Ureteral Catheter for Microinjection Tubing

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In the usual arrangement for microinjection with the Chambers or Fitz micromanipulators, the syringe is connected to the needle holder by fine metal tubing (*1*). This tubing works satisfactorily during the actual injection, where the amplitude of movement is small, but is subject to fairly frequent breakage during the greater flexions which occur in cleaning, filling, and adjusting the apparatus. When the microinjection apparatus is used separately from the micromanipulator, as it has been in many investigations involving semimicrotransplantation (*2*), or injection of materials into insects or embryos, the frequency of breakage is increased. In addition, the not inconsiderable mechanical resistance offered by the metal tubing interferes markedly with the delicacy of manual operation which can be attained. These obstacles are still more troublesome with a glass capillary connector (*3*). Furthermore, suitable metal tubing is difficult to obtain and repair and is expensive.

We find that the above difficulties can be overcome completely by substituting ureteral catheter tubing for the metal or glass connector. This type of catheter, which is made of plastic-impregnated nylon, is inexpensive and, though extremely flexible, is very resistant to pressure, stretching, kinking, and breakage.[1] Moreover, snug connections to both syringe and needle holder can be made very simply by way of No. 24 hypodermic needles (with the points cut off square and smoothed), providing the basal end of the needle holder is turned down to the standard taper of the hypodermic needle hub.

References

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Improving the Performance of the Flame Photometer

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The following modifications of the Model 18 Flame Photometer (manufactured by the Perkin-Elmer Corporation) have been necessary to bring the performance of the instrument up to an excellent degree of precision and reliability.

The flame characteristics were improved by placing a Bunsen burner inside the original Meeker burner and permanently removing the grid on top of the latter. The installation requires no machining of any kind (Fig. 1). The alteration resulted in a more steady flame with both bottled and Ohio natural gas, irrespective of the gas-jet size.

[1] Size "French 4" ureteral catheter, American Cystoscope Makers, Inc., 1241 Lafayette Avenue, New York City. These catheters are approximately 65 cm. long, 1.35 mm. o.d., and 0.5 mm. i.d.

Additional improvement was obtained by placing an ordinary 250-watt infrared lamp facing the right side of the photometer. The bulb serves to warm the air entering the atomizer by heating an improvized air filter and also warms

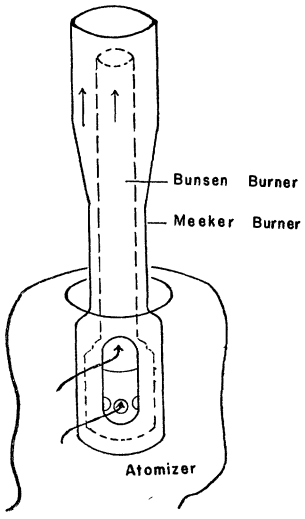

FIG. 1

the atomizer directly, as the door to its compartment is held permanently open. The increase in temperature permits a more rapid attainment of equilibrium within the vessel because of a greater degree of vaporization and a reduction in the excess condensation on the walls of the atomizer.

A Sensitive Cylinder-Plate Assay for Bacitracin

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A cylinder-plate assay is desirable for practically any type of antibiotic work because it is not readily disturbed by slight bacterial contamination, it readily reduces to routine handling by technicians, and it is less time consuming than other assays.

The assay of bacitracin previously described by Johnson, *et al.* (*2*) was a 72-hour dilution assay using *Streptococcus hemolyticus* (Chanin strain). For our preliminary research work on bacitracin, *Staphylococcus aureus* was used as a cylinder-plate assay organism, but its relative insensitivity and rather "fuzzy-edged" zones limited its usefulness.

During the examination of the spectrum of bacitracin four rather sensitive organisms were found which gave good growth on a plate: *Micrococcus flavus*, isolated from the laboratory air; *Gaffkya tetragena* ATCC #6007; *Sarcina flava* ATCC #540; and *Sarcina lutea* ATCC #272. Experiments using each one of these organisms showed *M. flavus* to be superior because of the smoothness of growth and sharp, even edges of the inhibition zones.

The assay as finally developed uses *M. flavus*[1] as the assay organism; a culture of this has been sent to the American Type Culture Collection (ATCC #10240). The inoculum is developed by inoculating 60 ml. of "Bacto Yeast Beef Broth"[2] (*1, 3*) in a 300-ml. Erlenmeyer flask by means of a loop from an agar slant culture. The flask is incubated at 37°C. while being shaken on a machine at 126 oscillations/minute with a 1½-inch stroke. This procedure gives a heavy growth of organisms; static inoculum culture gives poor and grainy plate growth.

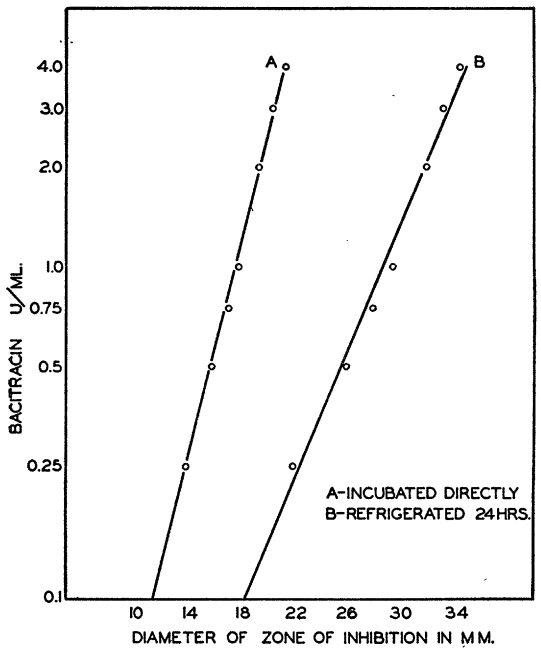

FIG. 1. Response of *M. flavus* to graduated doses of bacitracin on a cylinder-plate assay procedure: A, response curve when plates are incubated at 37°C. immediately after dosing; B, response curve when plates are held in refrigerator 24 hours before incubating at 37°C.

Plates are poured aseptically in the morning, using a Brewer Automatic Pipetting Machine, with 20 ml. of "Bacto Penassay Seed Agar"[3] (*3*). By this method a large number of plates can be rapidly and accurately poured.

In the afternoon the seed layer is poured, using the same kind of agar as the base layer, inoculated with 1 per cent of the 18-hour inoculum broth. Four ml. of seeded agar at 50°C. is used per plate, and the plate rapidly tipped to distribute the layer evenly. Stainless-steel cylinders are placed with a dispenser (made by Robert D. Shaw, Bloomfield, Connecticut) after the agar has gelled but before it has become very hard (15–20 minutes after pouring).

The cylinders are filled with the sample, diluted to approximately 2 units/ml. with sterile, 1 per cent phosphate buffer[4] of pH 6.5. Two cylinders on each plate are filled with a standard bacitracin solution of 2 units/ml., and two cylinders on the same plate are filled with the unknown solution, after which the plates are covered with "Coors" porous porcelain covers, not raised. The plates are incubated directly at 37°C. for 16–18 hours. The zones of inhibition are then read by projecting the image of the plate onto a screen by means of an overhead-type lantern-slide projector. The screen is ruled, and the diameters of the inhibition zones are read and recorded. The units/ml. for each sample are then calculated on a slide rule constructed from a standard curve prepared previously by plotting on semilog paper averages of 100 readings/dosage level, 0.1–4 units/ml. (Fig. 1). That the day-to-day variation on the curve is not usually excessive is demonstrated by Table 1. The variation when responses with different batches of media are compared may be slightly greater.

TABLE 1

DAY-TO-DAY RESPONSE OF *Micrococcus flavus* TO BACITRACIN

	Average diameter of zone of inhibition in mm.				
	0.1 u./ml.	0.5 u./ml.	1.0 u./ml.	2.0 u./ml.	4.0 u./ml.
1st day	11.3	16.2	17.7	19.3	21,0
2nd "	11.6	16.1	18.1	19.4	21.6
3rd "	11.2	16.3	17.9	19.3	21.4
Average	11.3	16.2	17.9	19.3	21.3

The slope of the curve is less than for penicillin, since there is approximately a 1.7- to 1.9-mm. increase in zone size per doubling of dose. This slope can be increased to 3.0 or better by refrigeration of the dosed plates 16–18 hours before incubation. The precision of the assay is not greatly increased, however, because the variation of zone diameters increases. The penicillin curve gives a slope of 3.2 without refrigeration. This difference in slope is a reflection of the relative speeds of diffusion of the two substances, the refrigeration procedure giving bacitracin a greater time for diffusion. For results in which reasonably high significance is needed, 4 plates (*i.e.* 8 zones) are averaged to give the assay; for final lots for clinical use, 8 plates are used.

The assay can be used for blood serum assays. Dilutions made up in fresh rabbit serum and in horse serum gave values within 10 per cent of the known. Attempts to extend the range of the assay below 0.1 unit/ml. by preliminary refrigeration have not given reliable results.

Common organic solvents used in recovery, such as butanol, ether, ethanol, etc., do not interfere with the assay when diluted 1/20 or greater. Bacterial contamination, unless excessive, does not interfere; bacitracin beer samples, for instance, are simply centrifuged for 20 minutes at 2,000 × g, which reduces the bacterial population in the supernatant sufficiently to allow a satisfactory assay.

[1] Identified by Robert S. Breed at the New York State Agricultural Experiment Station, Geneva, New York.

[2] Medium II of Schmidt and Moyer (*3*) as reported by Food and Drug Administration (*1*); manufactured by Difco Laboratories, Detroit, Michigan.

[3] Medium I of Schmidt and Moyer (*3*) as modified by Food and Drug Administration (*1*); manufactured by Difco Laboratories, Detroit, Michigan.

The composition of the buffer is: K_2HPO_4, 4.43 grams; KH_2PO_4, 5.57 grams; distilled water to 1 l.

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