AN EFFICIENT VACUUM APPARATUS FOR MICROTECHNIC

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From time to time notes on vacuum apparatus and its applications to microtechnic have been published. These papers, with the exception of very few instances, do not deal with the actual measurement and control of vacuum, the effect of vacuum on tissues and the pumping of tissues in reagents not miscible with water and water vapor. It seemed necessary, therefore, to check the above conditions and to determine, if possible, their relation to fixation, dehydration and infiltration of paraffin in plant material in the "in vacuo" process.

Lebowich (4) in 1936 developed a soap-wax medium for the simultaneous dehydration and infiltration of human tissues. In this technic he employed a vacuum apparatus consisting of a faucet aspirator and a wide-mouthed bottle placed in an oven automatically controlled for temperature.

Later Moritz (5) modified Lebowich's vacuum pump. Instead of using the expensive equipment which Lebowich had at his disposal, Moritz attained the same end with ordinary laboratory equipment obtainable by any technician. In the same paper he presented a modification of Lebowich's dehydration schedule. In the technics of both of these men a vacuum apparatus was used to definite advantage in regard to time and thoroughness of preparation of tissues.

In April 1940 Chermock and Hance (2) announced the use of a vacuum pump for general micrology. Their principle dehydration agent was "methyl cellosolve" which they used in a vacuum apparatus of simple and convenient construction. Their accessory devices were a thermometer and a water trap.

Johansen (3) illustrated a pump which he uses to extract the air from plant tissues in the process of fixation.

Sass (6) described a pump for speeding up killing and fixing of material. This apparatus again consists of the usual essentials of a vacuum pump with a finger valve for the manipulation of a slight increase or decrease of vacuum within the specimen bottle.

It must be remembered, however, that the less complicated vacuum systems used by many of these men were sufficient for their needs as they were not used for the complete processes from fixation through paraffin infiltration but were used only for one or another of these processes.

The vacuum pump is becoming more and more a necessary piece of equipment in microtechnical problems (Fig. 1). Before the initiation of the present pump system a faucet aspirator was used to which was attached a length of pressure tubing. A rubber stopper sealed this tubing directly to the individual specimen bottle. Most material pumped by this method, with the exception of some more woody stems, showed, when cut, one or more types of injury due to the extreme increase or decrease of vacuum. Some of these injuries were wavy cell walls, splitting of cell walls along the middle lamellae, abnormal vacuolization of the cytoplasm, absence of portions of the secondary walls, the crushed appearance of phloem cells, and the tendency of epidermal and peridermal tissues to break away from cortical tissues. At first these injuries were thought to be due to insufficient dehydration, insufficient infiltration or overheating while the material was in

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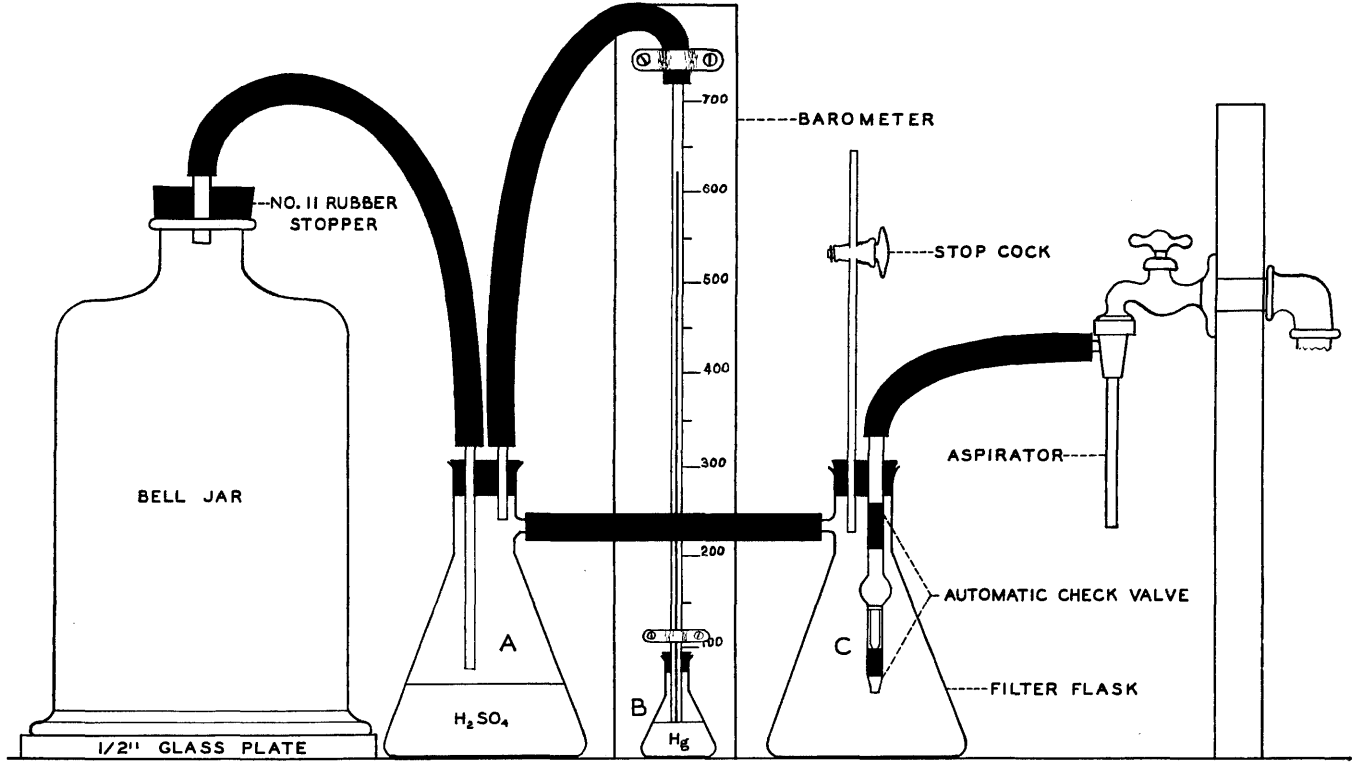

Fig. 1. Vacuum apparatus

paraffin. It was only when endeavoring to find the source of these injuries, that it was discovered that the very construction of the pumping system made it impossible to control the vacuum properly. Later, when the displacement of air was measured, the data showed a displacement rate of over 400 mm. mercury in one minute. This is a tremendous change of pressure vs. the pressure within the plant cell. As a result large amounts of air in the tissues rip through intercellular spaces and plant walls of softer tissues, rendering specimen material useless for a good finished preparation. Therefore, a pump system was constructed which would make impossible a rapid displacement of air and enable the operator to maintain a given displacement of air over a period of time. As a result of several experiments the present pump system evolved. The entire process of fixation, dehydration, and infiltration of paraffin were carried out under vacuum.

Some time ago Dr. D. M. Crooks devised an automatic check valve for a vacuum pump which has been in use at the University of Arizona. By means of this valve a critical control of the vacuum apparatus can be maintained. Although this valve has been modified slightly, the principle of operation is the same and the details of the valve are shown in the diagram (Fig. 2).

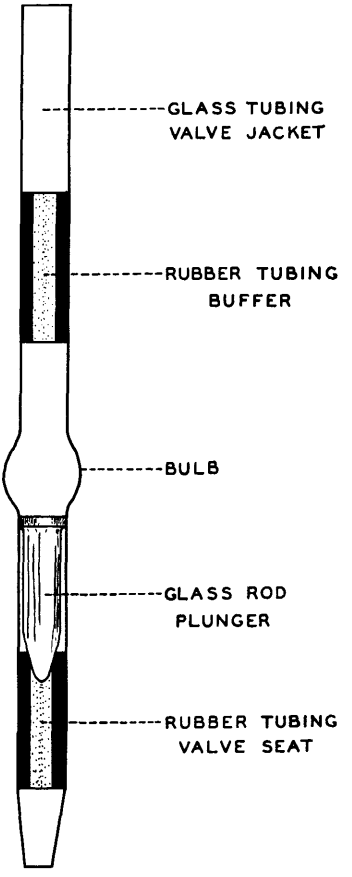

Fig. 2. Automatic check valve

To facilitate the rapid passage of air around the plunger of the valve, it was necessary to blow a bulb in the valve jacket. This bulb prevents the plunger from pushing the rubber tubing buffer out of the jacket. When constructing the glass tubing valve jacket, close one end by drawing it out with a twirling motion over a Bunsen burner. Next, heat evenly a section of the glass tubing one inch in length and three inches from the closed end. Continue the heating until the glass attains an even red glow, draw the tube away from the flame and immediately blow through the open end with quick, short breaths until the bulb is approximately one-half inch or more in diameter. A little practice may be necessary before an evenly blown bulb can be obtained. The closed end of the glass tubing should be cut where its diameter approximates three millimeters. In completing the jacket glaze the cut end until the opening is one or two millimeters in diameter. Another step in the construction of the valve is the insertion of the rubber tubing valve seat and buffer. First, cut two pieces of rubber tubing as specified in footnote 2, one and one-fourth inches in length. Moisten one piece and insert into the valve jacket. Then, with a glass rod which is slightly less in diameter than the inside diameter of the jacket, push the rubber tubing into the position shown for the valve seat as in Figure 2. Next, insert the glass plunger and push the second piece of moistened rubber tubing into the position shown for the buffer in Figure 2.

The list of materials mentioned below was used in the construction of the vacuum apparatus.[2] One or several bell jars may be used. The larger the capacity of the bell jar the better since the rate of increase of the vacuum is thereby reduced. These may be attached to the pump system by inserting one or more glass "Y" tubes in the exhaust line leading from the sulphuric acid flask (Fig. 1, A). With this arrangement a stop cock (Fig. 1) inserted on each bell jar and a hand check valve on the line leading to each bell jar enable the operator to remove the contents of one container without disturbing the others. This is a very worthwhile suggestion made by Dr. Crooks.

Aside from constructing the automatic check valve, the operation of the pump is probably of equal importance. If the faucet to which the aspirator is attached is turned wide open when using a 25 liter bell jar, the mercury column will rise approximately 250 millimeters the first minute, 150 millimeters the second minute and gradually decreases in rate each succeeding minute until a maximum of 700–720 mm. mercury on the barometer column is reached. It was found that the first two or three minutes of air displacement causes the damage to plant material. If the operator will reduce the excessive increase of air displacement during the first few minutes to not more than 150 mm. mercury per minute, the danger of damaging plant material is appreciably lessened.

All dehydration schedules tried were found to be easily cut in half with the aid of the pump. One reason for this is that diffusion in a vacuum takes place much more rapidly than under atmospheric pressure. Also, there is no air in the tissues to hinder diffusion. In a dehydration schedule where changes of reagents were made at two hour intervals, the change of the material under the pump was made at one hour intervals with exactly the same results.

Above 600–650 mm. mercury some damage of specimen material occurred. Just as important is the gradual reduction of the vacuum. The best results were obtained when the reduction did not exceed 90 mm. mercury per minute.

When the bottom flange of the bell jar (Fig. 1) was not vaselined carefully or vaselined at all, the decrease of the vacuum was allowed to take its own course through leakage. When a tight seal was maintained, it was necessary to reduce by opening the stop cock (Fig. 1).

A question might be raised as to the purpose of two pyrex filtering flasks in the pump system. The first (Fig. 1, C) was installed so that the automatic check valve could be held vertically and yet firmly in place. The second flask (Fig. 1, A) containing sulphuric acid was introduced to absorb most of the water vapor. The acid prevents the clouding of toluene and xylene which occurred before in dehydration steps in which these liquids were used.

In the process of paraffin infiltration the exhaust tubing which was originally attached to the bell jar was inserted through the side vent of a standard constant temperature oven. A number 11 rubber stopper was attached to a wide-mouthed 500 cc. bottle within the oven. Greater care in the operation of the pump should be exercised since a much smaller vacuum chamber is being used. The maximum millimeters of mercury should not exceed 400. The rate of increase or decrease of vacuum per minute was adhered to as previously mentioned. Five changes of

[2]One standard faucet aspirator, two 1000 ml. Erlenmeyer pyrex filtering flasks with side necks, one 30 ml. Erlenmeyer flask, one 18–36 liter bell jar with open top and ground glass bottom flange, one piece of ½ inch glass plate 16 inches sq., one glass stop cock with a 1 mm. bore, one piece of barometer tubing 3 ft. long with a 2 mm. bore, one piece of glass tubing 14 inches long with an outside measurement of 12 mm. and an inside measurement of 9 mm. for valve jacket, one piece of 7 mm. glass tubing 3 ft. long for connections, one piece of 8 mm. glass rod 5 inches long for the 1½ inch valve plunger, one No. 11 rubber stopper, two No. 8 rubber stoppers, one piece of 10 mm. black gum rubber tubing 2½ inches long for valve seat and buffer, and 6 ft. of rubber pressure tubing.

paraffin were used at intervals of three hours (25%, 50%, 75%, and two changes of 100% paraffin) before imbedding. An automatic temperature control oven regulated to oscillate between 52° C. and 58° C. resulted in the temperature in the vacuum bottle oscillating between 54° C. and 56° C. By this control the danger of overheating material was eliminated. A slight improvement as to the smoothness of texture of the paraffin was also observed.

Beside the actual use of the pump for the processes mentioned above, it is extremely useful when preparing demonstrations of the vascular system of the entire plant. *Coleus* plants cut off at one of the lower internodes and placed in an aqueous solution of basic fuchsin according to Camp and Liming's method (1) take from three to five days to clear properly in xylene. The clearing of these demonstration stems is a matter of several hours if the vacuum apparatus is used. This apparatus also permits the fixation of as much as three liters of plant material in a few hours. In permanent prestaining schedules, tissues pumped in the dye, stain very rapidly. The maceration of woody stems such as *Quercus, Liriodendron, Pinus*, and many others was obtained in one-half to one-third of the normal time otherwise required.

The following list of plant materials was used in this experiment: the roots, young and old, of *Liriodendron, Ranunculus*, nine species of the *Amaryllidaceae, Raphanus, Citrullus, Coleus*, and *Daucus carota;* the stems of *Helianthus, Zea mays, Vinca minor, Carica papaya, Echinochloa, Elymus, Pinus strobus, Hamamelis virginiana, Benzoin aestivale, Trifolium, Avena, Triticum, Phleum pratense, Picea;* the leaves of *Amaryllis, Hymenocallis, Trifolium, Aesculus glabra, Ranunculus serratus, Sarracenia purpurea;* the seed or flower parts of *Triticum, Coleus, Hymenocallis, Aesculus glabra* and *Ranunculus serratus.* The fungi used were *Poria, Polyporus, Coprinus*, and *Scleroderma.* The algae used included the genera *Sargassum, Fucus, Ulva* and *Chondrus.*

SUMMARY

1. The vacuum apparatus is a necessary piece of equipment for critical results.

2. The time element is cut to one-half normal time for most dehydration and infiltration schedules.

3. Air in the tissues is completely removed and this in turn accelerates diffusion.

4. Many difficulties in paraffin infiltration, such as temperature fluctuations, overheating and partial removal of air, are to a great degree eliminated.

5. A large amount of material may be carried through a given schedule at one time.

6. With this apparatus the factors affecting material while in a vacuum are more readily controlled by the operator.

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