July 22, 1969 D. M. BARTON ET AL 3,456,538

MICROTOME APPARATUS

Filed Oct. 31, 1966 4 Sheets-Sheet 1

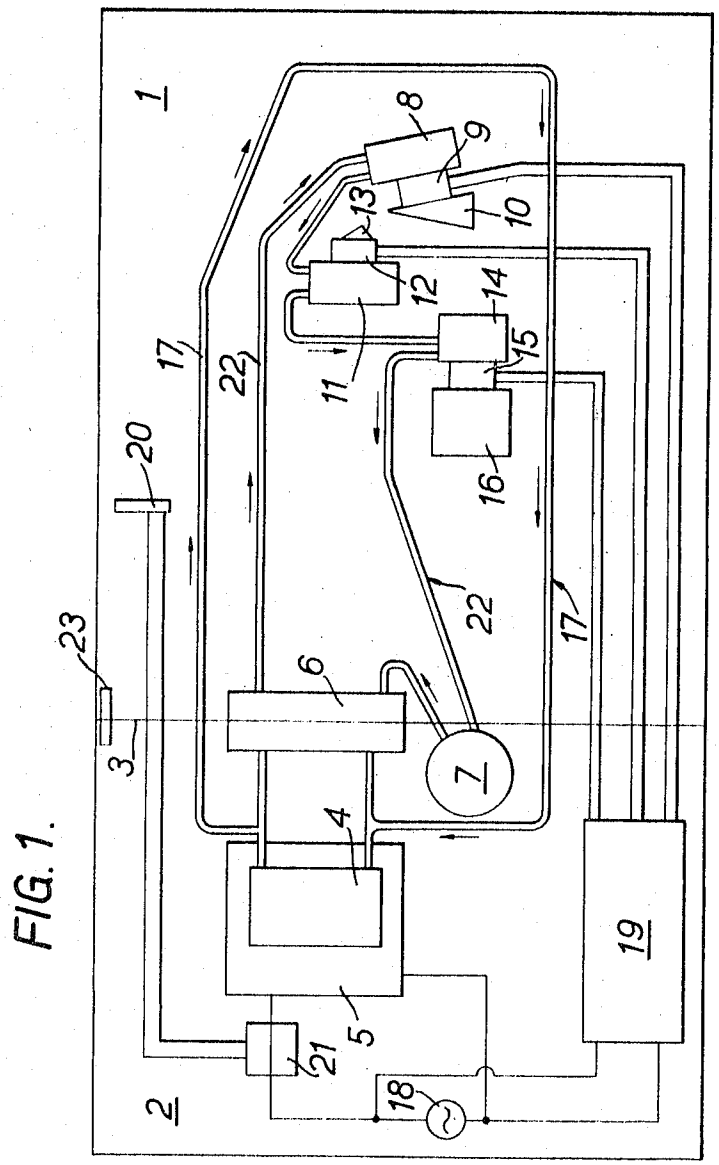


FIG. 1.

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MICROTOME APPARATUS

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FIG. 2.

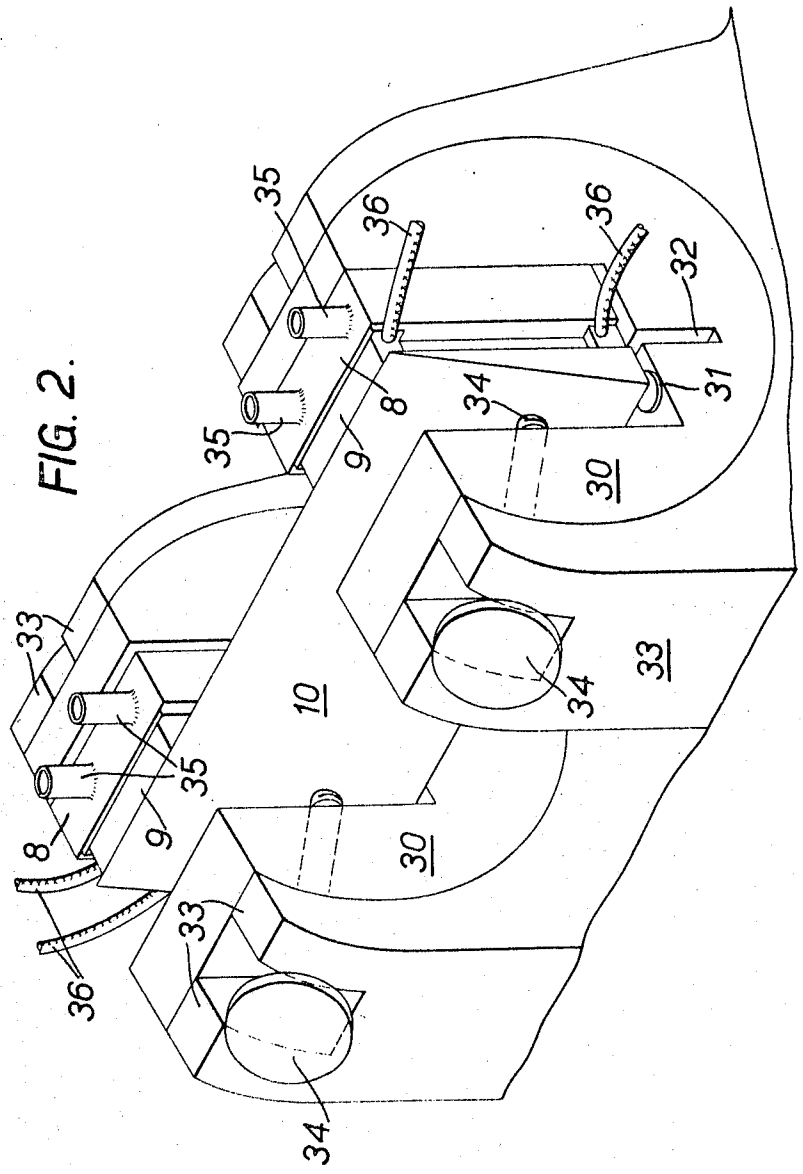


FIG. 3.

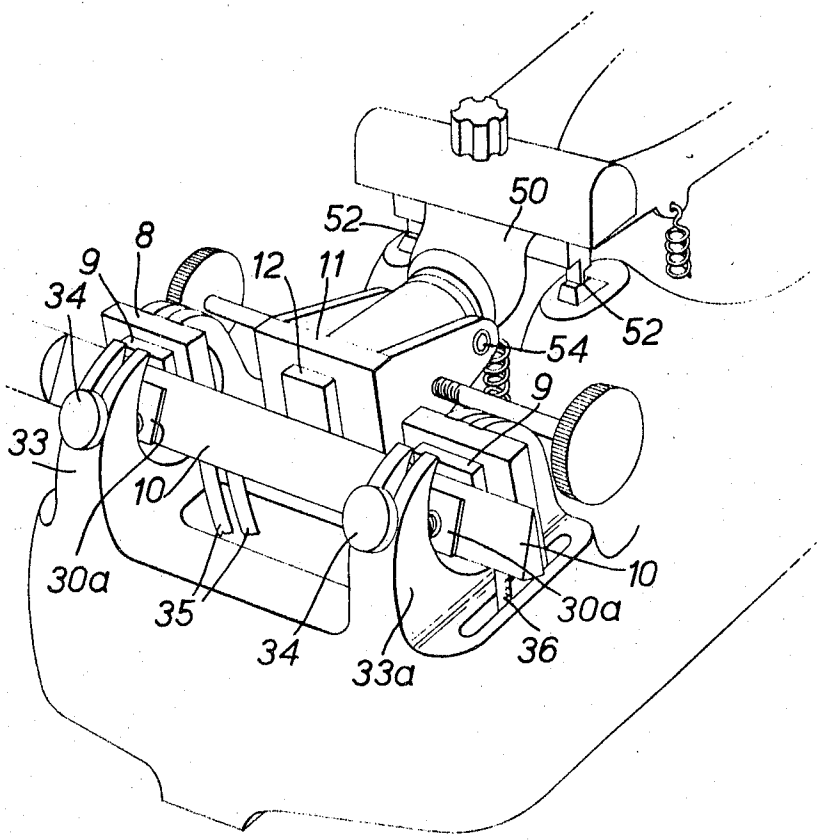


FIG. 4.

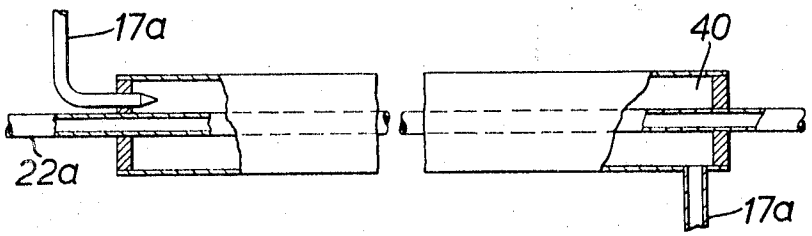
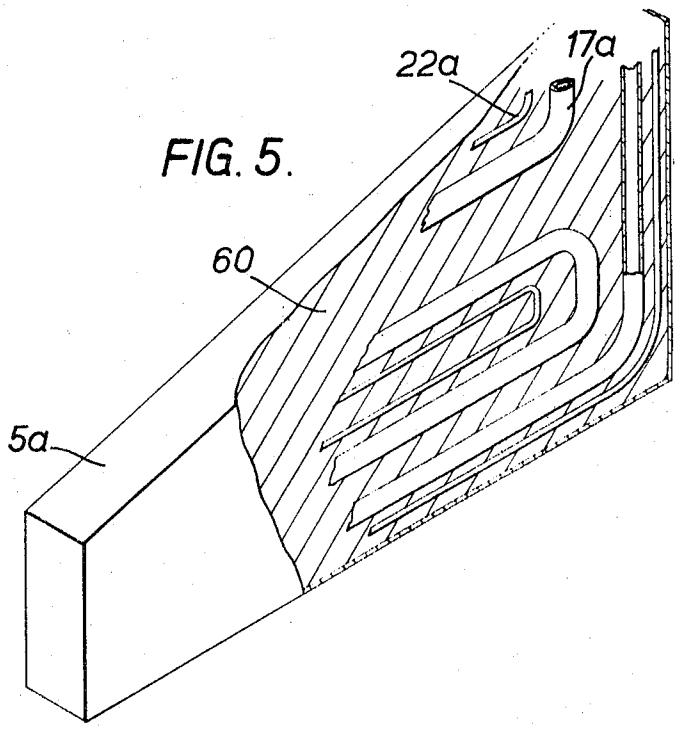


FIG. 5.



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3,456,538

MICROTOME APPARATUS

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Int. Cl. B26d 7/10
U.S. Cl. 83—171 7 Claims

ABSTRACT OF THE DISCLOSURE

This invention relates to microtome having independent thermoelectric cooling units for cooling the knife and the stage. The knife and stage are enclosed in a chamber so that the ambient air may be cooled. A single cooling circuit is also provided to cool the hot junctions of the thermoelectric cooling units and also to cool the air within the cryostat.

This invention relates to microtome apparatus for cutting, from a specimen, slices of material suitable for examination by microscope.

Microtomes may be used under room-temperature conditions, but it is also well known to mount them within refrigerated cabinets, provided with openable walls to give access, means for operating and adjusting the microtome from outside the cabinet when the latter is closed, and a transparent panel for observing the operations. Such refrigerated cabinets are known in the trade as cryostats and will be so termed hereinafter and, together with microtomes, they are currently available commercial items, and for this reason they will not be described in detail. Is using such apparatus, frozen sections of organic tissues and possibly other materials can be cut, the sections remaining frozen and capable of being handled after cutting owing to the refrigerated atmosphere. This permits sections to be cut without prior chemical fixing. However these instruments do not permit independent control of the temperatures of the various parts of the microtome and these are either approximately the same as the air temperature or vary from it in an arbitrary manner not susceptible to control. Further to obtain a low temperature at the knife, for example, one must provide refrigerating means sufficient to cool the whole apparatus to this low temperature.

It is also known to provide microtomes with thermoelectric cooling and temperature control of the knife and the stage and optionally the anti-roll bar so that they can be used to cut frozen unfixed specimens in non-refrigerated ambient air. British patent specification No. 992,979 describes and claims such an arrangement providing for independent temperature control of the various parts, since it has been found that the various parts of the microtome normally have different optimum temperatures for the cutting of any particular type of tissue. However, with this apparatus the ambient air is not cooled below 0° C., frosting occurs on the knife and it is difficult to obtain really low temperatures of the knife and stage.

According to the present invention there is provided a microtome apparatus for cutting, from a specimen, slices of material suitable for examination by microscope which apparatus includes a knife, a specimen holder stage and a closable chamber, primary cooling means for maintaining the temperature of the chamber at a temperature below the temperature of the air surrounding the chamber, a first thermoelectric cooling unit for controlling the temperature of the knife, a second thermoelectric cooling unit for controlling the temperature of the specimen

holder stage, means to enable the apparatus to be operated within the chamber with the chamber closed, and means to control separately and independently the supply of electric power to each of the thermoelectric cooling units.

The refrigerating apparatus used for cooling the chamber is preferably a conventional one and may be an absorption or a compression type refrigerator. Alternatively the chamber may be cooled thermoelectrically or by the expansion of carbon dioxide or by the use of solid carbon dioxide. Optionally a cold storage chamber is also provided for storing specimens and this may optionally be thermoelectrically cooled. The thermoelectric cooling means is in the form of units referred to hereinafter as modules.

The module comprises a sandwich of semiconductor blocks between metallic bridges, the bridge on one face being referred to as the hot junction bridges and those on the other face as the cold junction bridges. The constituents of the module are soldered together to connect them electrically in series, with a repeating unit of cold junction bridge, p-type semiconductor block, hot junction bridge, and n-type semiconductor block. The electric current is supplied via metallic hot junction terminals which occur at each end of the sequence where the sequence would lead one to expect hot junction bridges. Such thermoelectric cooling units are known articles of commerce e.g. they are supplied by De La Rue Frigistor Limited under the name Frigistor thermoelectric cooling modules. On passage of electric current one set of junctions becomes hot and the other set becomes cold. On reversal of the current the set of junctions which previously become hot now become cold. It is, however, convenient to refer to those junctions on the face adjacent to the article, surface substance or space whose temperature it is desired to change or control, as the cold junctions and the other set of junctions as the hot junctions. It is usual to arrange for the terminal members to occur in the position in the sequence where the hot junction bridges would occur since this avoids the electric current leads acting as a heat leak to the cold junctions and hence to the article, space or substance whose temperature it is desired to alter or control.

Onto the cold junction face is glued or otherwise attached a panel of thermally conducting electrically insulating ceramic (alumina or beryllium oxide) or of metal, preferably aluminium, whose face in contact with the cold junction face has been oxidised (anodised) or otherwise treated to render it electrically non-conducting. It is also possible to use a thin sheet of polymeric material of the type commonly referred to as "plastics." In the case of the stage this plate is the specimen plate to which the specimen may be attached suitably by water e.g. by thawing the rear surface of the specimen and freezing it on to the specimen plate. In the case of the knife and optionally the cold storage chamber thermal contact between the cold junction panel and the knife (or chamber wall) may be promoted by using a thin film of grease. In the case of the cold chamber the cold junction panel may alternatively be integral with the walls of the chamber e.g. if these are constructed of aluminium, anodised or otherwise rendered electrically insulating where they contact the cold junctions of the thermoelectric cooling module. Heat is removed from the hot junctions by heat sinks, preferably constructed of aluminium, each anodised or otherwise rendered electrically insulating on at least that face which contacts the hot junctions of the module. The heat sink is then preferably glued to the module using e.g. an epoxy resin. Each module with its anodised aluminium plate in thermal contact with the cold junctions and the anodised aluminium heat sink in thermal contact with the hot junctions can advantageously be

sealed with a layer of resin running all round the edges thus preventing ingress of moisture.

Other forms of heat sinks are possible, e.g. modules may be constructed in which the hot junction cooling fluid washes the hot junctions directly, but as we are unaware of such modules existing as articles of commerce we prefer to use standard modules and to attach heat sinks as described above.

Embodiments of the invention will now be described with reference to the accompanying drawings in which

FIGURE 1 shows in diagrammatic fashion one embodiment of the refrigerant and electrical circuits,

FIGURE 2 shows a perspective view of one form of mounting the knife and its thermoelectric cooling units,

FIGURE 3 shows a perspective view of a microtome apparatus with a slightly different form of mounting for the knife and its cooling units and showing also a specimen holder on a pivoted arm,

FIGURE 4 shows a section through an evaporator through which the circulating liquid is passed, and

FIGURE 5 shows a cut away perspective view of a heat exchanger containing a freezable mixture (matrix jelly) and the evaporator coils and coils for carrying the circulating liquid.

Referring to FIGURE 1 there is shown in diagrammatic fashion a closable refrigerated chamber **1** housing microtome apparatus. The electrical and refrigerant circuits, in so far as they pass through the walls of the chamber **1**, are shown passing through the line **3**. The chamber **1** is a Cryostat and is provided with suitable equipment for operating the microtome apparatus. The microtome apparatus has a knife **10** and a specimen holder including a heat transfer unit **11** with a specimen plate formed by a thermoelectric cooling unit **12** and having a specimen **13**. Outside the chamber there are provided a primary refrigerator **5**, a relay or switch **21** which controls the power supply to the main refrigerator in accordance with the temperature of air in the chamber **1**, as determined by a thermostat **20**, and the setting of a temperature control, in order to control the temperature inside the chamber. In addition a cold compartment **16** is provided with a thermoelectric cooling unit **15** and a heat transfer unit **14**. There are also provided a power supply **19** having three separate and independently controlled outputs each of which is connected as shown to a respective thermoelectric device **9**, **12** or **15**, and a thermometer for measuring the temperature of the air within the chamber. The refrigerator **5** may be of any suitable type compression, absorption, thermoelectric, or it may use carbon dioxide, whether solid, gas or liquid under pressure. It may provide a cooled fluid, which may be carbon dioxide (cooled by expanding and/or evaporating), which circulates through the pipe system **17** or otherwise cools the chamber **1** and/or circulates through a heat exchanger **6** to cool the hot junction cooling fluid which circulates through the pipe system **22** to cool the "hot junctions" of the thermoelectric cooling units **9**, **12** and **15**. Alternatively, the hot junction cooling fluid may be cooled by the refrigerator **5** in any suitable manner, e.g. if the refrigerator **5** is of the thermoelectric type, the hot junction cooling fluid may be circulated in thermal contact with its "cold junctions" (the term "hot junction" cooling fluid is used to describe the fluid cooling the hot junctions of at least one of the thermoelectric cooling units **9**, **12** and **15**). If the main refrigerator **5** is of the thermoelectric type the chamber **1** may optionally be cooled without the use of an intermediate cooling fluid, the inside of the walls of the chamber either being in thermal contact with the cold junctions of the refrigerator **5** or the air inside the chamber **1** being circulated past the cold junctions. In an alternative embodiment the hot junction cooling fluid and the chamber **1** are cooled by separate "main" refrigerator **5**, which need not be of the same type. The circulation of the hot junction cooling fluid is produced by a pump **7**, or it may be otherwise accomplished, e.g. by gravity where the cooling fluid is a volatile one, e.g. having a boiling point in the range of −30° C. to +30° C. cooling the hot junctions of the thermoelectric cooling units **9**, **12** and **15** by evaporation and condensation in the heat-exchanger **6**. The heat-exchanger **6** may be situated inside, outside, or in the walls of the refrigerated chamber **1** although in the preferred embodiment it is not inside. The pump **7** is preferably situated outside the chamber. The power supply to the pump is not shown. The pump **7**, and the compressor (not shown) if any, operating the refrigerating system **5**, are preferably mounted in such a way as to prevent vibrations from reaching the microtome. The hot junction cooling liquid is preferably a liquid of low melting point e.g. an organic solvent containing an oxygen atom such as a lower alcohol and may be e.g. ethyl alcohol or antifreeze such as is used in the cooling systems of motor vehicles. It passes through the heat transfer units **8**, **11** and **14**, which are in thermal contact with one set of junctions (hot junctions) of the thermoelectric cooling units **8**, **12** and **15** respectively; the other set of junctions (cold junctions) being respectively in contact with the knife **10**, and specimen **13** and the walls, base or lid of the cold compartment **16**. The cold compartment **16** and its attachments are not essential parts of the apparatus. In an alternative arrangement there is provided a cold compartment which is cooled directly with the hot junction cooling fluid.

The temperature of the chamber **1** may be controlled at any temperature desired. Normally it is sufficient to provide a main refrigerator capable of cooling the chamber **1** to −10° C. or even to −6° C. and the hot junction cooling fluid to −12° C. It is desirable that the chamber should be capable of being maintained at any temperature between −2° C. and −5° C. Conveniently, the cooled circulating fluid is passed first to the hot junctions of the thermoelectric heat pumps attached to the knife and then to perform its other functions. Further it is usually possible to allow the temperature of the chamber to vary ±2° or ±2.5° C. from the desired temperature. However it is possible to achieve closer temperature control of the knife and the specimen holder may reduce the need for finer temperature control of the air. In certain circumstances it may be necessary to prevent the air temperature from rising above 0° C. i.e. with a variation of ±2.5° C. it is desirable to set the air temperature at −3° C. or below.

The hot junction cooling liquid may be maintained at any suitable temperature during the operation of the microtome apparatus but in a preferred embodiment it is between −10° C. and −20° C. In one embodiment the main refrigerator **5** is kept in constant operation and the thermostat unit **20** actuates a relay governing a stopcock in the circuit of the refrigerant as it passes through the pipe system **17** rather than (as shown) controlling the supply of power to the main refrigerator **5**. In one alternative, two separate main refrigerators are used for the chamber **1** and the hot junction cooling fluid.

The temperature of the knife **10** and of the cold junctions of the cooling unit **12** in contact with the specimen **13** may be directly measured and indicated but if the air temperature within the chamber and the temperature of the auxiliary cooling fluid shown circulating through the pipe system **22** are kept constant to within ±2.5° C., this is, in general, unnecessary as the temperatures may be indicated indirectly by measuring the value of the current supplied to the respective thermoelectric cooling unit. In an alternative arrangement, the temperature of an "anti-roll bar" on the microtome is thermoelectrically controlled, but this is not essential, since both the knife and the air temperatures are controlled.

Referring to FIGURES 2 and 3, there are shown two forms of microtome apparatus having a number of features in common and in the description of these arrangements, the same reference numeral is used for similar parts. Each arrangement has a microtome knife **10**,

thermoelectric cooling units **9** arranged at points along the length of the knife and heat transfer units **11** in contact with the cooling units **9**. Adjacent to the knife there is (as shown in FIGURE 3) a thermoelectric unit **12** forming a specimen plate which is in contact with one surface of a heat transfer unit **11**. The heat transfer unit is attached to an arm **50**, which pivots about points **52**, so that the specimen plate may be moved in relation to the knife **10**. The heat transfer unit is also pivoted on the arm **50** in such a way that it may be rotated about the pivot point **52** irrespective of the position of the arm, and be brought away from the knife in order to enable an operator to have access to the specimen without danger from the knife **10**. The knife is supported within blocks **33**, in such a way that its angle with respect to the specimen plate may easily be adjusted. In the case of FIGURE 2, an inner block **30** is provided, the angle of which may be finally adjusted by means not shown within the block **33** in order to vary the angle of the knife **10**. The inner block **30** is secured in position by means of screws **34**. In the arrangement of FIGURES 3, the knife **10** is supported and held in position by means of screws **34** acting upon intermediate insulating plates **30***a*. In each arrangement the thermoelectric cooling units **9** are supplied with current through leads **36** and the heat transfer units **8** are supplied with hot junction cooling liquid through the pipes **35**. The block **30** in FIGURE 2 is shown with a slot **32** which enables the block **30** to be flexible to some extent so that when the screw **34** passing through a slot in the outer block **33** is screwed through a threaded hole in the inner block **30** against the blade **10** it is possible for the inner block **30** to expand against the block **33**. This mounting of the knife **10**, which is thermally insulated by means of insulating blocks **31** as shown in FIGURE 2, allows the knife to be easily removed for example for honing, without disturbing its cooling systems. The face of the heat transfer unit **11**, shown in FIGURE 3, to which the thermoelectric cooling unit **12** is attached, is anodised in order to provide an electrically insulating layer.

Referring to FIGURE 4, there is shown a cross section through a heat exchanger unit in which refrigerant is circulated via pipes **17***a* through an evaporator **40** and circulating liquid passing through the pipe **22***a* is cooled. Such a unit is suitable for use as a heat transfer unit **11**.

Referring to FIGURE 5, there is shown a view of a further heat transfer unit **3***a*, in which a refrigerant is circulated through a pipe **17***a* which is arranged in a zigzag fashion and a circulating liquid to be cooled is passed through a pipe **22***a* which is similarly arranged. Both of these pipes are immersed in a freezing mixture **60**, and the unit may conveniently be arranged in the wall **3** of the chamber **1**, referred to in FIGURE 1, as shown at **6**.

The usefulness of apparatus in accordance with the present invention is confirmed by the following results obtained using a Cambridge rocking microtome whose knife holder had been modified as shown in FIGURE 2 and whose specimen holder had also been modified to include a thermoelectric module for cooling the specimen. The knife was cooled by two F32 Frigistor (Registered Trademark) thermoelectric cooling modules and the block by one such cooling module. The chamber temperature was controlled between 0° and −5°, cooling being provided by conventional refrigeration. Cold alcohol (0° to −5°) was circulated continuously to the heat sinks of the thermoelectric cooling modules. A Teflon-coated glass anti-roll plate of the type described by W. T. West in Stain Technology 1963 vol. 37 p. 5 was used. With a current of 12 amps., and a chamber temperature of −5°, the minimum temperature of the midpoint of the knife is −55°. With a current of 6 amps. the tissue holder minimum is −38°. C. Heat conduction through frozen tissue is sufficiently good to ensure that the tissue itself is maintained at the temperature of the surface of the thermoelectric cooling module. Temperatures can be controlled to within ±1°.

The following table gives the optimum temperatures for the knife block (stage) and for the environment (the air inside the chamber).

	Knife temperature (°C.)	Block temperature (°C.)	Environment temperature (°C.)
Unfixed tissue:			
Liver	−18	−10	−5
Kidney	−15	−8	−5
Skin	−35	−10	−5
Keloid	−35	−12	−5
Patella	−40	−18	−5
Brain	−18	−15	−5
Thyroid	−20	−10	−5
Fixed tissue:			
Liver	−15	−8	−5
Kidney	−10	−8	−5
Skin	−35	−5	−5
Keloid	−35	−5	−5
Patella	−40	−20	−5
Brain	−15	−8	−5
Thyroid	−25	−12	−5

The most significant observation, which can only partly be expressed in tabular form, is that with the correct choice of temperature there is no mammalian or invertebrate soft tissue which cannot be cut serially at 4 microns (or less). Provided that hard steel knives (Jung AG) are employed, human skin, normally a problem tissue with the conventional cryostat, can be cut without difficulty. Invertebrate tissues (e.g. Helix, Limnaea, Patella, Limax) which provide insuperable problems if thin sections are required by conventional cryotomy, can be handled with similar ease.

The provision of optimal block and knife temperature reduces the damage attributable to compression and fracture (block too cold), or to an extended micro-melting zone (block too warm), to the absolute minimum. This is reflected in superior cytological detail in the stained preparations obtained from a wide variety of tissues. This is important at the light microscope level but even more so for the electron microscope, employing the section (cryostat) technique first described by Tranzer in Journale de Microscopie, 1965, vol. 4, pp. 419–336 and 409 et. seq.

Finally, as can also be seen from the table, it is now also possible to provide the correct set of temperatures for cutting tissues which have been fixed in formalin (cold or otherwise), glutaraldehyde, ethanol or, presumably any other fixative. There is thus provided a means of cutting thin serial frozen sections of fixed tissues which includes all the advantages of convenient handling afforded by the cryostat in contrast to conventional bench freezing microtome techniques. It is probable that, for all types of research requiring fixed frozen sections, bench freezing microtomes will become obsolete, even if they continue to be used in routine laboratory practice. The lowest temperatures ever likely to be needed are −50° C. for the cutting edge of the knife and −35° C. for the stage (tissue block holder) and these can readily be obtained.

Normally the cooling liquid for the hot junctions of the thermoelectric cooling modules and the cooling liquid for the cooling of the air in the chamber are both cooled by the evaporator of a conventional compressor. Preferably this is capable of maintaining itself at a temperature of about −12° C. with room temperatures of up to 35° C. (95° F.) so as to cool the hot junction cooling liquid at about the same temperature.

The evaporator is preferably of the type which is capable of being run dry as this facilitates the maintenance of constant temperatures in the chamber, but when using some of the other devices hereinafter mentioned (e.g. the cold reservoir), this requirement may prove unnecessary.

The amount of vibration from the compressor when running regularly is sufficiently little for it to be simple to prevent it from disturbing the cutting. However, the

starting up of the compressor produces more violent vibrations. To avoid this it is preferred to immerse both the compressor evaporator and coils of the cooling liquid (or liquids) which circulates through the chamber and the hot junction, in a "jelly" e.g. an ice-salt mixture (as described with reference to FIGURE 5) having a melting point of about −12° C. or other cold reservoir which will tend to even out temperature variations and thus permit more rapid recovery of optimum temperatures after this temperature disturbance e.g. those resulting from opening the chamber and allowing more even running of the compressor.

Alternatively the coolant may be fed through the centre of the evaporator. A further alternative is to intertwine coolant coils and evaporator coils.

A still further alternative is the use of twin-channels in a roll-bonded aluminium construction such as Alcanduct one channel for refrigerant forming part of the evaporator, and the other channel carrying the cooling liquid.

The cooling liquid (for cooling the chamber and the hot junction of the thermoelectric modules) may be lost during operation or storage of the device, and also it is often more convenient to transport the device without the cooling liquid in the pipes. Addition of cooling liquid initially or to replace losses is facilitated by the use of a self-priming pump (indicated by the numeral 7 in FIGURE 1) and an enclosed header tank.

The presence of thermoelectric cooling modules on the knife makes the knife cumbersome and difficult to remove and hence it is desirable to move the specimen support away from the vicinity of the knife to permit safe removal of the specimen. This accomplished by use of a pivot in the arm supporting the specimen block, as described with reference to FIGURE 3.

The microtome is preferably made removable from the chamber to facilitate cleaning, e.g. it may be fixed to the base of the chamber by a snap-action catch requiring a 5 lb. pull for release.

Since the cryostat optimum temperature is about −5° C., as compared with temperatures of −30° C. previously used, misting up of the observation window can be more simply avoided, e.g. by the use of a triple-glazed window, e.g. one composed of three parallel sheets of glass separated by two evacuated spaces.

Furthermore the temperature of −5° C. is not liable to cause frostbite so that it is possible to use bare hands for handling tweezers etc. to mount and demount the specimen. Hands and fresh specimens may be inserted through gaps in the wall of the chamber covered with elastomeric (rubbery) material which is self-closing and closes to form a seal round the wrists of the operator virtually eliminating the ingress of damp room air. Such self-closing gaps are well-known in incubators for babies, but modified material may be needed to meet the lower temperatures. Alternatively other known methods (air locks) may be used for inserting specimens without any appreciable ingress of air, and mounting and demounting can be accomplished with the aid of rubber gloves to fill gaps in the chamber wall. In either case where gaps in the wall are used during the inserting and/or mounting or demounting of specimens these gaps may be covered with thermally insulating covers during the cutting operations.

The chamber may be large enough to contain the whole microtome apparatus or it may simply consist of a comparatively small space surrounding only the knife and the stage to be cooled. Such a space may easily be cooled thermoelectrically or by a conventional absorption refrigerator without the need for excessively large power supplies. Thus not only may the vibration problem be reduced but the weight and bulk of the whole apparatus may be reduced thus enabling it to be handled comparatively easily.

In the case where the whole microtome apparatus is contained within the chamber, the base of the apparatus may be removed and the remainder of the apparatus may be mounted directly on to the wall of the chamber in order to reduce the weight of the overall assembly.

Where the arrangement has a comparatively small chamber enclosing only the knife and the stage to be cooled the chamber walls may be an envelope of flexible plastics material with thermal insulation properties and have areas including transparent material where necessary. Such an envelope may be inflated in order to maintain it in position. Alternatively a small chamber, isolating simply the space where the cutting takes place, may be formed by means of an electric curtain and the base and the knife and stage supports may be made of thermally insulating material so that the operating parts of the microtome are thermally insulated from any parts remote from the cutting area and so that the air immediately surrounding the cutting area is screened from any more remote air. Thus only the volume of air surrounding the cutting area need be cooled to about −5° C.

We claim:

1. In microtome apparatus for cutting, from a specimen, slices of material suitable for examination by microscope which apparatus includes a knife, a specimen holder stage and a closable chamber, a liquid cooling circuit, primary cooling means for maintaining the temperature of the air surrounding the chamber included in said liquid cooling circuit, a first thermoelectric cooling unit for controlling the temperature of the knife, a second thermoelectric cooling unit for controlling the temperature of the specimen holder stage, means to enable the operative part of the apparatus to be operated within said chamber with the chamber closed and means to control separately and independently the supply of electric power to each of the thermoelectric cooling units, said liquid cooling circuit being arranged to control the temperature within said chamber and the temperature of the hot junctions of said first and second thermoelectric cooling units.

2. Apparatus as claimed in claim 1 in which the primary cooling means includes a refrigerant which evaporates in an evaporator.

3. Apparatus as claimed in claim 2 in which circulating liquid which circulates through the primary cooling means is passed through the evaporator.

4. Apparatus according to claim 1 wherein the liquid cooling circuit includes heat exchanger means, said heat exchanger means being adapted to cool the liquid circulating through said liquid cooling circuit.

5. Apparatus according to claim 2 in which said liquid cooling circuit includes a heat exchanger, said heat exchanger being adapted to cool said refrigerant.

6. Apparatus according to claim 4 wherein said heat exchanger includes a freezable mixture, said freezable mixture being present in a quantity sufficient to stabilise the temperature of the circulating liquid.

7. Apparatus according to claim 1 which includes a supporting arm for supporting the specimen holder, said supporting arm being pivoted whereby the specimen can be removed readily from the immediate vicinity of the knife.

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U.C. Cl. X.R.

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