The differential staining of Schizomycetes in tissue sections and in dried preparations

1884 • Christian Gram

Gram, C. 1884. Ueber die isolirte Färbung der Schizomyceten in Schnitt-und Trockenpräparaten. *Fortschritte der Medicin*, Vol. 2, pages 185–189.

(I WISH TO THANK HERR DR. RIESS, Director of the General Hospital of the city of Berlin for the facilities and equipment to perform the following studies.)

The differential staining method of

Koch and Ehrlich for tubercle bacilli gives very excellent results either with or without counter-staining, since the bacilli stand out very clearly due to the contrast effect.

It would be very desirable if a similar method for the differential staining of other Schizomycetes were available which could be used routinely by the microscopist.

My studies—as associate of Herr Dr. Friedländer in the morgue of the city hospital in Berlin—have attempted to demonstrate cocci in tissue sections of lungs of those who have died of pneumonia as well as in experimental animals. As Friedländer has already mentioned briefly in his paper on the micrococci of pneumonia, I have discovered by experimentation a procedure for the differential staining of pneumococci. In my procedure the nucleus and other tissue elements remain unstained, while the cocci are strongly stained. This makes them much easier to locate than previously, since in ordinary preparations from pneumonia patients, where such a large amount of exudate occurs, they are impossible to see.

Further studies on the usefulness of this method for other Schizomycetes has gradually shown that this method is an almost general method for all tissue sections and dried preparations. . . .

For staining the ordinary aniline-gentian violet solution of Ehrlich is used. The appropriate sections must be carried up to absolute alcohol and taken from this directly into the dye solution. They should remain in the dye for 1–3 minutes (except tubercle bacilli, which should remain as usual 12–24 hours). Then they are placed in a solution of iodine-potassium iodide in water (iodine 1.0 part, potassium iodide 2.0 parts, water 300.0 parts) with or without a light rinse with alcohol and allowed to remain there for 1–3 minutes. During this time, a precipitate forms, and the previously dark blue-violet sections now become dark purple-red. (Footnote: This purple-red color is not soluble in water but dissolves very easily in alcohol. The chemical studies will be continued at a later time.) They are then placed in absolute alcohol until they are completely decolorized. It is well to change the alcohol once or twice during this step. Then the sections are cleared as usual in clove oil, in which the rest of the dye is dissolved. The nucleus and fundamental tissue is stained only a light yellow (from the iodine) while the Schizomycetes, if any are present in the preparation, are an intense blue (often almost black). The intensity of the staining has not been equaled by any of the current staining methods. This presents another great advantage of our method. It is possible after the decolorization in alcohol to place the sections for a moment in a weak solution of bismarck brown or vesuvine, and then dehydrate again with alcohol, in order to achieve a counter-stain. The nucleus will appear brown, while the Schizomycetes will remain blue.

In this way it is possible to prepare doubly-stained preparations that are just as excellent as those of the tubercle bacillus made after the method of Koch and Ehrlich. Permanent preparations in Canada balsam-xylene or gelatine-glycerol remain unchanged after 4 months.

This method is very quick and easy. The whole procedure takes only a quarter-hour, and the preparations can remain many days in clove oil without the Schizomycete cells becoming decolorized.

It is also useful for dried preparations. It is performed exactly as for

tissue sections, except that one treats the cover slip just like a section.

I have tried many times different aniline dyes (rubine-aniline, fuchsin-aniline and simple gentian violet solution), but without success. In addition, tincture of iodine or potassium iodide solution, as opposed to iodine-potassium iodide solution, are also ineffective, since the Schizomycetes then are also decolorized. When the sections are treated with water or dilute alcohol, the results are variable. . . .

I. After iodine treatment, the following forms of Schizomycetes are not decolorized by alcohol.

(a) The coccus of bronchial pneumonia (19 cases). . . .

(b) Pyogenic Schizomycetes (9 cases). . . .

(c) Cocci of a liver abscess . . . (1 case). . . .

(d) Cocci and small bacilli in circumscriptive infiltration of the lungs . . . (1 case). . . .

(e) Cocci of osteomyelitis (2 cases). . . .

(f) Cocci of suppurative arthritis following scarlet fever (1 case). . . .

(g) Cocci of suppurative nephritis following cystitis (3 cases). . . .

(h) Cocci of multiple brain abscesses following empyema (2 cases). . . .

(i) Cocci of erysipelas (1 case). . . .

(k) Tubercle bacilli (5 cases). . . .

(l) Anthrax bacilli (3 cases) (in mice). . . .

(m) Putrefactive Schizomycetes (bacilli and cocci). . . .

II. The following forms of Schizomycetes are decolorized in alcohol after iodine treatment.

(a) 1 case of bronchial pneumonia with cocci that formed capsules. . . .

(b) 1 case of bronchial pneumonia with cocci that did not form capsules. . . .

(c) Typhoid bacilli (5 cases) are decolorized either with or without iodine treatment very easily by alcohol. I have attempted to leave the sections in the dye for as long as 24 hours but without any better results.

I would like to make one closing remark. The behavior of the cell nucleus and the Schizomycetes to aniline dyes in other methods are almost identical, whereas with the present staining method a very distinct difference is visible.

Studies on Schizomycetes have been significantly improved by the use of this method. It is because of this that I publish my results, although I am well aware that they are brief and with many gaps. It is to be hoped that this method will also be useful in the hands of other workers.

Editor's note. I would like to testify that I have found the Gram method to be one of the best and for many cases the best method which I have ever used for staining Schizomycetes.

Comment

Presented here is the first report of the bacteriological staining method most widely used today. As first devised by Gram, the method was useful in staining bacteria in tissue sections. In his time this was an important discovery, because studies of the pathogenesis of different species of bacteria was just in its infancy. The first of Koch's postulates (see page 116) was that the suspected causal organism should always be found in association with the disease. However, this

presupposed a method for staining the minute bacteria in lesions so that they could be adequately visualized. Because of the fact that many bacteria exhibit the peculiar staining reaction which Gram describes here, it was possible to detect them much easier with his method.

For many years the main use of the Gram stain has been to differentiate species of bacteria. In the present paper, Gram describes several organisms that were not stained by his technique. We would call these Gram-negative, and the number of Gram-negative bacteria is probably larger than the number of Gram-positive bacteria. The Gram stain is one of the first procedures learned by beginning bacteriology students and is one of the first procedures carried out in any laboratory where bacteria are being identified. Its importance to bacterial taxonomy is therefore obvious.

The mechanism of the Gram stain is still a partial mystery. As Gram himself noted, the iodine-potassium iodide solution is essential in the reaction. We know that this solution must follow, and not precede, the gentian violet. We know that the iodine and the gentian violet form a complex inside the cell (Gram also noted this complex formation) which is insoluble in water but is soluble in alcohol. Apparently Gram-positive bacteria are those which are able in some way to keep the alcohol from reaching this insoluble complex. We know that the Gram stain is not an all-or-nothing phenomenon, but that quantitative variations in Gram-positivity exist between different species, and within the same species during different parts of the growth cycle or under different environmental conditions. We know that only intact cells are Gram-positive, so that cells which are even gently broken become Gram-negative. We know that bacterial protoplasts, devoid of cell wall, are still Gram-positive, indicating that it is probably the semipermeable membrane which is somehow involved in the reaction. Finally, we know that Gram-positivity is restricted almost exclusively to the bacteria, with only a few other groups, such as the yeasts, exhibiting this reaction. We can truly say that the implications of Gram's discovery have been widespread.