On the Differentiation of Leprosy and Tubercle Bacilli.

By

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In the course of an investigation of a case of tubercular leprosy the question arose as to the nature of certain lesions of the lungs. It is well known that patients suffering from leprosy frequently succumb to an affection of the lungs closely resembling ordinary phthisis. Whether this phthisis is the result of an intercurrent tuberculosis, or is of the same nature as the undoubtedly leprotic lesions of viscera other than the lungs, is still a matter of dispute. While certain observers, such as Hansen, Neisser, and Leloir, regard the phthisis as an intercurrent disease attacking a weakened patient, others, as Bonomé and Arning, think that the lesions are due to the spread of the leprotic process to the lungs. As a subsidiary branch of the inquiry it was thought advisable to examine the statements of previous investigators as to the differential staining and morphological differences of the bacilli present in the two diseases, and to test their results on material supplied by this undoubted case of leprosy.

The material, for which I am indebted to Dr. Delépine, consisted of various internal organs, glands, nerves, and skin from different parts of the body. In addition there were specimens of sputa obtained some three days before death.

Distinctions between the bacilli of leprosy and tuberculosis have been sought by many investigators, with the general result that one after the other the differential criteria have been shown to be untrustworthy or less constant than was originally supposed.
These criteria have been sought in differences in—
1. The size of the bacilli.
2. The shape.
3. The staining properties, including the resistance to decolorisation.
4. The numbers and distribution of the bacilli.

The differences based on the number and distribution will be found fully discussed in a paper written in association with Dr. Delépine ('Trans. Path. Soc.,' 1891).

While all observers speak of the B. lepræ as resembling in size the B. tuberculosis, yet some, such as Flügge, give measurements for the former which are considerably greater than those given for the latter. Bonomé, on the other hand, speaks of the leprosy bacillus as shorter and thicker than B. tuberculosis.

There is also a general consensus of opinion that the B. lepræ is less variable in length and more rectilinear (Koch, Flügge, Babes); but Baumgarten confesses himself unable to establish any fundamental differences on these grounds, and, considering the extreme variability of the B. tuberculosis in various specimens of sputa, &c., it would be impossible to lay much stress on these differences.

According to Neisser the B. lepræ possesses pointed extremities, while Bonomé, on the other hand, regards a swollen pole as a distinctive mark of the bacillus. In the specimens obtained from the present case there is a decided enlargement of the poles of the bacilli, and in rapidly stained specimens the extremities are more strongly stained than the intermediate portions.

The possession of a capsule seems to be common to both organisms.

It is to the variations in the staining properties that most attention has been paid, and differences sought in—
1. The different staining properties of various dyes, and of the various solutions of these dyes, whether made with aniline oil, alcohol, water, &c.
2. The rapidity of the staining.
3. The resistance to decolorisation.

Originally Koch considered that while the *B. tuberculosis* required for its demonstration one of the complicated methods devised by himself or Ehrlich, the *B. lepræ* could be readily stained by Weigert's method of nuclear staining; and also that while the *B. tuberculosis* was stained by alkaline methylene blue the leprosy bacillus remained unaffected.

Babes showed that various violets (gentian violet, &c.) in simple solution would stain the *B. tuberculosis*, but stated that this bacillus was not stained by simple solutions of red or violet fuchsine, methylene blue, or eosin, while all these colours would stain the *B. lepræ*.

Baumgarten pointed out that both the bacilli were stained by watery solutions of fuchsine, but in contradiction to Babes asserted that neither was stained by watery or alcoholic solutions of methylene blue or eosin.

Wesener found that it was possible to stain the *B. tuberculosis* with any dye which stained the *B. lepræ*, and agreed with Baumgarten that eosin and watery methylene blue stained neither. Alkaline methylene blue in diluted alcoholic solution, however, stains both organisms, as does also (but very badly) acid solution of eosin. Bismarck brown and vesuvin stain neither.

My own results agree in the main with those of Wesener. About such stains as fuchsine and the various violets all authors are agreed. It is not, however, easy to decide in all cases whether the stain is really taken up by the *B. lepræ* or by the material present in the lepra cell. If a section rich in bacilli or lepra cells is stained with simple methylene blue (2 per cent. diluted alcoholic), Löffler's solution, Friedlander's stain, logwood, or even Bismarck brown, it is perfectly easy to distinguish the lepra cells, and those parts of the section which can be shown by control staining to contain numerous bacilli. The cells are filled with a fairly strongly stained granular material, often of a different tint from the surrounding nuclei, and a similarly stained granular material interpenetrates those portions of the sections where bacilli are known to exist.
The cells contain numerous vacuoles apparently filled with colloidal material, and the web of granular material passing amongst the cells of the leprous granulomata seems to form the boundary of similar vacuoles. The granules are arranged in lines which suggest the course of bacilli. It is generally impossible to distinguish individual bacilli by the above stains, though Löffler's stain gives decided results; but the parts which are thus doubtfully stained are those which with fuchsine show definite bacilli. If the section be rapidly stained by fuchsine, decolourised by acid alcohol, and counterstained by methylene blue, the cells are seen to be filled with a similar granular blue-stained material in which a few red-stained bacilli are embedded. It would, therefore, appear probable that the granular staining material is in large part made up of altered cell contents and possibly degenerated bacilli.

It is impossible to distinguish with certainty these two bacilli by the use of various stains or by modifying the material in which they are dissolved, since the simple watery, the aniline oil, the alcoholic, and the carbolised solutions all stain.

The rapidity with which the bacilli are stained by such a dye as fuchsine has been tried as a means of distinguishing between the two bacilli. The statements of different observers are very contradictory.

Babes states that B. lepræ is alone coloured by staining for thirty minutes with simple Poirier fuchsine and decolourising by an acid. Baumgarten gives two methods for staining the B. lepræ and leaving the B. tuberculosis unstained. According to this author, dilute alcoholic fuchsine (5—6 drops of the alcoholic stain in a small watch-glass of water) stains the B. lepræ in twelve to fifteen minutes in sections, and on cover-glasses in five to six minutes, so as to resist decolorisation by acid alcohol (nitric acid 1 part, and alcohol 10 parts) for half a minute; or similar staining can also be effected in two to three minutes by Ehrlich's fuchsine solution. The B. tuberculosis is said to be left unstained.

Bonomé, who was attempting to solve the question as to the nature of some lung lesions in leprosy, used Baumgarten's
methods and considered them satisfactory, though at the same
time he pointed out various circumstances which might modify
the result, more particularly the thickness of the sections.

Wesener repeated Baumgarten's experiments with tubercular
material from various sources, and concluded that the methods
afforded no absolute criterion by which to differentiate the two
bacilli. Both the methods would stain tubercle as well as
leprosy bacilli.

My own experiments point in the same direction. The
material used was, for the leprosy, chiefly sections of skin
hardened in alcohol; while the tubercular material consisted of
cover-glass preparations of sputa, sections of bovine lung very
rich in bacilli, and human tubercular lung. It may be ob-
jected that results obtained with bovine tuberculosis are not
satisfactory, but bovine and human tuberculosis are usually
considered to be identical in origin, and it was important to use
sections containing large numbers of tubercle bacilli in order
to render them comparable with the leprosy sections. It was
impossible to find specimens of human tuberculosis which con-
tained sufficient numbers of tubercle bacilli to make the nega-
tive results certain.

This question of the number of bacilli present is highly im-
portant in estimating the rapidity of staining. As is well
known, the staining properties of bacilli in the same specimen
vary largely. Some bacilli are stained in a period which is
quite inadequate to stain others, and the process of decolori-
sation is also quite gradual. The most striking thing in a
leprosy section is the enormous number of bacilli present. Now
even if the two varieties of bacilli stain equally quickly, and are
exposed to the stain for such a period as is sufficient to colour
say 1 per cent. of the bacilli present, it is obvious that the
leprosy bacilli would appear to stain more quickly and strongly
than the B. tuberculosis, or even to stain in a time which is
insufficient to stain tubercle bacilli. The difference is really due
to the number, and not to the proper staining power of the
bacilli. Looking first at results obtained with cover-glass pre-
parations of sputa, it was found that the B. tuberculosis
was quite certainly stained in five minutes in cold diluted alco-
holic solutions of rubin, fuchsine, and magenta, prepared accord-
ing to Baumgarten's directions. The preparations were de-
colourised by acid alcohol (v. supra) and counterstained with
methylene blue. Cold concentrated aqueous solutions of these
two dyes also stained tubercle bacilli perfectly well, and they
resisted the same subsequent treatment as when stained by the
dilute alcoholic solutions.

The cover-glasses spread with the sputum from the
leper gave similar results. There was, however, in these
specimens a decidedly greater tendency for many of the bacilli
to have their red stain partially replaced by the methylene blue,
which, supposing them to be leprosy bacilli, accords with what
is subsequently noticed as to the behaviour of undoubted
leprosy bacilli in sections.

The sections were transferred to the stains either direct
from absolute alcohol or from water. Those transferred from
alcohol stained rather the more strongly, but the results were
not substantially modified.

It was found that in sections both the Bacillus lepræ
and B. tuberculosis were stained unmistakably by both
Baumgarten's methods.

They were also both stained in six minutes in cold concen-
trated watery solutions of rubin or magenta, with subsequent
treatment by acid alcohol. It was not possible to confirm
this last result in the case of human tuberculosis.

It is obvious, then, that there is no essential microchemical
difference in the behaviour of the two bacilli.

It is of interest to observe that in the two sets of sections
similarly stained, though the leprosy specimens showed a far
greater number of bacilli, yet on the whole the tubercle bacilli
were the clearer and took a purer stain. There was a strong
tendency for the leprosy bacilli to be purplish in colour—
partially stained by the methylene blue. It was noticeable,
too, that the general appearance of the section seen with a low
power was quite different in these rapidly stained specimens
from that presented by more fully stained sections. In these
latter the most striking features were the large strongly stained lepra cells and globi. In the former, on the contrary, it is the intercellular bacilli and those contained in the minute cells which are stained well, while the large lepra cells are inconspicuous, being, for the most part, stained blue with a few scattered red-stained organisms. This would seem to indicate that there are considerable differences in the age and probably also in the activity of the bacilli. From what we know of other bacilli, it would seem probable that the scattered intercellular organisms are the youngest and most active, while the large masses are composed of older and possibly degenerated bacilli, or those which have developed a considerable capsule.

The resistance to decolourising agents has also served as a basis of distinction between the two varieties of bacilli, but is not reliable as an absolute criterion. The B. lepræ apparently resists decoloration more vigorously than B. tuberculosis. The remarks made above as to the reaction between number and rapidity of staining apply equally to decolourisation.

Babes states that B. lepræ in cover-glass preparations will resist decolorisation by strong nitric acid for one hour, while the tubercle bacilli seldom resist for more than half an hour. Not having any cover-glass preparations of undoubted leprosy material, it was impossible to confirm this statement; but the B. tuberculosis is certainly decolourised in the time. The method is, however, a very severe one, often resulting in the detachment of the film, and is obviously inapplicable to sections.

Lustgarten proposed to distinguish between the bacilli by the greater resistance of B. lepræ to the decolourising action of 1 per cent. hypochlorite of sodium. Wesener and Bonomé, however, have examined this method and rejected it as useless.

Voltolini states that if, before staining, a cover-glass preparation of B. tuberculosis be exposed to the action of fuming nitric acid, the bacilli appear when stained as a row of
points—a streptococcus form. *Bacillus lepræ* does not give this appearance.

It is a method inapplicable to sections, and, bearing in mind the well-known action of strong acids in facilitating the production of a streptococcus appearance in *B. lepræ*, does not appear to be a reliable guide.

Unna and Lutz, by a modification of Gram's method, substituting nitric acid for alcohol as the decolourising agent, concluded that the true form of *B. lepræ* is a coccotrix. It does not appear to have been suggested as a method of distinction.

The conclusions arrived at from an analysis of the work previously done and my own observations are—

1. That any colouring agent which will stain the leprosy bacillus will also stain *B. tuberculosis*.

2. That the methods proposed to stain *B. lepræ*, while leaving *B. tuberculosis* unstained, are unreliable. There is no essential difference between the two bacilli in their relation to stains or decolourising agents.

3. That the apparent differences in respect to rapidity of staining and resistance to decolorisation are due to difference in numbers of bacilli present.

It has frequently been suggested that leprosy and tuberculosis are closely allied, and that the bacilli present in the two diseases only differ in their physiological activity. It seems certain that no distinctions can be based on morphological grounds or microchemical reactions.

As a rule, the pictures presented by the lesions of leprosy and tubercle are entirely different, and the nature of the affection can be certainly affirmed by a consideration of the anatomical nature of the lesion and the number and distribution of the bacilli. In the lung, however, we have scanty data for these conclusions, and any help that could have been derived from the individual characters of the bacilli would have been useful.

It may also happen that a diagnosis of the nature of a lung affection may be wanting during the life of the patient, and in
this case the staining reactions and morphology are almost the only data for a conclusion, as the distribution is less helpful here than in sections.

It must be borne in mind that it is the lack of difference in the reactions of the bacilli, and not in the lesions produced, which is insisted upon in the above paper. It is possible to argue from the character of the lesion as to the nature of the bacilli, but not from the morphology or staining of the bacilli as to the nature of the disease.

Note.—Stains coming from different manufacturers, and different samples from the same maker, are very variable in their staining properties. The two red dyes made use of were a magenta obtained from Messrs. Martindale, and a rubin-fuchsin from König, of Berlin.

Baumgarten's Methods.—a. Sections taken from distilled water, and stained for 12—15 minutes, at the most, in dilute alcoholic fuchsin, made by adding 5—6 drops concentrated alcoholic stain to a small watch-glass of water. Decolourised by acid alcohol (absolute alcohol, 10; nitric acid, 1) for \( \frac{1}{2} \) minute, washed in distilled water. Counter-stained by methylene blue for 2—3 minutes. Dehydrated by absolute alcohol for 3—4 minutes. Cleared and mounted in xylol balsam. Cover-glasses stained for 5—6 minutes.

b. Stain in Ehrlich fuchsin for 2—3 minutes, and treat as in a.

**Literature referred to.**

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