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DEMONSTRATIONS

A flicker photometer for trained animals

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Small active animals (at present grey squirrels, white rats, and fish) are trained to choose between a flickering and a steady light presented in a modified *Y* maze, automatically controlled, and to work continuously for a food reward.

The purpose of the apparatus is, like that of a conventional flicker photometer, to find the intensity of each monochromatic light, relative to a standard white, which gives no sensation of flicker when the two lights replace one another at speeds just below the critical flicker fusion frequency. This is a good approximation to the more difficult heterochromatic brightness match.

Each of the stimuli, flickering and steady, is composed of light from a standard white and a monochromatic source, falling on the back of opal glass behind which is a rotating disk of Polaroid filter which determines the flicker speed.

On the side which flickers, the white and monochromatic beams are polarized, one horizontally and the other vertically (by passing separately through Polaroid filters) before traversing the rotating Polaroid, which then cuts the beams out alternately, according to a $\sin^2$ law.

On the side which is steady, the rotating polaroid does not vary the intensity of beams which are not polarized in advance. In order to interchange the 'flickering' and 'steady' sides, it is only necessary to rock over a quiet vane which exchanges the pair of pre-polarizing filters on the 'flicker' side with the pair of compensating neutral density filters on the 'steady' side.

The apparatus sounds the same whichever side is set to flicker. Under

conditions where there is no sensation of flicker, the two stimuli have the same hue and brightness.

The automatic control unit (basically the same circuit which controlled previous absolute threshold experiments, e.g. Silver, 1966) rewards the animal for correct choices, and counts correct and wrong choices at each intensity level of the monochromatic light. The neutral density wedges varying the monochromatic light move backwards and forwards from end to end in steps.

After each choice the shutters close, the neutral wedges move on a step, and the vane controlling the side which flickers moves according to a randomized sequence. The animal can then return to the starting position, which automatically opens the shutters revealing the next stimulus.

In a successful experiment, the cumulative record of the counters shows an intensity level of the monochromatic light at which mistakes are made, flanked by higher and lower intensities where the animal can easily distinguish which side flickers.

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Receptive properties of retinal cells and tectal cells in the pigeon

By A. L. HOLDEN. *Institute of Ophthalmology, Judd St, London W.C.* 1

A study of the receptive properties of cells in the retina and tectum is being carried out in the pigeon, anaesthetized with urethane. Visual stimuli can be projected upon a screen, and moved in controlled velocity in any direction. Stimulus cycles are controlled from a timer unit and pulse generators designed by P. M. G. Bell, University Laboratory of Physiology, Oxford.

KCl-filled micropipette electrodes are advanced into the retina from the vitreous side. They record a sharply localized intraretinal electroretinogram (e.r.g.) from an area less than 4° in subtense. Stimuli larger than this area suppress the *B*-wave and 'off' response of the e.r.g.

Spike potentials of ganglion cells are recorded extracellularly, and are positive/negative in conformation and up to 1·5 mV in amplitude. Eighteen out of twenty-seven ganglion cell receptive fields have been uniformly 'on–off', the cell firing a brief burst of three to five spikes at the 'on' and 'off' of a 1° or $\frac{1}{2}$° spot anywhere in the receptive field. Like the local e.r.g., the spike responses of many cells are suppressed by spots of light larger than the receptive fields. Nine out of twenty-seven cells show a more complex organization, with a central area producing 'on–off' firing, and an 'off' periphery. Three of these show a specialized 'movement-centre', in which

'on–off' firing is particularly vigorous. Movement centres are also found in tectal cells, and have previously been described in the rabbit visual cortex by Arden, Ikeda & Hill (1967).

The field outlines are circular or elliptical, and in the region of visual space 20° posterior and superior to the optical axis, range in diameter from 2° to 17° (mean 5·6°). Twenty-three out of twenty-seven responded to movement of small spots or black targets through their receptive fields, with a preference for a particular velocity of movement. Eight out of twenty-three responded to motion in any direction. Fifteen out of twenty-three were directionally selective. These would respond to motion in one direction, but not to the opposite motion. Commonly the responsive axes extend through 180° of the field, with a maximal response at mid-axis, though more complex variants of directional selectivity have also been observed. The same preference is shown for spots of light as for black targets. Movement of two spots simultaneously through the field in opposite directions, one in the preferred axis, produces a smaller response than movement of one spot in the preferred axis. This would be expected on an inhibitory system of directional selectivity, as proposed by Barlow & Levick (1965). Preferred axes are for upward or anterior movement.

Receptive fields have been plotted for seventy-four tectal cells, some 20° above and below the optical axis of the eye. Fields are circular or elliptical in outline and are commonly uniformly 'on–off'. They range from 2° to over 45° in diameter. Small fields are found in superficial laminae of the tectum, and are of the same order of size as fields of retinal ganglion cells. Again, most cells respond to movement, either in all directions or with directional selectivity. Movement centres have been observed in both large and small fields. The preferred axes tend to be for upwards, downwards, or anterior movements. These cells could serve as tilt detectors during 'roll' in flight. They differ in their requirements for stimulus size. Some only fire when a small stimulus moves on a uniform background. Others are fired by stimuli extending considerably outside the outlines of the receptive field. The latter would signal the movement of large features in the visual field moving relative to the eye.

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Effects on the electroretinogram of change in the ionic composition of the fluid bathing the isolated avian and mammalian retina

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There have been several reports about the effects of ions on the electroretinogram (e.r.g.), but since this is a compound potential the results are difficult to interpret. We have therefore repeated these experiments on a fraction of the e.r.g., the PIII component, which is very likely to be developed only by the receptors, as a hyperpolarization of their distal ends.

A normal rat e.r.g. was first obtained with an incubation medium modified from Pautler, Murakami & Nosaki (1968). Omission or removal of calcium ions from this medium caused the '*b*-wave' to disappear, leaving only PIII. When calcium ions were restored, the *b*-wave returned at once. The effect of altering anions (e.g. phosphate) is consistent with the hypothesis that the effect on the *b*-wave is related to the availability of calcium ions within the retina.

In calcium-free high phosphate media, a constant flash elicits a constant PIII response for over 3 hr, provided the medium contains a small amount of plasma. In such media replacing all but 20 mM of the sodium chloride by equimolar choline chloride or tris chloride solutions, or by an equiosmotic sucrose solution, causes no immediate result other than that which can be explained by the change of conductivity. Though no immediate effect of sodium removal can be seen, over the subsequent half hour the PIII amplitude declines to a new stable level. When the retina is bathed in the original medium again, the response returns very rapidly to its former size. By contrast, it has been reported that the *b*-wave is abolished by low sodium solutions (Hamasaki, 1963).

Similar experiments have been performed on isolated pigeon retinas, which contain both rods and cones. Pigeon rods behave very similarly to rat rods, but the pigeon cone PIII is very different. A change from high to low sodium causes a very rapid reduction in the response. As with rods the effect is reversible.

Experiments are in progress to elucidate the nature of the delayed sodium effect on the rod PIII and to determine the reason for the differences between rod and cone responses. Evidently the role of sodium, in rods at least, may be different from that in many cells of the C.N.S.

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Experimental procedure for the study of circulatory changes in the iris vessels

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Rabbits are anaesthetized with urethane, thermocouples mounted in No. 27 needles are inserted into the anterior and/or posterior chamber of the eye and the temperature changes recorded. At the end of the experimental period the animal is killed and a cooling curve obtained with the thermocouple *in situ*. At a steady-state temperature, θ, the rate of heat dissipation from the circulating blood must be equal to the rate of heat loss to the environment and the latter is proportional to and may be calculated from the slope of the cooling curve at θ. At non-steady temperatures the heat dissipation is proportional to the slope of the cooling curve plus the rate of change of temperature.

Preliminary experiments were carried out using a model in which a metal cylinder was heated by warm water flowing across one face. The heat dissipation was calculated directly from the inflow–outflow temperature differential and rate of water flow and indirectly from the steady-state temperature of the cylinder and the cooling curve obtained at zero flow. Over a considerable range the indirect estimates of heat dissipation for steady states were, as a first approximation, proportional to the volume flow of water across the face of the cylinder.

Circulatory changes in the anterior uvea after close-arterial administration of catecholamines have been followed by calculating the relative rates of vascular heat dissipation before, during and after administration of the drugs. L-Noradrenaline and L-isoprenaline caused a marked fall of heat dissipation and this is tentatively ascribed to both substances exerting a vasoconstrictor effect on the iris vessels. In terms of equivalent amounts of base isoprenaline was the more potent in this respect but less effective as a mydriatic. The vascular action of noradrenaline was more susceptible than that of isopropylnoradrenaline to blocking by phentolamine methanesulphonate and, conversely, the isopropylnoradrenaline effect was the more readily inhibited by β-blocking agents such as propanolol and dichloroisoproterenol.

It appears that there exist adrenergic receptors of both the α- and the β-type capable of reducing heat dissipation from the iris blood vessels, presumably as a result of vasoconstriction, and that they are at least partially independent.

Possible mechanisms of the blue arcs of the retina

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The arcs, first described by Purkyně (1825), are evoked when the macular region of the retina is stimulated by a patch of light. Their loci (see Dolecek & de Launay, 1945 and earlier workers) are closely similar to the nerve fibre pattern of the papillo-macular bundle. The arcs are transient and can be generated at 'on' or 'off' or at both 'on' and 'off' by means of a suitably timed intermittent stimulus. Optimum conditions for demonstration have been described (Moreland, 1968*a*).

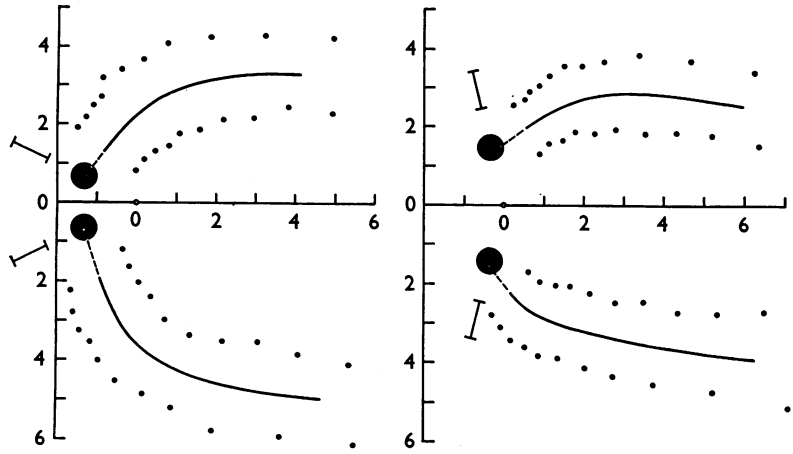

Fig. 1. Arcs plotted for the right eye of one subject using 40′ dia. stimulus (large circles). Axes are marked in degrees: abscissa is horizontal in the visual field. Origin is the fixation point. Small black circles indicate positions of a probe light used to locate the arc 'edges' in a forced choice experiment. Curves are graphically constructed centre lines of the arcs. Barred lines indicate theoretical limits between which the centre lines should pass if the photoreceptor layer is the site of arc excitation.

The hypotheses of arc generation which have been proposed (see reviews by Judd, 1929 and Moreland, 1965) are of three kinds. Arcs may be caused by light scatter in one or other retinal structure, or by a secondary emission of light (bioluminescence) by active nerve fibres that excite nearby photoreceptors, or by secondary electrical excitation of nearby neurones or nerve fibres by active nerve fibres.

The first may be discounted since the colour of the arcs is a constant reddish-blue independent of the stimulus colour (see Newhall, 1937). Evidence against the second hypothesis, that of bioluminescence, has been obtained from three independent experiments:

(1) It has been reported (Moreland, 1965, 1968*b*) that bioluminescence does not account for the threshold changes for arcs during dark adaptation.

(2) Attempts to photograph the arcs ophthalmoscopically were unsuccessful. Densitometry of the photographs indicated that whatever bioluminescence might have been present was at least 100 times weaker than a comparison light that subjectively matched the arcs.

(3) If the photoreceptor layer is the site of excitation then, from a consideration of the disparate location of photoreceptors and their associated neurones, it may be shown that there are certain extrafoveal stimulus positions for which arc loci would by-pass their stimuli by at least 1°. Careful plots of arcs generated by critically located stimuli showed no by-passing (Fig. 1). The photoreceptor layer was, therefore, not the site of excitation and hence bioluminescence was not the mechanism. The nerve fibre layer also was excluded since arcs were not seen between the raphé (or rather its entoptic projection) and the stimulus, but only between the stimulus and the blind spot (Fig. 1).

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Stereophotography of the human ocular fundus

By R. T. FLETCHER. *Institute of Ophthalmology, Judd St, London W.C.* 1

Stereoscopic photographs of the fundus can provide more information than the usual two-dimensional photographs in macular oedema, disk cupping, etc. However, conventional stereoscopic photography is practically impossible due to the diameter of the dilated pupil relative to the inter-lens distance in a stereo-camera. The purpose of this exhibit was to demonstrate the essential simplicity of fundal stereophotography. Three approaches are possible:

(1) To move the eyeball between two exposures, or to swing the camera in an arc with the cornea as the pivotal point of the camera's optical axis.

(2) To move the camera laterally by a set amount, usually about 3·5 mm, or to have two cameras mounted in tandem.

(3) To shift the optical axis of the camera laterally by using the refractive effect of a plane block of glass, mounted in front of the camera objective.

The method we have adopted is the third; it is (apart from the apparatus) the simplest, quickest and most satisfactory of the three known methods. Provided good fixation is obtained, the elapsed time between exposures is not important, although obviously the shorter the interval the better.

In use, the camera is aligned in the same manner as for taking two-dimensional pictures, but with the glass block swung to one limit of its movement. By swinging the block to the other limit we can determine whether the light-path impinges on the iris at either position. The two serial pictures can now be taken, one at each position of the block, and mounted and viewed in a conventional stereoscopic viewer.

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The effect of movement of the retinal image on visual resolution

By VERONICA M. READING. *Department of Physiological Optics, Institute of Ophthalmology, Judd St, London, W.C.* 1

Visual acuity is markedly reduced from its static value when a relative angular velocity exists between the object viewed and the eye. The question arises, can visual performance be quantized in the sense that to every target velocity there corresponds a momentary target exposure, the resolving power of the eye being equal in each case?

The apparatus is based on a binocular microscope. Targets of suitable angular subtense can be moved on the microscope stage at known velocities so that measurements on dynamic visual acuity can be obtained in circumstances which may have a bearing on some modern technological procedures. An electronic shutter permits the determination of flash visual acuity.

Entoptic halos

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Coloured halos have often been seen around bright lights. Some are caused by mist, suspended ice particles, etc. Others arise by diffraction from small structures in the eye itself.

There have been many reports of such entoptic halos due in particular

to the corneal changes in glaucoma, films of mucus on the corneal surface, and diffraction from the fibres of the crystalline lens.

In addition, at least three other halos may be seen in suitable circumstances, although observation may often be difficult. The cause again appears to be diffraction, but as yet the diffracting structures have not been identified.

In the demonstration, conditions favourable for viewing these elusive phenomena were provided.

A device for cutting ultra-thin unfixed frozen sections for electron microscopy

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Exploitation of electron microscopy in biology is limited by technical factors, resulting from fixation, dehydration or embedding the tissue in a plastic. We have developed a new method of preparing tissue for electron microscopy which obviates all these procedures. We freeze fresh tissue and cut ultra-thin sections at low temperatures. Some of the technical difficulties were presented and their solutions indicated. The premier difficulty is discovering a flotation medium on which to gather the sections. The best we have found is trichlorofluoromethane which is satisfactory in all respects except that its freezing point, $-111°$ C, sets a design limitation. We have also found that at lowest cutting speeds transient temperature rises of about 100° C may be expected in ultra-thin sections, indicating that the specimen block must be at lower temperatures than the knife during operation. Care must be taken to eliminate random thermal expansion in the system whilst preferably allowing rapid temperature equilibration. Our solution is shown in Fig. 1. In the specimen arm S, a constant temperature gradient is defined by a reservoir of liquid nitrogen (so arranged that its level is maintained above plane A) and a cooling coil outside the system. The knife K is alternately cooled by liquid nitrogen through N and heated by a current passing through resistor R. By setting a variable potentiometer in a bridge circuit against the platinum resistance thermometer P it is possible to maintain any desired temperature. The temperature of the knife at equilibrium includes a slight oscillation which may be minimized by a critical relative positioning of P, R and N. Further thermal movement of the knife edge is reduced by casting the holder in non-expanding invar steel and setting the knife edge in the null plane of expansion. After the sections are cut by a glass knife they are found to be electrostatically charged. To facilitate recovery, they are collected on a grid held by insulated forceps whose tips are connected through a power

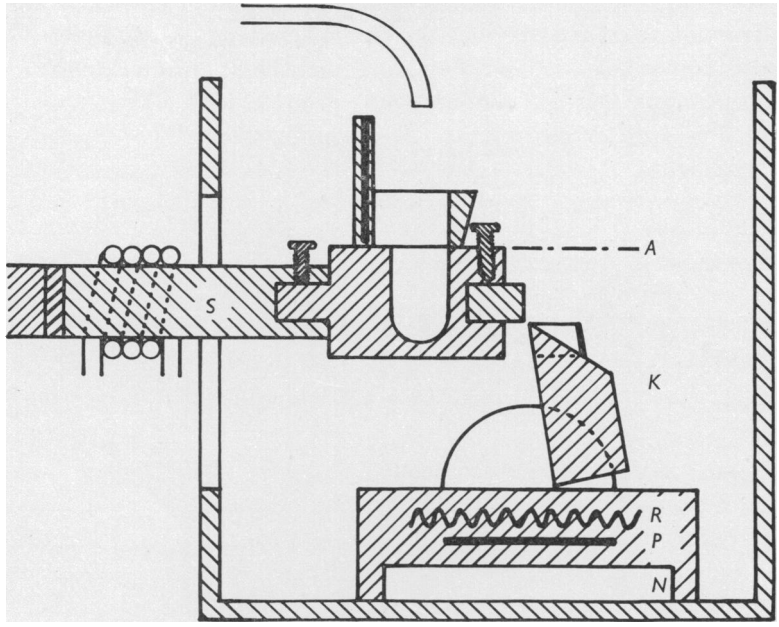

Fig. 1. A diagrammatic representation of the freezing attachment described in the text.

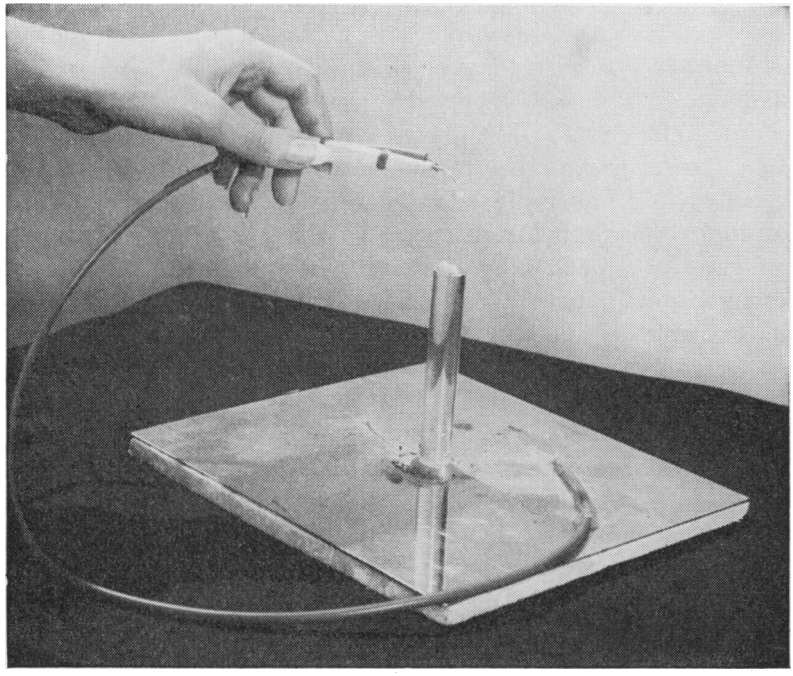

Fig. 2. Photograph of the insulated forceps and charging device required to collect frozen sections.

line to an electrophorus (Fig. 2). By causing an electron stream off the tips and later reversing tip polarity it is possible to recover all sections routinely. They are then freeze-dried at 10^{-4} torr and $-70°$ C in the presence of a cold finger and afterwards viewed in the electron microscope.

We wish to thank Dr T. A. Smith of I.C.I. Ltd., for his guidance in choosing a flotation medium, and Professor V. E. Cosslett for permission to use the 1 MeV electron microscope at the Cavendish Laboratory, Cambridge.

Two anomalous flow properties of blood

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Radio-isotope experiments with a thyroid circulation analogue

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An e.e.g. monitor incorporating simple pattern recognition

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The human scalp e.e.g. reveals well defined and often marked changes in a wide variety of systemic metabolic disturbances, drug and other intoxications, anaesthesia and cerebral anoxic states. These changes, though often non-specific to any particular causal agent, are commonly dose dependent. It is therefore possible, in a purely empirical way, to utilize them as indicators of the degree of intoxication or cerebral disturbance and more particularly to monitor changes in state with the elapse of time. A major hindrance to the use of this data up to the present has been the lack of a reliable monitor which could present information in a simple form meaningful to the relatively uninitiated. The demonstration is of a prototype machine designed to solve this problem and with illustrations of its use in several clinical situations.

Although some regions of the brain respond in different ways to different systemic disturbances, in general, the time course and dose dependency of any one region is characteristic for the particular disturbance in question. In practice, since changes in the occipital alpha rhythm are often a most sensitive early index of systemic disturbance, and other early and more particularly late changes are seen best in central regions, we have used a bipolar recording, occipital/fronto-central, to obtain information from both these areas on a single channel. The precise location of these electrodes, whether right or left hemisphere, or sagittal makes little difference to the results.

The changes seen may be readily characterized by the parameters, frequency, amplitude and time, which may be realized from a set of analogue filters. The filters described by Byford (1965) have been found to be most satisfactory. These are five band pass filters which cover the e.e.g. spectrum by octaval progression with centre frequencies at 1·5 (1–2) c/s, 3 (2–4) c/s, 6 (4–8) c/s, 12 (9–16) c/s and 24 (16–32) c/s, 3 db attenuation at crossover points between adjacent filters, and 30 db per octave cut-off. Additional low (< 1 c/s) and high (> 32 c/s) pass filters have been used in an effort to identify low and high frequency artifacts. All construction is solid state. The mode of use is as follows:

The e.e.g. signal from conventional differential amplifiers is passed through the filter bank, the outputs of each filter being rectified (full wave) and continually integrated by an operational amplifier. Additionally, the unfiltered e.e.g. is rectified and integrated to give a measure of total activity. The display is of two forms:

(i) A chart write-out in which the amplitude of the pen deflexion is proportional to the voltage of each integrator in sequence at set time intervals ($\frac{1}{2}$, 1, 2, 4 or 8 min) of integration. The pen write-out is thus a sequence of equal waves indicating total e.e.g., low pass, filters 1·5, 3, 6, 12, 24 c/s, and high pass. Chart stop and chart and integrator resets are automatically cycled.

(ii) An oscilloscope output in which the sequence above is continuously displayed as a standing wave form, the amplitude of each deflexion representing the instantaneous output at each integrator which now acts as a smoothing amplifier with a time constant of 2 or 4 sec. In either mode, the input signal is continuously monitored on a separate oscilloscope.

A simple pattern recognition system which may be used to operate a counter or automatic alarm is incorporated as follows: The outputs at the integrator (in either mode) are continuously sampled by an upper and lower level selector (set by the operator), one pair for each filter or total e.e.g. output. For each output, if the level is below that selected, a red light shows; if above, a white light; whilst if the signal is within the levels selected no light is seen. A switch is associated with each pair of level selectors so that they may be either operative or not operative thus allowing the operator to use all, one, or any combination of level selectors. The outputs of the selectors are combined so that the operator may choose either of two states, *all* levels *within* those selected, or, *any* level *outside* those selected, to operate a counter or alarm. When the machine is used in the chart display mode, the alarm or counter will operate only at the end of an integration cycle, in the continuous oscilloscope display mode it operates for every 1 or 2 sec for which the outputs are outside the levels selected. The system allows the operator a wide choice of conditions, and

permits, for example, the counting of ictal episodes in epilepsy, or an alarm when anaesthesia is too deep or too light or the occurrence of disequilibrium during renal dialysis. A number of these and other clinical uses were illustrated.

The author is indebted to H. B. Morton and A. E. Wynter, National Hospital for Nervous Diseases, and J. Green, Royal Free Hospital, for detailed design and construction of this equipment. This work is supported by grants from the Ministry of Health and Royal Free Hospital Endowment Research Funds.

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The design and management of a cat colony

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The excretion of anions, cations and water in the normal dog

By D. L. MATTHEWS. *Royal Free Hospital School of Medicine, Hunter Street, London, W.C.* 1

Inhibition and recovery of pituitary–adrenal function in rats on long-term steroid treatment

By J. R. HODGES and JANET SADOW. *Royal Free Hospital School of Medicine, Hunter Street, London, W.C.* 1

Characteristics of reflex jaw opening in the cat

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Vigorous electrical and mechanical stimulation of oral and facial areas will produce a transient jaw opening response. Kawamura & Fujimoto (1958) in reporting the patterns of e.m.g. activity in digastric and masseteric muscles in this reflex, concluded that the digastric was primarily responsible for the opening, the inhibition of elevator muscles only assisting the movement. The present study, using only mild stimulation in unanaesthetized decerebrate and hypothalamic preparations suggests otherwise. The responses were recorded as e.m.g. activity occurring in the digastric (anterior belly) and temporalis muscles and as jaw activity measured in terms of (*a*) movement (isotonic) or (*b*) force developed at certain predetermined positions of the jaw (isometric).

In the preparations described above, the jaw opening reflex, elicited by electrical stimulation of the exposed infraorbital nerve (150% threshold voltage), is characterized, under isotonic conditions, by e.m.g. activity in the ipsilateral digastric and temporalis muscles with latencies of 5 and 15 msec respectively. In the temporalis muscle, weak e.m.g. activity with an approximate latency of 4·5 msec may also be seen; this activity becomes more noticeable under isometric conditions. Furthermore, if the jaw is held wide open and the reflex elicited, a considerable increase of early (4·5 msec) temporalis e.m.g. activity and reduction of late (15 msec) activity usually occurs; this change is associated with a complete reversal of the reflex. The response measured isometrically becomes closure despite the fact that digastric activity still occurs with a latency of 5 msec.

An inhibitory component of the reflex can also be demonstrated, providing background temporalis e.m.g. activity is present; the approximate latency and duration of the temporalis inhibition is 8 and 7 msec respectively, corresponding to the interval between the previously noted bursts of activity.

Under the same conditions, inhibition of temporalis activity may also be produced by the application of light pressure to the maxillary mucosa. No activity occurs in the digastric muscle but a small maintained jaw opening results. The response can be obtained with slowly applied pressures as low as 300 g/cm^2. If under isometric conditions, firm finger pressure is used to elicit the response, a repeating tongue movement also results, bursts of activity occurring in the digastric muscle at approximately 210 msec intervals; this activity is, however, unrelated to jaw movement.

The results suggest a distinction between:

(1) A phasic and reversible jaw opening reflex characterized by digastric activity and by sequential activation, inhibition, activation of the temporalis muscle.

(2) A tonic jaw opening response in the absence of digastric activity related to jaw movement and involving, in the temporalis muscle, solely inhibition.

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

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Apparatus for estimating the rate of amino acid transfer by the human placenta *in vivo*

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The influence of repeated crush injuries on the nuclear population of peripheral nerve

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The cellular population of a peripheral nerve increases during Wallerian degeneration (Abercrombie & Johnson, 1946) and although it subsequently falls it does not return to normal when re-innervation occurs (Abercrombie, Johnson & Thomas, 1949). This primarily affects the Schwann cell population. A further increase follows a second crush (Abercrombie & Santler, 1957). In order to assess the influence of repeated degenerative and regenerative activity on the structure of peripheral nerve trunks, repeated crush injuries have been made on the peroneal nerve of the rabbit, performed at intervals of 4–6 weeks to allow intervening regeneration, up to a maximum of 9 times. A progressive increase in the cellular population of the distal stump results, giving rise to large clusters of Schwann cells. These are associated with multiple myelinated and non-myelinated axons.

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In vivo transillumination of the submucous plexus of vessels in the dog's stomach

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The submucous plexus of the stomach and duodenum can be transilluminated after suitable surgical preparation. At operation, muscle over a small area of the stomach or duodenum is removed leaving the submucous plexus of vessels exposed and the mucosa intact. Transillumination is effected by means of an intragastric light guide conducting light from a xenon source.

This method has been developed to enable the study of arteriovenous pathways, their reaction to nervous and chemical stimuli, and to assess local vascular dynamics. The value of observations is limited by surgical interference with Auerbach's plexus, unknown effects of absorption of light and general anaesthesia.

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A device called the Polgon for the measurement of the orientation of parts of the body relative to a fixed external axis

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The polarized-light goniometer (Polgon) is designed for the analysis of human movement. It measures the orientation of parts of the body to the true vertical as well as changes in angulation of joints. The Polgon consists of one optical projector and as many receivers attached to the parts of the body as required. The projector emits light from a mercury discharge lamp supplied by direct current. The light, which illuminates the subject, passes through a Polaroid screen rotating at uniform speed (*ca.* 50 c/s). A sensor on the motor shaft produces electrical pulses when the axis of the Polaroid sheet passes through the vertical.

Each receiver consists of two photovoltaic cells, mounted with their sensitive surfaces co-planar. They are fitted with Polaroid cover-slips whose axes of polarization are mutually perpendicular. The difference between the voltages across the cells reverses in polarity four times per revolution of the plane of polarization of the incident light. Every second reversal of voltage gives rise to an output pulse from an electronic circuit carried by the subject, i.e. electrical pulses are produced when the plane of polarization is symmetrical (45°) with respect to the axes of both cover-slips. The output is conducted from the subject by line.

The angle is measured by timing the interval (t msec) between the output pulses of the projector and receiver and comparing this with the period of rotation (T msec). Thus the angle to the vertical is given by $360\,t/T$ degrees. The time interval between the output pulses of two receivers permits measurement of the angle between adjacent or non-adjacent parts of the body.

The demonstrated device permits measurements of an angle 100 times per second although much higher frequencies are possible, comparing favourably with the information available from the analysis of cine-film. Measurement is most accurate when the Polaroid sheets in the projector and receiver are parallel. The differences between true and measured angles to the vertical are within 2 deg of arc for deviations of up to 20 deg from parallel. Like Elgon (Tipton & Karpovich, 1962), Polgon offers the possibility of 'on-line' recording of body movement. It is more easily attached because it does not require locating in relation to the instantaneous centres of rotation of the joints and it also measures angulation with reference to an external axis.

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Suppression of chromosomal RNA synthesis during mitosis

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Human blood lymphocytes respond to phytohaemagglutinin (PHA) *in vitro* by the production of transitional cells, blast cells and mitotic figures. Radioautographs of parallel cultures from a single blood sample, pulse-labelled with tritiated thymidine for 1 hr before termination, showed that DNA synthesis began after about 24 hr. By 72 hr approximately one third of the cells were in DNA synthesis. Mitotic cells were first seen at 30–36 hr; by 48–72 hr 3–6 % of cells exhibited mitosis. Using tritiated uridine as a specific RNA precursor, a similar pulse-labelling technique has shown that within 3 hr there was a significant rise in the proportion of labelled cells. At 24, 48 and 72 hr, 95 % of the cells were labelled; the intensity of labelling over-all per cell increased rapidly until 24 hr, when two thirds of the cells were heavily labelled. Initially, very few cells exhibited cytoplasmic labelling, the increase in labelling being restricted to the nucleus. Later, cytoplasmic labelling became apparent. Very few cells (i.e. < 0·5 %) were seen in mitosis at 48–72 hr, probably because heavy labelling largely obscured the form of these cells, preventing identification. In control smears, uncoated with emulsion, from the same cultures, the normal proportion of mitotic cells was observed. Mitotic cells identified in the radioautographs were all heavily labelled with no particular concentration of grains over chromosomes or cytoplasm (Winter & Yoffey, 1966).

When the period of pulse-labelling with tritiated uridine was restricted to the time taken to prepare the smears immediately after addition of the radioisotope (7–8 min), then in a 48 hr culture only about 80 % of cells were labelled. The intensity of labelling over-all per cell was markedly reduced and the location of the label was restricted to the nucleus—except in mitotic cells. Mitotic cells fell into two groups, unlabelled (Fig. 1) and labelled; the labelled ones exhibited grains randomly distributed over chromosomes and cytoplasm. Treatment with cold 5 % trichloracetic acid before coating and, in parallel smears, prior digestion with ribonuclease at pH 6·2, confirmed that the label was incorporated into RNA.

After 15 min in tritiated uridine, including time taken to prepare the smears, some blast cells showed slight cytoplasmic labelling. All the mitotic cells observed were labelled, several to a greater extent than mitotic cells labelled for the shorter period, but the pattern was similar.

This pattern of labelling in mitotic cells can be attributed to release of newly formed RNA into the cytoplasm on break-down of the nuclear membrane during prophase. Few cells in prophase, anaphase or telophase were found in control smears; the duration of these phases appears to be

very short in PHA blast cells. In coated smears from cultures labelled for the shortest period a small number of cells found in prophase (4), anaphase (5) and telophase (3) all exhibited labelling. More than 90% of mitotic cells examined were in metaphase, of which approximately 50% were labelled.

There appears to be a short period within the mitotic cycle when chromosomal RNA synthesis is suppressed, occurring in late prophase and early metaphase. The explanation advanced for this phenomenon, observed in other cells (Prescott & Bender, 1962; Muckenthaler, 1964), is that it is due

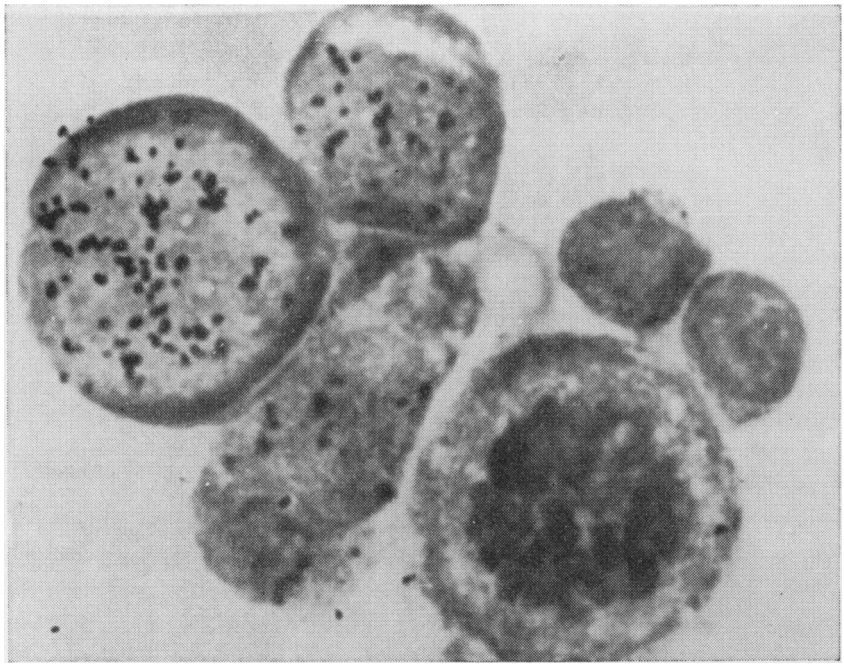

Fig. 1. Group of cells from 48 hr PHA culture to which tritiated uridine was added terminally for very short period. Blast cell and transitional cells exhibited labelling. Metaphase cell and small lymphocytes were unlabelled.

to chromatin condensation rendering transcription of the DNA inoperative. However, the occurrence of labelling in anaphase cells, where the chromatin is in a maximally condensed state, as in late metaphase, suggests that other mechanisms of RNA synthesis may be at work during the period of chromosomal suppression. The possibility exists of cytoplasmic RNA synthesis (Harris & La Cour, 1963). Resumption of chromosomal synthesis of RNA appears to take place rapidly. In smears of the shortest period of labelling, all the unlabelled cells, other than metaphase cells, were untransformed, relatively inactive small lymphocytes. Morphological studies

suggest that the progeny of dividing cells are not in this category. The daughter cells appear to be somewhat larger with a medium nuclear–cytoplasmic ratio. No unlabelled cells were found in this category.

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A demonstration of the negative pressure of interstitial fluid using Guyton's capsules

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Changes in the ultrastructure of gall-bladder epithelium in rabbits with experimental gall-bladder stones

By I. A. D. BOUCHIER, J. W. FRESTON and A. F. HAYWARD. *Royal Free Hospital School of Medicine, Hunter Street, London, W.C.* 1

The measurement of splenic blood flow using Xenon^{133}

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Isolated pig liver perfusion and storage

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Hepatic UDP-bilirubin glucuronyl transferase activity in liver disease

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Some drug-induced myocardial changes studied by electron microscopy

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Histochemical studies in early myocardial changes

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A study of mitochondrial and submitochondrial particles, isolated from either freshly removed or perfused guinea-pig hearts

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In order to determine whether long-lasting perfusion of the heart alters mitochondrial function, mitochondria were isolated, first, from freshly removed hearts and, secondly, from those which had been perfused for 2 hr with either Locke solution or a solution of organ culture medium ('Wellcome' brand Medium 199).

Mitochondria were isolated from ventricular myocardium by the method of Safer & Schwartz (1967), but using the medium of Tarjan & Von Korff (1967). An Ultraturrax TP 18/2 (Janke and Kunkel F. G.) was used for homogenization of the heart.

Respiration and phosphorylation of the mitochondria were measured at 30° C with an oxygen electrode ('Oxygraph', Gilson Medical Electronics). Suspensions of mitochondria, equivalent to 1 mg protein, were incubated with phosphate and various substrates (glutamate, pyruvate, succinate and palmityl carnitine) in a medium consisting of sucrose (0·25 M) and tris(hydroxymethylamino)methane hydrochloride (10 mM) at pH 7·4. The rate of oxidation of the substrate was then measured in the presence and absence of adenosine diphosphate (ADP) (Chance & Williams, 1956). The ADP:O ratio, a measure of the efficiency of adenosine triphosphate (ATP) synthesis, was obtained by measuring the oxygen consumed in the conversion of a given quantity of ADP to ATP.

The results showed that the rate of oxidation of all substrates examined was greatly reduced (about 50 %) in mitochondria isolated from perfused hearts, compared with mitochondria isolated from hearts which had been freshly removed; with some substrates there were indications that mitochondria isolated from hearts perfused with culture medium were less affected. The efficiency of ATP synthesis was, however, almost unimpaired, since there was little change in the ADP:O ratios.

A decrease in mitochondrial respiration rate could have been the result of an inhibition in the rate of phosphorylation since, in a coupled system, the two rates are interdependent. On the other hand, it is also possible that there was a decrease in the over-all rate of entry of substrate into the mitochondria. The first possibility, that of an inhibited rate of phosphorylation, was tested by measuring the rates of ATP-dependent reduction of

nicotinamide adenine dinucleotide (NAD^+) by succinate in submitochondrial particles.

The energy-linked reduction of NAD^+ by succinate is generally believed to involve a reversal of electron transport through the mitochondrial respiratory chain between the succinate-fumarate couple and the NADH-NAD^+ couple (Chance & Hollunger, 1961). Added ATP supplies the energy for this reaction. According to the method of Griffiths & Roberton (1966) submitochondrial particles were prepared from heart mitochondria by sonic oscillation for 40 sec at 0° C (at maximal output from the large probe of an M.S.E. 60 W cell disintegrator). Sonication ruptures mitochondrial membranes, but subsequent spontaneous vesiculation of the membrane fragments results in the formation of particles which are capable of carrying out oxidative phosphorylation (Ziegler, Linnane, Green, Dass & Ris, 1958). The reduction of NAD^+ by succinate in the presence of ATP was estimated by measuring the increase in absorption at 340 mμ with a Beckman DB recording spectrophotometer. The results showed that there was no difference in the rates of reduction of NAD^+ in submitochondrial particles isolated either from freshly removed hearts or from those which had been perfused.

Thus, the biochemical abnormality that causes the decrease in respiration rate of mitochondria isolated from perfused hearts remains obscure and requires further investigation.

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The ultrastructure of normal squamous epithelium of the human cervix uteri

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In vivo study of pathological disturbances of rat liver circulation by transillumination

By ELIZABETH K. MCLEAN. *Royal Free Hospital, Gray's Inn Road, London, W.C.* 1

Veno-occlusive disease of the liver can be induced experimentally in rats by feeding pyrrolizidine (senecio) alkaloids. Features of the course of the

disease are (1) severe centrilobular necrosis of the liver, (2) a block to the outflow of blood from the liver, (3) the later appearance of histologically demonstrable scars occluding many central and sublobular veins. The present experiments were set up to identify the site of the hepatic outflow block. Living circulation in a normal rat liver was demonstrated.

Output of prostaglandins from the rabbit kidney on renal nerve stimulation

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A simplified method for maintaining water balance in rats, and continuous recording of urine flow during the assay of vasopressin

By C. J. COURTICE, M. C. HARRIS and A. UNGAR. *Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.* 1

Simple methods for the display, processing and analysis of ultra-violet oscillograms

By C. J. COURTICE, D. KING, D. J. TULETT and A. UNGAR. *Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.* 1

The use of multi-channel ultra-violet recorders, associated transducers and electronic equipment for class experiments in physiology and pharmacology

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The metabolism by the lung of the prostaglandins E and A

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Changes in the geometry of the blood vessels supplying the carotid bodies of the cat studied by rapid freezing

By A. UNGAR. *Department of Pharmacology, School of Pharmacy, Brunswick Square, London, W.C.* 1

The method is based on that described by Storey & Staub (1962) for the measurement of pulmonary alveolar diameters.

In cats under pentobarbitone anaesthesia the tissue planes in which the carotid bifurcations lie are opened on both sides. The conditions of perfusion of one bifurcation are then altered by changing either the perfusion

pressure or the composition of the perfusing blood, or by stimulating the peripheral cut end of the cervical sympathetic trunk on one side, while the other bifurcation remains as a control.

After the alteration on one side, both carotid bifurcations are rapidly frozen by pouring into the open tissue planes 100 ml. of dichlorodifluoromethane at $-160°$ C. The neck is severed with a hack saw and the head and neck transferred to a mush of solid carbon dioxide with absolute alcohol containing 5 % picric acid and 5 % acetic acid at $-80°$ C. It is kept in this mixture at a maximum temperature of $-30°$ C. It is then transferred to absolute alcohol and gradually brought up to room temperature. The carotid sinus regions are excised and placed in absolute alcohol for 24 hr, cleared in xylene and embedded in ester wax.

Serial sections are cut at 10 μm thickness, and stained by Heidenhain's Iron-Haematoxylin method. Measurements of the calibre of sinusoids, and of sinusoid-glomus cell distances are made on enlargements of photomicrographs, the detailed structure in each field being checked during measurement by direct microscopy.

The method is being applied to the study: (i) of the effect of a reduction in perfusion pressure, (ii) of the effect of increasing the P_{CO_2} of the perfusing blood, and (iii) of the effects of sympathetic stimulation.

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The effects of prostaglandins on tremor

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COMMUNICATIONS

The distribution and metabolism of fast IgG immunoglobulin in the neonatal calf

By D. F. MACDOUGALL and W. MULLIGAN. *Department of Veterinary Physiology, The University of Glasgow*

The absorption by the neonatal calf of colostral immunoglobulins has been the subject of a number of recent studies (Gay, Anderson, Fisher & McEwan, 1965; Penhale, Christie, McEwan, Selman & Fisher, 1967), nevertheless, it has not yet been described in precise quantitative terms. For proper quantitation of the absorption it is necessary to know how the immunoglobulins are distributed between intra- and extra-vascular compartments, the rates of equilibration between compartments and the rates