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Cohen et al.

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[54] **ISOLATION AND FRACTIONATION OF ORGANS OF SMALL ANIMALS**

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[73] Assignee: **The Institute for Cancer Research,** Philadelphia, Pa.

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[52] **U.S. Cl.** **241/29,** 241/258

[51] **Int. Cl.** **B02c 18/10**

[58] **Field of Search** 209/172–173; 195/1.7, 1.8; 233/27, 28; 241/24, 29, 257, 258

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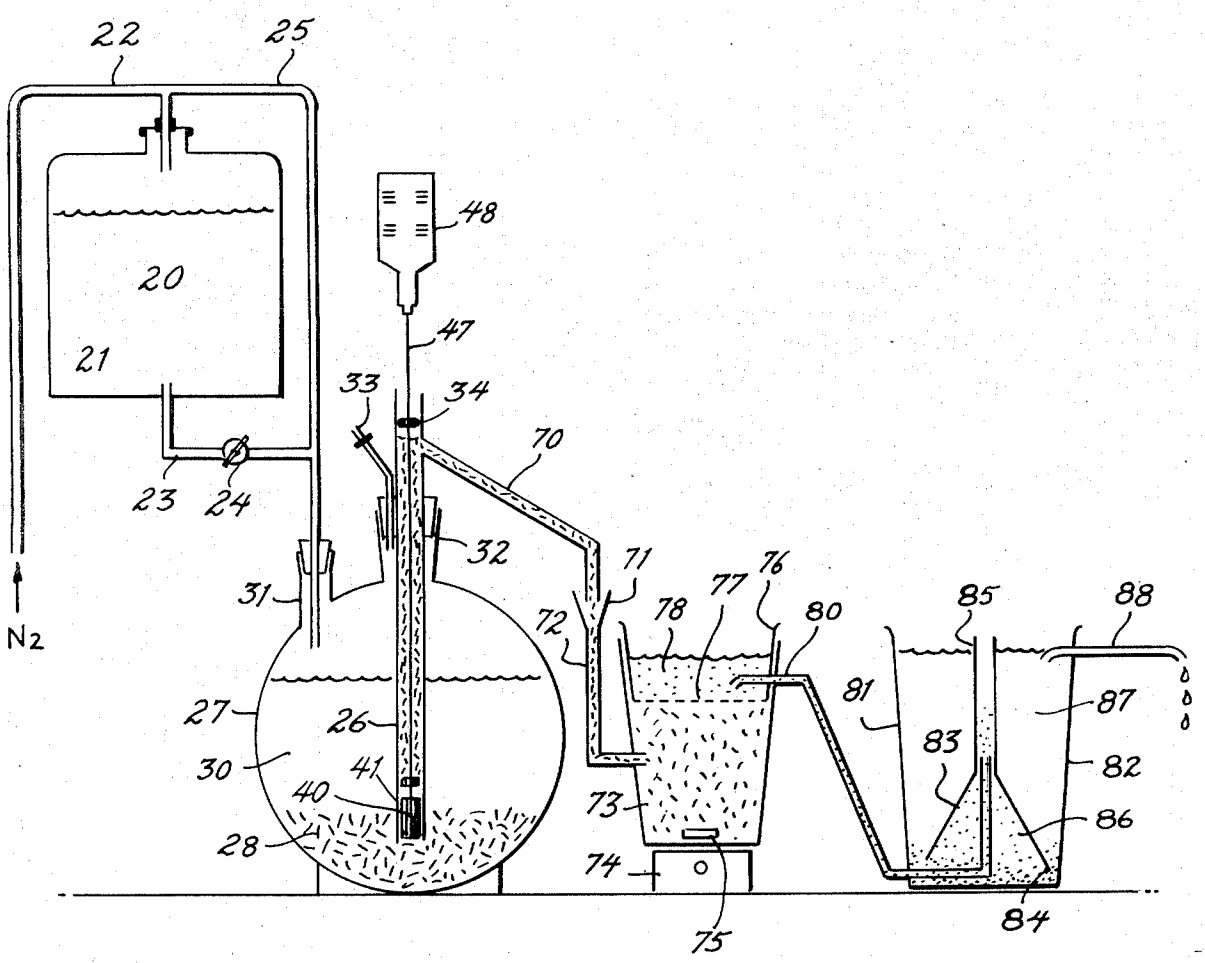
Primary Examiner—Granville Y. Custer, Jr.

Attorney—Joseph Gray Jackson et al.

[57] **ABSTRACT**

Methods and apparatus are described for isolation of diverse organs from small animals and fractionation thereof. The invention involves a continuous mechanical dissecting system, a centrifugal agitator for the separation of fibrillar from globular particles, and a settling chamber for the fractionation of organs at unit gravity with sedimentation velocities above the useful range for centrifugation. The invention is applicable to the isolation of polytene and non-polytene nuclei from larvae of Drosophila melanogaster (fruit fly) and is applicable to other small animals such as adult fruit flies or other insect larvae.

14 Claims, 13 Drawing Figures



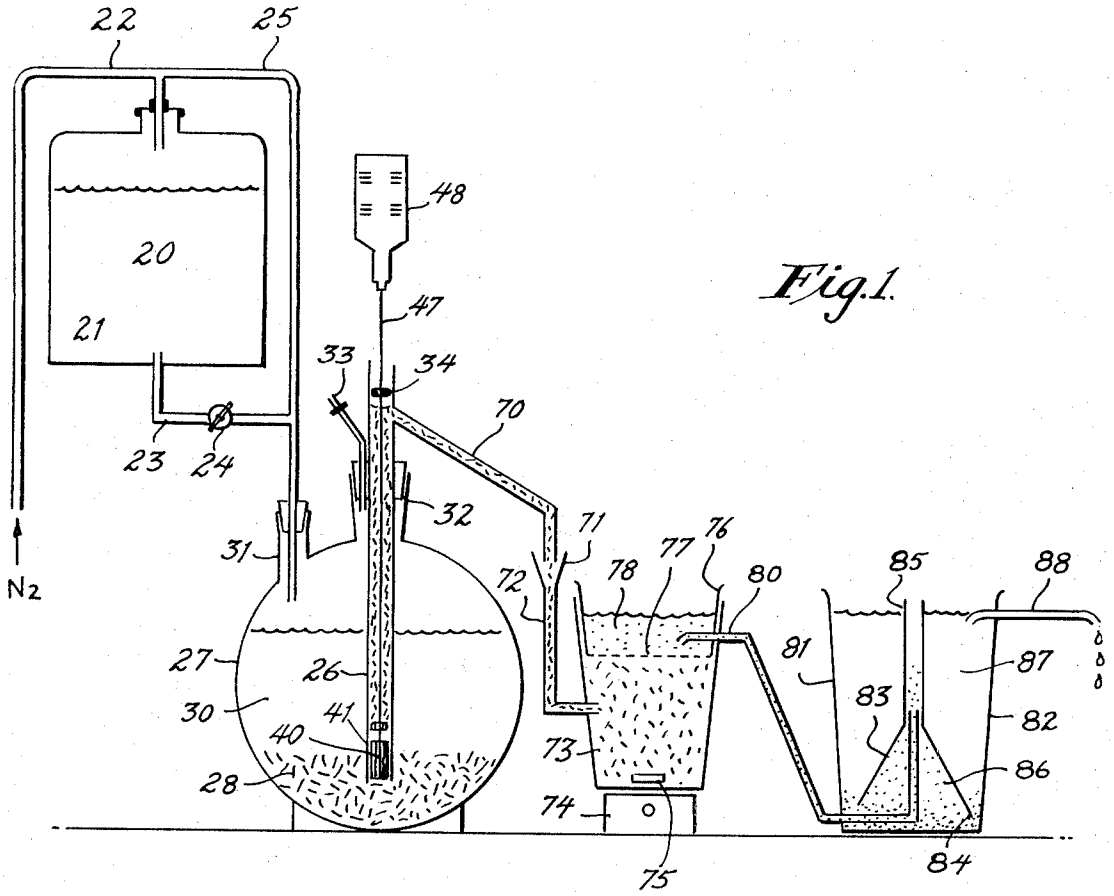

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Fig. 1.

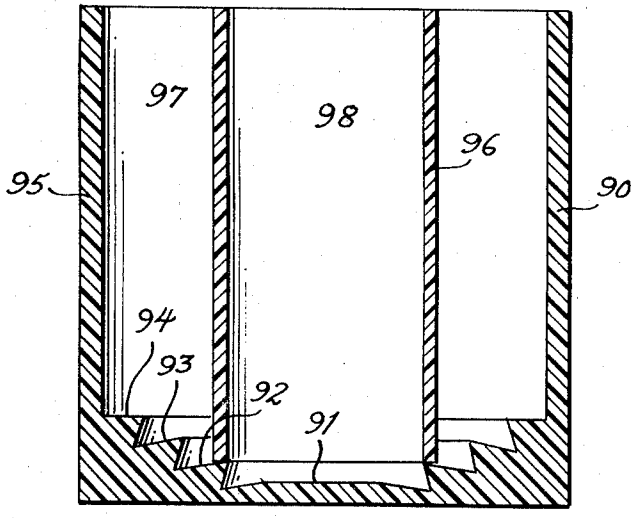

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Fig. 9

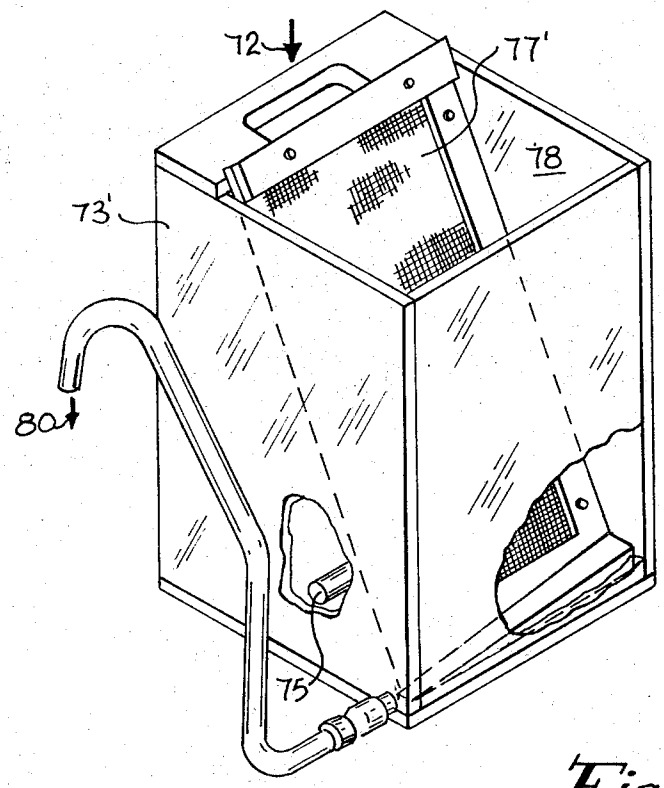
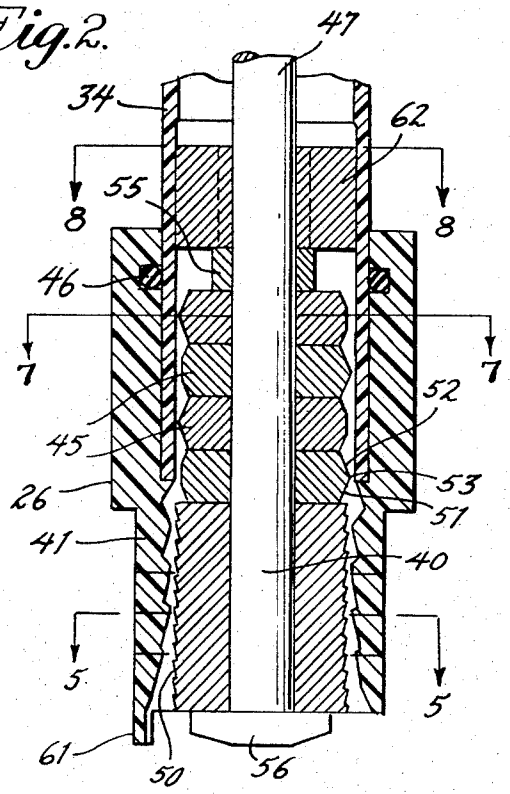


Fig. 1a

Fig. 2.



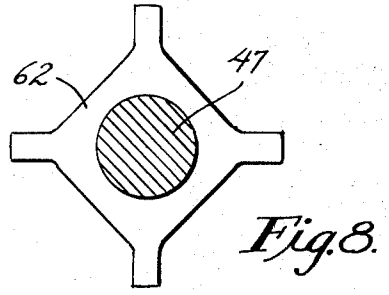


Fig. 8.

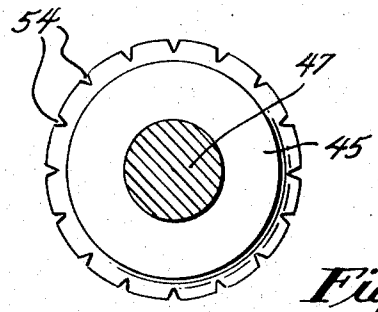


Fig. 7.

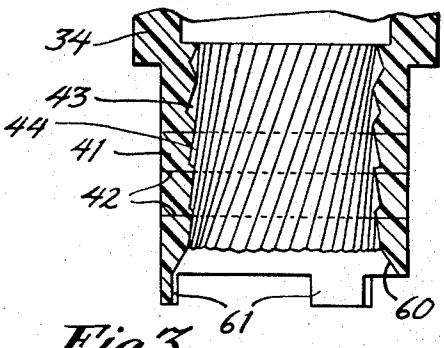


Fig. 3.

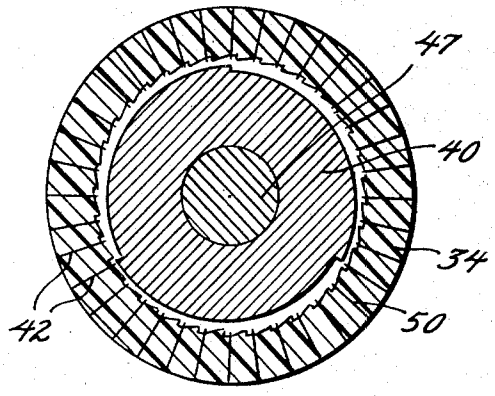


Fig. 5.

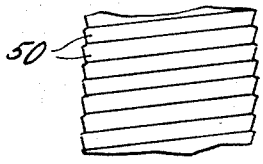


Fig. 4.

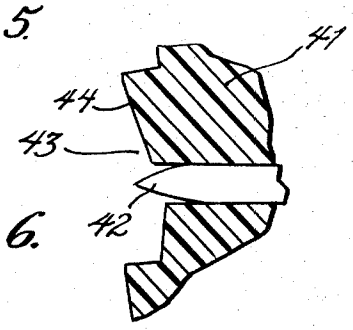
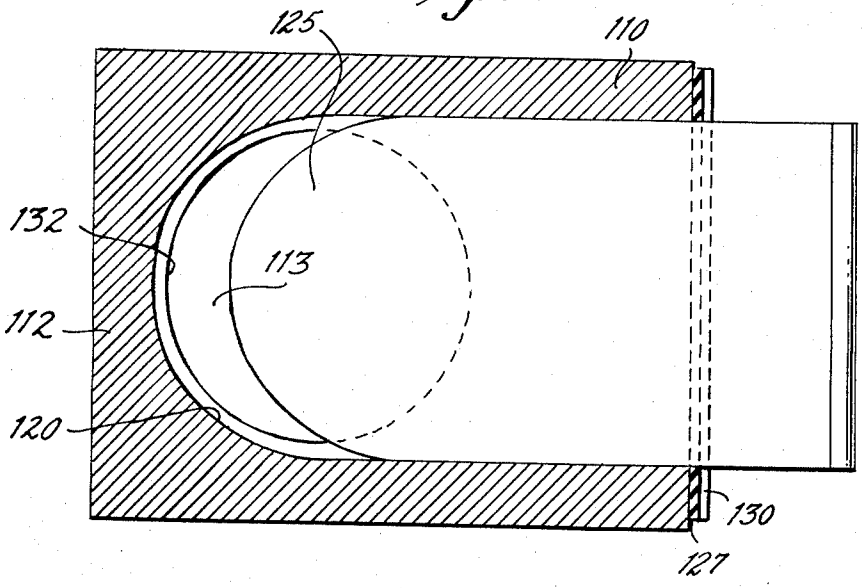


Fig. 6.

Fig. 11.


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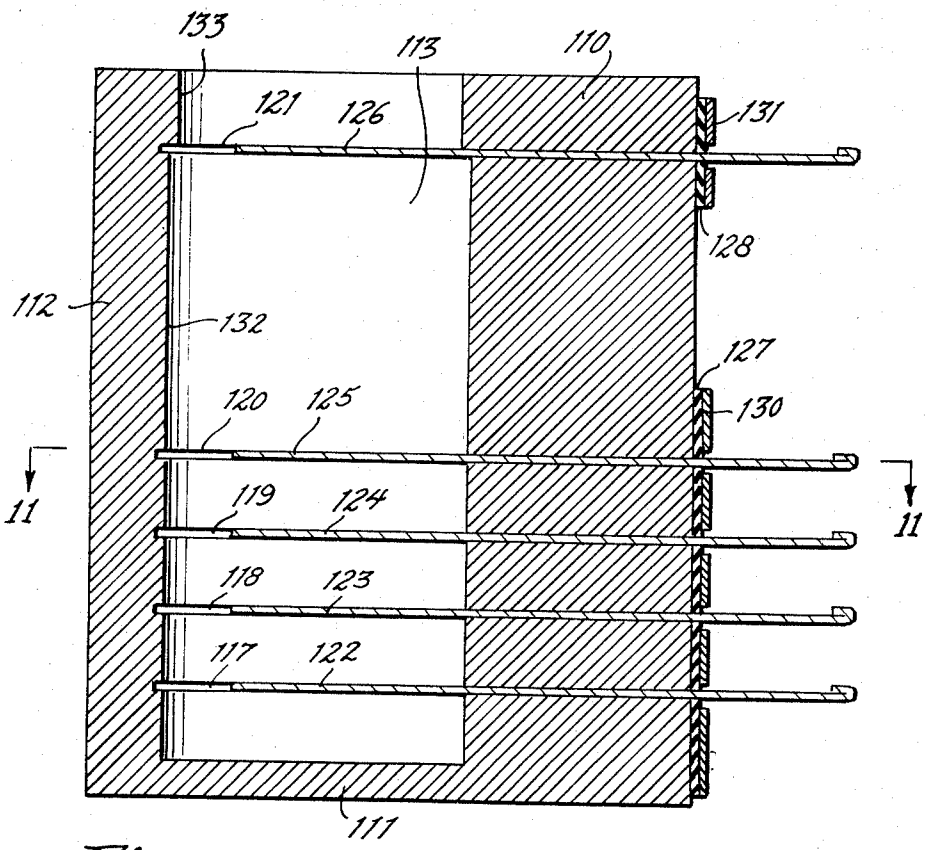

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Fig. 10.

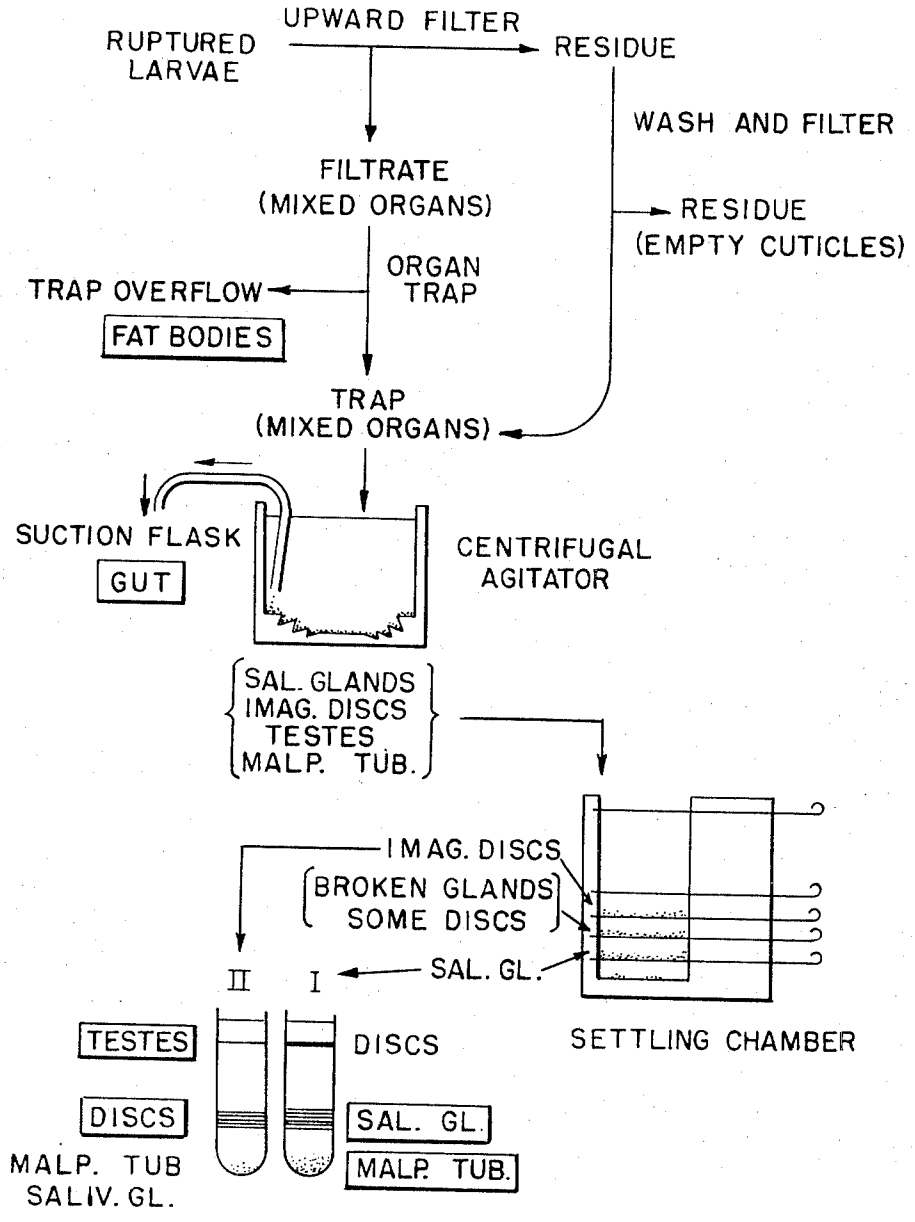

RUPTURED LARVAE
UPWARD FILTER
RESIDUE
WASH AND FILTER
FILTRATE (MIXED ORGANS)
RESIDUE (EMPTY CUTICLES)
ORGAN TRAP
TRAP OVERFLOW
FAT BODIES
TRAP (MIXED ORGANS)
SUCTION FLASK
GUT
CENTRIFUGAL AGITATOR
SAL. GLANDS
IMAG. DISCS
TESTES
MALP. TUB.
IMAG. DISCS
BROKEN GLANDS
SOME DISCS
SAL. GL.
II
I
TESTES
DISCS
MALP. TUB
SALIV. GL.
DISCS
SAL. GL.
MALP. TUB.
SETTLING CHAMBER

Fig. 12.

ISOLATION AND FRACTIONATION OF ORGANS OF SMALL ANIMALS

DISCLOSURE OF INVENTION

Many laboratories are doing fruitful research on larvae such as larvae of Drosophila melanogaster (fruit fly), since information derived therefrom is believed to be of broad application in the biochemical field. Biochemical studies of small organisms such as insects have been largely restricted to preparation of whole animals, because the amount of organs obtainable by manual dissection of these small organisms is small and the work is prohibitively tedious and expensive. In order to study proteins of polytene and non-polytene nuclei of Drosophila melanogaster, a method and apparatus have been devised for the isolation of pure organs in quantities measured in grams.

Fristrom and Mitchell, 1965, 27 J. Cell. Biol. 445 have devised a method which isolates the imaginal disks but is not suitable for other organs. Boyd, Berendes and Boyd, 1969 38 J. Cell. Biol. 369 have recently described a procedure for isolation of larval salivary glands, applicable to Drosophila hydei but not applicable to other species.

Cohen and Gotchel, 1971, 246 J. Biol. Chem. have devised a method for the rapid isolation of salivary glands of Drosophila melanogaster larvae. The isolation of imaginal disks can be carried out simultaneously, but the yield and purity of disks is very low.

The present invention comprises a method and apparatus for isolating large quantities of a variety of organs of small animals such as larvae with a good yield and a high degree of purity. In the procedure of the invention the larvae are automatically continuously dissected, then the various organ types are separated by centrifugal agitation, by settling at natural gravity, and then by isopycnic centrifugation in discontinuous density gradients. This makes it possible to do biochemical studies on specific kinds of tissues or organs of small animals.

In the general procedure in the preferred embodiment third instar larvae of Drosophila melanogaster, strain Oregon R. were thoroughly washed with distilled water and then drained. The larvae were then placed in ice-cold organ medium which in the preferred embodiment consists of 25 mM disodium glycerophosphate, 10 mM KH_2PO_4, 30 mM KCl, 10 mM $MgCl_2$, 3 mM $CaCl_2$, and 162 mM sucrose, pH 6.8. The weight of the larvae was approximately half the settled volume.

DRAWINGS

In the present invention the drawings diagrammatically suggest the apparatus.

FIG. 1 is a diagrammatic vertical side elevation in section of the apparatus for dissection and removal of body parts from organs.

FIG. 1A is a central vertical section of a variation in a component of FIG. 1.

FIG. 2 is a diagrammatic axial section of the dissecting apparatus including the rotor and housing.

FIG. 3 is an axial section of the housing with the rotor removed.

FIG. 4 is a fragmentary side elevation of the rotor with the housing removed.

FIG. 5 is a section of the rotor and housing on the line 5—5 of FIG. 2.

FIG. 6 is an enlarged axial section of a housing groove showing the position of a needle therein.

FIG. 7 is a section of the rotor on the line 7—7 of FIG. 2.

FIG. 8 is a section of the rotor on the line 8—8 of FIG. 2.

FIG. 9 is a central vertical section of the centrifugal separation device.

FIG. 10 is a vertical section of the sedimentation device under unit gravity.

FIG. 11 is a section of FIG. 10 on the line 11—11.

FIG. 12 is a flow diagram of procedure of the invention according to the preferred embodiment.

During all the operations to be described the fractions were kept at a temperature between 0° and 4° C. The larvae and the organs can be stored in the ice-cold organ medium for several hours without noticeable influence in the microscopic appearance. All of the reagents used herein were pretreated to remove trace metals.

DISSECTION SYSTEM

The dissection system combines high yield with minimal damage to the tissues. The dissection device first tears the cuticle open with a needle and then gently squeezes out the organs.

As illustrated in FIGS. 1 to 8 a buffer reservoir 20 contains organ medium 21 subjected to gas pressure, suitably nitrogen at 5 psi from piping 22 above the organ medium 21 and connected to the dissection device by piping 23 through an open stop cock 24 below the organ medium. The pressure is equalized by piping 25.

The dissection device 26 operates in an air-tight flask 27 having two necks and containing the larvae 28 mixed with organ medium 30. The entrance to housing 34 is below the level of the larvae 28 and the organ medium 30. In the side neck 31 of the flask is connected inlet piping 23 by a suitable rubber stopper. In the central neck 32 is connected a normally closed air vent 33 and housing 34 of the dissecting device by a two-hole rubber stopper. The flow of the pressurized organ medium and rotation of a rotor 40 of the dissecting device transports the animals upward in the grooves of a housing extension 41 over the points of needles 42 that protrude into housing grooves 43, where the bodies of the larvae are torn open. The collapsed carcasses then roll over ridges 44 in the upper parts of the grooves of the housing starting a gentle squeezing process that is continued by disks 45 on the upper part of the rotor, thus liberating the individual organs with little mechanical stress.

It has been found best to make the rotor of stainless steel, and the housing of a plastic such as methyl methacrylate. In the device according to the invention the main housing 34 is surrounded at its lower part by the housing extension 41 which is held thereon by a suitable rubber O-ring 46 in a suitable annular groove. By way of example the dimensions of the parts are given in a preferred embodiment applied to third instar larvae of Drosophila melanogaster.

The rotor at the upper end has a shaft 47 including a flexible coupling (FIG. 3, not shown) which is driven clockwise by a motor 48 on the suitable support. The motor is suitably a variable speed motor of high torque turning about 60 rpm.

The housing has grooves **43** which are left hand (in the case given). It will be seen by reference to FIG. **3** that these grooves are steeply sloping at the top. Each groove has at various positions upward three or four rows of tips of needles **42** inserted through the sleeve so that they protrude inward in the groove **43** but do not extend inward beyond an imaginary line connecting the high points of each thread. The needles are preferably No. 1 stainless steel fine intestinal needles which are preferably hand sharpened. The needles should protrude into the grooves between one-quarter and one-half the depths of the grooves. The rotor at the lower end opposite the grooves in the housing has a series of triple buttress threads **50** which are right hand in the case given and have steep groove walls at the lower side and gradual groove walls at the upper side (as shown in FIG. **4**). Thus rotation of the rotor clockwise produces a tendency to impel the larvae upward in the grooves and against the middle of the housing.

Above the groove portion of the rotor are a plurality of disks **45**, which have gradually sloping lower and upper portions **51** and **52** rising to a peak at **53** and interrupted circumferentially by axial grooves **54** which prevent piling up of organs on the disks. The groove portion of the rotor and the disks are united by a collar **55** on the shaft portion of the rotor and a nut **56** threaded on the shaft portion of the rotor at the lower end.

In the preferred embodiment the main housing is 16 inches long and is made from 1 inch outside diameter methyl methacrylate tubing, machined 2 inches at each end to an inside diameter of 0.875 inch so as to make the internal bore a precision surface. The lower 1 ½ inch of the housing is also machined at the outside so that it is perfectly round and aligned to the bore in order to make a proper fit with the housing extension **41**. The housing extension has **36** asymmetrical grooves (FIG. **5**) shown more in detail in FIG. **6**, in the interior wall which cuts straight at an angle of 21° to the axis, resulting in a change in the cross sectional slopes of the grooves along the length of the sleeve and an increase in the inner diameter of the sleeve toward the lower end (FIGS. **2** and **3**). In addition the lower end of the sleeve is beveled on the inside at **60** and provided with three feet **61** to facilitate entry of the larvae. Three or four sharpened needles **42** are at various levels in each of the grooves, and they protrude radially in about between one-quarter and half the distance between an imaginary line joining the inside of the grooves on the two sides and the depth of the groove as shown.

In the rotor the preferred embodiment has three leads, ¼ inch pitch, 0.008 inch maximum depth with the back surface at an angle of 5.5° to the axis of the cylinder.

Each of the squeezing disks **45** is of approximately one-quarter inch thickness in the preferred embodiment and each of the disks has a diameter at the high point which is progressively increasing, the disk diameter suitably being, counting from the bottom 0.815 inch, 0.820 inch, 0.825 inch and 0.830 inch.

At the point where the rotor comes into close proximity to the housing, that is, about half way up the threaded portion of the rotor, the largest space between the bottoms of the grooves in the housing and the bottoms of the grooves in the rotor is about the diameter of the animals being dissected, and the distance between the tops of the grooves in the housing and the tops of the grooves in the rotor is about one-quarter of the diameter of the animals so that when the needles dissect the animals, the tops of the grooves of the housing and the tops of the grooves of the rotor exert a gentle squeezing force squeezing the organs out of the bodies of the animals. The clearance between the squeezing disks at the high point radially and the housing is about half the diameter of the animals, and this exerts a supplemental squeezing action pressing out the organs. The dimensions of the grooves **54** in the squeezing disks are not critical.

The shaft portion **47** is centered within the housing by polytetrafluoroethylene spacer disks **62** (only one of which is shown).

It will be understood that the organ medium and the animals are forced between the rotor and the housing by a combination of upward forces exerted by the rotor and the housing and gas pressure differential which insures hydraulic flow between the rotor and the housing.

As a consequence organ medium and dissected animals flow up the space between the housing and the rotor and then through downward tube **70** through a pressure release **71** consisting of an open funnel into a tube **72** entering a vessel **73** having a magnetic agitator **74** rotating magnetizable rotor **75** in the lower part of the vessel **73**. There is a suitable conical ring support **76** whose lower end is closed by a filter medium **77**, preferably an open nylon mesh (mesh about 1 mm) which is designed to filter out the animals' bodies, and allow the organs to pass through. This is in effect an upward filtration system which eliminates most of the bodies from the organs.

FIG. **1A** shows a variation of the vessel **73** in FIG. **1**. The vessel **73'** is separated by a diagonal incline filter medium **77'**, suitably of nylon mesh, and it receives in that material from the tube **72** at one side of the filter medium and discharges it by the tube **80** drawing from the area **78** at the other side of the filter medium, passing the organs and retaining the bodies behind the filter medium.

The organs and organ medium pass through space **78** above the filter medium and then through tube **80** to the bottom of organ trap **81** which separates the organ medium from the organs. The trap is effective because of the reduced flow rate in the upper part which tends to keep the small organs in the bottom. The organ trap is a vessel **82** which has in the bottom a baffle consisting of a funnel **83** having legs **84** which allow a space for liquid circulation at the bottom and having a stem **85** extending above the level of the liquid. The tube **80** as shown transports the organs well up in the funnel making the baffle effective. The organs are kept within the trap at **86** and the organ medium, along with small fragments and fat bodies, flows into the space **87** outside and above the conical portion of the funnel and is carried by tube **88**.

After the larvae are dissected, any free organs remaining in the filtration system are decanted and washed out with organ medium through a double layer of the nylon net into a siliconized glass beaker. After settling for 20 minutes, the supernatants of the organ trap and the filter washes are removed carefully. The sedimented organs are then washed twice with at least 20 times their volume of fresh organ medium in large siliconized glass beakers, waiting about 15 minutes each time to remove the supernatant.

In later steps of purification the organs tend to stick to the glassware and this can be reduced by rinsing the glassware with the original supernate and then with organ medium.

CENTRIFUGAL AGITATION

Based upon experience that gut could be separated from salivary glands and imaginal disks in a rotated beaker, a special plastic beaker **90** shown in FIG. **9** preferably made of methyl methacrylate was constructed with the bottom having four levels, a central level **91**, a first step **92**, a second step **93** and a third step **94** joining the side wall **95**. A methyl methacrylate tube **96** rests upon first step **92**. In using this device with a tube **96** resting on the first step, the chamber **97** outside the tube is filled with organ medium and the chamber **98** inside the tube is filled with a suspension of organs or larvae. After 15 minutes the tube is carefully removed, and the vessel placed on a level surface of crushed ice and rotated suitably by hand about 1 inch in the circumference clockwise and 1 inch counter clockwise back and forth to facilitate the separation of different layers within the sediment by a very low centrifugal force. After 2 to 3 minutes the vessel is rotated suitably by hand with short but smooth hand strokes at a speed of about 10 to 15 rpm. Under this procedure the gut rises over the steps and up the side of the beaker, from where it is periodically transferred to a suction flask by means of a Pasteur pipette, without stopping the rotation. After the most easily disturbed gut is removed, the rotation is stopped and the pile of organs at the very center of the beaker gently is dispersed with a glass rod. Fractionation is resumed, first with a back and forth motion and then with rotation as described before. Under proper conditions all the gut can be removed within about 10 minutes leaving salivary glands, imaginal disks and most of the Malpighian tubules at the bottom of the beaker.

SETTLING AT NATURAL GRAVITY

The next step in fractionation of the organs is settling under natural gravity in a settling chamber **110** which has a bottom wall **111** and a side wall **112** which is preferably exteriorly square but which has a right cylindrical inner space **113**. At various horizontal levels the wall of the chamber has slots **117, 118, 119, 120** and **121** which are rectangular in horizontal shape and in which horizontal slidable partitions **122, 123, 124, 125** and **126** suitably of stainless steel sealed by gaskets **127** and **128**, suitably of rubber having slots which allow the partitions to slide in and out, held by gasket covers **130** and **131** secured by suitable screws (not shown) to the wall **112** of the vessel. The side wall **132** of the chamber is undercut below the partition **126** while the side wall **133** above it overhangs slightly to reduce the tendency of the organs to adhere to the walls of the vessel.

The horizontal partitions are lubricated with silicone oil. The principle of sedimentation under unit gravity through a stabilizing gradient has previously been used for cell separation, Lam, Furrer and Bruce, 1970, 65 Proc. Nat. Acad. Sci. U.S.A. 192. The high sedimentation rate of whole organs necessitated, however, the design of a special settling chamber shown in FIG. **10** to permit rapid layering of the sample on the gradient and rapid collection of the separate organ types. With the compartments almost closed, the chamber is filled with organ medium containing a stabilizing linear gradient consisting of Ficoll, a thickening agent of high molecular weight (molecular weight about 400,000) which is a polymer of sucrose. Instead of Ficoll, other sedimentation agents such as serum albumin can be used if desired. The Ficoll in the organ medium preferably has a concentration gradient from bottom to top of 2 to 5% by weight. After the mixture of organ medium and Ficoll fills the sedimentation chamber in the portion **132**, all the partitions are closed and then open to release bubbles. The top partition **126** is then closed to separate the sample compartment from the gradient. The organ suspension is poured in above the closed partition **126** and partition **126** is immediately opened. This creates a sharp boundary between the sample and the stabilizing medium below. After about 5 minutes the lowest partition **122** is closed and it separates small larvae, food particles and white Malpighian tubules from the organs above. After about 15 minutes partitions **123** and **124** above are closed and the space immediately above partition **122** contains the salivary glands and the space immediately above partition **124** contains the larvae imaginal disks. After about 25 minutes of settling partition **125** is closed. The Malpighian tubules being of variable size and density are distributed throughout the various chambers and are subsequently separated in isopycnic centrifugation. After the supernatant containing light particles has been poured off, the lower compartments are opened one at a time and their contents are rinsed out into siliconized glass beakers with organ medium. Each fraction is then washed once with organ medium to remove the Ficoll.

The imaginal disk fraction and salivary gland fractions are separately subjected to isopycnic centrifugation in density gradients suitable to the particular fraction as below described.

ISOPYCNIC CENTRIFUGATION

The discontinuous Ficoll gradients used by Fistrom and Mitchell, 1965, 27 J. Cell. Biol. 445 and by Boyd, Berendes and Boyd, 1968, 38 J. Cell. Biol. 369 were modified. Purified Ficoll was dissolved in organ medium and the density of the stock solution was adjusted with organ medium until it had a gravity of 1.1175 g/ml at room temperature (25° C.) The salivary gland fraction was layered onto a discontinuous gradient (Gradient I) consisting of 15 ml undiluted Ficoll stock solution (Ficoll A) and 20 ml of Ficoll B (Ficoll A diluted with ½ volume of organ medium) in 50 ml polycarbonate centrifuge tubes. For imaginal disks (Gradient II) Ficoll B was used as the lower layer and Ficoll C (Ficoll A diluted with one volume of organ medium) was used as the upper layer of the discontinuous gradient. All boundaries were broadened slightly by gentle stirring to prevent aggregation. After standing vertically for 2 minutes, the tubes were centrifuged at $40 \times g$ for 1 minute, then rotated 180° to prevent packing on one side during acceleration and centrifuged for another 3 minutes at $2500 \times g$.

The gradient for salivary glands separated the glands associated with parts of fat body together with some imaginal disks and gut pieces in the upper band from the main salivary gland fraction (lower band) and the Malpighian tubules as well as mouthparts (pellet). The gradient for imaginal disks separates testes associated with fat bodies as the upper band from the imaginal disks as the lower band and Malpighian tubules together with fragments of salivary glands (pellet). The

bands are recovered with a wide mouth, polypropylene pipette and washed twice with organ medium to remove the Ficoll.

The success of isopycnic centrifugation depends on the precise adjustment of the density of the different layers. The densities of the organs may vary with the size, age and growth conditions of the larvae. In addition, the previous exposure of organs to solutions containing Ficoll or sucrose tends to increase these densities. It is therefore recommended to run a pilot experiment to determine whether the density must be adjusted in the particular case.

RESULTS OF FRACTIONATION OF DROSOPHILA LARVAE

FIG. 12 is a flow diagram of the fractionation of organs of late third instar larvae of Drosophila melanogaster. In this diagram the printing indicates the organ type.

Salivary glands are produced in a yield of about 2 grams per 500 grams of animals, of which about half are broken, mainly near the neck. From microscopic examination, this fraction appears to contain about 2 to 5% of contaminating tissue mass, mainly attached fragments of fat body, mouthparts, and fragments of gut. Practically, all free contaminants can be removed by a rerun on the isopycnic gradient or by hand under a dissecting microscope. If desired, whole glands can be separated from gland fragments by appropriate fractionation in the settling chamber.

From 500 rams of larvae, about 0.4 grams of imaginal disks are obtained. From microscopic examination they are contaminated with about 20% by weight extraneous material. The yield of imaginal disks can be increased by slower and longer rotation of the centrifugal agitator. The purity can be improved by allowing the salivary glands to settle to the bottom of the settling chamber, leaving more of the chamber available for the separation of imaginal disks from other particles. This also permits separation of imaginal disks of different sizes. These changes can be made when the purity of the salivary gland fraction is not critical.

A small but quite pure fraction of testes was obtained at the top of the isopycnic gradient for imaginal disks, due to their association with small fat bodies. If desired, procedural variations recommended for imaginal disks may be applied to the testes fraction.

Other organs can be obtained in a lower degree of yield and purity. Malpighian tubules can be purified by isopycnic centrifugation in view of their high density. Thus the Malpighian tubule-rich fractions on top of the settling chamber and in the pellet of isopycnic gradient for both salivary glands and imaginal disks can be washed free of Ficoll and then banded in a discontinuous Ficoll gradient between layers with densities of 1.1175 and 1.22 g/ml.

The bulk of the gut is recovered from the centrifugal agitator by collection in the trap. It can be purified and fractionated in the settling chamber. Fat bodies can be concentrated from the overflow of the organ trap, either by continuous flow centrifugation or by floatation. The fat bodies are mostly small broken fragments.

Salivary gland and imaginal disk nuclei were isolated by using the previous procedure except that for the first homogenization, bovine albumin in a concentration of 1 mg/ml and a special organ medium (0.1 M Na_2 glycerophosphate, 50 mM sucrose, 4mM $MgCl_2$ and 0.4 mM $CaCl_2$ having a pH of 6.8) in order to minimize proteolysis was added. No detergent was used until the nuclei had been thoroughly washed to remove the albumin and cytoplasmic organelles. The nuclei were then suspended in 0.5% Triton X–100 (a non-ionic detergent consisting of an alkyl aryl polyether alchohol, widely used in Biology and water soluble), stirred for 1 minute and centrifuged at 164 × *g* for 1 minute. The Triton was removed by washing twice. This procedure produced polytene and nonpolytene nuclei.

When applied to Drosophila melanogaster larvae the times for the steps in FIG. 12 for separation of nuclei from salivary glands and imaginal disks from 500 gm of larvae were as follows:

1 ½ to 2 hours for dissecting the larvae;

1 hour for washing the crude organ mixture;

1 to 1 ½ hours to remove the gut using two centrifugal agitators alternately;

1 hour to separate disks and glands and to wash the fractions free of Ficoll;

½ hour to separate the salivary glands and imaginal disks from Malpighian tubules and testes by isopycnic sedimentation;

½ to 1 hour for final purification by resedimentation or under the dissecting microscope and,

1 hour for isolating the nuclei.

RESULTS OF THE INVENTION

It has always been a very difficult problem to obtain sufficient organs of small animals for biochemical experiments. Many laboratories are now investigating the biochemistry of the fruit fly, hoping to derive more knowledge of biochemistry generally and of human biochemistry in particular. It is very tedious and prohibitively expensive to separate organs by dissection in order to make analyses which are accurate within a reasonable range.

The invention is particularly recommended because of the gentle action separating the organs by minimal loss of soluble cellular components. As already stated, the maximum dimension in the separating part of the equipment between the rotor and the housing is about the diameter of the small animal, and the minimal clearance is about one-quarter of this diameter, to avoid cutting or slicing the organs. Also the needle protrudes into the groove about one-quarter the depth of the groove. Under these conditions over half of the Drosophila larvae are emptied of their organs.

Although it would be possible to obtain complete emptying by increasing the sizes of the squeezing disks, this is not to be recommended (except perhaps for the isolation of cuticles) because the yield would be mainly crushed tissue. If the device is set up with these limitations in mind, it can be used for other small animals provided the organs are easily liberated from the body once it is torn.

The three methods of separation of organ types described rely on different parameters, namely, shape, size and density, and the combined resolving power is much greater than any of them. Furthermore, the removal of gut by centrifugal agitation eliminates clumping in the subsequent steps while the preliminary separation of salivary glands and imaginal disks by means of the settling chamber permits the use of different density gradients for these two tissues in the isopycnic sedimentation step.

The efficacy of these procedures depends upon the species. For example, in the case of Drosophila virilis, which has long, narrow salivary glands, it has been found that neither the centrifugal agitation nor the settling chamber is very effective in separating salivary glands from gut although the settling chamber was effective in separating these two tissues from imaginal disks. In this species, therefore, the resolution of salivary glands and gut relies mainly on isopycnic sedimentation.

The methods and apparatus of the present invention have been used by other workers for studying nuclear proteins of the fruit fly, the nucleic acids of diverse organs of Drosophila larvae and have also been used to isolate mature eggs from adult fruit flies. They are also applicable to many other types of biochemical investigations.

In view of our invention and disclosure, variations and modifications to meet individual whim or particular need will doubtless become evident to others skilled in the art, to obtain all or part of the benefits of our invention without copying the process and apparatus shown, and we therefore claim all such insofar as they fall within the reasonable spirit and scope of our claims.

Having thus described our invention what we claim as new and desire to secure by Letters Patent is:

1. The process of dissecting small animals, which comprises progressing them between a grooved rotor having grooves inclined opposite the direction of rotation and a grooved housing having grooves inclined in the direction of rotation, the housing having needles projecting into each of the grooves approximately about ¼ to ½ the groove depth, the minimum clearance between the depths of the grooves in the rotor and the depths of the grooves in the housing being about the diameter of the animals and the clearance between the ridges of the grooves on the housing and the ridges of the grooves in the rotor being about one-fourth of the diameter of the animals, and progressing the animals in the clearance space between the grooves in the housing in the presence of liquid medium.

2. The process of claim 1, which comprises further progressing the animals between successively larger discs on the rotor and the housing, until the minimum clearance between the rotor and the housing is about ¼ the diameter of the animals.

3. The process of claim 1, which comprises subjecting the animals and the liquid medium to differential gas pressure which aids in flow of the animals and the liquid medium in the clearance space between the housing and the rotor.

4. A device for isolating and fractionating organs of small animals, comprising a vessel containing small animals in liquid medium, a housing extending upward in the vessel, a rotor within the housing and having clearance from it, means for rotating the rotor in one direction, the rotor having grooves which incline opposite the direction of rotation as the medium progresses in the clearance space and the housing having grooves which incline in this direction, needles projecting inward in the grooves of the rotor but not beyond the grooves, the rotor and housing together dissecting the animals and gently squeezing out the organs, and means for taking off the mixture of organs, bodies and liquid medium at a more advanced point of the clearance between the rotor and the housing.

5. A device of claim 4, having discs on the rotor located at a more advanced point than the groove portions of the rotor, the discs having progressively increasing maximum diameter and the clearance space between the rotor and the housing diminishing.

6. A device of claim 5, in which the needles project into the grooves of the housing between ¼ and ½ the depth of the grooves, the clearance between the depths of the grooves on the housing and the depths of the grooves on the rotor being about the diameter of the animals, and the minimum clearance between the ridges of the grooves on the housing and the ridges of the grooves on the rotor being about one-fourth of the diameter of the animals.

7. A device of claim 5, in which the minimum clearance between the discs and the housing is about one-fourth of the diameter of the animals.

8. A device of claim 4, in which the grooves on the rotor are gradually sloping with respect to the rotor axis and the grooves on the housing are steeply sloping in the opposite direction.

9. A device of claim 4, in combination with means for maintaining gas pressure on the animals and the liquid medium at the inlet between the rotor and the housing.

10. In a device for dissecting small animals, a vessel adapted to hold the small animals in a liquid medium, a housing having its inlet end below the level of the animals in the liquid medium, a rotor having a clearance between the housing and the vessel, means for rotating the rotor in one direction, the rotor having grooves inclined opposite to the direction of rotation and the housing having grooves inclined in the direction of rotation, and needles in the housing grooves projecting inward less than the depth of the housing grooves.

11. A device of claim 10, in combination with means for subjecting the inlet end of the animals and liquid medium to gas pressure which will promote flow between the rotor and the housing.

12. A device of claim 10, in which the grooves in the rotor slope gradually with respect to the axis and the grooves in the housing slope more steeply with respect to the axis.

13. A device of claim 10, in combination with discs of progressively increasing size on the rotor beyond the grooves, the housing opposite the discs being free from grooves.

14. A device of claim 13, having interruptions at circumferential points along the discs.

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