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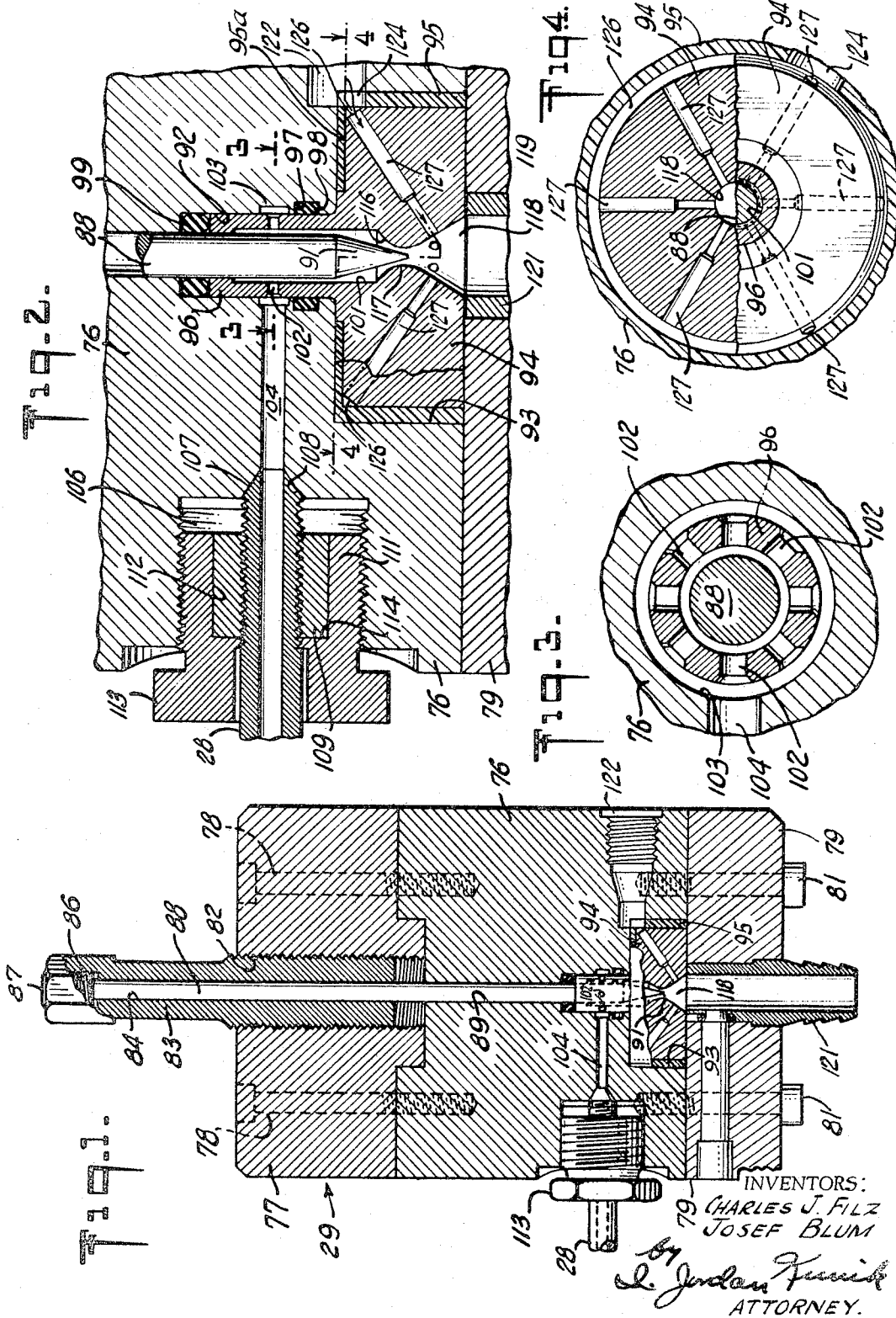
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CELL FRACTIONATOR APPARATUS

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2 Sheets-Sheet 1



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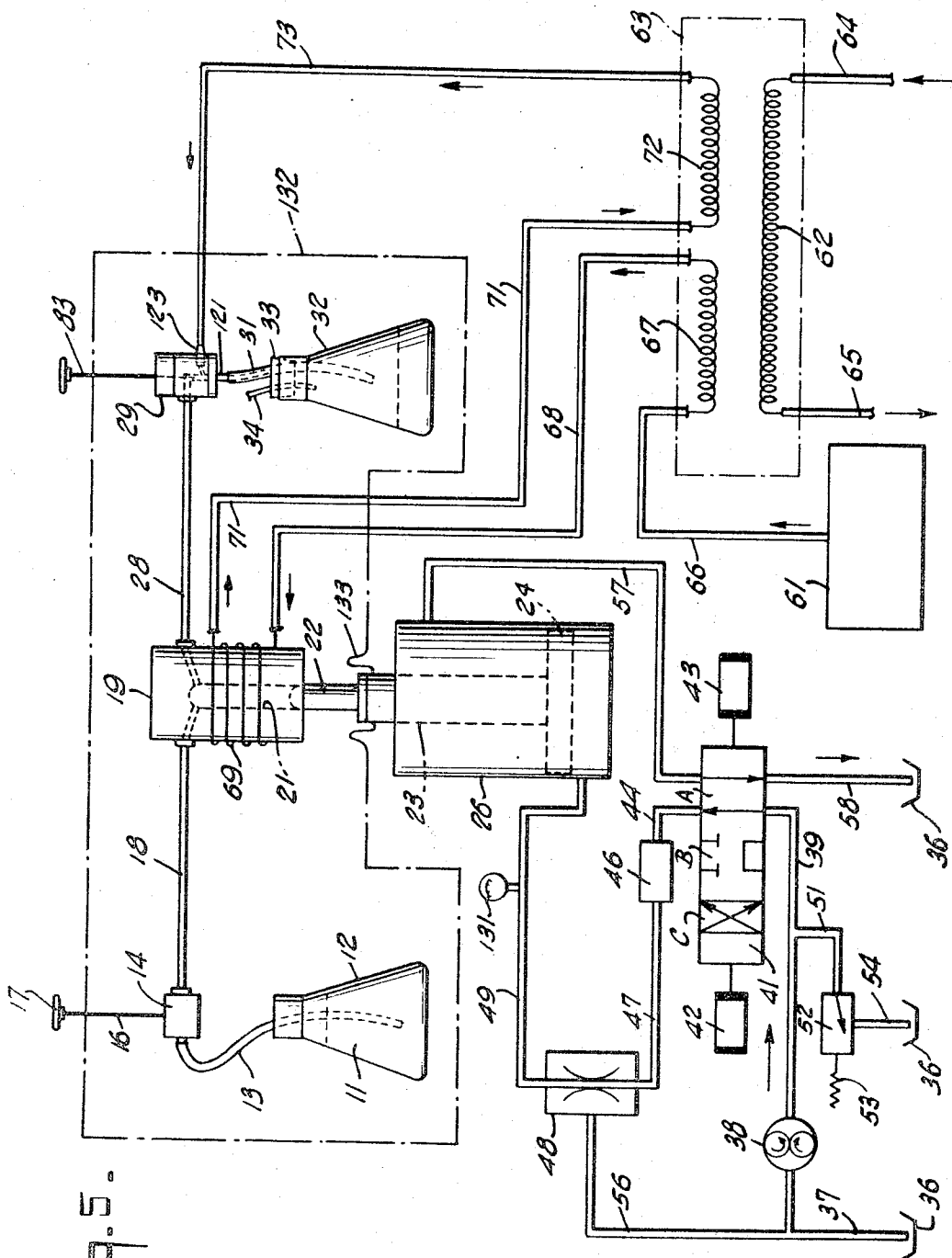


Fig. 5

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CELL FRACTIONATOR APPARATUS

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This invention relates to an improved apparatus for rupturing cells in order to study the structure and function of their constituent parts.

For a long time it has been realized that research in the area encompassing structure and function of microorganisms would be greatly facilitated if it were possible to rupture the cells and isolate the structural elements such as cell walls and protoplasm without significant alteration of these components. Recently, a great deal of attention has been directed toward development of tools and techniques for this purpose, and several instruments have been produced. Shaking and grinding devices, including the ultrasonic oscillator, have been used to disrupt microorganisms but when separation of the disruption products was attempted, a portion of the cell walls was fragmented to such an extent that it was impossible completely to remove them from the protoplasm by differential centrifugation. Thus, the yields of pure cell wall material were small in proportion to the amount of starting material, and protoplasm substantially free of cell wall material could not be produced. Neither cell walls nor protoplasm could be isolated in appreciable amounts from *Mycobacteria* (tubercle bacilli) by the use of these devices due to the tendency of the broken and unbroken cells and the protoplasm to aggregate to a butter-like consistency.

The French pressure cell, in which a pressurized microbial suspension is released at an orifice, seemed to be the most logical approach to the problem, since pressures could be selected and regulated according to the rigidity of the cell walls of a given microorganism. The cells would crack as they emerged through the orifice and would not be subjected to further disintegration. When this method was used, recovery of cell wall material was quite satisfactory, but temperatures at the orifice increased to a point where partial denaturation of certain protoplasmic constituents occurred, and this material adhered to the inner surface of the cell wall and could not be removed. Conventional cooling methods were not effective.

It was then proposed that the orifice and needle valve of the pressure cell be exposed to a stream of pre-cooled gas that was released from a separate needle valve. While this made it possible to produce somewhat better results in the form of purified cell walls and protoplasm, it was not until the introduction of the apparatus and method of the invention described and claimed in the copending U.S. patent application Ser. No. 171,674, filed Feb. 7, 1962, now U.S. Patent 3,165,266, issued Jan. 12, 1965, that suitably controlled results in quantity could be obtained.

The present invention comprises an improvement over the invention described in said copending application in providing more efficient means for cooling the valve seat and expansion or decompression chamber so that the yield of biological specimens suitable for analysis and experimentation is considerably increased.

The improved cooling means comprises the inclusion of a plurality of radially disposed channels surrounding the valve seat and the expansion chamber. The cooling medium is transmitted through said channels and expelled into the expansion chamber in close proximity to the valve seat. By arranging for the channels to be radially arrayed around the valve seat and around the expansion chamber, the block in which said valve seat and

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chamber are formed is also cooled, whereby heat generated at the valve seat is effectively counteracted. More efficient cooling of the expansion chamber is realized by introducing the cooling medium directly into the critical space of the chamber rather than by the previous means of indirect circulation.

Still other objects and advantages of the invention will be apparent from the specification.

The features of novelty which are believed to be characteristic of the invention are set forth herein and will best be understood, both as to their fundamental principles and as to the particular embodiments, by reference to the specification and accompanying drawing, in which:

FIG. 1 is a vertical central section view of the improved fractionating valve system and the outlet orifice cooling arrangement therefor, some parts being shown in partial elevation, and some parts being broken away;

FIG. 2 is a greatly enlarged section view of a portion of the apparatus shown in FIG. 1;

FIG. 3 is a further enlarged view of a portion of the apparatus, taken on line 3—3 of FIG. 2;

FIG. 4 is a view taken on line 4—4 of FIG. 2, partly in section and partly in elevation; and

FIG. 5 is a schematic representation, in reduced size, but not in relative scale, of the complete cell fractionating system of which the valve apparatus shown in FIG. 1 forms an integral part.

Referring to the drawings, the cell fractionating process is shown schematically in FIG. 5, where a specimen 11 constituting such materials as bacteria, fungi, spores, viruses, blood cells, and the like, in liquid suspension, is contained in a supply bottle 12. The specimen is cultured in the normal manner in nutrients which act as the liquid vehicle. Inserted into supply bottle 12 is one end of a flexible tube 13, made of rubber, neoprene, or the like, the other end of which is connected to the inlet port of high pressure one-way valve 14 which is adjusted by means of control rod 16, the outer end of which is provided with a manually or mechanically operable knob 17.

Connected to the outlet port of valve 14 is one end of transfer tube 18, the other end of which is connected to the inlet port of pressure cell 19. Pressure cell 19 has a cylinder 21 which slidably accommodates a plunger 22 connected to ram 23 mounted on piston 24 slidably positioned within hydraulic cylinder 26. Upon the outward motion of plunger 22 from pressure cell 19, suction is created whereby a specimen is drawn from a specimen supply bottle 12 through valve 14 and into the cavity of cylinder 21 of said cell. Upon the inward movement of plunger 22 into cylinder 21 pressure is applied to the specimen in the cavity of the pressure cell, causing the specimen to pass through transfer tube 23, one end of which communicates with cylinder 21, the other end of which communicates with the interior of the expansion valve assembly, generally designated 29, the detailed structure of which is shown in FIGS. 1, 2 and 3. Upon being processed through the expansion valve 29, the fractionated cells in the suspension medium pass through tube 31 into a receiver bottle 32. Thereafter the materials in the receiver bottle may be transferred to a centrifuge where the components are physically separated for such further study as may be required.

Tube 31 extends through a rubber stopper 33, or the like, in the mouth of the receiver bottle 32, said stopper also having a tube 34 extending therethrough to vent the cooling gas that had mixed with the effluent.

The control system for the hydraulic cylinder 26 comprises a sump or reservoir 36 of hydraulic liquid. A suction pipe line 37, extending into sump 36, is connected to a suitable pump 38 which causes the hydraulic fluid to pass by way of pipe line 39 through a three position valve 41, which is operated by a pair of solenoids 42 and 43.

The solenoids are actuated by suitable electric circuitry which is well known in the art, and which does not constitute any part of the present invention. Valve 41 is shown with the right hand section A permitting free flow of hydraulic fluid to and from hydraulic cylinder 26. The central section B of valve 41 is the no-go position, while the left hand section C has a cross-over arrangement for reversing the flow of the hydraulic fluid.

In the position of the valve 41 shown in FIG. 5, hydraulic fluid then passes under pressure by way of pipe line 44 through filter 46 and by way of pipe line 47 into a flow control valve 48 which determines the rate flow of the hydraulic fluid through pipe line 49 into hydraulic cylinder 26.

It will be noted that pipe line 39 is provided with relief pipe line 51 communicating with relief valve 52, the pressure control action of which is represented schematically by spring 53, whereby excessive pressure can be relieved by permitting flow of hydraulic liquid through outlet pipe 54 into the sump 36. Flow control valve 48 is also provided with an overflow pipe line 56 which also leads back to the sump 36.

The introduction of hydraulic fluid under pressure through pipe line 49 into cylinder 26 is operative by way of piston 24 and ram 23 to drive plunger 22 into pressure cell cylinder 21. This takes place when valve 41 is in the position shown in FIG. 5. Overflow of hydraulic fluid from cylinder 26 during the pressure stroke returns through pipe lines 57 and 58 to sump 36. A retracting or suction stroke of plunger 22 is produced by moving valve 41 to the right into the C position, whereby the flow of hydraulic fluid is reversed and pipe line 57 becomes the pressure line, pipe line 49 becomes the return line, and pipe line 56 is the overflow line into sump 36. In the B position of valve 41, the action of the hydraulic cylinder 26 is at a standstill, and if pump 33 continues to operate, the hydraulic fluid simply returns to sump 36 by way of pipe line 58.

On the suction stroke of plunger 22, a specimen is drawn from supply bottle into cylinder 21 of the pressure cell. On the pressure stroke of plunger 22, the specimen is transmitted through pipe 28 to the expansion valve 29, the return of the specimen to supply bottle 12 being prevented by one-way valve 14.

A double-purpose cooling system is provided in order to maintain pressure cell 21 and expansion valve 29 at the requisite low temperatures, since considerable heat is generated by the operation of both of these pieces of apparatus. The cooling medium consisting of dry nitrogen air, or other neutral medium, is stored in supply tank 61 which is provided with suitable pressure maintaining apparatus, not shown. The refrigerating circuit, shown only in part in FIG. 5, consists of a cooling coil 62 mounted in a suitable heat exchanger 63. Coil 62 is supplied with a continuous flow cooling medium, such as Freon, or the like, from a refrigerating source, not shown, through pipes 64 and 65.

The nitrogen is pumped through pipe 66 into cooling coil 67 in heat exchanger 63 after which the cooled gas passes through pipe 68 to a coil 69 that extends around and cools pressure cell 19. Since the nitrogen absorbs heat in coil 69, it is returned through pipe 71 to be cooled again in coil 72 in heat exchanger 63. The re-cooled gas then passes through pipe 73 into the expansion valve assembly 29 where it maintains the requisite low temperature at the exit orifice of the ruptured cells. Thence the gas and the cells pass through tube 31 into the receiver bottle 32, said gas then escaping into the atmosphere through vent tube 34.

As shown in FIGS. 1-3, the expansion valve assembly comprises a body 76, to the top of which is connected a nut 77 by means of a plurality of bolts 78, and to the bottom of which is connected a bottom plate 79, by means of a plurality of bolts 81. Body 76, nut 77, and bottom plate 79 are made of steel or the like, and may be circular

in cross-section. In one embodiment their diameter is in the order of about 2½".

Nut 77 has a vertical central threaded aperture 82 which accommodates a threaded valve stem screw 83, the upper portion of which extends above the top of said nut. Valve stem screw 83 has a central, longitudinal bore 84, the upper end of which terminates in a threaded coaxial aperture 86 of somewhat greater diameter, which accommodates set screw 87.

Bore 84 in valve stem screw 83 movably accommodates an elongated valve stem 88, which also extends into bore 89 in body 76, bore 89 being coaxially aligned with bore 84. The lower end of valve stem 88 terminates in a conical valve head 91, whose function will be described hereinafter. The upper end of valve stem 88 abuts the lower end of set screw 87 which can be adjusted to compensate for wear on valve head 91, and to maintain valve stem 88 in proper position in respect of stem screw 83. The relative dimensions of valve stem 88 and of bore 84 of stem screw 83 provide a barely running fit with extremely close clearance therebetween.

The lower end of bore 89 in body 76 terminates in a co-axial circular bore 92 of somewhat greater diameter. The lower end of bore 92 communicates with a still larger co-axial, circular recess 93 which accommodates expansion valve seat block 94 which is maintained captive therein by bottom plate 79. Interposed between the perimeter of block 94 and the vertical walls of recess 93 is a circular sleeve 95, made of Teflon or other suitable insulating material, on top of which is formed an inwardly extending integral collar 95a which mates with a corresponding recessed annular shoulder on top of said block.

Block 94 has an integral upwardly extending central tubular boss 96 that fits closely into bore 92. Bore 92 has an annular recess 97 which accommodates resilient O-ring 98 made of a suitable material such as rubber, neoprene, Teflon or the like, which serves as a seal between boss 96 and body 76. Positioned at the top of boss 96 is O-ring 99, also made of similar resilient material, which serves to seal valve stem 88 with adjacent parts. The sealing action of O-rings 98 and 99 is enhanced by the pressure conditions obtaining in the system when the apparatus is in operation as will be described hereinafter.

The lower end of valve stem 88 extends co-axially into the interior of valve chamber 101 in tubular boss 96 and block 94, there being a slight clearance between the surface of said stem and the interior wall of said chamber sufficient to permit liquid specimens to pass freely between said elements to fill valve chamber 101. Intermediate its ends, boss 96 has a plurality of radially and equidistantly arrayed ports 102 (FIGS. 2 and 3), the outer countersunk ends of which communicate with an annular recess 103 in body 76. Recess 103 serves to distribute specimen materials substantially equally through ports 102 into valve chamber 101. Distributor recess 103 communicates with a horizontal specimen transmitting channel 104 which extends to threaded inlet recess 106 in body 76.

Since the specimen is delivered from cell 19 (FIG. 5) to the expansion valve assembly 29 under high pressure, pipe line 28 is made of a substantially rigid material, which, in one embodiment, comprises a ¼ inch diameter pipe having a 16th inch inside diameter. Pipe 28 has a ground tapered end 107 which mates with a ground tapered seal 108 at the inner end of recess 106 in body 76.

In order to ensure an intimate leak-proof fit between pipe end 107 and seat 108, the end portion of pipe 28 is threaded to accommodate a threaded ferrule 109. The annular wall of inlet recess 106 is threaded to accommodate threaded gland 111 which has a circular aperture 112 into which ferrule 109 fits. Gland 111 has an outwardly extending integral rim 113 to facilitate rotation thereof whereby the interior wall 114 of aperture 112 is

forced against the end of ferrule 109 to cause tapered end 107 of pipe 28 to be urged into firm and intimate contact with seat 108. By this means a high pressure leak-proof connection is established between the interior of pipe 28 and channel 104 in body 76.

Similar high pressure, leak-proof connections are provided between pressure cell 19 and pipes 18 and 28, respectively, and between pipe 18 and high pressure unidirectional valve 14. These high pressure connections have a sufficient safety margin in strength to withstand the high pressure forces that are generated within pressure cell 19.

The lower end of transfer chamber 101 in block 94 has a conical or concave conoidal constriction 116 which terminates in a small circular neck 117 which, in turn, communicates coaxially with, and forms the smaller end of, a conical open-mouthed expansion chamber 118. When valve stem screw 83 is turned to cause the lower portion of conical valve head 91 to extend through neck 117, whereby an annular portion of valve head 91 bears firmly against an annular portion of constriction 116 which serves as a valve seat for said valve head, a leak-tight seal is formed which withstands the high pressures generated by pressure cell 19, thereby preventing the escape of the specimen from transfer chamber 101 into expansion chamber 118.

The widened exit end of expansion chamber 118 communicates with the upper end of outlet nipple 121 extending through bottom plate 79. Connected to the lower end of nipple 121 is one end of tube 31 the other end of which communicates with the interior of collection bottle 32.

Extending partially into body 76 is a horizontal aperture 122 (FIGS. 1 and 2) which threadably accommodates an inlet nipple 123 (FIG. 5) to which pipe 73 is connected. The inner end of aperture 122 communicates with an aperture 124 extending through an upper edge portion of sleeve 95. The upper peripheral corner of block 94 is beveled to form an annular transfer channel 126 with which aperture 124 communicates.

Extending obliquely and radially through block 94 is a plurality of cooling channels 127, the upper ends of which communicate with annular transfer channel 126, the lower ends of said channels 127 communicating in a circular array with expansion chamber 118 a short distance below neck 117 of valve seat 116. See FIGS. 2 and 4.

In operation, the specimen materials in supply bottle 12 are drawn into cylinder 21 of pressure cell 19 by the retraction of plunger 22, with valve 41 in the position where the C-portion thereof is arrayed between pipe lines 39-44 and 57-58. Thereafter, valve 41 is moved into the A position, as shown in FIG. 5, whereby the hydraulic system is operative to cause plunger 22 to produce its pressure stroke in cylinder 21, thereby bringing the materials in said cylinder under high compression. One-way valve 14 prevents the specimen materials from returning into supply bottle 12 while the forward end of the system including valve chamber 101 is closed by valve head 91 bearing firmly against valve seat 116.

When the requisite pressure has been achieved upon the specimen in the closed system, this is indicated by a suitable pressure gauge 131, or the like, mounted in pipe line 49. Gauge 131 is calibrated to read the pressures in the closed specimen system by simple computation that establishes the relationship between the pressures imposed upon the large piston 24 in hydraulic cylinder 26, and the pressure translated thereby through smaller plunger 22 in pressure cell 19.

Thereafter valve stem 88 is retracted very slightly by rotation of screw 83 to barely back off valve head 91 from constriction 116 by a distance, in one embodiment, in the order of about fifty-millionths of an inch, to form an annular orifice between valve head 91 and constriction 116 through which the specimen escapes from chamber 75

101. Upon passing through said orifice and through neck 117, the specimen is suddenly subjected to explosive decompression in expansion chamber 118 and immediate cooling by the cooling medium. Assisted by the flow of the cooling gas, the exploded cells then move by gravity into and through outlet nipple 121, and thence through tube 31 into receiving bottle 32.

Since tremendous energy is released in pressure cell 19 due to the compression of the materials in cylinder 21, which, in one embodiment, reaches as much as 57,000 pounds per square inch, the cooling medium from the refrigerating system is circulated around cell 19 through coil 69 to absorb the heat generated and to chill the cell, and to keep cylinder 21 cool.

It is vitally important to keep the specimen cool as it undergoes decompression; therefore, the cooling medium is conducted back into the refrigerating system through pipe 71 into heat exchange 63 for re-cooling, and thence it is passed by means of pipe 73 through inlet nipple 123, aperture 122, aperture 124, annular transfer channel 126 and down through cooling channels 127 whence it circulates into and through expansion chamber 118. In this manner the neck 117 of valve seat 116 as well as the space in the expansion or decompression chamber 118 are maintained at a low temperature, which, in some operations, does not exceed approximately 5° C., or lower.

The foregoing means for introducing and circulating the cooling medium close to the juncture between the tip of valve head 91 and neck 117 of valve seat 116 where the critical energy conversion takes place, considerably increases the yield of useful biological specimens.

Not only is the critical temperature controlled at or very close to the point of decompression and in the area of expansion, but the arrangement for extending cooling channels 127 obliquely and radially through block 94 also cools the latter whereby heat normally generated by the fractionating process is absorbed and conducted away from the valve seat which is an integral part of block 94.

Sleeve 95 and collar 95a made of a suitable insulating material, serve as an insulator to reduce or prevent heat transfer between block 94 and body 76. Said sleeve and collar also facilitate the assembly and disassembly of block 94 relative to body 76, when cleaning and replacement are necessary or desired.

By providing for a continuously circulating stream of the cooling medium within the critical valve area, and within the decompression chamber, said medium mixing intimately with the fractionated cells as they emerge from the valve chamber 101, it is possible, by means of the apparatus and system disclosed herein, to preserve the fractionated cell particles and constituents in a proper condition for further study, examination, analysis and experimentation.

The intimately mixed fractionated cells and cooling medium pass downwardly through outlet nipple 124 and through pipe 31 into receiving bottle 32, where the effluents in liquid suspension are collected.

By providing for the conical valve head 91 to protrude through constriction 116 and through neck 117 where the materials under high pressure escape through an annular orifice, directly into a coaxial expansion chamber, not only is the controlled retraction of valve head 91 facilitated, but also it is now possible to ensure the controlled cooling of the critical area in which the specimens under high pressure undergo explosive decompression or expansion. This was hitherto incapable of being achieved where the specimen under pressure flowed in a direction opposite to the valve head, thereby preventing access of a cooling medium to the valve head and the valve seat, in order to preserve the integrity of the specimen structures. Also, by providing for specimen flow in the same direction as the valve head, into a coaxial expansion chamber, the specimen does not impinge upon any obstruction to its flow which would otherwise denaturize the fractionated material.

The arrangement of making the valve stem structure in two parts, namely, stem 88 and screw 83, constitutes an improvement over a one-part structure. Due to the precision requirements of providing the controlled minute orifice between valve head 91 and constriction 116 with accurate concentricity of the conical end of said valve head in respect of valve seat and of neck 117 for even distribution of the specimen, it has been found that valve stem 88 and its head 91 can more readily be accurately machined as a part separate from valve stem screw 83, whose bore 84 can also be more accurately machined in relation to bore 89 in body 76. The relative positions of valve stem 88 and valve screw 83 to each other are established by set screw 87.

After the requisite pressure of the specimen has been achieved in the system, valve head 91 is very slightly retracted from constriction 116 by rotating valve stem screw 83 a small fraction of a turn. While the specimen is escaping through neck 117, the pressures within the system are sufficient to cause the specimen in valve chamber 101 to bear upon valve head 91 and to urge valve stem 88 upwardly against the lower end of set screw 87, whereby the requisite position of said valve stem 88 relative to the position of valve stem screw 83 is maintained. Leakage of specimen fluids from between the valve chamber 101 and body 76 is prevented by O-rings 98 and 99, the internal pressure of the system in actuality enhancing the sealing action of said O-rings.

Should any wear occur on either or both valve head 91 and constriction 116 due to frictional engagement therebetween, the position of valve stem 88 may be relocated in respect of valve stem screw 83 by adjusting set screw 87. Since the valve stem, valve head, and valve seat are accurately machined, any wear that takes place therebetween is substantially uniform, and, therefore, a re-setting of set screw 83 is sufficient to maintain the apparatus in working order.

The complete system, shown schematically in FIG. 5, may be enclosed in a suitable cabinet, or the like, not shown. In some embodiments it may be desirable to arrange for some of the components to be compartmented into a separate enclosure, indicated by the dotted lines 132, so that the processing of the specimens may take place in an environment which may be irradiated by ultraviolet or perfused with various materials, such as inert gases or bactericidal or germicidal sprays in order to protect the specimens from contamination. Furthermore, enclosure 132 served to protect the operator when certain types of infectious or otherwise dangerous materials are being processed by the apparatus.

Suitable leak-tight connections are provided in compartment 132, whereby the cooling medium may be introduced and withdrawn, the operation of the pressure cell piston facilitated by a flexible air-tight juncture 133 and for various external controls for setting the pressure value of one-way valve 14, and operating valve stem screw 83, and the like. Furthermore, such leak-tight connections serve not only to prevent contamination of the external controls and apparatus, but also serve to prevent the escape of contaminating materials into the atmosphere of the laboratory.

It is claimed:

1. Cell fractionating apparatus comprising a block, a compression chamber in said block, and expansion chamber in said block axially aligned with said compression chamber, a valve seat between said compression chamber and said expansion chamber, a valve member movable longitudinally within said compression chamber and cooperating with said valve seat, the entry portion of said expansion chamber being conical in shape and the apex thereof terminating in said valve seat, means for introducing biological specimens under high pressure into said compression chamber, a plurality of radially arrayed channels in said block, the inner ends of said channels communicating in a circular array with the

conical portion of said expansion chamber near said valve seat and means for passing a cooling medium simultaneously through all of said channels into said expansion chamber.

2. Apparatus according to claim 1 wherein said channels are arrayed obliquely within said block, the inlet ends of said channels being located in the upper portion of said block whereby said cooling medium is passed downwardly and inwardly toward said expansion chamber.

3. Cell fractionating apparatus comprising a block, a compression chamber in said block, an expansion chamber in said block axially aligned with said compression chamber, a constriction between said compression chamber and said expansion chamber forming a valve seat, a valve member movable longitudinally within said compression chamber and cooperating with said valve seat, a plurality of channels in said block radially arrayed around and communicating with the entry portion of said expansion chamber, the outlets of said channels forming a circular array near said valve seat, means for introducing biological specimens under pressure into said compression chamber, and means for transmitting a cooling medium through said channels, said cooling means entering said expansion chamber near said valve seat.

4. Apparatus according to claim 3 wherein said valve member normally cooperates with said valve seat to isolate said compression chamber from said expansion chamber, said valve member being controllably retractable from said valve seat when said specimens are under high pressure in said pressure chamber to release said specimens into said expansion chamber while said cooling medium passes into said entry portion of said expansion chamber from the inner ends of said channels.

5. Apparatus according to claim 3 and further comprising an annular channel in said block, the outer ends of said radial channels communicating with said annular channel, said annular channel transferring said cooling medium from said transmitting means simultaneously to said radial channels.

6. Apparatus according to claim 3 and further comprising a body, said introducing means and said transmitting means being located in said body, a recess in said body accommodating said block and an insulating sheath intermediate said block and said body.

7. Apparatus according to claim 3 and further comprising a body, said introducing means and said transmitting means being located in said body, a recess in said body accommodating said block, an insulating sheath intermediate said block and said body, an annular channel in said block, the outer ends of said radial channels communicating with said annular channel, an aperture in said sheath, said aperture establishing communication between said transmitting means and said annular channel for transferring said cooling medium to said radial channels.

8. Cell fractionating apparatus comprising a block, a compression chamber in said block, an expansion chamber in said block, a valve between said chambers, and a plurality of channels in said block, the outlets of said channels being located in a circular array around and communicating with the entry portion of said expansion chamber near said valve.

9. Cell fractionating apparatus comprising a block, a compression chamber in said block, an expansion chamber in said block axially aligned with said compression chamber, a valve between said chambers, the entry portion of said expansion chamber being conical in shape and the apex thereof terminating in said valve, means for introducing biological specimens into said compression chamber, and means for introducing and circulating cooling medium into said entry portion of said expansion chamber close to said valve.

10. Cell fractionating apparatus comprising a block, a compression chamber in said block, an expansion chamber in said block axially aligned with said compression

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chamber, a valve between said chambers, the entry portion of said expansion chamber being conical in shape and the apex thereof terminating in said valve, means for introducing biological specimens under pressure into said compression chamber, and means for transmitting a cooling medium through said block and into said entry portion of said expansion chamber to cool the latter and to increase the heat absorption of said block due to decompression of said specimens.

11. Cell fractionating apparatus comprising a block, a compression chamber in said block, an expansion chamber in said block, first means for controlling the passage of materials from said compression chamber into said expansion chamber, and second means for cooling said block and the entry portion of said expansion chamber near said first means.

12. Apparatus according to claim 11 wherein said first means comprises a normally closed valve between said compression chamber and said expansion chamber.

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13. Apparatus according to claim 11 wherein said second means comprises a plurality of channels, the outlets of said channels surrounding and communicating with said entry portion of said expansion chamber.

14. Apparatus according to claim 13 and further comprising a source of a movable cooling medium, said source being connected to the inlets of said channels.

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