Alpha rhythm and tremor (frequency range 8–12 Hz) can be recorded with a domestic recorder by employing a carrier. The choice between amplitude modulation (AM) and frequency modulation (FM) is made in favour of the former, because in these experiments more importance is attached to the fidelity of amplitude measurement than frequency; it is also simpler, as it only involves chopping the output of the pre-amplifier at 1 kHz. Such recordings can be displayed by replay into an amplifier, filters and a conventional hot stylus pen.

The arrangement of the component parts of the equipment is shown in Fig. 1. Analysis of the amplitude of the signal is performed by passing the output from the tape directly into a constant reset time integrator, consisting of a simple diode-pump circuit. The DC output from the integrator is proportional to the area under the input wave form.

We are grateful to Margaret Stevenson for her technical assistance, and to G. L. Read for advice. This work was supported by the Medical Research Council.

## A blood–brain barrier in foetal sheep

By C. A. N. Evans, J. Reynolds, Margaret Reynolds, N. R. Saunders and M. Segal. *Department of Physiology, University College London, Gower Street, London, W.C. 1*

## COMMUNICATIONS

## The effect of distending the junction between the superior vena cava and the right atrium in the dog

By C. T. Kappagoda, R. J. Linden and H. M. Snow. *Cardiovascular Unit, Department of Physiology, School of Medicine, University of Leeds*

Distension of the pulmonary vein–left atrial junction causes a reflex increase in heart rate (Ledsome & Linden, 1964, 1967). The present investigation was undertaken to ascertain the effect on the heart rate of distending the superior vena caval–right atrial junction.

Dogs were anaesthetized with chloralose and a right thoractomy performed. The superior vena cava was cannulated through the external jugular vein with a cannula which incorporated two balloons; one, positioned at the tip of the cannula, was placed at the junction between the right atrium and the superior vena cava. The second balloon when inflated acted as a cuff which occluded the superior vena cava at a point one centimetre proximal to the first balloon. A roller pump withdrew blood from the superior vena cava above the occluding cuff and returned it to the animal through a femoral vein; pressure in the superior vena cava above the cuff was maintained within narrow limits. Respiratory, femoral artery

and superior vena caval pressures and heart rate were recorded. The acid-base state of the animal was periodically assessed and maintained within normal limits.

The junction between the superior vena cava and the right atrium was stretched by distending the terminal balloon with 8 ml. of warm normal saline (range 4–10 ml.). Forty-seven distensions of the superior vena caval–right atrial junction in 14 dogs produced an increase in heart rate in every instance. The mean increase was 15·7 beats/min (range 5–51). There was no correlation between the changes in heart rate and the small concomitant changes in mean arterial pressure (mean change, +0·2 mm Hg; S.D., ±2·1) and the pressure in the superior vena cava (mean change, +0·4 cm $H_2O$; S.D., ±1·1) which occurred during distension.

In three dogs the right ansa subclavia was sectioned, and the response was abolished. Intravenous injection of propranolol (0·5 mg/kg; I.C.I. Ltd.) in five dogs abolished the response in two dogs and reduced it in three. Bretylium tosylate (10 mg/kg; Burroughs Wellcome and Co.) injected intravenously into two of the latter three dogs abolished the residual responses. Cooling or sectioning the right vagus nerve in the neck in four dogs diminished the response.

These results suggest that the increase in the heart rate obtained by stretching the junction of the superior vena cava and the right atrium is a reflex phenomenon. The efferent pathway of the reflex is solely in the sympathetic nerves and the afferent pathway is at least partially in the vagus nerves. The receptors most likely to be involved are the right atrial receptors. This reflex is in all respects qualitatively identical to that elicited from the left atrial receptors (Ledsome & Linden, 1964, 1967).

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## The response of arteries to nerve stimulation and to noradrenaline and the relationship of this to innervation density and wall thickness

By J. S. Gillespie and R. M. Rae. *Department of Pharmacology, University of Glasgow*

Most though not all arterial smooth muscle takes up noradrenaline, this uptake prolonging the response (Avakian & Gillespie, 1968; Gillespie & Muir, 1970). Adrenergic receptors may be involved in this uptake. The original purpose of the investigation was to see if the degree of uptake corresponded with the presence of these receptors as judged by the response

to noradrenaline and periarterial nerve stimulation. We have also examined the relationship between the response and the density of innervation of different arteries and the influence of wall thickness and luminal diameter. The saphenous, renal, splenic, superior and inferior mesenteric arteries and the central ear artery were examined in the rabbit, and comparable arteries, other than the ear, in the guinea-pig. Maximal responses to periarterial nerve stimulation and to noradrenaline were obtained using a frequency of 20/sec for nerve stimulation and appropriate doses of noradrenaline. The uptake of noradrenaline into smooth muscle was demonstrated by the Falck fluorescence technique and its amount measured using a Leitz MPV microphotometer.

Arteries differed in their response to noradrenaline. The most responsive (rabbit saphenous or ear artery) gave a rise in pressure some seven times the least responsive (rabbit superior mesenteric) and the rabbit arteries were always more responsive than their guinea-pig counterparts. There was a parallel variability in the response to nerve stimulation so that the order of responsiveness remained constant. The maximum response to nerve stimulation never exceeded the maximum response to noradrenaline and was often much less. If the response to noradrenaline involves the entire smooth muscle then the fraction of this achieved by nerve stimulation may indicate the fractional activation of the muscle. Such values vary from 38 to 98%. The responsiveness to noradrenaline and to nerve stimulation corresponded with the innervation density which shows a five-fold variation between the least and the most innervated. Neither the magnitude of the response nor the percentage activation of the muscle by nerve stimulation was related to wall thickness but was related to the wall thickness/lumen ratio. There was no relationship between the responsiveness to noradrenaline and the extent of accumulation of this substance inside the smooth muscle cells.

In summary, there appears to be considerable variation in the degree of adrenergic control of arteries. Some vasoconstrict strongly in response to noradrenaline, have high innervation densities, respond well to nerve stimulation and have a high wall thickness/lumen ratio. These may represent arteries involved in blood flow regulation. Others respond poorly to noradrenaline, are poorly innervated and have a low wall thickness/lumen ratio. The change from one type to the other may occur over a relatively short distance. A poor response to noradrenaline may be a reflexion of receptor density but other possibilities are under investigation.

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## Projections of the orbital cortex on the anterior lobe of the cerebellum

By D. S. Freedman and P. P. Newman. *Department of Physiology, School of Medicine, University of Leeds*

Responses were evoked in the anterior lobe of the cerebellum by electrical stimulation of the orbital cortex. These responses were observed along micro-electrode tracks that traversed the whole depth of the anterior lobe. The depth of the recording sites was of particular importance in distinguishing between mossy fibre and climbing fibre responses. The mossy fibre afferents produced excitation in the granular layers which was recorded as a negative wave. In contrast, climbing fibre excitation of Purkinje cells gave spike discharges imposed on negative waves in the molecular layers.

Experiments were performed on cats anaesthetized with thiopentone sodium, 45 mg/kg, administered intraperitoneally. A fine monopolar stimulating electrode was inserted stereotaxically through the cerebral hemispheres on each side until the uninsulated tip lay about 1–2 mm deep to the pia on the orbital surfaces. These tracts were afterwards identified in histological sections. Impulses set up by stimulating the ipsilateral or contralateral orbital cortex evoked maximal responses in lobules IV and V of the intermediate culmen. Elsewhere in the anterior lobe the responses were less clear. Early and late responses were recorded. The *early* responses had latencies ranging from 4·0 to 8·4 msec. The latencies were substantially longer than those observed following stimulation of the somatosensory cortex, and there was no significant latency difference between ipsilateral and contralateral projections (Hossain & Newman, 1969). The *late* responses had latencies ranging from 13·4 to 18·8 msec which is comparable to the latency range following somatosensory stimulation (Jansen & Fangel, 1961; Provini, Redman & Strata, 1968).

Symmetrical points in the two cerebral hemispheres were chosen for conditioning and testing stimuli. It was found that the conditioning stimulus had a long-lasting inhibitory effect on the early discharges of the test response. Comparable results were observed when the sequence of stimulation was reversed. Evidence of depression could sometimes be detected for periods lasting up to 400 msec. When the short and long-latency components appeared in the same response, it was possible to block the short-latency activity while the long-latency response remained intact. Interaction between the long-latency components gave typical inhibitory curves with maximal blocking effects occurring at intervals 10–40 msec between conditioning and testing stimuli.

These studies suggest that impulses from the orbital cortex are mediated

by two different kinds of afferent projections to the cerebellum. The short-latency responses arise from cells in the granular layer by activation of mossy fibres, possibly through reticulo-cerebellar projections. A monosynaptic pathway from the orbital cortex to the reticular nuclei of the brain stem had been previously reported (Newman & Wolstencroft, 1959). The long latency responses are interpreted as discharges along the climbing fibre projections from the inferior olive. This view is supported by the experiments in which selective blocking of the test responses was demonstrated.

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## The acute physiological, pharmacological and immunological effects of inhaled cotton dust in normal subjects

BY J. EDWARDS, PATRICIA MCCARTHY, MARGERY MCDERMOTT, P. J. NICHOLLS and J. W. SKIDMORE. *Medical Research Council Pneumoconiosis Unit, Penarth, Glamorgan, and University of Wales Institute of Science and Technology, Cardiff*

Dust previously airborne in a cotton mill was redispersed in a 640 cu.ft. room and thirty volunteers inhaled it for a total of 100 periods of 4 hr each. Dust concentrations ranged from 1·1 to 6·4 mg/m$^3$, and the cloud contained cotton fibres, particles of bract, pericarp and a little mineral. Lung function measurements were made before, during and for four days after inhalation.

The forced expiratory volume ($FEV_{1\cdot0}$) and vital capacity were reduced by 100–600 ml. accompanied by increases of up to 800 ml. in the residual volume and functional residual capacity, and in some subjects a smaller increase in total lung capacity. The airways resistance was increased up to threefold and the dynamic compliance became more frequency-dependent suggesting narrowing of the large airways with scattered narrowing of the peripheral ones.

There was no change in transfer factor for carbon monoxide or, for most subjects, in the static elastic properties of the lung. The changes in the $FEV_{1\cdot0}$ and dynamic compliance lasted several days, probably due to slow elimination of dust from the smaller bronchioles.

To investigate the suggestion of Bouhuys & Nicholls (1966) that these

changes are caused by histamine release in the lungs, estimates were made of excretion of the histamine metabolite 1-methyl-4-imidazole acetic acid (MeIAA) in 24 hr urine samples starting from the beginning of inhalation, and compared with the control level for each person. Dust clouds were produced in which the histamine releasing property of the dust had been reduced to 50% of its original level by washing. After inhalation of unwashed cotton the mean 24 hr MeIAA excretion in twelve subjects was 7·99 mg compared with a control value of 4·25 mg, a difference significant at the 1% level. With washed cotton which produced minimal lung function changes, comparable figures in eight subjects were 6·44 mg and 4·71 mg, a difference which is not significant.

Massoud & Taylor (1964) have postulated that allergy to a part of the cotton plant is involved in the development of symptoms in cotton workers and they demonstrated precipitating antibodies against cotton dust extracts both in cotton workers and those not previously exposed to raw cotton. In a group of fifteen subjects there was no mean change in antibody level or gamma globulin 10 days after cotton dust inhalation.

These results suggest that inhaled cotton dust produces narrowing of airways throughout the bronchial tree with no changes in the static elastic properties or transfer factor of the lung. There is some evidence that the changes are caused by histamine release in the lung, but none of antibody stimulation in normal subjects.

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## A comparison between adult and neonate porcine neurophysin

By S. E. Dicker and Venetia France (née King).* *Department of Physiology, Chelsea College, London, S.W. 3*

From electron microscopical and physiological evidence it has been suggested that if neurophysin is present in the new-born mammalian posterior pituitary glands (Rodeck, 1958; Heller & Lederis, 1959; Dicker, 1966) its properties of binding might be different from that of the adult animal. Having recently characterized the major protein components of adult porcine neurophysin (Dicker & King, 1969), it was thought of interest to attempt a direct comparison of the proteins of adult and new-born

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pig pituitary glands, on the basis of molecular sieve chromatography and starch gel electrophoretic analysis.

Pituitary glands from sixteen neonate (0–1 day) pigs were obtained from A. R. C. Babraham by courtesy of Drs M. W. Smith and Margaret Stanier. The whole glands were acetone-dried, homogenized in 0·1 M acetic acid and centrifuged at 30,790 *g* for 45 min. The residues were washed and re-centrifuged, the supernatant solutions being combined and freeze-dried. This material was then subjected to gel filtration on G 75 Sephadex in a Whatman 1 × 100 cm column equilibrated with 0·1 M acetic acid. A trace of eluant optical density at 254 m$\mu$ against eluant volume was obtained. The eluant was divided into five fractions which were freeze-dried and assayed for pressor activity. The protein components of each fraction were analysed by starch gel electrophoresis at pH 8. From 75 mg of starting material, 12·6 mg of soluble protein was eluted from the column.

The whole pituitary glands from adult pigs were subjected to an identical treatment to that described above, and data were obtained also for anterior and posterior pituitaries treated separately.

The U.V. optical density traces seem to indicate that the proteins from adult and new-born whole glands are not identical in their molecular weight distribution. This difference, however, is not correlated with the presence or absence of major components in the starch gel electrophoresis pattern, and it can only be concluded that neurophysin is probably present in the new-born pig pituitary. It is not possible, however, to ascertain at this stage whether there is sufficient protein to bind vasopressin and oxytocin in the gland, or whether the inability of the neonate to store these hormones has some other cause.

The authors would like to thank Drs M. W. Smith and Margaret Stanier for providing the new-born pituitary glands, Mr Jack Bush and his colleagues of T. Wall and Son for their most helpful co-operation and Miss Christine Morris for the biological assays. The work was supported by a grant from the M.R.C. which is gratefully acknowledged.

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## An electro chemical investigation of the inner ear fluids in the neonatal rat at the time of the development of the endocochlear potential

By S. K. Bosher and R. L. Warren. *Ferens Institute of Otolaryngology and S. A. Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W 1P 5FD*

## The forms of vitamin $B_{12}$ in the ileal enterocyte of the guinea-pig during fasting and absorption of cyanocobalamin

By A. V. Hoffbrand, J. C. Linnell, D. M. Matthews and T. J. Peters. *M.R.C. Intestinal Malabsorption Group and Department of Haematology, Royal Post-Graduate Medical School, London, W. 12 and Department of Chemical Pathology, Westminster Hospital Medical School, London, S.W. 1*

Using a chromatographic-bio-autographic technique for the separation and quantitation of individual cobalamins (Linnell, MacKenzie & Matthews, 1969) we have found that the predominant form of vitamin $B_{12}$ in either mitochondria prepared from the ileum or in the plasma of the guinea-pig, is 5′-desoxyadenosyl-cobalamin (coenzyme $B_{12}$).

Previous experiments (Peters, Quinlan & Hoffbrand, 1969) have demonstrated that there is a delay in the absorption of cyanocobalamin across the ileal mucosa of the guinea-pig, during which it is localized in the mitochondria. It has also been suggested (Latner, Hodson & Smith, 1962) that cyanocobalamin is converted to coenzyme $B_{12}$ during absorption and it therefore seemed possible that this conversion, which could be responsible for the delay, might occur in the ileal mitochondrion.

Table 1. Specific activity of $B_{12}$ analogues in ileum (cpm/mg protein)

| Time (hours): | ½ | 1 | 2 | 3 | 4 |
|---|---|---|---|---|---|
| Cyanocobalamin | 66·0 | 535 | 470 | 407 | 300 |
| Coenzyme $B_{12}$ | 17·4 | 70 | 121 | 185 | 219 |

Each figure is the mean result of two experiments.

To investigate this hypothesis, guinea-pigs were fed 10 ng of $^{57}$Co-cyanocobalamin by stomach tube and homogenates of ileal mucosa analysed at timed intervals after feeding. Table 1 shows the specific activity of the two forms of $B_{12}$ detected. Labelled co-enzyme $B_{12}$ appeared in the ileum before any radioactivity was detectable in the blood. These results indicate that there is a synthesis of coenzyme $B_{12}$ from cyanocobalamin by guinea-pig ileum. Preliminary data indicate that the mitochondrion may be the site of this interconversion.

Portal plasma obtained from animals fed $^{57}$Co-cyanocobalamin contained substantial amounts of labelled cyanocobalamin as well as labelled coenzyme $B_{12}$. Thus it is unlikely that the conversion of cyanocobalamin to coenzyme $B_{12}$ is the sole cause of delay in transport across the ileal enterocyte. Some absorption of unchanged cyanocobalamin from physiological doses also occurs in man (Linnell, Hoffbrand, Peters & Matthews, 1969).

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## The effects of amino acids and some related substances on isolated preparations of the sea anemone *Actinia equina*

BY R. F. CARLYLE. *Department of Pharmacology, King's College, W.C. 2*

Steiner (1957) offered *Actinia equina* pieces of filter paper soaked in various amino acids and found that glutamic acid was the most effective in producing a mouth opening response. In these experiments the actions of various amino acids have been tested on isolated electrically stimulated rings of muscle obtained from *Actinia equina*. Most of the experiments have used rings of supra oral sphincter. The pH of the sea water was 7·8.

The actions of some common amino acids and derivatives, applied in concentrations of up to $10^{-2}$ M, on the responses to electrical stimulation are listed in Table 1. Only DL-glutamic acid ($10^{-3}$–$5 \times 10^{-3}$ M) abolished the response of supra oral sphincter preparations to electrical stimulation. In many experiments glutamic acid also produced strong slow spontaneous contractions. D or L glutamic acid was equally active in producing these effects.

Glutaric acid, N-acetylglutamic acid, pyroglutamic acid, dimethylglutamic acid, 2,6-diaminopimilic acid or $\gamma$-methylglutamic acid, in concentrations of $10^{-2}$ M, were inactive. DL-DL-allo-3-hydroxyglutamic acid, glutamine or glutathione ($10^{-2}$ M) were only feebly active.

L-Glutamic acid ($10^{-3}$–$5 \times 10^{-3}$ M) also inhibited the response to electrical stimulation of the isolated oral sphincter, circular preparations of the upper, mid and lower body wall, and of the basal disk.

Removal of the tentacles from supra oral sphincter preparations did not reduce the inhibition produced by glutamic acid.

TABLE 1. Effect of amino acids on the contractile response of the supra oral sphincter preparation to electrical stimulation

| Effect on response to electrical stimulation | Amino acid or derivative | Molar concentration |
|---|---|---|
| No effect | Glycine, DL-$\alpha$-alanine, DL-$\beta$-alanine<br>DL-Ornithine, $\gamma$-aminobutyric acid<br>DL-Lysine, DL-cysteine, DL-histidine<br>DL-Cystine, DL-$\beta$-phenylalanine<br>DL-Valine, DL-leucine, DL-isoleucine<br>DL-Hydroxyproline, DL-lysine<br>DL-Threonine, DL-asparagine<br>DL-Tryptophan, DL-serine<br>2-Amino butyric acid, DL-aspartic acid<br>2-Amino-isobutyric acid, L-glutamine<br>6-Amino-n-hexoic acid<br>5-Amino valeric acid. Taurine | $10^{-3}$–$10^{-2}$ |
| | DL-Tyrosine | $10^{-3}$ |
| Increase of response (< 25 %) | DL-Proline DL-methionine<br>DL-Arginine | $5 \times 10^{-3}$–$10^{-2}$ |
| Variable inhibition of response (< 50 %) | Glutathione (reduced) | $10^{-2}$ |
| Total inhibition of response | DL-Glutamic acid | $10^{-3}$–$5 \times 10^{-3}$ |

Experiments have been made on supra oral sphincter preparations so arranged that glutamic acid could be applied to different parts of the sphincter while recording responses to electrical stimulation. These experiments suggest that glutamic acid does not act on sensory receptors to initiate inhibition, or on the conducting system. It seems likely that glutamic acid acts on the myoepithelial cells responsible for the contractile response to electrical stimulation.

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## Effects of lysine–vasopressin dosage on renal tissue and urinary composition in the conscious, water-diuretic rat

BY J. C. ATHERTON, R. GREEN and S. THOMAS. *Department of Physiology, University of Manchester*

The major effects of neurohypophysial antidiuretic hormone [ADH] on the mammalian kidney are generally considered to be increases in water-permeability of the distal tubule and collecting duct (Bennett & Berliner, 1967). However, continuous infusion of lysine-vasopressin

(15 $\mu$-u./min/100 g body wt.) in the conscious rat also results in progressive repletion of the corticomedullary osmolal gradient previously dissipated by sustained water diuresis (Hai & Thomas, 1969). Apart from the latter experiments, there is little information concerning the rapidity and magnitude of ADH-induced changes either in medullary–urinary osmotic equilibration or in the steepness of the corticomedullary osmolal gradient.

The present experiments were performed to investigate the influence of ADH dosage (2·5 to 60 $\mu$-u. lysine-vasopressin/min. 100 g body wt. given by continuous intravenous infusion for up to 4½ hr) on both urinary and renal tissue composition in the conscious, previously water-diuretic rat. The experimental procedure was similar to that used by Hai & Thomas (1969).

The results are summarized as follows:

(*a*) As with the changes in urinary osmolality, the rate and magnitude of increases in medullary osmolality were dependent on ADH dosage.

(*b*) Depending on ADH dosage, increases in medullary osmolality occurred almost as rapidly as those in urinary osmolality.

(*c*) Prolonged infusion of ADH caused increases in urea concentration in the urine greater than those in the medulla. However, initially, urea concentration in the papillary tip increased to values higher than those in the urine: i.e. the initial, rapid effect of ADH was to create a papillary–urinary urea concentration gradient incompatible with simple diffusion from the collecting duct into the papilla.

Thus, in addition to an increased collecting duct permeability to water, the effects of ADH must include other changes in renal medullary function.

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## Small amplitude displacement sensitivity of frog spindles during fast and slow muscle contractions

BY M. C. BROWN. *University Laboratory of Physiology, Oxford*

Matthews & Stein (1969) have shown in decerebrate cats that muscle spindle primary endings are relatively much more sensitive to small amplitude stretches than to large. The present experiments demonstrate a similar high sensitivity for small stretches in frog spindles and throw some light on its mechanism.

Fig. 1 shows results of an experiment on a spindle in the iliofibularis muscle. The muscle (23 mm long) was lengthened and shortened suddenly by 100 $\mu$ and the afferent discharge and tension were recorded during

stimulation of large and small motor nerves, innervating both the spindle and extrafusal muscle fibres (Katz, 1949). During the slow muscle contraction (A) there is 17 imp. $sec^{-1}$ modulation of the frequency, but in B, during fast muscle contraction, any change is inappreciable. The tension records also differ greatly. Although in neither case has the tension reached a steady state after the length excursions, there is a much greater modulation of the tension during slow muscle contraction (A) than during fast (B).

It appears that contracting slow muscle when subjected to small movements undergoes larger and longer-lasting tension changes than does fast muscle, and that similar differences in slow and fast intrafusal fibres will explain why only small motor nerve stimulation sensitizes the frog spindle to small movements. This difference between fast and slow muscle can be expected from a simple qualitative interpretation of the cross-bridge sliding filament model of muscle.

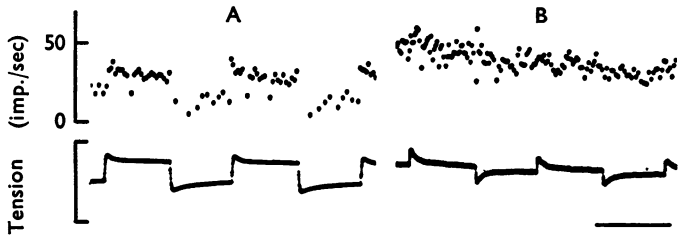


Fig. 1. Discharge (plotted automatically as instantaneous frequency) of a single spindle (above) and extrafusal tension (below) during stimulation of an unknown number of small motor nerves (A) at 29/sec or large motor nerves (B) at 33/sec. Throughout A and B 100 $\mu$ peak-to-peak 'square' stretches and releases applied. Bottom of tension calibration bar is zero tension, and the bar represents 40 mN for A and 20 mN for B. Time bar 1 sec. Temp. 20° C.

In the mammal the homologues of the slow and fast intrafusal fibres are the bag and chain fibres respectively, and the present results support Matthews's (1970) predictions about the effect of dynamic and static fusimotor fibres on the small amplitude sensitivity of mammalian spindles, and, more generally, that the character of the spindle discharge is importantly determined by the properties of the intrafusal fibres.

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## Heat production in twitches of stretched muscle

By I. C. H. Smith.* *Department of Physiology, University College London, Gower Street, London, W.C. 1*

Frog semitendinosus muscle can be reversibly stretched to a greater degree than sartorius muscle. This allows a study of heat and tension production at long sarcomere lengths where actomyosin overlap and, presumably, activity is progressively reduced. In this study the initial heat in isometric twitches of stretched muscles has been measured with thermopiles of both A. V. Hill's type and Wilkie's (1968) averaging type.

The heat decreased linearly with tension from its maximum, $H_0$, at $l_0$ (the length at which tension was a maximum) to a finite intercept, $A$, at zero tension. The intercept was estimated by the statistical linear regression of the heat against the tension. In 18 experiments at 0° C, using frog (*Rana temporaria*) and toad (*Bufo bufo*) dorsal semitendinosus muscle, and frog sartorius muscle, the mean ratio $A/H_0$ was 0·198 (S.D. 0·042). No difference was found between the different preparations, although accuracy was better with the semitendinosus muscle since it could be stretched till $P/P_0 \approx 0{\cdot}1$ compared with 0·25 for sartorius muscle. $A$ is more variable than the ratio $A/H_0$ since $A$ varies with the strength of the muscle. Mean values were, $A = 2{\cdot}4\ \mathrm{mJ.g^{-1}}$ ($0{\cdot}57\ \mathrm{mcal.g^{-1}}$) and isometric tension at $l_0 = 1{\cdot}2\ \mathrm{kg.cm^{-2}}$. Homsher & Ricchiuti (1969) have reported briefly that the heat produced in extremely stretched semitendinosus muscle of *R. pipiens* is $0{\cdot}30\ H_0$: without fuller details of their method it is difficult to explain their larger result.

Stretch causes a decrease in the rate of tension relaxation but the ratio $A/H_0$ was found to be independent of the size of this very variable effect. The time course of the heat produced in fully stretched muscle was of simple exponential form with a time constant ($\approx 400$ ms) between one and two times faster than the same muscle's tension relaxation rate.

It is supposed that the tension independent heat, $A$, measures the energy used in the control of activation—in particular by the reticular calcium pump. The linearity between heat and tension suggests that $A$ is independent of the muscle length. A. V. Hill's 'activation heat' is larger than $A$, the various techniques used giving values of 0·3 to 0·5 of $H_0$ (reviewed by Mommaerts, 1969). This supports Mommaerts's argument that actomyosin activity contributes to these measurements of 'activation heat'.

Assuming that the tension independent heat derives from an ATP consumption caused by the accumulation of 2 moles of calcium per 1 mole of ATP, it can be seen that the ratio $A/H_0 = 0{\cdot}2$ implies that at least 2 moles of ATP are used by the actomyosin ATPase per 1 mole of activating calcium

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in a normal twitch. If the alternative value of $A/H_0 = 0{\cdot}33$ is employed a similar calculation shows that only 1 mole of ATP need be used by the actomyosin per 1 mole of calcium.

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## On the 'filters' in the photophores of mesopelagic fish and on a fish emitting red light and especially sensitive to red light

BY E. J. DENTON, J. B. GILPIN-BROWN and P. G. WRIGHT. *The Laboratory of the Marine Biological Association, Plymouth and Makerere University College, Kampala, Uganda*

Photophores often have chambers with highly reflecting walls, within which light is generated, opening into structures with reflecting surfaces which distribute the light. Between these generating and distributing structures there is often a layer of coloured tissue and although the histology of some of these tissues has been well studied by Bassot (1966) their function is not certain.

Aboard R.R.S. *Discovery* photophores in freshly caught specimens of a number of mesopelagic fish were studied. These, when freshly trawled, often emit light spontaneously and the injection or local application of adrenaline usually increases and prolongs this light.

In general the coloured tissues were found to transmit a relatively small fraction of light, even for the wave bands which they transmit best, yet there was no indication that they were ever concerned in the production of light and when they were cut away the emission of light was always greatly increased. In photophores which shine mainly downwards these 'filters' usually have a band of transmission in the blue-green ($\lambda$ around 480 nm) and where such photophores emitted light, this light was always blue-green. In several species of *Argyropelecus* and *Sternoptyx* the 'pigment' in the 'filters' seemed, from its absorption spectrum, to be largely reduced cytochrome *c*.

Now it is thought (see e.g. Clarke, 1963) that the purpose of many photophores is to obliterate the shadows of their possessors' ventral surfaces and so make them difficult to see. If this were so the filters in fish like *Argyropelecus* would have the important function of making the light from the photophores match the colour of the daylight which penetrates to the depths where these animals live.

Close to the eyes of some fish there are large photophores which are

covered with red tissues which transmit only red light and in one such fish, *Pachystomias*, these photophores were seen to emit flashes of red light. The photosensitive retinal pigments of the eye of *Pachystomias* showed that it is probably exceptionally sensitive to red light. (The maximal retinal absorption was at wavelength about 575 nm with a maximal retinal optical density of about 1·0.) Another fish with red photophores, *Malacosteus niger*, has a bright red tapetum. Animals which can emit, and are exceptionally sensitive to, red light will have a considerable advantage in hunting, or avoiding, red and brown animals in the middle depths of the ocean.

The photophores on the ventral surfaces of *Pachystomias* have filters of exactly the same colour as those of *Argyropelecus* and they emit not red but blue-green light.

In all the fish examined there was then no evidence that the coloured tissues in photophores have any other function than acting as filters which determine the spectral properties of the light emitted.

We are very grateful to Mr P. M. David and his colleagues for very great help during a biological cruise of the National Institute of Oceanography in the winter of 1969.

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## Rapid transport of neurohypophysial hormones in the hypothalamo-neurohypophysial tract

By C. W. Jones and B. T. Pickering. *M.R.C. Group for Research in Neurosecretion, Department of Pharmacology, University of Bristol, Bristol BS8 1TD*

The concept of neurosecretion holds that the neurohypophysial hormones, oxytocin and vasopressin, are synthesized in the paraventricular and supraoptic nuclei of the hypothalamus and then transported along the axons of the hypothalamo-neurohypophysial tract to be stored in the neural lobe of the pituitary (Scharrer & Scharrer, 1954). Attempts to measure the time required for synthesis and axonal transport have in the past relied on autoradiographic techniques which have shown that labelled material can be demonstrated in the gland some 10 hr after intracisternal injection of a labelled amino acid (Sloper, Arnott & King, 1960; Flament-Durand, 1967). In an attempt to obtain more precise information, we (Pickering & Jones, 1970) have developed a method for isolating isotopically pure tritiated oxytocin and vasopressin from the pooled neural

lobes of four to five rats which had previously received intracisternal injections of [$^3$H]-tyrosine. Using this method, we have followed the arrival of tritiated hormones in the neural lobe of the rat.

Rats were anaesthetized with ether for the injection of the label (0·1 mc/rat in 0·1 ml.), and then allowed to recover from the anaesthetic so that the total time of anaesthesia was approximately 15 min. At the required interval after injection, the animals were decapitated, the neural lobes removed and their neurohypophysial hormones purified according to Pickering & Jones (1970). The amount of newly synthesized hormone present in the glands was estimated by determining the specific radioactivity (cpm/m-u.) of the purified hormones.

No radioactivity was associated with the hormones purified from rats killed 1 hr after injection of the label, but radioactive hormones could be isolated 2 hr after injection (oxytocin 0·26 cpm/m-u.; vasopressin 0·36 cpm/m-u.). The specific radioactivity of the purified hormones reached a plateau in 4–6 hr (oxytocin 1·2 cpm/m-u.; vasopressin 1·2 cpm/m-u. and remained almost unchanged for at least 72 hr. When neural lobes were isolated and incubated *in vitro* with [$^3$H]-tyrosine for 4 hr, the hormones subsequently isolated were not radioactive, although the glands had incorporated radioactive tyrosine into protein.

These results confirm the inability of isolated neural lobes to synthesize neurohypophysial hormones, and provide a minimum estimate of the time for transport from the hypothalamus to the neurohypophysis of 1–2 hr. Taking the mean length of the axons in the hypothalamo-neurohypophysial tract to be about 2 mm, the minimum rate of transport of the hormones becomes 24–48 mm/day. This rate is of the order found for 'rapid transport systems' in other central and peripheral neurones (Schmidt, 1968) and raises the problem of the mechanism of transport of neurosecretory granules.

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## Narrow 'tuning' of the responses of cochlear nerve fibres emanating from the exposed basilar membrane

By E. F. Evans. *M.R.C. Group, Department of Communication, University of Keele, Staffs*

Recent measurements of the threshold frequency response areas ('tuning curves') of cochlear nerve fibres in the cat (Kiang, 1965; Kiang, Sachs & Peake, 1967) and in the guinea-pig (Evans, 1970) have demonstrated that they are significantly narrower than the 'resonance' curves of the basilar membrane, derived for the guinea-pig by von Békésy (1944) and Johnstone & Boyle (1967).

On the other hand, determinations of the effective bandwidth of the cochlear fibres of both cat and guinea-pig using broad-band noise as the stimulus (Evans, Rosenberg & Wilson, 1970) indicate that the cochlea acts as a linear filter of bandwidth much narrower than that of the basilar membrane response, but corresponding to that of the frequency response area of the fibres themselves.

The simplest explanation of these findings is that suggested by Huxley (1969), that the techniques required to measure the motion of the basilar membrane deprive it of sharply tuned 'resonant' properties. In particular, Huxley implicated the surgical opening of the cochlea necessary to expose the basilar membrane in the experiments of von Békésy and Johnstone and Boyle. If this explanation were correct, then surgical exposure of the basilar membrane should destroy the sharp 'tuning' normally observed in cochlear nerve fibres.

Recordings were made from single cochlear nerve fibres of the pentobarbitone-anaesthetized guinea-pig, as described previously (Evans, 1970) After determining the threshold frequency response areas of fibres with optimal response to frequencies in the region of 14 kHz, the scala tympani in the first turn of the cochlea was opened widely, and most of the round window removed or reflected. This fully exposed that portion of the basilar membrane between 1·5 and 3 mm of the stapes, which subserves frequencies between 12 and 18·5 kHz (interpolation between von Békésy, 1944 and Johnstone, 1969). Further determinations of the threshold frequency response areas of fibres originating from this region were then made. There was no significant difference in threshold or shape of the response areas, nor was the cochlear microphonic frequency response or the $N_1$ threshold disturbed by the exposure of the basilar membrane. Furthermore, draining the perilymph from the exposed basilar membrane had no effect on these parameters.

This result therefore excludes the possibility that surgical exposure of the basilar membrane seriously interferes with its response. It is therefore

concluded that the sharpness of the frequency response exhibited by cochlear nerve fibres relative to the broadly 'tuned' response of the basilar membrane arises from processes intrinsic to the normal cochlea, which appear to act as a further, effectively linear, frequency selective mechanism in addition to that inferred from the vibration pattern of the basilar membrane (Evans, Rosenberg & Wilson, 1970).

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## Segmental integration of converging inputs to thoracic respiratory motoneurones

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During spontaneous breathing thoracic inspiratory and expiratory motoneurones are subjected alternately to reciprocal excitatory and inhibitory synaptic drives which generate the 'central respiratory drive potentials' (Sears, 1964). These motoneurones are also reciprocally activated following electrical stimulation of fibres in the pyramidal decussation and of reticulo-spinal tracts. This reciprocity has been attributed to a spinal system of interneurones engaged by the descending fibres (Sears, 1966).

The spinal integration of such reciprocal activities has been examined by studying the interaction between the spontaneous respiratory activity of thoracic motoneurones and their responses to electrical stimulation of the cerebral cortex or to reflex activation from intercostal nerves. Cats anaesthetized with sodium pentobarbitone were paralysed with Flaxedil. By monitoring efferent discharges (inspiratory) in an external intercostal nerve the spontaneous respiratory activity could be maintained at any desired level with appropriate artificial ventilation.

With the animal apnoeic, brief tetanic stimulation (3 shocks, 500 Hz, 0·2 msec duration) through a monopolar electrode applied to the contra-

lateral trunk area of the sensorimotor cortex evoked a brief latency (6–8 msec) discharge of expiratory motoneurones (intercostal and abdominal) in internal intercostal nerves; there was either no response of inspiratory motoneurones or a weak one of longer latency. A similar response pattern of 5–6 msec latency occurred with reflex activation from an internal intercostal nerve suggesting that the same spinal mechanisms might subserve these two inputs. Indeed, conditioning of an intercostal test reflex by a prior stimulus to the same intercostal nerve or to the cortex both gave conditioning curves showing facilitation of expiratory motoneurones at short intervals (3–25 msec) and an inhibition maximal at 40–60 msec and persisting for 100–200 msec with longer intervals.

If the animal was underventilated spontaneous inspiratory activity was evoked. Cortical or intercostal nerve stimulation now evoked a brief latency (6–8 msec) inhibition of the inspiratory activity for a period of 20–30 msec corresponding approximately with the phase of expiratory motoneurone facilitation; conversely, the actual discharge of expiratory motoneurones was inhibited during inspiration.

Whereas the spontaneous respiratory activity is abolished below a lesion of the ventral quadrant of the spinal cord the facilitatory effects of cortical stimulation are left intact. These facilitatory effects are abolished below a dorsal quadrantic lesion leaving the respiratory activity and reciprocal effects of reflex activation intact.

The present results clearly demonstrate the spinal integration of independent pathways regulating the activity of thoracic respiratory motoneurones. To explain the interactions between the reciprocal effects of these inputs a working hypothesis based on the operation of a spinal network of interneurones was discussed.

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## The resistance of a lymph node to lymph flow

BY N. L. BROWSE, R. L. DOIG and D. SIZELAND. *Department of Surgery, St Thomas' Hospital, London, S.E. 1*

High pressures develop in the peripheral lymphatics during exercise (Drinker & Field, 1933). It has been postulated that this is due to obstruction to the lymph flow by the lymph node (Browse, 1968). This paper reports the preliminary results of a study of the resistance of a lymph node to lymph flow.

Greyhounds were anaesthetized with sodium pentobarbitone (20 mg/kg). The lymphatics related to the external iliac vessels were displayed through an oblique abdominal incision. These lymphatics lead up to a single, occasionally double, lymph node lying on the lateral side of the iliac artery. Two of these lymphatics were cannulated with fine nylon cannulae (0·7 mm o.d. 0·45 mm i.d.) and the tips advanced to within 1 cm of the node. One cannula was used for recording pressure (Statham P 23 BB transducer) and the other was connected to a constant infusion pump containing heparinized dog plasma. Arterial and venous pressures were measured in the iliac arteries (P 23 De transducers).

The resistance of the node was calculated by dividing the pressure gradient across the node by the rate of plasma flow, assuming the pressure in the efferent vessels to be the same as that in the para-aortic cisterns, i.e. zero (Browse, Lord & Taylor, 1969). The effect of altering the arterial and venous pressure with balloon catheters, noradrenaline, acetylcholine and the carotid sinus reflex in the vessels draining the node was also studied.

The resistance of the node *fell* as the rate of lymph (plasma) flow increased, presumably due to the distension of the node. The lymph vessels themselves contributed almost no resistance, at the rates of flow used. The resistance of the node at a constant rate of lymph flow varied directly with the venous and arterial pressures but the effect of changes in venous pressure was far greater. The magnitude of the resistance changes were approximately the same whatever the method used for changing the arterial and venous pressure.

These experiments show that the lymph nodes do obstruct lymph flow but the obstruction gets less as the rate of flow increases and the nodes distend. It also emphasizes the close inter-relationship between lymph flow and blood flow within the node.

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## Stimulation of enzyme secretion from the perfused cat pancreas by potassium

BY B. E. ARGENT, R. M. CASE, LINDA M. POOLE and T. SCRATCHERD.
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It is known that hormones can be released from the neurosecretory terminals of the posterior pituitary gland (Douglas & Poisner, 1964) and from the chromaffin cells of the adrenal medulla (Douglas & Rubin, 1961) by raising the extracellular potassium concentration. Furthermore, Bdolah, Ben-Zvi & Schramm (1964) have shown that excess potassium stimulates enzyme secretion from parotid gland slices. It was therefore decided to test the effect of potassium on the pancreas, using a perfused preparation which we have recently described (Case, Harper & Scratcherd, 1968).

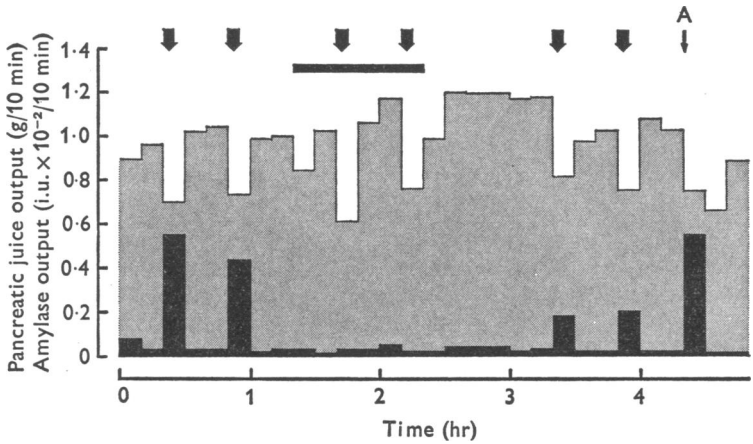


Fig. 1. The effect of atropine on the volume (stippled) and enzyme (in black) responses of the perfused pancreas to excess potassium. Secretion was maintained throughout by infusing secretin at a supra-maximal dose (8 $\mu$g/min). The perfusate contained atropine sulphate ($7 \times 10^{-7}$ M) for the length of the horizontal bar. The broad arrows indicate five minute periods during which the perfusate contained 50 mM-$K^+$. The final arrow (marked A) indicates a single injection of 5 $\mu$g acetylcholine.

In agreement with Douglas & Poisner (1964), little effect was noticed with potassium concentrations below 30 mM. However, higher concentrations of potassium caused the pancreas to secrete copious amounts of enzyme. Most of the enzyme appeared during the first few minutes of exposure to excess potassium, though an elevated output was maintained throughout the test period. Accompanying the enzyme stimulation, potassium also caused a constant reduction in the volume of pancreatic secretion.

Two mechanisms can be suggested to explain this release of enzymes. Either potassium has a direct action on the pancreatic cell or it acts by depolarizing the cholinergic nerve terminals, thus causing release of acetylcholine with consequent stimulation of the acinar cell. This latter hypothesis was tested by using atropine (Fig. 1). During perfusion with solutions containing atropine, the enzyme response to excess potassium was abolished. The reduction in volume produced by potassium, however, remained unaffected by atropine. These observations suggest that while the reduction in volume can be attributed to a direct action of potassium on the pancreatic cell, the enzyme secretion may be the result of acetylcholine release from nerve terminals.

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## The properties of the delayed rectifier as studied in voltage-clamped skeletal muscle fibres in isotonic potassium methylsulphate solution containing formaldehyde

BY O. F. HUTTER. *National Institute for Medical Research, Mill Hill, London, N.W. 7*

## Two phases of calcium entry during the action potential in giant axons of *Loligo*

BY P. F. BAKER, A. L. HODGKIN and E. B. RIDGWAY. *From the Laboratory of the Marine Biological Association, Plymouth*

The protein aequorin, which emits light in the presence of ionized calcium (Shimomura, Johnson & Saiga, 1962; Ridgway & Ashley, 1967) was injected into giant axons of *Loligo forbesi*. The luminescence of resting axons could be increased by raising external calcium, lowering external sodium or poisoning with cyanide, all of which should increase the free calcium inside the cell. The resting glow was reduced by injecting EGTA, but was little changed by injecting a 1:4 Ca, EGTA buffer, indicating that the ionized calcium concentration was roughly 0·5 $\mu$M (cf. Portzehl, Caldwell & Ruegg, 1964).

Conduction of as few as six impulses transiently increased the light production; this transient, which varied with $[Ca]_0$ and was abolished by injecting EGTA, is consistent with calcium entry during the spike (Hodgkin & Keynes, 1957) followed by uptake of calcium into an internal store. The effect of depolarization on calcium entry was studied with the voltage clamp technique using the light response as an index of calcium entry. The upper curves in Fig. 1 give the increment in brightness after 500 pulses as a function of pulse duration before, during and after treatment with tetrodotoxin. They show that the calcium entry may be divided

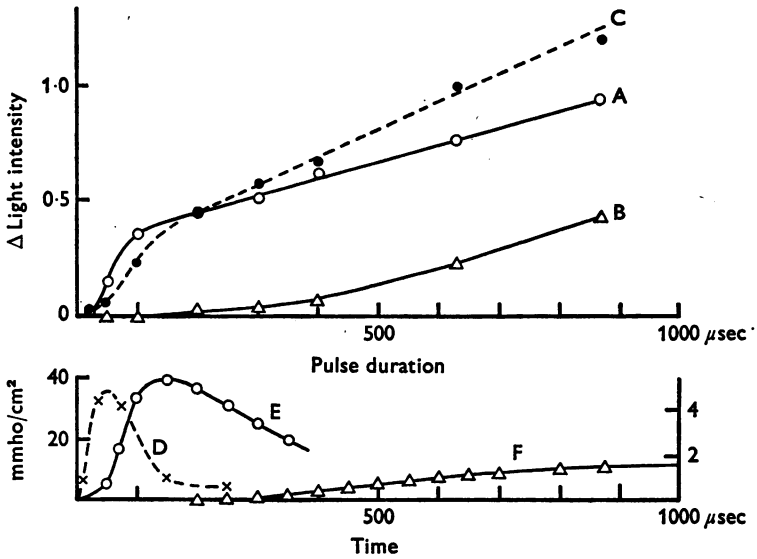


Fig. 1. Upper curves. Ordinate; increment in light intensity after 500 pulses relative to increment after 500 spikes, both pulses and spikes at 200/sec; A, before applying tetrodotoxin (TTX), B, in 0·8 μM-TTX and C, after removing TTX. Abscissa; pulse duration. Pulse amplitude 80 mV; 22° C; 112 mM-Ca and 400 mM-Na in external solution. Lower curves. D (right-hand scale), first derivative of curve A which gives Ca influx if all Ca entry occurs during pulse and Δ light ∝ Δ [Ca]. Curves E and F (left-hand scale) sodium conductance (E) and potassium conductance (F) at 80 mV in same experiment.

From tracer data (Hodgkin & Keynes, 1957) 1 unit in A, B, C corresponds to *ca.* 0·08 p-mole $cm^{-2}$, and 1 unit in D to *ca.* 80 p-mole $cm^{-2}$ $sec^{-1}$.

into an early phase, which is abolished by tetrodotoxin, and a late phase which is unaffected by it. Since the calcium influx (curve D) appeared to reach a peak before the sodium conductance (curve E) it is possible that the early calcium entry may be connected with the initiation of the increase in sodium permeability. The delayed calcium influx was somewhat similar in time course to the potassium conductance, but was not reduced

by injecting enough tetraethylammonium to block most of the potassium current. In this and several other respects (effects of Mg, Mn and pulse amplitude) the late phase resembles the calcium-entry mechanism involved in transmitter release (Katz & Miledi, 1967, 1969).

The voltage clamp equipment used in these experiments was lent to us by Dr E. Rojas to whom we are greatly indebted for advice and assistance. E.B.R. was supported by post-doctoral fellowships from N.A.T.O. and U.S. Public Health Service.

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## Effect of anticholinesterases on the response to stimulation of adrenergic fibres

BY J. H. BURN and K. U. MALIK. *Department of Internal Medicine, School of Medicine, St Louis University, U.S.A.*

Experiments have been carried out on the mesenteric arteries of the rat perfused with Tyrode solution at 30° C by the method of McGregor (1965). The sympathetic fibres running along the superior mesenteric artery were stimulated by maximal shocks at frequencies from 1/sec to 6/sec applied for 30 sec at intervals of 4 min. Further details have been described by Malik & Ling (1969*a*, *b*). The response to stimulation was recorded as the rise of pressure in the cannula tied in the superior mesenteric artery. Injections of noradrenaline were also made directly into the cannula by a Palmer pump. The response to sympathetic stimulation was not affected by the addition of hexamethonium to the perfusion fluid, but was abolished by the addition of bretylium.

When physostigmine sulphate ($6 \times 10^{-6}$ g/ml.), neostigmine ($2 \times 10^{-6}$ g/ml.) or dyflos (DFP) ($2 \times 10^{-6}$ g/ml.) was added to the perfusion fluid, there was an increase in the response which depended on the frequency of stimulation. Thus with DFP when stimulation was 1/sec, the mean percentage increase (10 expts.) was 386; at a frequency of 2/sec the mean percentage increase (6 expts.) was 154; at 3/sec it was 44 (6 expts.) and at 6/sec it was 17 (7 expts.).

Similar results were obtained with physostigmine and neostigmine. The

effect of each anticholinesterase was determined in 30 or more experiments, and for each of them the greatest increase was obtained at a frequency of 1/sec and the least at a frequency of 6/sec, which, as Folkow (1955) showed is near the top of the physiological range.

Neither neostigmine nor DFP modified the response to an injection of noradrenaline, but physostigmine produced a slight increase, much less than that in the response to stimulation at 1/sec.

The effect of the anticholinesterase was to increase the response to stimulation at 1/sec, so that it became equal to the response at 6/sec in the absence of the anticholinesterase.

These observations support the view that the acetylcholine which is released by adrenergic fibres plays an essential part in the release of noradrenaline.

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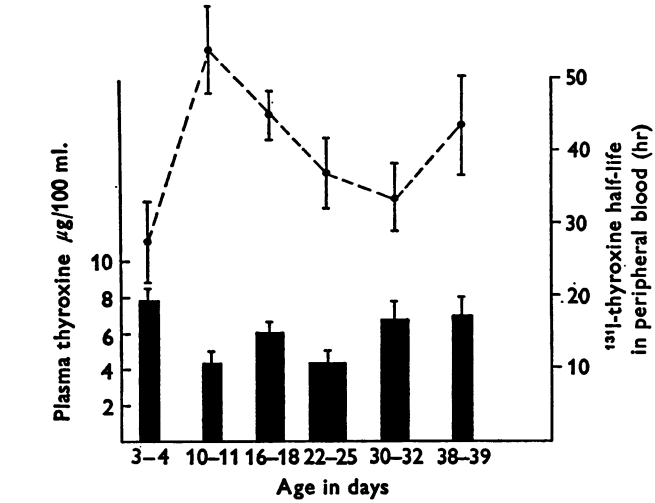
## Changes in thyroxine utilization by the young growing lamb

BY P. W. NATHANIELSZ. *Physiological Laboratory, University of Cambridge*

In the new-born calf the utilization of thyroxine during the 48–96 hr after birth is rapid (Nathanielsz, 1969). This is followed by a period in which the utilization decreases markedly. The young male Jersey calf does not begin to grow until 20–24 days after birth. 24–48 hr prior to the commencement of steady growth there is a pronounced increase in thyroxine utilization. In the present experiments, thyroxine utilization has been measured in the new-born lamb—a species in which the post-natal growth curve is very different (Fig. 1*b*).

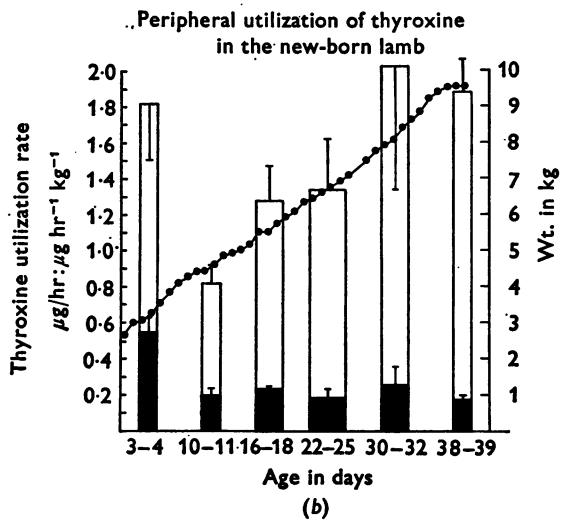
Seven lambs, suckled by their mothers, were investigated at weekly intervals from the third day of post-natal life. Fig. 1*a* shows plasma thyroxine levels and half-life of $^{131}$I-thyroxine in peripheral blood. Thyroxine levels on days 3 and 4 are significantly higher than days 10 and 11 and 22–25 ($P < 0{\cdot}01$). The differences between the other groups are not significant. Thyroxine half-life is shorter at 3 and 4 days than at 10 and 11 and 16–18 ($P < 0{\cdot}01$).

Total thyroxine utilization rate (open histogram, Fig. 1*b*) on days 3 and 4 is greater than days 10 and 11 ($P < 0{\cdot}02$). Thereafter steady increase in utilization occurs. This rise is not significant until days 38 and 39. When utilization is calculated per unit body weight (closed histogram, Fig. 1*b*), the initial period is significantly faster than all the others investigated




Half-life of $^{131}$I-thyroxine in plasma and plasma thyroxine levels in the young lamb

(a)




(b)

Fig. 1. Peripheral utilization of thyroxine in the growing lamb, age 1–39 days. (*a*) plasma thyroxine levels μg/100 ml. plasma ± S.E.M. (closed histogram) and $^{131}$I-thyroxine half-life in peripheral blood. (*b*) wt. in kg. ●—●; total thyroxine utilization/hr ± S.E.M. (open histogram) and relative (per kilogram) utilization of thyroxine ± S.E.M. (closed histogram).

($P < 0{\cdot}01$). After the initial fall, there is no significant variation in utilization/kg.

These results show certain similarities to those reported in the calf (Nathanielsz, 1968, 1969). A decrease in thyroid activity after initial hyperactivity at birth is again apparent. The lamb, however, shows no secondary increase in utilization/kg, as demonstrated in the calf. The steady post-natal growth (Fig. 1*b*) of the lamb contrasts with the calf's post-natal growth curve. It is suggested that utilization of thyroxine in the processes associated with growth may prevent the fall in utilization to the low levels demonstrated in the calf around 8–18 days.

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## The effect of intraventricular noradrenaline on plasma unesterified fatty acids in the ox

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Intraventricular noradrenaline (1 mg/animal) reduces the heat production and rectal temperature of the ox when in a cold ($-1°$ C) environment (Findlay & Thompson, 1968). Since the extra heat production of oxen in the cold results from the metabolism of fat (Blaxter & Wainman, 1961), the present experiment was undertaken to find if intraventricular noradrenaline inhibits the mobilization of unesterified fatty acids in the cold.

Five oxen, each with a lateral ventricle of the brain and a jugular vein cannulated, were exposed to an environment of $-1°$ C and wind speed of 120 m/min. Oxygen consumption was measured using a face mask and an open circuit; rectal temperature was measured with a thermocouple, and blood samples (20 ml.) were taken from the jugular cannula. The lipids were extracted from the plasma of each blood sample and the unesterified fatty acids (U.F.A.) separated from other lipid classes by thin layer chromatography. The methyl ester derivatives of the fatty acids were then separated by gas liquid chromatography and quantitated by comparison with a heptadecanoic acid internal standard.

Between 15 and 45 min after injecting sterile water into the lateral ventricle the average total U.F.A. concentration was 12·22 mg/100 ml., rectal temperature averaged 38·70° C and oxygen consumption 4·70 ml./kg.min. At the same time after similarly injecting 3 $\mu$g/kg noradrenaline

the total U.F.A. concentration fell by $4{\cdot}02 \pm 0{\cdot}63$ (S.E.M.) mg/100 ml. ($P < 0{\cdot}01$), oxygen consumption fell by $0{\cdot}78 \pm 0{\cdot}17$ ml./kg.min ($P < 0{\cdot}01$) and between 30–60 min after injection rectal temperature fell by $0{\cdot}32 \pm 0{\cdot}06°$ C ($P < 0{\cdot}01$). The decrease in total plasma U.F.A. resulted from decreases in all the major individual fatty acids.

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## Electron microscopy of frog muscle fibres in extreme passive shortening

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When a muscle is passively shortened below its slack length, the myofibrils become wavy and $C_M$ contraction bands fail to appear (Huxley & Gordon, 1962; Gonzalez-Serratos, 1966*a*, *b*). From observations on isolated fibres, set in gelatine, compressed longitudinally (Gonzalez-Serratos, 1966*a*) and fixed with glutaraldehyde, we have obtained three pieces of evidence that individual sarcomeres shorten by sliding until the thin filaments meet at the *M*-line, while shortening beyond this point occurs by bending, not shortening, of the myofibrils:

(1) In the living fibres, waviness begins when the striation spacing is 1·97–2·02 $\mu$, close to the estimated length of the thin filaments.

(2) In T.S., there was no double overlap of thin filaments at the *M*-line (Fig. 1).

(3) Fibres were compressed at one end; the other end, slightly stretched, served as a control. In L.S., the mean sarcomere length ($L$), measured parallel to the fibrils in the wavy part, was not shorter than the mean thin filament length ($I$) in the control (Fig. 2). In this fibre, $L = 1{\cdot}859\mu \pm 0{\cdot}016$, and $I = 1{\cdot}852\,\mu \pm 0{\cdot}013$ (S.E. of mean). Allowing for shrinkage (estimated from the 38·8 nm periodicity on the thin filaments) these values correspond to about $1{\cdot}99\,\mu$ in the fresh material, agreeing closely with the spacing mentioned in (1).

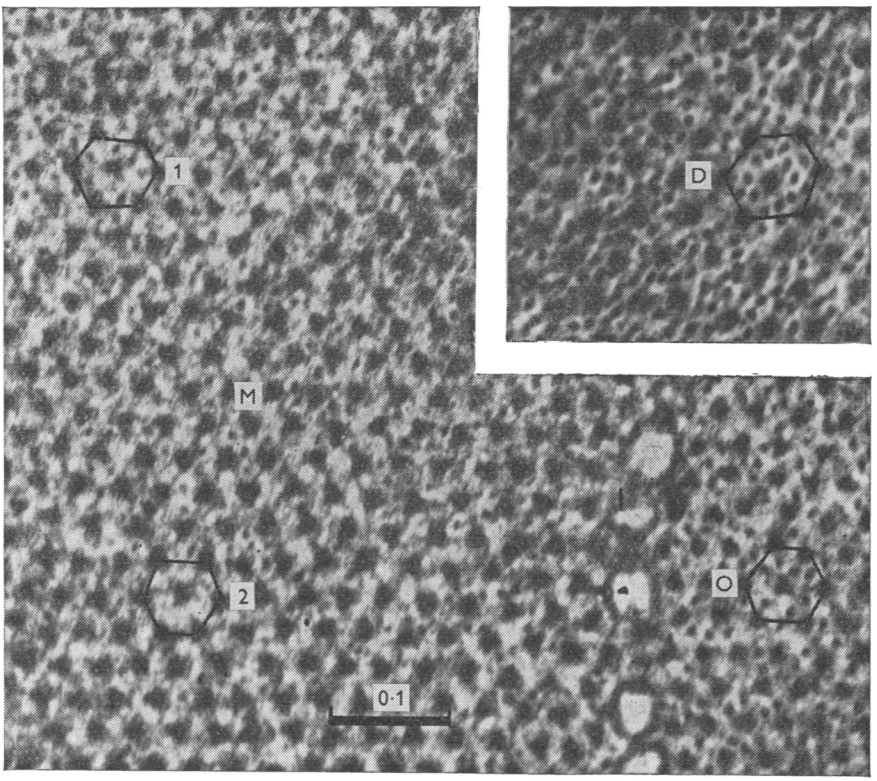


Fig. 1. T.S. of *M*-line region (M) of a wavy fibre to show single array of thin filaments (1) or none at all (2). O, single array in overlap zone. Inset: actively contracted fibre (kindly supplied by R. Rüdel and S. R. Taylor) for comparison. D, double array of twelve thin filaments. Scale: 0·1 $\mu$.

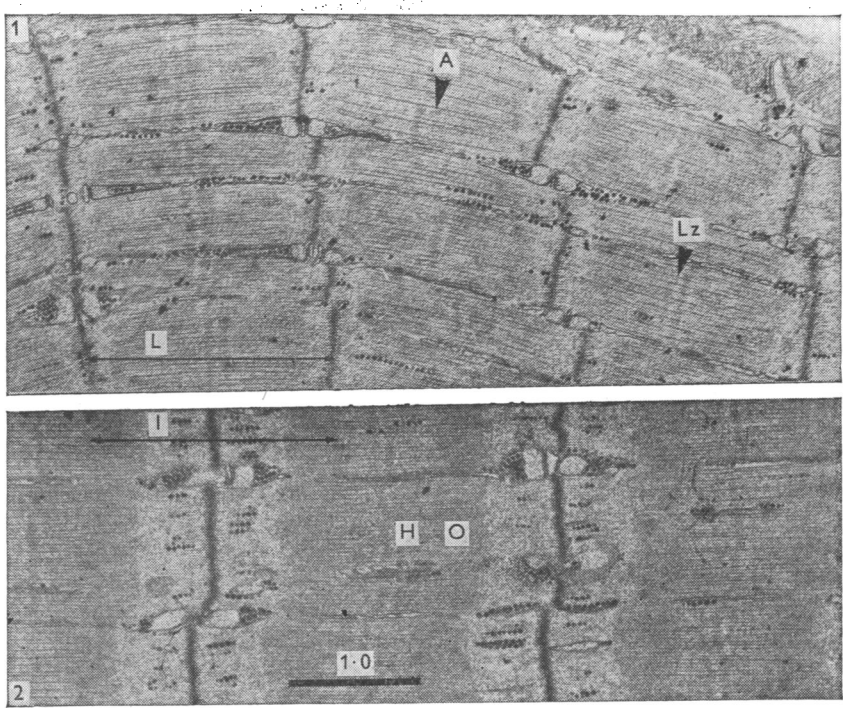


Fig. 2. L.S. of fibre compressed at one end. (1) Wavy; (2) control. L, sarcomere length parallel to fibril; I, thin filament length; A, *A* filament; Lz, *L* zone; H, *H* zone; O, overlap zone. Scale: 1·0 $\mu$.

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## Morphological changes associated with inhibition of fluid transport in the rabbit choroid plexus

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Diamond & Bossert (1968) have suggested that the primary process in the solute linked transfer of water across secretory epithelia is the active

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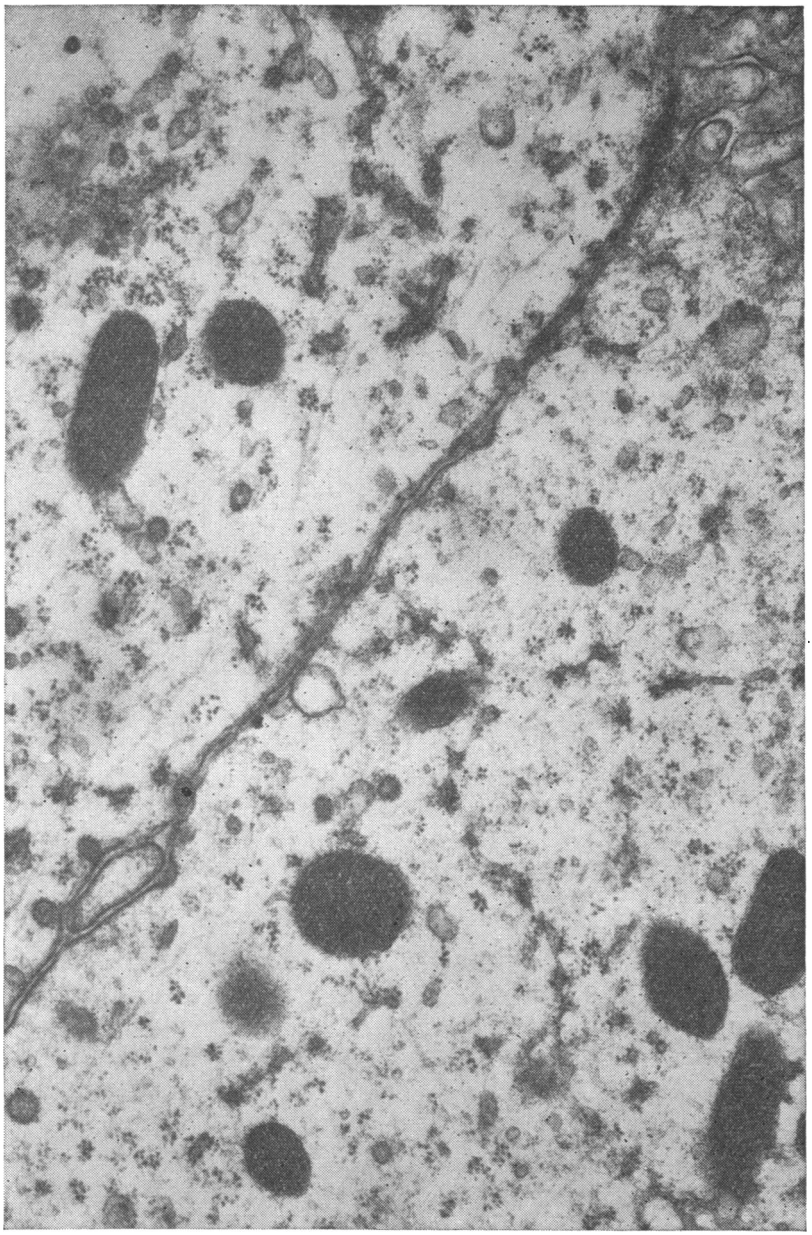

Fig. 1. Epithelial cleft of normal choroid plexus ( × 30,000).

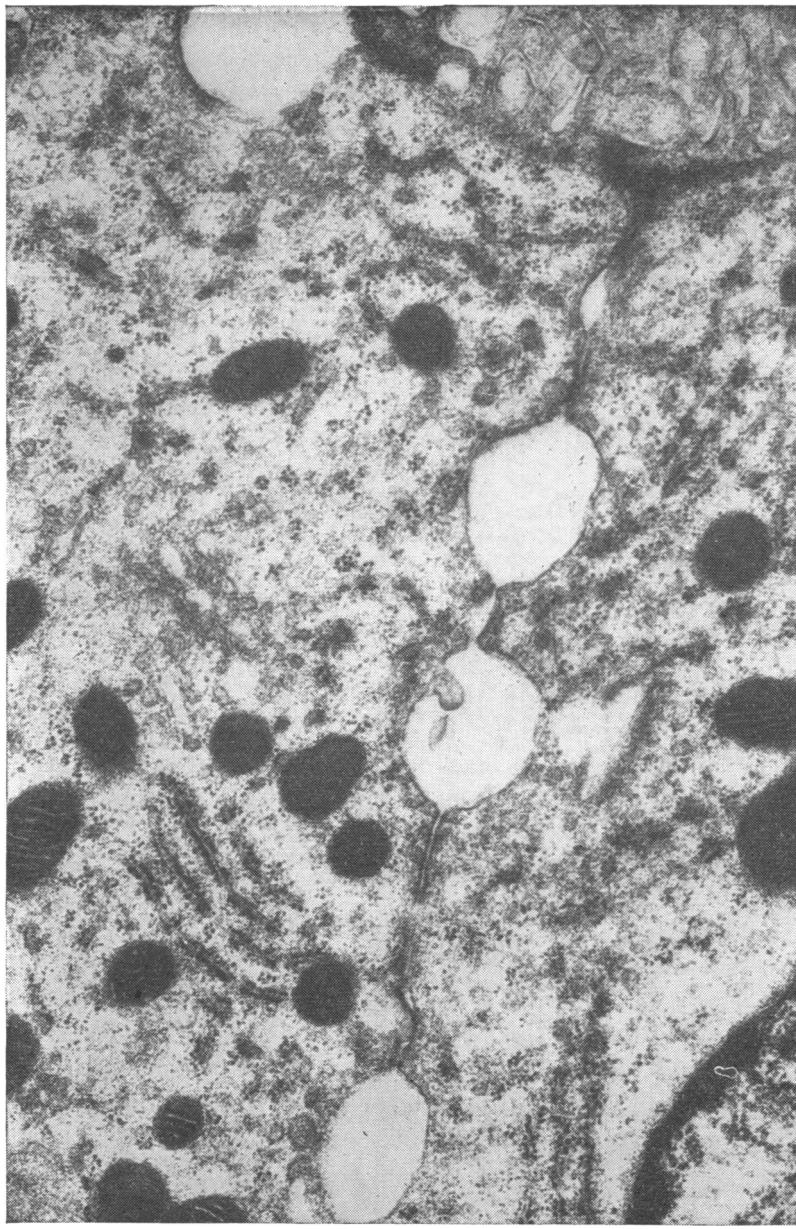

Fig. 2. Epithelial cleft of normal choroid plexus, but poisoned with Diamox.

transport of solute into the intercellular clefts of these epithelia. Transfer of water in the gall-bladder caused dilatation of these clefts; they are collapsed when the transport is inhibited. We have studied the clefts in the rabbit choroid plexus, which although morphologically similar to the gall-bladder, transports in the opposite direction. Control and choroid plexuses poisoned with Diamox were fixed *in situ* and compared. As Figs. 1 and 2 show, the clefts in the inhibited plexuses are highly swollen, while those in the controls are not. It is thought possible that the choroidal capillary pressure may be sufficient to dilate the clefts in the non-transporting plexuses.

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## Analysis of horizontal eye movements during visual pursuit

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When an object moves in relation to the eyes it is harder to resolve its detail than when it is stationary. This could be due to imperfect pursuit movements leading to blur. This could reduce retinal flux and contrast below that to be expected when both target and eye are virtually stationary. Horizontal eye movements were recorded utilizing the principle of electro-oculography; this is based on changes in electrical potential produced between two electrodes one on either side of the moving eye (Mowrer, Ruch & Miller (1936)). At the lower angular velocities (22°/sec and 43°/sec) after an initial latent period of average duration 200 msec (Fig. 1) a saccadic, i.e. rapid, movement is followed by accurate and synchronous pursuit, without further saccades, presumably because fixation has been achieved. As the target velocity increases (83°/sec) the incidence of saccadic movements rises, presumably because of a failure to achieve fixation by the initial saccade. At 167°/sec the eye movement responses disintegrate into a series of saccades, probably because the time available for seeing and the latent period were commensurate. Whilst the extraocular muscles can produce smooth pursuit velocities approaching 100°/sec such a fast visual stimulus cannot be tracked. Thus the velocity of the image was matched with considerable precision over a range of only low velocities, permitting good resolution. At the higher angular velocities visual acuity deteriorates markedly because of the persistence of saccadic movements owing to fixation failure. It follows that Ludvigh & Miller's description of their own data (1953) on dynamic visual acuity in terms of the reciprocal of the cube of the angular velocity of the target in fact covers two physiologically distinct situations.

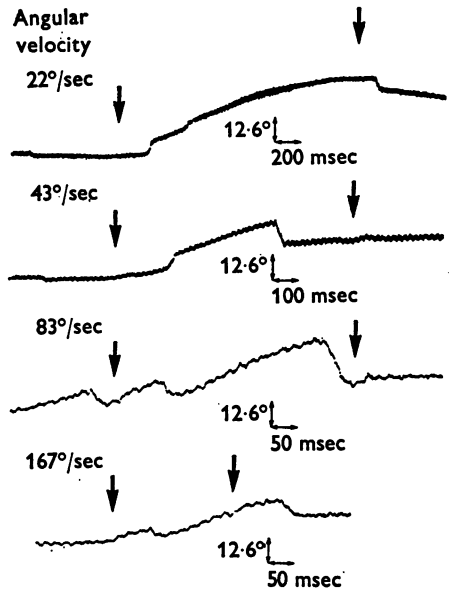


Fig. 1. At each angular velocity, the arrows indicate the point of entry and exit of the stimulus from the field of view; the trace denotes eye movements.

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## Input–output relations of the inferior olive

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Inputs to the inferior olive were provided by stimulating the head of the caudate nucleus, the mesencephalon, the juxta-fastigial region of the cerebellum and the skin of the forepaw dorsum in cats anaesthetized with thiopentone sodium. The olivary output was monitored at the cerebellar cortex by recording evoked climbing fibre responses of Purkinje cells (*reflex* responses, Eccles, Llinás & Sasaki, 1966) in the case of juxta-fastigial stimulation) with 4 M-NaCl-filled glass micropipettes.

Experiments were performed in which test responses evoked by stimulating one site were conditioned by prior stimulation of either the same or a different site. The resulting interactions were of two kinds:

(*a*) *immediate block*, where the test response was blocked for a period beginning immediately after the response to the conditioning stimulus and

(*b*) *delayed block*, where the onset of the block of the test response was delayed for 15–30 msec after the conditioning response.

Regardless of whether their onsets were immediate or delayed, the blocks of any *individual* test response produced by various conditioning stimuli persisted until a similar period had elapsed after the conditioning response (range for all experiments 60–140 msec).

An immediate block was the most likely outcome when both conditioning and testing stimuli evoked the same *single unit*, or when the background spontaneous activity was intense; either type of block could occur when the conditioning stimulus evoked a field potential only. However, in the short term (10–20 min) the type of block produced by any one conditioning stimulus remained constant. Except for caudate–caudate interactions, where the block was always immediate, no correlations were detected between type of block and site of stimulation.

Occasionally, a reflex response to juxta-fastigial stimulation was blocked by stimulating the caudate nucleus at approximately half the intensity required to evoke a cerebellar response, this threshold difference persisting for stimulations along 2–3 mm of vertical depth through the caudate nucleus. This suggests that perhaps a greater degree of convergence of caudate efferents is necessary for excitation than for inhibition in the olivary nucleus.

An apparent facilitation of the test response occurred during the first 30–50 msec following the blocking period, when the amplitude of the field potential evoked by the test stimulus frequently exceeded that of control responses. Also a juxta-fastigial stimulus which by itself did not evoke a reflex response, frequently did so if applied during the period of facilitation.

The fluctuating responsiveness of Purkinje cells to climbing fibre activation which is manifested by these inhibitory interactions seems, in reality, to depend on control of the olivary output, as suggested previously (Latham & Paul, 1970). Since the mechanism controlling the olivary output operates on reflex responses to juxta-fastigial stimulation it is likely to be located within the olive itself.

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