Measurement of cell aggregation and shape change by transmitted and scattered light through a cell suspension (Dual Channel Cell Monitor)

R.T. HUTCHINGS, GRETA E. LIEBERMAN & Z.T. SABIKOWSKI (introduced by G.P. LEWIS)

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

A low cost dual channel Cell Monitor has been developed to measure aggregation of various cells as well as their shape change by transmitted and reflected light through the cell suspension. The instrument permits examination under identical conditions of control and test samples or two test samples simultaneously. The circuit and sampler holder are a modification of those used in the Born Aggregometer (Born & Hume, 1967).

The apparatus consists of a brass sample holder fitted with four photocells and lenses, one variable AC stirring motor with a magnet, two tungsten lamps and an electronic unit with power supplies and four Wheatstone bridge circuits.

The sample holder (Figure 1) consists of a brass block (a) 100 × 60 × 60 mm which is drilled and plugged to provide a water circulation pathway (b). At the centre of the block are two vertical holes into which

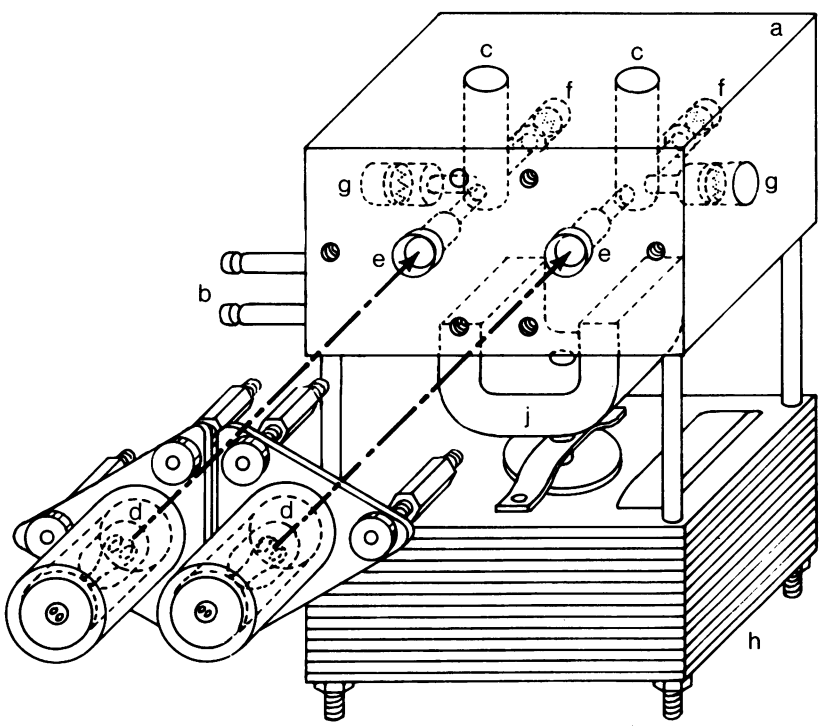

Figure 1 Sample holder
a Brass block
b Water connectors
c Sample tubes with flea magnets
d Tungsten lamps
e Lenses
f Photocells – aggregation
g Photocells – shape change
h AC stirring motor
j Magnet

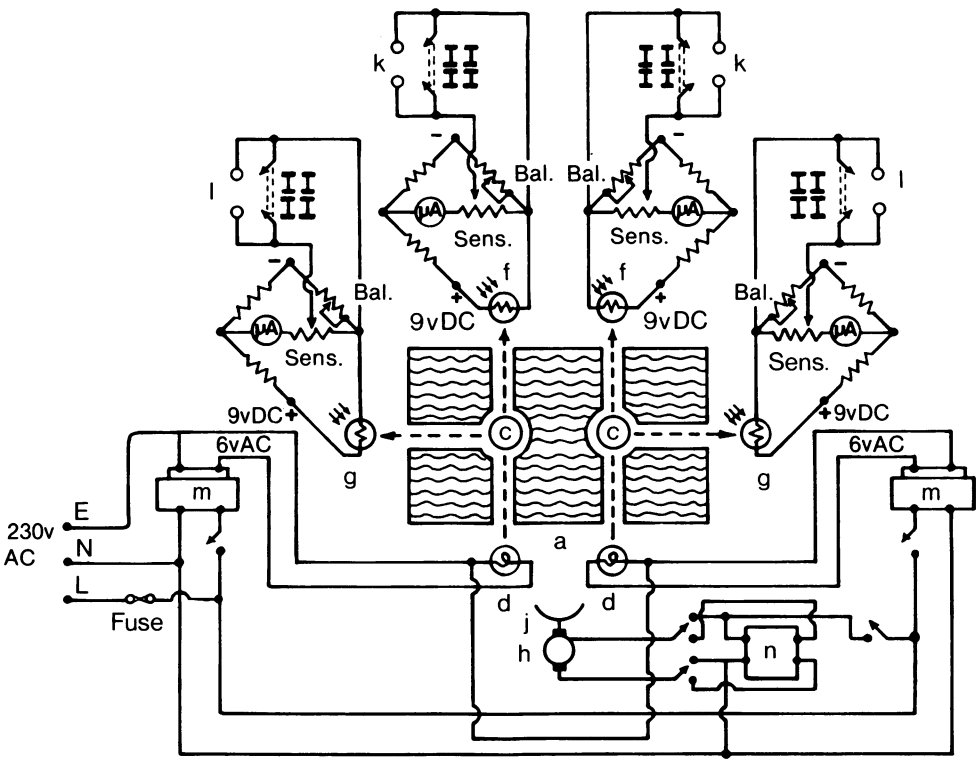

Figure 2 Electronic circuit

a Brass block
c Sample tubes with flea magnets
d Tungsten lamps 6V 15 Watt
f Photocells – aggregation
g Photocells – shape change
h AC stirring motor
j Magnet
k Output – aggregation
l Output – shape change
m Constant voltage transformer 6V 15 Watt
n Motor speed controller

are placed the 100 × 10 mm sample tubes (c). Two tungsten 6v 15 Watt lamps (d) are fitted onto an adjustable lampholder which is mounted on two brass plates and screwed to the block with six adjustable screws. The light passes through two lenses (e) at right angles to the sample tubes and impinges on two Cds RPY 33 (Mullard) photocells (f) to measure aggregation. Two similar photocells (g), placed at right angles to the beam and covered with filters polarized in the vertical plane, record shape change of cells.

Constant temperature of the samples is maintained by circulating water from an external thermostatically controlled source. The cell suspension is stirred with an AC motor (h) which can be variable or set to 1200 rpm and which is fitted with a magnet (j) arranged to rotate a flea magnet inside each of the sample tubes.

In the circuit diagram shown in Figure 2, the four photocells are connected each to its own Wheatstone bridge circuit with multiturn potentiometers and edge-indicators for zero balance adjustment. Adjustment of the output voltage level is provided by multiturn sensitivity controls with duo-dials so that the apparatus can be used with a wide choice of pen recorder input. All outputs are provided with an RC filter circuit and a switch to minimize excessive signal fluctuation.

Addition of aggregating agents to the stirred cell suspension produces a variation in light intensity. This results in conductive changes of the photocells which unbalance the Wheatstone bridge circuits to produce a voltage. The output of the two photocells measuring cell aggregation (transmitted light channels) might exceed 100 mv d.c., but that from the photocells measuring shape changes (light scatter channels) might reach only about 20 mv d.c. The outputs can be finally connected to a four channel potentiometric pen recorder.

We wish to acknowledge the advice of Professor G.V.R. Born and Professor F. Michal who, with RTH and ZTS, constructed the single channel Aggregation and Shape Change Monitor in 1971.

Reference

BORN, G.V.R. & HUME, M. (1967). Effects of the numbers and sizes of platelet aggregates on the optical density of plasma. *Nature (Lond.)*, **215**, 1027–1029.

Production of acid secretion in the mouse isolated stomach by electrical field stimulation

J.A. ANGUS & J.W. BLACK

Department of Pharmacology, University College, London

In the mouse isolated stomach preparation, the absence of blood-borne endocrine feedback between antrum and body offers a simplified way of studying the coupling between vagus nerves and oxyntic cells. We have investigated the effect of electrical stimulation and its neural dependence in this preparation.

Mice of either sex (Charles-River, 23–26 g) having had free access to food and water were anaesthetized with ether. Polyethylene cannulae were tied into the pyloric and fundic parts of the stomach, the oesophagus ligated and the stomach lumen washed with warm oxygenated mucosal solution (in mM, NaCl 135; KCl 4.8; $MgSO_4$ 1.2; $CaCl_2$ 1.3 and glucose 31.6). The stomach was placed in a 40 ml organ bath containing buffered serosal solution at 37°C (in mM, NaCl 118; KCl 4.8; $MgSO_4$ 1.2; KH_2PO_4 0.14; Na_2HPO_4 15.9; $CaCl_2$ 0.65; and glucose 31.6) and gassed with 95% O_2 and 5% CO_2. The stomach was continuously perfused with mucosal solution at 1 ml/min and the perfusate passed over a pH electrode system adjusted to raise the intragastric pressure to 18 cm H_2O. A pair of platinum ring electrodes (ring diam. 5 mm, wire diam. 0.5 mm) was lowered to lie either side of the stomach to stimulate the region of the fundic glands. The ring electrode was preferred to a plate electrode to reduce the current requirement. Field stimulation was achieved with square wave pulses, 0.5 ms, 10 v and 10 Hz from a Grass 5D9 stimulator and monitored on an oscilloscope.

Characteristically, stimulation for 10 min increased gastric acid secretion with a latency of 3–4 min and reaching a plateau after 7–8 minutes. Compared to the effects of histamine added to the bath, the latency and plateau were not significantly different although time to reach plateau was considerably shorter for field stimulation (Figure 1). Field stimulation could be repeated at 30 min intervals at least 3 times without showing a decline.

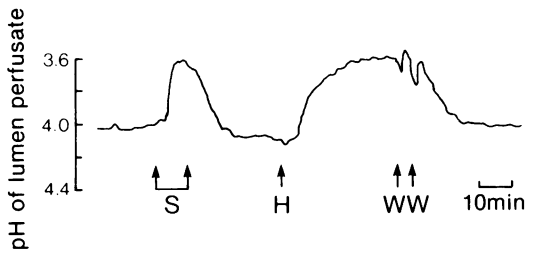

Figure 1 Continuous pH recording of the lumen perfusate from an isolated whole mouse stomach perfused at 1 ml/min. At S, field stimulation was applied across the stomach at 10 Hz, 10 v, 0.5 ms for 10 minutes. At H, histamine (10^{-5}M) was added to the organ bath and washed out at W.

Pharmacological analysis of field stimulation was investigated using two responses in the same stomach separated by 30 min while the stomach was equilibrated with antagonists added to the bath. The response to the second stimulation expressed as a percentage of the first was 115.2 ± 13.7% (mean ± s.e. mean, $n = 13$) in control experiments. Tetrodotoxin (10^{-7}M) completely blocked the second stimulus ($n = 3$) without reducing the response to histamine (10^{-5}M) indicating that field stimulation involved a neural process. Hexamethonium (5×10^{-5}M) reduced the second stimulus significantly to 23.7 ± 7.0% ($n = 6$) suggesting the involvement of ganglia. Atropine reduced the response to 70.7 ± 13.7% ($n = 6$) at 10^{-8}M and to 2.8 ± 2.3% ($n = 6$) at 10^{-7}M.

Field stimulation therefore apparently stimulates gastric acid through a cholinergic, presumbaly vagal, mechanism.

We thank Dr S. Szelenyi from Chemiewerk Homburg for assistance in some of the experiments.

J.A.A. is a holder of a National Health and Medical Research Council of Australia, C.J. Martin Fellowship.