DEMONSTRATIONS

Photometric method for the measurement of cell migration inhibition

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The inhibition of cell migration *in vitro* has been widely used for the assessment of cell-mediated immunity and as an assay of proposed mediators of cellular immunity (Bloom & Glade, 1971). Lymphoid cells produce a soluble factor in culture capable of inhibiting leucocyte migration. Measurement of the degree of inhibition of cell migration is usually achieved by determination of the area of migration from either a projected image or a photograph of the migrating cells. This demonstration describes a device for photometric estimation of cell migration. The migrating cells are viewed under a microscope using dark field illumination, the image being projected on to a ground glass screen situated in front of a cadmium sulphide photodetector, whose resistance change is displayed on either a galvanometer or a pen recorder. Oil-induced guinea-pig peritoneal exudate cells are packed into capillary tubes and cultured in chambers on microscope slides at 37°C for 18 hours. The cell migration for a range of concentrations of the inhibitory material has been assessed both by area measurement and by the photometric method. The photometric method gives a comparable dose-response relationship to that obtained by area measurement and has the advantage of speed and convenience. Its use is illustrated in both guinea-pig macrophage migration and human peripheral leucocyte migration.

REFERENCE

BLOOM, B. R. & GLADE, P. R. (1971). In In vitro *Methods in Cell Mediated Immunity*. London & New York: Academic Press.

Non-traumatic blood collection from rat tail vessels. Application in *Mycoplasma arthritidis*

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Withdrawal of blood from the retro-orbital plexus, or from a cut in the tail, or by cardiac puncture, involves tissue damage which renders these procedures unsuitable for repeated use in the same rat, and may spoil the blood for certain tests. A simple technique of injecting or withdrawing fluids via the tail vessels was therefore devised. The mount is removed from an injection cannula, and the cut-off end of the cannula inserted into one end of a polythene or nylon tube (3–30 cm). The other end of the tube is connected via another cannula to a syringe. Short-bevel 25 gauge (0·5 mm) needles and 0·5 mm bore tubing are used for intravenous injections or infusions, and 23 gauge (0·65 mm) needles and 1·5 mm bore tubing for aspiration of blood. Under light ether anaesthesia, the tail is placed in warm water and then shaved. One of the lateral veins is compressed by an assistant, and the cannula inserted by hand or with a smooth forceps. Insertion into the ventral artery is slightly more difficult, and used mainly for collecting larger blood samples ($>0{\cdot}5$ ml) and for B.P. recordings.

Blood (0·5 ml) was collected twice weekly from rats infected with *Mycoplasma arthritidis*. W.b.c. counts, percentage of granulocytes, E.S.R., serum lysozyme, total haemolytic complement (C'H50) and C'3, increased with the developing polyarthritis. Metabolic inhibition antibodies developed only after 3–4 weeks, when the polyarthritis was already subsiding. Rats receiving salicylates (200 mg/kg twice weekly) developed a more severe polyarthritis; some of the blood changes were more pronounced.

Implantation of electrodes in the dentine of an upper canine tooth in the dog

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Electrical stimulation of the dental pulp causes predictable and characteristic pain responses in the conscious dog. Suppression of these pain responses by drugs gives an estimate of their analgesic activities (Heng & Domino, 1960; Mitchell, 1964). In the development of this technique in our laboratories it has been necessary to modify the original test, both in the surgical procedure of implanting electrodes in the dental pulp and in the method of evaluating the analgesic activities of new drugs.

A mature Beagle dog was anaesthetized with pentobarbitone intravenously. A gum flap was deflected from the base of an upper canine tooth. Two cavities were drilled approximately 4 mm apart above the gum line in the labial surface of the tooth using a round plain cut (No. 2) bur. The cavities almost reached the dental pulp and were undercut using an inverted cone (No. 37) bur. A flexible hollow probe was introduced under the gum margin and passed subcutaneously to a skin incision over the calvarium. The electrode leads were two diamel-coated stainless steel wires (external diameter 0·2 mm) sheathed in polythene tubing (external diameter 1·2 mm) connected to an Amphenol strip connector (Type 221-2), mounted on a winged, polytetrafluoroethylene base plate. The polythene tube was passed down the probe which was then withdrawn and the base plate positioned under the skin. The ends of the wires were bared, coiled and placed separately into the tooth cavities, which were then packed with amalgam (fine grain solila alloy) to form the electrodes. The gum flap and the external skin incision were sutured.

In the testing of drugs for analgesic activity the following procedure was adopted. Dogs were trained to sit in individual boxes (81·7 × 66·6 × 53·5 cm). The external electrode assembly was connected to a Palmer stimulator by leads and an Amphenol strip connector (Type 221-1). The dogs were viewed through a one-way mirror. Every 30 min, for a period of 3 h after the animals received drug or placebo by mouth, a series of transient but increasing stimuli were applied to the dental pulp. In each stimulation schedule the frequency, pulse width and duration of the stimulus remained constant at 10 Hz, 50 ms and 10 s, respectively; only voltage was varied. Increasing stimuli (0·5–30 V) were applied to the dental pulp at intervals of 30 s to determine the threshold voltages for individual reactions, for example snarling or head jerking. After the initial schedule, selected stimuli were used so that each subsequent schedule lasted for only 90 seconds. In this test the minimal oral doses of aspirin, codeine and pentazocine required to suppress pain responses in the dog were 15, 3·5 and 5·0 mg/kg, respectively. These dogs may be used repeatedly for about 6 months during which time