STAINING OF *MYCOBACTERIUM LEPRAE* BY THE RIO HORTEGA SILVER METHOD IN FROZEN AND PARAFFIN SECTIONS

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It has been recognized for some time that *Mycobacterium leprae* has the property of staining with the silver methods (2). However, there has not been described a simple, quick and easy technique for staining the bacillus in that way, applicable to either frozen or paraffin sections. It is my purpose to present a method which satisfies these requirements, and which also shows a new aspect of this staining property of the leprosy bacillus.

TECHNIQUE

1. Fix the material in 10 per cent neutral formalin for 24 to 48 hours or more.
2. Make frozen sections at 8 to 12 microns, or paraffin sections at 7 or 8 microns.
3. Wash twice in distilled water. With frozen sections this is done directly in small staining dishes. Paraffin sections are deparaffinized and handled in the usual way.
4. Transfer the sections to 2 per cent silver nitrate solution to which 2 drops of pyridine are added for each 15 cc. Place on heater at 56°C. until they turn brownish (tan color); this will take about 20 minutes.
5. Wash in distilled water.
6. Put the sections in Rio Hortega's silver carbonate (5) with 2 drops of pyridine per 15 cc. Place on heater until they turn dark brown; this will take about 30 to 45 minutes.

Rio Hortega's silver carbonate:

(*a*)	Sodium carbonate	4.5 gm.
	Distilled water	90 cc.
(*b*)	Silver nitrate	3.0 gm.
	Distilled water	30 cc.

Add the sodium carbonate solution to the silver nitrate solution, whereupon a yellow precipitate will form. Then add ammonia, drop by drop, shaking well until the solution is clear. Put the solution in a dark bottle and add 280 cc. of distilled water.

7. Wash in distilled water.
8. Transfer the sections to 0.2 per cent gold chloride solu-

tion at room temperature and let stand for 20 minutes, then place on heater and let stand for another 20 minutes.

9. Transfer to sodium hyposulfite, 5 per cent, for a minute or so.

10. Wash in tap water, mount (frozen sections), dehydrate and apply coverslip.

RESULTS AND COMMENT

Leprosy bacilli appear black. Nuclei and connective fibers are light red. Melanin is also black.

There are two things that I wish to point out: first, the limitations of the method, and, second, the property of the bacillus in staining with this particular process.

Since the bacilli and melanin are stained the same color, the method is only applicable in cases where it is easy to make a clear distinction between these two elements, as in lesions of lepromatous leprosy with or without treatment. Lesions from cases of the tuberculoid or the indeterminate type of leprosy, with few bacilli, are very difficult to interpret. Ulcerated lesions with secondary infection are subject to error, since the pyogenic bacteria are also stained with the silver carbonate.

Working with this method, which Rio Hortega calls "double impregnation without reduction," I reviewed most of the possible variants of the silver carbonate process (panoptic, double and triple impregnation, reticulin, etc.), and only with this method, in which there is no reduction of silver with formalin, were the bacilli demonstrated. This fact suggests the property that *M. leprae* has in taking the silver, the property called argentaffinia.

Another important thing which must be mentioned is the great number of bacilli revealed by this method as compared with the other classical techniques—Ziehl-Neelsen (1) and Gram-Weigert (4). Several cases which were negative with these routine staining methods have been found positive with the double impregnation method (3).

This argentaffinia of the leprosy bacillus seems to remain unchanged by the action of therapeutic drugs or of the lipid solvents (alcohol, acetone, xylol) used in the embedding process. I cannot, however, explain satisfactorily which of the components of the bacilli are responsible for this property.

RESULTADOS Y COMENTARIO

Los bacilos de la lepra aparecen negros. Los núcleos y las fibras conjuntivas son rojo pálido. La melanina también es negra.

Hay dos cosas que deseo aclarar: primero, las limitaciones del método, y segundo, la propiedad del bacilo de teñir con éste proceso especial.

Puesto que los bacilos y la melanina aparecen teñidos del mismo color, el método puede aplicarse solamente en aquellos casos en que éstos dos elementos se pueden diferenciar claramente, como en casos lepromatosos, con o sin tratamiento. Lesiones de casos tuberculoides, o indeterminados, con pocos bacilos, son de muy difícil interpretación. Lesiones ulceradas con infección secundaria son sujetos a érror pues las bacterios piogénicas también tiñen con el carbonato de plata.

Trabajando con éste método, que Rio Hortega llama "doble impregnación sin reducción," yo repasé la mayoria de las posibles variantes del método del carbonato de plata (panóptico, doble y triple impregnación, reticulina, etc.), y sólo con éste método, en el cual no hay reducción de la plata con formalina se demostraron los bacilos. Este hecho sugiere que el bacilo *M. leprae* posee una propiedad llamada argentofinia.

Otra cosa que debe mencionarse es el gran número de bacilos demostrados por éste método en contraste con otras técnicas clásicas, Ziehl-Neelsen (1) y Gram-Weigert (4). Varios casos que fueron negativos usando éstos métodos rutinarios, resultaron positivos con el método de doble impregnación.

Esta argentofinia del bacilo de la lepra parece permanecer estable aún despues de drogas terapéuticos y do los solventes lipoidicos (alcohol, acetona, xyol) usados en el proceso de impregnación (con parafina). No puedo, sinembargo, explicar cuales de los componentes de los bacilos son los responsables de ésta propiedad.

REFERENCES

1. De Faria, J. L. Valor do método de Faraco para coloração do bacilo de Hansen em cortes. Rev. brasileira Leprol. **17** (1949) 18-26.
2. León Blanco, F. and Fite, G. L. Silvering of lepra bacilli in tissues. Arch. Path. **46** (1948) 542-549.
3. Martínez Baez, M. Personal communication.
4. Portugal, H. and Azulay, R. D. Vantagems de coloração pelo método de Gram-Weigert dos cortes de lesões lepromatosas. Rev. brasileira Leprol. **18** (1950) 206-207.
5. Del Río Hortega, P. El método del carbonato argéntico; revisión general de sus técnicas y aplicaciones en histología normal y patológica. Arch. Histol. Normal y Patológica, Fasc. I-II, 1943.

DESCRIPTION OF PLATE

PLATE (8)

FIG. 1. Frozen section of a lesion from a case of lepromatous leprosy. Numerous Virchow cells laden with black-stained bacilli. (B.1506 ×97.)

FIG. 2. Lepromatous lesion in a case under sulfone treatment, frozen section. Routine staining methods revealed very few bacilli in this specimen. (B.1447 ×97.)

FIG. 3. Paraffin section of a lepromatous lesion in a case under sulfone treatment. Numerous bacilli are present in the cytoplasm of Virchow cells. (B.1331 ×97.)

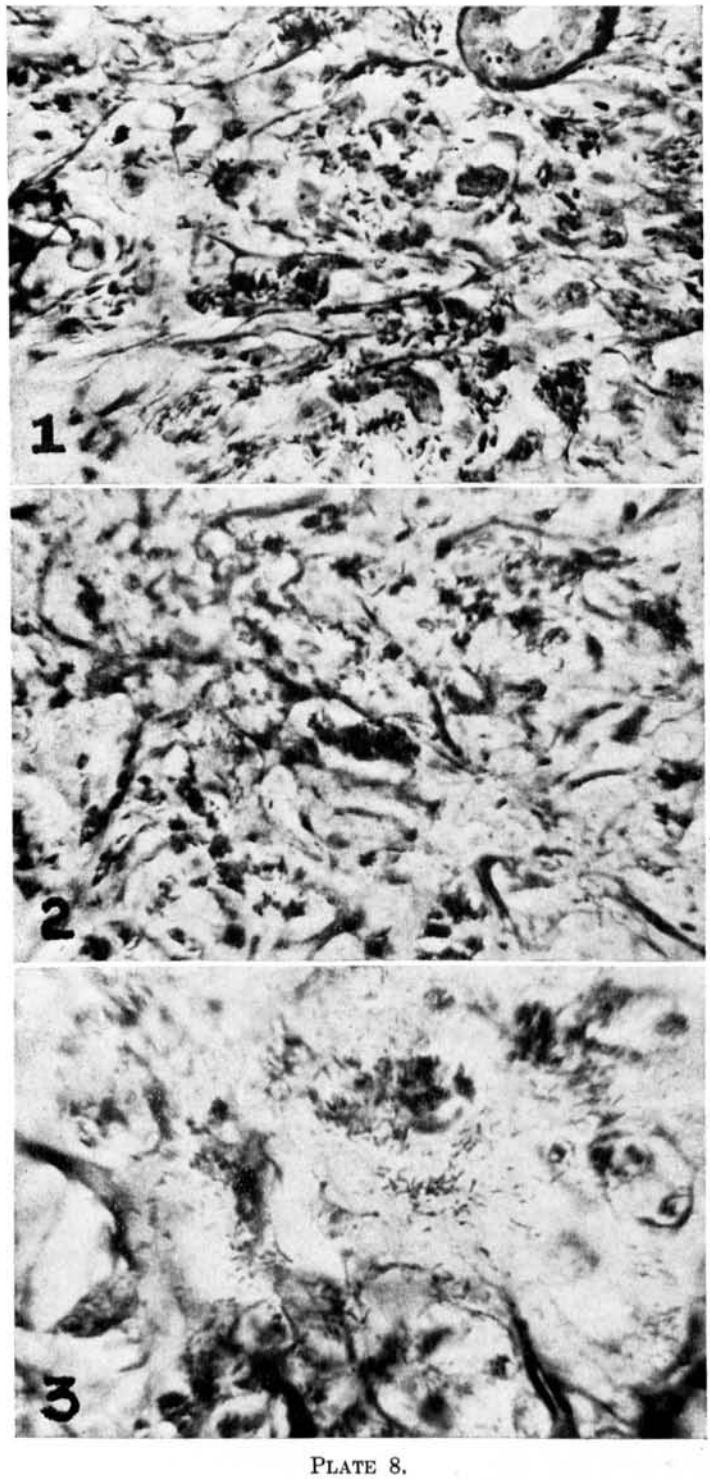

PLATE 8.