A Constant Temperature Tissue Bath. (25189)

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Use of isolated tissue preparations for study of their physiological and pharmacological reactions necessitates some device for maintenance of a stable environmental temperature, usually in the vicinity of 38°C. This is usually accomplished by means of a large reservoir of water containing a thermostat, heater and stirrer; the tissue bath is either immersed in this or water is pumped through a water jacket surrounding the bath. Both systems are expensive, and usually cumbersome because of the large water reservoir required for accurate temperature control.

Method. The tissue bath described here (Fig. 1) is compact, occupies little space and is inexpensive. It consists of a central compartment (a), in which the tissue is to be suspended in an appropriate saline solution, surrounded by a space (b) continuous below with a solvent reservoir (c) and closed above except for an air vent (d). The upper part of this space is surrounded by a condenser jacket (e) with inflow and outflow tubes (f and g). A drainage tube (h) passes from the bottom

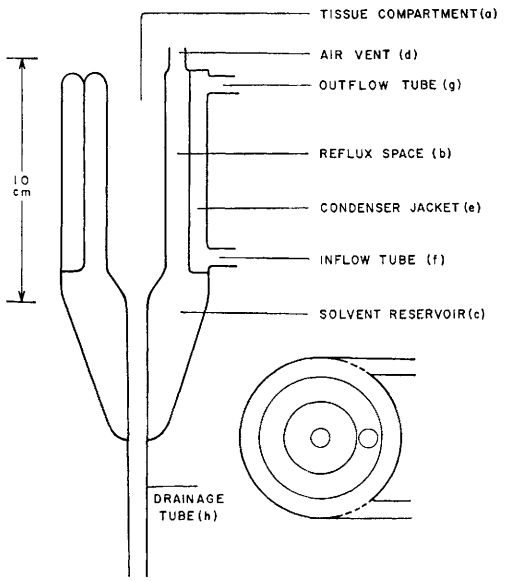

FIG. 1. Tissue bath.

of the central compartment through the solvent reservoir, which is enclosed in a perforated Glass-Col heating mantle designed for hot filtration (110-volt mantle) with top diameter of 2.5 inches. *Rate of reflux* must be controlled to prevent vigorous bumping and rapid dissipation of solvent, both of which will occur with low boiling solvents when the heating mantle is employed at full voltage. The voltage may be conveniently regulated by connecting a light bulb of suitable wattage in series with the mantle.

Temperature of the saline solution may be regulated by a suitable choice of solvent, which should present a negligible hazard from explosion or toxicity. For the majority of experiments it is desirable to maintain the temperature of the saline solution as close as possible to 38°C. With a 100-watt bulb in series with the mantle and employing methylene chloride (b.p. 40.1°C at 760 mm) as the refluxing solvent, a temperature of 38.9°C is obtained for saline solution through which a rapid stream of oxygen is passed. Over a period of 5 hours the variation is ± .03°C. Commercially available methylene chloride (Matheson catalogue No. 5509) gave consistent results without further purification. Saline solutions must be preheated for bath changes, since approximately a 10-minute period is required to reach the experimental temperature from the initial room temperature of the saline. Preheating may be conveniently accomplished by employing a second tissue bath. For a temperature in the vicinity of 20.0°C an azeotropic mixture of methyl formate and trichloromonofluoromethane (Freon-11, DuPont) may be employed. Temperatures in the range of 24.0°C to 35.0°C may be obtained with an appropriate solution of trichloromonofluoromethane and trichlorotrifluoroethane (Freon-113, DuPont). These 2 compounds do not azeotrope, but when mixed in proper proportions will form a constant boiling solution (Table I).

TABLE I. Temperatures of Saline Solutions with Different Proportions of Freon-11 and Freon-113.

Wt % Freon-11	Wt % Freon-113	B.p. °C
90	10	25.0
80	20	26.4
70	30	28.0
60	40	29.7
50	50	31.7
40	60	34.2
30	70	36.7

Summary. A compact and inexpensive tissue bath enabling the study of isolated biological materials has been developed. Temperature control of the saline solution is achieved by means of a refluxing solvent. When employed at a temperature of 38°C accurate control to ± .03°C is possible. By appropriate choice of solvents it is possible to study isolated tissue preparations over a wide range of temperatures.

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Cytochemical Demonstration of Diphosphopyridine Nucleotide-Diaphorase Activity in Cells of Vaginal Secretions of Mice.* (25190)

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Our previous studies have indicated the presence of at least 2 oxidative enzyme systems in the vaginal epithelium of rodents both of which have been shown to be under the influence of circulating titres of ovarian hormones during normal cyclical events(1,2). The enzyme systems described were the succinic dehydrogenase (SDH) and the diphosphopyridine nucleotide diaphorase (DPN) systems, histochemically demonstrable by using neotetrazolium as an indicator dye. The use of such methods has made localization of oxidative enzyme systems such as these possible at the tissue or histologic level of study. Recently, however, newer more sensitive and suitable tetrazolium salts have been advanced, especially nitro-blue tetrazolium (NBT), which now make possible enzyme localizations at the cellular or cytochemical level of study both with the light microscope(3) and the electron microscope(4). The purpose of this report is to show the cytochemical distribution of one such oxidative enzyme system (DPN) with use of NBT in the cellular population of vaginal secretion during the estrous cycle. It is our intent to show that: (1) cytochemical localization of such oxidative enzymes is possible through use of smear preparations, and (2) that the various cell types resident in vaginal secretions show distinctive enzyme localization patterns each related to degree of cytomorphological change (cornification) in the cell.

Methods and material. Adult, female albino mice (Swiss strain) were used in these studies. The animals were studied through at least 3 consecutive estrous cycles during which time the vaginal lumen was smeared by the use of a cotton-tipped applicator previously moistened in cold Ringer's solution. Smears were prepared immediately on glass slides which were air dried and quickly immersed into the following preheated incubating medium:

.1 M Na phosphate buffer, pH 7.6	14 ml
1.0 M sodium lactate	4 "
Coenzyme I (1 mg/ml aq.)	2 "
Nitro-blue tetrazolium (DAJAC)	20 mg

Following incubation at 38°C for 30 minutes and fixation in 10% neutral formalin, slides were rinsed in water and counterstained in eosin with subsequent mounting in synthetic resin. The insoluble dye, dinitroformazan, precipitated as a result of enzymatic activity has been shown to resist dissolution in the higher alcohols and xylene(3). Under the conditions of these experiments smear preparations incubated in the absence of lactate contained no detectable amounts of dinitroformazan. Parallel studies of each smear were also

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