IN THE LABORATORY

Methods of Automatic Watering of Plants

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The usual method of surface watering of bench crops and potted plants is a time-consuming operation, and, because of lack of sufficient labor, plants in many research investigations are not adequately watered. In recent years several methods (*1–5*) of automatic watering of bench crops and potted plants have been developed for research and commercial use in the field of floriculture. The constant water level system is the simplest and has worked well in the production of all types of florists' crops.

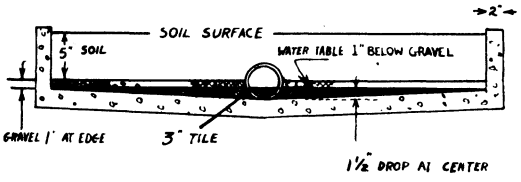

FIG. 1. Arrangement of bench for automatic watering of bench crops.

Bench crops. Tile is placed lengthwise in the middle of a water-tight bench (Fig. 1). Pea gravel to a depth of 1″ at the side of the bench is leveled, and the bench is filled with soil. A constant water table is maintained in the bottom of the bench about ¾–1″ below the soil by means of a float valve in a tank on the side of the bench. The water moves through the soil by capillarity.

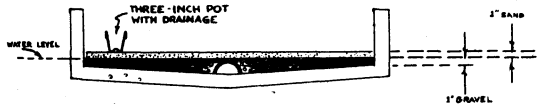

FIG. 2. Bench with pot on sand for automatic watering by the constant water level method.

Potted plants. The plants are placed on a layer of sand in a water-tight bench with a constant water table 1″ below the pot (Fig. 2). The water table should not contact the bottom of the pot. Water moves from the water table in the bottom of the bench through the sand, through the walls of the pot, and through the soil in the pot. As water is removed from the soil by plants and by evaporation, more water moves into the soil by capillarity. If the plants do not obtain sufficient water automatically, more sand is added and the pots are plunged; large pots generally have to be partly plunged.

Several other automatic and semiautomatic methods of watering plants are also described in the references presented.

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Use of Thin Kidney Slices and Isolated Renal Tubules for Direct Study of Cellular Transport Kinetics

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The techniques described here were developed with the intention of examining active cellular transport by a simple, direct method which would obviate the more complicated tissue culture procedures. Thin slices of kidney or kidney fragments were obtained as for the Warburg manometric techniques, but here the kinetics of phenol red concentration in the lumina of renal tubules was observed *in vitro* rather than the nature of gas exchange by the excised tissue. It was hoped that this method would be useful in rapid screening to test the possible effects of chemical and physical agents on renal tubular secretion and also permit an examination of the nature of the secretory process itself through a study of metabolic competition and an analysis of specific stimulants and depressants of dye transport.

Active cellular transport is characterized as the performance of work empowered by the expenditure of some fraction of the energy in cellular metabolic reactions. It seems to be a general property of living organisms to produce striking differences in concentrations across their boundaries by the constant expenditure of energy to maintain steady states sometimes far removed from chemical equilibrium. Hill (*3*) states: "Throughout we are involved, not with genuine equilibria, but with conditions maintained constant by delicate governors and by a continual expenditure of energy. How that energy is supplied, how it is utilized to maintain the structure and the organization, is, I think, the major problem of biophysics." Such examples of steady state in the renal tubule are found in the selective elimination of metabolic end-products as urea and uric acid in the lower vertebrates, and of diodrast, *p*-amino hippuric acid, and phenol red by all the vertebrate kidneys. The selective reab-

sorption of amino acids, and particularly of sugars, by the cells of the renal tubules is well known. In the latter instance glucose concentrations of 250–300 mg% in the glomerular filtrate are reduced to zero in the collecting ducts as the urine travels the length of the renal tubule. Cells in the distal convoluted tubule finally achieve a steady state in which the concentration of sugar on one side in the tubular urine is zero, while the peritubular fluid on the other side of the cell may contain glucose in concentrations up to 300 mg%.

The selective transport of phenol red from peritubular circulation to tubular urine has been described by perfusion experiments and clearance techniques in representatives of all the vertebrate classes. Its tubular maximum has been carefully measured, and in every animal studied the essential characteristics of its secretory process are similar. As such, it constitutes a good example for study as a representative of those substances selectively eliminated by active cellular transport (*2*).

The procedures employed here are extremely simple. Thin slices of kidney tissue were prepared by free section with a razor blade or by guided section with the tissue lightly compressed between frosted glass plates (*6*). The slices were then placed immediately in a solution of precisely known chemical constitution containing between 1 and 2.5 mg% phenol red and actively oxygenated. Customarily, at the end of 5, 15, and 30 min, the slices were removed temporarily from the solution and examined without a cover slip under low power of the microscope to detect whether phenol red had or had not been concentrated in the tubular lumen and, if so, to what degree.

A satisfactory preparation was obtained by sectioning the kidneys of small frogs to obtain 4 or 5 longitudinal slices from each kidney. The following solution has been found to produce optimal phenol red concentrations in controls: 0.580% NaCl (100.0 mM), 0.019% KCl (2.5 mM), 0.022% $CaCl_2$ (1.5 mM), 0.020% $MgCl_2$ (1.0 mM), 0.126% $NaHCO_3$ (15.0 mM), 0.007% NaH_2PO_4 (0.5 mM), 0.054% $C_6H_{12}O_6$ (3.0 mM), phenol red: 1–2.5 mg %. Each of the foregoing components has been individually tested for optimal concentrations. The relatively high bicarbonate content is particularly important in inducing active phenol red transport. The solution without glucose is stable indefinitely. Customarily, glucose is added to the previously prepared solution on the day of the experiment. Kidney slices from frogs and other cold-blooded animals show definite phenol red concentration in some tubular lumina within 5 min after immersion in this medium at room temperature with oxygen actively bubbling through. Progressive increases both in number of tubules concentrating the dye and in intensity of the red color within the lumina will be noted for hours. The cells of the renal tubule remain colorless or take on a light yellow cast, while the color in the lumen ranges from light pink to very deep red, depending on the level of the tubule being examined, the distance of the tubule from the exposed surface, etc.

Microcolorimetric readings have been made with known concentrations of phenol red in glass capillary tubes drawn to the approximate diameter of renal tubules. When placed alongside the slice preparation, these comparison standards indicated that in the distal portions of the tubule concentrations as high as 50 times or 250 mg% were obtained.

In a second procedure it was found that renal tubules could be isolated for individual study. Fish kidneys seem to have little cementing substance and it was noticed that upon transferring fragments of fish (sculpin, flounder, and trout) kidneys to the sustaining medium the individual tubules separated from one another as a result of the mechanical agitation of oxygen actively bubbling through the solution. This proved to be a very satisfactory preparation characterized by a high rate of dye transfer, presumably due to the relatively complete exposure of the individual tubules to the solution.

Three variations in procedure have been employed: (1) The excised tissue was placed directly in the oxygenated phenol red solution, which already contained the experimental agent, and the effect of the variable on the kinetics of dye transport in inhibiting or stimulating phenol red concentration in the renal tubule lumen was noted by comparison with a control. (2) Phenol red was allowed to be concentrated in the tubule lumen before the tissue was exposed to the experimental variable and the effect on dye retention in the lumen subsequently noted. (3) The kidney tissue was exposed to the experimental factor before being immersed in the dye-containing solution. The first of these was found to be most useful.

The method has been used in this laboratory in a variety of problems. Some of these are: the effects of ionic unbalance, disturbances in osmotic equilibrium, temperature effects, narcosis and anoxia, competition and substitution in the dye transport system, iodoacetate, pyruvate, succinate, and lactate effects for energy source analyses and for screening the actions of specific pharmacological agents.

Kidneys from representatives of all classes of the vertebrates have been studied with these techniques. The method has not yet been successfully adapted to slices from the kidneys of mammals (rabbits, rats, and mice).

The chief criticism of the method described here relates to the unphysiological nature of the preparations. These same objections can be raised against measurements of respiration rates *in vitro* by kidney slices, minces, and homogenates. Renal clearance techniques have been developed which measure with great accuracy the various aspects of kidney function in normal unanesthetized animals and man. However, in the intact animal it is obviously impossible to test the effects of toxic enzyme inhibitors such as iodoacetate, cyanide, and fluoride, and it is frequently very difficult to separate the extrarenal side effects which might cause variations in renal blood flow, blood pressure, chemical composition of the blood, or other factors difficult to control with the kidney an integral part of the intact organism. This method obviates the necessity of tissue culture techniques so fruitfully utilized by Chambers and his group in studies made on cystic explants of the mesonephros of the chick and other embryos (*1*), and it more closely approximates

the high rate of dye transport characteristic of the intact kidney than is obtained with studies made on excised whole organs (*4, 5*). It can be recommended as a simple and useful procedure which might be profitably employed in a wide variety of studies on the physiological and biochemical nature of cellular function in renal tubules.

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Magnetic-Flea Agitating Device for Microtitration

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It is often desirable in microtitration studies to agitate the mixture while adding an ingredient. This can be accomplished in admirable fashion by the "magnetic flea"—a small iron powder magnetic core, surrounded by glass and caused to move up and down in the titrating vessel by an interrupted magnetic field.

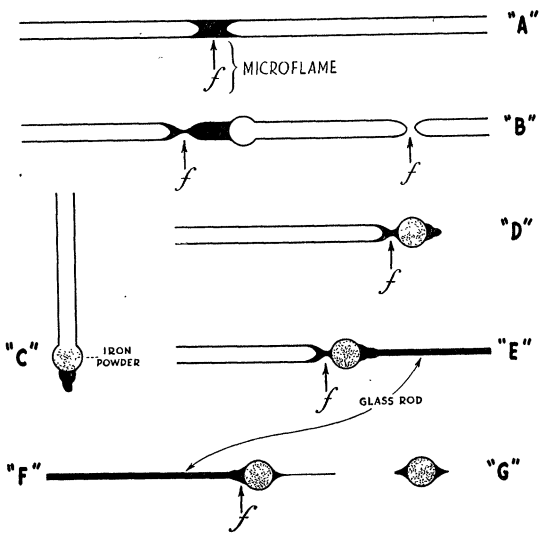

FIG. 1

The "magnetic flea" is made by taking a length of thick-walled Pyrex glass capillary tubing, which may vary in thickness from 0.75 to 1.5 mm o.d., and collapsing a portion of it near one end over a microflame (Fig. 1A). A small bubble is blown just above the collapsed portion (B). The open end of the capillary is now pushed into a bottle of iron powder (not filings) and inverted to allow the powder to fall through the tube and completely fill the bubble (C). The bubble is then sealed off from the rest of the capillary (D). By sealing a small glass Pyrex rod to alternate ends of the bubble, the excess glass may be heated and removed from the opposite ends of the glass bubble (E, F).

A very easy alternate method for making the "magnetic flea" is to proceed through the steps taken in making a Cartesian diver in a diver jig (*1*).

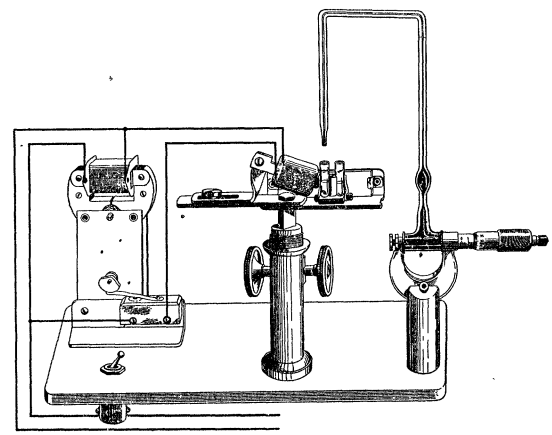

FIG. 2

Before the neck of the Cartesian diver is cut, it is essentially as shown in Fig. 1C. The capillary is cut approximately 50 mm from the bubble, filled with iron powder, and sealed off near the bubble. The rest of the procedure is as outlined. The finished "flea" should look like Fig. 1G.

The agitating device consists of a synchronous motor,[1] the shaft of which is fitted with an eccentric cam which operates a microswitch[2] which in turn opens and closes an electric circuit, passing through an electromagnet (Fig. 2). This provides an interrupted magnetic field which causes the "magnetic flea" to rise and fall in the titration vessel. The electromagnet may be fashioned from a coil of a 110-v, 60-cycle relay. The electrical circuit is outlined in Fig. 2, in which a convenient assembly incorporating a Scholander microburette (*2*) is shown. The entire assembly placed in front of the opal glass of an X-ray viewer gives maximum comfort in matching colors during titration.

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[1] Available in several r.p.m. from Hansen Manufacturing Company, Inc., Princeton, Indiana.

[2] Mu Switch, Canton, Massachusetts.