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DEMONSTRATIONS

Some properties of bipolar cells in the retina of dogfish and rays

BY J. F. ASHMORE and G. FALK. *Department of Biophysics, University College London, WC1E 6BT*

The retinal photoreceptor cells of a number of elasmobranch species consist almost exclusively of rods, and their bipolar cells have relatively large perikarya (Witkovsky & Stell, 1973). The responses to flashes and to steps of light have been recorded in bipolar cells in the eye-cup preparation of the ray, *Raia clavata* and the spotted dogfish, *Scyliorhinus canicula* with intracellular micro-electrodes. Gently tapering electrodes are drawn from commercially available borosilicate tubing containing a small capillary fused to the inside wall (Clark Electromedical Instruments, Pangbourne, Reading), and have resistances of 200–300 MΩ when filled with electrolyte. After a cell is penetrated in the dark-adapted retina, the position of the impaled cell in the framework of the optical system is obtained by the response elicited as a flashing, dimly illuminated edge is moved in orthogonal directions over the retina. Spots, annuli and slits may then be centred upon this position. Such a procedure maintains dark adaptation and yet is not too time consuming.

The only class of bipolar cells found thus far in the ray and dogfish is that which responds to a centred spot of light by a depolarization, as has also been reported briefly by Kaneko (1971) for the smooth dogfish, *Mustelis canis*. These cells have receptive fields of about 150–200 μm in diameter for their centre response and in the dark-adapted state only a barely detectable antagonistic effect of illumination of more distant regions of the retina. The maximum depolarization in response to light is about 25 mV from a resting internal potential of -45 to -60 mV. The responses to steps of light of various intensity are illustrated in Fig. 1. The response to a moderately strong light consists of a phasic peak depolarization followed by a plateau of depolarization outlasting the duration of the stimulus. During the plateau, there is often a reduction in the noise

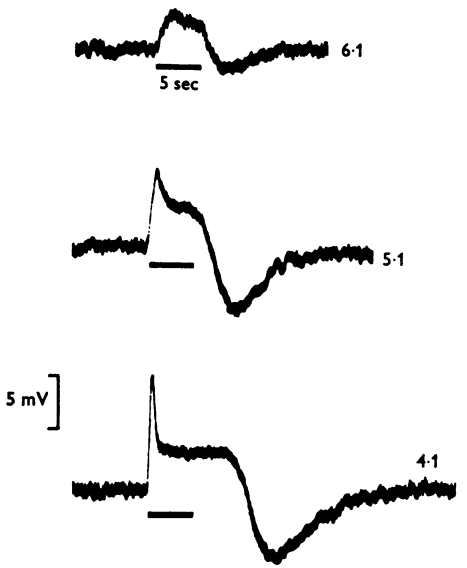

Fig. 1. Responses of a bipolar cell in the retina of the dogfish, *Scyliorhinus canicula*, to long duration pulses of blue-green light. An upward deflexion of the trace indicates an increase of internal potential (depolarization) from a resting potential of -54 mV. The light stimulus was a circular spot 540 μm in diameter, the duration of which is indicated by the horizontal bar under each trace. The number on the right of the trace indicates the attenuation in $\log_{10}$ units provided by neutral density filters. The unattenuated light had an irradiance at the retina of $3{\cdot}2 \times 10^5$ photons $\mu m^{-2} sec^{-1}$ at 495 nm, the wave-length of maximum transmission of the blue-green colour filter. The cell was located at a depth of 80 μm from the vitreal surface of the retina.

level of the trace, analogous to that found in the hyperpolarizing bipolar cells of the turtle by Simon, Lamb & Hodgkin (1975). When the light is turned off there is a transient hyperpolarization. Comparison of the flash and step sensitivities (Baylor & Hodgkin, 1973) yields an integration time of 0·8–1·8 sec at 16° C.

This work was supported by the Medical Research Council.

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A technique for studying the metabolism of muscle

By P. M. Daniel, O. E. Pratt and E. Spargo. *Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, London SE5 8AF*

In investigating the factors which cause the release of amino acids from muscle (which include hormones) and studying artificially induced muscular dystrophy, it was necessary to obtain blood which is derived almost solely from muscles. We found that in both the rabbit and the

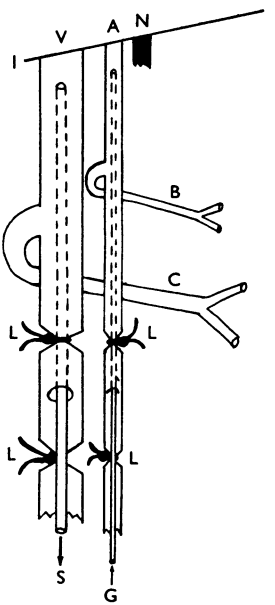

Fig. 1. Diagram to show vessels supplying and draining the extensor muscles of the thigh and the arrangement of catheters for injecting substances intra-arterially and for sampling venous blood draining these muscles; A, femoral artery; B, arterial branch to thigh muscles; C, vein draining thigh muscles; G. fine catheter for injecting substances into arterial blood going to thigh muscles; I, inguinal ligament; L, ligatures holding catheters in place and occluding vessels; N, femoral nerve; S, catheter for sampling blood from thigh muscles; V, femoral vein.

baboon a single branch of the femoral artery supplies the bulk of the extensor muscles of the thigh and that this group of muscles is usually drained by a single vein which enters the femoral vein. To obtain blood from these muscles (Fig. 1) a polythene catheter (Portex, 1·34 mm o.d.) is inserted into the femoral vein some 3·5 cm below the inguinal ligament, is advanced up the vessel for some 3·0 cm and tied in. A fine catheter (Portex 0·63 mm o.d.) is passed up the femoral artery to the same level

and also tied in. Arterial blood is sampled via a catheter in any convenient artery. To study the effect of hormones on the muscles a continuous injection of a selected hormone, e.g. glucagon (Daniel, Pratt & Spargo, 1975) is given into the femoral artery via the fine catheter by means of the injection apparatus described by Daniel, Donaldson & Pratt (1975) and Pratt (1974). The hormone is thus carried down the artery directly to the muscles and during its injection blood samples are taken from the catheter in the femoral vein and from the arterial sampling catheter in order to measure arterio-venous differences of amino acids. When diseased muscle is being studied the in-going catheter into the artery is not needed. This system has proved useful for studying the metabolism of stimulated or denervated muscle.

The collateral circulation is so good that the leg below the level of the ligatures does not suffer damage.

The work during which this technique was developed was supported by a grant from the Muscular Dystrophy Associations of America, Inc.

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A current pump monitor for micro-iontophoresis circuits

BY C. J. COURTICE. *Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London WC1N 1AX*

This instrument (Fig. 1) was developed to measure iontophoretic currents generated by Howland current pump circuits (Geller & Woodward, 1972; Kelly, Simmonds & Straughan, 1975). Its main advantages are low cost compared with electronic analogue meters and the fact that it does not introduce interference into the recording system as digital meters do.

The principle is to measure the voltage drop across a resistor in series with the output from the iontophoresis panel. The system is kept floating at the voltage generated by the iontophoresis pump circuit. One input terminal of the amplifier is connected to the common, i.e. virtual zero for the amplifier circuit. This maintains the common mode voltage at the amplifier inputs well within the amplifier's maximum value. The amplifier is powered by rechargeable batteries rather than a mains operated power supply. This avoids 50 Hz interference caused by capacitative coupling across the windings of a power supply transformer, which becomes a problem with the relatively high output impedance of the iontophoresis pump circuit.

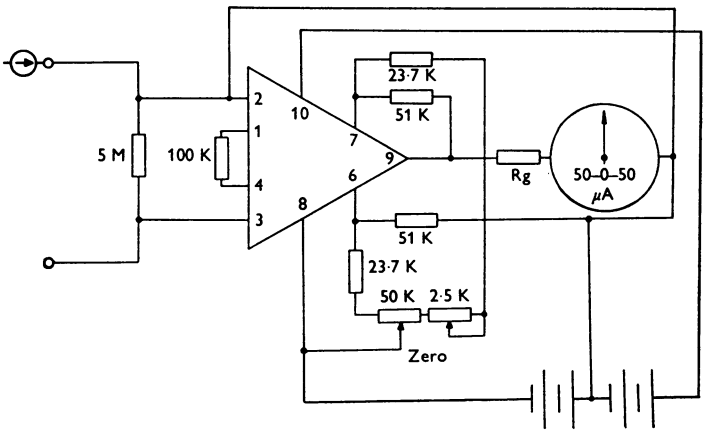

Fig. 1. Circuit for current measurement. The amplifier is a Burr–Brown 3670J used at unity gain. Output of the amplifier is 5 mV/nA and Rg is selected according to the range of currents which are to be measured. Rechargeable batteries providing ± 7 to ± 20 V may be used.

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A robust transducer suitable for measuring forces of 1 μN

BY P. W. BRANDT, E. CHAPPELL and B. R. JEWELL. *Department of Physiology, University College London, London WC1E 6BT*

This transducer was developed for use with bundles of chemically-skinned cardiac muscle fibres (Brandt & Hibberd, 1976), which develop tensions of 10–100 μN (~ 1–10 mg wt) with a rise time of many seconds. The transducer element (AE 800 series, Akers Electronics, Horten, Norway) is a bar of silicon with planar-diffused resistors, which function as strain gauges: these are connected as a half-bridge and energized by 5 V d.c. (Fig. 1*b*). The silicon bar is a cantilever with a compliance of 3·3 nm/μN and a natural frequency of about 7 kHz. We have used an aluminium lever (Fig. 1*a*) to couple the muscle to the transducer element, which is not brought into contact with the lever until the fibre bundle has been fixed in the Perspex clamp (C, Fig. 1*a*). The lever is pivoted on a knife edge (K) and the lever ratio is 10:1, so the compliance felt by the muscle is ten times greater than the value quoted above. The sensitivity of the system (including amplification of about × 70 in the bridge amplifier) is 2 mV/μN and the drift rate appears to be negligible; for

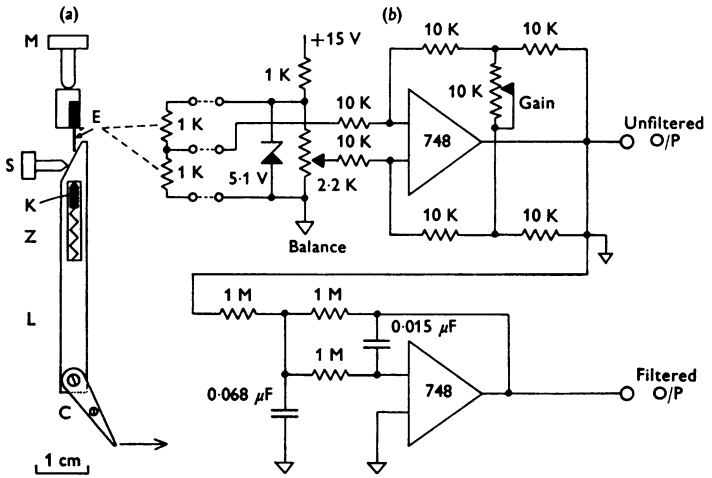

Fig. 1. (*a*), Schematic diagram to show the arrangement of the transducer element (E), the lever (L), and a stop (S) which prevents excessive movement of the lever. A micrometer (M) is used to advance the element towards the lever, which is pivoted on a knife edge (K) and held in place by a weak spring (Z). The direction of pull at the muscle clamp (C) is indicated by an arrow. (*b*), Circuit diagrams of the variable-gain bridge amplifier (top) and 2nd order filter (bottom): both are based on designs in the Philbrick Applications Manual (Philbrick Researches, Dedham, Mass. 02026). The operational amplifiers (from Radiospares, London EC2P 2HA) have specially good temperature stability.

example, no drift in base line was detectable over a 6 hr test period when the output of the system was displayed at 10 μN/cm. The 2nd order filter (-3 dB at 5 Hz) is used to eliminate high frequency noise.

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A rapid method of F-wave analysis, using a three-dimensional plotting technique

BY P. FURNESS* and JENNIFER JESSOP.* *Department of Physiology, University College London, Gower Street, London WC1E 6BT*

The F wave (Magladery and McDougal, 1950; Dawson and Merton, 1956) normally shows variations in latency, amplitude and probability of occurrence. In recent experiments, the causes of these variations have

* M.R.C. Scholar.

been sought. The direct response, or M wave, acts as a control and must also be analysed.

In a situation such as this, where there are several variables, data reduction can become both tedious and time-consuming.

Useful data compression has been obtained automatically by plotting successive sweeps of e.m.g. three-dimensionally, using hidden-line suppression (Fig. 1). This may be produced, by a LINC-8 computer, either

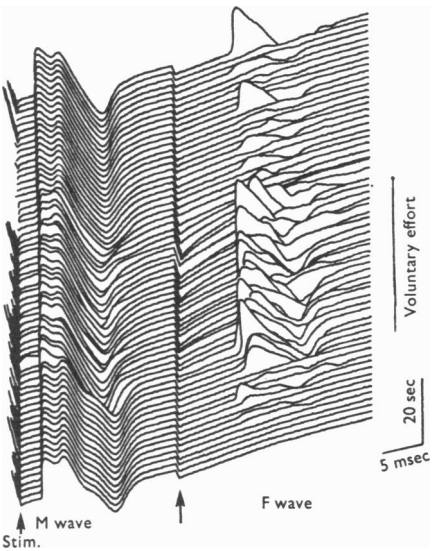

Fig. 1. Arrow shows point at which gain was increased × 5.

off-line on an incremental plotter, or on-line on an oscilloscope (Furness, 1974). Variations in both M and F waves then become obvious, and further data reduction and measurements can be suitably planned.

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Isotachophoretic analysis of muscle extracts

By D. C. Gower and R. C. Woledge. *Department of Physiology, University College London, Gower Street, London WC1E 6BT*

Isotachophoresis, an electrophoretic technique, separates ions according to their mobilities in an electric field. In the LKB Tachophor separation takes place in a 0·5 mm diameter Teflon tube. The technique is different from the more conventional zone electrophoresis in that, in isotachophoresis, most of the current is carried by the ions being separated, rather than by a buffer present in excess. A sample is introduced between two

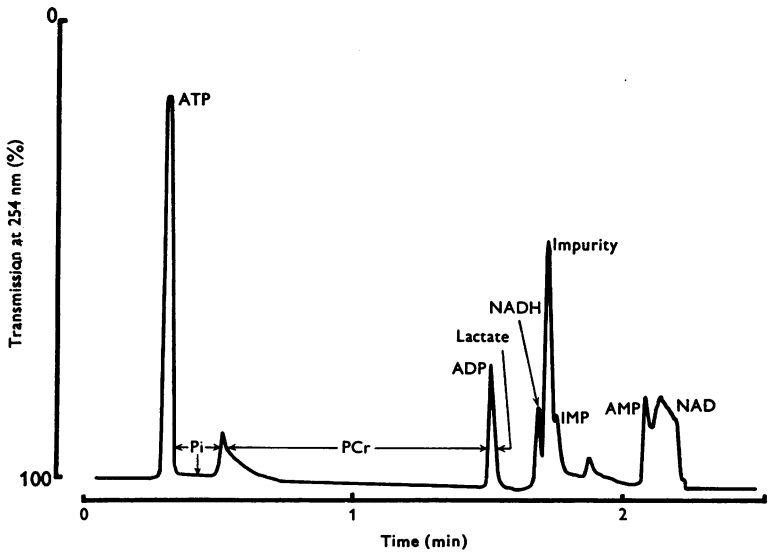

Fig. 1. U.V. record from isotachophoretic analysis of muscle extract. 10 μl. of extract made by method (2) was used, which is equivalent to about 1 mg of muscle. The analysis was made with an LKB Tachophor, using as leading electrolyte: 5 mM-HCl, 20 mM β-alanine, 0·5% methylcellulose, pH 3·92; and as terminating electrolyte 5 mM caproic acid. The column length was 46 cm, electrophoretic current 50 μA. The run time was about 20 min.

electrolytes (referred to as 'leading' and 'terminating') having anions of very different mobilities. During the run those sample anions having a mobility between that of the leading and terminating electrolytes remain between them and segregate into adjacent zones, each containing only one type of anion. The zones are stabilized by the fact that each has a characteristic voltage gradient, depending on the mobility of the anion species in it. Zones of sample anions cannot separate from each other, but remain adjacent.

Samples for analysis were prepared in two ways: (1) frozen muscles were ground, extracted with perchloric acid, filtered and neutralized; (2) flattened, frozen muscles were soaked for 4 days in 1·25 mM EDTA (pH 7·6) in 50% methanol at −30° C. Either of these extracts may be used directly for analysis but the best results have been obtained by freeze drying the extracts and redissolving in 0·2 times the original volume. In either case the amount of extract required for analysis is equivalent to 0·5–2 mg of muscle.

U.V. absorbing and non-U.V. absorbing zones, as shown in the figure, can be identified by the comparison of records from muscle extracts with and without the addition of standard solutions. Amounts of metabolites from 1 to 50 nmol can usually be estimated by measurements of the zone length, as the concentration is uniform throughout the zone. However, in the case of U.V. absorbing materials, the area under the peak on the record, after transforming from a transmission scale to an optical density scale, is linearly related to the amount of material in the range 0·01–50 nmol. Using these calibration methods we have measured ATP, ADP, AMP, IMP, NADH, P_i, PCr, lactate, and glucose-6-phosphate in muscle extracts.

Polarographic measurement of O_2 consumption in frog sartorius muscle at 0° C

By R. J. Paul.* *Department of Physiology, University College London, London WC1E 6BT*

Recent refinements of both chemical and myothermal techniques have shown that in *R. temporaria*, the initial phosphorylcreatine (PCr) break-down in an isometric tetanus cannot be scaled to the environmental energy exchange (heat + work) using a single constant for the enthalpy of PCr break-down. Using the *in vitro* value of −34 kJ/mol, one finds more energy release than can be accounted for by PCr splitting. This has led to the suggestion of a 'missing' reaction associated with muscle contraction; i.e. the known initial reactions may not measure the total chemical energy cost of contraction (Gilbert, Kretzschmar, Wilkie & Woledge, 1971). As this hypothesis is of fundamental significance to muscle physiology, a test independent of both the standard biochemical and myothermal measurements was performed, utilizing O_2 consumption to measure the total chemical energy required for contraction (Kushmerick & Paul, 1976*a*, *b*).

The design features and operation of the polarographic system developed to measure recovery O_2 consumption following single isometric tetani in frog sartorius are presented in this demonstration. To achieve a clear

* British-American Heart Association Research Fellow.

separation of initial and recovery processes as well as for comparison to standard works, it is necessary to work at 0° C. This constraint increases the technical difficulty as the extent and rates of metabolism are considerably reduced. Most materials commonly used in design of physiological apparatus cannot be used as their absorption and consumption of O_2 is of similar magnitude to the rate of metabolism. The chamber is constructed of glass and stainless steel. To achieve the required sensitivity, the volume (4 ml.) has been minimized within the constraints imposed by muscle size and the vigorous stirring required for stable electrode performance. Access into the closed chamber, required for mechanical measurements, is accomplished by using a long diffusion path as an effective seal against leakage to the atmosphere. This system can resolve differences in O_2 content of approximately 5 nmol. It has been used to measure from a basal rate of 7 nmol/(min g) the recovery O_2 consumption following a 1 sec tetanus. Although commercial systems for measurement of O_2 are available, this system achieves better sensitivity and stability. As the resting O_2 consumption of frog muscle at 0° C almost represents a lower limit for physiological studies, this system could be readily adapted to other muscles and tissues. Total cost of the polarographic system (not including a recorder) is less than £100.

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The effect upon fusimotor neurones of small, brief stretches of their muscles

BY P. H. ELLAWAY, J. E. PASCOE and JUDY R. TROTT. *Department of Physiology, University College London, London WC1E 6BT*

Autogenetic reflex excitation of alpha motoneurones, by primary endings of muscle spindles (the stretch reflex) has long been recognized. In contrast, it was not appreciated until recently (Trott, 1975, 1976) that impulses in spindle primary axons could also excite homonymous fusimotor neurones. Trott used muscle vibration of an amplitude (50–100 μm) sufficient to excite powerfully the primary endings but which did not affect secondary endings or tendon organs. The response to vibration was a gradual increase in frequency of the background discharge of some fusimotor neurones. In order to investigate the central pathway of this reflex we needed a technique which would allow us to measure central

delay. Essentially, two techniques have been combined. Single, brief, small amplitude stretches of the muscle are used to excite selectively a volley of impulses in primary endings. Any response of a fusimotor neurone is then detected on constructing a post-stimulus histogram of action potentials. The methods are described below and were demonstrated during an experiment.

Decerebrated cats which are paralysed and artificially respired have their left hind limb denervated except for the triceps surae. Tonically firing fusimotor neurones are recorded in isolated filaments of one fascicle of the triceps surae nerve. A large proportion of the nerve is preserved intact to provide an adequate afferent pathway for the experiment. The Achilles tendon is attached to an electro-magnetic device designed to stretch the muscle. With the muscle near its maximum natural length stretches of up to 50 μm (rise time 3 msec, decaying within 5 msec) excite a single impulse in many primaries but do not modulate the firing of secondary endings or tendon organs.

Such selective driving of primary endings excites those fusimotor neurones which can also be excited by vibration. The excitation is not pronounced enough to be seen on a single oscilloscope sweep but is evident on repeating the stimulus and constructing a post-stimulus histogram. A Biomac (Data Laboratories) or a LINC 8 computer (Digital) is used on line for this purpose.

Conduction times between the periphery and the spinal cord in both the fusimotor and afferent axons have been recorded. In preliminary results these indicate central delays of 6–8 msec. Such delays are considerably longer than for the pathway from primary endings to alpha motoneurones suggesting a separate, and possibly supraspinal, connexion to fusimotor neurones.

We are grateful for financial support from the M.R.C.

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Changes in the probability of firing of human motor units following cutaneous nerve stimulation

BY J. A. STEPHENS and T. P. USHERWOOD. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH*

Differences have been shown to exist in the synaptic organization of the afferent inputs to motor units of different mechanical type in the cat (Burke, Jankowska & Bruggencate, 1970). The technique now described promises to allow the study of such differences in man.

The hand is immobilized in Plasticine and the force of abduction of the index finger produced by contraction of the first dorsal interosseous muscle (1DIM) recorded using a strain gauge positioned against the lateral aspect of the second proximal interphalangeal joint. A monopolar e.m.g. needle is inserted into the 1DIM for recording motor unit action potentials. Two spring electrodes are placed around either the little finger or the index finger for electrical stimulation of the digital nerves. The anode surrounds the distal interphalangeal joint and the cathode is 1 cm proximal. When stimulating the little finger, the size and timing of the orthodromic sensory nerve volley can be measured by averaging the surface electroneurogram recorded from two 10 mm diameter silver disk electrodes attached to the skin overlying the ulnar nerve at the elbow.

A single motor unit action potential is isolated using the intramuscular electrode, and the twitch characteristics and threshold of the unit measured using the spike triggered averaging technique (Milner-Brown, Stein & Yemm, 1973; Stephens & Usherwood, 1975). The subject is then required to maintain a contraction such that the unit fires at a steady rate. At the same time the digital nerves are stimulated at 3/sec (pulse width 50 μsec) and a post-stimulus time histogram of the occurrence of motor unit spikes following each stimulus is accumulated over 1024 sweeps (bin width 5·12 msec).

At stimulus strengths of approximately four times sensory threshold, the major features of the response are a pronounced increase in the probability of motor unit firing at a latency of about 50–60 msec and lasting 15–20 msec, followed by a more prolonged reduction in probability reaching a minimum at a latency of approximately 80–100 msec and with a total duration of up to 100 msec. In many instances this reduction in probability is succeeded by another period of raised probability reaching a maximum at a latency of 150–200 msec returning to control about 100 msec later. For some motor units the initial increase in probability is less marked or absent.

Using bin widths of 1·28 msec we have on occasion been able to distinguish a reduction in probability of shorter latency (48–49 msec) lasting 11–12 msec which for some motor units was preceded by an increase in probability with latency 38–40 msec and duration about 10 msec.

Changes in the membrane potential of cat motoneurones following cutaneous nerve stimulation in low spinal animals (Burke *et al.* 1970) show a sequence of excitation and inhibition broadly similar to the shorter latency changes in probability of motor unit firing described above. It may emerge that changes in the probability of motor unit firing can provide essentially the same information as that obtained by intracellular recording in situations where this is impossible (cf. Harrison & Pascoe, 1975).

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The role of sympathetic nerves in nasal secretion in the cat

BY H. WILSON and M. S. YATES. *Department of Pharmacology and Therapeutics, Universtity of Liverpool, Ashton Street, Liverpool L69 3BX.*

Inhibition of nasal secretion in the dog by the sympathetic nervous system (Jung, Tagand & Chavanne, 1927) was confirmed in man by Fowler (1943) who observed vasomotor rhinitis after left stellate ganglionectomy. In the cat (Eccles & Wilson, 1973, 1974), however, stimulation of the Vidian nerve for 3 min induced nasal secretion whereas stimulation for 15 sec produced vasoconstriction in the nasal cavity because of stimulation of post-ganglionic sympathetic fibres in this nerve. The role of sympathetic nerves in nasal secretion was therefore examined in anaesthetized cats in the first instance, by observing the duration of vasoconstriction evoked by Vidian nerve stimulation during 3 min stimulation. In other experiments, frequency/secretion response curves were obtained by stimulating the nerve for 3 min in normal cats and in others after unilateral removal of the superior cervical ganglion 10–14 days previously. The techniques did not allow measurement of nasal secretion and vasoconstriction in the same cat.

The head of the animal was rigidly fixed and the lacrimal gland and nictitating membrane removed. Exposure of the Vidian nerve, measurement of nasal secretion and recording of nasal vascular changes were carried out as previously described (Eccles & Wilson, 1973; Wilson & Yates, 1975).

After atropine sulphate (50 μg/kg I.V.) to abolish nasal secretion, vasoconstriction evoked by stimulating the cut peripheral end of the Vidian nerve at 5 and 7 Hz was reduced to between 60 and 90% of the original value after 90 sec and from 20 to 60% at the end of 3 min stimulation. At 15 Hz, vasoconstriction was between 30 and 60% of the initial value after 90 sec, and undetectable after 3 min stimulation. The frequency/secretion response curves of six normal cats were similar to those from six with a denervated nasal cavity in that the watery nasal secretion appeared after 30 sec and at a frequency of between 2 and 5 Hz. Secretion increased with increasing stimulation frequency to reach a maximum value at 15 Hz. At 2 and 5 Hz, secretions in the denervated

animals were significantly less than those of the normal cats ($P < 0.05$). Vidian nerve stimulation in denervated cats caused only vasodilation.

These results suggest that a degree of vasoconstriction occurs during the time nasal secretion is being formed and that secretion in the cat is not inhibited by the activation of sympathetic nerves. These findings are supported by the histological studies of Dahlström & Fuxe (1965), who failed to detect adrenergic fibres around the glands of the nasal mucosa of the cat, rat, guinea-pig and rabbit, and by those of Änggård & Densert (1974), who reported only a sparse adrenergic innervation of the nasal mucosal glands of the cat.

This work is being undertaken with the aid of a Medical Research Council project grant to Dr H. Wilson.

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The space-clamped retina

BY P. FATT. *Biophysics Department, University College London, WC1E 6BT*

A method has been devised to allow the controlled application of current across a defined area of retina in the perfused frog's eyecup, while extracellular micro-electrodes probe the retina (Fig. 1). The scheme uses concentric central and guard electrode compartments in contact with the back of the eye and an operational amplifier, the effect of which is to nearly abolish tangential current at the edge of the central compartment (for the principle see Marmont, 1949). This is necessary, owing to the relatively high resistance offered by the tight packing of cells within the retina as compared with the sclera, so that, in the absence of a guard system, tangential current through the sclera would exceed many-fold the current across the area of retina overlying the central electrode.

The isolated eye is cleared of adhering tissue and the stainless-steel collar *f* is fixed to its front with butyl-cyanoacrylate (Histoacryl Blau, B. Braun, Melsungen, W. Germany). The eye is positioned at this stage so that the optic nerve stump is well off the axis of the collar, while the optic axis of the eye is closer to it. The portion of the eye protruding above the collar is cut away and the lens removed. The collar with attached eyecup is dropped over the lower electrode assembly *c*, the spherical

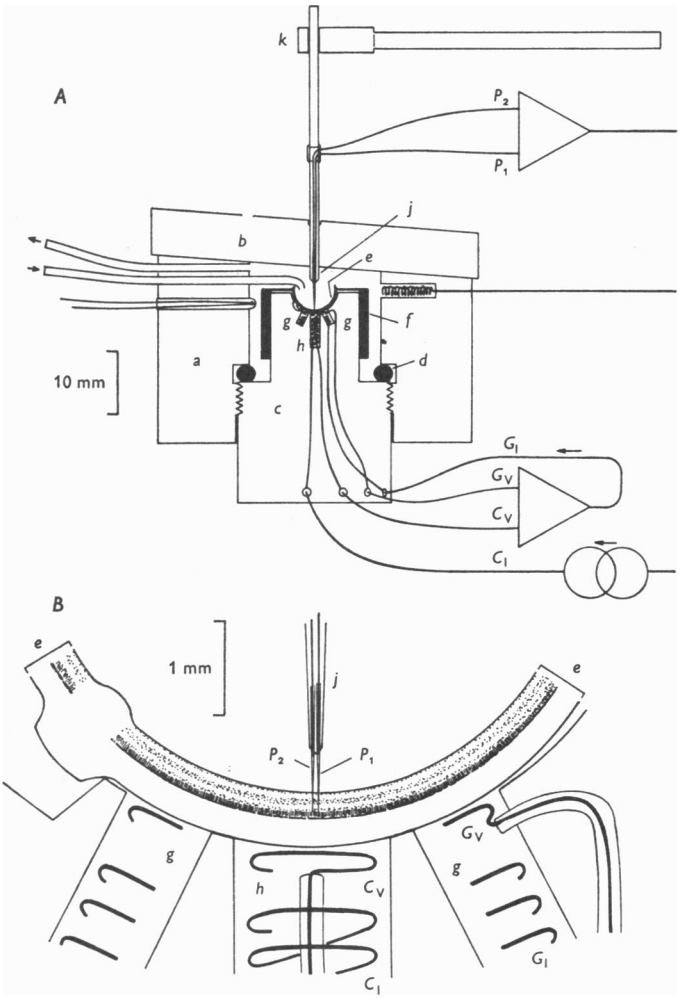

Fig. 1. *A*, cross-sectional view of measuring cell. The cylindrical main body of cell *a* has fixed to its upper, slightly angled end, the window *b*; while screwed into it from beneath is the lower electrode assembly *c*. These parts are of Perspex. A water-tight seal is made by the O-ring *d*. A spherical depression centred in the upper end of *c* contains the eyecup preparation *e*, to which is cemented the stainless steel collar *f*, in the form of a cylindrical tube with a thin annular disk attached to its upper end. The weight of this collar holds the eyecup against the narrow ridge separating the guard electrode compartment *g* from the central electrode compartment *h*. The former is in the form of an annular groove. A pit just peripheral to it accommodates the optic nerve stump. The double micro-electrode assembly *j* is sealed into glass pipette tubing which slides in a close-fitting hole in *b*, concentric with *h*. Fixed water-tight seals are made with paraffin wax, sliding seals with Apiezon grease. The clamp *k* on the micromanipulator arm allows some play in horizontal position and angle at which the shaft of *j* is held. The wall of *a* contains an electrode for earthing the solution filling the eyecup, a thermistor, and inlet and outlet tubes for perfusion.

B, enlarged view in the region of the central and guard electrode compartments and the retina penetrated by micro-electrodes. All electrodes use platinum black for metal-to-electrolyte contact.

depression of which is filled with Ringer solution. This is then screwed into the remainder of the measuring cell, and perfusion is begun.

The micro-electrode pairs used for probing within the retina are of glass-coated tapered tungsten wires with exposed tips coated with platinum black (Merrill & Ainsworth, 1972).

The method was devised for the purpose of carrying out impedance measurements on the layer of receptor outer segments, thus extending to the intact preparation the study of light-evoked changes in passive electrical properties of rod outer segments previously carried out on suspensions of isolated outer segments (Falk & Fatt, 1973). The same arrangement could be used with advantage for the study of intra-retinal currents evoked by light.

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Auto-instruction in histology and cell biology as a substitute for traditional laboratory activities

By T. Bauer, N. F. Metcalfe, W. K. Metcalf and E. D. Prentice. *Department of Anatomy, University of Nebraska Medical Center, Omaha, Nebraska 68105, U.S.A.*

In recent years, there has been a trend towards the reduction of traditional histology laboratories in the medical curriculum with greater reliance placed upon the use of coloured atlases, and assorted kodachromes as learning aids. In view of these events in medical histology teaching, it was decided to develop a comprehensive histology and cell biology (HCB) teaching programme which could serve as an effective substitute for traditional laboratory activities. The HCB programme employs an auto-instructional format which emphasizes mastery level-student centered learning. Instructional material consists of over 400 kodachrome slides which include photomicrographs, transmission electron micrographs, and scanning electron micrographs. Slides are accompanied by scripts in written and audio-cassette forms.

The HCB programme was validated by means of learner gain scores on individual units, analysis of questionnaire data, and student performance on written and laboratory examinations. Using an experimental group of 17 physical therapy students who used the HCB in place of a traditional histology laboratory, and a control group of 145 medical students, no

significant difference was found between the groups in mean performance levels in six written examinations employing multiple choice questions and three laboratory examinations involving the identification of various structures, both on standard microscopic glass slides and kodachrome slides. It is, therefore, concluded that the HCB program is an effective substitute for a traditional histology laboratory.

Distribution and localization of tritiated amino acids by autoradiography in the cat spinal cord *in vivo*

By M. E. Dennison, C. C. Jordan and R. A. Webster. *Departments of Anatomy and Pharmacology, University College London, Gower Street, London WC1E 6BT*

Autoradiography of rat spinal cord slices incubated with [^{3}H]glycine has shown it localized in inhibitory nerve terminals (Matus & Dennison, 1971, 1972) although after injection directly into the cat cord *in vivo* (Ljungdahl & Hokfelt, 1973) it occurs in other cellular elements. We have studied the distribution and localization of [^{3}H]glycine in the cat cord compared with that of GABA glutamic acid and the non-transmitter leucine.

[^{3}H] Amino acid (glycine-20 μM, 2·0–3·8 Ci/m-mole; GABA-20 μM, 1·3 Ci/m-mole; glutamic acid-40 μM, 0·65 Ci/m-mole; or leucine-20 μM, 1·0 Ci/m-mole) in artificial cerebrospinal fluid (c.s.f.) was perfused through the central canal of the lumbosacral cord of spinalized cats (Jordan & Webster, 1971) for 45 min followed by basic artificial c.s.f. for 15 min. The cord was then fixed *in situ* by vascular perfusion with phosphate buffered (pH 7·4) 4 % glutaraldehyde. Some samples of cord were divided into grey and white matter and digested for liquid scintillation counting and others were processed for light and electron microscopic autoradiography (Matus & Dennison, 1972).

[^{3}H]Glycine diffused throughout the grey matter but produced substantially lower levels of radioactivity in white matter. Electron microscopic autoradiography revealed activity in all cellular elements, but of synapses associated with exposed grains, 82 % contained 'flat' vesicles. The relative concentration of grains (RCG) amongst cellular elements in ventral horn was determined with a 10 mm grid on a montage (0·4 m^2, magnification $\times$ 9750) as:

$$\text{RCG:}\ \frac{\text{no. grains over element}}{\text{total no. grains}} \times \frac{\text{montage area}}{\text{area occupied by element}}.$$

Thus ratio > 1 indicates a concentration process.

Mean ($\pm$S.E.) RCG values (n = 11 montages) for glycine were:

synapses (1·72 ± 0·3), neurone cell bodies (1·62 ± 0·14), nuclei (1·65 ± 0·15), dendrites (1·04 ± 0·14), axons (0·91 ± 0·07), astrocytes (0·95 ± 0·06), oligodendroglia (0·31 ± 0·08). Adding 10^{-5} M *p*-chlorophenylmercurisulphonate to artificial c.s.f., to inhibit glycine uptake (Balcar & Johnston, 1973), before and during loading with [^{3}H]glycine, reduced total activity. A higher proportion was concentrated in white matter than in controls, with reduced activity in nerve terminals but not cell bodies.

Unlike glycine [^{3}H]leucine was confined to a discrete region around the canal. Activity was concentrated in cell bodies but not in the neuropil. The picture resembled that for glycine after *p*-chlorophenylmercurisulphonate.

[^{3}H]Glutamic acid showed a widespread distribution with high concentrations in the meninges. A general distribution was also seen with [^{3}H]GABA. It differed from other amino acids in its absence from ependymal cells but, like them, was associated with certain sub-ependymal cells which resembled the 'c.s.f.-contacting neurones' described by Vigh & Vigh-Teichman (1973) although we found no morphological evidence of a neuronal identity.

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The fine structure of the living cell – a reappraisal. Television film

By H. Hillman. *Unity Laboratory, Department of Human Biology and Health, University of Surrey, Guildford, Surrey*

A brief historical review was given from Hooke's description of the cell in 1665 until the modern use of the electron microscope. Intracellular movements of living cells, including streaming, pinocytosis, phagocytosis, mitochondrial movements, vacuolation and nuclear rotation, were shown. These photographs have been taken under low-power light microscopy, and are incompatible with the presence of a fine net – the endoplasmic reticulum – believed to be attached to the cell membrane and the nucleus, and requiring to be seen with 100–1000 times the magnification which is needed for viewing these intracellular movements. The

geometry of the endoplasmic reticulum, the 'unit membranes', and the nuclear pores, were shown to be two dimensional, and a truly three dimensional model of them could not be envisaged. An explanation for the appearance of the endoplasmic reticulum was offered. A fine structure of the cell, which is totally compatible with observations on living tissues, was proposed.

Pressure measurements in the terminal lymphatics of the cat mesentery

By Geraldine Clough* and L. H. Smaje. *Department of Physiology, University College London, London WC1E 6BT*

Subatmospheric tissue pressures have been measured using implanted perforated capsules (Guyton, 1963) and saline impregnated cotton wool wicks (Scholander, Hargens & Miller, 1968). This pressure appears to result from the interplay of osmotic forces created in the interstitial gel, mechanical tissue forces and possibly a sucking action of the lymphatics. The interstitium consists of a meshwork of collagen and elastin fibres embedded in a mucopolysaccharide gel which is unsaturated, and tends to imbibe water (Laurent, 1970). The terminal lymphatics embedded in the gel appear to be in hydraulic continuity with the interstitium (Leak, 1970) and it might be expected that their hydrostatic pressure would be subatmospheric, reflecting that found in the gel itself. In fact hydrostatic pressures measured in terminal lymphatics in superfused preparations of mesentery and omentum have been found to be positive, ranging from 0 to 2·5 cm H_2O (Zweifach & Prather, 1975). A possible explanation for this apparent contradiction is that continuous superfusion of these preparations with physiological salt solution leads to imbibition of fluid by the gel, thus causing the normal subatmospheric pressure to become positive.

The present experiments were performed on the exteriorized mesentery of anaesthetized cats essentially as described by Zweifach & Prather (1975). Lymphatic pressures were measured by direct puncture, using sharpened glass micropipettes (4–8 μm tip diameter) attached to a null-balance servo pressure measuring system. The pipettes were filled with 1 M dm^{-3} NaCl coloured with 1·5% Evans Blue and injection of small volumes of the dye were used to show up the lymphatic network and ensure that the pipette tip was free in the lymphatic lumen. The main difference between our methods and those of Zweifach & Prather is that we used warmed FC-80 Fluorocarbon, a fluorinated hydrocarbon, to prevent the tissue from drying whereas they used a physiological salt solution. Thus water imbibition

* M.R.C. Scholar.

could not occur in our preparation and it was hoped that under these circumstances the gel would remain unsaturated and the pressures in the mesenteric lymphatics would more nearly reflect those in the normal *in vivo* state.

The measurement of pressures in the lymphatic vessels was demonstrated, and data obtained in mesenteries superfused with physiological salt solutions and with FC-80 Fluorocarbon Liquid will be presented.

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Vascular permeability in the perfused cat salivary gland using single passage multiple tracer dilution

BY G. E. MANN,* L. H. SMAJE and D. L. YUDILEVICH.† *Department of Physiology, University College London, London WC1E 6BT, and Department of Physiology, Queen Elizabeth College,† London W8 7AH*

Permeability in the cat submandibular salivary gland has been studied using the single passage multiple tracer dilution technique (Yudilevich & Smaje, 1976). A reference tracer, which remains within the vasculature, is injected close-arterially together with 'diffusible' tracers, [^{51}Cr]EDTA and [^{86}Rb]Cl. The dilution curves for these radioisotopes are obtained by sampling the venous effluent at 1 sec intervals and counting these samples in a Panax gamma spectrometer. Since the 'diffusible' tracer leaves the vessels, its concentration in the outflow will be proportionately less than that of the reference tracer. The maximal loss occurs initially and as the 'diffusible' tracer passes back into the capillaries the concentration ratio will be increased. The permeability of the microvasculature (permeability × surface-area, PS) and that of the extravascular barriers to transport can be determined from the dilution data and plasma flow (Martín de Julián & Yudilevich, 1964).

In glands with an intact vascular supply parasympathetic nerve stimulation caused an increase in PS (Fig. 1*a*). However, there was also a marked increase in glandular blood flow, and it is known that PS can increase with flow (Alvarez & Yudilevich, 1969). To differentiate the flow effect from a change in permeability as such, perfusion at constant flow

* M.R.C. Scholar.

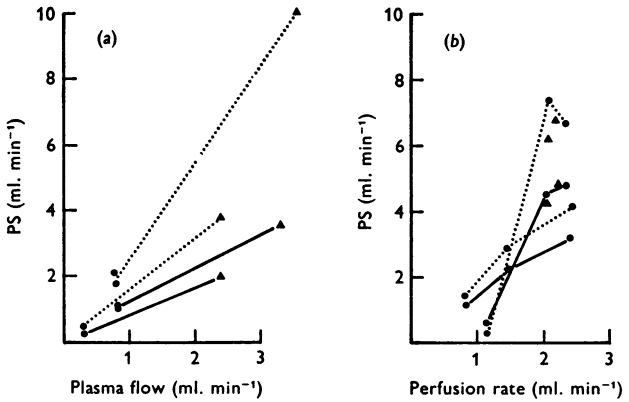

Fig. 1. Relationship of PS against flow for (*a*) glands with an intact vascular supply, (*b*) glands perfused with Krebs–Ringer albumin solution at different flow rates. Two separate experiments are shown in each case. In (*a*) changes in flow are due to changes in resistance induced by parasympathetic nerve stimulation (▲) while in (*b*) they are produced by increasing pump output either with (▲) or without (●) parasympathetic stimulation. Points for Rb are joined by a dotted line and those for Cr with a solid line.

was used to investigate the relationship between PS and flow in non-stimulated and nerve-stimulated glands (Fig. 1*b*). The perfusion medium consisted of an oxygenated modified Krebs–Ringer albumin solution. At the highest flows used the perfusion pressure never exceeded 70 mmHg. Experiments so far show that in the perfused gland also, with the labelled molecules used, PS increases with flow.

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A warning device indicating the stoppage of paper in an ink-writing recorder

BY M. W. JOHNS and E. S. REEVES. *M.R.C. Environmental Physiology Unit, London School of Hygiene and Tropical Medicine, London WC1E 7HT*