

## [54] APPARATUS FOR TISSUE PREPARATION

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[58] Field of Search .... 34/5, 15, 92

### [56] References Cited

#### UNITED STATES PATENTS

|           |         |                |        |
|-----------|---------|----------------|--------|
| 3,009,258 | 11/1961 | Taylor .....   | 34/92  |
| 2,803,888 | 8/1957  | Cerletti ..... | 34/5 X |
| 1,884,429 | 10/1932 | Warner .....   | 34/5 X |

### FOREIGN PATENTS OR APPLICATIONS

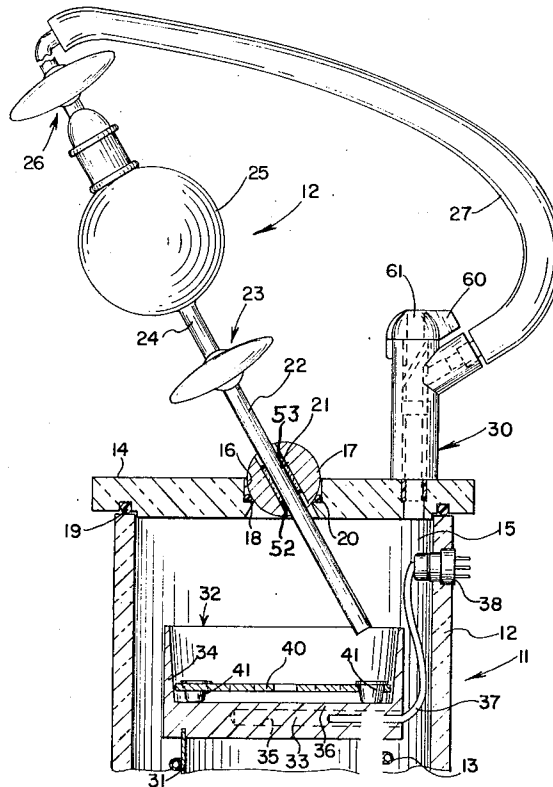
932,043 7/1963 Great Britain ..... 34/5

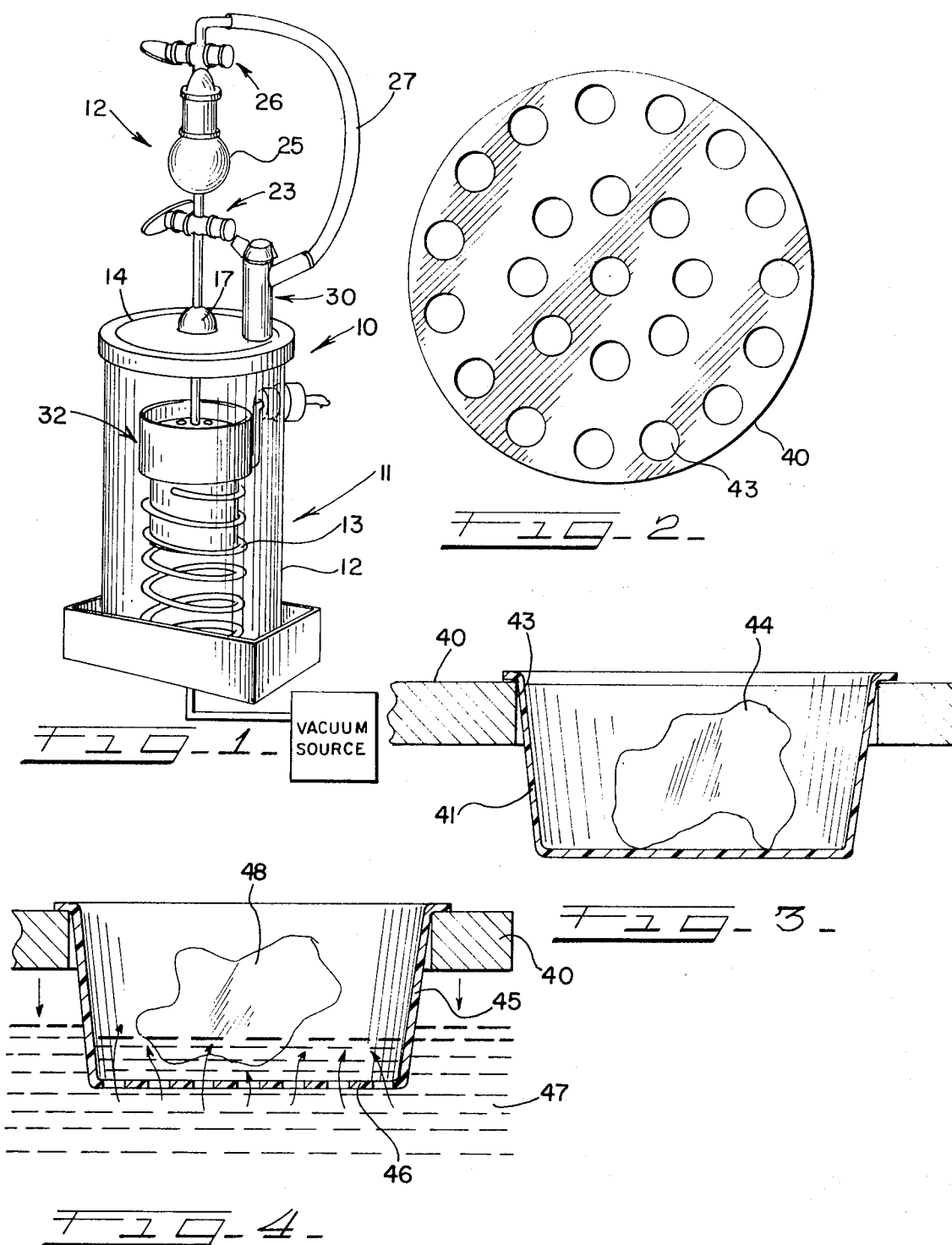
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### [57] ABSTRACT

A tissue-embedding assembly is shown which is convertible to a resin option from a paraffin option. The tissue-embedding assembly permits the simultaneous embedding of multiple-tissue specimens with degassed embedding material. Full observation of the process as well as manipulation of the specimens is available during and up to solidification of the degassed embedding material. A novel heat transfer arrangement is available which permits the heating of paraffin to cause melting and embedding without disconnection of the refrigeration.

5 Claims, 6 Drawing Figures





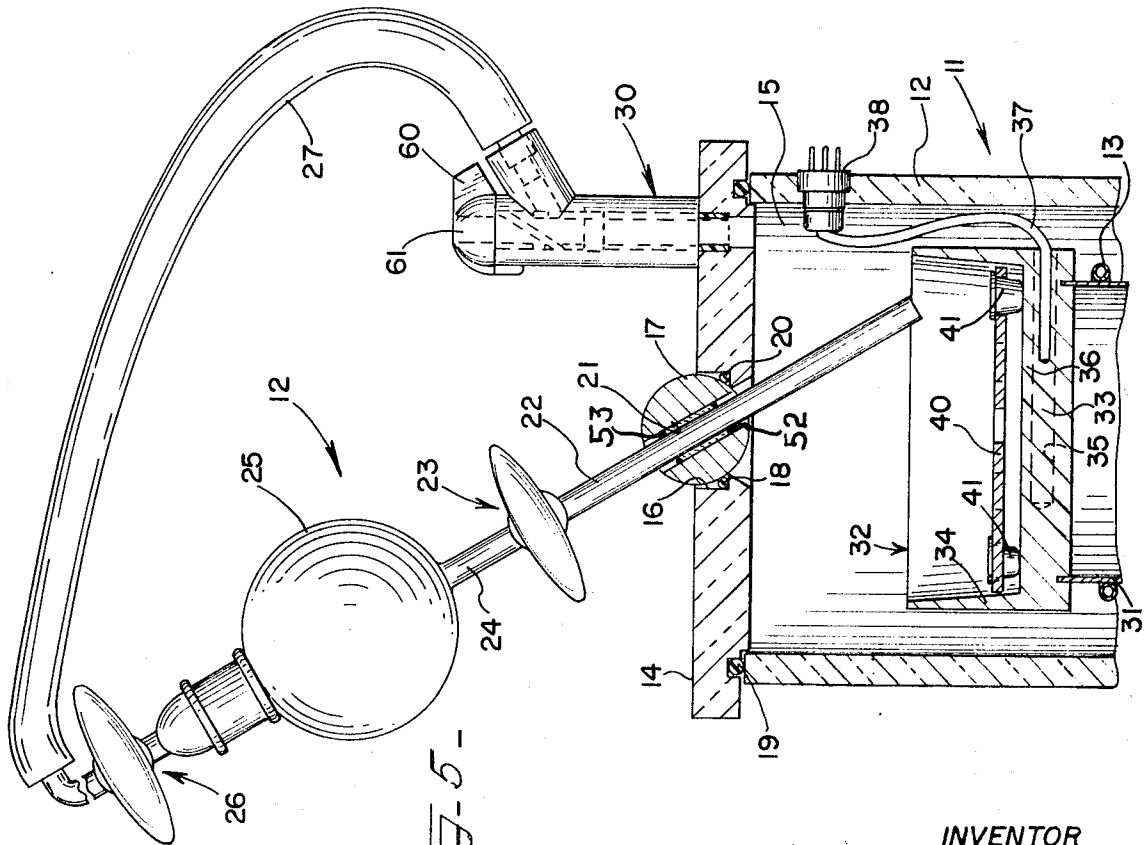
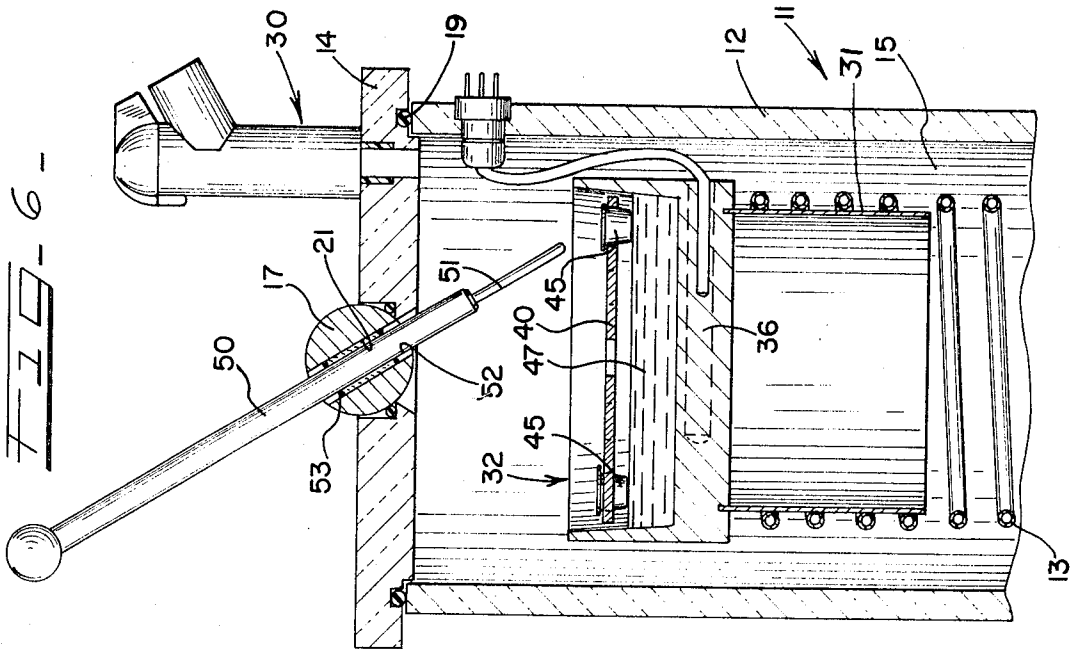
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## APPARATUS FOR TISSUE PREPARATION

This invention relates to improvements in tissue preparation and, more specifically, is directed to a new and improved apparatus for tissue drying and embedding as well as a novel method for performing tissue embedding.

A great deal can be learned from a pathologist's examination of a small portion of tissue. In order to effectively carry out such examination, the orientation of the cells in the tissue sample should be undisturbed. This is accomplished through drying the specimen and then filling the voids or spaces between the fibers or cells with liquid paraffin or resin which is allowed to solidify, usually through cooling. Thereafter, the embedded tissue is cut into thin section to permit microscopic examination and other study, including such subjects as histology, parasitology, bacteriology, botany, zoology and kindred subjects. Such tissue study techniques of this general type have been employed in the past with considerable success, however, certain areas require improvement if effectiveness of this type of examination is to be increased.

Generally, in the past, the process has included the selection of a proper tissue, dehydration with one of several dehydrants, clearing of the tissue with a soluble oil or acceptable clearing agent and thereafter infiltration of the tissue with the paraffin wax or combination of wax and resinous material which is thereafter allowed to harden. An instrument commonly known as a "microtome" is used to slice thin sheets of the impregnated tissue in the block of paraffin so that it may be used on a slide for microscopic study.

Problems have been encountered in the past with prior art designs because of the poor visibility during drying and embedding afforded by prior art dryers. Poor heat transfer between the cooling or heating medium and the specimen was an added problem which was brought about by the type of support for the tissue. Allied problems were encountered in that when the specimen was required to be manipulated during the embedding step, only one specimen could be embedded at a time. Small specimens were oftentimes apt to be lost or damaged because of the nature of the drying and embedding operation.

The present invention overcomes the difficulties encountered in the prior art through the provision of a new and improved apparatus for tissue preparation which is of greatly simplified construction. The present design uses a known and versatile type of refrigeration and vacuum source, having an upstanding evaporator coil which is in intimate transfer contact with a support structure which receives and supports the specimens to be embedded. A unique cassette permits the embedding of a large number of tissue specimens simultaneously, thereby expediting the preparatory process and making the most efficient use of the machine. While manipulation of the tissue is not ordinarily required, means is provided to permit manipulation of the tissue within the individual cuplike holders prior to or during embedding and while under vacuum. The manipulative means also includes additional metering means to permit resin or other types of embedding substances other than paraffin to be used and includes means to evacuate or degas the embedding resin prior to use. Several advantages are provided by the present design including single-step embedding, permitting the use of irregular chunks or discs of paraffin. Embedding is performed under vacuum with degassed paraffin or resin. Conversion from the resin to paraffin option can be conveniently accomplished through replacement of the movable probe. The containers for embedding are relatively inexpensive, permitting them to be discarded after each experiment to avoid any problems of contamination of future specimens. The samples may be observed throughout the embedding step through the use of a transparent chamber which permits 360° observation. Breaking of the vacuum is easily accomplished and removal and setup for the next embedding run is completed with unequal ease through the novel but simple structural arrangement.

It is an object of this invention to provide a new and improved method and apparatus for tissue preparation.

It is a further object of this invention to provide a new and improved apparatus for tissue preparation of simplified design which permits embedding of multiple samples without necessitating manipulation under ordinary circumstances, however, means is provided to permit manipulation should it become desirable.

It is a still further object of this invention to provide a new and improved apparatus for tissue preparation having a novel support arrangement having improved heat transfer characteristics which permit heating to melt the embedding material without terminating the refrigeration.

Objects in addition to those specifically set forth will become apparent upon reference to the accompanying drawings and following description.

In the drawings:

FIG. 1 is a fragmentary perspective view of the apparatus of the present invention having the resin option;

FIG. 2 is a top plan view of the cassette which is adapted to carry the cups supporting the tissue samples;

FIG. 3 is an enlarged cross-sectional view of the cassette and a cup for use in the resin option and with a tissue sample in full elevation;

FIG. 4 is an enlarged view of a modified form of cup containing a tissue sample undergoing embedding in the paraffin option;

FIG. 5 is an enlarged cross-sectional view of the form shown in FIG. 1 with the resin probe in position to feed resin to one of the tissue holding cups; and

FIG. 6 is a cross-sectional view similar to FIG. 3 illustrating the paraffin option.

Referring now to FIG. 1, the tissue drying and embedding accessory is indicated generally by the reference numeral 10 consisting of a closed chamber indicated generally at 11 and a resin metering assembly 12 positioned on the chamber 11. The closed chamber 11 consists of a transparent cylinder or sleeve of plastic or the equivalent 15 which is sealed with a vacuumtight fit along its lower margin to the top of a freeze dryer known in the art as Model No. 10-010 sold by The Virtis Company, Inc. of Gardiner, N.Y. An O-ring slotted to receive the lower end of the sleeve 15 is satisfactory to provide sealing with the top of the freeze dryer.

The transparent sleeve 15 is coaxially arranged with a stainless steel condensing coil 13 helically wound in the manner indicated. The upper end of the transparent cylinder 15 is closed off by a transparent cover 14 with a seal 19 interposed and serves to fluidtightly support the resin embedding assembly 12. The details of this arrangement will be given in conjunction with the description of the enlarged views of FIGS. 5 and 6.

As best seen in the enlarged fragmentary view of FIG. 5, the cover 14 is provided with a shouldered central opening 16 which receives a sphere 17 of nylon or the equivalent. The sphere is sealed for free movement in the opening through an O-ring 18 which is supported on an axially facing portion or shoulder 20. A cylindrical bore 21 is formed through the center of the sphere 17 and receives the lower end of a glass tube 22 forming a part of the resin-embedding assembly 12. The tube is sealed for movement relative to the sphere through a pair of spaced O-rings having high-vacuum grease in between. The lower end of the tube 22 is located within the chamber and the opposite end is integral with a conventional stopcock valve 23 which, in turn, is in communication through a tube 24 with a resin reservoir 25. The upper end of the reservoir is connected to a flexible tube 27 through a stopcock valve 26 with the opposite end of the flexible tube 27 in fluid communication with the chamber 15 through a three-way valve which is the subject of a copending patent application, Ser. No. 650,134, filed on June 29, 1967 and now U.S. Pat. No. 3,509,909. The three-way valve 30 permits the resin reservoir 25 to be placed in communication with the drying chamber for degassing of the resin prior to the embedding procedure and also permits the vacuum to be broken for ease in removing the cover.

The condenser coil 13 receives a sleeve 31 with a tight fit to provide for good thermal conductance and mechanical support. The sleeve 31 supports a cup-shaped member 32 having a thickened base portion 33 and upstanding sidewalls 34. The sleeve 31 may be joined through the cup 32 by any suitable means which will provide good heat transfer. The bottom of the cup 32 is provided with an opening 35 which receives a heating element 36 having a conducting wire 37 joined to a suitable plug 38 which is mounted in the wall of the transparent sleeve 15 with a vacuum-tight fit. The cup 32 supports a cassette 40 which receives a plurality of flexible tissue cups 41 (only two shown) which are manually pressed into the cassette plate. The cups may be formed of plastic or the equivalent and are readily available commercially.

As seen in FIG. 2, a typical cassette may consist of a flat disc having a plurality of openings 43 which are sized so as to receive the cup 41 which is shown in enlarged cross section in FIGS. 3 and 4. In the form of cup shown in FIG. 3, the tissue specimen 44 is supported in the plastic cup 41 which is press-fitted in any one of the 24 openings in the plate 40. Any number of openings may be provided in the plate 40, limited only by mechanical geometry or the capacity of the system. As is evident, a number of diverse samples may be dried simultaneously.

In FIG. 4, a modified form of tissue cup is shown at 45 having a plurality of perforations 46 in the bottom wall to allow wax or other liquid embedding material 47 to flow into the cup from the bottom and envelop the tissue specimen 48. This will be described in greater detail in connection with the paraffin option shown in FIG. 6.

In the paraffin option of the invention which is shown in enlarged cross section in FIG. 6, the glass tube 22 may be replaced by a solid glass rod 50 which extends through the nylon sphere 17 and has a reduced diameter at its end to form a manipulative probe 51. The rod 50 is sealed to the central bore 21 of the nylon sphere 17 through the use of O-rings held within the bore 21 through shoulders (not shown) formed at opposite ends of the bore 21. The area intermediate the O-rings 52 and 53 is filled with a suitable sealing compound such as a high-vacuum grease to assure a vacuumtight seal as well as relatively free movement of the probe 50 relative to the sphere 17. From the foregoing, it is evident that conversion from the resin to paraffin option is accomplished through substitution of the resin assembly for the manipulative probe.

In the paraffin option shown in FIG. 6, the container 32 is filled with chunks or blocks of paraffin which are heated to a temperature sufficient to cause them to melt and form a pool as shown at 47 in FIG. 6. Throughout the melting process, a vacuum is drawn in the chamber 15 to remove any entrained gas from the paraffin. Refrigeration is continuously applied and the heating means 36 deenergized, causing the paraffin to solidify. When the paraffin 47 reaches the solid state, the three-way valve 30 may be opened to admit air, permitting removal of the cover 14 and insertion of the cassette 40 containing perforated cups 45. The cups 45 contain tissue specimens which may have been previously dried or, in the alternative, which have been prefrozen and may be dried within the tissue preparation chamber 15 in advance of the embedding step. The cover 14 is replaced while the refrigeration is continuously applied. A vacuum is then applied to the chamber 15. The radiant heat transmitted through the walls of the transparent chamber 12 and cover 14 is sufficient heat input to cause sublimation of the tissue specimens to be embedded, causing the water to vaporize so the vacuum will cause its removal from the chamber. When drying is completed, the heater 36 is energized, causing the paraffin 47 to liquefy. Throughout this step, the refrigeration is continued, however, the proximity of the heating element to the container is sufficient to override the effect of the refrigeration and cause melting of the paraffin 47.

In FIG. 4, the paraffin is in the liquid state and the cassette 40 and perforated cups 45 sink slowly into the liquid paraffin, allowing the paraffin to enter the cup 45 in the direction

shown by the arrows and thereby envelop and permeate the dried specimen 48. If at any time during the process it is necessary to manipulate the tissue specimen, the probe 50 may be moved to any position to bring the end 51 into contact with the specimen in any one of the cups to permit the same to be oriented relative to the cup for best results in embedding.

After the specimen 48 is fully enveloped and embedded, the heater is deenergized and the refrigeration becomes effective to cause solidification of the paraffin 47. The application of vacuum is discontinued, the valve 30 opened to vent the chamber and the cover is removed. The cassette plate 40 with the specimens embedded in paraffin may then be removed from the container for preservation and the study procedures outlined above. The process may be repeated as necessary.

In operation, the resin embedding option functions quite similarly to the paraffin option, however, the embedding resin is metered into the cup under the influence of gravity. The tissue cup 41 of necessity must be of the form shown in FIGS. 3 and 5. The processing steps through freeze drying are the same as in the paraffin option, however, during the drying process the resin is degassed. To accomplish this, valve 26 is opened as is the three-way valve 30 to apply vacuum to the resin reservoir 25. When the tissue is ready for embedding, valve 23 is opened after the probe has been positioned over the cup 41 containing the tissue to be embedded. The resin in the reservoir 25 flows under the influence of gravity into the cup and envelops the tissue specimen 44. The valve 23 is closed when the cup is filled and the resin probe 22 moved from cup to cup with each cup filled to a level above the tissue specimen.

As shown in dotted lines and more particularly described in the patent on the three-way valve 30 alluded to above, the vacuum may be broken by rotating the handle 60 to a position to admit air through the end 61 permitting the cover 14 to be removed to remove the specimens. The use of the isolated cooling coil 13 as the sole support of the sleeve 31 reduces the mechanical vibrations transmitted to the cassette 40 and tissue receiving cups 41 or 45. This damping of the vibrations is important to prevent mechanical abrasion of the tissue samples while they are being sublimated and otherwise unsupported, since it is conceivable that excessive vibrations could reduce these tissue samples to powder if operation at a resonant frequency occurred.

It will be appreciated from a consideration of the foregoing that the freeze drying and embedding accessory provides great versatility for existing freeze dryers. The simplified chamber and embedding procedure of the present invention permits the use of chunks of paraffin as opposed to predimensioned discs. Moreover, conversion of the system from paraffin to resin is easily accomplished through substitution of the manipulative probe 50 with the resin assembly 12. Multiple embeddings can be made simultaneously and the process observed continuously through a full 360° due to the transparent chamber. Moreover, the use of a transparent chamber functions to provide sufficient radiant energy to eliminate the need for a controlled heat input during the freeze drying process. The unique location of the heating means permits the heating step to be performed without cutting off the refrigeration, thereby expediting the entire embedding process.

A further important aspect of this invention is that in either of the paraffin or resin options, the tissue embedding process is carried on with the reservoir or cup-shaped member in direct metallic contact with the condenser coil. In other words, there is no discontinuity between the heated reservoir and the condenser coil. Nevertheless, the condenser temperature will be maintained, even though the reservoir is sometimes at a temperature of approximately 70° C. since the heat transferred is limited by the thin wall sleeve 31 which connects the reservoir to the coil. This sleeve provides a relatively slow heat transfer path so that what heat does get through to the coils can be dissipated within the capacity of the refrigeration system. This unique structure permits the continuous operation of the refrigeration system.

Upon a consideration of the foregoing, it will become obvious to those skilled in the art that various modifications may be made without departing from the invention embodied herein. Therefore, only such limitations should be imposed as are indicated by the spirit and scope of the appended claims.

I claim:

1. A tissue dryer for drying and embedding tissue samples and the like comprising a cooling coil, support means including an axially extending sleeve disposed within and contiguous to said cooling coil, in direct heat transfer relationship with said cooling coil said support means being mounted on said cooling coil, and supporting container means and means to elevate the temperature thereof to permit heating of embedding material, a cassette mounted on said support means having a plurality of tissue receiving cups thereon, closure means enveloping said support means and cooling coil in a vacuumtight manner, and means to evacuate said closure means.

2. A tissue dryer for drying and embedding tissue samples and the like comprising a cooling coil, support means disposed in heat transfer relationship with said cooling coil, a cassette mounted on said support means and having a plurality of tissue-receiving cups thereon, said support means including a reservoir, and each of said tissue receiving cups being perforated at its lower extremity to admit embedding material, said reservoir being filled with embedding material, and heating means to soften said embedding material to permit flow

into said tissue receiving cups, closure means enveloping said support means and cooling coil in a vacuumtight manner, and means to evacuate said closure means.

3. A tissue dryer for drying and embedding tissue samples and the like comprising a cooling coil, support means disposed in heat transfer relationship with said cooling coil, a cassette mounted on said support means and having a plurality of tissue receiving cups thereon, said cooling coil being the sole support for said support means thereby to reduce the mechanical vibrations transmitted to said cassette and said tissue receiving cups, closure means enveloping said support means and cooling coil in a vacuumtight manner, and means to evacuate said closure means.

4. A tissue dryer for drying and embedding tissue samples and the like comprising a cooling coil, support means disposed in heat transfer relationship with said cooling coil, a cassette mounted on said support means and having a plurality of tissue-receiving cups thereon, closure means enveloping said support means and cooling coil in a vacuumtight manner, and means to evacuate said closure means, said closure means including means to permit manipulation of the tissue in said tissue receiving cups during drying thereof without breaking the vacuum in said closure.

5. The tissue dryer of claim 4 wherein said means to manipulate said tissue in said cup includes means to supply embedding resin under vacuum to each of said cups.

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