
In the Laboratory

A Photographic Method for Recording Ureteral Kinetics in Situ

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A device for recording ureteral kinetics in situ has been described by Trattner (3). During a series of experiments on dogs using a similar apparatus, several undesirable features became apparent. A membrane tambour yields a tracing which is approximately a logarithmic function of the pressure applied; therefore, the sensitivity is reduced appreciably when working with increasing pressures. The elasticity of a membrane exerts a similar effect on fluid displacements so that successive increments or decrements of liquid are not recorded isometrically. The fling of a lever arm further adds to the inaccuracy of the method. Friction of the writing style on a smoked drum became perceptible when working with small animals. The purpose of this paper is to describe a recording unit to replace the membrane tambour with its lever and writing style.

Fig. 1 shows schematically the construction of such a unit, which consists of a small test tube to which two soft-glass tubes of about a 2-mm. bore are affixed.

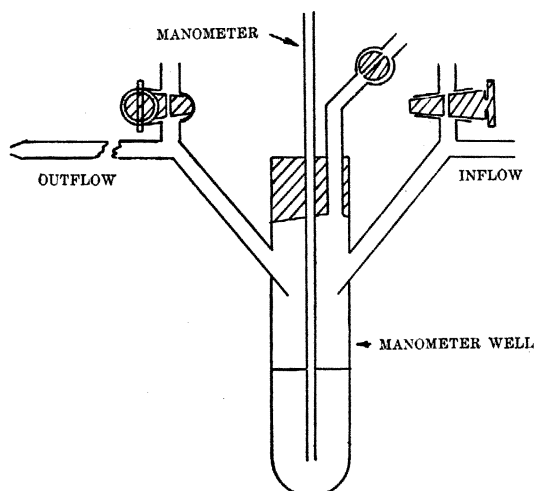


FIG. 1.

These serve as inflow and outflow conduits, respectively. As in Trattner's apparatus, the outflow orifice is constricted so that the ureter is working against a resistance which can be altered at will by using different-sized hypodermic needles, or an aperture of constant bore connected by a rubber tube a few centi-

meters long so that it can be raised or lowered. Valves are placed at strategic points to facilitate expulsion of air bubbles so that an all-liquid system can be realized. The principle of this unit is based on Pascal's Law, which states that pressure exerted at any point upon a confined liquid is transmitted undiminished in all directions. A rubber stopper in the open end of the test tube supports a 1-mm., even-bore, soft-glass tube of convenient length which serves as a manometer. Such a small-caliber manometer allows measurement of 1 mm. or less of liquid displacement. The stopper is designed with a depression in the bottom so that a valve for releasing air bubbles from the manometer well (test-tube section) can be incorporated in the unit. The manometer well contains bromobenzene (phenyl bromide), this compound being chosen because it has a density of 1.4991 (1) and a surface tension of about one-third that of water; furthermore, it is immiscible with water (0.0446 grams are soluble in 100 cc. of water at room temperature).

An excellent method for recording the excursions of a liquid in a manometer photographically has been reported by Kirchhof and David (2). However, a method which has been found suitable is to saturate bromobenzene with methyl red. This solution is photographically opaque to bromide paper and will cast a shadow when the manometer is juxtaposed to a thin slit in a light-tight box and adequate illumination is provided. For this purpose a 500-watt, clear glass tungsten bulb is satisfactory. To obtain a continuous tracing the bromide paper is fastened to a kymograph drum within the box with scotch tape. A record secured in this fashion is shown in Fig. 2.

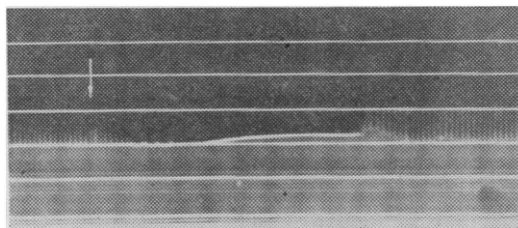


FIG. 2.

Prior to recording, the optical system is aligned and adjusted by trial and error. The distance between the light source and the manometer, as well as the distance between the manometer and the paper, must be regulated to achieve sharp contrast. The unit is filled with physiological saline solution. Hydrostatic pressure from the liquid in the inflow and outflow arms forces bromobenzene into the manometer, the meniscus of

which establishes a base or zero pressure line. A catheter from the ureter filled with either physiological saline solution or urine is connected to the apparatus; any bubbles which appear during this maneuver can be evacuated by means of the valve on the inflow conduit.

A more complete study of the ureter is possible when an automatic drop recorder is appended to the unit.

It has been found that intravenous infusion of a 5- or 10-per cent dextrose solution during the course of an experiment insures a motile ureter, whereas hypodermic administration does not always promote a diuresis adequate to stimulate the ureter.

References

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The Estimation of Streptomycin in Body Fluids

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Increasing interest in streptomycin as a chemotherapeutic agent has made apparent the need for a simple method for estimating the concentration of this drug in body fluids.

Stebbins and Robinson (3) proposed an agar cup-plate method of assay employing *Staphylococcus aureus* SM as the test organism. This method, which measures concentrations of from 1 to 20 micrograms/ml., requires materials and techniques which may not be readily available in a small laboratory.

A 3-hour turbidimetric assay, which had been giving accurate and reproducible results when aqueous or buffered solutions of streptomycin salts were assayed, was investigated. This method, which employs a non-encapsulated strain of *Klebsiella pneumonia*, was found to be unsatisfactory because of the stimulating effect exerted by body fluids on the test organism.

A survey of our stock cultures demonstrated that several were sensitive to streptomycin when examined by the serial dilution method, employing a modified medium consisting of peptone 1 per cent, beef extract 0.5 per cent, and sodium chloride 0.25 per cent adjusted to pH 7.8-8.0 with NaOH. Further investigations revealed that one of these, *Bacillus circulans*, was the most sensitive and gave accurate, reproducible results with various body fluids. Consequently, it was chosen as the test organism.

B. circulans is a mesophilic, motile, aerobic spore-bearing microorganism. It grows well at temperatures between 30° and 37° C., forming floccules which make the end point in the serial dilution test relatively easy to determine. It is sensitive to 0.15 microgram/ml. of streptomycin base. Broth cultures are quite stable and may be preserved in screw-cap bottles under refrigeration for periods of one month with no appreciable loss in sensitivity.

Technique of the test: Amounts (0.5 ml.) of the modified nutrient broth are placed in sterile Wasserman tubes and serial dilutions by halves made by adding 0.5 ml. of the fluid being tested to one of the tubes and carrying 0.5 ml. by serial dilution for the desired number of tubes. The first tube in the series contains 0.5 ml. of the solution under test only. A standard is prepared for comparison by diluting a streptomycin salt of known potency in broth to contain 10 micrograms of the base per milliliter. This standard is serially diluted in the same manner as the body fluid under test. One and one-half milliliter of a 1:100 dilution of the test organism in broth is then added to all tubes, after which they are incubated overnight. The last tube in which no growth occurs is considered the end point.

The concentration of streptomycin in the unknown is then determined by comparing the end point with that of the standard. An example is given in Table 1, in which it will be noted that the standard completely inhibited growth of *B. circulans* in the fifth tube.

TABLE 1

Fluid	Tube No's.						
	1	2	3	4	5	6	7
Standard	0	0	0	0	+	+	+
Serum	0	0	0	+	+	+	+
Urine 1:50	0	0	0	0	+	+	+

Since this represents 10 micrograms, the serum tested contains one-fourth as much, or 2.5 micrograms; the urine which caused complete inhibition in the fourth tube contained 5 micrograms \times 50 or 250 micrograms/ml. To determine lower potencies it is necessary to vary the dilution series of standard and unknown.

In a similar method (2), employing *Bacillus subtilis* for determining penicillin in body fluids, it was found that unexplained inhibitory substances often interfered, necessitating a control series for proper evaluation (1). To determine whether similar inhibitory factors were present against *B. circulans*, the sera from approximately a hundred individuals were tested. Only one of this number possessed inhibitory properties in a serum dilution of 1:4 for *B. circulans*. In a second series, the sera of 40 individuals being treated