VII. PHYSIOLOGY MATERIALS

A. KYMlGRAPH

A wide range of,physiological experiments using larger organisms may be performed using the kymograph.

B. VOLUMETER

In addition to identifying pressure changes, one may calculate the volume of gas exchanged with this equipment.

C. FERMENTATION TUBES

Fermentation rate is measured indirectly by the measurement of carbon dioxide.

D. MANOMETER

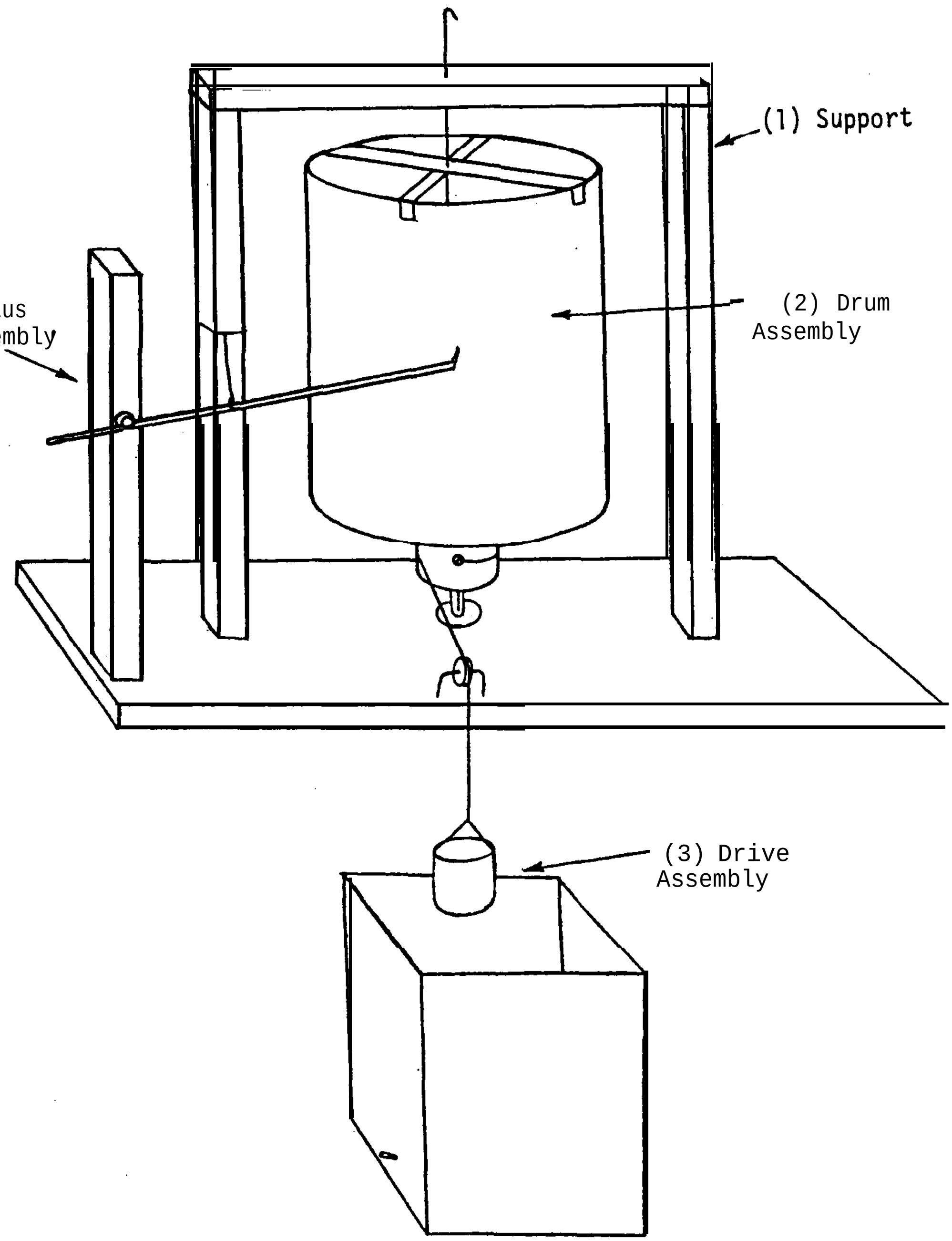
This apparatus enables one to identify changes in pressure within a biological system.

E. CHROMATOGRAPHY APPARATUS

Chromatography gives students useful insight into the techniques scientists use in investigating the biochemical composition of organisms.

A. KYMOGRAPH

Al. Kymograph



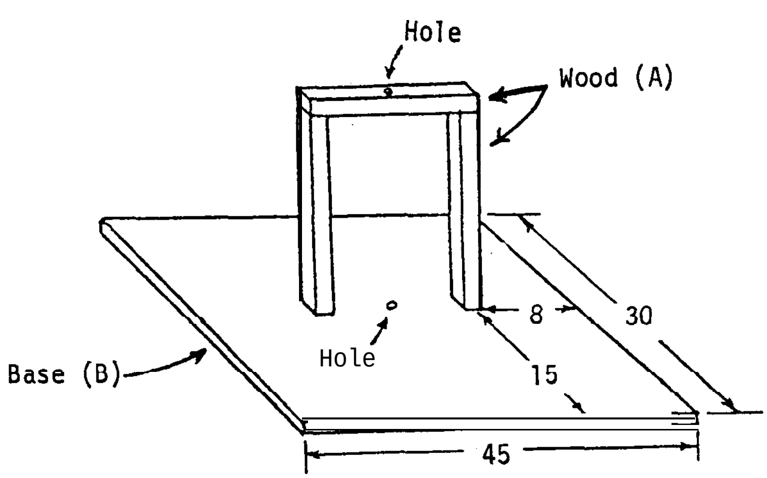
a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Support	3	Wood (A)	25 cm x 2 cm x 2 cm
	1	Wood (B)	45 cm x 30 cm x 1.0 cm

	1	Sheet Metal (C)	2 cm diameter
(2) Drum Assembly	1	Tin Can (D)	4 liter (i.e., 15 cm diameter, 17 cm high)
	2	Sheet Metal (E)	17 cm x 2 cm x 0.05 cm
	1	Stiff Wire (F)	30 cm long, 0.2 cm diameter
	1	Tin Can (G)	5 cm diameter, 6 cm high
	3	Sheet Metal Screws (H)	1 an long
	1	Glass Tubing (I)	0.4 cm diameter, 1.0 cm long
(3) Drive Assembly	1	Wood (J)	3 cm diameter, 0.5 cm thick
	1	Stiff Wire (K)	16 cm long, 0.2 cm thick
	1	Tin Can (L)	1 liter capacity
	1	Sand (M)	600 g
	1	Stiff Wire (N)	20 cm long, #20 gauge (0.1 cm diameter)
	1	String (O)	100 cm
	1	Tin Can (P)	4 liter capacity
	1	Pencil Stub (Q)	--
(4) Stylus Assembly	1	Wood (R)	25 cm x 2 cm x 2 cm
	1	Stiff Wire (S)	30 cm long, 0.2 cm thick
	1	Nail (T)	1 cm long, 0.2 cm thick
	1	Rubber Band (U)	6 cm long

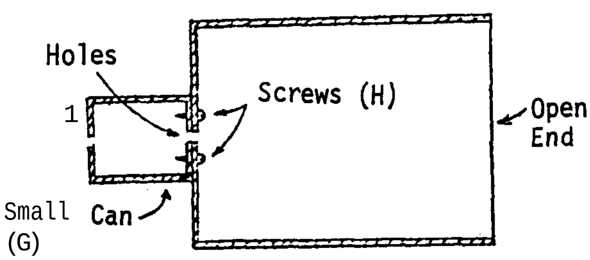
b. Construction

(1) Support



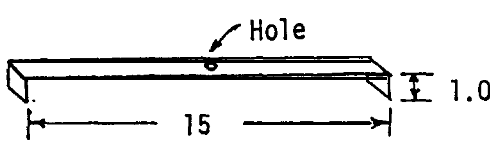
Drill a hole through one of the pieces of wood (A) directly in its center. Drill another hole in the base board (B) directly below the hole in the upper strip of wood. Each hole should have a diameter of about 0.3 cm. The hole in the base should be drilled only halfway through. Nail two of the strips (A) to the base and nail the crosspiece (A) so that the two holes are aligned. Finally, drill a 0.3 cm hole through the center of the disc of sheet metal (C) and nail the sheet metal to the base so that the hole in the base and the hole in the sheet metal are aligned.

(2) Drum Assembly



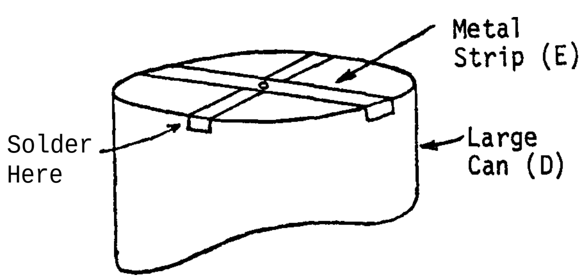
Side view (Cross-Section)

Try to select two tin cans (D,G) for the drum assembly which have both ends more or less intact. Otherwise, adjustments must be made to compensate for the open ends. At any rate, drill holes 0.3 cm in diameter in the center of both ends of each of the tin cans. Solder the two cans together making certain the holes in each align. If one of the ends of one or both cans has been removed, then the two cans can be screwed together with two sheet metal screws (H). Here, the illustrations show the situation when the small can (G) is intact and the large can (D) has one end removed.

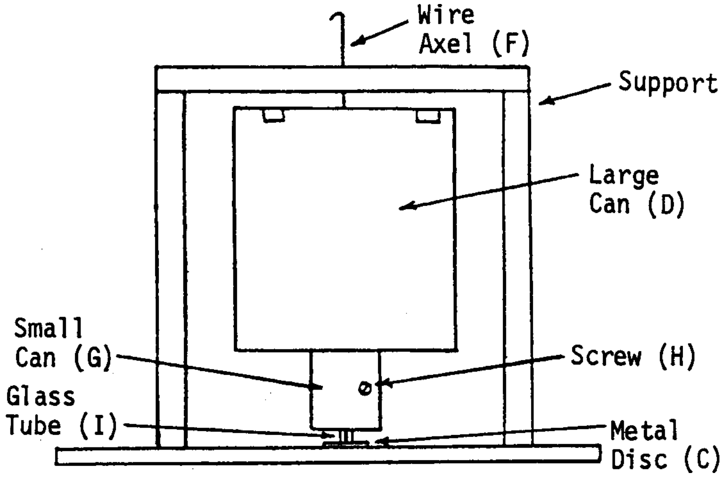


Metal Strip (E)

To solve the problem of the open end of the large can (D), take the two sheet metal strips (E) and bend a flap down 1.0 cm from each end. Drill a 0.3 cm hole through each strip in its center. Put the two metal strips across the top of the can so that they are at right angles (90°) and their holes are aligned. The flaps can be adjusted so that the strips are



held tightly in place, or they may be soldered to the sides of the can.

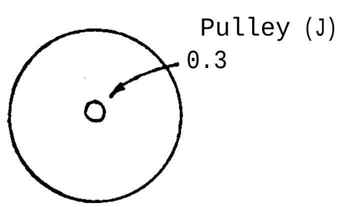


Front View

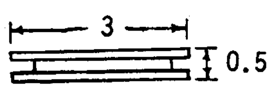
Screw a short sheet metal screw (H) partway into the small can (G) to serve as an attachment point for the drive assembly string (0). Then, take the stiff wire (F) and insert it through the hole in the support, through the drum and through the glass tubing (I). Fire polish the ends of the glass to make them smooth. Finally, make sure the end of the wire rests in the hole in the base. The exposed end of the wire axle (F) may be bent for safety. The whole drum assembly should turn freely now.

(3) Drive Assembly

Saw a groove all around the circumference of the wood disc (J) to make it act as a pulley. Drill a hole 0.3 cm in diameter through its center. Make the pulley mount from the stiff wire (K) by bending it to a "U" shape.



Top View



Side View

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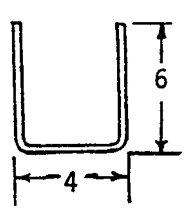
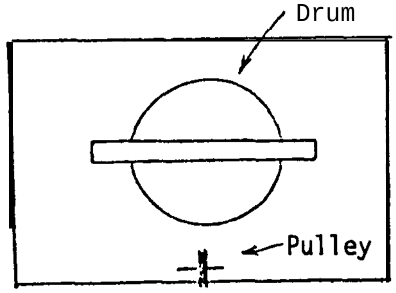
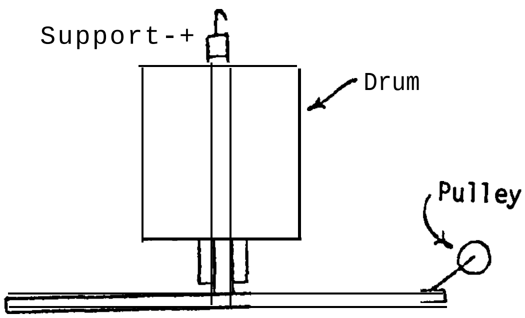


Pulley Mount (K)

Hammer the pulley mount into position on the base of the support after the pulley (J) has been slipped into place on the mount. It may be necessary

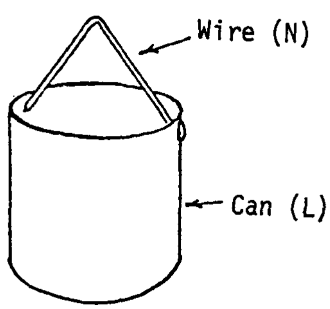


Top View

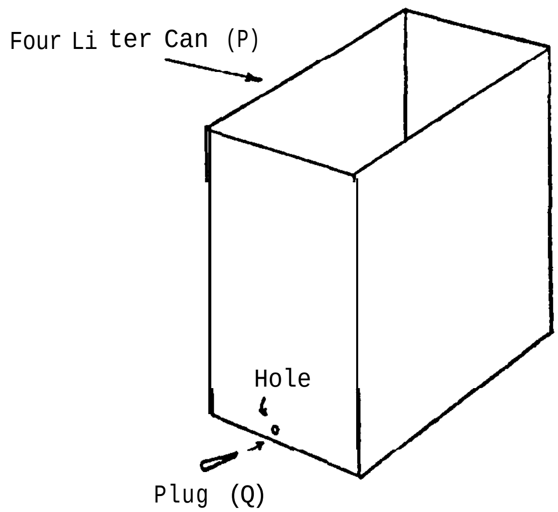


Side View

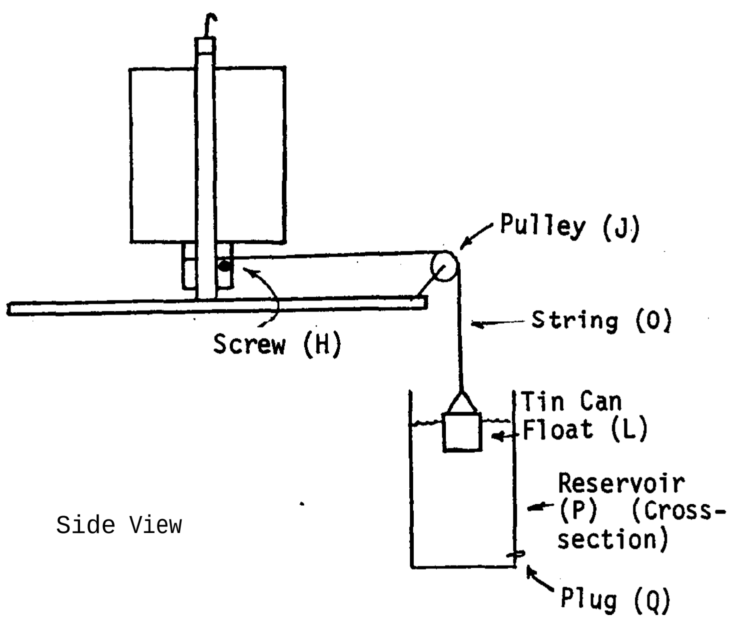
to drill small holes in the base for the pulley mount to fit into. The pulley and mount must be positioned at the front of the base directly in line with the drum assembly, and the pulley must extend beyond the edge of the base. The pulley mount can be bent over to insure that the pulley will extend out beyond the edge of the base, or alternatively, the holes into which the mount is inserted can be drilled at an angle.



Use the wire (N) to make a handle for the 1 liter can (L). Simply drill or punch two holes (0.2 cm diameter) near the top edge of the can, insert an end of the wire through each hole, and bend the ends up. Add the sand (M) to the can to act as ballast. This much weight should cause the can to float with only about 1 cm sticking above water level.



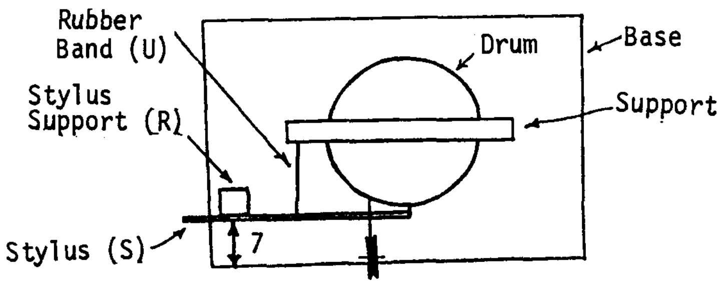
Make the 4 liter tin can (P) into a reservoir by removing its top. Tape the cut edges to prevent students from being cut. Drill or punch a small (0.15 cm diameter) hole at the bottom of the reservoir. Plug this hole with a pencil stub (Q) or piece of wood.



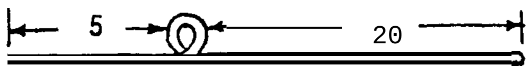
Side View

To set up the drive assembly, plug the hole in the reservoir (P) and fill it with water. Attach one end of the string (O) to the handle of the can (L) and make a small loop in the other end of the string. Put this loop around the screw (H) in the small tin can (G) under the drum, and wrap one turn of string around the small can. Run the string across the pulley (J), and float the tin can in the reservoir. Properly done, the float should lower gradually when the reservoir plug (Q) is pulled and water leaks out. As the float lowers, the string pulls on the drum causing it to slowly turn.

(4) Stylus Assembly

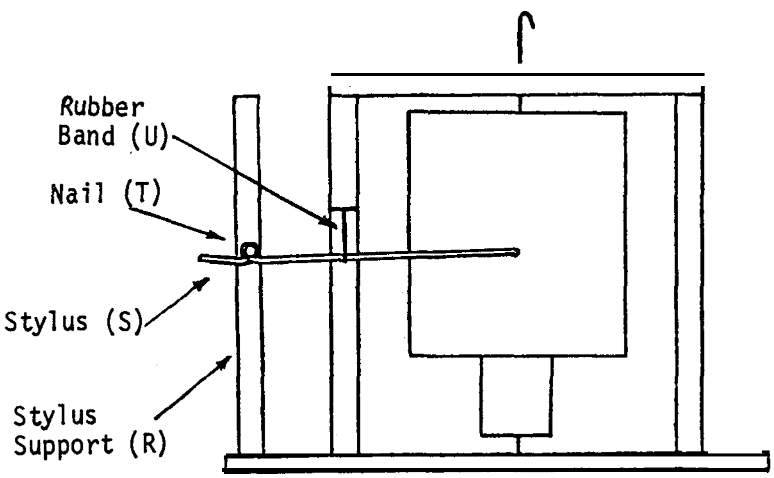


Top View



Side View of Stylus (S)

Top View



Front View

Nail or screw the stylus support (R) into position about 1.0 cm from one edge of the base, and 7 cm from the other edge. Make the stylus itself from the stiff wire (S) by making a loop in it about 5 cm from one end. Bend about 1 cm of the other end to a 90° angle to form the point. This point may be filed sharp to make a finer line. Position the stylus on the support by driving a nail (T) with a large head through the loop in the stylus. Drive the-nail in only enough to allow the stylus to pivot freely without twisting a great deal. The position of the stylus point on the drum depends upon where on the stylus support the stylus is nailed, i.e., the higher the pivot point on the support, the higher on the drum the point of the stylus will strike. Finally, tie the rubber band (U) (break it at one point) to the upright support and to the middle of the stylus. Adjust this rubber band so that the stylus point strikes the drum firmly, yet lightly enough not to interfere with the rotation of the drum.

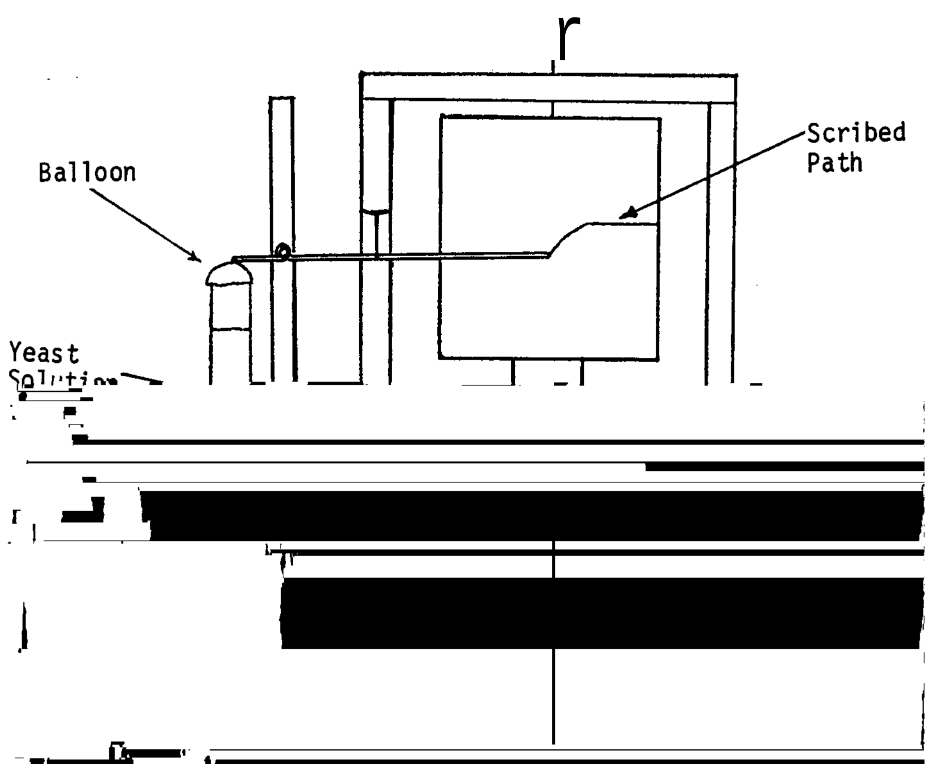
C. Notes

(i) To operate the kymograph, the drum must first be covered with a sheet of 17 cm x 50 cm glossy paper. Remove the drum from the support and attach the paper, glossy side out, to the drum with tape at the top and bottom. See that the seam where the two edges of the paper overlap is positioned in such a way that the stylus point will not catch on it (the seam) as the drum rotates.

Rotate the drum over a burning kerosene lamp. Hold the drum high enough so that the paper will not be scorched. Continue rotating it in the smoke until the drum is completely covered with carbon black. It takes about five minutes to cover the paper with carbon, requiring about 10 ml of kerosene. Handle the drum carefully since the carbon is easily scratched and rubbed off.

Replace the drum in the support, holding the stylus out of the way until the drum is in position. When the string to the float in the reservior is taut, the apparatus is ready to use.

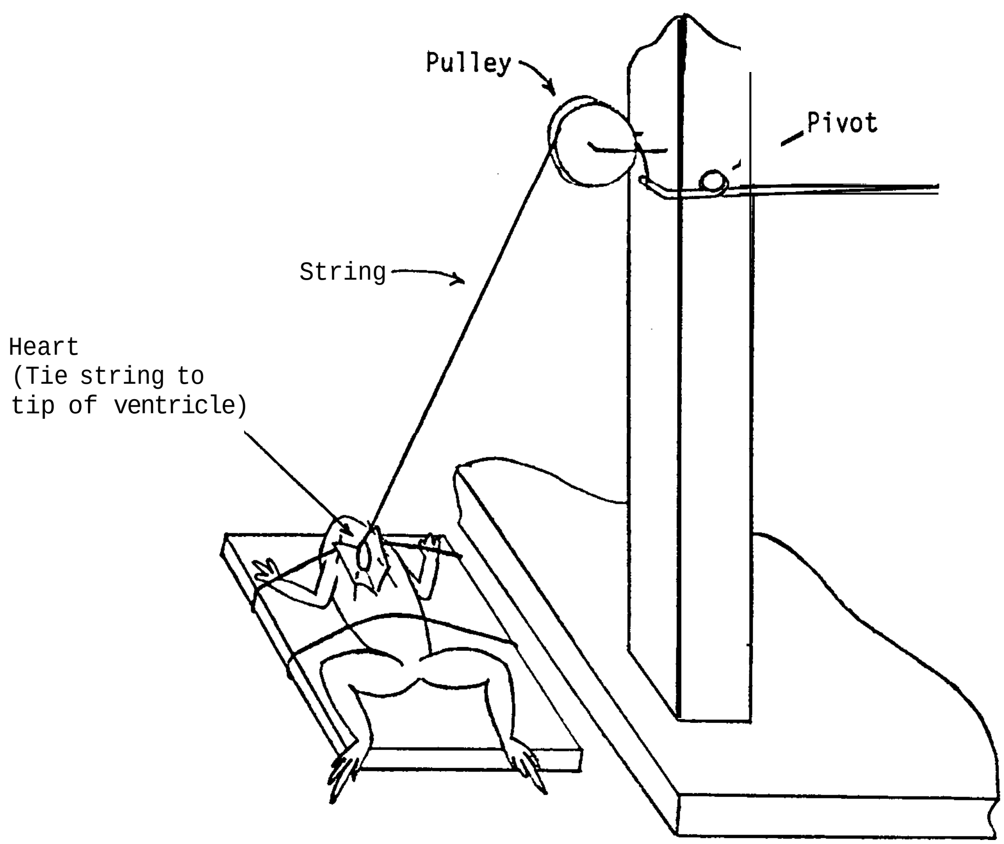
(ii) One example of the use of the kymograph will be given here. Refer to the drawing below:



Put some warm water, yeast, and sugar in a bottle or test tube and seal the end with an expandable membrane (a piece of balloon rubber works well). Place the solution under the end of the stylus so that the tip of the stylus rests on the balloon. Start the drum rotating by pulling the plug from the bottom of the reservoir. As the yeast respire, carbon dioxide gas is given off, gradually causing the balloon to expand, pushing the tip of the stylus up and its point on the drum down, leaving a scratch on the smoked paper. The slope of the scratched line indicates the rate of respiration of the yeast.

(iii) Use a clock, watch or other timing device to record time intervals (e.g., 30 seconds) and record these intervals by making a small mark on the drum each interval. These marks must be made as the drum revolves since the drum doesn't turn at a constant speed. This is because the velocity of the drum depends on the rate of flow of water from the reservoir which is not constant since the water pressure lowers as the depth lowers, thus causing the drum to slow down.

(iv) The stylus assembly may be altered to conform to requirements of other experiments. For example, the following illustration suggests how the stylus might be connected for studies of the heartbeat of an anesthetized frog:



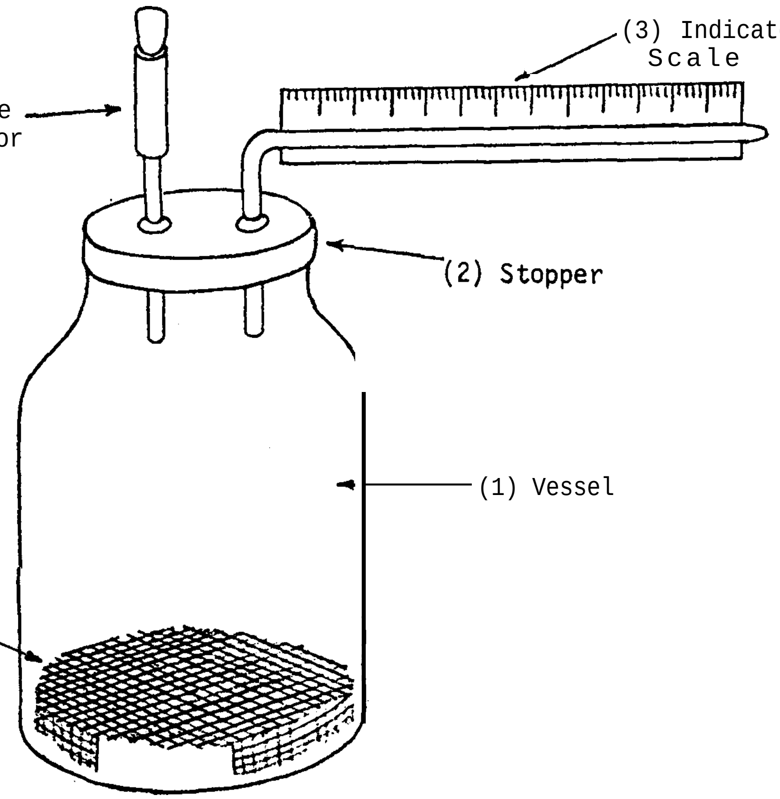
Here, as the frog's heart contracts and expands, its motion is translated into up and down movements of the stylus. Remember, the stylus acts as a lever, and the amount of movement of the pointer depends upon the relative lengths of the portions of the stylus to either side of the pivot point.

(v) The rate at which the drum revolves depends directly upon how fast the float lowers in the reservoir. Therefore, in order to make the float, and thus the drum, go faster, it is necessary either to enlarge the reservoir outflow hole or make several such holes. Conversely, to make the float and drum slower, a reservoir with a large cross-sectional area is needed. In this case, even though the float still drops the same distance per one revolution of the drum, more water must flow out of the larger can to cause it to drop the same distance as in a smaller can.

(vi) If, for any reason, it is necessary that the drum turn two or more consecutive revolutions, remember that the reservoir must be deep enough to allow the float to drop the additional distance required. To be precise, for each revolution of the drum, the float must lower a distance equal to the circumference of the small can which the drive string is wrapped around.

B. VOLUMETER

Bl. Volumeter



a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Vessel	1	Wide Mouth Glass Jar (A)	Size depends on organism to be studied
(2) Stopper	1	2-Hole Rubber or Plastic Stopper or Screw Cap (B)	To fit vessel
(3) Indicator and Scale	1	Glass Tubing (C)	30 cm long, 0.75 cm outside diameter, 0.5 cm inside diameter
	1	Stiff Paper Strip (D)	20 cm x 2 cm
(4) Pressure Regulator	1	Glass Tubing (E)	4 cm long, 0.75 cm outside diameter, 0.5 cm inside diameter
	1	Rubber Tubing (F)	4 cm long, 0.75 cm inside diameter
	1	Tapered Wood or Glass Rod Plug (G)	To fit rubber tubing

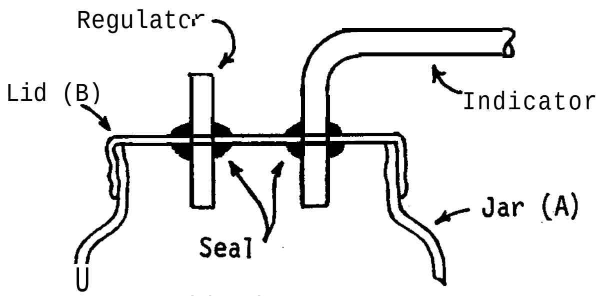
(5) Mesh Stand 1 Wire Mesh (H) To fit vessel

b. Construction

(1) Vessel

Almost any container (A) from a vial, to a test tube, to a jar will suffice. It must be a convenient size for the organism to be studied - a liter jar would not be used for small insects - and should have a tight-fitting lid or stopper.

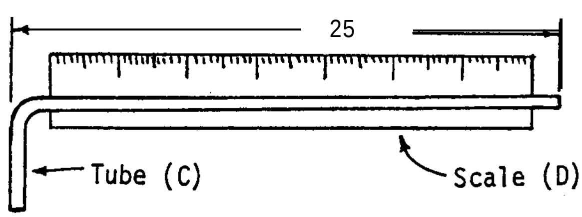
(2) Stopper



Side View
(Cross-section)

Depending on what vessel is used, the stopper (B) could be a two-hole rubber stopper or a tight-fitting screw cap with two holes drilled in for glass tubing. If a jar lid is used, the openings must be sealed with clay or paraffin after tbe indicator and pressure regulator have been inserted to prevent gas leak. Seal the underside, also.

(3) Indicator and Scale

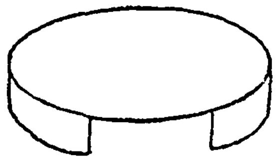


Bend a piece of glass tubing (C) at a 90° angle as indicated. Cement a paper scale (D) to the long arm, and add a drop of colored detergent solution to serve as an indicator.

(4) Pressure Regulator

This is a piece of glass tubing (E), topped with a section of rubber tubing (F) and a plug (G). To regulate the position of the indicator, one simply releases the plug for a short period of time.

(5) Wire Mesh



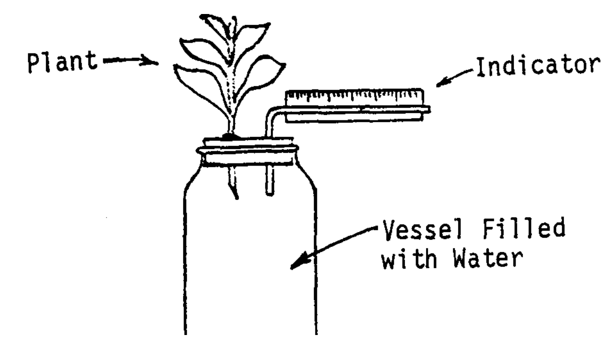
Fold to
This Shape

Cut the wire mesh (H) slightly larger than the diameter of the vessel. Bend the ends down to form a support on which the organism will be placed.

C. Notes

(i) If one wishes to study the oxygen uptake per unit time of an animal, a CO_2 absorber such as KOH should be placed in the vessel under the wire mesh support. As the animal respires, O_2 is taken up and pressure in the tube falls, causing the bubble to move toward the vessel. If one knows the size bore in the tubing, then one can compute the volume of gas being exchanged by noting the distance that the indicator moves per unit time.

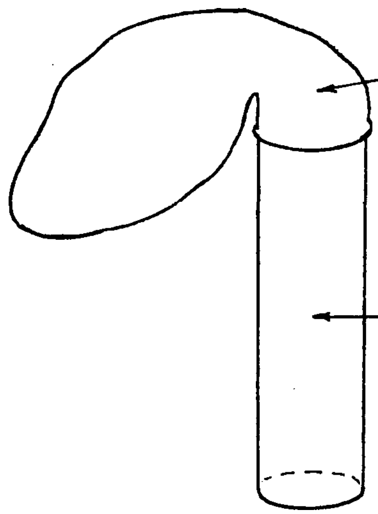
(ii) Transpiration may be measured by removing the pressure regulator from the top and inserting a broad-leaved plant cutting into the opening and sealing the joint with clay. Fill the container with water. Have the indicator bubble start at the open end of the sidearm tube. Allow the water to reach room temperature before setting the indicator bubble.



Volumeter Used as a
Transpirometer

C. FERMENTATION TUBES

Cl. Balloon Fermentation Tube



a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Vial	1	Medicine Vial, Test Tube, or Small Bottle (A)	50 ml capacity
(2) Balloon	1	Balloon (B)	To fit vial opening

b. Construction

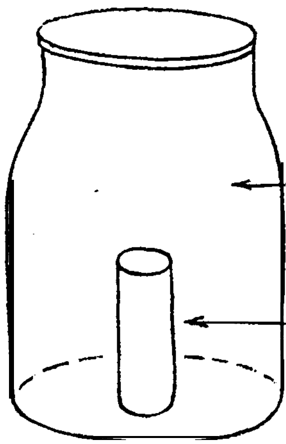
(1) Vial — Any small container (A) with a mouth narrow enough to stretch the open end of the balloon over will suffice.

(2) Balloon — Fit the open end of the balloon (B) over the bottle.

c. Notes

(i) Fill the vial with a yeast-sugar solution before attaching the balloon. As CO_2 is given off, it collects in the balloon from which it can be taken for analysis.

C2. Durham Fermentation Tube



a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Jar	1	Wide-mouthed Glass Jar or Beaker (A)	500 ml capacity
(2) Vial	1	Medicine Vial or Test Tube (B)	50 ml capacity

b. Construction

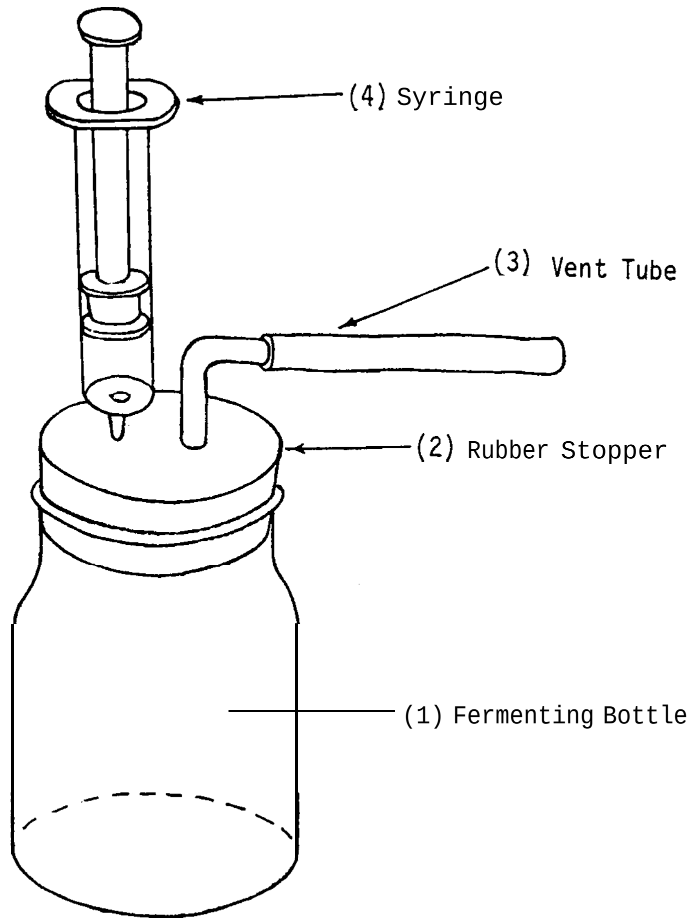
(1) Jar — Any large wide-mouthed jar (A) or beaker will do. It must be large enough so that the small vial (B) can be covered completely with fermenting solution.

(2) Vial — The vial (B) should easily fit inside the jar where it can be completely submerged in solution.

C. Notes

(i) Fill both the jar and vial with a sugar-yeast solution. Place a finger over the open end of the vial, and invert it into the solution in the jar. As carbon dioxide is given off, some will be collected in the vial. This is useful for measuring relative amounts and rates of CO_2 production.

C3. Syringe Fermentation Tube



a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Fermenting Bottle	1	Wide-mouth Jar (A)	300-500 ml capacity
(2) Rubber Stopper	1	Z-Hole Rubber Stopper (B)	To fit fermenting bottle opening
(3) Vent Tube	1	Glass Tube (C)	6 cm long, 0.5 cm outside diameter
	1	Rubber Tube (D)	100 cm long, 0.5 cm inside diameter, 25-50 ml
	1	Plastic Syringe (E)	25-50 ml capacity

b. Construction

(1) Fermenting Bottle

Select a large glass or plastic container (A) with a wide mouth.

(2) Rubber Stopper

A two-hole rubber stopper (B) is needed to seal the bottle opening.

(3) Vent Tube

Bend the glass tube (C) to a right (90°) angle and insert it into one of the holes in the rubber stopper (B). Attach the rubber tubing (D) to the other end of the glass tube.

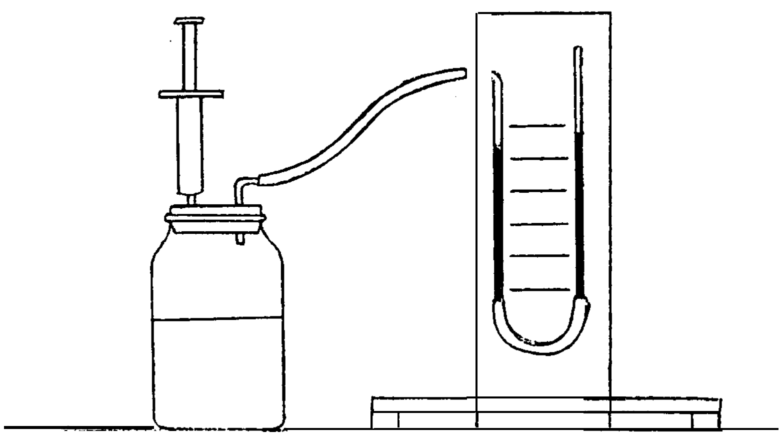
(4) Syringe

Insert the syringe nozzle (E) into the remaining hole of the stopper.

c. Notes

(i) One method of use for the syringe fermentation tube is as follows: Seal off the vent tube with a clamp or wood plug. Put about 250 ml of yeast solution in the fermenting bottle and put a known amount and concentration (e.g., 25 ml of 0.1 M) of glucose solution in the syringe. Inject the sugar water into the yeast solution and collect the carbon dioxide given off in the syringe.

(ii) Since it is difficult to accurately measure the amount of gas given off by the method described in (i) above, a further refinement is as follows: Connect the free end of the vent tube to a U-tube manometer (see VIII/Dl). Fill the fermenting bottle with yeast solution, and add a measured amount of sugar water. As carbon dioxide is given off, continually raise the syringe plunger so as to keep the two columns of the manometer equal height. Continue this until gas is no longer evolved. When the gas has stopped evolving, the amount of gas trapped in the syringe will be a very accurate measure of the total amount of gas given off since use of the manometer insures that pressure in the rest of the system is maintained at the original level.



Fermentation Tube and
Manometer Combination

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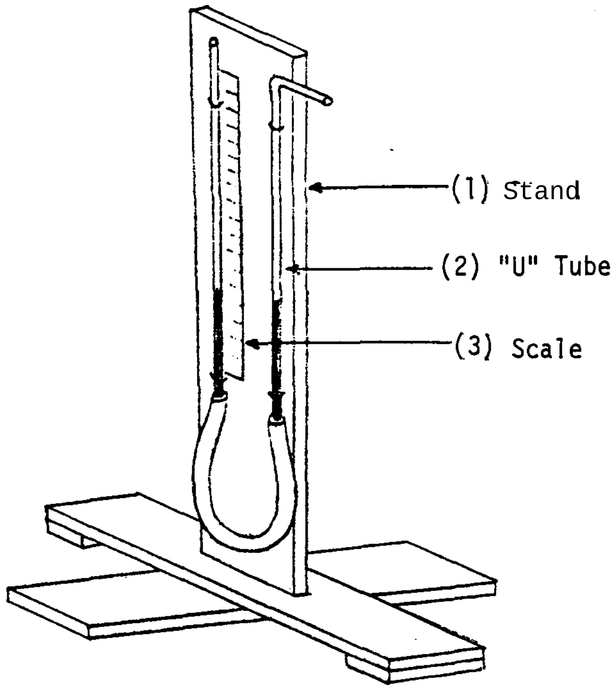
D. MANOMETER

D1. Manometer

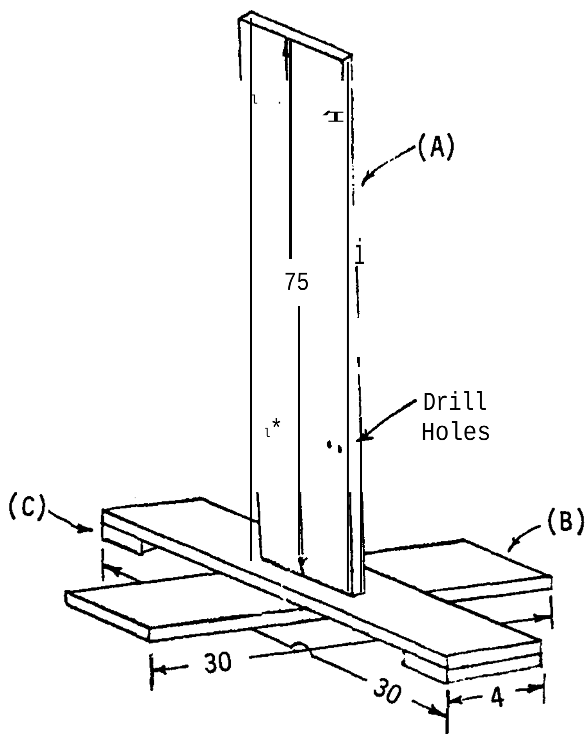


a. Materials Required

Components	Qu	Items Required	Dimensions
(1) Stand	1	Wood (A)	75 cm x 8 cm x 2 cm
	2	Wood (B)	30 cm x 4 cm x 2 cm
	2	Wood (C)	4 cm x 4 cm x 2 cm
(2) "U" Tube	2	Glass Tubing (D)	60 cm long, 0.75 cm outside diameter, 0.5 cm inside diameter
	1	Rubber Tubing (E)	50 cm long, 0.7 cm inside diameter
	4	Fine Wire (F)	8 cm long
(3) Scale	1	Graph Paper (G)	40 cm long, 2 cm wide

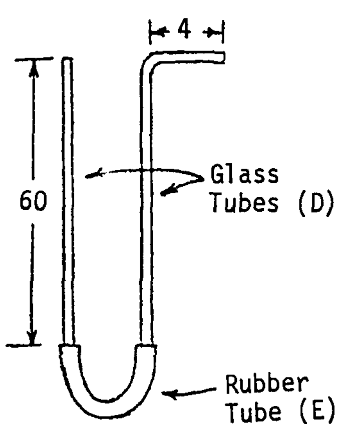
b. Construction

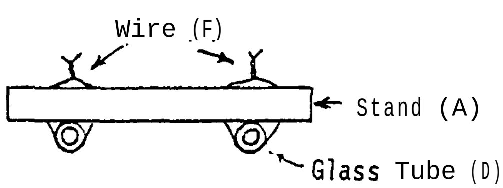
(1) Stand



Nail two pieces of wood (B) together at right angles to form the base of the stand. Nail the square blocks (C) under the upper board to provide stability. Finally, nail the other board (A) into an upright position on the base. Drill four pairs of small holes (0.2 cm in diameter) into the upright in such a position that each pair of holes will be in line with the position of the "U" tube when it is in place.

(2) "U" Tube





Top View

Heat one of the pieces of glass tubing (D) about 4 cm from one end and bend it to a right (90°) angle. Attach the rubber tubing (E) to the end of each piece of glass tubing (D). Fasten this "U" tube to the stand upright (A) by passing the fine wires (F) around the tubing, through the holes in the upright, and twisting the wires tight to hold the tubes in place. Do not fasten the straight tube too tight in order to allow it to slide up and down to adjust the height of the indicator liquid,

(3) Scale

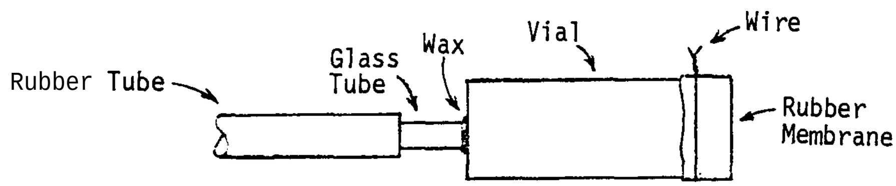
Glue or tape a piece of graph paper (G) between the two tubes to serve as a scale. Suitable scales can also be made by hand with plain paper and a rule.

C. Notes

(i) Use the manometer to detect and measure changes in pressure. To do so, it must be half filled with an indicator solution like colored water (use food coloring or ink) which serves as well as anything as an indicator. In normal usage, a rubber tube is used to connect the manometer to a closed system in which the pressure is changing (e.g., a jar containing a yeast-sugar solution or a jar containing a respiring animal with KOH to absorb the CO_2 given off. With the yeast solution, pressure in the jar will increase as the yeast oxidize the sugar. See VIII/C3 for further detail. In the case of the respiring animal, pressure will decrease as it takes up O_2 and gives up CO_2 which is taken up by the KOH.). As pressure changes, the indicator solution will move up or down depending on the direction of the pressure change.

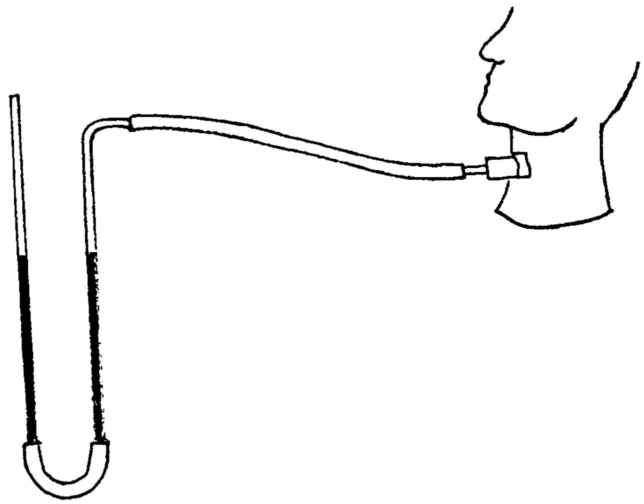
(ii) A detailed, specific example of the use of the manometer is as follows:

Drill a hole in the bottom of a plastic medicine vial. This hole needs to be large enough to insert a short piece of glass tubing (5 cm long). Attach a piece of rubber tubing (100 cm) to the glass tube, and insert the glass tube into the hole in the bottom of the vial. Seal the joint with melted wax from a candle. Stretch a rubber membrane or piece of toy balloon over the open end of the vial and fasten it securely with a string or rubber band to hold the membrane on the vial.



Side View

Attach the end of the rubber tubing to the bent piece of glass tubing on the manometer "U" tube. Slide the straight tube up or down to make the height of the indicator solution the same in both tubes. Place the rubber membrane against the carotid artery of the throat. A pulse can be seen by the rythmic rise and fall of the indicator solution. (See illustration on next page.)

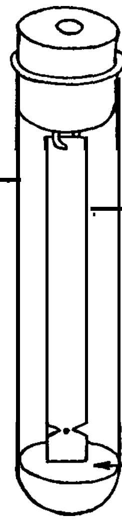


(iii) The manometer may be made from a single piece of glass tubing by bending it in a flame to a 180° angle. While this eliminates the need for a rubber tube, it also eliminates the possibility of adjusting the heights of the indicator solution.

(iv) Further instruction in the use of the manometer may be found in the Nuffield O-Level Biology, Teacher's Guide III, p 34, and the BSCS Blue Version text, p L8, L95, among other sources.

E. CHROMATOGRAPHY APPARATUS

El. Chromatographic Device



a. Materials Required

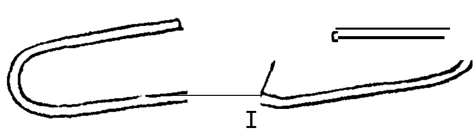
Components	Qu	Items Required	Dimensions
(1) Test Tube	1	Test Tube (A)	15 cm long, 2 cm diameter
(2) Paper Holder	1	l-Hole Stopper (B)	To fit test tube
	1	Paper Clip (C)	--
(3) Paper	1	Filter Paper (D)	1 or 2 cm shorter than the length of the test tube
(4) Solvent	--	Acetone (E)	2 ml
	--	Petroleum Ether (F)	23 ml

b. Construction

(1) Test Tube

Use a rack or holder to support the test tube (A).

(2) Paper Holder



Open up the paper clip (C) and cut it as shown. A short piece of wire can be bent to the same shape, too. Punch the U-shaped piece of clip or wire through one end of the paper (D) and force the ends of the wire up into the one-hole stopper (B) until it is held fast.

(3) Paper

Use standard filter paper (D) or chromatography paper if it is available. Cut it about 1.0 cm wide.

(4) Solvent

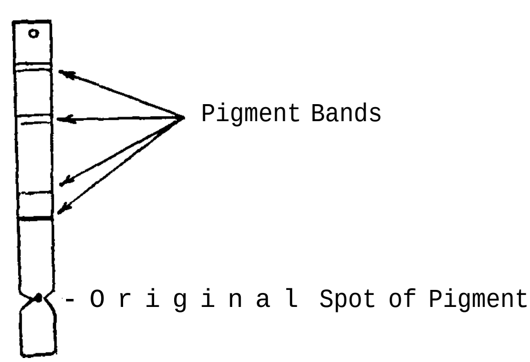
Mix the acetone (E) and petroleum ether (F) and add the mixture to the test tube.

C. Notes

(i) This chromatographic device is used to separate plant pigments. To prepare the sample of pigments, grind several heavily pigmented plant leaves together with some fine sand and about 5 ml of acetone. When thoroughly ground, filter this mixture through filter paper. Alternatively, heat several finely chopped leaves in about 5 - 10 ml of alcohol in a water bath. Do not heat the alcohol directly. Heat this mixture until the liquid is dark green.

Avoid both touching the surface of the paper with the fingers (oil affects the results) and having the paper touch the table where the pigment's to be placed. Thus, support the paper strip between two pencils or other small objects. About 1 cm from one end of the paper, place a small drop of pigment. This is most easily done with a fine-pointed pipette or a hypodermic syringe. When the first drop is dry, add another. Try to make the spot as small and as densely colored as possible. At least four drops should be placed one atop the other.

Make a notch in the paper on each side of the spot to mark its position. Attach the paper to the wire and put the wire into the stopper. Insert the paper holder in the test tube so that the end of the paper is in the solvent with the spot about 0.5 cm above the level of the liquid. It may be necessary to adjust the paper holder to keep the paper at the proper level. When the upper level of the solvent has soaked into the paper almost to the paper holder wire, remove the chromatogram and allow it to dry. The bands of color can be studied when the chromatogram is thoroughly dry. A number of excellent references exist describing additional exercises and information for chromatography.



Finished Chromatogram