Method For Recording Electrical Activity of the Isolated Heart

By MARVIN L. ZATZMAN and ROBERT L. RUSSELL

The simultaneous recording of electrical activity from two sites on an isolated mammalian heart by a simple and effective method is described. The use of agar-filled polyethylene tubing containing cotton wick electrodes offers distinct advantages compared to the usual platinum hook electrodes.

THE USUAL METHOD for recording electrical activity from isolated hearts has involved the use of platinum hooks (1, 2). An alternate method which has several advantageous features is described. While recording the atrial and ventricular ECG from isolated rabbit hearts in the Anderson coronary perfusion apparatus, it was found that platinum hook electrodes had limitations. Not only was there injury to the tissue but also breaks between the leads and the platinum hooks occurred repeatedly. To alleviate these occurrences, it was decided to employ cotton wick electrodes. Accordingly, the lower chamber of this unit as described by Anderson and Craver[1] (3) was altered to allow the introduction of these wicks (Fig. 1).

Two electrodes were positioned in each stopper. The wicks were made of cotton tufts saturated with the perfusing solution. Accordingly, each wick was tamped lightly into the end of the polyethylene tubing (PE 350, o.d. 0.157 in., i.d. 0.125 in.) which had been filled previously with agar (3–4% in mammalian Ringer's). Stainless steel wires or tantalum wires (0.025-in. diam.) were inserted into the agar-filled tubing for connection to a recorder with alligator clips. A piece of warmed PE 100 was crimped to the wire as a sleeve to keep the wire in place. Another adaptation is shown in the upper right corner of the figure. This was a hypodermic needle (No. 18, 1.5-in. length) inserted in the agar instead of the stainless steel wire, thereby making it possible to couple the electrode to the recorder with a banana plug.

In addition to the two openings in the lower chamber for the introduction of the stoppers containing the polyethylene tubing, a third opening or port was provided in the original apparatus. Consequently, the experimenter is enabled to insert a probe or forceps through the port in order to position the cotton wicks on specific areas of the heart as desired. After positioning the wicks, the port can be closed by inserting a stopper.

Appropriate recording apparatus was used to record atrial ECG and ventricular ECG. Coronary flow and heart rate may also be recorded simultaneously (Fig. 2). To determine any possible distorting effect of the wick electrodes on the ECG as compared to the record obtained with the usual hook electrodes, a simultaneous recording was obtained from a frog heart using both types of elec-

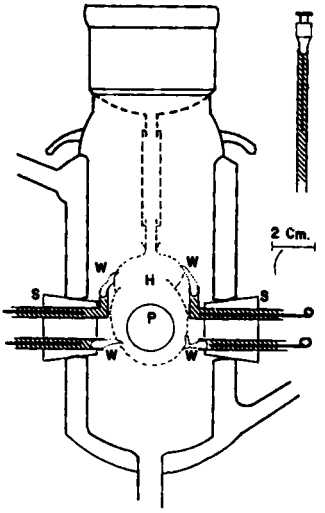

Fig. 1.—Modified lower chamber for recording of ECG from an isolated heart with wick electrodes. Key: W, cotton tuft; H, heart; S, stopper; P, port. Insert: Alternative connector to agar-electrode. (See text for details.)

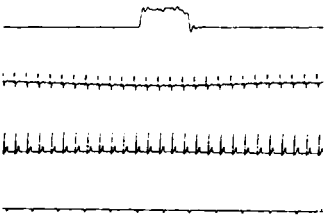

Fig. 2.—Electrical activity and coronary flow in an isolated rabbit heart. From above downward the record presents coronary flow, atrial electrocardiogram, ventricular electrocardiogram, and time in seconds. The coronary flow is indicated by the change in air pressure in a venous Statham pressure gage each time the coronary flow-meter empties.

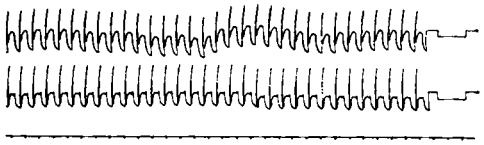

Fig. 3.—Simultaneous recording from the same sites on a frog heart. From above downward: ventricular electrical activity recorded through platinum hook electrodes, ventricular electrical activity recorded through wick electrodes, and time in seconds. Calibration indicates 2 mv.

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[1]Available from Metro Industries, Mineola, L. I., N. Y.

trodes (Fig. 3). Although the amplitude of the recorded potential was reduced somewhat using the wick electrodes, the wave forms are essentially the same. The mass of the cotton wick electrodes is extremely low and is a very flexible system, permitting the tip of the tuft to follow the movements of the heart. The wave form of the ECG of a control preparation indicates no change even after 1.5 hours of continuous recording.

Using the system described, it is possible to denote the difference between a control recording of an isolated cardiac preparation and that following experimental procedures, illustrating that the cotton wicks retain their position and that no injury is produced from their use.

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Microbiological Assay of Aspartocin in Pharmaceutical Preparations Containing Chlortetracycline

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An agar diffusion procedure, using *Staphylococcus epidermidis* ATCC No. 12,228 and 0.1 *M* calcium chloride solution as diluent for aspartocin standard and samples, was evaluated. Satisfactory standard curves for aspartocin were obtained with concentrations of compound ranging from 0.5 mcg./ml. to 50 mcg./ml.; chlortetracycline (CTC) did not interfere in the assay of aspartocin-CTC mixtures.

THE FERMENTATION, isolation, and antimicrobial properties of aspartocin have been previously reported (1–4). It is active primarily against Gram-positive bacteria; and although somewhat similar to amphomycin (5), aspartocin may be separated from this compound and identified by paper chromatography or electrophoresis (4).

For microbiological assay, an agar-plate method using *B. subtilis* and alkaline conditions was proposed (4); however, the tetracyclines are highly active against this test organism, and aspartocin-tetracycline mixtures would be difficult to assay under these conditions. With this in mind, the application of a tetracycline-resistant test organism was investigated.

Staphylococcus epidermidis ATCC #12,228 is routinely employed in this laboratory for neomycin assays, particularly with mixtures containing streptomycin and tetracycline, where resistance to these antibiotics (6) would be essential. Since the aspartocin antibacterial spectrum indicates that low concentrations inhibit staphylococci and streptococci *in vitro* (2), *Staphylococcus epidermidis* ATCC #12,228 was evaluated for use in aspartocin microbiological assays.

EXPERIMENTAL

The method outlined is a modification of FDA cylinder-plate procedures generally used in our laboratory for antibiotic assays; a more detailed description of antibiotic cylinder-plate methods appears in Grove and Randall (7).

Test Organism.—*Staphylococcus epidermidis* ATCC #12,228 (available from the American Type Culture Collection) is subcultured every 2 weeks on nutrient agar slants, incubated overnight at 30–35° and stored at 4°.

For inoculum, suspend the growth from a stock slant in sterile 0.9% saline solution and inoculate a Roux flask containing 200 ml. sterile Difco Penassay seed agar; incubate overnight at 30–35°. Harvest this growth with approximately 50 ml. sterile saline solution and store at 4°; this concentrated suspension may be used at least 5 to 6 days. Before the actual assay, determine what dilution of the concentrate will give 80% light transmission using a Lumetron model #400 colorimeter and 650 mμ filter. Use trial plates to determine the optimum concentration of diluted suspension for the assay (usually 0.5 to 1%).

Assay Medium.—Difco Penassay seed agar (antibiotic medium #1) is autoclaved 15 minutes at 121°, cooled, and adjusted to pH 8.0 with 4% NaOH solution.

Diluent.—The diluent was calcium chloride 0.1 *M* in distilled water for aspartocin standard and unknowns; anhydrous $CaCl_2$ (reagent grade) 11.1 Gm. dissolved in distilled water to make 1,000 ml. of solution (pH 5.8); it was autoclaved for 15 minutes at 121°.

Aspartocin Standard Curve.—Sodium aspartocin (Lot #31460-165A) with an assigned potency of 1,000 mcg./mg.[1] was used for the standard.

Weigh 50 to 100 mg. of the standard and dissolve in sufficient distilled water to obtain 1,000 mcg./ml. stock solution. Dilute further in calcium chloride solution to obtain 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78, and 0.39 mcg./ml.; the reference point solution is at 10 mcg./ml. The stock solution is held refrigerated and may be used at least 7 days.

Preparation of Test Plates.—The assay agar is cooled to about 45° and inoculated with the test organism. A 6-ml. volume of inoculated agar is distributed evenly into each flat-bottomed Petri dish (Pyrex #3162) equipped with an unglazed ceramic cover, and allowed to solidify on a perfectly level surface.

The agar is allowed to harden 15 to 20 minutes at room temperature before placing six stainless steel Penicylinders on each plate. These cylinder plates

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[1] Obtained from the Biochemical Research Section, Lederle Laboratories Division, American Cyanamid Co.