

An Effective Method of Infiltrating Tissues With Paraffin in Vacuo¹

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Several methods of infiltrating tissues with paraffin have been described in the literature (1, 2). This paper is concerned with a deviation from the conventional method. A similar technique is described by Lillie (2). The method to be described here involves the infiltrating of tissues with paraffin in a temperature-controlled vacuum oven utilizing paraffin from which the air has previously been evacuated. It is designed to save the investigator time by reducing crystallization of the paraffin in the tissue which would then have to be re-heated and hardened again. It is well known that prolonged heating results in hardening of the tissues with subsequent difficulty in sectioning (1). This method was devised in the process of research on embryonic chick tissues.

Tissues which have been killed, fixed, and dehydrated are placed in a toluol-paraffin or xylol-paraffin solution. From this solution the tissue is placed in a small Stender dish filled with pure paraffin which has had the air evacuated. Air evacuation from the paraffin is accomplished by placing a beaker of melted, filtered paraffin in the oven and reducing the pressure to a vacuum of approximately 30 inches for 30 minutes. This stock can be kept in the oven at all times. By careful pouring, small glycerine-coated Stender dishes can be filled without introducing very much air and if necessary can be evacuated in the same manner as the stock paraffin. The Stender dishes are used in preference to paper boxes so that air bubbles can be seen more easily. The dish or dishes are placed in the vacuum oven with the temperature regulated to the melting point of the paraffin. The vacuum is then applied, slowly reducing the pressure about five inches at a time to avoid cellular distortion caused by convection currents. Reduction of pressure is continued to approximately 25 inches of vacuum for from 15 to 45 minutes, depending upon the type of tissue being infiltrated. Air bubbles are inevitably introduced when transferring the tissues from the toluol-paraffin solution to the pure paraffin. The removal of the air bubbles can be ascertained by viewing the material through the glass window of the oven. It is not necessary to repeat the pure paraffin step because the toluol is vaporized, nor is it necessary to immerse the blocks immediately in ice water to prevent crystallization. The possibilities of crystallization have been greatly reduced in the process of evacuation of the air from the paraffin. This indicates that probably the presence of air, dissolved in the paraffin, is in part responsible for crystallization of paraffin. It is desirable to use ice water after the paraffin has hardened at room temperature to remove the block from the Stender dish. The paraffin contracts from the walls of the dish and floats free in the ice water.

In most moderately priced 8- by 12-inch vacuum ovens it was found that the temperature varied from 4° to 5° C. from the front of the oven to the back. By the installation of a small fan, the temperature can be equalized throughout the oven. The fan was constructed from a 1-3 volt motor and propeller operated on a 1.5 volt dry cell battery. The wires to the motor were inserted through a vent in the top of the oven and were sealed in by sealing wax. The battery remained outside the oven. Such a motor and propeller can be purchased at a hobby shop. It was found

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that a motor enclosed in plastic was more desirable than one enclosed in metal. The plastic covered model was completely enclosed except where the shaft protruded. The metal covered model had open seams and the vapors in the oven tended to disrupt the function of the brushes. The pressure in the oven was not reduced enough to interfere with the operation of the fan.

This infiltration technique can be used for most delicate tissues without fear of overheating or cellular distortion. It is especially useful when air bubbles are hard to remove from tissues involving a cavity or lumen, as in the eye, lung, or intestine. Dense connective tissue and muscle tissue are also more easily infiltrated by this method. Since the temperature can be controlled and the time element is reduced, histochemical research involving thermolabile enzymes can be carried out. Except in cases where a more firm infiltrant is desired, this method of infiltrating can in most instances replace the time-consuming celloidin technique. Tissues can be infiltrated successfully in 15 to 45 minutes, depending upon the delicacy, density, or porosity of the tissue involved.

SUMMARY

A method of infiltrating tissues with paraffin by use of a vacuum oven is described. This method permits more rapid infiltration than do other methods. Crystallization of the paraffin in the tissues is considerably reduced by removing the dissolved air from the paraffin stock by evacuation. The installation of a small fan in the vacuum oven was found to equalize the temperature within the oven. This helped to prevent overheating of tissues which may cause their shrinkage and hardening.

LITERATURE CITED

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