Demonstration of *Treponema pallidum* in tissues By a modification of the rapid method of silver impregnation of Ito, Ohtani, and Haba (1968)

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A new method for the demonstration of *Treponema pallidum* in tissues was described by Ito, Ohtani, and Haba (1968). Silver impregnation of treponemes could be carried out in a simple and rapid way, using their new method, which was based on the same property of silver nitrate as the older methods. Treponemes have been demonstrated by this method, subsequently modified by Ohtani (1969a), in papular lesions of secondary syphilis and in experimental syphilis in the rabbit.

We have used essentially the same technique in five cases of recent syphilis, using experimentally-induced syphilis in the rabbit testis as a control.

Materials and methods

The cases investigated may be classified as follows:

- (1) Primary syphilis, lower lip;
- (2) Primary syphilis, inguinal lymph node;
- (3) and (4) Secondary syphilis, papular lesions of the trunk;
- (5) Secondary syphilis, corymb-like lesions.

Rabbit testis, inoculated with Nichols strain treponemes, was used as a control.

Biopsy specimens were fixed in alcohol and/or in Bouin liquid, and embedded in paraffin. Sections 5–10 μ thick and frozen sections were used for the staining procedure.

Technique

The method described by Ohtani was used first; with experience we made slight modifications to the original procedure. The following solutions remained active for months, stored under refrigeration:

(1) Uranyl nitrate 1.0 g.	
Alcohol 70 per cent. 100 ml.*	
(2) Silver nitrate 1.0 g.†	
Polyoxyethylene sorbitan mono-oleate 1 ml.	
Distilled water 100 ml.	
(3) Sodium bisulphite	6.0 g.
Sodium sulphite (anhydrous)	1.0 g.
Hydroquinone	5.0 g.
Phenidone (Geigy)	0.2 g.
Sodium lauryl-benzene-sulphonate	0.3 g.
Pyridine	20 ml.
Acetone	25 ml.
Distilled water	300 ml.

Procedure

At room temperature:

- (1) after deparaffinization, bring sections down to water;
- (2) Solution (1) 5 min.;
- (3) wash in two changes of distilled water;
- (4) Solution (2) 20–40 min.;
- (5) wash in two changes of distilled water;
- (6) Solution (3) 10 min.;
- (7) wash in distilled water; dehydrate, clear in xylene and mount in Canada balsam. Harris haematoxylin and eosin were used on some occasions as a counterstain.

Results

Treponema pallidum was identified both in the primary and in the secondary syphilitic lesions; only the so-called 'corymb-like' secondary syphilis was completely devoid of treponemes. The typical spiral

*Ito and other (1968) and Ohtani (1969a), added laurylpyridinium chloride, 0.3 ml. as a cationic surface active agent. In a first attempt, we used 0.3 ml. of a 10 per cent. solution, but we later discarded it, observing no substantial differences in the staining properties. On the other hand, Ohtani (1969b) maintained that laurylpyridinium chloride is 'not very active' in this phase of the procedure.

†Ito and others used 2 g.; Ohtani (1969a) used 0.2 g. silver nitrate.

formation was recognizable in the majority of the sections. However, the structure of the treponeme was not fully identifiable in sections from the epidermis of the lower lip in a case of primary syphilis (Fig. 1); we believe that this could be due to the fact that the patient had previously treated the lesion with an antibiotic ointment.

In the inguinal lymph node of primary syphilis, treponemes were typically arranged near to or within the walls of small vessels; some of them were detected entering the blood stream of large vessels (Fig. 2).

In secondary syphilitic papular lesions, treponemes were usually encountered among the cells infiltrating

FIG. 1 *Treponemes, altered by topical antibiotic treatment, among the epidermal cells of the lower lip, in a case of primary syphilis. × 540*

FIG. 2 *Treponemes in the walls of a large blood vessel, some of which are entering the blood stream. Inguinal lymph node from a case of primary syphilis. × 540*

the lesion or in the proximity of small blood vessels (Fig. 3).

Many treponemes were present in the interstitial tissues of the rabbit testis, thus providing good control

material for the various modifications of the procedure which we tried (Fig. 4).

Surprisingly, no treponemes were found in the corymb-like lesions of secondary syphilis. We were

FIG. 3 *Treponemes lying in the cellular infiltrate of the dermis in a case of secondary syphilis.* $\times 600$

FIG. 4 *Abundance of treponemes in the interstitial tissue of the rabbit testis, not counterstained.* $\times 800$

unable to explain this by our own experience or by reference to the literature.

Comment and conclusions

Walter, Smith, Isaac, and Gager (1969) described a new modification of a silver stain impregnation method for *Treponema pallidum*. Their entire procedure, though demonstrably valuable, seems to be over complicated compared with the method presented here. It involves more than twenty steps, and six solutions which cannot be stored; moreover, the method works at temperatures ranging from 60° to 70–75°C. At all events it indicates the measure of interest in this matter and justifies attempts to evolve new procedures. The opportunity of confirming data obtained in different laboratories seemed to us another good reason to have undertaken the present work.

In substantiating all the conclusions of the Japanese workers who first described this method, we agree that the advantages can be summarized as follows:

- (1) This staining method is more rapid and much simpler than those described in the past.
- (2) The entire procedure can be performed on ordinary histological sections and not on the whole specimen as in other methods, thus obtaining a better homogeneous staining.
- (3) It offers the prospect of employing counter-stains.
- (4) The staining procedure was successful using either alcohol or formalin or Bouin liquid for fixing; the method also worked on frozen sections.
- (5) Both the silver nitrate and the reducing solutions can be used at room temperature, and all solutions can be preserved for months without loss of activity.

In conclusion, we believe that the method described above represents a significant improvement on the older techniques and deserves wider recognition. The slight modifications to the original technique proposed here, though not essential, gave an equally distinct and clear impregnation of *Treponema pallidum*.

Summary

The authors describe the results and advantages offered by their modification of a method of demonstrating treponemes in tissues by silver impregnation. The reliability and simplicity of this method has been proved in cases of human syphilis and in experimental syphilis of the rabbit testis.

References

- ITO, K., OHTANI, M., and HABA, T. (1968) *Bull. pharm. Res. Inst. (Osaka)*, no. 72, p. 1
 OHTANI, M. (1969a) *Ibid.*, no. 82, p. 1
 — (1969b) *Ibid.*, no. 70, p. 1
 WALTER, E. K., SMITH, J. L., ISRAEL, Ch. W., and GAGER, W. E. (1969) *Brit. J. vener. Dis.*, **45**, 6

Mise en évidence de *T. pallidum* dans les tissus par une modification de la méthode rapide d'impregnation à l'argent d'Ito, Otaha, et Haba (1968)

SOMMAIRE

Les auteurs exposent les résultats et les avantages que présente la modification qu'ils ont apporté à une méthode de mise en évidence de tréponèmes dans les tissus par une imprégnation argentique. Le degré de confiance et la simplicité de cette méthode ont été démontrés dans des cas de syphilis humaine et sur les testicules de lapin au cours de la syphilis expérimentale.