neighbor. Of the wounded rats, six were in the group which received neither food nor water, and eight were in the group which received water alone.

Fifty per cent. of the injured rats in both of the groups continued their own destruction by eating either their fore paws or their tails, depending upon which had been wounded. If the tail was eaten, the fore paws were left unmolested, whereas, if the fore paws were eaten, they were attacked simultaneously and removed somewhat symmetrically, while the tail was left undisturbed. In extreme cases of autophagia almost two inches of the tail was eaten and the forelimbs were completely removed up to the elbows. The eaten parts were removed evenly leaving clean wounds which presented no evidences of infection. The autophagia was not completed in one meal but was continued over a period of several days, the rats apparently finally being halted in the autophagia by their extreme weakness, which was evidently due to their inability to secure sufficient nourishment from this source.

Since an equal proportion of injured starved rats in the group to which water was available and in the group which lacked a water supply, namely, 50 per cent., ate portions of their bodies, it appears that the availability of water did not affect the incidence of autophagia.

A control group of 15 male adult albino rats traumatized in the same manner while on a supermaintenance diet exhibited a tendency to mouth their wounds, but there were absolutely no evidences of autophagia. Since neither unwounded starved rats nor wounded well-fed rats eat themselves, and since 50 per cent. of the wounded starved rats practiced autophagia, it is evident that neither the hunger drive nor the desire to mouth the wounded areas is strong enough alone to produce autophagia, but that in combination the two drives were strong enough in 50 per cent. of the cases to cause the rat to eat the wounded member.

Evidently the starved traumatized rat is originally attracted to its wound by its desire to mouth it, and, when this occurs, the already existing hunger drive causes the rat, in 50 per cent. of the cases, to begin an actual eating process of the wounded member. Apparently the mouthing of the wounded area is a necessary stimulus to set off this process. It is possible that the 50 per cent. of the injured starved rats which failed to practice autophagia were wounded before the hunger drive was strong enough to induce autophagia, and that by the time the strength of the hunger drive had reached such a proportion, the desire to mouth the wounds had ceased to exist due to the lack of irritation at these places.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

SOME USES OF VACUUM IN MICROLOGY

AT the Columbus meetings of the American Association for the Advancement of Science we demonstrated some of the advantages that obtained through the carrying out of various micrological procedures under partial vacuum. The apparatus required is simple and can be put together from materials available in any biological laboratory. An ordinary filter pump is adequate for lowering the pressure. A motor-driven vacuum pump has been tried, but it must be closely watched to prevent the production of too high vacuum. The rather low partial vacuum that we have used has not been injurious to delicate tissues.

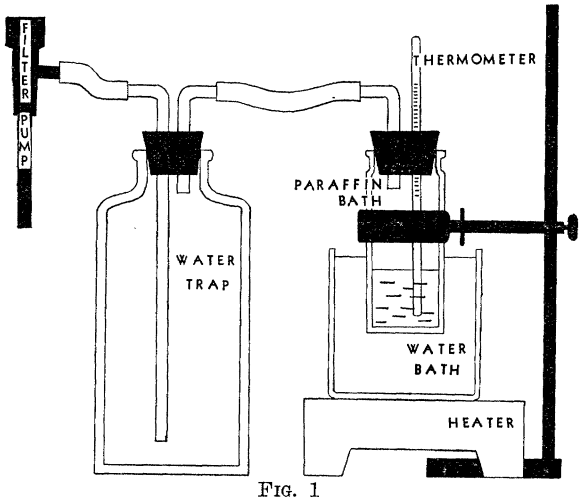

FIG. 1

The construction of the apparatus can be understood by referring to the diagrammatic figure.

The time required in the various solutions will vary with the size and density of the tissue. In general 15 to 30 minutes under vacuum will help the penetration of the killing agent, although the tissue should remain in this solution for several hours to complete fixation; 30 minutes will dehydrate the tissue when "Cellosolve" is used (tissue should be changed to fresh "Cellosolve" once during this time); 15 minutes in chloroform; 15 to 120 minutes in melted rubber-beeswax-paraffin.[1]

Of particular value is the marked reduction of the time required for the infiltration of the tissue with the paraffin mixture and the automatic indication of the completion of this process. The chloroform used for clearing leaves the tissue in a stream of bubbles when

[1] R. T. Hance, SCIENCE, 77: 353, 1933.

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 catalogue[2] 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.

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