Measurement of changes in the microvasculature

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This inexpensive instrument was designed to record *in vivo* any changes in a microvessel which would result in a change of optical density, e.g. alteration of vessel diameter, white thrombus formation, white cell rolling.

The apparatus consists of an optical adaptor, photocell and amplifier (Figure 1). It can be used in normal daylight with any research microscope having a phototube and a magnification factor of up to 100-350 x and a tungsten light source.

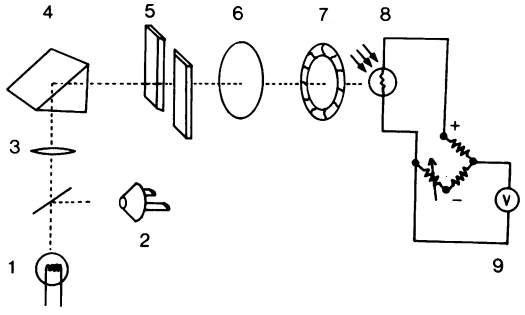

Figure 1

1. Tungsten light source
2. Binocular viewing tube
3. Phototube lens
4. Prism
5. Adjustable knife edge
6. Ground glass screen
7. Adjustable iris diaphragm
8. Photocell
9. Pen recorder

The optical adaptor consists of a prism attached above the projecting eyepiece of a standard Leitz camera tube. The beam is turned through 90° and projected onto a small ground glass viewing screen. A CdS photocell (cadmium sulpho selenide photoconductive cell – Mullard RPY 33) is placed in front of the screen after the experiment has been set up. To reduce glare and increase contrast, an optical knife edge is placed behind the screen and an iris diaphragm in front. Manipulation of the masks enables a square or rectangle to be formed. The photocell is recessed into a black perspex disc and when placed into position excludes all extraneous light. The experiment can be monitored using the binocular viewing head of the microscope. The pen recorder can be calibrated by aligning the vessel with the graticules in the eye-piece and on the ground glass screen.

The high sensitivity CdS photoconductive cell used is connected to a Wheatstone bridge circuit. The circuit is adjusted to a zero balance with a multi-turn potentiometer and balance meter. A local variation of light intensity results in a conductivity change in the photocell which unbalances the bridge circuit and produces an output voltage in excess of 50 mV at 2.5 kΩ impedance. The voltage is then filtered out with an RC filter and recorded on a continuous potentiometric pen recorder.

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A simple perfusion system for the study of histamine release from rat peritoneal mast cells

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Rat mast cells are extensively used to investigate the processes involved with the release of inflammatory mediators from cells. In the present study, a simple and rapid perfusion technique has been developed for the determination of histamine release from rat peritoneal mast cells.

Mast cells were obtained by gentle lavage of the rat peritoneal cavity with 12 ml of a modified buffer solution at pH 7 (Uvnäs & Thon, 1961) containing bovine serum albumin (0.1% w/v). The solution was withdrawn from the cavity and stored on ice until required. Aliquots (1-4 ml) were passed through a nylon membrane (duralon, 1 μm pore size) encased in a millipore filter-holder (Swinnex-25). A heating jacket was used to control the temperature of the filter-holder. In 20 experiments, the histamine content of the perfusate, before and after filtration, was comparable (within 5.8 ± 2.9%, mean ± s.e. mean), suggesting that this procedure did not disrupt the cells. Further, comparison of the total histamine content of the perfusate (using 0.4 *N* perchloric acid to disrupt any cells present) indicated that over 90% of the mast cells were trapped on the

membrane, a finding confirmed by microscopy. The cells were then perfused (2 ml/min) with albumin-free buffer solution, and the perfusate collected at timed intervals. The histamine content of the perfusate was determined by alkaline condensation with O-phthalaldehyde followed by acidification and measurement of the fluorescence (excitation 350 nm, emission 440 nm; Shore, Burkhalter & Cohn, 1959) or by bioassay on the superfused guinea-pig ileum. In further experiments, the perfusate was led directly to a mixing coil and to a flow-cell spectrofluorimeter which enabled the chemical histamine assay to be carried out automatically, with the fluorescence intensity being displayed on a chart recorder.

The cells appeared to retain their functional integrity since the low resting histamine output could readily be increased up to 200-fold by chemical or immunological challenge. In 12 experiments, administration of the polymer 48/80 (0.1 ml volume) into the perfusion medium gave a reproducible and dose-dependent release of histamine from the cells. The threshold (0.05-0.1 μg) and maximal (1-2 μg) doses of 48/80 were similar to those observed with incubated mast cells. With initial high doses of 48/80 (0.5-2 μg), subsequent administration in the same experiment gave a very diminished histamine release, presumably due to depletion of the stored mast cell histamine. However, with lower doses of 48/80 (0.05-0.2 μg), several comparable responses could be obtained.

This method, which can readily be adapted to study other mediators such as 5-hydroxytryptamine, and other cell types, should give a better understanding of the kinetics of mediator release from specific inflammatory cells and provide a simple technique for the rapid screening of drugs which alter such release.

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The inhibitory transmission to the internal anal sphincter

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Inhibition of this sphincter by the sacral nerves is atropine-resistant (Langley & Anderson, 1895a; Garrett & Howard, 1975). Our results show that this sacral inhibitory post-ganglionic transmission resembles that present in the retractor penis (Luduena & Grigas, 1966, 1972; Ambache, Killick & Zar, 1975). It is likely that the other atropine-resistant sacral inhibitory effects described by Langley & Anderson (1895b) are similarly mediated.

Procedure: The anal canal is excised by a wide perineal incision. The anus is slit and a 1-2 cm width of mucosa, adjoining the skin, is dissected off the underlying sphincters. A plane of separation is found between the pink-brown external sphincter (skeletal) and the white smooth muscle band of the internal sphincter. Strips from > 50 rabbit, pig, dog, cat and guinea-pig sphincters were suspended in Krebs-Henseleit (35° C) and stimulated transmurally (1-20) pulses at 1 min intervals; supramaximal voltage; 0.2 ms 10 Hz).

Results: Transmural stimulation elicited repeatable inhibitions unaffected by pentolinium (93 μM) but abolished by tetrodotoxin (0.63 μM). Pure relaxations were obtained without 'rebound' (e.g. Figure 1) in most preparations; only a few had an additional (adrenergic) motor component which disappeared after guanethidine, 10 μM, or phentolamine, 5.3 μM.

That acetylcholine is not the inhibitory parasympathetic transmitter was shown by: (a) its opposite, contractile effect, except once (cat sphincter; cf. monkey, Rayner, 1971) when, in high doses (277 μM), acetylcholine, like nicotine, produced a pentolinium-susceptible inhibition. (b) Persistence of these neurogenic inhibitions in atropine (0.029-29 μM); and (c) failure of physostigmine (7.7 μM) to potentiate the atropine-resistant inhibitions.

The inhibitory transmission is not adrenergic or tryptaminergic, persisting after propranolol