the immunity which guinea-pigs possess against that particular kind of virus. To say that the animal does not die because the destruction of bacilli by migrating cells is more marked when the weak virus is used than when the strong virus is injected is not really solving the problem, for, although this is undoubtedly true, the question would then be: "Why do leucocytes show a preference for the bacilli contained in the weak virus?" I think that the question is to be satisfactorily answered by supposing that the bacilli contained in the weak virus either secrete the same poisons but in lesser quantities, or other poisons which are less toxic than those secreted by the strong virus.

The activity of leucocytes is notably lessened by the action of some poisons—for example, quinine—and their movements are even totally arrested by such poisons. It is not too much to suppose that the bacilli contained in the strong virus secrete a substance which has a paralysing influence on the migrating cells present in the inflammatory exudation, and that the bacilli of the weaker virus secrete less of this substance, or perhaps none at all. It is impossible to prove or to disprove this theory at present, but it must not be fogotten that other micro-organisms may, by artificial means, be made to secrete certain substances or not. The bacillus pyocyaneus, for instance, which secretes a green colouring matter in the media in which it grows, and the bacillus or micrococcus prodigiosus, which secretes a red colouring matter, if cultivated at a high temperature loses the property of secreting colouring matter at all. The function of these micro-organisms can therefore be altered by artificial means, and this alteration in function is inherited through countless generations. It may be assumed, therefore—though this is merely a hypothesis—that the weaker virus (B) is so altered by the manipulations (drying, etc.) to which it is subjected as to lose the power of secreting a large quantity of toxic substances. That the weak virus does not completely lose the power of secreting toxic substances is proved by the fact that though life is greatly prolonged the inoculated animals do perish ultimately when a large quantity of the virus is injected.

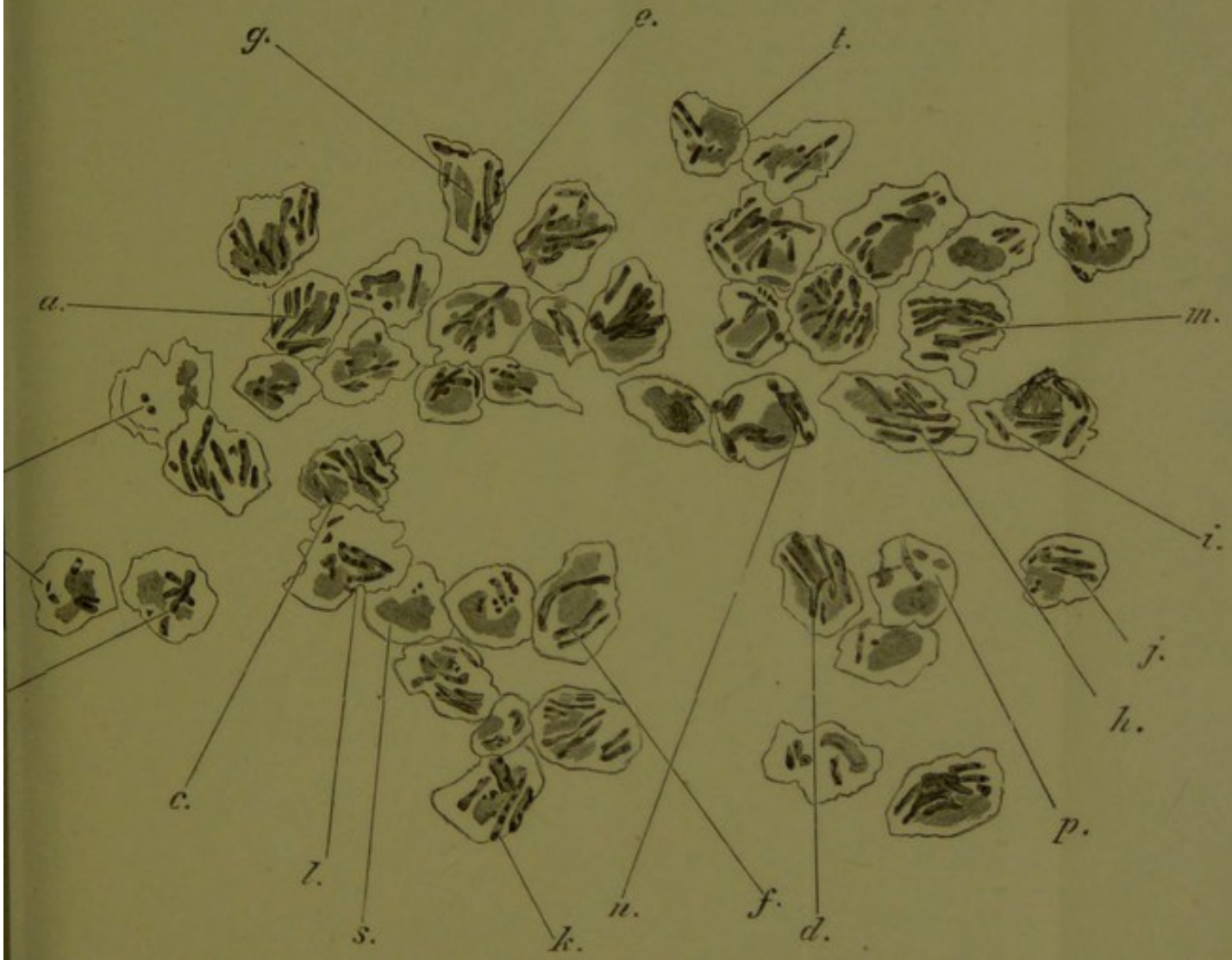
Conclusions.—1. The inflammatory process consecutive to the introduction of the bacilli of quarter-evil under the guinea pig's skin is a protective process, and serves a useful purpose.

2. The destruction of micro-organisms at the point of inoculation is carried out entirely by the amœboid cells present in the inflammatory exudation.

Note.—I beg to be allowed to express my best thanks to my friend, Mr. T. C. Beadles, who kindly drew most of the original pictures for me from my preparations.



B.—Section of part of the abscess wall of a guinea pig which died forty-eight hours after the inoculation of virus A. Carmine and gentian-violet stain. Vérick microscope; oc. 1 × $\frac{1}{18}$; oil immersion; Abbé's condenser. M.F. = muscular fibres. Microph. 1, 2, 3, 4 = microphages containing bacilli. *f.b.* = bacilli lying free in the exudation fluid. For other particulars, see text.



g. C.—Section at spot of inoculation of a guinea pig. Killed forty-eight hours after the inoculation of virus B. Alum-carmine and gentian-violet stain. Vérick microscope ; oc. 1 × $\frac{1}{12}$; oil immersion ; Abbé's condenser. For other particulars see text.

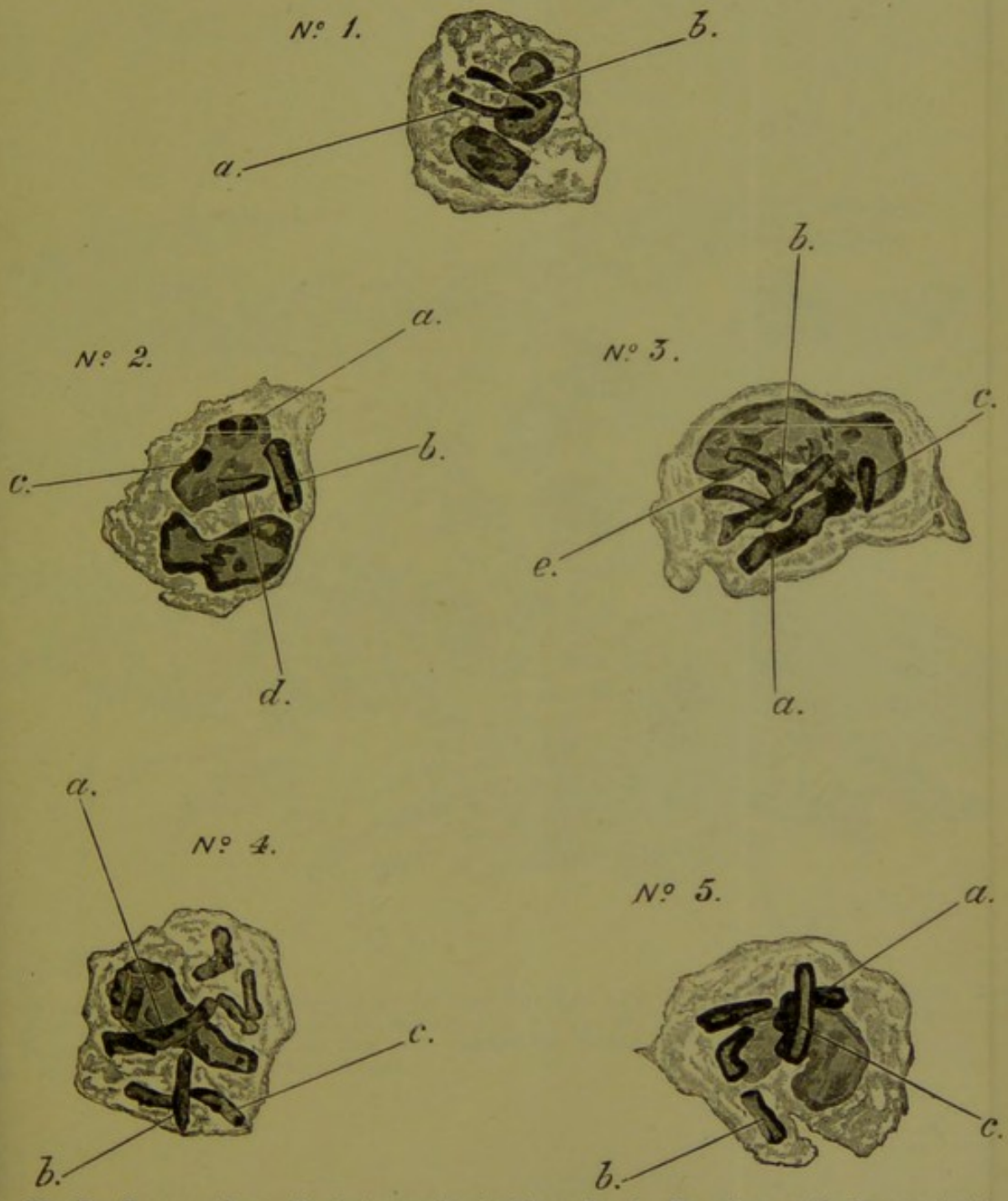
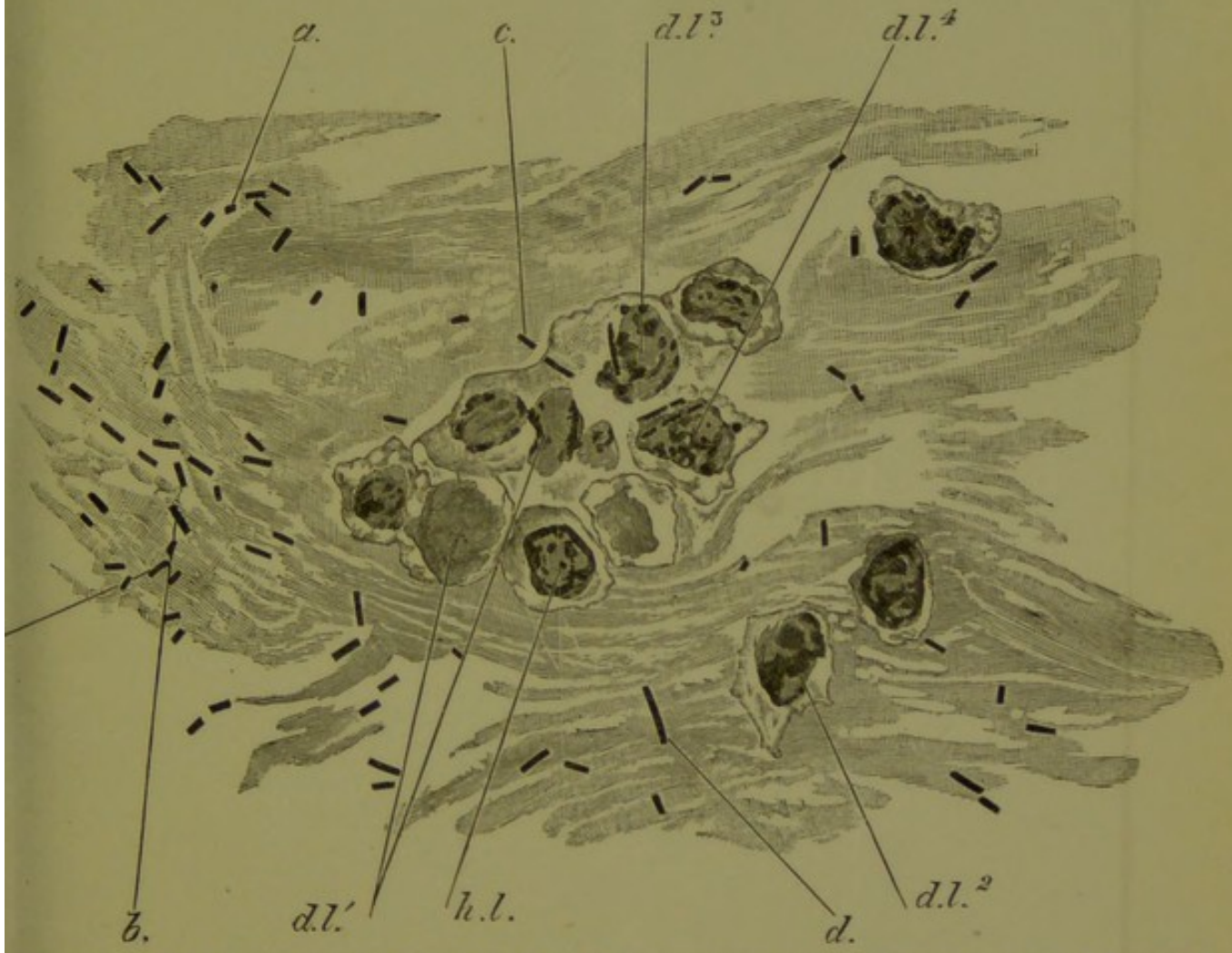


Fig. D.—Same section as the last, examined with oc. 3 × $\frac{1}{12}$. For other particulars see text.



. E.—Section of œdema of guinea-pig which died five days after the inoculation of virus B. Logwood and gentian-violet stain. Vérick's microscope; oc. 1 × $\frac{1}{12}$; oil immersion, and Abbé's condenser. *d.l.*, 1, 2. Empty pus cells. *d.l.*, 3, 4. Pus cells containing bacilli. *h.l.* Healthy leucocyte. *a*, *b*, *c*, *d*, *e*. Micro-organisms. For particulars see text.