The use of multiple choice questions in examining and teaching physiology

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COMMUNICATIONS

Changes in mammary gland permeability at the onset of lactation in the goat: an effect on tight junctions?

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The aqueous phase of colostrum, besides being rich in immunoglobulins, has more Na and Cl and less K and lactose than milk. This could be accounted for in three ways: (1) by a qualitative change in the disposition of ion pumps in the secretory cells, (2) by extracellular fluid entering milk in pinocytotic vesicles which may carry immunoglobulins, (3) by the partial equilibration of extracellular fluid and the fluid in the secretory alveoli, i.e. paracellular as opposed to transcellular movements.

In contrast to lactating animals (Linzell & Peaker, 1971*a*, *c*) [^{14}C]lactose, introduced into the teat, passed from colostrum to blood, and [^{14}C]sucrose from blood to alveolar colostrum in significant quantities; as in lactation (Linzell & Peaker, 1971*b*), the ducts were impermeable. Lactose movement from alveolar lumen to blood could not occur by mechanism (2), but mechanism (3) could account for both lactose and sucrose movements. This suggests that there is some continuity between extracellular fluid and colostrum and that paracellular movements of Na, K and Cl as well as of other ions, down their concentration gradients, occur in late pregnancy. The most likely explanation for these paracellular movements would be that the 'tight junctions' between secretory cells are not physiologically tight at this stage.

By infusing I.A. ^{24}Na, ^{36}Cl, [^{14}C]sucrose and ^{3}HOH we were able to calculate the total flux of these ions into alveolar colostrum, removed after an injection of oxytocin. If it is assumed that the passage of [^{14}C]sucrose represents the movement of ions from extracellular fluid into colostrum then the paracellular fluxes of these can be calculated; the transcellular flux can be obtained by difference. The total flux of both Na and Cl was greater in animals in late pregnancy than during lactation but the additional quantity was entirely due to an increase in paracellular, rather than transcellular, movements. This implies that the secretory cells may have similar properties in the two states and that the difference in milk composition can be accounted for by movements between cells.

In the goat, normal milk composition is established within 2–3 days after parturition. Therefore the present results indicate that the mammary gland may be an organ that exhibits the two types of 'tight junctions' (Frömter & Diamond, 1972) at different phases of its activity. The mechanism responsible for this rapid change at the start of lactation is of obvious interest.

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The role of individual sections of the mesenteric microvasculature in constrictor responses

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It has been generally accepted that the smallest resistance vessels play the most important role in controlling the rate of blood flow through the vascular beds of most organs during increased sympathetic discharge. However, on the basis of direct observation of rat mesenteric vessels during increased sympathetic discharge, Zweifach (1961) concluded that the precapillary arteriolar vessels are the least sensitive to vasoconstrictor stimulation. With the aim of more closely analysing the behaviour of consecutive sections of the vascular bed, individual vessels of the exteriorized rat mesentery have been observed directly on sympathetic nerve stimulation and after the topical application of noradrenaline.

Paravascular nerves were stimulated using a pair of silver wire electrodes hooked under the major vessels of a mesenteric arcade. Square wave pulses of 0·5 msec duration were used, at frequencies between 0·5 and 6 Hz, the length of each train of pulses being 45–60 sec. Noradrenaline was applied topically at concentrations between 10^{-10} and 10^{-5} g/ml.; the mesentery was observed for 60 sec and then washed with fresh solution. Internal diameters of vessels were measured using a calibrated micrometer eye piece and changes in flow were noted in vessels of less than 40 μm diameter.

Paravascular nerve stimulation constricted all arterial vessels of greater than approximately 18 μm internal diameter. The constrictions were graded with stimulus frequency and, at 6 Hz, arteries of the main arcade attained a maximum constriction of 50–70 % and small arteries and terminal arterioles were constricted by 40–60 %. Precapillary arterioles,

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vessels of less than 18 μm diameter, from which the capillaries branch were not affected by nerve stimulation. Blood flow was much reduced in all the vessels of the microcirculation during nerve stimulation and usually ceased at stimulus frequencies greater than 3 Hz. In response to topically applied noradrenaline, however, all arterial vessels, including precapillary arterioles, were constricted.

Histochemical studies, using the fluorescence method for localizing monoamines (Falck, 1962), showed that all arteries up to the end of the terminal arterioles have a dense adrenergic innervation. At the end of the terminal arterioles single fibres branch into the fat and the precapillary arterioles are not innervated.

Thus it has been established that there is good correlation between the responses of individual vessels of the mesentery to paravascular nerve stimulation and their adrenergic innervation. It seems that the arteries of the main arcade and the small arteries of the microvasculature play a major role in controlling blood flow through this vascular bed during sympathetic nerve stimulation. Precapillary arterioles are not innervated and are not constricted during increased sympathetic discharge.

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Blood flow and oxygen extraction in ischaemic and normal regions of the myocardium following acute coronary artery ligation

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Little information is available concerning the metabolic and blood flow changes which occur in the myocardium following coronary artery ligation. The purpose of these experiments is to outline a method which allows comparisons to be made of changes in blood flow, oxygen extraction and consumption in normal and ischaemic regions of the myocardium and to relate these to changes in general haemodynamics.

Dogs were prepared as previously described (Ledingham, McBride, Parratt & Vance, 1970) and ventilated with 100% oxygen. After thoracotomy, a Longdwell Teflon catheter was inserted into the main vein adjacent to the descending branch of the left coronary artery (l.a.d.). Blood

TABLE 1. Metabolic changes in blood draining normal (coronary sinus) and ischaemic (coronary vein) regions of the myocardium before, and at various times after acute coronary artery ligation

(Mean of 15 experiments ± S.E.)

	Pre-ligation	Post-ligation				
		0–10 min	11–20 min	21–40 min	41 min–1 hr	2 hr
Coronary sinus						
P_{O_2} (mm Hg)	28 ± 2	28 ± 2	26 ± 2	27 ± 2	29 ± 2	26 ± 2
P_{CO_2} ((mm Hg)	46 ± 2	47 ± 3	48 ± 4	51 ± 4	47 ± 3	48 ± 2
pH (units)	7·360 ± 0·016	7·352 ± 0·017	7·345 ± 0·019	7·327 ± 0·023	7·351 ± 0·016	7·326 ± 0·016
Oxygen extraction (%)	56 ± 3	60 ± 4	60 ± 3	61 ± 4	49 ± 6	54 ± 5
Lactate extraction (%)	42 ± 5	42 ± 6	41 ± 7	29 ± 6	—	—
Coronary vein						
P_{O_2} (mm Hg)	27 ± 1	26 ± 2	25 ± 2	26 ± 2	25 ± 2	26 ± 1
P_{CO_2} (mm Hg)	45 ± 2	54 ± 4*	57 ± 5*	60 ± 3*	57 ± 3*	54 ± 2*
pH (units)	7·361 ± 0·014	7·313 ± 0·019*	7·298 ± 0·023*	7·265 ± 0·023*	7·318 ± 0·022*	7·302 ± 0·017*
Oxygen extraction (%)	54 ± 3	65 ± 3*	65 ± 3*	69 ± 3*	53 ± 4	57 ± 3
Lactate extraction (%)	40 ± 4	−0·3 ± 11*	−12·4 ± 9*	−7 ± 8	—	—

* Significantly different from pre-ligation values ($P < 0·01$).

samples were taken anaerobically from this venous catheter and from the coronary sinus, right atrium and aorta before, and for up to 4 hr after ligation of the l.a.d. Samples were analysed for P_{O_2}, P_{CO_2}, pH, oxygen content, lactate and pyruvate as previously described (Ledingham *et al.* 1970). Peripheral coronary pressure and flow were measured from a catheter in the ligated artery. This catheter was also used for the injection of ^{133}xenon into the developing infarct for the assessment of blood flow. Flow in the unligated branch of the left coronary artery was measured using a non-cannulating electromagnetic flow probe.

Before ligation there were no significant metabolic differences in blood taken from the coronary sinus and vein (Table 1). After ligation there was a marked increase in coronary venous P_{CO_2}, in lactate production and in oxygen extraction. The time course of these changes (which were not seen in coronary sinus blood) are summarized in Table 1. Ligation also decreased cardiac output, myocardial blood flow in the region supplied by the ligated vessel (from 82 ± 8 ml./100 g.min to 17 ± 2 ml./100 g.min) and oxygen consumption in the ischaemic region (from $12 \pm 0{\cdot}7$ to $2{\cdot}9 \pm 0{\cdot}4$ ml./100 g.min). Blood flow (79 ± 6 ml./min) and oxygen consumption in the normal myocardium were unchanged.

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On the descending 5-hydroxytryptaminergic pathway controlling the stretch reflex

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The tonic stretch reflex of extensor muscles is absent in the spinal cat but it does occur after intravenous injection of the 5-hydroxytryptamine (5-HT) precursor 5-hydroxytryptophan (5-HTP) (Ahlman, Grillner & Udo, 1971). These authors also found that the drug potentiated the discharge of secondary endings of soleus muscle spindles but only when the gamma efferent supply was intact. Their conclusion was that 5-HTP increased the resting discharge of static gamma motoneurones which, by increasing activity in primary spindle endings, facilitated the tonic stretch reflex. We now have direct evidence that activation of gamma motoneurones by

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5-HTP does occur but that this process is probably of secondary importance in facilitating the stretch reflex.

Intravenous injection of 5-HTP (50 mg/kg) in the acute spinal animal gave a prolonged increase in the resting discharge of gamma motoneurones, identified by their conduction velocity, to the triceps surae muscles. The latency of excitation, however, was less than two min. Furthermore, 5-HT itself (0·1–0·5 mg/kg I.V.) was found to excite discharges in gamma motoneurones. The short latency action of 5-HTP and the fact that 5-HT does not cross the blood–brain barrier suggest that these actions are peripheral. A preliminary investigation has shown that neither drug excited a significant discharge in skin nerves known to have powerful reflex connexions to gamma motoneurones. In disagreement with Ahlman *et al.* (1971), however, we do find that both drugs can excite some primary and secondary afferents from de-efferented spindles; this could contribute to facilitation of the stretch reflex.

We re-investigated the action of 5-HTP on the stretch reflex of the soleus in the spinal cat and found the latency of facilitation to be 6–10 min. Injection of 5-HT, however, at a dose which can excite gamma motoneurones, had no effect. The difference in latency of action of 5-HTP on gamma discharges and the stretch reflex, and the lack of effect of 5-HT on the stretch reflex suggest that neither excitation of gamma motoneurones nor direct action on the spindles by 5-HTP are the prime causes in facilitating the reflex. We have now recorded the reflex discharge from ventral rootlets in response to stretch of the soleus muscle in the decerebrate cat with ventral roots L6 to S3 sectioned. One hr after a spinal section the reflex was no longer present but could be elicited again after injection of 5-HTP. The reflex disappeared on cutting the muscle nerve.

We conclude that 5-HTP facilitates the stretch reflex by a central mechanism, as yet unknown, independently of its excitatory action on gamma motoneurones.

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Appearance of prostaglandins in the renal venous blood of dogs in response to acute systemic hypotension produced by bleeding or endotoxin

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Prostaglandin concentrations in renal venous blood of the dog are increased by renal arterial infusions of noradrenaline (McGiff, Crowshaw, Terragno, Malik & Lonigro, 1972) or angiotensin II (McGiff, Crowshaw, Terragno & Lonigro, 1970; Aiken & Vane, 1971), stimulation of renal sympathetic nerves (Dunham & Zimmerman, 1970) or reduction in renal arterial pressure (McGiff, Crowshaw, Terragno, Lonigro, Strand, Williamson, Lee & Ng, 1970; Herbaczynska-Cedro & Vane, 1972). We now show that prostaglandin-like activity appears in renal venous blood in response to an acute fall in blood pressure, that the appearance can be abolished by indomethacin, and that the prevention of prostaglandin output is associated with a recovery of blood pressure.

Dogs of either sex weighing 14–31 kg were anaesthetized with pentobarbitone (30 mg/kg) and given heparin (1000 units/kg I.V.) Hypotension was produced either by arterial bleeding, or by the intravenous injection of endotoxin (*E. coli*, Difco 0111 B4). Prostaglandin-like activity was estimated by means of the dialysis modification of the blood bathed organ technique of Vane (Collier, 1972). Blood for assay was continuously withdrawn (20 ml./min) from the vascular bed under study, dialysed against Krebs solution (which superfused the assay tissues) and then returned to the animal intravenously.

When animals were bled (nine trials in seven dogs), or given endotoxin (1–5 mg/kg; seven trials in seven dogs) the prostaglandin-like activity in renal venous blood increased to 2–8 ng/ml. (assayed as E_2) and most closely resembled E type prostaglandins. The output of activity was maintained throughout the period of hypotension in all endotoxin experiments but in only six out of nine haemorrhage studies. After hypotension was established, indomethacin (1 mg/kg I.V.) always abolished output of prostaglandin-like activity into renal venous blood, and at the same time there was a recovery of arterial blood pressure. The rise in blood pressure was such that in ten experiments (four haemorrhage studies and six endotoxin) the final pressure was equal to or greater than that prior to hypotension. Prostaglandin-like activity also disappeared from renal venous blood in two haemorrhage experiments when the systemic blood pressure was restored by retransfusing shed blood; the activity reappeared after a subsequent bleed.

The output of prostaglandin-like activity into renal venous blood of the dog in response to endotoxic and haemorrhagic hypotension has not previously been reported. That the substances detected were most likely prostaglandins is based primarily on the specificity of the system used for bio-assay (Gilmore, Vane & Wyllie, 1968) but is reinforced by the disappearance of activity after indomethacin, a specific inhibitor of prostaglandin biosynthesis (Vane, 1971).

In the dog, both E and A-type prostaglandins cause hypotension so that a rise in blood pressure would be expected when their formation is prevented. That indomethacin will cause such an increase has previously been reported for the endotoxin-treated dog (Erdös, Hinshaw & Gill, 1967) but not in haemorrhagic hypotension.

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The distribution of vasopressin and neurophysin in the hypothalamo-distal-neurohypophysial and hypothalamo-infundibular neurosecretory systems of normal and scrapie-affected sheep

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Livett & Parry (1971), using immunofluorescence (Livett, Uttenthal & Hope, 1971), demonstrated neurophysin in the hypothalamo-distal-neurohypophysial neurosecretory system (HDNS) of normal and scrapie-affected sheep. Semi-quantitative estimates of neurophysin content based on immunofluorescence and immunodiffusion have been correlated with vasopressor activity.

With fluorescein isothiocyanate as the fluorophore, neurophysin-specific immunofluorescence appeared in normal sheep as an evenly granular-textured green coloration throughout the HDNS and in the median eminence-pituitary stalk (MES) within the external infundibular zone as well as the internal, but not in the pars tuberalis.

In natural scrapie the intensity of the specific immunofluorescence was reduced markedly throughout the HDNS, but in the external infundibular zone the intensity was increased at least two-fold and some fluorescence occurred in the pars tuberalis.

Sectors of the HDNS were assayed for pressor activity using Dekanski's (1952) method as modified by Dean & Hope (1968) with a three-point design of assay (Gaddum, 1959). One scrapie-affected and one paired normal sheep were killed under standard conditions, the brains and pituitaries removed within 30 min, the whole MES taken for pressor assay, and the remainder cut sagittally, one side being used for the pressor assay and the other for neurophysin assessment.

The respective vasopressin contents (i.u.) of the normal and scrapie samples were: supraoptic nuclear region 0·016 and 0·007, paraventricular nuclear region 0·012 and not detectable, distal neurophypophysis 6·40 and 2·28, MES 0·186 and 0·154.

Thus in scrapie the vasopressin content of the HDNS is reduced more than twofold, while that of the MES is little changed. Since the reduction of neurophysin immunofluorescence of the internal infundibular zone is similar to that of other sectors of the HDNS, the presumption is that its vasopressin content is reduced similarly. Hence the vasopressin content of the remaining MES i.e. the external infundibular zone (and possibly pars tuberalis), will be increased approximately twofold, which corresponds closely with the observed increase of neurophysin immunofluorescence.

The presence of vasopressin with the neurophysin in the external infundibular zone supports the view that vasopressin is involved in the normal control of adenohypophysial function, through its effects: (i) on membrane permeability (Leaf & Sharp, 1970), (ii) on portal system blood flow (Porter, Kamberi & Grazia, 1971), (iii) as a hormone-releasing factor (HRF), especially for adrenocorticotropin (McCann & Porter, 1969), and (iv) potentiating other HRFs (Yates *et al.* 1971).

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Hepatic ketone body metabolism in the foetal and neonatal sheep

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The ketone body concentrations in sheep foetal plasma remain very low even when the glucose concentrations are near to zero or when the foetus is isolated from the mother and perfused without glucose supplement (D. P. Alexander, H. G. Britton & D. A. Nixon, unpublished). However, the ability to produce ketone bodies may be present shortly after birth since the new-born lamb develops severe ketotic diabetes if treated with streptozotocin (Alexander, Britton, Cohen, Mashiter, Nixon & Smith, 1971). To investigate the development of ketone body production liver perfusions based upon the technique of Andrews, Britton, Huggett & Nixon (1960) have been carried out on foetal livers of 108–144 days gestational age and a 16-day-old lamb, with oleate as substrate.

Foetal livers of 108, 115 and 130 days were perfused with maternal sheep blood for periods of up to 3 hr. In each case there was a small fall in the endogenous level of the blood ketone bodies but this corresponded to an uptake of less than 10 μmole/hr. This fall continued even when the oleate bound to plasma albumin was infused to give high levels ($\sim$ 3–6 m-equiv/l.) of free fatty acids. In the 108- and 115-day animals and in an additional liver from an animal of 110 days sodium acetoacetate was also administered to give total ketone body concentrations of about 20 mg acetone/100 ml. Conversion to β-hydroxybutyrate occurred but there was virtually no net uptake of ketone bodies.

When the livers of three older animals of 141, 142 and 144 days were perfused there was a marked production of ketone bodies during the oleate infusion (giving concentrations of ~ 1–5 m-equiv/l.), although little production was seen during the initial control periods. From the rate of rise of concentration a production rate of 0·6 mmole/hr was calculated for the most active preparation (141 days, foetal wt. 3·06 kg, liver wt. 57 g). In comparison, a liver taken from a 16-day-old lamb showed appreciable ketone body production during the control period probably related to the higher endogenous plasma free fatty acids, and when oleate was infused to give a level of about 3 m-equiv/l. the production rose to about 1·5 m-mole/hr (wt. of lamb 6·16 kg, liver wt. 143 g).

The livers from the animals of all ages removed free fatty acids from the circulation. Glucose concentrations were higher in the older animals.

It is concluded that the sheep foetal liver develops the ability to produce ketone bodies at about 1–2 weeks before term but production *in utero* is likely to be negligible because of the low free fatty acid levels in the foetal circulation. The lack of uptake of ketone bodies by livers from the younger animals is to be compared with the rapid uptake by the whole foetus at these ages (Alexander, Britton & Nixon, 1966).

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Line, edge and grating detectors in human vision

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Kulikowski (1969) has shown that the threshold contrast for a fine dark line may be reduced by superimposing it on a dark striation of a *subthreshold* sinusoidal grating. Apparently, the 'detector' for the fine line is also sensitive to the grating; we can thus study the contrast sensitivity of the 'line detector' to gratings of different spatial frequency. Similarly, we may derive the 'grating sensitivity' of 'edge detectors' and 'grating detectors' (Fig. 1*a*).

Using Fourier transform techniques (Campbell, Carpenter & Levinson, 1969) we can transform these 'grating sensitivity' functions into 'line

sensitivity' functions, i.e. the contrast sensitivity to a fine line as a function of the distance of the line from the centre of the 'receptive field' of the detector (Fig. 1*b*).

We have confirmed these line sensitivity functions by superimposing the test stimulus (line or edge) on a background pattern of two subthreshold lines as represented in Fig. 1*b*. These results are represented by the squares in Fig. 1*b*, and they fit the predicted functions reasonably well. R. Shapley & D. J. Tolhurst (in preparation) have made similar

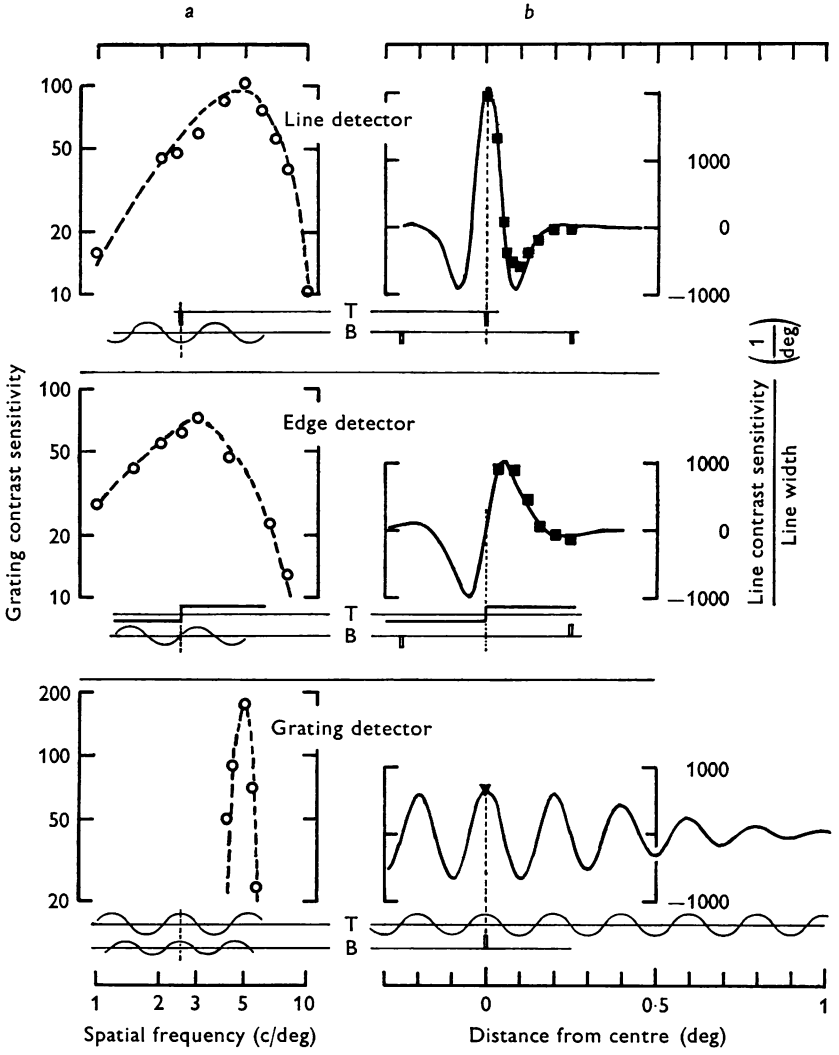

Fig. 1. For legend see opposite page.

measurements for the edge detector. We also measured the maximum line sensitivity of the grating detector (Fig. 1*b*, triangle) which agreed with the value predicted by the Fourier transform.

The spatial properties of the line and edge detectors (Fig. 1*b*) resemble those of cortical cells described by Hubel & Wiesel (1962). However, evidence for the grating detector is only psychophysical (Sachs, Nachmias & Robson, 1971). The grating sensitivity function of the line detector is similar to that of ganglion cells (Enroth-Cugell & Robson, 1966) and many cortical cells (Campbell, Cooper & Enroth-Cugell, 1969).

The detectors described above are the most sensitive in their respective classes; finer and coarser detectors may also be revealed.

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Legend to Fig. 1.

Fig. 1. Contrast sensitivities of 'line', 'edge' and 'grating' detectors determined by subthreshold summation. (*a*) Contrast sensitivity to gratings of variable spatial frequency. 'T' and 'B' represent the luminance distribution of the test and subthreshold background stimuli respectively. (*b*) Contrast sensitivity to a fine line as a function of its distance from the centre of the 'receptive field' of the detector. The continuous curves are derived by Fourier transformation of the 'grating sensitivity' curves of Fig. 1 (*a*). The squares and the triangle represent more direct measurement of 'line sensitivity' derived from the test and subthreshold background stimuli represented by 'T' and 'B'.

In all the experiments the screen, subtending $2\frac{1}{2}$ deg in diameter, of space average luminance – 5 cd/m^2, was viewed binocularly through natural pupils. Subject J.J.K. (37 years). Contrast sensitivity is the reciprocal of contrast threshold, and contrast is defined by $(L_{max} - L_{min})/(2\bar{L})$, where L_{max}, L_{min} and $\bar{L}$ are the maximum, minimum and space-average luminances of the pattern.

Sensitivity to a given background (grating or line) was calculated from $(C_{OT} - C_{BT})/C_{OT}$. C_B where C_{OT}, C_{BT} are contrast thresholds for test only and test plus background and C_B = background contrast.

Effects of urea infusion on water and electrolyte excretion in the hydropaenic rat

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It is generally accepted that urea plays a unique role in the elaboration of concentrated urine by the mammalian kidney (Berliner, Levinsky, Davidson & Eden, 1958). Yet, paradoxically, it has been claimed that urea can act as an osmotic diuretic agent, i.e. that infusion of urea causes an increased excretion of water and electrolytes, as does mannitol (e.g. Koike & Kellogg, 1963). The present experiments were performed to ascertain whether urea could indeed be considered to act as an osmotic diuretic in the rat.

Conscious, hydropaenic rats (48 hr water-deprived), were infused with a solution of 5% (w/v) urea in 2·5% (w/v) dextrose, at a rate of 75 μl./min over a period of 6 hr. During this period, changes in urinary flow and composition were studied, and compared with the changes produced in control animals, by infusion of 2·5% dextrose (75 μl./min) alone.

The following changes in urinary flow and composition were observed:

(*a*) In the urea-loaded animals, urine flow increased progressively during the first 3–4 hr of the infusion. From 3 to 4 hr onwards, an essentially steady state existed, with the urea excretion rate close to the rate of administration (62·5 μ-mole/min), and the urine flow approximately equal to the rate of water infusion. Infusion of dextrose alone produced almost identical changes in urine flow.

(*b*) The increased urine flow in the urea-loaded animals was accompanied by an increased osmolal output, from a pre-infusion level of $5{\cdot}00 \pm 0{\cdot}48$ (S.E.M.) μ-osmole/min, to a final (6 hr) value of $67{\cdot}02 \pm 6{\cdot}90$ μ-osmole/min. This increase was almost entirely due to urea.

(*c*) In the urea-loaded animals, sodium output increased slightly over the 6 hr period, from an initial $0{\cdot}305 \pm 0{\cdot}031$ μ-equiv/min, to $0{\cdot}572 \pm 0{\cdot}197$ μ-equiv/min. This increase was not statistically significant when compared to the sodium output of the control series. Similarly, no significant increase in potassium output was observed.

It is concluded that, in the hydropaenic rat,

(*a*) the diuresis arising from the infusion of urea solutions is largely, or entirely, attributable to the infusion vehicle, and not to urea *per se*.

(*b*) unlike true osmotic diuretic solutes, such as mannitol, urea has little effect on non-urea solute excretion.

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The effects of schistosomiasis, anaemia and malnutrition on the responses to exercise in African children

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Iron deficiency anaemia, schistosomiasis and malnutrition are endemic to the African continent but there are regrettably few studies of the physiological effects of these debilitating diseases.

The present investigation is concerned with the responses to exercise performed on a stationary bicycle ergometer of forty East African children aged 7–16 years who were diagnosed, at the time of measurement, as suffering from iron deficiency anaemia (Hb $< 8{\cdot}5$ g/100 ml.) or schistosomiasis (from urinary egg output) or malnutrition (from the criteria of Jelliffe 1966). The data have been compared to those of normal African children. In addition, a group of ten children who had been rehabilitated from a state of malnutrition, has been included in the analysis.

The results showed that whereas anaemia and malnutrition produced a marked decrease in maximum aerobic power and an increased cardiac response at a given oxygen intake, Bilharzia was without effect on the physiological responses to exercise. The decreased $\dot{V}_{O_2}$ max of the malnourished children was associated with the loss of lean body mass and leg (muscle plus bone) volume. The findings from the previously malnourished children suggest that the changes of exercise function associated with undernutrition could be reversed by treatment.

It was concluded that iron deficiency anaemia and malnutrition together constitute a more serious physiological problem than does Bilharzia in children, and their treatment must be given urgent and continued priority in East Africa.

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Visual control of human muscular movement

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The effect of visual feed-back in the control of voluntary muscular movement has been the subject of considerable attention, leading to models such as that proposed by Navas & Stark (1968). Most of these models have been derived from mechanical measurements on the muscle control system. In the present study, electromyography has been used to obtain further information about the nature of the control mechanism.

The supine subject was presented with a visual display of the position of his forearm and instructed to keep it in a vertical position by keeping a line centred in the display screen. A step shift was then introduced into the display to make the subject assume a new arm position. The arm displacement and surface e.m.g.s from the muscles controlling the arm were recorded. The e.m.g.s were then rectified, averaged and filtered to provide a time histogram of the electromyographic activity associated with the movement. Fifteen subjects aged between 18 and 30 have been tested.

After five to ten practice trials, changes in electromyographic activity were found to occur in two distinct phases in all subjects tested. A decrease in any existing antagonist activity preceded any agonist activity increase by 30–40 msec. Successive bursts of activity were then observed in agonist and antagonist muscles at 150 msec intervals until the arm reached its final position.

Increasing the load applied to the arm had no effect on the time course of the changes in activity. The loop gain of the visual display could be varied over a 10:1 range without affecting the subject's performance. No difference could be observed between the left- and right-armed responses of any subject.

These results suggest a high degree of adaptability in the visual control system consistent with the findings of Stephens & Taylor (1972). They also provide the basis for a model of the visual control system that does not contain the 'Input-synchronized sampler' proposed by Navas & Stark.

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Static and dynamic fusimotor action in isolated cat muscle spindles with intact nerve and blood supply

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Boyd (1971) showed that 89% of fusimotor fibres to isolated cat tenuissimus muscle spindles specifically operate either nuclear bag or nuclear chain intrafusal fibres. The fusimotor fibres could not be identified as 'dynamic' or 'static' (see Matthews, 1972) since the preparation was removed from the cat.

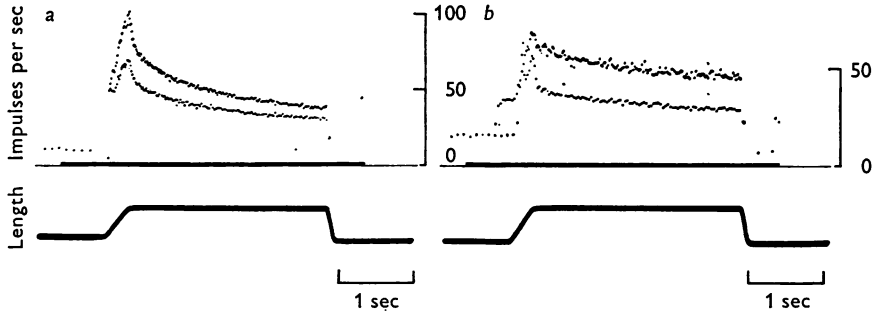

Fig. 1. Comparison of the action of (*a*) a dynamic and (*b*) a static fusimotor fibre on the response of the primary sensory ending of an isolated spindle (upper traces) to a 0·8 mm stretch at 2·5 mm/sec (lower trace). Frequency recordings show firing in the Ia afferent fibre during (above) and in the absence of (below) repetitive stimulation of the γ fibre for the period indicated by thickening of the zero frequency line.

(*a*) Maximal effect of the dynamic γ fibre; conduction velocity 24 m/sec; stimulation at 75 pulses/sec. Dynamic index (decay in Ia frequency in first 0·5 sec following completion of stretching) increased by 16 impulses/sec.

(*b*) Maximal effect of the static γ fibre; conduction velocity 25 m/sec; stimulation at 150 pulses/sec. Dynamic index reduced by 12 impulses/sec.

We have now succeeded in isolating muscle spindles with an adequate blood supply and with their nerve fibres from the spinal roots intact. The abductor digiti quinti medius muscle of the cat, with a pedicle of blood vessels and nerve, was reflected into a bath. The Ia fibres and γ fibres to this muscle were isolated in 'single fibre' spinal root filaments and the γ fibres classified as 'dynamic' or 'static' according to their action on the Ia afferents. A spindle was then isolated and the response of its intrafusal

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fibres to fusimotor stimulation observed directly and recorded on moving film. The mechanical behaviour of nuclear bag and nuclear chain fibres in the seven spindles studied so far was the same as previously demonstrated (Boyd, 1970).

Four γ fibres had a clear-cut dynamic action (Fig. 1*a*). All four produced focal contraction at one pole of nuclear bag fibres only. In two cases only one of the nuclear bag fibres contracted. None of these dynamic γ fibres produced driving of the afferent discharge.

Eight γ fibres had a clear-cut static action (Fig. 1*b*), three operating both spindle poles, and the remainder one pole only. Four of these static γ fibres produced local contraction in nuclear chain fibres only. The principle action of the other four static fibres was to produce local contraction in nuclear chain fibres, but some movement in one of the nuclear bag fibres occurred in addition. Seven of the static fibres produced driving of the primary afferent discharge.

We acknowledge the valuable technical assistance of Miss Jess Wilson.

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Interlimb co-ordination in stepping in the cat

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Stereotyped movements of all four limbs resembling those of natural stepping may be elicited in the high spinal cat (Sherrington, 1910), thus indicating that the principal reflex systems for the co-ordination of the limbs in locomotion lie within the spinal cord. Neurophysiological investigations in the high spinal cat have revealed that long ascending propriospinal pathways linking the lumbo-sacral and cervical segments can exert excitatory actions on different groups of forelimb motoneurones (Miller, van der Burg, van der Meché & Reitsma, 1971). The effects are stronger ipsilaterally and occur at latencies of 6–15 msec. Transmission in the pathways can be greatly facilitated and modified by activation of descending bulbo-spinal projections (Bergmans, Miller & Reitsma, 1972). In the present study an attempt has been made to determine if the wide range of natural patterns of co-ordinated limb movements seen in normal

awake cats could be related to the functional organization of the ascending propriospinal pathways demonstrated neurophysiologically.

The patterns of joint movements and of activation of different muscles of the limbs have been studied in normal cats trained to walk, trot and gallop on a treadmill. Under brief Fluothane anaesthesia thin insulated wires were inserted into various muscles. Electromyograms were tape-recorded and synchronized with moving film sequences of the cats.

Two principal patterns of stepping have been observed: (1) Alternate stepping, as in all forms of walking and trotting, where the hind limbs step alternately. (2) In-phase stepping, as in all forms of galloping, where the hind limbs step more in phase, and the muscles of the back contract bilaterally to contribute to the forward thrust of the body. In both types of stepping there is a consistent time-locking of joint angle displacement between hind limbs and forelimbs. The first extension phase of the hind limb in its swing phase is followed closely by the first flexion of the ipsilateral forelimb at the end of its stance phase. At all speeds above a 'slow walk' (above 1 m/sec) the time interval remains remarkably constant at about 40 msec. A significant time-locking between the electromyograms of the appropriate hind limb and forelimb muscles was also found with an interval of 30–50 msec. In addition this interval was significantly less variable than the total step period.

The hypothesis is proposed that in all forms of quadrupedal stepping in the cat which are faster than a 'slow walk', co-ordination between hind limbs and forelimbs may be accounted for by one main central programme of neural activity, in which long ascending propriospinal pathways play an important part.

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The action of adrenaline on the reliability of firing of primary endings of muscle spindles in the rat

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The suggestion by Banker & Girvin (1971) that a smooth muscle component can be seen in the intrafusal musculature of the mammalian

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muscle spindle makes appropriate a re-examination of the action of adrenaline on the excitability of spindle primary afferents. Previous studies of the effects of adrenaline on muscle spindles (Paintal, 1959; Hunt, 1960; Calma & Kidd, 1962) showed that it was difficult to differentiate between a direct action on the muscle spindle, and one secondary to changes in muscle blood supply.

Using a modification of the rat caudal muscle preparation (Gladden & Kidd, 1969), C. H. Vaillant (1971, personal communication) succeeded in blocking the blood supply of the muscle by injecting 0·3 ml. of soft Vaseline intra-arterially into the vascular system (Gurumurthy & Kidd, 1967). When the caudal muscle was bathed in oxygenated Krebs solution at 35° C, the blockade of the circulation had no effect on the excitability of the spindles in the muscle. Vaillant's observation of this was confirmed by a similar one (Kayaap, Kučera & Smith, 1972) during experiments in which the same muscle preparation was used.

As the excitability of the spindles in the preparation is independent of the blood flow, it seemed a useful one for the study of a possible direct action of adrenaline on their excitability.

An adrenaline-in-Krebs solution was prepared (5×10^{-8} M) and stabilized with ascorbic acid (1×10^{-3} M). The muscle spindles were stretched to a little over their threshold length in which position they discharged action potentials unreliably. The replacement of the control Krebs solution by one containing adrenaline always increased the reliability of firing of the receptor. A phase of depression was never seen.

The application of the adrenaline increased the reliability of discharge of the primary endings to an extent equivalent to that given by an 0·2–0·3 mm of additional stretch. The normal range of movement for the muscle is 2·0–3·0 mm (Gladden, 1970). The effect appeared 1·0–1·5 min after application, and was fully developed in 2·5–3·0 min. It persisted unchanged for up to 1·5 hr when the adrenaline solution was periodically changed. Curarization of the muscle had no effect on the response of the muscle spindle to adrenaline.

We conclude that adrenaline does have a direct action on the muscle spindle, and that it is most easily seen as an increase in the reliability of discharge of the primary ending stretched to a length just above threshold.

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Evidence that the inhibitory effect of burimamide on gastric secretion is not due to decreased gastric mucosal blood flow

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Black, Duncan, Durant, Ganellin & Parsons (1972) introduced burimamide as a drug acting specifically on those histamine receptors (H_2) which are insensitive to mepyramine. The H_2 receptors include those in the stomach and these workers demonstrated that burimamide decreases gastric acid secretion stimulated by histamine or pentagastrin. Gastric secretion is dependent on high mucosal blood flow, and is decreased when mucosal blood flow falls. Black and his colleagues did not exclude the possibility that the antisecretory activity of burimamide was secondary to decreased gastric mucosal blood flow. We have demonstrated that this is not so.

TABLE 1. The maximum percentage changes in acid secretion, mucosal blood flow (M.B.F.) and the ratio of M.B.F. to acid secretion caused by burimamide

Dose of burimamide	2×10^{-5} mol/kg^{-1}			4×10^{-5} mol/kg^{-1}		
Stimulus	Acid secretion	M.B.F.	M.B.F./ acid secretion	Acid secretion	M.B.F.	M.B.F./ acid secretion
Histamine	−40	−22	+36	−65	−61	+100
Pentagastrin	−40	−16	+45	−72	−49	+95
Feeding	−58	−43	+31	−82*	−64*	+311*

* Mean of two results.

Four healthy bitches (12–24 kg) with long-established Heidenhain pouches were used. Acid secretion (60–70% maximal) was stimulated by pentagastrin (4 μg kg^{-1} hr^{-1} I.V.), histamine acid phosphate (2 μg kg^{-1} min^{-1} I.V.) or a standard meal. Gastric mucosal blood flow was estimated by radioactive aniline clearance (Curwain & Holton, 1971; Curwain, 1972). Burimamide (2 or 4×10^{-5} mole kg^{-1} I.V.) was injected during the secretory plateau.

As shown in Table 1, both doses of burimamide decreased acid secretion and gastric mucosal blood flow in response to each stimulus. The inhibitory

effect usually lasted 45–75 min but on one occasion the effect of the large dose was still apparent for more than 2 hr. In every experiment the ratio of mucosal blood flow to secretion increased after burimamide. These results show that the inhibitory action of burimamide on gastric secretion is not due to decreased blood flow and support the claim of Black *et al.* (1972) that it is a specific H_2 receptor antagonist.

We are grateful to Dr Black of Smith, Kline & French Ltd for a supply of burimamide and to the Medical Research Council and the Wellcome Trust for support.

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Changes of capillary density and enzyme pattern in fast rabbit muscles during long-term stimulation

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It has been shown that muscle fibres with slow contraction times and high activity of oxidative enzymes are surrounded by more capillaries than those with fast contraction times and high glycolytic activities. This had led to the conclusion that the capillary density and blood flow are related to the activity of oxidative enzymes. When the type of metabolism is changed by cross-innervation the number of capillaries and type of metabolism change accordingly (Romanul & Pollock, 1969). It is possible that the changing enzyme pattern is induced by an increased supply of substrates, in which case it would be expected that the change of capillary density would precede the change in oxidative metabolism. A suitable situation for studying this possibility is provided by experiments in which fast muscles in adult rabbits are subjected to chronic stimulation with a pattern of activity that causes a transformation towards the slow type of muscle.

Electrodes were implanted under pentobarbitone anaesthesia for stimulation of the lateral popliteal nerve (Salmons & Vrbová, 1969). Tibialis anterior and extensor digitorum longus muscles were stimulated at 10/sec for 8 hr a day. The contraction times, capillary density and enzyme activities were examined after different periods of stimulation in extensor digitorum longus.

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At 4 days there was a slight slowing of the muscle, but no change in the activity of oxidative enzymes (as measured by the citrate synthase activity) was noticeable at this time. There was an increase of activity of hexokinase and decrease of triose-phosphate-dehydrogenase. The activity of oxidative enzymes only started to increase after 7 days of stimulation, and reached the value comparable to that found in slow muscle after 28 days. In contrast, at 4 days, the number of capillaries per muscle fibre was already increased by 20 %. The capillary density continued to increase, by as much as 55 % at 28 days after the beginning of stimulation. The muscle fibre diameters showed an over-all decrease, hence there was an absolute increase in the number of capillaries per unit area. In muscles stimulated for 4 days the number of capillaries per mm^2 was 690 ± 23 (S.E.) as compared to the value of 530 ± 40 of the contralateral muscle. In muscles stimulated for 28 days the number of capillaries per mm^2 was 1200 ± 60 (as compared to 540 ± 50).

A change of a fast muscle to a slower type by chronic electrical stimulation by a frequency normally occurring in the nerve to a slow muscle thus induces a growth of new capillaries which precedes the changes in the activity of oxidative enzymes.

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Cardiorespiratory response to dangling on a rope in simulated rock-climbing accident

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It has been estimated that, in Britain, 50,000 people actively engage in the sport of rock-climbing. The usual method of attaching the rope which, in free climbing, provides protection only, is by a waist loop. Dangling from a waist loop after a fall can result in death within 20 min, even without obvious trauma. This study was designed to investigate the effects of rope dangling from a waist loop in three fit males, and to compare this with other types of commercially available harness.

Systolic blood pressure, pulse rate, electrocardiogram, tidal volume and respiratory rate were recorded. The maximum period tolerated was $5\frac{1}{2}$ min, and all subjects experienced severe fatigue as the pain produced by the

waist loop was relieved by holding the rope. In two subjects blood pressure and pulse rate rose progressively, and in one subject the blood pressure fell to an unacceptable level. Dangling from a shoulder harness was too painful for any measurements to be made; all subjects developed petechial haemorrhages in the underlying skin. Dangling from a pelvic harness was comfortable and produced insignificant changes.

Changes of functional residual capacity (FRC) induced by dangling from waist loop, pelvic harness, shoulder harness and combinations of pelvic and shoulder harnesses were determined by rebreathing from a bell-spirometer using CO_2 absorption and measuring shifts in end-tidal point. Both waist loop and pelvic harness reduced FRC by approximately 1 l. and the shoulder harness increased FRC by approximately 1 l. Vital capacity was not significantly reduced.

Oxygen consumption and CO_2 elimination were determined by collecting mixed expired gas before, during and after three minutes' waist loop dangling. Although minute volume increased greatly, a marked oxygen debt developed. CO_2 elimination was disproportionately larger than oxygen utilization, and this resulted in a respiratory exchange ratio of greater than 1·2 during dangling.

The results suggest that the excessive isometric (static) work load associated with waist loop dangling causes fatigue and the development of an oxygen debt. When muscle activity is reduced due to fatigue, and muscle blood flow increases, lactic acid and potassium enter the systemic circulation (Donald *et al.* 1967). It is possible that cardiac arrest could occur under these circumstances, due to the combined effects of metabolic acidosis and hyperkalaemia on an already stressed myocardium.

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The influence of oxygen tension, glucose and anaesthesia on the oxygen consumption of the jejunum from normal and hypothyroid rats

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Although hypothyroidism induces numerous changes in the functions of the small intestine (Levin, 1969) the effect on its oxygen consumption has received little study. Bronk & Parsons (1965*a*) using a sensitive polarographic method (Bronk & Parsons 1965*b*) reported briefly that there

was an insignificant increase between hypothyroid and euthyroid intestine. The intestine, however, was removed from decapitated animals, sliced into rings and then incubated in a medium gassed with 20% oxygen, factors that could affect oxygen-dependent differences between euthyroid and hypothyroid intestine. Because of this we have reinvestigated by polarography the effects of hypothyroidism on jejunal oxygen consump-

TABLE 1. Oxygen consumption of sacs of everted jejunum made from intestine removed from control (euthyroid) rats and rats made hypothyroid by giving them 0·5 mM 6-propyl-2-thiouracil in their drinking water for approximately 28 days. The results are given as the mean ± S.E. The number of animals used is shown in brackets. The group designation 'Nembutal' indicates that the intestine was removed from these animals after anaesthesia was induced by I.P. injection of sodium pentobarbitone, while 'Killed' indicates that the intestine was removed after the rat had been killed by a blow on the head. The incubating medium maintained at 37° C was Krebs bicarbonate saline, gassed with either 95% O_2/5% CO_2 or with 20% O_2/5% CO_2/75% N_2. When glucose was added it was present in both mucosal and serosal solutions

Thyroid status	Group	Initial level (%) oxygen in medium	Oxygen consumption μl./mg dry wt. hr	
			Glucose present (28 mM)	Glucose absent
Controls (euthyroid)	Nembutal	95	10·05 ± 0·79 (13)	6·04 ± 0·41 (9)
Hypothyroid	Nembutal	95	6·48 ± 0·30 (12)	6·83 ± 0·22 (13)
Controls (euthyroid)	Killed	95	8·10 ± 0·47 (15)	—
Controls (euthyroid)	Nembutal	20	3·11 ± 0·32 (9)	2·53 ± 0·09 (6)
Hypothyroid	Nembutal	20	3·31 ± 0·23 (6)	2·83 ± 0·19 (6)
Controls (euthyroid)	Killed	20	2·72 ± 0·14 (9)	—
Hypothyroid	Killed	20	3·48 ± 0·22 (6)	—

tion in the presence and absence of glucose at 95% oxygen and 20% oxygen with tissue taken from dead and from anaesthetized rats.

A comparison of jejunal oxygen consumption at 95 and 20% oxygen reveals the rate-limiting effect of the lower tension (Table 1). Removing intestine from dead rather than anaesthetized euthyroid animals reduces further its oxygen consumption at both oxygen tensions. At 95% O_2 and in the presence of glucose, hypothyroidism caused a 36% decrease in oxygen uptake but in the absence of glucose there was no significant difference from controls. At 20% oxygen, hypothyroidism had no effect on the oxygen uptake of intestine from anaesthetized animals but with

that from dead rats the hypothyroid values were greater than the controls. Thus incubating intestine *in vitro* in a low oxygen tension after removal from dead animals creates conditions that reverse the effects of hypothyroidism observed at 95% oxygen with tissue from anaesthetized animals. The previous finding that intestine appears refractory to hypothyroidism is probably due to these methodological differences.

In brief, hypothyroidism does reduce greatly the consumption of oxygen by jejunal tissue in the presence of glucose and good oxygenation.

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Inhibition of glucose-induced electrical activity in pancreatic islet cells by phloridzin, mannoheptulose, and anoxia

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In the β-cells of the pancreatic islet phloridzin blocks the uptake (Hellman, Lernmark, Sehlin & Täljedal, 1972) and mannoheptulose the metabolism (Coore & Randle, 1964) of glucose; both substances inhibit insulin release. In view of the correlation between insulin release and membrane electrical activity of the β-cell (Matthews & Dean, 1970), we have studied the effects of these inhibitors, and anoxia, on the bio-electrical activity evoked in β-cells by D-glucose.

Intracellular recordings of membrane electrical events in mouse pancreatic islet cells were made *in vitro* with glass micro-electrodes (Dean & Matthews, 1970).

Mannoheptulose, 20 mM, rapidly antagonized the electrical activity induced in islet cells by D-glucose, 28 mM, and hyperpolarized them (Fig. 1). On removal of mannoheptulose the cells again partially depolarized and electrical activity reappeared (Fig. 1). In other experiments prior treatment of the islets with mannoheptulose completely prevented the appearance of electrical activity on subsequent exposure to D-glucose.

Electrical activity induced by D-glucose, 28 mM, was partly inhibited by phloridzin 10 mM, or anoxia, if the cells were exposed to D-glucose and inhibitor simultaneously, and completely abolished if exposed to inhibitor for 30–60 min before D-glucose.

D-Galactose, 20 mM, which in many systems shares the transport system of its epimer, D-glucose, is not metabolized by β-cells and does not induce

them to release insulin, depolarize, display electrical activity or block activity induced by D-glucose, 28 mM.

These results point to the conclusion that the induction of electrical activity requires both the uptake and metabolism of the glucose molecule in the β-cell. However, the stereospecific activation of a membrane-located

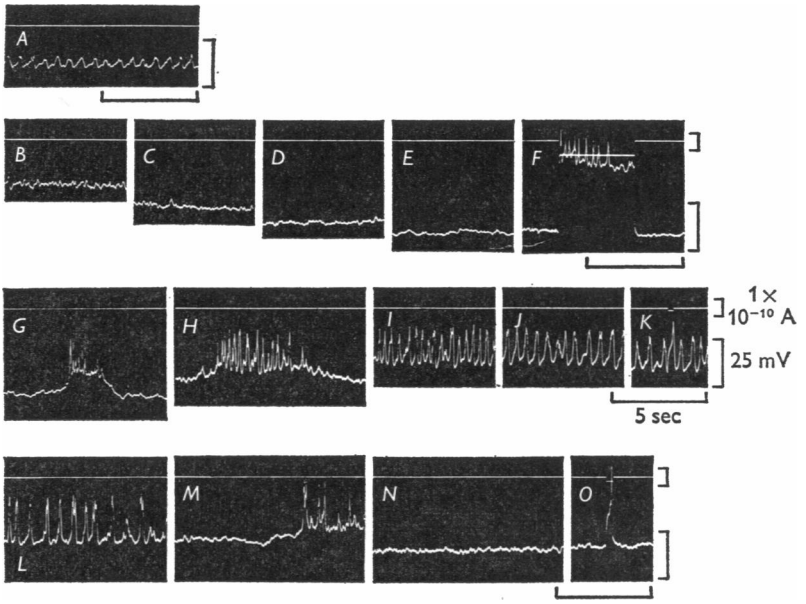

Fig. 1. The effect of mannoheptulose on glucose-induced electrical activity in a pancreatic islet cell. Intracellular records, all from the same cell; the horizontal white line indicates the zero potential. Records *A* to *K* in presence of D-glucose, 28 mM, and *L* to *O* in Krebs solution. Records *B* to *F*, 5, 15, 20, 30 and 35 min after addition of mannoheptulose, 20 mM; *G* to *K*, 18, 35, 45, 60 and 60 min following its removal; *L* to *O*, 20, 30, 60 and 60 min after changing to Krebs solution alone. Depolarizing current injected through the recording micro-electrode in *F*, *K*, and *O*; current duration and magnitude indicated by deflexion on zero potential level.

glucoreceptor, or transport process, for initiation of the ionic flux responsible for electrical activity and insulin release cannot be ruled out, especially since it now appears that mannoheptulose uptake is mediated by a glucose-sensitive transport site in the β-cells (Hellman, Idahl, Lernmark, Sehlin, Simon & Täljedal, 1972).

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Variability of properties of units in cat visual cortex

BY I. M. L. DONALDSON and J. R. G. NASH. *University Laboratory of Physiology, Oxford*

Binocular responses of 35 units in cortical area 18, and 15 units in area 17, of adult cats under light pentobarbitone anaesthesia, immobilized with gallamine, were studied quantitatively using extracellular recording with glass micro-electrodes. The receptive fields were 10° or more from the area centralis. Average-response histograms (ARH) of the responses to a moving bar of light in each of four orientations (see Hubel & Wiesel, 1959, 1965) were constructed 'on-line' and, for some units, repeated sequential examinations of the orientations were made for periods of up to 2 hr.

The ARH usually showed responses to stimulus movement in both forward and reverse directions. These responses were defined using criteria based on the peak bin count and the total number of spikes. Units were 'orientated' if the largest total response (forward + reverse) in one orientation was at least twice that in some other orientation. Frequently, the best and worst responses were not at right angles. 'Directional' units were those whose response in one direction was at least twice that in the other, in at least one orientation. With these criteria 94% of the area 18 and 75% of the area 17 units were 'directional' and 74% and 80% respectively were 'orientated'.

Changes in preferred direction occurred during the period of observation in 18 of 19 units adequately tested in area 18 and in 8 of 9 in area 17. 'Directional' units lost, often temporarily, their directional preference (Fig. 1) or, occasionally, this preference reversed. Similar changes in preferred orientation occurred in 7 of 11 units in area 18 and 6 of 7 units in area 17.

Sometimes these changes followed intentional variation of the anaesthetic level but they were also frequent when the level was kept as constant as possible.

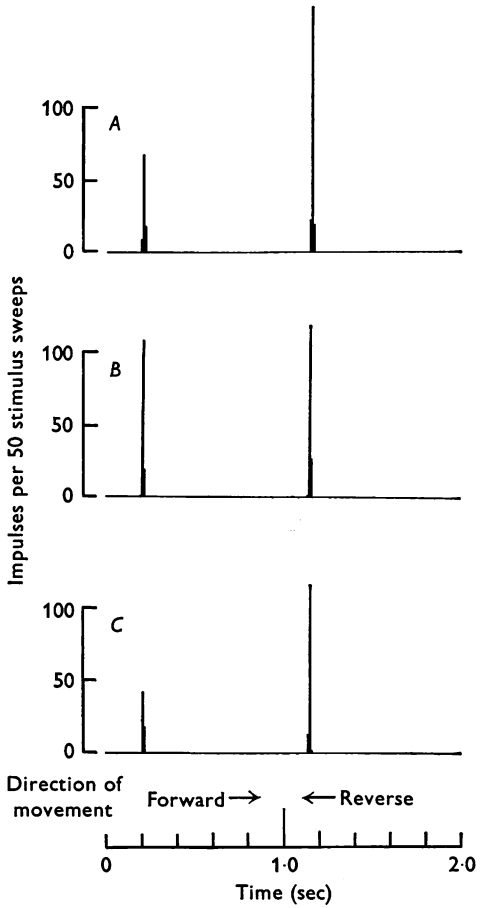

Fig. 1. Average response histograms for a unit in area 18 of cat cortex driven by a bar stimulus in the preferred orientation (10–4 o'clock) which remained constant for the 2 hr for which the unit was studied. Bin width 10 msec; response summed over 50 stimulus sweeps.

A, 23 minutes after isolation of the unit; *B*, 48; and *C*, 75 min after isolation. A supplementary dose of hexobarbitone (20 mg) was given 14 min before *C*. Note that in *A* the unit was directional in 'reverse' direction: in *B* it was non-directional, and in *C* it had regained its original directional preference.

We are grateful to the Medical Research Council for support and for providing a PDR-12 computer.

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Prevention by angiotensin II antiserum of drinking induced by intracranial angiotensin

By Alan N. Epstein, James T. Fitzsimons and Alan Kim Johnson.
Department of Biology and Institute of Neurological Sciences, University of Pennsylvania, and the Physiological Laboratory, Cambridge

Angiotensin is the most potent dipsogen known when applied directly to limbic-hypothalamic structures in the rat and other mammals (Fitzsimons, 1972). Specific antibodies block other physiological actions of angiotensin. In these experiments we examined the effect of an angiotensin II antiserum (Worcel, Meyer, d'Auriac & Milliez, 1969) applied directly to the brain at the same loci from which drinking was induced by angiotensin II (Hypertensin CIBA in 0·9 % NaCl) and by synthetic tetradecapeptide renin substrate. Male rats, that drank after a short latency to 1 or 5 p-mole of angiotensin injected through a permanent intracranial cannula in the anterior forebrain, were given 10–25 μl. of anti-angiotensin II rabbit serum (0·2 ml. neutralizes the pressor response to 180–200 p-mole of angiotensin II in the rat) and were tested for angiotensin-induced drinking from 4 to 6 min afterwards and at irregular intervals thereafter until the dipsogenic response returned. Five rats were studied. One was tested twice. As shown in Fig. 1, the blockade was effective in all six tests.

The animals did not drink to supraoptimal doses of angiotensin (10 or 100 p-mole) for an average of 64·3 min (range 23–206 min) after antibody and when drinking resumed it was attenuated. In control experiments, drinking was unaffected by prior injections of 10 μl. of non-immune rabbit serum. The same antibody also attenuated or completely blocked drinking to 10 p-mole of renin substrate given intracranially ($N = 8$; five drank $2{\cdot}4 \pm 0{\cdot}89$ ml. after antibody, eight drank $5{\cdot}8 \pm 1{\cdot}72$ after non-immune serum; $P < 0{\cdot}02$).

These results show that (1) drinking induced by antigenically active angiotensin II can be prevented by prior injection of anti-angiotensin II, (2) the dipsogenic effect of renin substrate in the brain is mediated, in part at least, by local generation of angiotensin II, and (3) antibodies to angiotensin can be used for the further analysis of the hormone's role in the neural mechanisms of thirst.

Supported by USPHS NDB 03469 and a grant from the Nutrition Foundation to A.N.E., a grant from the MRC to J.T.F., and USPHS GM 218 to the Institute of Neurological Sciences. A. K. Johnson was supported by USPHS MH 49, 314–02.

We are grateful to our colleagues Philippe Meyer and Mary Osborne of the Hypertension Laboratory, Hôpital Broussais, Paris, for generously providing the anti-angiotensin II serum.

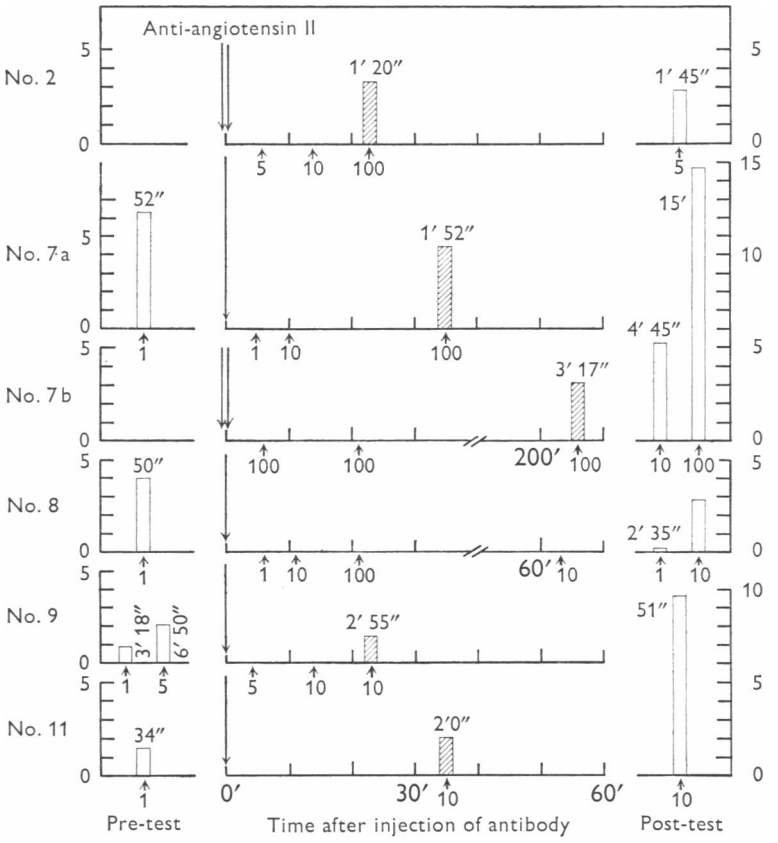

Fig. 1. The failure to drink to intracranial angiotensin II after intracranial injection of anti-angiotensin II serum. The amounts drunk in millilitres are shown before injection of antiserum (pre-test, far left), immediately after antiserum (hatched columns, centre), and at least one full day after antiserum (post-test, far right). The dose of angiotensin II in p-moles is given beneath each arrow and the latency to the onset of drinking is given above or next to the columns. Animal no. 7 was tested twice with an interval of 4 days between the two tests. All injections of antiserum were given unilaterally (single arrows) except for animal no. 2 and the second test in animal no. 7 where the injections were bilateral (double arrow). The volume injected through a single cannula was 10 μl. except in the second test with animal no. 7 where 12·5 μl. was given through each cannula. Angiotensin was always given unilaterally and through the same cannula as the antiserum. In control experiments (not illustrated) angiotensin-induced drinking was unaffected by prior injection of non-immune rabbit serum.

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Motion at the wrist induced by rhythmic forces

By E. Geoffrey Walsh. *Department of Physiology, University of Edinburgh, Edinburgh EH8 9AG*

A printed motor concentric with the wrist has been used to supply sinusoidally changing forces to the hand through a light crank (cf. Walsh, 1968, 1970). Torques of up to 0·8 Nm at rates up to 15 Hz have been available. The frequency has been programmed to rise in an exponentially increasing manner whilst the position of the hand has been monitored by a plastic potentiometer, a velocity signal being obtained by differentiation.

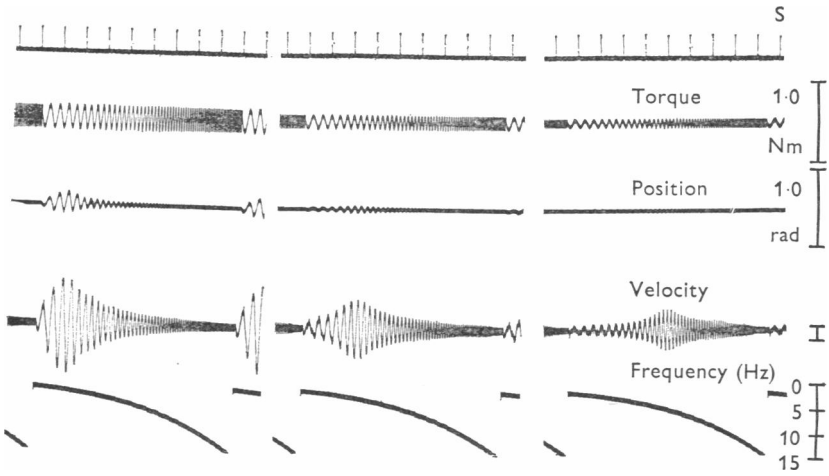

Fig. 1. Frequency sweeps at different levels of force. Torque in middle example is one half that on left-hand side, that on right side is one half of that in middle. Velocity trace shows shift of resonance to higher frequencies as force is reduced. Amplification of velocity trace is automatically increased as force is reduced, the envelope accordingly reflects the admittance of the system (reciprocal of mechanical impedance). Velocity calibration 1 rad s^{-1} at highest force, $\frac{1}{2}$ rad s^{-1} for middle record, $\frac{1}{4}$ rad s^{-1} for right-hand traces. Subject P.C. male aet. 20.

For motion in the horizontal plane the movements are greatest at the lower frequencies (e.g. 2 Hz) if the subject is relaxed and the force relatively large (e.g. 0·5 Nm). With voluntary stiffening the resonant frequency rises according to the degree of the effort and may reach 9 Hz or more: a value of 13 Hz was obtained in a professional pianist. If the wrist is stiffened whilst being subjected to low frequency torques the motion is almost abolished. If, however, the stiffening occurs when higher rates (e.g. 9 Hz) are being used, the oscillations may become greater. Changes in the frequency characteristics are seen also on changing the level of applied force.

With decreasing torques the resonant frequency is elevated (Fig. 1). The system behaves in a non-linear manner; at low frequencies it is substantially stiffer for small forces than for those which are larger.

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Vibration sensitivity of muscle spindle endings in a rat hind-limb muscle and its relationship to conduction velocity

BY G. C. LESLIE. *Department of Physiology, The University, Dundee DD1 4HN*

Low amplitude vibration, applied at the tendon, at frequencies above 100 Hz has been considered, as a stimulus, to stimulate selectively the primary endings of muscle spindles; the reports of Bianconi & van der Meulen (1963) and Brown, Engberg & Matthews (1967) stressed the insensitivity of secondary endings in cat hind-limb muscles to this form of stimulation. However, Andrew, Leslie & Thompson (1973) found that vibration sensitivity of muscle spindle endings in rat caudal segmental muscles was correlated with conduction velocity of the afferent fibre and that a continuous range of vibration sensitivity between 40 Hz and 400 Hz was demonstrable.

The present experiments were made on Sprague Dawley rats (200–300 g) to see if such a correlation exists in M. peroneus longus. Under urethane anaesthesia (1·5 mg/g) functionally single fibre dorsal root filaments, connected to muscle spindles (de-efferented by ventral root section), were prepared and their conduction velocity measured. Vibration was applied longitudinally to the cut distal tendon and the ending's maximum frequency of following was ascertained.

Observations from eight experiments, with a minimum exposure of tendon and muscle, are shown in Fig. 1*A*. One may state, as a measure of the obvious relationship, the correlation coefficient $r = 0{\cdot}88$ (significantly different from $r = 0$ at $P = 0{\cdot}01$). Fig. 1*B* shows the results from four experiments when tendon and muscle were dissected clear from surrounding tissues. Again there is a relationship but one with noticeably increased sensitivity of the primary endings. Apart from this effect of freeing the muscle, temperature too has been shown to influence vibration sensitivity; cooling the muscle decreases sensitivity.

In concluding that a relationship exists and that certain factors affect

it one may reflect on the applicability of these results rather than those from cat when using vibratory stimuli in human muscle studies.

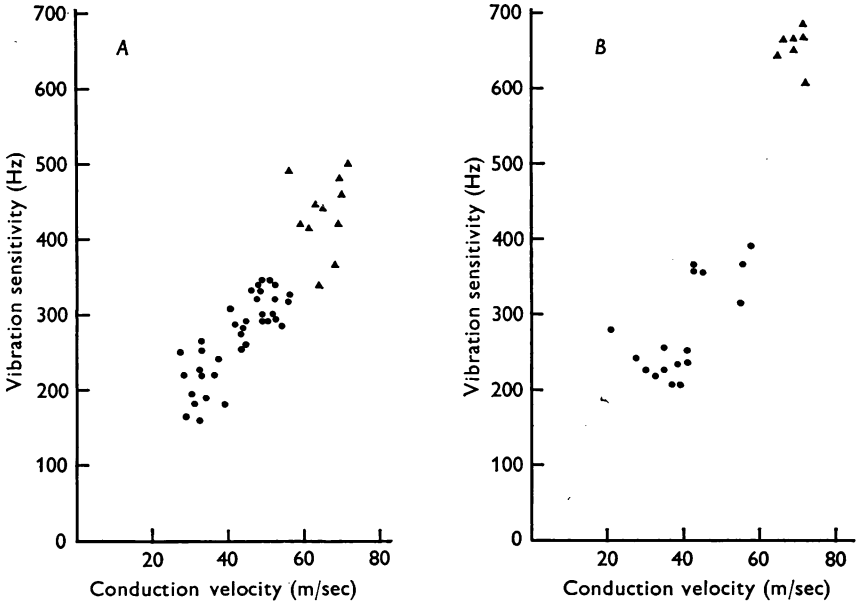

Fig. 1*A*. Shows the vibration sensitivity (Hz) of an ending plotted against the conduction velocity (m/sec) of its fibre for eleven primary endings (▲) and thirty-seven secondary endings (●) in eight experiments when there was minimum exposure of tendon and muscle; vibration sensitivity is taken as the maximum frequency of vibration to which the ending can be driven to respond, without failure, with one action potential per oscillation of the vibrator. The coefficient of correlation (r) for these data is 0·88 (significantly different from $r = 0$ at $P = 0{\cdot}01$).

B. Shows the same for seven primary and seventeen secondary endings in four experiments when the tendon and muscle were dissected free from surrounding tissues. $r = 0{\cdot}91$ (significant at $P = 0{\cdot}01$).

The maximum amplitude of movement available from the vibrator used was as follows: 200 Hz, 250 μm; 300 Hz, 80 μm; 400 Hz, 40 μm; 500 Hz, 20 μm; 600 Hz, 11 μm; 700 Hz, 9 μm.

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Acetylcholine and the pulmonary circulation

BY GWENDA R. BARER and BERNICE THOMPSON. *University Department of Medicine, Royal Hospital, Sheffield 1*

Acetylcholine may increase or decrease pulmonary vascular resistance. We need to know if these actions: (1) occur in the same or different vessels, (2) depend on dose, vascular tone or bronchomotor effects.

In isolated strips of pulmonary artery only contraction occurred (rats, cats, rabbits).

In open-chest cats with a lobe of lung perfused at constant blood flow, acetylcholine infusions caused vasodilatation and vasoconstriction, both abolished by atropine. Dilatation was commoner with low doses and high

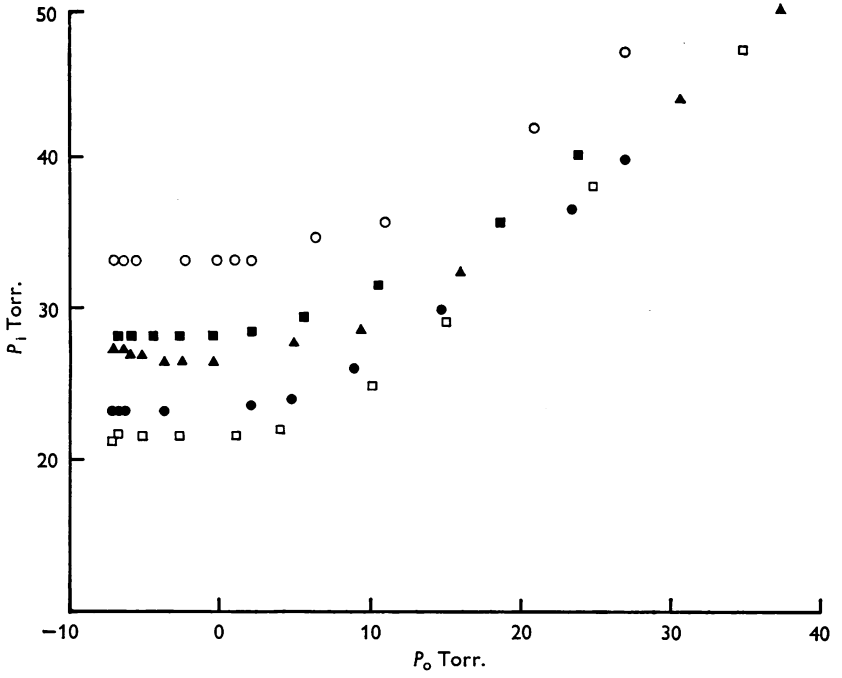

Fig. 1. Vasodilator and vasoconstrictor effect of acetylcholine (ACh). Cat, 2·3 kg. Isolated lung perfused at constant blood flow in a forward direction (247 ml./min). Alveolar pressure constant (P_A, 6·5 Torr). Inflow pressure (P_i) measured on the arterial side, is plotted against outflow pressure (P_o) measured on the venous side. P_o raised in steps. P_i begins to rise when pressure in post-alveolar vessels exceeds P_A. This inflexion point is less than P_o because of resistance in veins. 1, ●; control curve. 2, □; infusion ACh to pulmonary artery (39 μg/min). 3, ■; increased infusion rate (3·9 mg/min). 4, ○; later control curve, P_i has risen. 5, ▲; infusion ACh (390 μg/min). Curves 2 and 5 show dilatation and 3 constriction of pre-alveolar pulmonary vessels.

pre-existing tone (caused by hypoxia or hypercapnia). Constriction occurred with large doses and low tone and was accompanied by an increase in lung blood volume. Both actions occurred in collapsed airless lobes and are thus independent of changes in airway pressure.

To separate actions on pre- and post-alveolar vessels we perfused isolated cat lungs at constant blood flow in forward and backward directions under waterfall conditions. Alveolar pressure (P_A) was constant. Outflow pressure (P_O), irrespective of direction, was initially less than P_A and was slowly raised. Inflow pressure (P_I) was unchanged until pressure in post-alveolar vessels exceeded P_A, giving a plateau (Fig. 1). Changes in plateau level indicate changes in resistance in pre-alveolar vessels irrespective of flow direction. Acetylcholine both raised and lowered this plateau during forward and backward perfusions (Fig. 1). It can therefore constrict and dilate both arteries and veins.

Early and late facilitation of transmission through a sympathetic ganglion and the influence of preganglionic B and C fibres

By D. I. Wallis and B. Woodward. *Department of Physiology, University College, Cardiff*

Modulation of transmission through the superior cervical ganglion is believed to reflect complex underlying processes, while the role of preganglionic fibres in modulating transmission is still unclear. We have investigated this further, recording preganglionic and post-ganglionic compound action potentials from rabbit excised ganglia. The post-ganglionic compound action potential has two major components. The first, Sa, is evoked by stimulating preganglionic B fibres, while the second, Sb, is evoked by stimulating preganglionic C fibres.

When two stimuli were applied to the preganglionic trunk at differing intervals, the Sa component of the second response was facilitated or inhibited depending on the stimulus interval and the stimulus intensity. Thus if two stimuli submaximal for the Sa component were used, a period of early facilitation (40–75 msec) was followed by a prolonged tail of facilitation persisting for many seconds. If the stimulus intensity was increased the magnitude of the early facilitation decreased and a phase of inhibition develped which was maximal at a stimulus interval of 200 msec. This inhibitory phase separated the facilitation into early and late components. If the effect of stimulus intensity is expressed in terms of the type of preganglionic fibres excited, the stimulation of C fibres reduced early facilitation and increased the magnitude of inhibition.

By using a test stimulus submaximal for the B fibres and varying the conditioning stimulus (CS), early facilitation was seen to be maximal

when CS excited about 50% of the B fibres. When all of the B fibres were activated by CS, facilitation was reduced; C fibre activation first reduced then abolished early facilitation.

By contrast, late facilitation of Sa was unaffected by preganglionic C fibres. Facilitation of a similar magnitude and time course was observed by Larrabee & Bronk (1947) in the cat stellate ganglion; they concluded that a presynaptic mechanism was involved.

Submaximal Sa responses were more strongly inhibited than maximal Sa responses 200 ms after a CS. Preganglionic C fibres seem able to modulate the degree of inhibition. When this inhibition was maximal, facilitations of submaximal Sb responses was observed.

These results are consistent with the concept of a subliminal fringe of Sa cells, in which the e.p.s.p. is insufficient to cause spike discharge at low stimulus intensities. However, when CS excites the rest of the B and the C fibres, these cells are progressively removed from the subliminal fringe. This suggests a high degree of convergence, as well as divergence, of preganglionic fibres on to ganglion cells in the superior cervical ganglion. Further, it is clear that C fibres can modulate transmission and that the Sb response is in part due to the Sa cells firing to their C fibre input. A neuronal model in which the preganglionic input is able to vary the size of the subliminal fringe is consistent with these findings.

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Receptor potential and impulse activity in isolated mammalian spindles

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Primary and secondary afferent fibres have been identified by size difference in isolated spindles from cat tail muscles. The spindle, intact from tendon to tendon, was tied to nylon rods connected to a puller which provided symmetrical stretch of controlled velocity and amplitude. The nerve, with one cleaned axon entering the sensory region, was raised into paraffin oil. D.c. recording was carried out with one lead in the Locke's solution containing the spindle and the other on the nerve in oil.

Stretch of ramp and hold configuration produced a graded receptor potential and impulse activity. The discharge pattern of primary endings was similar to that seen in Ia fibres from mammalian spindles *in situ*

(Harvey & Matthews, 1961; Bessou & Laporte, 1962). Primary endings showed a marked dynamic response during the ramp stretch as well as a static discharge during maintained stretch. Secondary endings usually showed less dynamic response although they varied considerably in this respect.

Receptor potentials, following block of impulse activity by tetrodotoxin, were similar in primary and secondary endings, and showed a dynamic component during the ramp portion of stretch and a static component maintained during the plateau. The dynamic component varied in configuration and amplitude with the velocity of stretch. With stretches of about 0·5 mm amplitude and a rise time of 10 msec the peak dynamic response was approximately double the static level. The initial rising phase of the receptor potential decreased markedly when a second identical stretch was delivered after a short interval. This component appears responsible for the 'initial burst' of the discharge pattern to stretch.

Firing level for impulse initiation was fairly constant during maintained stretch but during rapid stretch it was lower, particularly in primary endings. This suggests that impulse initiation in these endings may be sensitive to the rate of change of the receptor potential.

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Birth-weight distribution patterns in relation to estimated duration of pregnancy in normal infants, spina bifida and Down's syndrome

BY J. K. BURNS. *Department of Physiology, University College, Galway*

It is generally accepted that a trigger mechanism is responsible for initiation of human labour. Such a factor is considered to precipitate a high incidence of births at a fixed interval following the beginning of the last menstrual cycle. The duration of human pregnancy is usually stated to be about 40 weeks from the beginning of the last cycle and the timing of parturition is thought to be related temporally to the beginning or the middle of this cycle.

Birth weights of over 1000 infants were plotted individually against durations of pregnancy as estimated from the last menstrual period. The distribution was examined in relation to vertical (birth weight) and horizontal (duration of pregnancy) density patterns. Mean birth weights showed an approximately linear relation (correlation coefficient 0·91) to duration of pregnancy during the last 2 months of the third trimester.

Mean durations of pregnancy were linear in relation to different birth weights until about the end of the 40th week. The distribution of birth weights at term is consistent with a carry forward of a temporal variation of about 3 weeks. It is proposed that variation in fertilization times explains the horizontal scatter of birth weights at term. There is a marked decrease in incidence of births beyond 42 weeks pregnancy duration or with weights above 140 oz. In normal infants, in Down's syndrome and in spina bifida, timing of parturition in the vast majority of cases is from 39 to 42 weeks. Data for spina bifida and Down's syndrome are compatible with fertilization at various times during the menstrual cycle. There is not a high density of births of these infants during the 42nd week which should result from late fertilization.

I wish to thank the authorities and staff of the Western Regional Hospital, Galway for permission to abstract these data from their records.

Interaction of Arvin with erythrocyte flexibility

By P. Myers, M. W. Rampling and J. A. Sirs. *Department of Biophysics, St Marys' Hospital Medical School, London W2 1PG*

Arvin, a purified fraction of the venom of the Malayan Pit Viper, is used clinically to reduce the plasma fibrinogen concentration in patients. Its action is specific, in that it activates the formation of fibrin from fibrinogen, without involving any other component of the clotting mechanism. According to Rampling & Sirs (1972), a fall in fibrinogen concentration should be accompanied by the erythrocytes becoming less flexible. This is also suggested by the extremely low (approximately zero) E.S.R. of patients treated with Arvin, but E.S.R. measurements involve other factors as well as erythrocyte flexibility. The present experiments were undertaken to confirm that it is specifically fibrinogen, and not other components in the clotting chain, which is linked to erythrocyte flexibility and that the flexibility is lowered by treatment with Arvin.

A 20 ml. sample of blood was collected by venepuncture. This was divided into three main portions as follows:

(1) A 6 ml. sample which was defibrinated with glass beads. This was then further subdivided into three 2 ml. portions: (A) to which nothing further was done, (B) to which was added 0·1 ml. of Arvin in saline, to give a final concentration of 0·5 u. of Arvin per ml. of blood, (C) to which was added 0·1 ml. of heparin in saline, to give 6 i.u./ml. of blood.

(2) A 4 ml. sample to which was added 0·2 ml. of Arvin in saline and the clot removed. This was divided into two 2 ml. samples: (D) to which nothing further was done, (E) to which was added heparin at a concentration of 6 i.u./ml. of blood.

(3) A 10 ml. sample to which was added 0·5 ml. of heparin in saline. Two 2 ml. samples were taken from this: (F) no further treatment, (G) 0·1 ml. of Arvin in saline added to give 0·5 u./ml. of blood, and the clot removed. The remaining 6 ml. sample was diluted in its own plasma to give a series of controls to check the packing rate at different haematocrits.

The rate of packing of each sample was then measured, under a centrifugal force of 200 *g*, using the Automatic Recording Centrifuge method of Sirs (1970). Sample F, the heparinized normal control, packed at a rate of 4·2 %/min. All the other samples packed at an average rate of 2·1 ± 0·1 %/min. This relative difference was the same after storage for up to 24 hr after completing the preparation. This series of controls and treatments indicates that it is the lowering of the fibrinogen content of blood, either by direct defibrination or treatment with Arvin, that produces the slower rate of packing which is indicative of an increased rigidity of the erythrocytes.

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The absence of post-hyperventilation apnoea in the wakeful state

BY KAREN M. ASHBRIDGE,* SHEILA JENNETT and J. B. NORTH.† *Departments of Physiology and of Neurosurgery, University of Glasgow*

Seven out of ten current physiology text-books reproduce the classical account of apnoea following hyperventilation in normal subjects as described by Haldane & Priestley in 1905. Yet Boothby (1912) and Mills (1946) elicited this phenomenon in very few and Fink (1961) in none of the normal subjects studied. Fink suggested that in the wakeful state a cerebral drive over-rides the chemical control of respiratory rhythm. In 1962 Plum, Brown & Snoep studied the effect of brief hyperventilation (five deep breaths) which lowered the P_ACO_2 by at least 6 torr, in large groups of both normal subjects and patients with brain damage. They concluded that apnoea of longer than 12 sec was sufficiently abnormal to be virtually diagnostic of bilateral forebrain damage. When apnoea does follow hyperventilation, it is usually assumed that its incidence and duration are related to the magnitude of the reduction in P_ACO_2.

We have studied fifty normal subjects, aged 10–73, only four of whom had read text-books of physiology. Each was told only that routine recordings of breathing were to be made. Three times during continuous

* Student vacation scholarship.
† Commonwealth Medical Scholar.

recording of tidal volume by pneumotachograph and of CO_2 concentration at the mouth, they were instructed to take five deep breaths.

The reduction in P_{A,CO_2} over 150 tests averaged $7{\cdot}3 \pm 0{\cdot}3$ (S.E.M.) torr, from a base line of $36{\cdot}1 \pm 2{\cdot}4$ torr. The duration of the interval from the end of the last deep inspiration to the beginning of the subsequent inspiration averaged $3{\cdot}9 \pm 0{\cdot}1$ sec, which was not significantly different from the longest corresponding interval occurring spontaneously before the test.

In order to exclude the possible effects of the usual respiratory apparatus, ten of the subjects had a preliminary hyperventilation test with only chest electrodes applied for impedance pneumography. To achieve a greater reduction in P_{CO_2} than in the standard test, twelve subjects were asked also to overbreathe maximally for 30 sec and twelve for 2 min. No individual showed a longer interval than he did after the routine five deep-breath test.

Fifty patients with brain damage were studied by identical methods to the normal series. In one or more of the three tests, over half of them showed apnoea more than 5 sec longer than their greatest spontaneous interval. The incidence of apnoea was related to drowsiness or disorientation. However, it was notable that neither the value to which end-tidal P_{CO_2} was reduced, nor the amount by which it was reduced, bore any relation to the duration of apnoea.

These results confirm that there is not normally a cessation of breathing after voluntary hyperventilation, and indicate that when this does occur abnormally, it is not related simply to a subthreshold P_{CO} .

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The effect of sodium salicylate on bile secretion in the dog

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Current views on bile formation indicate a primary secretion of bile salt by the liver cell into the bile canaliculus and the subsequent passage of water and electrolytes (Sperber, 1959). In some species secretin can stimulate the bile ductules to produce a bicarbonate rich solution (Preisig,

Cooper & Wheeler, 1962). In rodents there is a third mechanism involved in formation of part of the bile which is independent of bile salt excretion. It is postulated that this fraction of bile depends on active transport of sodium by the liver cell into the canaliculus (Erlinger, Dhumeaux & Benhamou, 1969; Erlinger, Dhumeaux, Berthelot & Dumont, 1970; Boyer, 1971; Forker 1967). The formation of this fraction can be inhibited by substances such as ouabain or ethacrynic acid which are known to interfere with sodium transport or the Na-K activated ATP-ase.

Sodium salicylate, administered intravenously in doses up to 100 mg per kg body weight, to the anaesthetized dog with cannulated bile duct, produces a pronounced and prolonged choleresis. This choleresis, of the order of 4 to 5 times control secretion, is not accompanied by concomitant increase in bile salt output nor does it result in bicarbonate rich secretion. The choleresis is not influenced by bilateral cervical vagotomy nor by post-ganglionic cholinergic blockade with atropine. Salicylate choleresis is not inhibited by portal intravenous injection of ouabain in doses up to 1 mg, nor can the amount or concentration of salicylate in bile account for the extra water. The biliary concentration of cations and the osmotic pressure of bile, as determined by flame photometry and freezing-point depression, both decline during the salicylate choleresis, but the electrolytes proportionately more than the osmotic pressure.

It has been suggested that the addition of water at the canaliculus would result in the potentiation of Bromsulphalein maximal transport into bile (O'Maille, Richards & Short 1966). Experiments with salicylate have failed to give clear-cut evidence of potentiation. However there is an addition of water to bile which does not appear to be related directly to bile salt or sodium excretion.

It is conceivable that salicylate acts as a choleretic by preventing the formation of micelles in bile and allowing the ions present to become more osmotically active.

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Development in the kitten of control of contact placing by sensorimotor cortex

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In most one- to two-day-old kittens, following contact of the dorsum of the paw with the side of a solid, the forelimb places, although inconsistently, on top of the solid. Contact placing (CP) to stimulation of the ulnar aspect of the paw usually develops later, by the sixth day. The latencies of the initial lifting-withdrawal phase and of biceps activation are several fold those in the adult (Amassian, Weiner & Rosenblum, 1972). The onsets of the directed landing phase and of the activation of triceps and the long

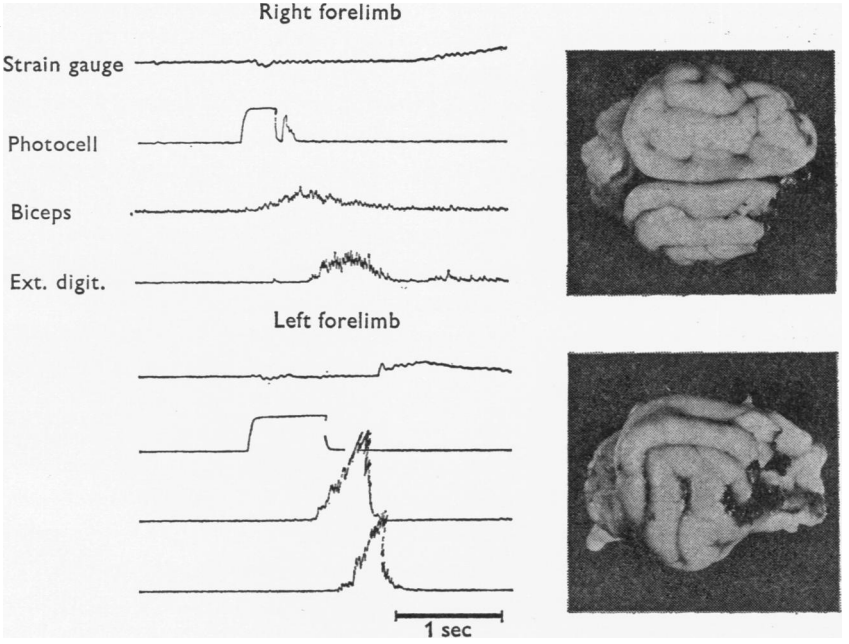

Fig. 1. Contact placing of the forelimbs after unilateral removal of sensorimotor cortex. Contact of the radial aspect of a forepaw with the side of the platform interrupts a light beam incident on a photocell (upward deflexion). Lifting-withdrawal of the paw restores the output of the photocell. A strain gauge signals the subsequent landing of the paw on top of the platform (upward deflexion). The electromyograms were recorded by bipolar pins which were inserted percutaneously into the biceps and the long extensors of the digits; they were integrated with a decay time constant of 10 msec. The right sensorimotor cortex was removed 27 days post-natally (Photographs at right). Both recordings were obtained the following day. Contact placing was present to stimulation of the radial and dorsal aspects of the left forepaw, but was less probable than on the intact side.

extensors of the digits are delayed, resulting in a large vertical displacement of the paw.

When the sensorimotor cortex, including the adjoining medial surface, and most or all of SII are removed unilaterally prior to the fourth postnatal week, CP fully returns within several hours or even within minutes, but subsequently is lost by one to two months of age. Such findings resemble the temporary retention of CP following hemispherectomy in infant rats (Hicks & D'Amato, 1970). The retention of CP in kittens does not depend on ipsilateral sensorimotor cortex because it occurs after extensive bilateral lesions including such cortex. Unilateral removal of sensorimotor cortex in a kitten 5 weeks or more in age virtually abolishes CP of the contralateral forelimb and results in an extended posture of this limb. A lesion made during the intermediate period (Fig. 1) abolishes CP only to stimulation of the ulnar aspect. Thus, CP initially can be managed by a subcortical circuit; this circuit subsequently becomes ineffective and the sensorimotor cortex, as in the adult (Bard, 1938), is normally an essential part of the control circuit. Whether the circuit that includes sensorimotor cortex initially is non-functional or is functional but redundant remains to be determined.

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Effects of prostaglandins E_1, E_2, A_1 and A_2 on resistance and capacitance vessels in the hind limb of the dog

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Although the potent vasodilator properties of the prostaglandins (PG) are well known, comparative studies of their relative potencies are conflicting. Likewise PGE_1 is known to relax capacitance vessels (Greenberg & Sparks, 1971; Daugherty, 1971), but little is known concerning this effect for the other compounds and, furthermore, the relationship between the effects on resistance and capacitance vessels is unknown.

The responses to four prostaglandins have therefore been studied after intra-arterial injection into the hind limb of the dog, perfused at constant flow. Changes in perfusion pressure indicated the degree and duration of resistance effects and changes in limb volume, measured by a mercury-in-rubber plethysmograph, the capacitance vessel changes.

The relationship of log-dose and percentage change in hind-limb resistance for the four compounds was linear and parallel. The regression

lines of resistance response to dose gave a potency ratio for PGE_2 of 0·42 relative to PGE_1 with 95% confidence limits of 0·26–0·68. For PGA_2 the relative potency was 0·06 with 95% confidence limits of 0·037–0·098. In a separate series of experiments the relative potency of PGA_2 to PGA_1 was 0·36 with 95% confidence limits of 0·17–0·78.

The duration of action to a single dose of PGE_1 and PGE_2 did not differ greatly from PGA_1 or PGA_2 and although the tendency was for PGE_1 or PGE_2 to show the greater duration for a given resistance response this had no obvious physiological significance. All the compounds dilated capacitance vessels with relative potencies of the same order as for the resistance vessels. However, for a given degree of arterial dilatation the capacitance effect of the PGA series was significantly greater than that of PGE_1 or PGE_2 ($P < 0·001$).

In order to estimate the possible haemodynamic consequence of these findings, the capacitance responses of the prostaglandins were compared with nitroglycerine. For a given degree of arterial dilatation, the prostaglandins produced a greater effect on capacitance vessels than did nitroglycerine ($P < 0·01$).

These findings demonstrate the importance of the capacitance responses of these prostaglandin compounds particularly of the A series. These effects must be considered in assessing any rôle they may have in the control of the circulation.

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The maturation of toad visual units

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In adult *Xenopus laevis* the neuronal responses of single ganglion cells recorded from their terminal arborizations in the optic tecta can be classified into three distinct unit types; 'sustained', 'event' and 'dimming' units. The development of these specific unit responses has been studied in *Xenopus* tadpoles of various stages. Animals were prepared as described previously (Gaze, Chung & Keating, 1972) and responses in the optic tecta were recorded using metal-filled glass micro-electrodes.

The first visual responses could be recorded at the end of embryonic development (stage 44). Responses consisted of a burst of spikes at the onset of a very bright light (2×10^4 lm/m^2). Sensitivity to visual stimuli increased rapidly with maturation and by stage 47 the majority of units responded to 'off' as well as 'on' of a 2° spot of light. Units usually had

an oval-shaped receptive field with 'on–off' centre surrounded by an 'off' region. The size of the receptive field decreased progressively from 30° to 40° of the visual angle at stage 47 to 10° at stage 56, at which time the units were very similar to adult 'event' units.

Near the end of premetamorphosis (stage 49–50) new kinds of units appeared, characterized by their acute sensitivity to small changes in total light flux and an extremely high resting discharge. These units gave bursts of spikes to stepwise brightening as well as dimming of light and the resting discharge was unaffected by the steady ambient illumination. As the tadpoles approached metamorphic climax these units became more like those in adult animals in that they responded to dimming only and the resting discharge was suppressed by the background illumination.

Around stage 60 we were able to record units that gave a sustained discharge to an object moved into the field. These only responded to relatively large stimuli (20°–30°), and responses fatigued quickly. The size of the optimum stimulus decreased gradually throughout metamorphosis.

During metamorphosis the segregation of unit types to different layers of the tectum was observed. 'Sustained' units were found in the superficial layer of the tectum while 'event' and 'dimming' fibres extended their terminals beneath this layer. Although at the close of metamorphosis the acute response characteristics of tadpole 'event' and 'dimming' units resembled those in adult animals, they differed in that the receptive field organization was simpler and there was no distinct endogenous chatter associated with each type of unit. The 'sustained' units were the last to mature, acquiring adult finesse one month or so after metamorphosis. Two months after metamorphosis the evolution of all three types from the simpler units was complete and they became indistinguishable from adult units in all respects.

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Latency measurements compatible with a cortical pathway for the stretch reflex in man

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If, while it is actively flexing, the top joint of the thumb is extended by an electric motor at rates of up to, say, 360°/sec, a brisk stretch reflex is elicited in the long flexor muscle with a latency, in C.D.M., to the start of the response in the electromyogram of some 45 msec (Marsden, Merton & Morton, 1971, 1972; cf, for biceps, Hammond, 1960). Measurements of stretch reflex latency have now been made in a similar manner on the

masseter muscle of the jaw and on the long flexor of the big toe. In C.D.M., the latency was 13 msec for the jaw and 75 msec for the toe, approximately. These latencies are roughly twice as long as the conduction time from the muscle to the spinal cord (or brain stem) and back, using as an estimate of this time the tendon jerk latency in the same or a nearby muscle. In C.D.M., recording through the same electrodes as were used for the stretch reflex, the latencies were: for the jaw jerk 8 msec, for the finger jerk 23 msec and for the ankle jerk 37 msec. Jerks were elicited with a tendon hammer incorporating an electrical contact. The stretch reflex times are thus in excess of the jerk times by some 5 msec for the jaw, 22 msec for the thumb and 38 msec for the toe. Clearly, this excess latency of the stretch reflex might reflect the distance from the brain of the motoneurones in question. Similar results were obtained on another subject.

The stretch reflex is usually thought of as spinal; but, from these measurements, there is sufficient time for it to go via the cerebral cortex, as conjectured by Phillips (1969). He asks whether, in the course of evolution, this transcortical servo-loop has come to dominate the segmental loop. Our results, so far as they go, suggest that in the muscles we have used it may well have done so.

Evarts (1973) has been led to a similar point of view. By recording in the motor cortex of unanaesthetized monkeys, he has obtained direct evidence for an appropriately short latency effect of hand displacement on pyramidal motoneurones.

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The use of repetitively applied sharply rising mechanical pulses to excite spindle primary endings and thus minimize the 'double driving' occasionally produced by high frequency sinusoidal stretching.

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High frequency vibration is a potent stimulus for the primary endings of the muscle spindles and is widely used to investigate the central effects of their excitation. Brown, Engberg & Matthews (1967) studied the effect of longitudinally applied sinusoidal oscillation at 100–500 Hz on de-efferented soleus endings and found that peak-to-peak amplitudes of 25 μm regularly excited a spike on every cycle ('driving'); but, when tested, increasing the amplitude to 200 μm did not excite two or more spikes per cycle ('double driving', etc.), and we confirm this. However, we find that in the decerebrate cat with intact ventral roots, cut dorsal roots, and appreciable spontaneous fusimotor activity sinusoidal stretching at 100–175 Hz occasionally elicits double driving for amplitudes below 200 μm. Double driving is usually only prominent when the vibration is applied at the same time as the muscle is being dynamically stretched, and has only been seen in preparations with appreciable dynamic fusimotor activity, as judged by measurement of the dynamic index.

When double driving occurs the spikes are discharged in pairs synchronized with the oscillations. The two spikes of a pair are appropriately separated for both to be presumed to be excited by the rising phase of the same cycle of stretch. When double driving is poorly developed some cycles excite one spike and some two. Double driving is most marked for lower frequencies of vibration and has not been observed for frequencies above 200 Hz. At lower frequencies the interval between the two spikes of a pair has never been less than 1·7 msec. It thus seemed likely that double driving could be minimized, yet secure single driving still achieved, by reducing the duration of the rising phase of the stretch while prolonging its falling phase to produce an approximately sawtooth wave form. Such asymmetrical wave forms with a rising phase of 1·5 or 2·0 msec were generated at 140/sec by supplying Digitimer pulses to an electromechanical stretcher. Double driving was then often eliminated and was always markedly reduced in comparison with that produced by symmetrical vibrations of the same frequency and amplitude. Moreover, on using 1·5 msec pulses we have only observed double driving during the dynamic phase of a ramp stretch, and not the static phase, and this only occurred when the stretch alone excited the ending to discharge at above the repetitive frequency of the mechanical

* M.R.C. Scholar.

pulses; sometimes the rapid pulses actually reduced the frequency of firing by only producing single driving. Thus, by comparison of their action with that of sinusoidal stretching, sharply rising pulses may be used to test whether sinusoidal vibrations are producing a significant central action by virtue of any double driving that they may be eliciting.

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Calcium release and vasopressin action in toad bladder

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The mechanism of the final effector process by which vasopressin (ADH) increases the permeability of amphibian bladders to water and to sodium ions is unknown. However, some characteristics of the response are known with certainty. The hormone acts only when placed in the serosal solution, while the permeability change to water (Hays & Leaf, 1962) and to sodium (Civan & Frazier, 1968) occurs at the mucosal surface. The way in which the stimulus is translocated across the cell is unknown, but may involve the formation of cyclic adenosine monophosphate (cAMP) (Handler & Orloff, 1971).

In previous studies it has been suggested that permeability changes may result from the displacement of calcium from crucial binding sites in the mucosal surface (Cuthbert & Wong, 1971; Schwartz & Walter, 1968). In the experiments reported here we have measured the washout of ^{45}Ca from the mucosal and serosal surfaces of bladders previously loaded with isotope. After initial washing with Ringer solution to remove the loading solution the efflux of ^{45}Ca from both surfaces had a double exponential form. Addition of ADH (100 mu./ml.) to the serosal solution caused an increase in calcium efflux from the mucosal surface only. The hormone increased the amount of calcium released by $82{\cdot}4 \pm 27\%$ (seven measurements). Theophylline, 10 mM, which also increases water and sodium permeability, caused an increase in calcium efflux from both the mucosal and serosal surfaces by $76{\cdot}8 \pm 24\%$ (six measurements) and by $71{\cdot}7 \pm 20\%$ (four measurements) respectively.

By contrast, cAMP (5 mM) inhibited calcium release from both surfaces. When applied to the serosal surface the calcium efflux from that side was reduced by $52{\cdot}5 \pm 4{\cdot}7\%$ (six measurements). The nucleotide reduced mucosal efflux by 43% (two measurements) when applied to that side.

It is not known whether the ^{45}Ca entering the mucosal and serosal bathing solutions during washout comes from within the cells, is displaced from

binding sites in the membrane or is derived from both these sources. If bladders are bathed on the serosal side only with Ringer containing ^{45}Ca the serosal to mucosal flux is unaltered by ADH, suggesting calcium permeability is unaltered. However, EGTA (5 mM) added to the Ringer bathing the mucosal surface of ^{45}Ca loaded bladders causes a large efflux, presumably by release from the cell membranes, but there still remains a low level efflux after the chelator is removed. This perhaps indicates that some of the ^{45}Ca is derived from intracellular sources.

The release of calcium into the muocsal bathing solution by ADH may be relevant to the final effector process. Treatment of the serosal surface of bladders with phospholipase C not only blocks the calcium releasing action of ADH but also the hydro-osmotic and natriferic effects of the hormone (Cuthbert & Painter, 1971).

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X-ray diffraction patterns from mammalian heart muscle

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We have obtained meridional and equatorial X-ray diffraction patterns from papillary and trabecular muscles of rabbits, cats and guinea-pigs. The muscles used were either living (freshly dissected in mammalian Ringer solution) or glycerol-extracted (extracted for several weeks and then placed in a solution of composition: 5 mM-$MgCl_2$, 100 mM-KCl, 10 mM imidazole, pH 7·0), and were studied at room temperature.

Both living and glycerol-extracted muscles show a meridional pattern similar to that obtained from vertebrate skeletal muscle in rigor (see Huxley & Brown, 1967). We observed the usual actin pattern at moderate angles (beyond 27 Å), and clear actin layer-line reflexions at 45, 51, 59 and 70 Å. A meridional reflexion at 143 Å was present as well as a strong near-meridional layer line at 376 Å and a much weaker one at

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about 188 Å. Weak myosin layer lines were seen in some preparations. As in the case of skeletal muscle (Huxley & Brown, 1967), these observations suggest an attachment of some projections from myosin filaments to the actin filaments. In addition, a strong collagen pattern (several orders of 645 Å) was found on the meridian, showing that there is a substantial amount of this protein oriented parallel to the muscle fibre axis.

All the muscles gave equatorial reflexions which could be indexed as the 1:0 and 1:1 reflexions from a double hexagonal lattice. This is consistent with electron micrographs of heart muscle which show such an arrangement of thick and thin filaments (Spiro & Sonnenblick, 1965). In living muscles near *in vivo* sarcomere lengths (2·1–2·5 μm) the 1:0 spacing ranged from 340 to 390 Å. As in skeletal muscle (Huxley, 1953), this spacing varied with muscle length, showing that at longer lengths the filaments were closer together.

From our results we conclude that mammalian heart muscle has actin and myosin filaments in a double hexagonal array, but that in contrast to fast skeletal muscles there is some cross-attachment of myosin and actin filaments in the living muscle under the conditions of our experiments. This is consistent with the observation of a high resting tension in heart muscle that can be partially relaxed by agents such as procaine (Luisada & Weiss, 1954).

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The effect of glycine-conjugated bile acids on net water absorption and potential difference across isolated rat ileum

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Jejunal water absorption is inhibited by glycine-conjugated dihydroxy bile acids in rat, hamster, and man. Comparable studies on ileum have not been reported, although Harries & Sladen (1972) found that taurodeoxycholate did not reduce water uptake in rat ileum *in vivo*.

The present experiments were designed to measure net water and glucose transport, and transmural potential difference (p.d.) across isolated rat ileum. Experimental methods were identical with those described in jejunal studies (Wingate, 1973), and the same pure bile acids were used. These were the dihydroxy conjugates, glycodeoxycholate (GDC) and glycochenodeoxycholate (GCDC), and the trihydroxy conjugate, glycocholate (GC).

The results are given in Table 1. Compared with the jejunal studies, there was little difference between trihydroxy and dihydroxy conjugates. The addition of glucose induced significant net water movement ($P < 0{\cdot}01$), which was abolished by all three bile acids. Likewise all three conjugates reduced glucose uptake, and abolished glucose translocation.

TABLE 1. Krebs-bicarbonate Ringer fluids at 38°; gas phase 5% CO_2 in O_2; duration of each experiment: 1 hr. Glucose, when present, initially 28 mM/l. in mucosal and serosal fluids. Bile acid, when present initially 5 mM/l. in mucosal fluid only. Each value is the mean of 6 experiments, given as mean ± S.E.M.

Glucose	Present	Absent	Present	Present	Present
Bile acid	—	—	GC	GCDC	GDC
Water transport (μl./mg dry wt)					
Mucosal	−1·7 ± 0·3	−0·2 ± 0·3	−0·2 ± 0·2	0·2 ± 0·1	−0·1 ± 0·2
Serosal	1·6 ± 0·3	0·2 ± 0·2	0·3 ± 0·1	−0·1 ± 0·05	−0·04 ± 0·1
Glucose transport (μM/mg dry wt)					
Mucosal	−0·20 ± 0·03	—	−0·08 ± 0·02	−0·05 ± 0·01	−0·05 ± 0·01
Serosal	0·08 ± 0·02	—	−0·02 ± 0·02	−0·05 ± 0·03	−0·06 ± 0·03
Transmural potential difference (mV: serosal-mucosa)					
5′	8·8 ± 0·6	4·8 ± 0·5	9·1 ± 1·4	3·6 ± 0·2	3·0 ± 0·4
15′	6·3 ± 0·4	2·7 ± 0·5	2·2 ± 0·3	2·6 ± 0·2	1·7 ± 0·2
25′	5·7 ± 0·4	2·7 ± 0·4	2·0 ± 0·3	2·9 ± 0·3	1·9 ± 0·3
35′	5·3 ± 0·4	2·9 ± 0·4	2·9 ± 0·3	3·0 ± 0·3	1·9 ± 0·3
45′	5·0 ± 0·5	2·7 ± 0·5	4·0 ± 0·3	2·8 ± 0·5	1·5 ± 0·2
55′	4·7 ± 0·4	2·7 ± 0·4	4·5 ± 0·3	2·6 ± 0·5	1·2 ± 0·2

A glucose-dependent increment in p.d. was demonstrated which was initially abolished by all three bile acids, but whereas the p.d. remained depressed with GDC and GCDC, the glucose-dependent p.d. returned in the later stages of GC experiments. This may reflect falling mucosal fluid concentration of GC due to mucosal uptake.

These inhibitory effects may be of significance since the rat, lacking a gall-bladder, stores its bile acid pool mainly in the intestinal lumen, and GDC is the principal dihydroxy conjugate in this pool (Weiner & Lack, 1968). Moreover, dihydroxy glycine conjugates induce secretion of water in perfused intact human ileum (S. F. Phillips, personal communication). Nevertheless, there is an apparent and unexplained discrepancy between such experimental observations and the co-existence, in health, of intraluminal bile acids and 'normal' water absorption. Perhaps the use of steady state 'physiological' conditions in absorption studies is essentially non-physiological.

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Effects of sugars and diols on enzyme-potentiated hyposensitization

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β-Glucuronidase has been shown to cause hyposensitization when given with a small dose of antigen to patients suffering from asthma or rhinitis (McEwen, Ganderton, Wilson & Black, 1967). The enzyme also shows hyposensitizing activity in guinea-pigs, rats and mice (McEwen & Starr, 1972), but variability in the immunological effects of different samples of enzyme could not be correlated with differences in β-glucuronidase activity nor with differences in purity. This problem has been investigated using pinnal anaphylaxis in mice to assess the immunological

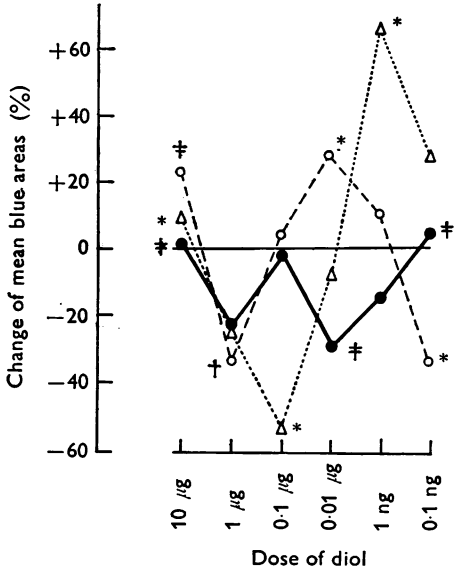

Fig. 1. Mouse pinnal anaphylaxis: groups of seven mice sensitized with 250 μg horse serum. Treated after 3 weeks with 1 μg horse serum + 10 u. β-glucuronidase + various doses of diols and challenged 8 days later. Results expressed as % of result after treatment with antigen + enzyme. ●—● Propylene glycol. ○---○ Propane 1,3-diol. △····△ Butane 1,3-diol. Significance: * $P = < 0{\cdot}001$; † $P = < 0{\cdot}01$; ‡ $P = < 0{\cdot}05$.

effect of a small second dose of horse serum with or without enzyme given 7 days before challenge. It was found that glucose and other pyranose derivatives added to the enzyme/antigen mixture 1 hr before injection determined the immunological effect. This action was shared by propylene glycol but propane 1,3-diol was found to have similar effects at greater dilution (see Fig. 1). Butane 1,3-diol was even more active, while butane 1,4-diol was less so, and pentane 1,5-diol had no significant effect.

The dose–response curves for both sugars and diols were W-shaped: two doses caused hyposensitization while an intermediate dose had little effect or induced hypersensitization. Hypersensitization might also result from a dose of sugar or diol less than that required to produce the low dose zone of hyposensitization.

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