

OHLMACHER (A. P.)

Some suggestions in
Bacteriological technique



*Reprinted from the New York Medical Journal
for March 2, 1895.*



SOME SUGGESTIONS
IN BACTERIOLOGICAL TECHNIQUE.

BY A. P. OHLMACHER, M.D.,
CLEVELAND, OHIO.

THE following subjects are here considered :

- I. A simplified method of making a bacteriological autopsy on small animals.
- II. The use of methyl violet in staining the diphtheria bacillus.
- III. A note on methylene blue.

I.

The object of these modifications in the technique of bacteriological autopsies on the small animals used in experimental laboratory work is to do away with certain troublesome features of the ordinary methods. As practiced by the usual methods, a properly conducted bacteriological autopsy is a tedious task, especially in those cases in which pieces of the solid organs are required as material for culture experiments. It is necessary to have at hand several sets of sterilized cutting instruments—a set for each particular organ—or the instruments of a single

set must be heated to a damaging degree in the free flame before and after the removal of each piece of tissue.

The novel feature of the modified method here proposed lies in the use of ordinary commercial *benzene* as the disinfecting agent, both in solution and in ignition. The benzene is employed as a bath for the instruments used in the autopsy, and also to disinfect the surface of the animal's body before the final incision. Benzene was chosen as the agent for these purposes because of its cheapness, its active disinfecting power, and its property of burning off of the surface of metallic or other substances without creating a great amount of heat. The details of an autopsy performed with the aid of benzene are as follows :

Two small artery catch forceps (Péan's forceps), a medium-sized dissecting forceps, and a pair of medium straight dissecting scissors are placed in a suitable beaker glass two thirds full of benzene. An extra dish of benzene and a medicine-dropper are also provided.

The animal (mouse, rat, guinea-pig, rabbit, etc.) is secured on its back to the dissecting board by tacks driven through the extended fore and hind legs. The skin of the ventral surface is incised in the median line from the symphysis pubis well on to the neck; lateral cuts are made on both right and left sides at the cephalic and caudal extremities of the median incision. The flaps of skin are carefully dissected from the underlying tissue throughout the entire length of the primary incision so as to thoroughly expose the subcutaneous tissues of the thorax and abdomen; then the flaps are secured with tacks so as to be well stretched out on each side of the body. This primary dissection is done with a coarse dissecting forceps and a pair of straight scissors, no particular efforts being made to avoid contamination of the exposed surfaces. Care must be taken, however, in this dissection that only the skin is re-

moved, and that the abdominal and thoracic walls are not penetrated. With the medicine-dropper benzene is now applied to the exposed ventral wall of the body. The benzene is then ignited and allowed to burn until the flames spontaneously subside. Should any particular area not appear properly singed, the benzene may be again applied to this spot and set afire. Some care must be taken to keep the benzene from running over the flaps and into the hair of the animal, for here the fire does not subside so harmlessly as on the exposed moist surfaces. The dissecting scissors and forceps are now removed from the dish of benzene, and the benzene clinging to them is ignited and allowed to burn off. A fold of the abdominal wall in the median line is raised with the forceps and cut through with the scissors so as to avoid the underlying organs. As soon as a moderate cut has been made the edges of the incision are grasped with the artery forceps which have been flamed on their removal from the benzene; then the incision is continued down to the pubes and up through the thoracic cavity in the middle line, while the abdominal walls are held well up with the artery forceps. The artery forceps are now changed so as to grasp the edges of the deep cut at the diaphragmatic border, while the diaphragm is cut on each side so as to allow the incised ventral walls to be held well apart, exposing freely the thoracic and abdominal contents. If an assistant is available, the artery forceps can be intrusted to him; but in the absence of an assistant the operator can secure a comfortable exposure of the abdominal and thoracic organs by making lateral incisions at the ends of the deep incision, when the weight of the artery forceps will usually be sufficient to hold the walls well apart. The scissors and forceps are now wiped free of blood and tissue, replaced in the benzene, then removed and the adhering benzene ignited, and the removal of

pieces of the organs desired for bacteriological study is begun. Of course, it will be necessary to sterilize the forceps and scissors as each particular organ is dealt with, so as not to carry infectious material from one organ to another. All that is necessary for sterilization is to wipe the instruments, plunge them a moment in benzene, and burn off the benzene. It will also be necessary, especially in early autopsies, to avoid contamination of the remaining organs from the blood which flows from those already cut. This can be avoided by following a proper order in attacking the various organs, depending, of course, on the object of the examination.

From a considerable practical experience with this method, the author is ready to conclude that it is simpler, more cleanly, more expeditious, and more saving of instruments than the usual methods of bacteriological autopsy. The proportion of accidental contaminations after this method is certainly smaller than I have ever obtained with any other procedure, and this occasional contamination is doubtless due to air infection and not to imperfect sterilization of instruments and body surfaces. Control experiments with culture media, made after benzene sterilization of scissors and forceps, show the method to be a safe one. Perhaps this contains a suggestion for surgeons, especially in emergencies when ordinary sterilizing appliances are not on hand.

II.

A very powerful and brilliant stain for the diphtheria bacillus, and one which strikingly shows the irregularities of the "typical" form, may be obtained by the use of methyl violet. The aniline which I have employed was obtained from Grübler, and is specified by him as *methyl violet, 5 B*. It is important to obtain this particular brand.

To make the staining solution a saturated alcoholic

solution of the dye is added to water in the proportions of about one to ten. The exact proportion is not a matter of great moment, and a little experimenting will settle this question, and enable any one to make the staining solution at a moment's notice. This mixture keeps remarkably well.

The technique of staining with this solution differs in no essential feature from the common method practiced by bacteriologists. A fixed cover-glass preparation from the diphtheria culture is stained, with heat, for half a minute, then washed with water, dried, and mounted. Or the stain may be employed without heating by allowing it to remain in contact with the film of the cover-glass preparation for one minute, though the results are not quite so good as in the heated specimens. In any case the resulting preparations are stained with a brilliancy and beauty that quite surpass the more commonly employed stains, like Loeffler's methylene blue.

III.

For some time I have had considerable trouble in making a methylene-blue solution which would stain bacteria in a satisfactory manner. The stain was always too faint and imperfect to be satisfactory. This was true of the aqueous solution, Loeffler's alkaline solution, Gabbet's decolorizing solution, and carbolic acid solution, of methylene blue. Finally, settling upon the aniline as the source of the trouble, I tried other brands, and discovered that an aniline which is recommended for another purpose was remarkably well suited to bacteria staining, while the dye recommended for bacteriological use was unsatisfactory. In these experiments I have confined myself to the use of Grüber's anilines. The unsatisfactory methylene blue is the brand which he recommends for bacteria staining (*Methylen Blau für Bacillenfärbung*, Koch), while the blue which gave positive

results is the one proposed by Ehrlich for blood work, especially for staining *intra vitam* (*Methylen-Blau nach Ehrlich*). The Ehrlich blue is considerably more expensive than the blue after Koch, but its solutions are much more powerful as staining agents, so that in the end it is an economy to use this brand when methylene blue is called for. As the same trouble has occurred in three different lots of aniline obtained from Grüber in original packages, I am sure the failure is not accidental.

BACTERIOLOGICAL LABORATORY, MEDICAL DEPARTMENT,
UNIVERSITY OF WOOSTER.



