

THE GRANULAR FORMS OF THE LEPROSY BACILLUS¹

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The existence of granular forms of acid-resistant bacilli such as Koch bacillus has been known for some time, but it has not been possible to explain the real nature and importance of these granules. It is now recognized that the well-known special tissue modifications provoked by the Koch bacillus itself represent only one form of the disease. It is entirely possible that other forms of tuberculous infection exist which are not yet recognized sufficiently. The granular forms of the bacillus may be of considerable importance in connection with the pathology and the phenomena of immunity of tuberculosis, and therefore also the epidemiology of the disease, especially as regards inheritance and transmission. This can well be assumed from the results of experimental investigations hitherto conducted, though because of the reduced size and difficulty of preparation of these granular and invisible forms they cannot be demonstrated by the present methods of investigation.

Two hypotheses have been formulated concerning the nature of the granules: (1) Some investigators believe that we are dealing with simple degeneration and disintegration forms—that is, phenomena of cellular extinction. (2) Others believe that we are dealing with enduring forms, and forms of multiplication that might be compared to spores. However, it has not yet been possible to arrive at a definite conclusion.

To form a conclusion concerning such forms of the leprosy bacillus is especially difficult since we do not have at our disposal an easy method of cultivation, or animals susceptible to leprosy; we are restricted solely to observation and clinical experience. Nevertheless, it is evident that the granular forms are of great importance in leprosy, and for that reason investigations, at least on the patients, concerning the nature of these granulations are necessary and urgent.

For some years I have been conducting bacteriological examinations in the state hospital for lepers in Havana, dealing with more

¹ Translated from the Spanish by Anna B. Banyea.

than two hundred patients. Among the possibilities at my disposal I concentrated attention upon these granular forms, which are found very frequently when cases of leprosy are carefully observed.

The morphology of the completely developed leprosy bacillus, apparently well conserved and stained, is not always that of an undivided, rigid rod. Individuals are readily recognized that possess certain special and characteristic peculiarities of structure and external form. In some, nodular dilatation in circumscribed places suggests granular forms. Bacilli are also readily found in which between four or five of these dark red, slightly enlarged places, there are thin intercalated sections. Bacilli are also seen which appear to be disintegrated into four to six granules held together by an invisible sheath or substance, so that the form of the original rod from which they separated remains recognizable. But there are also instances in which only from one to three such granules become detached from the bacterial body, usually not colored, and which, to judge by their uniform arrangement, must have some relationship to it, though it has not been possible for us to stain the fundamental substance suspected by us. Possibly in such cases we might obtain better results by other staining methods, or by ultra-microscopic examination. The details are clearly shown in the photomicrographs made from my original preparations and presented here.²

Before going further into this matter I wish to make some general observations on the method which I use in staining the leprosy bacilli, especially for coloring the granules. The Ziehl-Neelsen method of staining the Koch bacillus is not suitable for leprosy bacillus; with it one must proceed with greater caution, as it is considerably less acid-resistant. Therefore, in examining suspected cases in which no bacilli are found I consider it important to stain control specimens from cases that do contain bacilli.

STAINING METHOD

1. After cleaning the ear lobe with alcohol and ether, make an incision parallel to the folds of the skin. Express a little lymph from this by light pressure, avoiding as far as possible mixing lymph with blood. Remove the lymph with the edge of a slide and spread a light smear over a second slide. Dry and fix with heat as usual.

2. Pour Ziehl's carbolfuchsin plentifully over the preparation³ and heat carefully in the usual manner. Wash in water.

²For these photomicrographs I am indebted to the courtesy and technical ability of the celebrated Prof. J. Novak, of the University of Crakovia, to whom I am very grateful for his great interest in the matter.

3. Apply, drop by drop, absolute alcohol or a mixture of alcohol and acetone until the excess red coloring has disappeared for one or two seconds. Wash quickly.

4. Apply by drops 30 per cent nitric acid for one or two seconds, to obtain uniform coloration. Wash. If the red stain is still visible, apply the acid again and wash quickly. By this means the preparation will become permanently and completely decolorized.

5. Counterstain for a few seconds with Löffler's methylene blue solution diluted to one-third. Wash thoroughly and dry.

When staining of the granules is especially desired, proceed in the same manner but shorten the time of decoloration, for sometimes the acid-resistance of the granules is not much greater than that of the cells of the tissue. Just as soon as the tint of color of the smear disappears decoloration must be stopped.

In the microscopic examination attention is first fixed upon the nodules visible in the bacilli themselves. Then will be noticed the bacilli that are in the disintegration stage, of which there remain only one or two granules. Finally may be observed acid-resistant granules that are free from the bacilli.

Frequently small round bodies are seen, of the size of a red blood cell, which are composed of massed bacilli supported by a non-acid-resistant substance that may be the remains of the disintegrated lipid coverings. In these bodies the granular disintegration of the bacilli may be observed very clearly. Often only a few partially decolorized bacillary forms are conserved. These bodies often contain only granular forms. At other times there are small, irregular groups in which at first glance the arrangement of the bacilli seems to be that which we usually see in leprosy, but which close observation reveals to be composed entirely of granules. Sometimes the granules are so small as to be barely within the limit of visibility, and their coloration is frequently indistinct and pale.

In not-too-thick, well stained smears from treated cases, large numbers of such small granulations may be seen distributed in the form of a fine powdery cloud, forming small irregular groups. The manner of their arrangement sometimes shows that they are groups that had been confined in leucocytes, and undoubtedly set free by the disintegration of them. Well preserved leucocytes are also encountered which contain such groups of granulations in the protoplasm, the nucleus remaining free.

In some cases the granular forms may predominate so completely that no bacilli are found. Sometimes the groups are disposed in

the same manner as the bacilli are arranged ordinarily. On the other hand, the form of the bacilli may be so greatly altered that nothing can be seen but a fine irregular powder composed of very small granules.

Just as the size of the granules in material from treated cases varies from that of small cocci to scarcely visible dots, so without doubt the acid-resistance diminishes with progressive cure. In properly stained preparations granules are found that have been decolorized completely, giving the impression that this loss of color can be due only to a difference of acid-resistance of these bodies. I am distinctly of the impression that the succeeding stage in progressive disintegration is that the granules completely lose their acid-resistance and therefore become imperceptible to the eye, or at last can not be recognized positively by present staining methods.

This is confirmed perhaps by an observation made by comparing smears from the same cases stained by both the Gram and Ziehl methods. The impression was gained that with the Gram stain the granules were unmistakably larger and in greater number; also that the intensity of staining varies, decreasing until only "silhouettes" remain—perhaps an expression of progressive disintegration. Whereas the largest are the size of small cocci and are suggestive of such bodies, the smallest are only within the limits of visibility, suggesting fragments of bacilli in disintegration, or colloidal particles of the serum.

The rod form is rarely demonstrated as strongly by Gram's as by the Ziehl method, because the interstitial substance remains completely unstained. The disintegrating bacilli appear like streptococci in short chains of four or five links, the centers of which are usually broader than the ends. Frequently the granules occur in the form of diplococci; also, they may be in small bunches like staphylococci. Very rarely three or four granules fuse to form a small rod.

The recognition of all these granules as such is not always easy. Even though the Ziehl staining be sufficiently clear and certain, the eye perceives nothing in the red coloration except the acid-resistant remains of the disintegrated leprosy bacilli. In the Gram stained material, on the contrary, one cannot be sure whether all that is considered granular forms of the Hansen bacillus is really such, or whether some of the granules that retain the Gram may perhaps have some other significance. It would be desirable for the elucidation of

this important question if special staining procedures might be evolved which would avoid all possibility of error.

The supposition is undoubtedly well founded that the granular forms described, all of which have been observed in properly treated cases or in cases that present evident improvement and progressive cure, are probably forms of degeneration. This can be deduced from the visible differences in size and coloration, and because their presence depends upon the treatment, progressive improvement, and cure.

I also consider it very probable that, besides these, there exists a second class of granules which are fundamentally different and are capable of life and proliferation, and therefore may play an important part in the cycle of development of the leprosy organism. With our present means it is difficult to obtain definite proof of this conception, but it is easy to imagine that in the future, with new working methods, we may be able to prove it.

Granules are especially numerous in microscopic preparations of tissue sections, whether the Ziehl or the Gram stain be employed and regardless of the treatment method. Therefore, the belief that these granules are solely forms of degeneration is unnatural and without foundation, even when we take into account the natural defenses of the organism. These granules are encountered in recent, progressing inflammatory pathologic foci and in an enormous stage of proliferation. By study free from prejudice one is led by their appearance more to the conclusion that they must constitute a special form of the living leprosy bacilli, and that they probably serve as a means of multiplication and proliferation; or that they are special, resistant forms which successfully withstand in the tissues the action of the defensive substances of the organism, possibly in this way explaining the long duration of the illness. I sometimes have seen granules that gave the impression that a new germ, which at first did not take the Gram stain, had originated in them.

The above statement seems to be confirmed by the fact that granulation forms are not seen exclusively in smears from treated cases; I have also seen them in large quantity even at the first examination of new cases which, from all indications, had not yet been treated.

In these cases, undoubtedly, some of the granules can be considered disintegration forms. It is well known that in leprosy spontaneous cure plays a considerable part, and that the defense of the organism is capable of arresting the disease for many years and may even bring about local cure, and therefore may damage the bacilli considerably and prevent their proliferation. However, in these untreated cases one is especially inclined to think of resistant forms, or development forms, and less inclined to make the positive assertion that all these granules may be disintegration forms. Undoubtedly, the supposition presents itself that precisely these granules may be the specific forms that resist the tendency of the organism to lasting and definitive cure, inasmuch as they may grow anew and produce new bacilli in proportion as the antibodies become exhausted, and thus produce the exacerbations of the disease.

In Gram-stained sections my attention was attracted to the fact that, as a general rule, the granules are within cells which have stained intensely in a special manner, and that also many very small forms exist there which may have originated from the granules. All the cells are full of these granulations, and there are many free ones outside of cells. Sometimes it seems as if the young leprosy bacillus might be a cellular parasite which has its own center of proliferation within the cells. This supposition seems to be confirmed by the fact that the tissue cells, especially the large endothelial cells, frequently contain masses of bacilli disposed regularly and especially radially, which undoubtedly must have some special significance. In these they can well be considered centers of proliferation of new bacilli.

Perhaps here originated the characteristic globi, and especially the groups resembling bundles of cigars, that are frequently encountered free in smears and which strongly recall the radial disposition of the intracellular groups. Under antileprosy treatment these intracellular forms disappear gradually. Therefore, they cannot be satisfactorily explained on the grounds of phagocytosis of the dead or debilitated bacilli; if this were the case, they would have to occur more frequently as a result of the treatment. We do not deny that there may be intracellular localization independently of these forms, due to true phagocytosis. Undoubtedly we need other methods of staining and preparation to enable us to identify transition or

development forms which cannot be demonstrated by the usual methods.³

I should like to state here especially that I have also frequently found such granulations in cases that presented allergic reaction, as is observed in treated cases when, because of the sudden disintegration of many bacilli, large numbers of antibodies are set free and exercise their stimulus on the tissue which was formerly leprosy and therefore desensitized. Frequently patients interpret these phenomena as a new exacerbation or a relapse of their illness and are inclined to discontinue the treatment, for they consider them as an unfavorable reaction to it. In reality, their allergic reaction is a favorable sign, and completely disappears in a few days as rapidly as it appears. I have often examined such allergic foci and have always found them free of bacilli. The physician should not be led into a natural error by these phenomena, which are often very intense, but should confidently continue treatment because he is upon very favorable ground.

The case is recalled of a patient who for a whole year received regularly every week one or two intravenous injections of antileprol and found himself in very good condition, and from whom for some time no bacilli had been obtained, only granular forms. This case was studied by me with special care. Suddenly intense phenomena again presented themselves in the skin in the form of papular eruptions and intense reddening over the entire body, with nocturnal fever and severe pains and tumefaction in the joints. The patient himself considered this as a relapse of his illness and refused to continue treatment.

This case was peculiar, and inexplicable in view of the good results that had been obtained with antileprol treatment in all the others. I was convinced that this was not a case of relapse but one of marked allergic reaction, the expression of a favorable curative action. This view was soon confirmed by the fact that it was impossible, on examining material from the swollen and

³ Since the above was written I have examined briefly a recently-arrived new case, in which the symptoms were of recent occurrence and limited principally to tumefaction of the face and the ears. In this case the lymph from an incision in the ear lobe had a milky-white appearance, and microscopically was composed entirely of an emulsion of leucocytes, considerably increased in volume, generally two or three times greater in diameter than normal, the protoplasm of which was completely filled with leprosy bacilli, without granulations. This prematurely developed case confirmed my opinion that this condition cannot be considered phagocytosis, but that the leprosy bacilli are actual intracellular parasites. It is easy to suppose that the bacilli in the cells are neither devoured nor destroyed by the supposed phagocytosis but that, protected from the antibodies, they find there conditions favorable for their growth and nourishment. Therefore the term phagocytosis would not be considered correct, at least in cases of this kind.

✦ painful ear lobe, to find Hansen bacilli. However, there were granules so tiny that only after long and careful search were some very small groups of them found. This stage, which had been interpreted as a retrogression of the illness, was beyond a doubt caused by the toxins set free by the bacilli destroyed in large numbers during the treatment. As was expected, there was a sudden retrocession of all the grave symptoms, which would have occurred sooner had the patient himself not interrupted the plan of treatment and had he not hidden himself.

Two months later the patient was in very good general condition, presenting no visible clinical phenomena. Nevertheless, I was now able to find in the lymph from the ear, from which the swelling had entirely disappeared, enormous quantities of leprosy bacilli. Particularly numerous were short forms which probably were young forms developed from the resting granules because of the abandonment of the treatment for several months.

This late bacteriological finding, in conjunction with the allergic reaction offers, to my mind, important data for the treatment of the disease. The action of carbonic acid snow and the effect of iodine in treatment are attributed to identical processes. The formation of young forms is stimulated. These are more affected by treatment, and by their disintegration they provoke an intense formation of defense substances.

The granulation forms, or the granules—which are so extraordinarily small that they can be seen only after careful search—indicate that there may exist other forms of the bacillus—forms which are filterable and invisible. Possibly they serve partly for the conservation of the species, and perhaps also play a part in the transmission of the disease, concerning which we still know very little. It certainly cannot be said that all granular forms which we encounter are of one and the same nature, and though the majority of them are degeneration forms others may serve for proliferation.

The importance of the granules in the diagnosis of leprosy should not be lost to sight. In new cases seen for diagnosis which present the usual cutaneous lesions, bacilli in sufficient quantity are generally encountered. However, there are cases in which temporary improvement sets in, either due to spontaneous cure or as a result of treatment, in which the finding of the bacilli is difficult. In such cases it is necessary to search for and recognize the acid-resistant granules, and to take them into account. I consider the Gram stain alone not suitable for the examination, for it is more difficult to form an exact opinion of the Gram-resistant granules than of the acid-resistant ones, the latter being much clearer and more distinct.

More important than in the new cases, it seems to me, is the finding of granules as a criterion of the result of treatment. As stated, I have frequently observed that the bacillary forms have completely disappeared after treatment, but that it was still possible to find granular forms in varying numbers. Naturally, such cases must not be considered as being free of bacilli or as cured; further careful treatment is necessary if complete success is to be obtained.

Therefore, I believe the granular forms to be of the greatest importance to the physician who has to treat leprosy cases, permitting him to form an accurate opinion of the progress of the treatment. This may be of value in judging new drugs and treatment processes.

The cure of leprosy is at present a complicated and difficult problem for us to understand completely, comprising in part the immediate toxic action on the microbe of our present drugs, and necessarily also the action of the substances of defense and resistance produced by the organism. During the disintegration of the bacilli into granules, and later during the disintegration of the granules themselves, there must undoubtedly be set free in abundance antibodies which in many cases will produce the allergic reaction that greatly facilitates treatment. It is possible that certain remedies favor and accelerate granular disintegration, and also that the granules may be more efficaciously attacked by one product than by another, and in greater proportion than the bacilli themselves. Thus perhaps may be explained the fact, now well proved, that certain medicinal products of gold and of antimony, used in conjunction with antileprol, show a very pronounced curative action, which is manifested also by increased granule formation.

SUMMARY

The leprosy bacillus, like other acid-fast bacilli, produces in its evolutionary cycle great numbers of granular forms which are found both within the bacilli and as free-lying bodies. These granules constitute an essential phase in its evolution.

Among the free-lying forms are those of all sizes down to the limits of visibility, so that it is probable that still smaller, perhaps invisible and filterable forms exist, which may be of special though as yet unknown importance in the pathology and epidemiology of the disease.

The supposition is feasible that many of these granular forms are phenomena of degeneration and disintegration, which are deter-

mined in part by the defensive substances of the organism and in part by the action of our medicinal products, and especially by the chaulmoogra oil; therefore, their presence can be considered a favorable sign.

However, a careful study of the granular forms suggests that they should not be considered solely as degenerative forms. Evidence is given that in other cases they seem to be especially resistant or young forms, essential for the preservation and perhaps propagation of the microorganism of leprosy. Obviously, the importance of these forms for scientific study and practical work in leprosy cannot be overestimated.

It is no less possible that, besides these granular forms, other forms exist which are stable and serve as means of propagating and preserving the species, and which may be considered responsible for relapses, failure to check the progress of the disease, its long duration, and its supposed incurability. Both forms may exist simultaneously and perfectly.

With the means now at our disposal it is not possible to solve the difficult question of the nature of the granules. To do so improved procedures of staining and of cultivation are required and especially of experimentation upon animals. As yet we are restricted mainly to observations on patients to enable us to study these singular and no doubt very important granular formations.



DESCRIPTION OF PLATE

PLATE I

The figures represent photomicrographs of material from cases of leprosy in preparations stained with carbolfuchsin as described in the text. As reproduced they are magnified about 2,000 times.

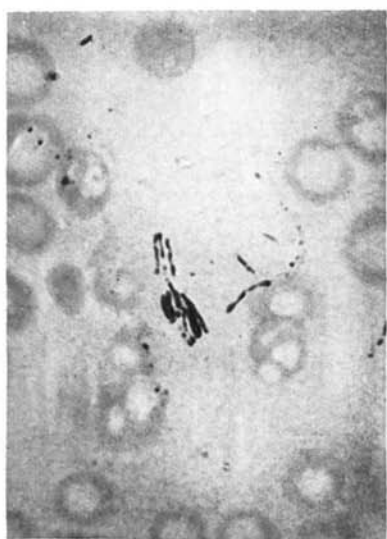
Intrabacillary granular forms can be seen, as well as the free granular forms.



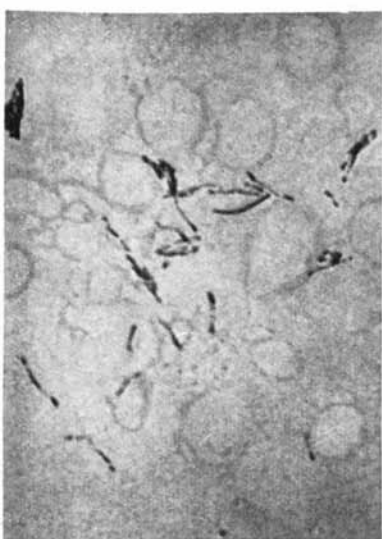
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