DEMONSTRATION OF ACID-FAST BACILLI IN TISSUE SECTIONS*

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I

Procedures for Paraffin Sections

Satisfactory demonstration of acid-fast bacilli in paraffin sections is not easily accomplished. Whether one is studying their distribution in a frankly positive lesion or searching for them where they are few, the objective is not merely to reveal some of those present but all of them as nearly as possible, the old and deteriorated organisms as well as the relatively invulnerable young forms. The difficulty is a general one,[1,2] although it is more serious with the relatively labile leprosy bacillus than with that of tuberculosis. An adequate explanation of this difficulty has not been seen.

The trouble is due to the liability to extraction of the waxy complex upon which acid-fastness depends, in the first place by the sequence of reagents involved in the imbedding and deparaffinizing process, or later, if the bacilli have survived to that point, by the final dehydrating and clearing sequence. The principle involved may be stated as follows: When acid-fast bacilli are exposed to a reagent which affects the integrity of the waxy complex but does not of itself extract it, that complex may nevertheless be "conditioned" in some way so that on subsequent exposure to another such reagent, which likewise, by itself, would not cause extraction upon primary exposure, many if not most of the bacilli are rendered unstainable. This sequence constitutes, to give it a name, the double jeopardy condition. The reagents involved need not both be active lipid solvents; one, for example, may be the cedar oil used for clearing the tissue before paraffin, the other the xylene used to deparaffinize the sections.

For one example of this effect, smears or sections in which the bacilli have faded cannot be restored by cleaning with xylene, passing to water through alcohol, and restaining; few, if any, will then retain the stain. Again, if the bacilli are suspended in chloroform, as in the Dharmendra method[3] of preparing lepromin, their acid-fastness is not lessened; but when, after the chloroform has been evaporated, the residue is taken up in ether to permit separating the bacillary bodies by centrifuging, few of them remain acid-fast or even distinguishable as blue-staining ghost forms.

Two principles are involved in efforts to overcome this difficulty with

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sections before staining. One is the protection from extraction of the conditioned and vulnerable bacilli while removing the paraffin. The other is the restoration of the acid-fastness of bacilli which have been extracted in the deparaffinizing process. Most difficult to accomplish, apparently, is the demonstration of bacilli which have degenerated and become non-acid-fast in the lesion, or in the paraffin block during storage.

Applications of the Principle of Protection

In the earlier days of the study of the histopathology of leprosy, some workers avoided the harmful effects of the dehydrating-clearing sequence by drying the sections before mounting the coverglass. The protection principle was applied in a unique manner in a staining method which I devised 25 years ago. The technic was never published, because I was never fully satisfied with it, but it has been used continuously in this institution and exclusively until the advent of Fite's new-fuchsin-formaldehyde method,[4] which stains the bacilli deep blue.

The essential feature of this method is that the paraffin is removed at the outset, and the water at the end, by essential oils which exert minimal effects on the bacilli. To deparaffinize, the slides are immersed in a suitable oil for twice as long as is necessary to remove the visible wax from around the sections. Origanum or bergamot oil usually has been used, but cedar oil is better for tissues which have been subjected to that oil in imbedding. As much of the oil as possible is blotted away and the sections are washed in running water for 1 or 2 hours, after which they are stained, decolorized, and counterstained in the usual way. The sections are then blotted until dull-dry and covered with a few drops of anise oil. By pouring this off, blotting again, and replacing the oil two or three times the sections will clear without shrinkage, ready for mounting the coverslip.

Applications of the Principle of Restoration

The principle of restoration of acid-fastness of bacilli which have been damaged by reagents may be illustrated by two simple situations from recent experience. The defatted leprosy bacilli in the Dharmendra[3] lepromin suspension can be made fully acid-fast again if the ether solution of the lipids from the bacilli and tissue is emulsified in the suspension and the ether boiled off.[5] With a suspension of bacilli which had been more or less damaged by the concentration method described by Henderson,[6] restoration has been effected in smears by treating them with paraffin oil (liquid petrolatum), of which more is to be said.

Faraco's Method. The restoration principle was introduced in 1938

by Faraco,[2] who applied it first to defatted tubercle bacilli in sputum and then to leprosy bacilli in sections. The xylene used to remove the paraffin is replaced with several drops of olive oil or a mineral lubricating oil and the slide is heated for some minutes, after which the sections are blotted to opacity and placed in carbol-fuchsin. This procedure, as well as the after-treatment, is cumbersome and not clean, nor is it always successful.*

Fite's 1947 Method. A procedure avowedly suggested by that of Faraco, and designated the Fite-Faraco method by Lillie,[7] was introduced in 1947 by Fite, Cambre, and Turner.[8] In this method the paraffin is removed by a mixture of two parts of xylene and one part of cottonseed oil,† applied for 2 to 4 minutes in two changes. The sections are blotted to opacity and stained for 15 to 30 minutes without heating, decolorized with 1 per cent hydrochloric acid in 70 per cent alcohol, and counterstained with methylene blue. The sections are then dried before mounting.

The use of the paraffin solvent in combination with an oil constitutes a most important new maneuver in dealing with the problem, effective not only at the stage of removing the wax but also at that of preparing the section for mounting the coverglass, for the oil retained by the tissue protects it from excessive shrinkage. In my experience this technic has proved very good with fresh tissues from active lesions but it has not been all that was desired when working with difficult material, and in neither case will it demonstrate all of the bacilli that can be stained.

A Modification of Fite's Method

Recent attempts to arrive at an improved method were based on the observation that high-test gasoline is more sparing of "decrepit" bacilli than most other solvents, and on the restorative effect of paraffin oil for damaged bacilli. Other materials tested with smears of such bacilli, and, in part, with sections, comprised chicken, hog, and human fat, and the total lipids extracted from leproma tissue and bacilli by the chloroform and ether sequence, all of them applied both hot and cold; coconut, olive, and cottonseed oils; and several essential oils. The best of them was much inferior to paraffin oil.

In attempting to apply this material in a simple and practicable method for staining sections, it was for a time used alone to dissolve the paraffin wax from them. No thoroughly satisfactory and dependable

* Some Brazilian workers prefer chicken fat, which Faraco also mentioned. In a demonstration with that material given me by an experienced technician in São Paulo the bacilli failed to stain.

† Peanut and olive oils are mentioned as alternatives, and it is stated that almost any non-volatile oil will serve the purpose, including liquid petrolatum.

procedure was arrived at, however, until the oil and gasoline were employed in combination, as in Fite's method. Later it was found that rectified turpentine is at times, but not always, in some ways better than gasoline.

Procedure

1. Deparaffinize the sections by immersion for 1 or 2 minutes in a 1:2 mixture of paraffin oil (either light or heavy grade) and high-test gasoline (aviation grade without lead; color of no consequence), or of the oil and rectified turpentine. If multiple slides are to be stained, they may be immersed and removed in turn at 1-minute intervals, or, with practice in the next step, at 30-second intervals.

2. Blot to opacity and place in water until the series is ready.

3. Stain in carbol-fuchsin at room temperature, 15 to 30 minutes (20 minutes being ample in summer weather). Wash.

4. Decolorize the sections one by one until they, or at least the portions not loaded with old fuchsin-retaining foamy cells, are of the proper pink color. For regular use, 20 per cent aqueous sulfuric acid is preferred, but if the tissue retains too much of the dye, 2 per cent hydrochloric acid in 70 per cent alcohol may be better. Place in water until all are ready for counterstaining.

5. Counterstain lightly in Loeffler's methylene blue, one-fourth or one-eighth strength. Wash.

6. From the water, wipe the slides clean, and allow the sections to dry in the air.

7. Mount the coverglass with a good synthetic mounting resin.

Special Restorative Methods

Although Fite's recent method has been regarded as a development of that of Faraco, it is actually a protective rather than a restorative one. The short period of application of his solvent-oil mixture is insufficient to restore acid-fastness to bacilli which have been extracted, and no such effect has been obtained by prolonging the exposure; indeed, the bacilli are sometimes rendered thinner and paler. The modification of his method given above is similarly a protective procedure. Although the regular method given will ordinarily demonstrate more acid-fast forms than any other simple one, still more can be revealed in most specimens by special treatment with paraffin oil.

For this purpose a heavy grade of paraffin oil, some four times as viscous as the light grade, is somewhat the better. No advantage has been found in heavier oils such as those mentioned by Faraco, nor in the application of heat up to 100° C.

Prolonged Treatment of Ordinary Slides. Sections mounted on slides in the usual manner are immersed at room temperature in the gasoline-oil or the turpentine-oil mixture, or in pure paraffin oil, for 2 to 3 hours (extremes, 1 to 6 hours). Thereafter they are blotted and processed as described, preferably using acid alcohol after the gasoline-oil mixture, and aqueous sulfuric acid after the turpentine-oil preparation.

Mounting Sections with Oily Materials. For this purpose it is necessary to use slides on which a thin layer of the egg albumin mixture previously has been dried. Several drops of the gasoline-oil or the turpentine-oil mixture are spread on the slide so prepared and the paraffin ribbon is laid on it. The wax dissolves slowly and wrinkles straighten out if enough of the solvent fluid is used. After the wax has dissolved, the fluid is first wiped* and then drained off. The sections are then treated with a little more of the mixture, to ensure complete removal of the wax, and, after draining is complete, they are blotted. The slides may be dried in the paraffin oven for 2 or 3 hours or longer at lower temperatures, and are stained on the same day. It is well to blot them again after drying, to ensure affixation.

Pure paraffin oil may be used, but it dissolves the wax much more slowly and often incompletely, and the sections do not position as readily. Because the gasoline evaporates rapidly, the mixture with that solvent is in effect equivalent to pure oil in a more practicable form.

The first of these restorative methods will commonly reveal more bacilli than the regular technic, and often many more. This holds true not only for old foamy and other lepra cells in which there obviously has been much deterioration, but also for those of younger lesions which contain numerous invulnerable bacilli. The second method has often given maximum demonstration of bacillary forms, and at times the results have been extraordinary. There is, however, a disadvantage in that the clusters of bacilli often show an effect of loosening and spreading, so that they are not as well confined to their original locations as when the first method is used. After either method the intracellular clusters of bacilli often are found to be arranged in radiating, stellate fashion instead of being in the more familiar packets, a condition noted by Leon-Blanco and Fite[9] after impregnation with silver.

Comments

Of primary importance in work with the leprosy bacillus, is the fact that no single procedure can be expected to give the best results in all cases. There are too many variables with respect to the condition of

* For wiping, facial tissue is most practicable.

the bacilli and the nature and condition of the cells which contain them.

In the macrophage type of leproma the cytoplasm of the older lepra cells, the familiar Virchow foamy cells, is so tinctured by the products of the bacilli contained in it that it tends rather strongly to retain the fuchsin. On the other hand the elongated cells of the histiocyte type of leproma, which are typically crowded with nude bacilli without globar material and which consequently have relatively little tendency to vacuolation even when old, are much less retentive of the dye; and that holds true for the epithelioid cells of the tuberculoid lesion of leprosy.

In the lesions of an untreated or unsuccessfully treated case, the lepra cells of whatever type usually are crowded with bacillary forms of ordinary appearance. In treated cases which have improved, especially in these days of sulfone therapy, the bacilli may be relatively few, and when stained they may be smaller, thinner, and paler, or appear only as granules. In either case some of the bacilli are relatively resistant to adverse conditions and are both acid-fast and alcohol-fast; others retain the dye against acid but not alcohol, while still others are non-resistant and may not be demonstrable except after special restorative treatment. If, after the usual procedure, there is good demonstration of bacilli in a young area of an active lesion, one should not conclude that the few which are seen in older, foamy-cell areas are the only ones present. Large numbers of tinctorially degenerated but undigested bacillary forms may actually be present, illustrating the poor capacity of these cells to destroy bacilli completely.

With regard to fixation, Mallory[10] recommended Zenker's fluid for tissues in which bacteria are to be demonstrated, and Leon-Blanco and Fite[11] recently have shown it to be best for the leprosy bacillus. The tissues should be thoroughly washed after fixation; otherwise the tissue elements may hold the fuchsin excessively. After-treatment with alcohol should not be shortened. Treatment of the tissues or sections with iodine is not necessary, nor in fact desirable.

The over-all effects of the two solvent-oil mixtures specified differ materially in certain respects. After the gasoline mixture the foamy cell areas hold the fuchsin more strongly than after the turpentine mixture. Often this is beneficial, but if too much is held the bacilli are not properly differentiated. With a similar mixture of petroleum ether and paraffin oil this tendency usually is greater, for that solvent is even less active in extracting from the cells the element which makes them acid-resistant. In either case the bacilli are typically a clear, brilliant red.

After the turpentine-oil mixture the sections decolorize more quickly and much more uniformly, although in the regular method the foamy

cells usually retain some red, and the counterstain is taken more deeply. After prolonged exposure, the general effects are the opposite of those imparted by the hydrocarbon solvents. The connective tissue, especially, tends to retain the fuchsin, while the foamy cells may give it up and take the counterstain; yet with proper treatment the bacilli in them remain well stained and in correspondingly strong contrast. With either procedure, the bacilli after turpentine are darker and duller, which may or may not be an advantage.

Any usual carbol-fuchsin may be employed. For the leprosy bacillus, 20 minutes at ordinary room temperatures is ample; for the tubercle bacillus, 1 hour in the paraffin oven is needed, or proportionately longer at a lower temperature. In neither case is undue prolongation of the time beneficial, and after a certain point the tissue elements retain too much of the dye.

No one decolorizer should be depended upon exclusively. Ordinarily, acid alcohol decolorizes the sections more uniformly than do the aqueous acid solutions. This is often an advantage with respect to contrast, but not with respect to the demonstration of the greatest possible number of bacillary bodies unless the foamy cells are retentive of the fuchsin, as after the restorative procedures. Aqueous sulfuric acid has less effect on these cells; nitric acid has the least effect, and has little to recommend it.

Contrary to a prevalent idea, the sections should not be exposed to the decolorizer longer than necessary, and one must often choose whether to treat for the more readily decolorized areas of a section or the resistant ones. Furthermore, the treatment should be active, the slides being sloshed back and forth in a dish of the reagent and not merely immersed in it. After the turpentine-oil mixture the time required is usually less than 15 seconds, and after the gasoline-oil mixture it is seldom more than 20 seconds. Stop-watch control is desirable to ensure uniform treatment of a batch of slides.

The expedient of drying the sections before mounting is permissible because the tissue elements retain enough of the oil through the staining process to give much protection from shrinkage. This can readily be demonstrated by including in a set of slides some that have been deparaffinized in the usual way.

With regard to the medium for mounting coverslips, it is difficult to choose between permount* and the H.S.R.† product. Certain others which have been tried seem not to preserve the stained bacilli as well.

Study of sections processed in the ways described will give to many a

* Obtained from the Fisher Scientific Co., New York, N.Y.

† Hartman Leddon Co., Philadelphia.

new idea of the tremendous numbers of bacilli, of various degrees of vitality, that are present in the full-blown lepromatous lesion, and a new appreciation of the task faced by the clinician when he undertakes by therapy to eliminate them from the patient.

Summary

The difficulty encountered in the adequate demonstration of acid-fast bacilli in sections is ascribed to extraction by serial treatment with reagents, wherein one of them affects the integrity of the waxy component upon which acid-fastness depends and the next one removes it.

The difficulty can be overcome in various degrees, before staining, by methods which either protect the "conditioned" bacilli from extraction or restore acid-fastness after that has occurred. The protective method devised by me and used in this institution for many years, based on the use of essential oils for removing the paraffin and for dehydrating and clearing after staining, is given in summary. The restorative principle, as introduced by Faraco, involves treatment of the sections with an oil after deparaffinizing.

The revolutionary method recently devised by Fite, in which paraffin is removed by a mixture of xylene and cottonseed or other similar oil, was derived from that of Faraco but is actually a protective and not a restorative one. An improvement of that method employs mixtures of paraffin oil (liquid petrolatum) and aviation gasoline or rectified turpentine, the two preparations giving somewhat different effects.

This improved method is also essentially of a protective nature. However, by treating sections mounted in the ordinary way for some hours with the mixtures specified, or by mounting sections on dried albuminized slides with those mixtures, a considerable degree of restoration can be obtained, and the results are sometimes remarkable.

No single procedure or schedule can be expected to give the optimum results with all specimens, and hence the various factors are considered in some detail.

II

Application of the Carbowax Technic

To avoid the harmful effects, on acid-fast bacilli in tissues, of the sequence of reagents involved in paraffin imbedding, a matter discussed in part I of this article, some workers have resorted to frozen sections, with or without infiltration with gelatin or agar. Good results have been obtained, but such methods are neither convenient nor entirely practical.

Recently introduced into histologic technic are certain waxy sub-

stances, polyethylene glycols called carbowax compounds,* in which tissues can be imbedded directly from water or alcohol. When it was learned that it is feasible to section tissues such as skin in carbowax mixtures, some of them were acquired for trial. They have proved entirely practicable, once the technician becomes accustomed to the characteristics of this odd medium, and eminently useful for the demonstration of leprosy bacilli. The process is protective to a considerable degree, and the sections are susceptible to the restorative measures that have been found useful for paraffin sections.

General Considerations

The carbowax method, being new and not yet standardized, presents certain problems peculiar to itself which are considered from a general point of view in another paper.[12] The different grades of carbowax, which are designated by numbers representing their average molecular weights, differ widely in hardness and in hygroscopicity. The 1000 grade is moderately firm and the 1540 grade more so, whereas the 1500 grade is of the consistency of petrolatum because it is a mixture of 1540 and a liquid polyethylene glycol; the 4000 wax is quite hard. The 1000 grade is so hygroscopic that on exposure to atmospheric moisture in this locality it becomes fluid in a day or so, while the 4000 grade has shown no such effect over a period of several months. There are indications that different lots of the same designation may differ somewhat in their hardness. An important fact is that the manner in which these materials are heated and cooled affects their mode of crystallization and their consistence.

The usual embedding mixture used[13-15] consists of 1:9 parts of the 1500 and 4000 grades, although it was said[14] that that is too soft for summer conditions in Maryland. By fortunate inadvertence, instead of the soft 1500 grade, I was supplied the firmer 1540 variety. A 1:9 mixture of it and 4000 is sometimes too hard to ribbon properly even in warm weather. A 2:8 proportion usually has been better, but a 15:85 mixture has proved best when the temperature reached 90° F. or higher. Firminger's[14] procedure of heating a newly prepared mixture briefly to 175° C. has been found at times disadvantageous. Melting should be done in the paraffin oven, and not over the open flame.

With regard to infiltration, fat is not penetrated by carbowax, hence the subcutis cannot be studied as well as in paraffin sections, and even intracellular fat, if excessive, interferes with penetration. Infiltration

* Products of Union Carbide and Carbon Corporation, 30 East 42nd St., New York 17, N.Y.

times specified vary from 1 to 3 hours, but for old leproma specimens with foci of lipid-rich foamy cells a longer period is better. Some trouble has been met with on this account in dealing with caseous lesions of tuberculosis.

There are problems in connection with the flotation of the ribbons and the affixing of the sections to the slides. No material has been found which permits the floating and stretching of carbowax ribbons intact as is done with paraffin ribbons, and apparently Mayer's egg albumin as used with such ribbons has not been found reliable for affixation. The procedure here given has proved satisfactory in extensive experimentation in this laboratory.

Procedure

Preparation of the Tissue. Blocks of Zenker-fixed and thoroughly washed tissues, cut to the usual thickness and trimmed of gross fat, may be transferred directly to the melted carbowax mixture from the wash water, as may be done from formalin if that fixative is used. It has been found better, however, to treat Zenker-fixed tissues overnight with 80 per cent alcohol, from which also transfer can be made directly to the wax.

Preparation of the Carbowax Mixture. A 15:85 mixture of the 1540 and 4000 grades is recommended. The ingredients are melted together in a beaker in the paraffin oven (56° to 58° C.), and the mixture may be kept there indefinitely.

Infiltration. Infiltrate in the melted carbowax mixture, 2 changes, for 2 to 6 hours according to the size and character of the specimen. The tissue blocks should be stirred about occasionally, for the material is viscid and does not take up water with avidity.

Blocking. This is done as usual in paper boats, in wax not hotter than the temperature of the paraffin oven. The blocks are solidified in the refrigerator, after which they are allowed to warm to room temperature before the paper is removed.

Sectioning. Preparing the blocks for sectioning is done precisely as with paraffin blocks, with care to make the upper edges exactly parallel. Cutting is done at room temperature. If the blocks are too hard to form ribbons, or if ribbons break up too badly on handling, that condition may correct itself spontaneously in a day or two. It can usually be corrected at once by "doping" the upper and lower surfaces with 25 per cent beeswax in chloroform painted on with a fine brush and allowed to become dull-dry. Plain water also may serve this purpose. The ribbons are laid out on paper or in a suitable container and cut into short lengths.

Preparation of Slides. Difficulty in affixing sections to the slide is avoided by the use of dried albuminized slides, as used by Mann.[16] A drop of Mayer's egg albumin solution is rubbed over the slide with the finger and the excess wiped off with a firm stroke or two of the side of the hand, and the slides are then dried overnight at room temperature or for 2 or 3 hours in the paraffin oven. If too much albumin is left on the slide, the glycerin prevents proper drying. There is little danger of removing too much.

Flotation of Ribbons. Because the wax dissolves immediately on all watery flotation media and on most others, leaving the sections nude, it is best to spread a few drops of the flotant on the prepared slide and then lay a suitable length of ribbon upon it. After the wax is dissolved the sections are held in position with the teasing needle (the needle held below and not on them) while the slide is drained first sideways and then obliquely. The slides are dried in the paraffin oven for 2 or 3 hours, or otherwise overnight.

The most satisfactory flotant of many which have been tried is a 10 per cent solution of carbowax 1540 in distilled water containing 0.005 per cent of turgitol 7.* This wetting agent abolishes the violent and often injurious surface tension effects of plain water. The carbowax which remains in the sections lessens the danger of shrinkage on drying, during which the sections become bone-white.

Preparation for Staining. Before applying any ordinary staining method, it is well to treat the sections with water for a few minutes to remove any residual wax. Sections to be stained for acid-fast bacilli may, alternatively, be immersed in a 2:1 mixture of aviation gasoline and paraffin oil for a few minutes, after which they are blotted to opacity and transferred to water. After this treatment the bacilli may be stained better, and little trouble results from such carbowax as may be present. Treatment of Zenker-fixed tissue for removal of iodine is neither necessary nor advisable.

Staining for Acid-Fast Bacilli. 1. Stain with carbol-fuchsin for 20 minutes at room temperature. Wash.

2. Decolorize, preferably with 20 per cent aqueous sulfuric acid. Less time is required than with paraffin sections, often no more than 5 or 6 seconds.

3. Counterstain lightly in dilute (one-fourth to one-eighth) Loeffler's methylene blue. Wash.

4. Wipe the slides clean and allow the sections to dry in the air.

5. Mount the coverslips with a synthetic resin.

* Union Carbide and Carbon Corporation.

Restorative Treatment with Paraffin Oil

As with paraffin sections, more bacilli often can be made to stain well by restorative treatment with paraffin oil, which can be done in either of two ways.

Oil Treatment of Mounted Sections. With carbowax sections the gasoline-oil mixture seems generally preferable to turpentine and oil. The slides are left in it for 2 to 3 hours, or longer, before blotting and staining.

Mounting of Sections with Oil Medium. With some specimens the optimum effect has been obtained by using the turpentine-oil or the gasoline-oil mixture for the flotant. Although the sections become translucent, the wax does not dissolve in either of these mixtures, and wrinkles do not disappear spontaneously as they do on aqueous flotants. However, the consistency of the wax is somewhat modified, so that by gentle application of the teasing needle any wrinkling present can be considerably reduced. After draining the slide for some minutes the sections are blotted to opacity and dried in the paraffin oven for 3 hours or so, or longer at a lower temperature.

Slides so prepared are best stained the same day. After another precautionary blotting, they are treated with water to remove the wax and transferred to carbol-fuchsin. Little shrinkage occurs, but there is some danger of loosening.

Comment

In most instances the simpler staining procedure with carbowax sections reveals many more bacilli than are demonstrated in paraffin sections of duplicate tissue blocks by the same staining procedure. It has happened that with specimens from cases under active sulfone treatment the results have been disappointing and no better than with paraffin sections. However, with such difficult material the restoration methods have given better results in carbowax sections than in their paraffin opposites.

In many cells of active lesions, especially cells of the elongate histiocyte type, the bacilli will be found after restorative treatment to have the radiate arrangement that can be shown also in paraffin sections (part I). The numbers that will be found in old lesions, especially after the restorative treatment, are often amazing. Old foamy cells which ordinarily are regarded as containing mostly detritus with few actual bacilli may appear simply crowded with them.

It cannot be said that the carbowax method is entirely harmless, at least for bacilli in poor condition. If, for example, carbowax sections are treated with chloroform, which has no primary denaturing effect on

fresh bacilli from lesions or those in heat-killed tissues used for making lepromin, fewer acid-fast forms will be found than in controls, indicating that some degree of conditioning for extraction has occurred. However, the carbowax process is decidedly less harmful than the paraffin sequences. Furthermore, the restorative procedures are more effective with carbowax than with paraffin sections.

Firminger[14] has pointed out that the carbowax method is admirably suited to the study of intracellular lipids, although Lillie[17] said that this medium dissolves some lipids to a considerable extent, with specific mention of those of the adrenal gland. Whether this method of sectioning will be of value in studying with fat stains the lipid elements of the leproma has yet to be determined.

Summary

The carbowax method of sectioning tissues, which involves no harmful sequence of reagents either in the imbedding process or in dewaxing the sections, has been found eminently satisfactory for the demonstration of leprosy bacilli in lesions.

The carbowax method involves certain peculiar problems and has not yet been fully standardized. Having in view the essential features, a practicable technic has been worked out. Without special treatment, carbowax sections have usually given decidedly better results with carbol-fuchsin staining than have comparable paraffin sections, and the results of the restorative procedures have been still better in comparison.

Whether or not this method will be equally superior for the demonstration of tubercle bacilli in lesions has yet to be determined. Also to be determined is the possible value of the method for the study of intracellular lipids in the lesions of leprosy.

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