NOTES

ON

THE DESTRUCTION OF MICRO-ORGANISMS
BY AMŒBOID CELLS.

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THE task of the pathologist studying the lesions produced by infectious diseases has, in the course of the last few years, been greatly modified by the important discoveries of bacteriological science. Some years ago, the student simply described the microscopical and naked-eye changes in the anatomical elements of patients who had died during the course of an infectious malady. Here his task ended, for there was but little possibility of explaining the causation of symptoms by the pathological alterations found in the cells of the body. With the discoveries of Pasteur, Lister and Koch, a new era set in, and pathologists strove to find the microscopical germs which are the cause of infectious disease, and the study of cellular alterations present in such affections fell into apparent neglect. This was not to last long, however, for with Metschnikoff's researches a new impetus was given to the study of cellular pathology, and the struggle of the cells of the animal organism with the lower parasites is now the most interesting problem of pathology.

The following is an account of a few facts which I have observed whilst investigating this problem.

1. If a Peyer's patch of a healthy rabbit¹ be removed with all antiseptic precautions, immediately after death, and plunged at once into absolute alcohol, it presents, on section and after careful staining (alum-carmin and gentian-violet), the following remarkable appearances.

The lumen of the intestinal tube formed by the fence-like layer of epithelium cells covering the patch is lined by numerous micro-organisms of various shapes and sizes. A number of leucocytes, wandering out from the lymphoid tissue beneath, find their way into the layer of epithelium cells, push some of these forcibly aside, and emigrate into the lumen of the intestinal tube. Examination with a high power shows that some of the leucocytes present between the epithelium cells contain micro-organisms in their interior, which, being stained dark violet, stand out sharply from the red background.

The interior of the patch is riddled everywhere by an enormous number of micro-organisms, which, without exception, are contained in the interior of the lymphoid cells of the tissue. The latter may be roughly divided into two kinds.

Some are small, with one or many nuclei, surrounded by a small amount of protoplasm. Others are of greater size, with a large clear vesicular nucleus. In the latter the amount of protoplasm surrounding the nucleus is larger, and the cell often contains peculiar-looking yellow bodies in its interior. The smaller cells are evidently the ordinary lymphocytes found in all lymphoid tissue, but what are the larger ones?

The nucleus of some of the ordinary small lymphocytes of the Peyer's patch, instead of staining almost uniformly with carmine, contains nucleoli and a well-marked intranuclear network. In other cells again, the nuclei are much larger. In the latter cells the intranuclear net-work becomes more delicate, the amount of surrounding protoplasm increases, and, at last, an enormous cell is formed, which bears but little resemblance to the lymphocyte from which it originated. These large cells are nothing more than the *macrophages* described by Metschnikoff in pathological structures, whilst the smaller ones are true *microphages*. By histologists, similar cells have been described in lymphoid tissue under the name of epithelioid cells.

Many macrophages, as I shall now always call these large cells, contain in their interior distinct and unmistakable *microphages*, sometimes in the interior of a vacuole. The nuclei of some of these *intracellular* microphages² may still appear to be normal, whilst others show signs of degeneration, and look as if they were being digested in the interior of the macrophages. In that case they present the following remarkable appearances.

In the first stage of disintegration the nuclei become irregular or tri-radiate even. Later on, they lose the property of staining with the usual colouring-matters and become of a yellowish dirty-brown colour, the protoplasm swelling up at the same time. The nuclei of the *intracellular* microphages finally disappear, and nothing is left in the interior of the macrophage but a large yellow body, which, itself becoming vacuolated, is slowly digested, until it is represented merely by small round, irregular masses of dirty-yellow protoplasm. Sometimes, however, the protoplasm of the cell is digested before the nucleus, and when that is the case, fragments of nuclei are left scattered through the protoplasm of the macrophage.

The micro-organisms present in the cells, that is, in the micro- or macrophages, also undergo characteristic changes, showing that they are undergoing a process of destruction.

In the first place, they do not retain colouring matters like normal microbes, but stain diffusely and unequally, or present a mottled appearance. In a later stage a peculiar change in the chemical reaction of the intra-cellular micro-organisms takes place, so that their protoplasm no longer greedily takes up aniline dyes, but picks up carmine or logwood more or less readily, the micro-organisms being coloured red partly or as a whole, whilst later on the parasites lose their sharp contours, etc., and break up into small refracting granules.

The conclusion I draw from these facts is, that the lymphocytes (microphages) of the rabbit's Peyer's patch wander to the surface of the intestine, seize on the micro-organisms present in that situation, and carry them into the interior. Here both microphages and micro-organisms fall an easy prey to the more voracious epithelioid cells (macrophages).

There is one great objection to this theory, namely, the fact that although the leucocytes have been seen loaded with micro-organisms at the surface of the Peyer's patch, they have not actually been watched returning into the depths of the tissues. It might be argued further, that micro-organisms being endowed with power of movement, they might possibly find their way between the epithelium cells and so penetrate into the strata beneath.

My answer to these objections is the following:—In the first place very careful examination of the epithelium border never reveals the presence of micro-organisms inside or between the epithelium cells, except when such parasites are contained in the interior of amœboid cells. Secondly, it can be proved that inorganic, solid, non-motile particles penetrate into the lymphoid tissues of the alimentary tract; and it is impossible to account for their presence in that situation except by supposing that they have been carried inwards by cells endowed with the power of movement.

It is easy to prove the penetration of foreign inorganic particles into the lymphoid tissue of the alimentary tract. All that it is necessary to do is to examine the lymphoid organs of another part of the alimentary canal of dogs. Every one knows that these animals swallow all kinds of dirty refuse which they come across in their roamings through the streets. Some of the dust and charcoal particles necessarily sticks to the animals' tonsils as it passes with the food over these organs. Examination of the tonsils of dogs reveals the presence on their free surface of a number of leucocytes crammed with charcoal particles. These, however, are present, not on the surface of the tonsils only, but they return loaded with charcoal particles into the interior of these organs. Here charcoal and leucocytes fall an easy prey to the large epithelioid cells—that is, macrophages—of the dog's tonsils. In the rabbit's tonsils, indeed, the macrophages wander to the very surface, and take not only leucocytes and charcoal into their interior, but also various kinds of micro-organisms which undergo, in the interior of the macrophage, the same process of intracellular digestion, as in the Peyer's patches.

These researches throw some light on a fact which has been known for centuries, namely, that most infective processes taking place in the alimentary tract originate in the lymphoid structures (tonsils, Peyer's patches), present in that canal. We have seen that it is in these organs chiefly that germs are carried after having been fetched from the free surface by amœboid cells, and that, in some animals at least, a struggle is continually taking place between the amœboid cells and the parasites. In the majority of cases the former are successful in destroying the micro-organisms and preventing their entrance into the system, but there can be little doubt that under certain conditions (cold, insufficient food, &c.), the latter may gain the victory, and set up a local or general infective process.

¹ Quarterly Journal of Microscopical Science, January, 1890.

By intracellular microphage I mean a leucocyte contained in a macrophage.

I conclude from these researches that: 1. The wandering cells of the lymphoid tissues of the alimentary canal have the power of proceeding to the free surface of such tissues and of taking into their interior micro-organisms and foreign matter (charcoal, etc.) 2. There are two kinds of wandering cells in the lymphoid tissues of the alimentary canal: *a*, microphages (small mono- or multi-nucleated cells); *b*, macrophages (large mono-nucleated cells). 3. The macrophages are developed from the small mono-nucleated lymphocytes. 4. Macrophages are able to swallow microphages (leucocytes), and to destroy and digest them. 5. The micro-organisms are rapidly destroyed in the interior of the micro- and macrophages. 6. Micro-organisms are never found lying free between the cells, or in the blood vessels and lymphatics. 7. The destruction of micro-organisms taking place in the normal lymphoid tissues of the alimentary tract, resembles in all particulars the destructive process following on the inoculation of pathogenic organisms into resistant animals.

II. It is interesting to see that the epithelioid cells of other lymphoid organs and of pathological new formations possess the same power of amoeboid movement, and I may be allowed to give here the result of a few observations and experiments bearing on that point.

In the spleen are found large mono-nucleated cells which are generally termed epithelioid, from their great resemblance to an epithelium cell. These possess a single clear oval and vesicular nucleus with a well-marked intranuclear network and nucleoli. This is surrounded by a varying amount of coarse, often vacuolated, protoplasm. Kölliker has in such cells seen the normal destruction of red blood-corpuscles, and, according to Metschnikoff, they take an active share in the destruction of micro-organisms.

These facts are almost sufficient to convince the observer that they are endowed with the power of independent movement, but I have lately been able to bring forward additional evidence to prove that this is indeed the case, and as these facts have not been published as yet, I am glad to have this opportunity of saying a few words about them.

According to my observations, these cells originate in the spleen just as the same cells originate in the lymphoid tissue of the Peyer's patch. They are undoubtedly derived from the small lymphoid cells of the spleen and are amoeboid, for they are met with not only in the lymphoid tissue, but also in the interior of the venous sinuses, where they often assume shapes most astonishing in their irregularity.

Some of these cells contain small lymphocytes in their interior. The intracellular lymphocytes occasionally appear to be normal, but in most cells they show distinct evidences of being partially digested by their host. Notice, however, that instead of swelling up and forming yellow bodies, as in the Peyer's patch, the protoplasm of the intracellular lymphocyte always disappears first, being eaten away, so to speak, from the periphery. Soon nothing is left in the cell but the nucleus, which, becoming more and more irregular, finally breaks up, and what remains of the intracellular leucocyte is represented by fragments of digested nuclei, which, however, still take up logwood or gentian violet.

Thanks to Professor Bouchard's kindness, I have been able to prove directly—namely, by experiment—that these large macrophages have the power of taking foreign particles into their interior. To do this I prepared aseptically an emulsion of finely divided vermilion in distilled water. Several cubic centimetres of this mixture (15 to 20 centimetres) were then injected slowly into the lateral vein of a rabbit's ear on two consecutive days, and the animal killed twenty-four to forty-eight hours after the last injection. The rabbit recovered perfectly—in fact, appear to suffer no evil effects either during or after the injection. Moreover, the lateral vein being quite superficial, the operation is less difficult than an ordinary hypodermic injection. The spleen, after a successful injection, is of a brick-red colour, owing to the vermilion which has been arrested in that organ.

Microscopic examination of sections of the spleen after staining with logwood and auramine reveals the fact that some of the vermilion had been taken into the interior of these large cells, and I show you here one of the large macrophages crammed with vermilion and digested leucocytes. The large epithelioid cells of the spleen, therefore, are true phagocytes, have the power of destroying red and white corpuscles, and of taking into their interior inorganic particles such as vermilion, as well as living micro-organisms. They are, therefore, amoeboid cells.

By the same experiment it may also be proved that the so-called epithelioid cells of the lungs—*Staubzellen* of German

authors—are really amoeboid cells, for the vermilion may be traced into their interior also. They are, therefore, true macrophages, a fact which had already been demonstrated by Tschistowitsch.

Lastly, the epithelioid cells of lymphatic glands also possess the power of absorbing small foreign bodies—that is, are amoeboid structures—for in sections of the bronchial glands of cats, the macrophages present in these organs are found crammed with coal particles, which they have fetched from the surface of the lung, or which have been brought to them by the wandering leucocytes present in these organs.

III. Turning now to the relation of these cells to pathology, we know that similar cells were long ago described in infectious granulomata.

It is easy to show that the epithelioid cells of infectious granulomata have functions similar to those of the same cells in lymphoid organs. In tubercle, for instance, such cells not only take into their interior the specific bacilli and digest them, as Metschnikoff had noticed, but, according to my observations, such cells absorb and digest the smaller lymphocytes which enter into the formation of the tubercle nodule. In glanders, also, I have seen the same process taking place in the epithelioid cells entering into the formation of the chancre in the Schneiderian membrane of the horse. But there is one disease in which the destructive action of these cells can best be studied, namely, in actinomycosis. Professor Crookshank, in his researches of this disease in men and animals, described large mono-nucleated cells, which he rightly, in my opinion, compared and regarded as identical with the epithelioid cells of the tubercle.

If, instead of examining the centre of each microscopic nodule, which consists of the central mass of hyphen-like bodies lying in a nest of epithelioid cells surrounded by inflammatory material, the periphery of the nodule be examined, a number of epithelioid cells are found, often containing in their interior one, two, three, or more—sometimes as many as eight or ten—lymphocytes in various stages of degeneration. They, like the epithelioid cells of tubercle, of glanders, lymphatic glands, spleen, etc., are easily recognised by their large clear, vesicular, generally single nucleus, and the large amount of vacuolated protoplasm surrounding it.

The power which such cells possess of taking into their interior and destroying pathogenic micro-organisms has already been dwelt on by Metschnikoff. As a rule, however, epithelioid cells are concerned chiefly in the destruction of other cells which have been weakened, or have died, as the result of their struggle with pathogenic parasites. Notice also, that these cells do not pre-exist at the point where the struggle takes place, but that time must elapse—several days in most cases—before such cells are formed.

Of the larger multi-nucleated cells (so-called giant-cells) which are found in certain chronic forms of inflammation, namely, tubercle and actinomycosis, I have little to say that is new. According to Metschnikoff, such a cell is formed either by karyokinesis (the protoplasm of the cell not dividing) or by the fusion of several epithelioid cells. In any case I agree with Metschnikoff in thinking that, far from a giant-cell being a structure in any way weakened or diseased, it is on the contrary a most active body—in other words, a *fighting* cell. Everyone who has successfully isolated one, and has seen its long slender processes often prolonged in the interstices between many groups of cells, cannot doubt that it is an amoeboid structure. Its contents, again, show it to be a combatant, for in it are found numbers of tubercle bacilli, in various stages of destruction—a fact already described in masterly fashion by Metschnikoff, whose observations on that point I can only confirm.

I have lately, though quite accidentally, observed one fact which illustrates this destructive function of giant-cells. In the spleen of many animals, more especially of guinea-pigs, the uni-nucleated epithelioid cells often contain a quantity of pigment, which is really the remainder of red corpuscles destroyed in the interior of these cells. I have lately shown that the number of these cells is greatly increased in certain infective diseases, that is, quarter-evil. In the spleen of tuberculous guinea-pigs, in which the tubercle has invaded that organ, the destruction of red blood corpuscles in the macrophages of the spleen is an extremely active one, but—and this is the most interesting point—the giant-cells of tubercle take an active share in this process, and I show you here two giant cells absolutely crammed with blood pigment and debris of partially-digested leucocytes—a further proof that giant cells are amoeboid, and, like other amoeboid structures, have the power of taking into their interior, and digesting, red blood corpuscles, leucocytes, and micro-organisms.

Considering, therefore, that the tubercle-nodule consists of a number of extremely active amoeboid cells, which all have the power of seizing, destroying, and digesting tubercle bacilli, I am of opinion that the nodule is really a barrier raised up against the invasion of the whole organism by the specific bacilli, and that the processes taking place in the nodule, as long as the anatomical elements composing it are alive, serve a useful purpose.

IV. Lately³ I have published facts showing that processes going on at the point of inoculation of the virus of quarter-evil are really protective in their nature. I endeavoured to show that in this disease, as soon as the virus was introduced subcutaneously, an emigration of leucocytes took place, which, surrounding the virus, prevented the specific bacilli from getting into the system. Moreover, the leucocytes, taking the bacilli into their interior, destroyed and digested them. The conclusions I drew from those experiments were that:—

(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; and (2) the destruction of micro-organisms at the point of inoculation is carried out entirely by the amoeboid cells present in the inflammatory exudation.

V. More lately still⁴ I have brought forward similar facts relating to diphtheria, but, as they have only just been published, I must restrict myself to repeating the conclusions at which I have arrived.

1. The bacilli of diphtheria are present in the most superficial part of the membrane only, that is, in a place where they are well within reach of medicinal agents, an observation not without interest from the point of view of treatment.

2. In the diphtheritic membranes there is an active struggle taking place between the amoeboid cells in the membrane and the micro-organisms. In other words the diphtheritic membrane is a battlefield for amoeboid cells and the pathogenic microbes of diphtheria.

3. The reason why the bacilli do not actually penetrate into the tissues is probably that, as soon as they try to do so, they are arrested by the amoeboid cells present in the diphtheritic membrane.

A DISCUSSION

ON

THE ETIOLOGY OF TYPHOID FEVER.

In the Section of Public Medicine at the Annual Meeting of the British Medical Association, held in Birmingham, July, 1890.

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To judge from what one occasionally sees in the lay press, no subject would appear to be more trite, or to have been more thoroughly threshed out than the causation of typhoid fever. Those whom I am now addressing are, however, well aware that there are several important points connected with the natural history of typhoid fever upon which the medical profession are by no means agreed. On the important question of the immediate origin of typhoid fever, it is most desirable that we should be able to state some definite conclusions as representing the opinion of the bulk of the profession. From a sanitary point of view it is important, because no excuse is more frequently put forward for existing defects than the fact that they have not produced typhoid fever; from a legal standpoint it is also important now that actions are frequently brought to recover damages from owners of houses where typhoid outbreaks have occurred. Quite lately I have been called upon to give evidence in the High Court of Justice in connection with a house where typhoid fever and structural sanitary defects had admittedly been coincident, and the pivot upon which the case turned was the question—Did the typhoid result from the sanitary defects?

The surest way of arriving at sound conclusions in the matter will, I believe, be found in the careful investigation into, and record of facts in connection with, actual outbreaks rather than in theories based on abstruse questions in biology, and my object on the present occasion is to endeavour to show what light can be thrown on the subject by the results of some seventeen years, pretty constant personal investigation into outbreaks of the disease, fre-

quently under conditions exceptionally favourable for studying its etiology.

All the cases to which I propose to refer have been officially inquired into by myself as medical officer of health, and I have notes of every one of them. The outbreaks occurred in various urban and rural districts in seven different counties, and frequently under widely different physical conditions. The number of cases of which I have notes amount in the aggregate to 2,451. As 743 of these occurred in towns where enteric fever may be considered to be more or less endemic, and as in a purely etiological inquiry such cases could only afford unreliable evidence I have excluded them from present consideration, and for the same reason I do not propose to include various statistical details of information which the above series of cases might be made to convey, but to give certain general conclusions arrived at after careful and precise inquiry into the individual cases. With reference to the remaining 1,708 cases, I have notes of all of them, made under conditions sufficiently uncomplicated to enable a reasonable conclusion to be arrived at as to their origin. In by far the largest majority of cases the outbreaks could be distinctly and indisputably attributed to direct importation from other districts, or to the revival of old germs of infection from previous outbreaks on the same premises. The cases were imported either in the stage of incubation or in the stage of preliminary *malaise*, or even in the early stages of the established disease when the patient is able to make an effort to get home, or is sent home from a situation by an employer. These cases came, as a rule, from those centres of population where typhoid fever is endemic, or from any town where epidemic typhoid fever was prevailing, as during the severe epidemic in Kidderminster some few years ago I had a number of cases imported thence into different parts of my district. Owing to the comparatively lengthy period of incubation cases often come from long distances, and I have notes of typhoid being imported into my district from Egypt, Italy, and Germany. In at least one instance the infection was attributable to urban manure brought from a distant town for agricultural purposes. As so large a proportion of cases of typhoid fever occurring in rural districts are domestic servants, *employés*, or others who have contracted the infection elsewhere, death returns are utterly misleading as indications of its local connection, and are equally fallacious as indications of the amount of such disease, in consequence of the exceedingly variable percentage of mortality to cases.

Although it is only on inferential evidence that cases can be said to owe their origin to the revivification of old germs, in many such cases the evidence is overwhelming, whilst in others such a source can only be suspected. I am confident that this is a much more frequent source of outbreaks of typhoid fever than is generally suspected, and that it is the true explanation of many of the so-called spontaneous cases. For instance, such a case as the following might, if investigated by anyone uninformed of the previous history of the house, have been put down as an inexplicable case. A boy, aged 11 years, got thoroughly wet through going to school, and was allowed to remain all day at school until his clothes had dried on him, when he returned home at night. Eighteen days after he sickened with typhoid fever. The house was in every way unobjectionable from a sanitary point of view, and here was a case in which the popular explanation, so commonly volunteered, that the fever came from catching cold, would seem to be the only one admissible. I was, however, acquainted with the history of the house. This family had only lived in the house four months, and three years previously a case of typhoid fever had been imported into the house, and had been investigated by myself. The cottage had been disinfected by sulphur fumigation and lime-washing as far as applicable, but the wall paper in the bedrooms had not been stripped, and there or elsewhere about the premises the infection had remained, and had revived under favourable conditions of season and subject. Such cases are by no means uncommon, and are specially liable to occur in the case of new comers to a house; indeed, it would seem that the partial protective inoculation, which those acquire who live in localities where typhoid fever is endemic, extends to the occupiers of infected houses.

There yet remain a certain number of outbreaks to which, without an undue straining of evidence, neither of the above explanations can be made to apply. Some proportion of these, especially when occurring in houses with no objectionable sanitary features, may be presumed to be the result of infection conveyed through some unsuspected channel or connected with some previous un-

³ BRITISH MEDICAL JOURNAL, May 24th, 1890.

⁴ BRITISH MEDICAL JOURNAL, July 26th, 1890.