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DEMONSTRATIONS

An application of cumulative sum technique (cusums) to neurophysiology

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During a recent demonstration to the Society a cusum technique was applied to post-stimulus time histograms (PSTH) used to detect responses of a neurone to a stimulus (Ellaway, Pascoe & Trott, 1976). A PSTH is a correlogram between stimulus and action potentials and is formed from the sum of a number of trials. The count in any one bin is randomly determined and this 'noise' in the PSTH may obscure any change in the mean count following a stimulus. A cusum reduces the noise in a series of counts and emphasizes any change in the mean by producing a clearly visible slope or trend over the period of change.

Cusums are constructed simply by subtracting a reference level from each datum point and accumulating the differences. Taking a reference level equal to the mean of a control stationary period gives a cusum of zero slope (see Fig. 1). If the mean level subsequently changes, even by a small fraction of the standard deviation of the points, the cusum changes slope dramatically. Having thus identified the response period, conventional statistics may be used to test the change in mean level.

Cusums have yet to be used widely in physiology where they can provide a powerful tool to anyone examining a series of values occurring at regular intervals of time (cf. Woodward & Goldsmith, 1964).

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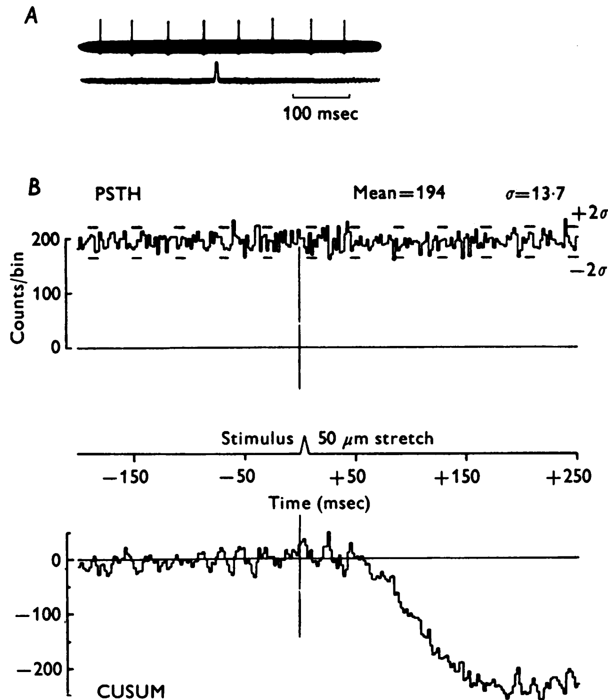

Fig. 1. *A*, train of action potentials (upper trace) from a single nerve cell (fusimotor neurone) typical of the records used to construct the PSTH below. Lower trace indicates the length of a muscle and the timing of the stimulus, a brief 50 μm stretch. *B*, PSTH constructed from 1792 sweeps similar in nature to that above. The mean control count of 194 in each 2 msec bin is not indicated, but horizontal dashed lines have been placed at + and − two standard deviations from the mean. Time and the timing of the stimulus are indicated below the PSTH. CUSUM constructed from the PSTH data above (as explained in the text). Note the negative slope, between 50 and 150 msec following the stimulus, clearly indicating that the neurone was inhibited at this time even though this was not evident from the PSTH.

A device for measuring the McCollough effect using polarized light

By C. C. D. Shute. *Physiological Laboratory, University of Cambridge*

Three mains-operated slide viewers are used. The first viewer holds the adapting slide with vertical and horizontal gratings backed by Wratten coloured filters. The gratings are viewed alternately for 5 sec during a period of 5 min. The second viewer holds the achromatic test slide with gratings arranged centrally and peripherally: a 0·3 neutral density filter can be added. The third viewer holding a blank slide is used for matching.

Over the lens a device is fitted enabling a $1\frac{1}{2}\lambda$ birefringent compensator to be rotated through a reduction gear between parallel polars. When a match is obtained, the angle between the slow direction of the compensator and the transmission axis of the polars is read to $\frac{1}{2}°$ from a graduated scale. Suitable compensators are made by combining different thicknesses of transparent adhesive tape ('Caribonum polytape': the retardations of $1\frac{1}{2}$, 1, $\frac{3}{4}$ and $\frac{1}{2}$ in. wide tape, measured with a Babinet variable compensator, were found to be 345, 275, 240 and 110 nm respectively). A compensator made from two thicknesses of $1\frac{1}{2}$ in. tape and one of $\frac{1}{2}$ in. tape to give a retardation of 800 nm yields a red interference colour that will closely match the McCullough effect (ME) colour produced by a Wratten filter no. 58 (peak transmission 530 nm). If the angle between compensator and polars at the match point is $c°$, the percentage extinction of green light $= 100 \sin^2 2c$, which may be taken as a measure of the ME. Since c usually lies between 15° and 30° and the curve is nearly linear between these points, an acceptable approximation is ME $= 3\frac{1}{3}\ (c - 7\frac{1}{2})$. The spectral composition of the match when ME $= 50$, i.e. when $c = 22\frac{1}{2}°$, is shown in Fig. 1.

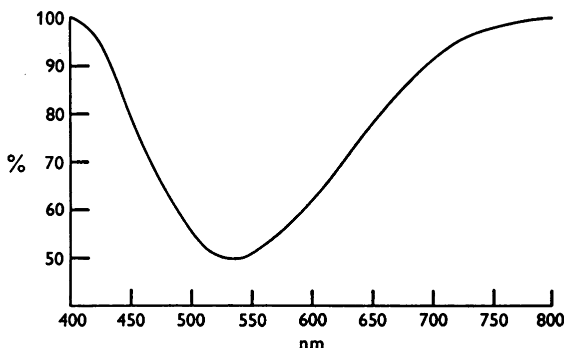

Fig. 1. Percentage transmittance (t) of 800 nm compensator in $22\frac{1}{2}°$ position between parallel polars given by $t = 100\ (1 - \frac{1}{2} \sin^2 \frac{1}{2}\theta)$, where

$$\theta = \text{phase angle} = \frac{\text{retardation}}{\text{wave length}} \times 360°.$$

The equipment is simple, portable and does not require darkness. The ME is measured in units that may reflect the percentage inhibition occurring in the visual system. The method is sensitive enough to show changes produced by varying environmental conditions, e.g. stress, fatigue and drugs.

A high-resolution thermometer/differential thermometer

By C. M. Laws. *Department of Pharmacology, Charing Cross Hospital Medical School, London, W. 6*

Modern integrated circuit technology has made possible a simple yet accurate electronic version of the well-known Beckmann differential thermometer with a resolution of 1/100° C and response time of ~ 1 sec. Such a thermometer may be built at considerably less cost than commercially available equivalents, and is easily assembled by technical staff with experience of soldering.

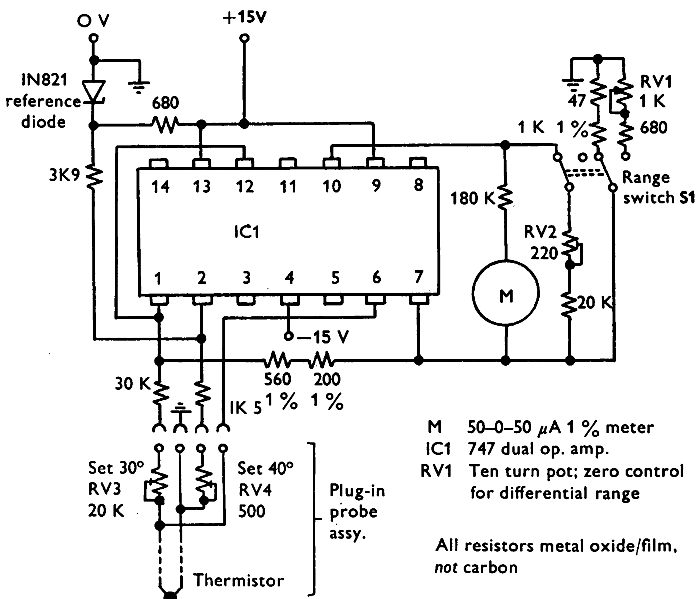

Fig. 1. Thermometer circuit.

The unit demonstrated (20–40° C ± 0·1 °C) has a differential − 1° C–0–+ 1° C range (± 3 %) with selectable reference temperature, and interchangeable probes. Each probe requires one initial calibration procedure following construction; the temperature-sensing element may either be a type F thermistor (ITT Components Ltd) which resembles a small clinical thermometer, or a type GM thermistor (ITT Components Ltd) which is a bead 5 mm long × 1 mm diameter suitable for miniature probe applications. Both thermistors are glass encapsulated.

In the circuit diagram shown RV2 is set up so that the resistance between pins 7 and 10 is divided accurately by 10 when S1 is closed (using a

digital multimeter, IC1 unplugged). Calibration of probes is made against reliable references at (i) 30° C-adjusting RV3, (ii) 40° C-adjusting RV4. The specification above is quoted for calibration standards accurate to ± 0·05° C.

The unit needs only a simple bridge-rectifier ± 15 V power supply, and may be easily extended to monitor many probes by addition of further probe sockets and a suitable selector switch.

An ion selective electrode for the determination of potassium

By D. M. Band, J. Kratochvil and T. Treasure. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London, SE1 7EH*

Advances in ion-slective membranes based on various organic carriers has made possible the construction of a range of electrodes suitable for biological measurements.

The electrode demonstrated was developed to study the relationship between conventional concentration measurements of potassium and the activity as indicated by the electrode in biological solutions. The membrane chosen was a solid plastic based PVC containing Valinomycin and potassium tetraphenylborate (Moody, Oke & Thomas, 1970; Band & Kratochvil, 1974). The composition is shown in Table 1. The membrane was cut up

Table 1. Constituents of PVC based electrode membrane. The quantities are dissolved in approximately 3 ml. of tetrahydrofuran and poured into a Teflon container with a diameter of 2 cm. As the solvent evaporates the membrane polymerizes and is formed on the base. This quantity will make 20–30 electrodes

Valinomycin	0·0015 g
Bis-2-ethylhexyladipate	0·15 g
Nitrobenzene	0·05 g
Potassium tetraphenylborate	0·000025 g
High molecular weight PVC	0·075 g

into disks and cemented on to the end of 3 mm o.d. PVC tubes, which were filled with 3 mM/l. KCl and fitted with internal silver/silver chloride electrodes. The electrode jacket and cuvette were designed to maintain small samples ($< 200\ \mu$l.) anaerobically and at blood temperature. Any activity electrode measurement in blood is complicated by the indeterminacy of the liquid junction potential formed between the blood and the bridge solutions. The Radiometer K497 liquid junction – free diffusion saturated KCl type – was chosen since this junction has been extensively used in blood acid–base measurements.

The electrode was calibrated using (*a*) pure potassium chloride activity standards and (*b*) potassium chloride standards adjusted to an ionic strength of 0·15 M with NaCl. Preliminary work indicated that the

apparent activity coefficient for potassium in the blood plasma was similar to that in the KCl/NaCl standards. This was confirmed by a larger series (> 150) of samples from patients in intensive care. The electrode was calibrated in concentration terms using the KCl/NaCl standards and the measurements on whole blood samples compared with the results from flame photometry. A systematic discrepancy of 0·2 mM/l., the electrode indicating a higher concentration than the flame photometer, was detected. The flame photometer measurements were calculated per litre of plasma and no allowance was made for the volume of the plasma proteins. This correction would be of the order of the discrepancy found. It could be argued that the effect of a residual liquid junction potential between the bridge and the blood and standards respectively was being offset by a coincident discrepancy in the activity coefficients, but it seems more reasonable to assume that the activity coefficients are similar and that the liquid junction potential between saturated KCl and blood is negligible.

The PVC membranes show a high rejection of interfering ions and freedom from poisoning when exposed to biological solutions. The lack of buffering of potassium in the solution necessitates a cuvette that flushes readily. Control of temperature and the free diffusion liquid junction help in achieving highly reproducible results.

The cell is in clinical use in St Thomas's Hospital where its cheapness and rapid direct reading of potassium on whole blood samples are advantages. The reproducible free diffusion junction with saturated KCl could prove useful in the measurement of ions when the calculation of electrochemical gradients is required. The basic cell can be adapted to the measurement of other ions by altering the composition of the plastic membrane.

This project is being supported by St Thomas's Hospital Endowments Fund (research).

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A denervated dog's kidney preparation for studying the actions of drugs or hormones on renal function and renal lymph formation

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A method has been developed to measure the effects of a substance injected directly into the renal circulation, on the flow rates and composition of blood, urine and, usually, lymph. It also displays any acute changes produced in renal volume. The kidney is denervated by resection and

reanastomosis to the animal's neck vessels by a method similar to that described by Reinhardt, Klose, Ellinghaus, Brechtelsbauer & Behrenbeck (1967) and Lichardus & Nizet (1972).

Greyhounds are anaesthetized with pentobarbitone sodium. A carotid artery and jugular vein are prepared to receive the transplanted kidney. Their distal ends are transected, pulled through and everted back over thin-walled, stainless-steel tubes. The experimental kidney is approached through the abdomen. Surrounding connective tissue is cut between ligatures to minimize bleeding. Lymphatics also caught in these ligatures become distended and are opened. The renal vessels and ureter are cut and the kidney removed to the neck where the vessels are ligated over the everted ends of the prepared neck vessels. The ureter is ligated over a third stainless-steel tube. All tubes are screwed into a plate from which the kidney is supported on a nylon net. A box is advanced over the kidney and bolted in position on to the plate. The box is maintained at 37° C by an external water jacket. It is filled with light liquid paraffin which is connected to a beaker suspended from a force transducer. The quantity of paraffin displaced into the beaker reflects lymph collected within the box or a change in renal volume. Samples of lymph are drained from the box as required. Renal venous blood is sampled from a tributary of the jugular–renal venous anastomosis. Steady-state values of renal blood flow are measured by clearance techniques and acute changes followed by a Biotronics blood-flow transducer. The substance under investigation is infused directly into the blood supplying the experimental kidney.

Some results obtained using this preparation have been published (Wilcox, 1976).

The work was supported in part by grants from the Wellcome Trust and the National Kidney Research Fund.

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A mechano-electronic working model of the muscle spindle

BY A. W. S. BROWN,* F. G. HARRISON and M. J. LAB. *Department of Physiology, Charing Cross Hospital Medical School, Fulham Palace Road, London, W6 8RP*

Pre-clinical medical students can find the subject of the function of the muscle spindle difficult to visualize. To alleviate some of the difficulties, a

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working model of a muscle spindle was built which demonstrates some of the responses of both the muscle spindle and the Golgi tendon organ, using strain gauges as the mechanoreceptors and audiovisual signals for the nerve impulses.

Construction

Corrugated rubber hose, covered with thin coloured sponge rubber, is used to represent the extrafusal fibres, the intrafusal fibres with swollen sensory zone, and the tendon. The fibres are attached at their ends to two horizontal bars. The length of the muscle fibres, e.g. vertical distance between the bars, is controlled by modified cycle brake-cable mechanisms, concealed, giving independent control of the 'extrafusal' and 'intrafusal' elements. The cables are also concealed in the rubber hoses, as are the strain gauges.

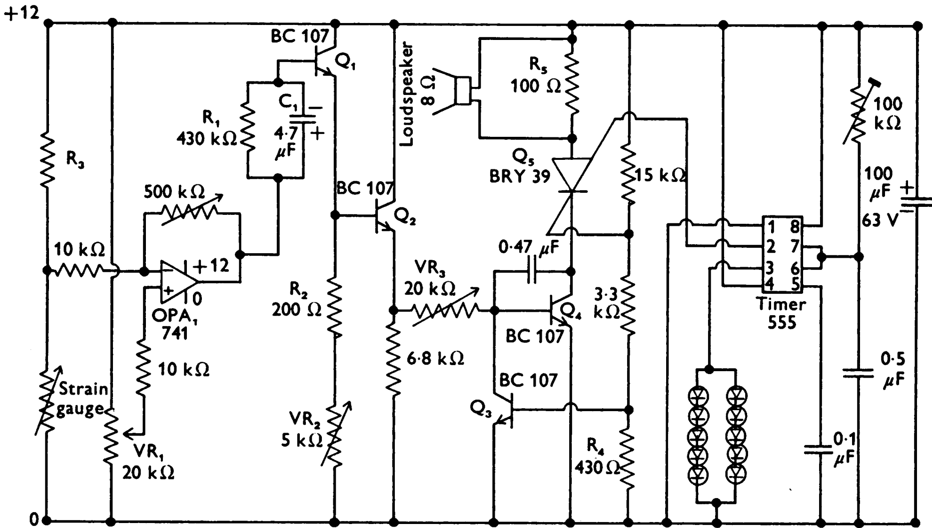

Fig. 1. Circuit diagram for the muscle spindle or Golgi tendon organ.

Motor and sensory 'nerves' are of plastic sleeving and spiral cable wrapping with light emitting diodes (LEDs) spaced along them indicating the frequency of 'discharge' by variable rate of flashing. The sensory and motor 'nerves' run from a diagrammatic representation of a spinal cord transverse hemi-section. The whole model is mounted vertically in a 19 in. rack.

Circuitry

The circuit (Fig. 1) is designed to give audiovisual signals via loud-speakers and light emitting diodes. One such circuit is required for each 'receptor'. The frequency of both sound and light signals follows the time course of the transistor, Q_1, output wave form, modified by the resistance of the strain gauge. The audiovisual signals thus simulate the passage of action potentials along the '1a' or '1b' afferent nerves. Both static and dynamic properties of the receptors can be demonstrated by appropriate adjustment of variable resistance VR_2, whilst sensitivity is adjusted by VR_3 and threshold by VR_1. The gain of the operational amplifier OPA_1 and the value of resistance R_3 are chosen to suit the characteristics of the strain gauge. The duration of the LED flashes, to be clearly seen, is controlled by a pre-set variable resistor.

The model has been used in a tutorial-demonstration to small groups for several years, as well as for practical illustration in lectures. Student response in a recent questionnaire was very favourable, and indicated that the model was successful in creating interest and expediting understanding of the working of the muscle spindle.

Method for internal recording from diaphragm in a vertical position

By L. D. Mitchell. *Department of Physiology, St Mary's Hospital Medical School, London, W2 1PG*

For internal recording the rat diaphragm is usually mounted horizontally in a bath with flowing saline (e.g. Liley, 1956). The muscle can also be mounted vertically without flow in experiments designed to conserve drugs, and in situations when the oxygenation of the whole tissue is necessary. The rib was secured to one leg of a vertical holder, and the micro-electrode approached at an angle. The present method has been used for end-plate recording (Creese, Franklin & Mitchell, 1976), and is modified from Creese, Scholes & Whalen (1958).

This research was supported by a grant from the Medical Research Council.

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Evaluation of an isotonic apparatus for muscle recording

By D. L. Birdsall, J. A. Hodgson* and D. M. Lewis. *Departments of Physiology and Aeronautical Engineering, University of Bristol, BS8 1TD*

An apparatus for measuring shortening velocity should present the muscle with a constant load. The design of this isotonic apparatus presents problems in that inertial and damping forces are added to the load which is required. It is therefore essential to estimate these forces so that any necessary corrections may be made to the results of experiments in which the apparatus is used. Damping forces have received little attention in this context even though they will add to the load on the muscle at constant shortening velocity. In this demonstration we illustrated the methods which we have used to quantitate inertial and damping forces in an isotonic apparatus designed for rat limb muscles.

We have considered two damping forces: Coulomb friction producing a force independent of velocity, and viscosity producing a force proportional to velocity. In order to measure these the isotonic apparatus was set up with loads clamped to the free end of the vertically hung lever. The shape of the oscillation decay envelope will depend on the nature of the damping forces. Coulomb friction will reduce the oscillations linearly (Den Hartog, 1940). The decay in amplitude depends on the constant force opposing rotation (damping moment, D) and on the mass (M) of the lever and attached load.

Thus

$$\frac{a_0 - a_n}{n} = \frac{4D}{Mg},$$

where a_0 is the amplitude peak at time t sec and a_n is the amplitude after n cycles occurring at time $(t+T)$ sec. If the frequency is f Hz and the length of the lever is x then the frictional force is given as:

$$\frac{(a_0 - a_n)\,Mg}{4Tfx}.$$

Viscous damping results in an exponential decay of oscillations, described by the expression:

$$a_n = a_0 \exp\left(-\frac{kT}{2M}\right),$$

where k is the coefficient of viscosity (Den Hartog, 1940).

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The mean velocity of the lever (V) was calculated as $\sqrt{2\pi f(a_0+a_n)/2}$. The damping force ($V.k$) is then given by the expression:

$$\sqrt{2\pi f(a_0+a_n)}\,\frac{M}{T}\,.\ln\left(\frac{a_0}{a_n}\right).$$

These expressions were used to estimate the damping force at different times of the decay. The values of additional load due to damping were estimated using both expressions but the results were similar in each case.

The damping force added 1 mN to a 30 mN isotonic load and this additional force increased to 5 mN with a 2·5 N load.

The lever was then moved to a horizontal position with the free end supported by a spring then allowed to oscillate. Weights added to the lever modify the frequency of oscillation (f) according to the expression:

$$f = \frac{1}{2\pi}\sqrt{\frac{S}{m_i+m}},$$

where S is the stiffness of the spring, m_i is the apparent mass of the lever and spring and m is the mass of the added weight.

The linear form of this equation:

$$m = \frac{S}{4\pi^2 f^2} - m_i$$

provides an easy method of estimating the inertial mass from a sequence of frequency measurements under different loads. This inertial mass may then be substituted into the expression derived by Wilkie (1949) to describe the acceleration of the lever under the influence of a contracting muscle.

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Self-instructional materials in medical physiology

BY MARY DAVIES and PAMELA HOLTON. *Department of Physiology, St Mary's Hospital Medical School, London W2 1PG*

This demonstration represents a progress report of a project undertaken by the Audio Visual Sub-Committee of the Board of Studies in Physiology, University of London. Our aim is to provide learning materials in Physiology which are efficient and effective. The learning materials cover difficult topics in physiology, and can be used for revision or first time study.

Each unit of Self-Instructional Materials consists of an audiotape cassette and booklet. Recording scripts are also available.

The material has the following characteristics:

(1) It is based on learning objectives which specify what the student will be able to do as a result of his study. The learning objectives are agreed by the Sub-Committee.

(2) It is designed to involve the student actively.

(3) It is modified in response to criticism from academic staff throughout the University. By this means we hope to ensure that the material is accurate and up-to-date.

(4) The relevance to medicine is emphasized.

(5) A test with answers is included.

The effectiveness of the materials has been demonstrated by the achievement of the learning objectives. Students' attitudes towards the material were favourable.

This work was supported by grants from the World Health Organization and the Nuffield Foundation.

A method for collecting the fluid from the Graafian follicles of the ovary or fluid from other small spaces

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Some years ago we investigated the insulin content of various body fluids (Daniel & Henderson, 1967) and are now extending this work. In the course of a study of the insulin content of Graafian follicles we tried to collect the fluid by means of a fine needle. We found that we could only withdraw a small percentage of the fluid, and this was often contaminated by blood as a result of damage by the needle tip. We therefore devised a method in which the ovaries are frozen and the follicular fluid is dissected out as solid, rounded, frozen pellets. This method may be used for obtaining all the bile in the gall-bladders of small animals such as hamsters, as well as the fluid from the anterior chamber of the eye, and fluid from other small, cyst-like spaces.

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A device for inducing cardiac arrest and then re-establishing the heart beat

BY P. M. DANIEL, F. L. PRATT, O. E. PRATT, E. SPARGO and D. E. M. TAYLOR. *Department of Applied Physiology, Institute of Basic Medical Sciences, Royal College of Surgeons, Lincoln's Inn Fields, London WC2A 3PN, and Department of Neuropathology, Institute of Psychiatry, De Crespigny Park, London, SE5 8AF*

In order to study some of the effects of temporary cardiac arrest on the brain we have devised a combined stimulator-defibrillator. Rats are anaesthetized with I.P. sodium pentobarbitone. The instrument is connected to two needle electrodes, insulated except at the tip, one of which is inserted through the chest wall so that the tip lies near the apex of the heart and the second near the atrium. Cannulae for recording arterial and venous pressure are inserted.

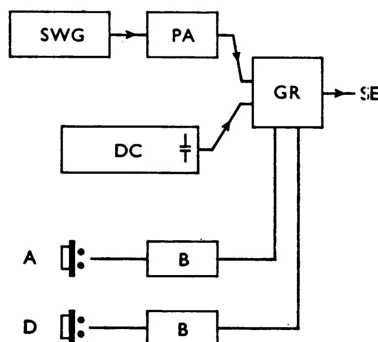

Fig. 1. Arrangement of apparatus: SWG square wave generator; PA power amplifier; GR gating relays; DC defibrillator capacitor; SE stimulating electrodes; A, D, push buttons for inducing cardiac arrest and defibrillating respectively; B, bistables to control relays. The relays make the appropriate connexions to the electrodes or alternatively to recharge the capacitor. Pulses were generated from a Devices No. 252 isolated stimulator driven by a Digitimer D 121 and amplified by a high-voltage power transistor connected as an emitter follower.

When the 'Arrest' button (A, Fig. 1) is pressed a stimulus is applied between the electrodes (100 Hz, 5 msec pulse width, square wave, approx. 30 V, 10–20 mA). Ventricular fibrillation occurs at once, the arterial pressure falling, usually within 5 sec, to about 5–15 mmHg; the venous pressure rises simultaneously to a roughly similar level. After a suitable interval, e.g. 1–2 min, the 'Defibrillate' button (D, Fig. 1) is pressed. This stops the square wave stimulus and when the button is released a capacitor discharge (100 V, 50 μF) is given automatically. This capacitor discharge

can be repeated as often as required by pressing and releasing this button. Usually ventricular fibrillation can be aborted and a normal rhythm can be re-established.

The determination of myo-inositol in micropuncture samples from the testis and epididymis of the rat

By B. T. Hinton, B. P. Setchell and R. W. White. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT*

The presence of high concentrations of free myo-inositol within the male reproductive tract has been known for several years (see Setchell, Dawson & White (1968) for references) but precise measurements of the concentrations of this sugar alcohol in the testicular and epididymal tubular fluid have not yet been made. We have developed micropuncture techniques and a yeast microbioassay to obtain such values.

Approximately 100–300 nl. of fluid can be collected from rat testicular and epididymal tubules by using sharpened glass micropipettes of tip diameter 30–40 μm (Levine & Marsh, 1971; Tuck, Setchell, Waites & Young, 1970; Howards, Johnson & Jessee, 1975). The sample is kept between liquid paraffin in the micropipette to prevent its evaporation. To remove spermatozoa, the sample is withdrawn into very fine siliconized constant-bore capillary glass tubing (120 μm o.d., 88 μm i.d., kindly provided by Professor K. J. Ullrich, Max-Planck Institut für Biophysik, Frankfurt, W. Germany), the end sealed ('Seal-Ease', Clay Adams, or Resin, B.D.H.), placed into a similarly sealed glass capillary (1·0 mm o.d., 0·7 mm i.d.) and finally inserted into a sealed standard haematocrit centrifuge tube. The sample is centrifuged at 12,000 *g* for 15 min using a microhaematocrit centrifuge (Hawksley). The spermatozoa pellet is discarded and the clear supernatant stored under water-saturated liquid paraffin prior to analysis.

For the measurement of free myo-inositol we have scaled down an established bioassay (White & Black, 1975); this uses a yeast *Kloeckera apiculata* which has an absolute requirement for inositol and thiamine. Six myo-inositol standards ranging from 2 to 64 ng are prepared with each assay. After the introduction of the sample into test glass tubes (length 50 mm; diameter 6·5 mm), the samples are dried at 80° C for 20 min and then heat sterilized at 120° C for 2½ hr. To each tube we then add 50 μl. of assay medium (see White & Black, 1975) containing approximately 100,000 yeasts. The tubes are heat-sealed using a gas oxygen flame and the samples incubated at 25° C for 3 days. Then, the yeasts in a 10 μl. sample are counted in a haemocytometer chamber or a Coulter counter. Incubating the yeasts with ^{3}H-thymidine to detect cell division proved in-

sensitive. We have also tested several other sugars, sugar alcohols and inositol derivatives to ensure that the assay is specific for free myo-inositol.

Since the free myo-inositol concentration is about 0·4 mg ml.$^{-1}$ in seminiferous tubular fluid, 0·5 mg ml.$^{-1}$ in rete testis fluid and 0·6–9 mg ml.$^{-1}$ along the length of epididymis (3–4 mg ml.$^{-1}$ initial segment; 1–3 mg ml.$^{-1}$ proximal caput; 0·8–1·5 mg ml.$^{-1}$ distal caput; 0·6–1 mg ml.$^{-1}$ corpus; 4–6 mg ml.$^{-1}$ cauda; 7–9 mg ml.$^{-1}$ vas deferens) it was possible to assay 25 nl. samples of seminiferous tubular fluid or 5–10 nl. of epididymal tubular fluid. Blood serum contains about 0·01 mg ml.$^{-1}$ and therefore samples of at least 1 μl. are needed for assay.

This investigation received financial support from the World Health Organization.

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Gastric secretion and gastric mucosal blood flow in the conscious rat

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ADH and prolactin in the sheep foetus

BY D. PAULINE ALEXANDER, H. G. BRITTON, H. L. BUTTLE and MARY L. FORSLING. *Department of Physiology, St Mary's Hospital Medical School, London W2 1PG*

A modified technique for placental perfusion in the guinea-pig

BY ENID FENTON. *Department of Physiology, St Mary's Hospital Medical School, London W2 1PG*