

## TECHNICAL NOTE

### ZENKER vs FORMALIN FIXATION FOR THE HISTOPATHOLOGY OF LEPROSY TISSUES AND OTHER DESIRABLE FEATURES OF TECHNIQUE

H. W. WADE, M.D.<sup>1,2</sup>

*Leonard Wood Memorial Laboratory  
Culion, Philippines*

After long years of observation of the shortcomings of formalin as a general-purpose tissue fixative for histopathology, especially for study of the skin specimens of leprosy, and of experience with the advantages of Zenker's fixative for such work, I have been led to discuss this matter while there is still an opportunity to do so. The value of Mallory's special staining procedures for which Zenker fixation is required is also discussed, together with certain other matters of technique, i.e., the use of thin cedar oil for clearing the tissue blocks after dehydration, and of embedding mixtures instead of pure paraffin wax for sectioning.

As Mallory (<sup>14</sup>) and many others have pointed out, the choice of the proper fixing reagent for a given tissue depends on the purpose for which it is preserved. Formalin is, of course, the required fixative for many special histologic procedures, such as the demonstration of fat in frozen sections, or of nerve elements by silver impregnation, or for many histochemical examinations. Such examinations, however, are not a part of general histopathology, for which a fixative that will give precise pictures of the details of the structures and cytology of lesions is much to be desired. For the special examinations which require formalin or some other special fixative, duplicate specimens, or aliquot parts of any single specimen that is sufficiently large for adequate study, should be prepared.

Formalin is a "soft" fixative and, as said by Lillie (<sup>13</sup>), it "often does not harden certain cytoplasmic structures adequately for paraffin imbedding." More or less marked shrinkage occurs during the processing of the tissue blocks and sections, generally more with specimens of skin lesions than of the visceral organs. Shrinkage effects are evident in all too many of the reproductions of photomicrographs seen in publications, especially dermatology periodicals.

In work with leprosy skin lesions, shrinkage after formalin is often conspicuous, most marked in sections of the succulent lesions of reactional conditions in the tuberculoid and borderline forms of the disease. It is often impossible to distinguish between vacuolation due to edema

<sup>1</sup> With the cooperation of Avelino San Agustin, formerly technician of the Leonard Wood Memorial and at present with the Pathology Laboratory, Culion Sanitarium.

<sup>2</sup> This article has been prepared with aid of the National Science Development Board, Manila, Project 2.24.

and the distortion due to shrinkage, and the foamy cells—often called Virchow cells—are also likely to be affected, or even imitated.

The relative softness of formalin-fixed tissue is a drawback in staining acid-fast organisms in sections of bacillus-rich lepromatous lesions, when it is desired to make a total demonstration of their numbers and locations. During the process, due to reagent "turmoil" (Hanks), considerable proportions of the bacilli may be washed out of the cells of formalin sections, as compared with those of duplicate specimens fixed in Zenker's. Negative findings in tuberculoid lesions are most reliable in Zenker-fixed material.

An important consideration is that success in applying the valuable aniline-blue and phosphotungstic-hematoxylin stains of Mallory depends on Zenker fixation. It has been said that sections of formalin-fixed tissues can be prepared for such stains by mordanting, or "zenkerization," but that is a makeshift procedure and in our experience the results are not generally satisfactory. In our practice those two stains, plus hematoxylin-eosin and one or two for bacilli, are routine for all specimens of research material.

One feature of Zenker fixation that is not mentioned in the literature is that the potassium bichromate component of the solution seems to have a mordanting effect on the lipids of the leprosy bacillus. It is certainly a fact<sup>(18)</sup> that the relative acid-fastness of the lepra cells, evidently due to their saturation with the lipids of the bacilli they contain, can be demonstrated in sections deparaffinized by especially mildly-acting solvents after Zenker fixation but not after formalin.

A few authorities on the subject of technique are cited. Mallory, in 1938<sup>(14)</sup>, stated that:

"The best general fixative yet devised for all kinds of tissue is, in my opinion, Zenker's fluid. It is recommended above all others after 40 years of constant trial."

Krajian<sup>(10)</sup> is of the same opinion:

"... after many years of constant trial, Zenker's fluid stands out as probably the most reliable and effective of the many fixing reagents."

Cowdry<sup>(4)</sup>, while paying respects to the many uses of formalin, says that Zenker's is "perhaps the most popular fixative," and that tissues so fixed give better contrasts of acidophilic and basophilic components than those fixed by formalin.

As for citations from the leprosy literature, Leon Blanco and Fite<sup>(11)</sup> found Zenker's fluid to be the best of the fixatives tested for the demonstration of the bacilli. Cochrane once wrote from his London laboratory that he would examine only specimens that had been fixed in Zenker's fluid, and he still prefers that fixative (personal communication). However, because too many specimens had been sent him in Zenker's fluid without washing and "had the consistency of old leather," he has come to recommend a mixed fixative containing formalin—but

only because he cannot rely on those who send him material to use Zenker's solution properly. Khanolkar (8), in a note on biopsy technique, recommends Zenker's fluid without discussion. Carpenter and Naylor-Foote (3), in the chapter on bacteriology of Cochrane's book, also recommend Zenker's fluid; about formalin fixation, they simply say that it is not satisfactory. These writers doubtless were thinking of specimens processed in their own laboratories.

The only special article on the subject which I recall is that of Serial (15). After noting that Lever (12), of Harvard, used Helley or Zenker almost exclusively for his skin work, he reported that after formalin there was vacuolization of the cells of tuberculoid leprosy lesions (not speaking of reactional lesions) that was not present after other fixatives. "The exaggerated vacuolization is produced only by fixation in formalin."

After more than fifty years' experience with Zenker's fluid since I was taught its use in Mallory's laboratory at the Boston City Hospital, I strongly endorse the recommendations cited.

#### ZENKER FIXATION OF BIOPSY SPECIMENS

The formula of the stock solution of Zenker's solution is simple:

Mercuric bichloride (to saturation).....	70 gm.
Potassium bichromate .....	25 gm.
Water, distilled .....	1000 cc.
Dissolve with heat, cool and filter.	

Another ingredient, glacial acetic acid, is added in 5 per cent concentration when a portion of the fixative is to be used. Thus, for the 20 cc. of solution needed for a 1 gm. tissue specimen, use 1 cc. of acetic acid.

Biopsy specimens should be fixed for 4 to 6 hours and not much longer; very small specimens will be thoroughly fixed in even less time. The time usually given in the textbooks is 12 to 24 hours, which in practice means overnight. For general work with no element of haste, as with autopsy tissues, that time is quite satisfactory for general purposes.

However, in the studies that led to improved methods of staining leprosy bacilli in sections (16, 17) it was found that over-long fixation often mordanted the acid-fastness of the bacillus-containing tissue elements to the point that, when the sections were deparaffinized with mildly-acting solvents such as aviation gasoline or petroleum ether the bacilli might be entirely obscured (18). When it was found by experimentation that passable fixation could be obtained in as little as 2 or 3 hours, we shortened the time as indicated; since then there has been no difficulty from overstaining the tissue elements with carbol-fuchsin.

Biopsy specimens usually come to the laboratory from the operating room during the forenoon. They can be fixed and put to wash by the end of the work day, and transferred to 80 per cent alcohol the next morning. Thus if necessary they can be processed as quickly as forma-

lin-fixed tissue.<sup>3</sup> For any situation where running water is not available, repeated changes of water would serve; if the 80 per cent alcohol should become yellow because of insufficient washing it should be changed. Better, it would be a simple matter for over-night washing to rig up a large bottle of water on a shelf above a sink, with a glass-tubing siphon and a rubber tube with a suitable pinchcock to control the rate of flow.

Largish specimens, to be split to make two or more tissue blocks, are given special treatment in this laboratory. To cut a specimen while it is raw is liable to cause more or less distortion of the pieces, and distortion is liable to persist even if the pieces are placed on blotting paper or the like before immersion in the fixative. To avoid that trouble, the entire specimen is dropped into the fixative for an hour or so. So rapid is the penetration that the specimen becomes sufficiently hardened in that time to be sliced without distortion. The flat-cut surfaces will later need little if any trimming before embedding, and sections can be cut with minimal loss of tissue. This is particularly important for specimens of which serial sections are to be made.

Zenker fixation introduces one step not involved in formalin fixation. That results from the deposition in the tissue of crystals of the mercuric salt, which must be removed from the sections after deparaffinizing. Apply Lugol's iodine, or a 0.5 per cent alcoholic solution of iodine, for 5-10 minutes. Then, after rinsing in water, remove the iodine by treating for 5 minutes or so in a 5 per cent solution of sodium thio-sulfate ("hypo"). Wash in running water for 10 to 20 minutes and proceed with the staining process.

#### CEDARWOOD OIL AND "TISSUEMAT" IN EMBEDDING

Many reagents may be used for clearing the tissue blocks, in the step between the dehydrating and impregnation baths. Benzene is often specified and sometimes chloroform. In the textbooks, thin cedarwood oil is generally mentioned along with other substances, but seldom more than that.

Mallory wrote that he had used chloroform for many years but then shifted to cedarwood oil, which Bolles Lee preferred to all others, and that it had proved very satisfactory. Lillie recommends it highly for such difficult objects as human skin, uterus, and the like. He points out that after it has been used for some time it may be restored by filtering and then heating to 60°C *in vacuo* for 30-60 minutes to remove contaminating alcohol and water. I have used cedar oil since beginning of my work with leprosy specimens, with complete satisfaction.

<sup>3</sup> For the occasional specimens received in our laboratory in the afternoon, since we lack a night guard the technician takes them home at the end of the day. At the end of the fixation period he puts them—with his wife's consent—to overnight wash in the bathroom. Thus there is no delay in processing.

It is especially suitable for skin specimens, which are naturally tough and liable to become leathery, for it causes no undesirable hardening. Consequently, with blocks embedded in a proper paraffin mixture, long, smooth, intact ribbons of serial sections can regularly be obtained.

An important advantage of cedar oil over most other reagents in leprosy is in connection with the demonstration of leprosy bacilli: it does not tend to extract the liquids upon which their acid-fastness depends. It does, however, share with them the effect of in some way modifying the bacillary liquid complex so that on later exposure to a fat solvent, as for example xylene in the deparaffinizing step, the acid-fastness of many of the more "decerepit" bacilli is lost. Preliminary treatment (of the tissues) with a fat solvent or an oil in embedding, followed by another treatment (of the sections) with an ordinary paraffin solvent, is the "double jeopardy" situation which affects bacilli so badly. Fortunately, it can easily be overcome by the protective method of adding paraffin oil to the deparaffinizing solvent, for which we employ turpentine exclusively. Such a protective method was first used by Fite and associates (<sup>6</sup>) in a process which is often mistakenly called the "Fite-Faraco" method, and is the basis of my improved methods of staining bacilli in sections.

A word of warning about the grade of cedar oil needed for the purpose. It is *not* the thick oil used with the oil-immersion objective, but the thin product described as "cedar oil, for use as clearing agent in microscopy (Fritzsche 2053)."<sup>4</sup> It is somewhat expensive, but as it does not evaporate with repeated use and can be restored, the final cost is hardly more than that of chloroform.<sup>5</sup>

As regards the embedding medium, the paraffin wax supplied for the purpose is not very satisfactory. Paraffin with an admixture of 10 or 20 per cent beeswax is much better, and so is the Histowax of the Coleman and Bell Company, but in our experience the best is the product of the Fisher Scientific Company called Tissuemat,<sup>6</sup> and we use that

<sup>4</sup> As listed by the Arthur H. Thomas Company, of Philadelphia, at \$3.72 per pound. Dealers in Manila seem never to have heard of it and insistently supply the immersion oil. We therefore have to import the substance, by parcel post.

<sup>5</sup> Tetrahydrofuran has recently come into use to some extent for both dehydrating and clearing tissue blocks, and is said (<sup>7</sup>) to prevent shrinkage even of formalin-fixed specimens. It is not, however, available locally and cannot be mailed, so we have not employed it. There is apparently no assurance that it would be better than other more ordinary reagents with respect to the staining of leprosy bacilli in sections.

<sup>6</sup> On inquiry of the manufacturer about the composition of "Tissuemat," which is of course a trade secret, they wrote that: "The most important additive . . . is related to a petroleum-base plastic, and the other one" [meaning the other component?] "is a paraffin-wax extract."

*Added in proof.*—Since this article was prepared it has been learned that the Arthur H. Thomas Company now sells for tissue embedding a material called Paraplast, described as "purified paraffin compounded with plastic polymers of regulated molecular weights," melting point 56-57°C. This material is priced to compete with Tissuemat and presumably would give equally good results.—*Editor*



entirely. In this climate, the 56°-58°C MP grade, obtained some years ago, has been used at all seasons. The manufacturer now lists, for the harder grades, one melting at 56°±0.5°C and one at 61.0°±0.5°C.

#### MALLORY'S SPECIAL STAINS

Two special stains devised by Mallory, constantly used in this laboratory, are highly recommended for regular use with research specimens. In our language, all biopsy specimens from leprosy patients, whether obtained for microscopic study or primarily for making lepromin, are research specimens. So in fact is autopsy material, although that is virtually a thing of the past in this institution.

*Mallory's aniline blue stain.*—Two staining solutions are required, one of acid-fuchsin and the other an aniline blue-orange G combination. The formulae:

(1) Acid fuchsin	0.5 gm.
Distilled water	100 cc.
(2) Aniline blue, water soluble	0.5 gm.
Orange G	2.0 gm.
Phosphotungstic acid	1.0 gm.
Distilled water	100 cc.

#### Staining procedure:

1. Deparaffinize sections with xylene and remove mercury deposit as usual. Wash in running tap water and rinse in distilled water.
2. Stain sections in the acid fuchsin solution for 3 to 5 minutes or longer. (Shorter staining, sometimes prescribed, does not give satisfactory results in our hands.)
3. Transfer directly, without washing, to the aniline blue solution for 3-5 minutes.<sup>7</sup>
4. Transfer directly to 95 per cent alcohol, two or more changes, to remove excess stain.
5. Dehydrate with absolute alcohol, clear with xylene, and mount, preferably with a synthetic balsam.

**Results:** Collagen fibers, clear deep blue and sharply distinct; reticulin and various hyaline substances, varying lighter shades of blue. Nuclei red and cytoplasm in lighter shades; also red are fibrin, axis cylinders, and various special fibers. Erythrocytes, nucleoli, and myelin are supposed to stain yellow and elastic fibrils, pale pink or yellow, but actually we seldom attain that degree of differentiation.

With lepromatous leprosy tissues, whose chronic lesion-masses contain a "basketwork" arrangement of collagen and a network of reticulum fibers, the latter element can usually be distinguished from the former because of its lighter color and a less-sharp, rather blurred, appearance. The red staining of the cell cytoplasm is often useful because, where vacuolated or foamy cells are massed together, their outlines (the outer limits of cytoplasm) are usually distinct, whereas they are often not distinguishable in hematoxylin-eosin sections.

<sup>7</sup> Mallory prescribed 30-60 minutes, although apparently it was 10-20 minutes in his original article. The longer period was required in this laboratory until the fixation time was reduced to a few hours. Surprisingly, it was then found that a few minutes was sufficient and that more time resulted in an overwhelming density of the blue in the collagen.

This stain is indispensable, with us, in the study of tuberculous lesions. Often, in hematoxylin-eosin sections, small dermal nerve branches within the masses of cellular infiltrate are quite indistinguishable because of dispersal of the perineurium by the infiltrate. In aniline-blue sections, however, such nerve twigs are distinguishable as characteristic small groups of wavy, blue-stained endoneural fibers lying denuded in the cell masses.

To cite an unusual example of the usefulness of this stain: A question arose about the identity, in a frankly tuberculous lesion, of what appeared in the hematoxylin-eosin section to be a foamy cell in a small subcutaneous focus of infiltrate. Another one was found in one of the infiltrate areas in the dermis. The objects did look very much like foamy cells, although on second thought the vacuoles were unusually uniform in size. Oil-immersion examination of the aniline-blue section, however, revealed the structures to be small nerves. There was a tiny circle of blue collagen, the neurolemma, around each "vacuole" (myelin sheath), centrally within which was a tiny red dot (axis cylinder).

*Mallory's phosphotungstic-acid hematoxylin.*—For this stain only one dye solution is required, which has to be ripened. The sections are given a preliminary oxidizing treatment with potassium permanganate and oxalic acid.<sup>8</sup>

The staining solution:

Hematoxylin	1 gm.
Phosphotungstic acid	20 gm.
Water, distilled	1,000 cc.

Dissolve the ingredients in separate portions of the water, the hematoxylin with the aid of a little heat, and combine when cool. Spontaneous ripening, said by some to be best, requires several weeks (less time if exposed to the sun), but satisfactory ripening can be accomplished immediately by Mallory's method of adding 177 mgm. of potassium permanganate.

Staining procedure:

1. Deparaffinize sections, remove mercuric crystals, and wash as usual.
2. Treat with potassium permanganate, 0.25 per cent aqueous, 5 minutes.
3. Rinse in water.
4. Apply oxalic acid, 5 per cent aqueous, 10 minutes.
5. Wash in water, 1-2 minutes.
6. Stain in the phosphotungstic-hematoxylin solution overnight (14 to 18 hours).
7. Transfer sections directly to 95 per cent alcohol briefly and then quickly to absolute alcohol, because the first alcohol readily extracts the red part of the stain.<sup>9</sup>
8. Clear in xylene and mount.

<sup>8</sup> This treatment is prescribed in Mallory's and Lillie's texts, but is omitted in the AFIP manual (1). We have not tried the stain without it.

<sup>9</sup> Staining several slides at a time, the Coplin jar or larger slide dish, drained thoroughly of the staining solution, is filled with 95 per cent alcohol, which is immediately poured off and replaced very briefly by a second wash of the same fluid. Absolute alcohol is then poured on in its turn, 2 changes, but not hastily. It affects the stain but slightly, and unless the sections are properly dehydrated they are liable to fade badly after some years.

Results: Nuclei and fibrin are blue, as are the contractile elements of striated muscle and various fibrils described by Mallory. Collagen, reticulum, and elastin are yellowish to brownish red.

Although not stated in the texts, the cell cytoplasm also takes the blue color considerably less deeply than the nuclei, and the demonstration of cytology thus effected is the principal reason for our interest in the stain. Cells in close arrangement are usually clearly defined where, in hematoxylin-eosin sections, and often even in aniline-blue preparations, they may not be individualized. Foamy lepra cells are particularly well demonstrated, as are foci of epithelioid cells.

A further virtue of this stain in leprosy work is that, like fibrin, areas of fibrinoid necrosis stain deep blue, almost black, and that effect is often useful in studying reaction lesions. Not infrequently have such areas been found in these sections, which were so inconspicuous after other stains as to be quite overlooked.

In a recent study, Kraft (<sup>9</sup>) applied this stain to a study of certain intestinal lesions of mice to determine that the nuclei in what appeared to be multinucleate cells ("balloon" cells) were not separated by cell membranes, as they would be if they were mere cell aggregates. On inquiry she said (personal communication), that Zenker fixation had been used, and remarked that in such work "the fixation is just as important as the staining technique, and should be practised with as much care."

#### DISCUSSION

The remark just quoted might be made the subject of a dissertation. To say with respect to the study of leprosy lesions that if it is worth doing at all it is worth doing well would of course be platitudinous. Not so to say that for the research pathologist it is a primary responsibility to fix his tissues for the best possible demonstration of cytology. If good fixation were widely used, and good staining procedures beyond the habitual hematoxylin and eosin, the various lesions of leprosy would be better understood than they actually are.

Advocacy of Zenker's fluid over formalin for fixation is not merely a matter of personal preference or prejudice. In consultations with Dr. Chapman H. Binford, an experienced pathologist long with the Armed Forces Institute of Pathology in Washington, D. C., and now serving there as the research pathologist of the Leonard Wood Memorial, he has repeatedly spoken favorably about the sections turned out in this laboratory. In writing about some that had been sent him recently he said, "In order to answer some of [your] questions . . . we had the case brought to our staff conference. . . . We were again impressed by the beauty of your histologic demonstrations and the excellence of the stains." The comment on that might be that any competent technician could regularly produce such preparations if he were to use Zenker's fluid for fixation, cedar oil in the embedding sequence, and a good embedding mixture for sectioning.



In a report of a recent meeting of the American Dermatological Association (<sup>5</sup>) it was stated that one of the participants, Becker (<sup>2</sup>), had told of the results of a questionnaire sent to all dermatology training centers in the United States, one of the questions being about the fixative used for biopsy specimens. Becker has said (personal communication) that, of the replies received, 96 per cent reported that formalin was used! Only two institutions reported the use of Zenker's fluid. This is a shocking report, indicating almost universal acceptance of second-rate sections for diagnostic examination. If general pathology laboratories should be canvassed, I venture to say that the results would be quite different.

Laboratories serving widespread clientele which send in specimens by mail, such as the Instituto de Leprologia in Rio de Janeiro, the Instituto Conde de Lara in São Paulo, the Dermatology Service in Buenos Aires, the Research and Training Centre in Chingleput of which Dharmendra is the director, and Cochrane's service already mentioned, have little or no control of the matter except for specimens arising within their own services. They could, however, at least put the formalin specimens received from outside into Zenker's for a few hours and obtain similar results. Where specimens are so numerous that processing machines like the Technicon are used, after-treatment with Zenker's could be done and the specimens washed in the machine as a part of the sequence, according to information received from the manufacturers of the machine referred to.

Rural clients might be encouraged to use the Zenker fixative by distributing to them small packets of weighed quantities of the salts of that fluid, to permit ready preparation of small quantities of the solution as needed; presumably glacial acetic acid would be obtainable almost anywhere. A physician sufficiently interested to remove a specimen surgically should be willing to treat it carefully.

As for sectioning, it has been a matter of astonishment how many slides received from elsewhere have carried sections not cut in ribbons, and placed haphazardly on the slides. Smooth ribboning is essential for the production of serial sections to permit examination of given areas of a lesion at different levels and after staining by different methods.

In presenting these technical notes, no expectation is entertained that the practice of leprosy workers who are doing histopathology will be revolutionized thereby. Professional pathologists are liable to be set in their ways, and reluctant to employ unfamiliar methods. Other workers doing leprosy histopathology may, it is hoped, be more open to suggestion. Anyone, it is submitted, would profit from employing the methods described.

## SUMMARY

Zenker fixation of leprosy specimens for histopathologic study is advocated because of the excellence of the structural and cytologic pictures which it makes possible, because it is required for certain staining processes that are particularly useful, and because it is best for the demonstration of bacilli.

It has been observed, in work as yet not reported, that the potassium bichromate of Zenker's solution has a mordanting effect on the bacillary lipids. This effect permits, after the use of especially mild deparaffinizing reagents, the demonstration of the relative acid-fastness of the bacillus-containing lepra cells.

Formalin fixation of such material is deprecated because it is a "soft" fixative after which there commonly is more or less marked and disturbing shrinkage of the tissue elements, especially in sections of tuberculoid skin lesions and most marked in those of reactional lesions of such nature, and also because in staining for bacilli there are liable to be considerable losses of the microorganisms from the sections.

Formalin is of course required for many special purposes, but if the histopathology is also to be studied, duplicate specimens should be used, or a given specimen should be divided into aliquots before fixing.

For clearing the tissue blocks after dehydration, thin cedarwood oil made for the purpose is superior for all specimens and particularly so for skin, and it does not directly harm the bacilli. For embedding, some additive preparation of paraffin wax is recommended for good sectioning.

Mallory's aniline-blue staining process, primarily for the demonstration of collagen and reticulum, and his phosphotungstic-hematoxylin method, particularly valuable for the demonstration of cytologic details, are described.

## RESUMEN

Se aboga por la fijación de Zenker para los ejemplares leprosos dedicados a estudio histopatológico debido a la excelencia estructural y citológica que permite obtener, debido a que se requiere para ciertos procedimientos de coloración y debido a que es mejor para el descubrimiento de los bacilos.

Se ha observado, en estudios todavía inéditos, que el bicromato potásico de la solución de Zenker ejerce efecto mordante sobre los lípidos bacilares. Este efecto permite, después del uso de reactivos desparafinizantes leves, el descubrimiento de la relativa ácidosresistencia de los bacilos que contienen células leprosas.

Se protesta contra la fijación con formalina de ese material por ser un fijador "blando," después de lo cual hay comúnmente contracción más o menos acentuada y perturbadora de los elementos histológicos, sobre todo en los cortes de lesiones tuberculoideas de la piel y más acentuadas en las lesiones reaccionales de esa naturaleza, y además porque al colorear en busca de bacilos sobrevendrán probablemente considerables pérdidas de microbios de los cortes.

Se necesita, por supuesto, formalina para muchos fines dados, pero si va a estudiarse

también la histopatología, deben usarse ejemplares duplicados o debe dividirse un ejemplar dado en partes alcuotas antes de fijarlo.

Para despejar los bloques de tejido después de la deshidratación, este aceite de cedro preparado para este fin resulta superior para todos los ejemplares y en particular para la piel y no perjudica directamente los bacilos. Tratándose de la inclusión, se recomienda el uso de algún aditivo de cera parafinada para la buena preparación de cortes.

Se describen el procedimiento colorante del azul de anilina de Mallory, primordialmente para la observación del colágeno y del retículo, y su método de fosfotúngstico-hematoxilina, en particular útil para la observación de pormenores citológicos.

#### RESUMÉ

La fixation par le Zenker des spécimens de lépre destinés à l'examen histopathologique est recommandée pour plusieurs raisons: à cause de la perfection des images morphologiques et cytologiques que cette méthode permet, parce qu'elle est indispensable pour certain procédés de coloration qui sont particulièrement utiles, et enfin parce qu'elle est la meilleure technique pour mettre les bacilles en évidence.

Dans un travail non encore publié, il a été observé que la bichromate de potassium de la solution de Zenker a un effet mordant sur les lipides bacillaires. Grâce à cet effet il est possible, après usage de réactifs permettant de déparaffiner avec une délicatesse particulière, de démontrer l'acido-résistance relative des cellules lépreuses contenant des bacilles.

La fixation au formol de ce matériel n'est pas prisée. En effet, celui-ci est un fixateur "faible"; il entraîne généralement un ratatinage plus ou moins marqué, et désagréable, des tissus, spécialement dans les coupes de lésions cutanées tuberculoïdes, et plus particulièrement dans celles de lésions réactionnelles de tout nature; il est aussi responsable du fait qu'un nombre considérable de microorganismes, présents dans les coupes, ne peuvent plus être colorés par les méthodes de coloration bacillaire.

Le formol est évidemment requis pour de nombreux examens spéciaux. Si l'on veut toutefois étudier aussi l'histopathologie, il faut prélever les spécimens en double, ou bien diviser les prélèvements avant de procéder à la fixation.

Pour dégager les blocs après déshydratation, l'huile de cèdre épaisse destinée à cet usage est supérieure quel que soit le spécimen dont il s'agit, et particulièrement pour les préparations de peau; elle n'abîme pas les bacilles. Pour enrober les blocs, une préparation supplémentaire de paraffine est recommandée car elle permet de bonnes coupes.

On décrit la technique de coloration de Mallory par le bleu aniline, conçu d'abord pour la démonstration du collagène et du reticulum, ainsi que sa méthode à l'hématoxyline-phosphotungstique, spécialement utile pour la mise en évidence de détails cytologiques.

ADDENDUM: After this paper was sent to press, there came to hand a report by D. G. Reddy and K. R. Krishnamurthy (*Indian J. Med. Res.* **50** (1962) 692-697) on a study of the pathology of the nerves in leprosy. The study involved the demonstration of the bacilli in tuberculoïd lesions, and for that purpose they found Zenker fixation to be "definitely superior" to formalin fixation; and, for the staining, better than the ordinary Ziehl-Neelsen method was a modification (by Beamer and Firminger) of Fite's first (1938) stain. With that combination of methods they had succeeded in demonstrating bacilli in most of the tuberculoïd lesions examined, whereas with Ziehl-Neelsen staining after formalin fixation those lesions "failed to show any bacilli." It is to be noted, however, that the staining method used involved

deparaffinization of the sections by xylene, so only a part of the bacilli present—the younger and more “resistant” ones—could have survived the “defatting” action of that reagent on bacilli after paraffin embedding.

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