A NEW STAINING TECHNIQUE FOR POLAR BODIES

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Received for publication November 12, 1949

When Gomori's method for the histochemical demonstration of acid phosphatase (1941) was applied to various microorganisms, prominent staining of the polar bodies of corynebacteria was noticed. Gomori's method depends upon the action of the enzyme to separate the phosphate radical from the glycerophosphate in the incubation mixture, which in addition contains lead nitrate and acetate buffer, pH 5.0. The liberated phosphate combines with the lead at the site of the enzymatic activity. Subsequent treatment with ammonium sulfide converts the lead phosphate into dark, easily visible, lead sulfide. When the glycerophosphate was omitted from the incubation mixture, however, the same number of stained polar bodies were observed. It was obvious, therefore, that this staining property did not depend on any specific enzymatic action. This observation led to a simple and efficient staining procedure for the identification of corynebacteria.

STAINING TECHNIQUE

(1) Fix and dry films in the usual manner by heat.

(2) Either put slides in a 10 per cent lead nitrate solution at room temperature for 15 minutes or longer, or cover slides with the lead nitrate solution, hold over flame, and heat gently for 2 to 3 minutes.

(3) Wash thoroughly with tap water.

(4) Immerse from 1 to 2 minutes in a 1 per cent aqueous solution of sulfurated potash (this solution will keep for several weeks in the icebox) or in freshly prepared dilute ammonium sulfide in a coplin jar (approximately 1 ml of light ammonium sulfide in 50 ml of water).

(5) Wash.

(6) Counterstain for 30 to 60 seconds with a 1 per cent aqueous solution of safranine O.

Result. Polar bodies appear black, bacterial cytoplasm red.

RESULTS AND DISCUSSION

Corynebacterium diphtheriae. Twelve strains of corynebacteria were obtained from the Division of Laboratory and Research, State Department of Health, Albany, New York. The strains were grown on blood agar and Loeffler's medium. Polar bodies could be demonstrated in all strains (figure 1). There was considerable variation in the number of stainable polar bodies in the various strains examined. In two of them, only a few were found. Polar bodies were demon-

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strated in 24-hour cultures as well as in cultures that had been allowed to remain up to 7 days in the incubator.

Other corynebacteria. Strains of the following corynebacteria were likewise available for study from the same source: *C. hemolyticum*, *C. hoffmanii*, *C. ovis*, *C. renale*, *C. ulcerans*, and *C. xerose*. The staining reaction resembled closely that found in *Corynebacterium diphtheriae*. There was considerable variation in the number of stainable polar bodies; one strain of *C. xerose* showed many polar bodies whereas another strain revealed very few.

Other bacteria. Among many strains of various common bacteria studied, a few showed demonstrable polar bodies. These included three strains of *Escherichia coli*, one strain of *Shigella dysenteriae*, one strain of *Salmonella typhosa*, and two

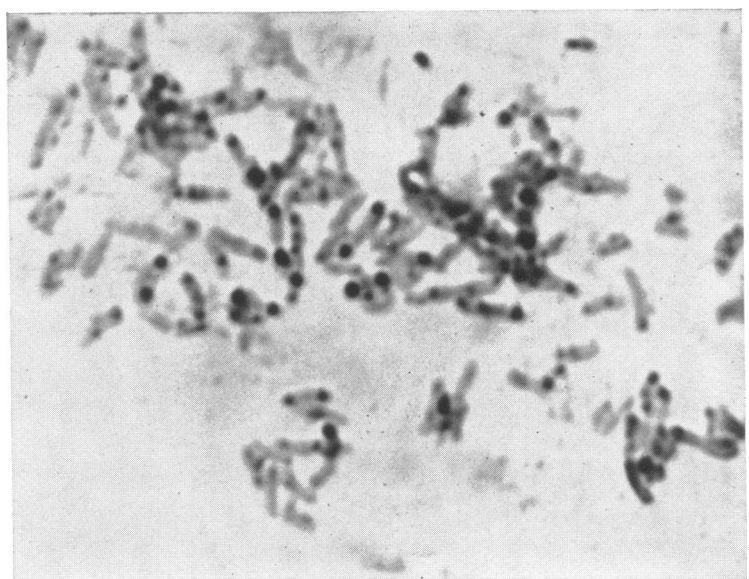

Figure 1. Corynebacterium diphtheriae. Polar bodies are demonstrated by their selective absorption of lead salts. 1,900 ×.

strains of *Gaffkya tetragena*. Granular black deposits were also seen in several strains of baker's yeast as well as in several organisms isolated from sputum.

It is well known that polar bodies are encountered not only in corynebacteria but in other microorganisms as well. The method presented here makes possible a reliable and simple method for the demonstration of these structures. In our hands it gave better and more constant results than those obtained by the use of metachromatic dyes.

Although the histochemical phosphatase techniques have been applied not only to animal tissues and plants but also to bacteria (Bayliss, Glick, and Siem, 1948), doubt has been expressed as to the specificity of the staining technique for acid phosphatase (Heinzen, 1947). It has been particularly noted that in nerve tissue, under certain conditions, a nonspecific absorption of lead solutions

may occur (Bartelmez and Bensley, 1947; Lassek, 1947). To our knowledge, however, no other cellular structures so far examined have shown such a specific absorption of lead as do the polar bodies. The only reference to a staining technique using lead is that of Ghoreyeb (1910). Spirochetes as well as bacteria stain dark when treated first for 30 seconds with osmic acid and then for 10 seconds each with lead acetate and sodium sulfide. This procedure has to be repeated three times. We were able to confirm this finding. If, however, the osmic acid treatment was omitted, spirochetes and bacterial cytoplasm remained almost unstained, but polar bodies showed the typical prominent lead absorption. It is therefore obvious that Ghoreyeb's method is primarily dependent upon the reduction of osmium tetroxide (osmic acid).

The chemical composition of the polar bodies, identified by various names such as Babes-Ernst granules, metachromatic granules, or volutin bodies, is not definitely known. According to Grimme (1902) and Meyer (1912), they readily dissolve in hot water and in dilute solutions of alkali and acids, but are insoluble in lipoid solvents. They are resistant to digestion by trypsin and pepsin and are not stained with Millon's reagent. They are supposed to function as a storage food product and to consist of, or at least contain, considerable amounts of a nucleic acid compound (Lewis, 1941; Dubos, 1947).

For further elucidation of this point, films were stained by the Feulgen technique. In agreement with Lillie (1947) we did not obtain a positive reaction, thus excluding desoxyribonucleic acid. In order to find out whether these polar bodies contained ribonucleic acid, a bacterial suspension was subjected to the action of ribonuclease. The procedure used was similar to that of Bartholomew and Umbreit (1944). Several strains of corynebacteria grown for 24 hours on blood agar were suspended in small test tubes in physiological saline solution and boiled for 3 minutes. Following this procedure, a slight reduction in the number of stainable polar bodies took place. One-half ml of the bacterial suspensions was mixed with the same amount of a freshly prepared solution of crystalline ribonuclease obtained from the Worthington Biochemical Laboratory, Freehold, New Jersey. In two sets of experiments a concentration of 0.8 mg per 1 ml distilled water was used, and in two others a concentration of 0.4 mg per 1 ml distilled water. The final concentration of ribonuclease to which the bacteria were exposed was therefore 0.4 mg and 0.2 mg per 1 ml fluid.

Controls were mixed with the same amount of distilled water. The test tubes were left in the incubator at 45 C for up to 2 hours. No difference in the amount of the stainable polar bodies was noticed between the bacterial suspensions with or without the added ribonuclease. Their number diminished slightly in both sets after 30 minutes' incubation. The good quality of the used enzymatic preparation was proved by the complete disappearance of cytoplasmic ribonucleic acid in sections of rat pancreas fixed in Bouin's solution under identical conditions. In these experiments distilled water has proved as effective a solvent for ribonuclease as the previously advocated buffer solutions (Brachet and Shaver, 1948).

Grimme and Meyer, as previously mentioned, found that trypsin and pepsin

did not digest polar bodies and that they did not give a positive tyrosine (Millon's) reaction. Another recently published test for protein was tried. Properly fixed bacterial films were stained with Serra's method for arginine (1946). Both polar bodies and the cytoplasm remained unstained.

SUMMARY

A simple and reliable staining technique for the demonstration of polar bodies based on their selective absorption of lead salts is presented. This staining reaction is not prevented by the exposure of bacterial suspensions to the action of ribonuclease. Polar bodies do not stain with the Feulgen technique for desoxyribonucleic acid or with Serra's method for arginine.

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