RANSOM'S STAIN FOR TUBERCLE BACILLI[1]

CHARLES G. RANSOM
BACTERIOLOGIST, NASHVILLE GENERAL HOSPITAL

The science of bacteriology is continually feeling the need of new methods for differentiating microorganisms. The author has a new stain for tubercle bacilli possessing certain advantages over the present carbol fuchsin stain. These advantages may be enumerated as follows:

1. The ease with which the solution is prepared.
2. The new stain is more stable and keeps well for a relatively long time without precipitating.
3. The likelihood of overlooking tubercle bacilli in liquid sputum is much lessened.
4. Tubercle bacilli may be stained satisfactorily in much less time.

This stain is named Ransom's stain for tubercle bacilli. In experimenting with this stain Safranin was made up in various strengths ranging from a one per cent solution to that of complete saturation. It was found that a solution of one per cent to a supersaturated solution was capable of producing the desired stain so as to withstand the decolorizing agent but that the shade of color was too faded to make the results worth while. Hydrochloric acid ranging from .05 per cent to 10 per cent was experimented with. Other mineral acids were tried but were discarded. Effort was then turned toward developing a satisfactory stain in an alkaline solution. Preparation of the stain which was most suited for this purpose was as follows:

Preparation of the Stain

100 cubic centimeters of distilled water.
10 cubic centimeters of N/10 NaOH or 1cc. of N/1 NaOH.
3 grams of Safranin.

Add the NaOH to the distilled water and dissolve the Safranin in the hot solution at from 80 degrees to 100 degrees centigrade. Then filter.

Method of Staining

Cover smears with Ransom's stain and gently heat in a steam dish or by steaming directly over the flame, but do not let the stain boil. At the end of this time decolorize with 2 per cent hydrochloric acid in 95 per cent grain alcohol until the washing alcohol has no red color. Dry at room temperature, or by blotting, but do not flame. Then stain with methylene blue[2] for ten seconds, wash and dry by blotting or

[1]Read before the Tennessee Academy of Science, April 26, 1929.
[2]Brilliant green (2 grms. in 100cc. of water) may be used in place of methylene blue.

by flaming. The technique may be shortened by staining with the Ransom stain for one minute.

In studying this stain one thousand two hundred and fifty samples of sputum were obtained from hospitals and examined. The organisms were present in variable numbers. Comparative staining studies were made with the Ziehl-Neelson carbol fuchsin stain and the Ransom stain. As is well known, carbol fuchsin applied to tubercle bacilli stains them a deep red color. Ransom's stain gives a more brilliant but less deep red color. This is especially valuable in very fluid sputums which are semi-transparent and have a reduced number of organisms. It is also of value in those organisms which have a slight tendency for retaining the red stain when decolorized with acid alcohol. No organisms belonging to the various strains of tubercle bacilli fail to take the brilliant red stain. An effort was made to obtain the best possible field of organisms, but this was difficult due to the variable thickness of the smears. The smears then were termed smears as a whole. In ninety per cent of sputum smears carbol fuchsin stain gave identical results with the Ransom stain. In the other ten per cent the results varied, with some bacteria taking the Ransom's stain more easily and some the carbol fuchsin stain.

Pure cultures from human sources gave most excellent results when stained by Ransom's method. They retained their staining ability for more than three months, but different strains varied in staining quality just as they do with other staining techniques. Bovine cultures also took the stain most excellently, but different strains varied somewhat, though not as much as with human cultures. Avian cultures stained excellently with this stain, but varied some in the few cultures that were tried. Smegma bacillus cultures retained the stain but they did not give as satisfactory results as did the tubercle bacilli.

In the examination of these specimens there were many strains having their own peculiarities of staining. The difficult problem is to determine the causes of these peculiarities.

In conclusion, it might be said that in most cases the Ransom stain gave best results but in other cases the two stains gave similar results. Each appears to have advantages over the other. This may be due to the reaction of the stain.

The Chemical Analysis of Tubercle Bacilli

About eighty-five per cent of the tubercle bacillus consists of water. Twenty to twenty-five per cent of the residue is soluble in ether or alcohol. This latter material consists of fatty acids and waxy substances. The residue after alcohol-ether extraction is composed chiefly of nitrogenous constituents. These can be extracted with dilute alkaline solutions, and consist chiefly of nucleoproteins. After removal of the so-called nucleoproteins that is, the material which comes down on treatment with dilute acetic acid in the cold, there remains a small amount of coagulable protein. I also have recently observed small amounts of a substance that reacts like Bence-Jones protein. The final residue con-

tains alcohol-precipitable substances similar to those of tuberculin reactions. Cellulose is also found and is supposed to represent the frame work of the cell membrane. In addition there is an ash rich in calcium and magnesium.

Many hospitals and Public Health Departments over the United States and some foreign countries have adopted this method as a routine procedure in connection with carbol fuchsin for the staining of tubercle bacilli.

Summary

1. A new method for staining tubercle bacilli is here considered.
2. It appears to have advantages over carbol fuchsin stain in many cases as is here pointed out.
3. In some few cases carbol fuchsin stain gave better results than Ransom's stain, but in most cases the Ransom stain was best.
4. It seems wise, therefore, to utilize the new stain as a supplement to the usual carbol fuchsin stain[2].

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[2]Ransom, Charles G., 1929. New stain for tubercle bacillus. Tenn. Med. Jour., Feb., 1929; review in Jour. Amer. Med. Ass. for April 10, 1929. Also see note in Jour. Amer. Med. Ass. for July 25, 1929, under "Staining of Tubercle Bacilli."