A SIMPLE APPARATUS FOR PARAFFIN INFILTRATION UNDER REDUCED PRESSURE (IN VACUO).¹

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SEVERAL different types of apparatus for paraffin embedding under reduced pressure are at present on the market. Their cost is so high, however, as to put them beyond the reach of the average histologist. To meet this objection various workers have constructed apparatus at a comparatively small cost, which have been quite as efficient as the more costly shop-made ones. To all these, whether of trifling or high cost, there is the objection, that a special form of oven or water-bath is required. With a view to overcoming this I have employed the apparatus described below, with most satisfactory results.

The pattern of paraffin oven found in the majority of laboratories is fitted with but one opening, other than the door, for the insertion of a thermometer passing through a perforated stopper (Fig. 1, F and F¹). In order to utilise this opening as a passage for the exhaust tube as well as the thermometer the following connection is necessary: A piece of glass-tubing, about 15 cms. long and having a bore sufficiently large to admit the thermometer readily, is obtained; to the side of this tube (Fig. 1, A), about 4 cms. from one end, another tube about 5 cms. in length and of smaller bore is annealed (Fig. 1, B). The other extremity of tube A must be slightly constricted just above the extreme end (Fig. 1, E), in order to afford a hold for connecting-tubing. The thermometer (Fig. 1, C) is now passed into the main tube until its bulb reaches just above the constriction, when it is held in position by a rubber sleeve (Fig. 1, D)—really a short length of heavy rubber tubing—which encircles both it and the upper extremity of tube A. It is thus possible to read the thermometer through the glass-tubing, and to raise or lower it with ease should occasion require. A stopper (Fig. 1, F) is now perforated to receive this part of the apparatus, and is fitted into the opening F¹. The glass tube (constricted end) must extend into the oven for at least 2.5 cms., and to this end is fitted a sufficient length of rubber pressure-tubing to reach to the paraffin bottle (Fig. 4, I), to which it is eventually connected by means of a perforated rubber stopper and glass tube.

The oven being thus fitted (*vide* Fig. 1), connection is made with the side tube (Fig. 1, B) and a three-way glass stop-cock (Fig. 1, H), by means of composition piping and a suitable rubber union. The stop-cock is best placed at some distance from the oven, because the heat tends to dry up the lubricant on the tap. The stop-cock is now connected by its second arm to a manometer (Fig. 1, K) and a mercury trap (Fig. 1, L) by means of composition tubing, a glass T-piece, and rubber unions; by its third arm it is joined to a sufficient length of pressure-tubing to reach the work-bench, where connection may be

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effected with another bottle (Fig. 1, II) when thorough penetration of substances without the application of heat is desired.

The object of the mercury trap is to prevent regurgitation of water into the paraffin bottle should the water supply, for any reason, be inadvertently shut off. The glass tubes of the manometer and of the mercury trap must be of more than barometric height, and in both cases must reach well below the surface of the mercury. In the case of the manometer, the tube should be rather a loose fit. From the mercury trap a short tube leads out, and is connected with an ordinary water filter pump.

The method of using this apparatus is as follows: The pump is started, and the stop-cock is opened for all three ways. The specimen to be impregnated with paraffin is placed, after sufficient dehydration, in the bottle containing melted paraffin, and is put into the oven; a few minutes are allowed to elapse so that the specimen may acquire the same temperature as the oven, then the stop-cock is turned to the position shown in Fig. 2. Almost immediately

bubbles appear, rising from the specimen and also from the paraffin if it is being used for the first time. As soon as the rush of bubbles tends to subside the stop-cock is gently turned to permit a re-entrance of air, and is then closed; exhaustion is continued until no more bubbles rise (in about 10 minutes from the start)—thus indicating the removal of all air, and a consequent thorough saturation with paraffin. The specimen is now ready to be blocked in the ordinary way.

If it is required to carry a fixative, such as mercury, which is slow to penetrate rapidly yet thoroughly, into a tissue such as lung, this apparatus is especially convenient. The mode of procedure is the same as for paraffin, except that the specimen is placed in bottle II (Fig. 1) instead of in bottle I (Fig. 1), which is in the oven, and which must be disconnected in order to provide an avenue of entrance for air to the bottle in use; the stop-cock is set as shown in Fig. 3.

Both bottles must *not* be exhausted simultaneously, as under such circumstances there would be no other means of readmitting air, except by providing a four-way stop-cock, or by disconnecting some part of the apparatus between the trap and the bottles—a procedure not always easy when the pressure is so

much lower within than without. Bottles of large capacity (150 to 200 c.c.) should be used, since from small bottles some of the penetrating medium is apt to be sucked over into the tubes, thus soiling and, in the case of paraffin, even completely blocking them.

In preparing specimens for this method of paraffin infiltration it is essential to employ a volatile vehicle, such as chloroform, for the paraffin. If acetone is used as a dehydrant the specimen may be placed directly into the melted paraffin.

I have found that the following routine in preparing material gives uniformly good results:—

1. Fix and harden in formalin alcohol (40 per cent. formalin, *i.e.* commercial strength, 1 part; 70 per cent. alcohol, 3 parts) for twenty-four hours.

2. Dehydrate in acetone, changing each half-hour for first two changes, followed by two subsequent changes each an hour apart.

3. Infiltrate with melted paraffin—under reduced pressure (-80 cms. Hg.)—for ten or fifteen minutes; specimens are then ready for blocking.

Among the advantages accruing from this method of paraffin imbedding are—

- (1) Thoroughness of saturation of the specimen with paraffin;
- (2) shortness of exposure to heat in oven during the actual procedure;
- (5) rapidity in preparation of tissue for examination.

For the apparatus I claim—

- (1) Simplicity and economy in construction;
- (2) small cost;
- (3) results equal to any obtained by the use of much more expensive appliance.