

placed in melted paraffin under partial vacuum. When the bubbling ceases, infiltration is complete. The short time required for infiltration of the paraffin makes it possible for the micrologist to watch and to control the temperature of the bath so that it is kept but a few degrees above the melting point of the paraffin.

Since the demonstration at Columbus our attention has been called to equipment for paraffin impregnation *in vacuo* listed in an English apparatus catalogue² and to a description by C. E. Moritz of equipment similar to that here described.³

Aside from the cost of the English apparatus it obviously has certain disadvantages not possessed by the outfit we use. The English vacuum imbedding oven is all metal, which prevents viewing the tissue during the process of infiltration. Since, as noted above, the cessation of the flow of bubbles from the tissue indicates that infiltration is complete, it is desirable to be able to constantly see the tissue. It can then be removed and blocked at once. The English apparatus reduces the pressure on the paraffin with a hand pump. We have found it advantageous to keep the paraffin bath on the pump throughout the period required for infiltration which serves to remove all traces of chloroform and other volatile fluids that may be introduced with the tissue. This results in a paraffin of superior texture for cutting.

Moritz has redescribed and added to technique developed by Lebowich in 1936.⁴ He uses acetone for dehydration preparatory to infiltration with a soap-wax mixture.

Our equipment permits the ready application of pressure reduction not only to the processes of paraffin infiltration but to fixation, dehydration and clearing as well. The apparatus is so simple and inexpensive that one can be assigned to each pair of students.

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A METHOD FOR PRESERVING TRYPANOSOMA EQUIPERDUM

TRYPANOSOMA EQUIPERDUM has been kept viable and infective for a period of fourteen months by freezing and storing infected rat blood in a dry ice alcohol bath. The procedure used in preserving the blood was as follows: Rat blood heavily infected with *T. equiperdum* was citrated with 0.2 per cent. sodium citrate or defibrinated by stirring with a sterile glass rod. Three to 5 cubic centimeters of the blood so treated were then introduced into a 50-cc sterile Pyrex vial. The vial was then stoppered with a sterile one-hole rubber

cork into which a glass rod one foot long had been inserted. The vial was then lowered into the freezing bath of dry ice and alcohol, rapidly twirling it during and after submersion by rolling the glass rod back and forth between the palms of the hands. The blood froze almost instantaneously in the form of a thin thimble in the bottom of the vial. The cork and rod were then removed without removal of the bottom of the vial from the bath. A new sterile aproned rubber cork was then inserted into the vial and the apron of the cork turned down and securely fastened with rubber bands. The vial was then allowed to submerge in the dry ice alcohol bath. Sixteen vials of infected rat blood were prepared in this manner.

The bath used was a wide-mouthed thermos bottle of five gallons capacity into which two gallons of ethyl alcohol and twenty pounds of dry ice had been placed. Additions of dry ice were made once to twice weekly to maintain the bath.

The viability of the trypanosomes was tested after forty-six days and after fourteen months by removing vials from the dry ice bath and allowing them to thaw either at room temperature or by submersion in cold tap water. The blood, when thawed, was hemolyzed but actively motile trypanosomes were present and on inoculation into young white rats produced fatal infections of *T. equiperdum* which could be transmitted in series.

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² Charles Hearson and Co., Catalogue, London, England.

³ C. E. Moritz, *Stain Tech.*, 14: 17-20, 1939.

⁴ R. J. Lebowich, *Arch. Path.*, 22: 782-805, 1936.