COMMUNICATIONS

The morphology of Group Ib muscle afferent fibre collaterals

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Micro-electrodes filled with a horseradish peroxidase solution were used to impale single Group Ib axons near their entrance to the spinal cord in chloralose-anaesthe-

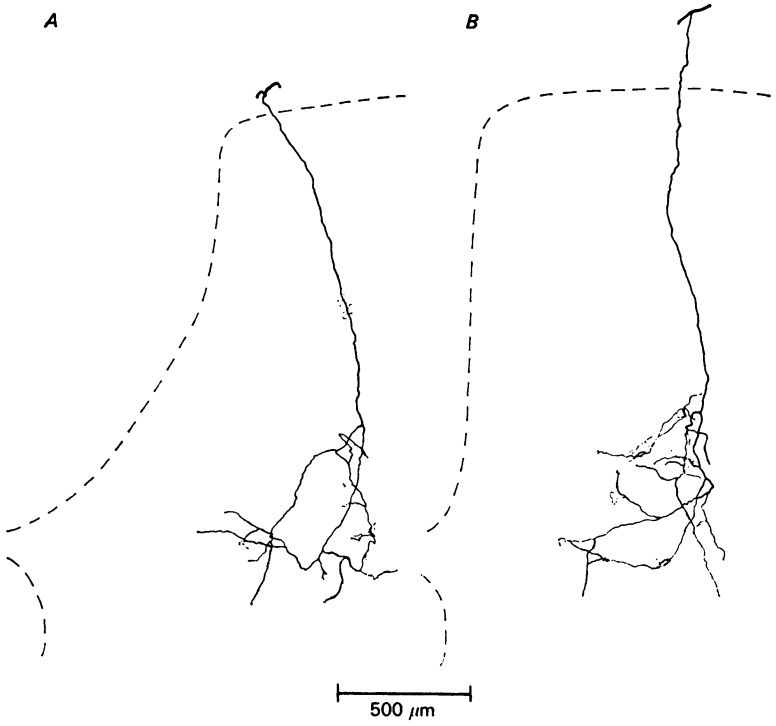

Fig. 1. Reconstructions from transverse sections of the spinal cord of two Ib collaterals from lateral gastrocnemius–soleus muscle afferent fibres in different cats. The outline of the dorsal horn and the central canal are indicated by dashed lines.

tized cats. The enzyme was injected by iontophoresis (Snow, Rose & Brown, 1976) and subsequent histochemistry allowed the morphology of axons and collaterals in the lumbosacral cord to be determined. All axons had peripheral conduction velocities greater than 80 $m.sec^{-1}$, no ongoing activity when first recorded and required noticeable muscle stretch to excite them.

All Ib collaterals had a characteristic morphology (Fig. 1) that differed from all other muscle and cutaneous collaterals identified so far (Brown & Fyffe, 1978; Brown, Rose & Snow, 1977, 1978). Ib collaterals pursued a direct course through the dorsal horn to the intermediate region (laminae V, VI) where they divided and

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arborized widely. Occasional minor branches arborized more dorsally in laminae IV and V. The main arborization was in lamina VI and ventral parts of lamina V.

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Coupling of hip and knee movement during forwards and backwards stepping in man

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There is considerable evidence that spinal segments in the cat can generate the control signals underlying locomotor movements of single limbs and of all four limbs in functional patterns (Miller & van der Meché, 1976). Most previous gait studies in man (e.g. Carlsöö, 1972) have concentrated on forwards stepping in which onset of knee extension characteristically leads to onset of hip extension. The question raised in this study is how the couplings of movements and also of electromyograms of different muscles are shifted during walking backwards. Large phase shifts would have considerable implications for the functional and anatomical organization of the underlying spinal networks.

Eight subjects, male and female, naïve to the procedures required, stepped steadily forwards and backwards on a motor-driven treadmill running at constant speeds. They also made forwards and backwards stepping movements in the air while supporting their weight on their arms. Movements at hip and knee joints were recorded by a polarized light goniometer, and electromyograms were obtained from muscles acting at these joints.

Stepping backwards differed from stepping forwards in that onset of knee extension lagged onset of hip extension. Stepping in the air differed from stepping on the treadmill in two respects. The duration of knee extension for both directions was about 180° compared with about 270° obtained on the treadmill. The resulting wave forms of movement in the air are thus approximately sinusoidal. The second difference was in the size of the phase shifts between hip extension and knee extension. When stepping backwards, both in the air and on the treadmill, onset of knee extension follows onset of hip extension by 45°. When stepping forwards in the air, onset of knee extension precedes onset of hip extension by 45°. In contrast, when stepping forwards on the treadmill, knee extension precedes onset of hip extension by 135°. Thus, switching from backward to forward stepping requires a phase shift of knee extension to hip extension of 90° in the air, but 180° on the treadmill. Comparable systematic phase shifts were also observed in the appropriate electromyograms.

These results imply that systematic phase shifts in the coupling of movements at the hip and knee occur when reversing to backward stepping and that the coupling

employed when stepping forward in the air is different to that used stepping forward on a treadmill.

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Purkinje cells in the Lurcher mutant mouse

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The mouse heterozygous for the Lurcher gene lurches from side to side when walking and also tends to run backwards. It has a cerebellar lesion which affects Purkinje cells, granule cells and olivary neurones (Caddy & Biscoe, 1975, 1976). In sections of tissue impregnated using the Golgi–Cox technique the Purkinje cell in the normal mouse is easily recognized (Fig. 1*A*). Fig. 1*B* shows a Purkinje cell from

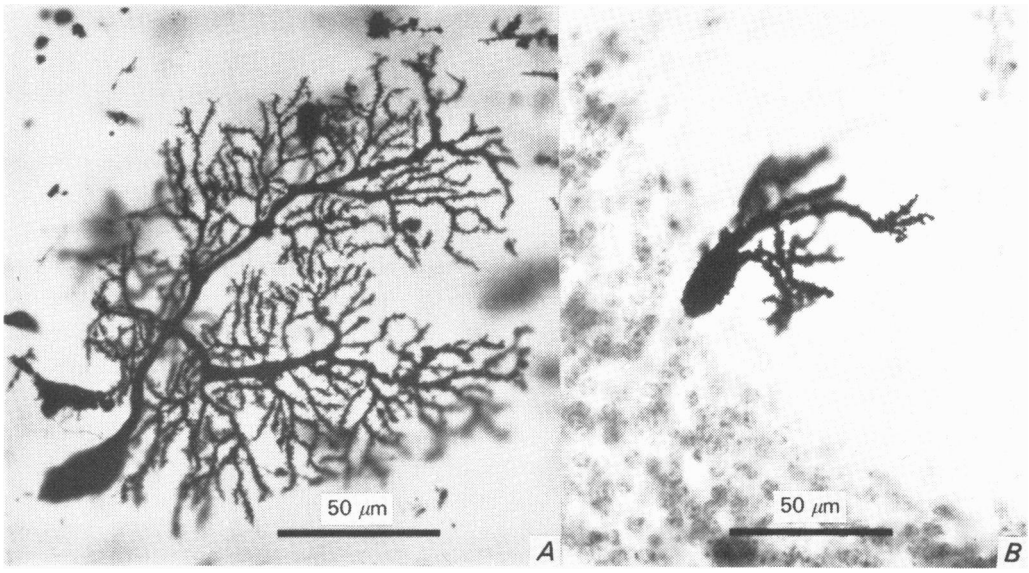

Fig. 1. Tissue impregnated with the Golgi–Cox method. *A*, Purkinje cell from a 15 day post-natal normal mouse. *B*, Purkinje cell from a 14 day post-natal Lurcher mouse.

a Lurcher mouse. The obvious differences are the diminution of the dendritic tree, the thickening of the distal parts of the dendrites and the increase in number of primary dendrites in the Purkinje cells of the Lurcher mouse. The changes in fine structure mainly involve the mitochondria. In the Purkinje cell of the normal mouse the mitochondria are sausage shaped but in the P-cells from the Lurcher they gradually become spherical as degeneration proceeds. Similar abnormal mitochondria also occur in P-cells from another mutant mouse called nervous (Landis, 1973). It is

hoped to determine which is the primary lesion by studying the development of the phenotype.

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Facilitation of raphespinal and other bulbar raphe neurones by stimulation of the sensorimotor cortex

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Anatomical studies have shown the presence of degenerating terminals in raphe nuclei following lesions in the sensorimotor cortex in the cat (Brodal, Walberg & Taber, 1960). We have therefore investigated the influence of stimulation of this region of the cerebral cortex on neurones in nucleus raphe magnus and pallidus.

Experiments were made on cats anaesthetized with chloralose, 50 mg/kg. The frontal cortex was exposed, the cerebellum removed and micro-electrode recordings made from neurones in the mid line raphe nuclei of the pons and medulla. Raphespinal neurones were identified as previously described (West & Wolstencroft, 1977). Single shock stimulation of the sensorimotor cortex with silver-ball or needle electrodes produced one or more spikes in raphespinal and other raphe neurones with latencies of 4–40 msec. The raphespinal neurones tested had conduction velocities ranging from 1·6 to 52 m/sec; many were excited by stimulation of areas 4 or 6.

Raphespinal neurones presumably mediate the analgesia and the inhibition of dorsal horn neurones produced by stimulation in nucleus raphe magnus (Fields, Basbaum, Clanton & Anderson, 1977). Thus the present results suggest that a descending projection from the sensorimotor cortex may inhibit spinal neurones responding to sensory inputs via a relay in the bulbar raphe nuclei.

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Objective typing of mouse ankle-extensor muscle fibres based on histochemical photometry

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Most populations of skeletal muscle fibres are groupable, on the basis of physiological, ultrastructural or histochemical properties, into three or four fairly distinct 'fibre types' (review: Spurway, 1978). However, there has been cause for disquiet

about histochemical classifications in that they have almost always, until now, been founded on subjective visual assessments of reaction intensity. This communication reports an attempt to make multivariate histochemical typing objective.

Microphotometric measurements are made with equipment previously demonstrated (Kerr & Spurway, 1977). Values of the mean light absorbance of each sampled fibre in a histochemical preparation are expressed relative to those of one randomly chosen 'standard' fibre. From serial transverse sections, values representing ten variables are recorded for each fibre. The variables are: activities of seven enzymes, all demonstrated by standard methods (Pearse, 1972), contents of lipid (Sudan stain) and glycogen (PAS), and mean diameter. The enzymes are: succinate dehydrogenase (SD), NADH-tetrazolium reductase (NADH-TR), α-glycerophosphate dehydrogenase (αGPD), glycogen phosphorylase (GP), and myosin ATPase in its native form or preincubated in either alkaline or acid media.

Two hundred fibres from the gastrocnemius, plantaris and soleus muscles of an adult, white mouse have been examined in this way, and the data processed to answer two questions:

(1) Are subjectively defined categories confirmed by objective measurements of the same variables? (Typically, three variables have been considered: any one indicator of oxidative capacity, any one of glycolytic, and any one of the myosin characteristics.)

(2) How closely correlated are reactions, usually taken to be equivalent indicators of a given metabolic pathway?

In answer to the first question, results of previous subjective classifications are encouragingly confirmed. Where there have been divergent claims before, they were evidently due at least as much to differences between the reactions employed as to differences between observers. This is because of the unhappy second finding that markers previously assumed effectively equivalent are not so: correlation coefficients between αGPD and GP or PAS readings are each about 0·67, and that between SD and NADH-TR is only 0·41.

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The effect of denervation on the mechanical and electrical properties of cat skeletal muscle

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In the rat the isometric twitch of skeletal muscle becomes prolonged on the third or fourth day following denervation (Finol & Lewis, 1975) and the prolongation approximately coincides in time with a prolongation and other changes in the membrane action potential (Redfern & Thesleff, 1971; Lewis, 1972). A reasonable

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hypothesis is that the mechanical changes are a consequence of a prolonged action potential which could release more intrafibrillar Ca^{2+}, resulting in an increase in the amplitude and duration of the twitch.

In the cat the twitch changes have an onset later than in the rat (Lewis, 1972). If the explanation is to stand, the onset of changes in the action potential should also be delayed in the cat in comparison with the rat. We have therefore examined the early effects of denervation on the isometric twitch and the membrane action potential in fast and slow twitch muscles of the cat hind limb. Recordings from the muscles *in vivo* were taken three to fourteen days after denervation in one hind limb. Isometric contractions in response to direct stimulation were recorded first. Membrane potentials were measured in samples of 40–60 fibres using glass micropipettes of 15–20 MΩ resistance. Action potentials were evoked by local stimulation of fibre fascicles through a concentric needle electrode. We measured the duration, amplitude and the maximum rate of rise of voltage of the action potentials.

The twitch time to peak was within normal limits for seven days after denervation. After nine days all muscles showed the prolonged twitch contraction typical of denervation. The onset of the change is much later than in the rat although earlier than reported by Lewis (1972). Prolongation of the action potential and reduction in both amplitude and rate of rise of voltage occurred over the same period.

These results are compatible with the suggestion that the early twitch changes of denervation are a direct consequence of changes in the muscle action potential. They do not rule out alternative hypotheses – for example, that the mechanical changes depend on changes in the T-tubular system or the sarcoplasmic reticulum. In such cases the time course of changes in these internal membranous systems would have to be the same as in the external membrane.

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Effect of the oestrous cycle and exogenous steroids on metabolism of 5-hydroxytryptamine by monoamine oxidase from rat lung

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Monoamine oxidase (MAO) activity in rat tissues varies during the oestrous cycle and after pretreatment with exogenous 17β-oestradiol and progesterone (Holzbauer & Youdim, 1973). Lung was not amongst the tissues studied. As lung extensively metabolizes 5-hydroxytryptamine (5-HT) (Bakhle & Vane, 1974), the effects of the oestrous cycle and exogenous steroids on MAO activity in rat lung using 5-HT as substrate were investigated.

Virgin female rats exhibiting at least two consecutive oestrous cycles of 4-day length (as assessed from vaginal smears) were used. Lungs were removed from the animals, perfused via the pulmonary artery until free of blood, and homogenized in

0·3 M buffered sucrose. The homogenate was filtered through gauze. MAO was assayed by incubating 0·5 ml. of the filtrate (approximately 500 μg/ml. protein) with [^{14}C]5-HT in 0·1 M phosphate buffer (pH 7·4) at 37 °C for 60 min. The reaction was stopped with 0·5 M perchloric acid, and after neutralization the radioactive metabolites separated by ion-exchange chromatography (Bakhle & Youdim, 1976).

Metabolism of 5-HT by the lung extracts varied during the different phases of the oestrous cycle. MAO activity, measured initially at a fixed concentration of 5-HT (200 μM), was lowest during pro-oestrus (0·94 ± 0·03 pmole product.min^{-1} μg protein^{-1}; mean ± S.E. of mean; n = 3) and highest during met-oestrus (1·41 ± 0·09; n = 4). These values are significantly different ($P < 0·05$). Activities at di-oestrus (1·08 ± 0·07; n = 4) and at oestrus (1·04 ± 0·05; n = 5) were significantly less than at met-oestrus. Over a range of substrate concentrations (10–1000 μM 5-HT), estimates of apparent K_m and $V_{max.}$ were obtained. It was clear that the change in activity reflected changes in K_m, from 92 μM at met-oestrus to 210 μM at pro-oestrus.

Treatment of female rats with 17β-oestradiol (0·5 mg.kg^{-1}.day^{-1}, in saline; injected intraperitoneally for 8 days) gave MAO activity (at 200 μM) of 0·8 ± 0·08 pmole (n = 4) and an apparent K_m of 92 μM. Progesterone (1·0 mg.kg^{-1}.day^{-1} for 8 days) produced higher MAO activity (1·26 ± 0·13 pmole; n = 4) and a low K_m (85 μM).

These results suggest that, although the lung is not usually considered a 'target organ' for sex steroids, the MAO activity in lung homogenates is affected by changes in endogenous and exogenous steroid levels.

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Non-acclimatization of man to cold

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Twelve men of the Trans-Antarctic Expedition spent 14 months in Antarctica and crossed the continent via the South Pole in a 2158-mile journey at altitudes up to 3015 m and lasting 99 days. They formed a highly self-selected group of white Caucasian males (average age 34, range 27–49).

Each man kept a daily record of clothing worn and work done on a 'sleep card' which also carried much other data. A full meteorological record was kept. During the 14 months considered here the maximum temperature at Shackleton Base was −0·3 °C, the minimum −55·1 °C and the mean temperature was −23·6 °C. During the traverse the maximum temperature was −1·0 °C, the minimum was −35·9 °C and the mean was −20·6 °C.

The cold stress was severe, and a detailed computer analysis has been made of the relationship between the clothing worn and the relevant climatic data using the layer-counting technique (Rogers & Sutherland, 1974) in a search for evidence of

acclimatization of man to cold. The general assumption was made that if acclimatization to cold occurred then less clothing would be worn subsequently under conditions of similar cold stress (Rogers & Sutherland, 1971).

No such evidence could be found in this large mass of data (approx. 1 million bits). The clothing worn was clearly shown to be more closely correlated with temperature than with wind-chill. The clothing worn increased as temperatures fell, increased with the passage of time, and failed to decrease by the expected amount when higher temperatures occurred or when similar climatic conditions occurred after an interval of time (Rogers, 1973).

The entire mass of data is consistent with the thesis that under conditions of cold stress man creates and controls his own micro-climate, having already reached his limit of adaptation to cold in moving from a tropical to a temperate climate.

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The survival of the adrenalectomized fetus in sheep

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Drost & Holm (1968) adrenalectomized sheep fetuses in thirty-seven pregnancies at 110–120 days of gestation and observed prolongation of gestation in eight ewes, although only two fetuses were alive at 158 and 159 days *post coitum* when Caesarean delivery occurred. Barnes, Comline & Silver (1977) adrenaloctomized fetuses at 123–132 days gestational age in twenty-one pregnant ewes and seven fetuses received no subsequent treatment. Three died *in utero* just before normal term and four remained alive until artificial termination of the pregnancies after normal term.

In this study of thirty-nine pregnancies in thirty-five ewes, thirty-one single fetuses and one each of eight pairs of twins were adrenalectomized at 112–124 days of gestation (median, 120 days) and injected I.M. with 5 mg cortisone acetate and, to correct the mineralocorticoid deprivation of adrenalectomy, 10–50 mg DOC pivalate (Percorten crystules; Ciba Laboratories). Fetal heart rate was monitored by Doppler ultrasound fetometer or by radiotransmitter attached to fetal e.c.g. leads. In eleven cases, permanent catheters enabled daily sampling of amniotic fluid for electrolyte analysis and bacteriological examination to be made.

The survival of adrenalectomized fetuses was very variable. Nine fetuses (seven single, two twin) survived from 24 to 42 days after surgery but only five of the single fetuses survived beyond normal term (median, 143 days; range 141–147 for twenty-one laboratory-housed ewes with unoperated fetuses). Three of these fetuses died suddenly *in utero* at 149-151 days *post coitum* and the other two were delivered

alive by Caesarean section at 156 and 161 days of gestation. The two twin fetuses survived until spontaneous delivery of the unoperated fetus at term.

Fifteen fetuses survived between 3 and 20 days after surgery and some of them were found to have a fungal infection which presumably caused abortion. Fourteen fetuses died within 48 hr of operation; in two cases, strangulation of the cord occurred and one adrenalectomized fetus died on the day after surgery.

One ewe with twins was induced at 135 days of gestation by I.M. injection of 25 mg dexamethasone and delivered live female lambs 34 hr later; they have survived with the adrenalectomized lamb receiving daily injections of adrenal steroids.

These results emphasize the fragile state of the adrenalectomized fetus and confirm the requirement of a fetal adrenal component for successful delivery at normal term in the sheep.

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Growth of the kidneys in the lactating mouse

BY B. F. MATTHEWS. *Department of Physiology, University of Manchester, Manchester M13 9PT*

Pregnancy in mice results in an increase of approximately 25 % both in the weight of the animals and the fresh weight of the kidneys (Matthews, 1977). The total renal content of RNA, as of protein, was significantly increased in the pregnant mouse (when compared with the virgin animal), while the amount of DNA was unchanged. The growth of the kidneys was therefore due to enlargement of existing cells without accompanying hyperplasia.

Lactation is associated with changes in the kidneys similar to, and even greater than, those already described in pregnancy. The observations were made on nine lactating mice, aged 74–77 days, approximately 14 days after parturition. Nine virgin mice of the same age were used as controls. The average litter size was nine pups and all dams had free access to food and water.

Compared with the virgin mice, the lactating group showed an increase of approximately 35 % in body weight with an increase in the fresh weight of the kidneys of almost 60 %. As in pregnancy, no change was seen in the total content of DNA (estimated by the method of Burton, 1956) in the kidneys of the lactating animals. In contrast, compared with control values, there was an increase of approximately 35 % in the amount of RNA (estimated by the method of Munro & Fleck, 1966), while the protein content increased by 60 %. In that cell numbers remain constant, the renal changes in the lactating mouse resemble those found during pregnancy. However cell size, which had increased in pregnancy, increased even more during lactation. There was no evidence of connective tissue proliferation since the total collagen content (measured as hydroxyproline after acid hydrolysis by the method of Stegemann & Stalder, 1967) was not influenced by lactation.

Seven days after the end of a 3-week period of lactation there was clear evidence of involutional changes in the kidneys with fresh weight and cell size approaching control values.

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Effects of pregnancy on renal handling of glucose in the rat

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Pregnancy is associated with changes in renal handling of salt and water in women (Hytten & Leitch, 1971) and in rats (Atherton & Pirie, 1977). Experiments were performed to determine whether changes in glucose handling, as observed in human pregnancy, also occur in the pregnant rat.

TABLE 1. Effects of pregnancy on renal handling of glucose in the rat

	Virgin [$n = 8$]	Pregnant [$n = 10$]	P
Saline loading			
Glomerular filtration rate (ml. min^{-1})	1·76 ± 0·13	2·12 ± 0·11	< 0·05
Plasma glucose (mmole l.$^{-1}$)	5·59 ± 0·40	5·78 ± 0·25	NS
Glucose reabsorbed (μmole min^{-1})	10·0 ± 1·1	12·2 ± 0·8	NS
Glucose excreted (nmole min^{-1})	18·9 ± 2·6	34·3 ± 6·2	< 0·05
Glucose loading			
Glomerular filtration rate (ml. min^{-1})	1·83 ± 0·13	1·96 ± 0·08	NS
Plasma glucose (mmole l.$^{-1}$)	28·0 ± 2·3	19·9 ± 1·3	< 0·005
Glucose reabsorbed (μmole min^{-1})	39·6 ± 3·4	30·3 ± 2·5	< 0·05
Glucose excreted (μmole min^{-1})	11·5 ± 1·9	7·7 ± 1·7	NS

Eight virgin and ten 7- to 8-day pregnant rats, all aged 12–13 weeks, were anaesthetized with 100 mg kg^{-1} Inactin [5-ethyl-5-[1′-methyl-propyl]-2-thiobarbiturate] and infused at 200 μl. min^{-1} with 0·9 % NaCl for 4 hr followed by 5 % glucose for 3 hr, both solutions containing [^{3}H]inulin. From 90 min after the start of saline infusion, urine samples (from a suprapubic bladder catheter) and plasma samples were obtained every 30 min and analysed for glucose [^{3}H]inulin.

There was no significant variation of plasma or urine constituents with time during either saline or glucose infusion; the average values for each animal during each infusion were calculated and their means and standard errors are presented in Table 1.

The data indicate at least two changes in glucose handling during pregnancy in the rat: (*a*) during saline loading more glucose is excreted; (*b*) during glucose loading less glucose is reabsorbed.

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Richard Bright's 'Reports of Medical Cases' (1827) in the development of renal physiology

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By the eighteenth century, urinary secretion could be understood as a separation of the more watery part of the blood by the transmitted force of the heart beart (e.g. Boerhaave, 1743). Uncoupling of the secretion of different substances is first reported by Bright (1827) in his observations that in kidney disease albumen was lost in the urine while water, as oedema, was excessively retained in the body. The further observation by Bright and his younger colleague Rees, that the blood urea concentration is raised in renal disease pushed wide open the gate into a new field of thought. No existing concept of urinary secretion could account for retention of urea and loss of albumen as simultaneous consequences of renal damage. After Bright the central question in renal physiology became what it is still: how, in the formation of urine, are the constituents of blood reproportioned and controlled in amount?

Richard Bright was born on 28 September 1789 at 28 Queen Square, Bristol, and the present slight deviation from what might have been a sesquicentennial commemoration of his *Reports of Medical Cases* allows the Society to consider Bright's achievement at a meeting in his native city. As one example of his wide interests and avid curiosity, we may note that, in 1814 and 1815, Richard Bright toured on the Continent, spending some months in Hungary (Bright, 1818), where his visit and exchange of medical ideas are still regarded warmly. Bright's renal studies were published while a full physician of Guy's, a post which he held from 1824 to 1843. He died in 1858.

One of the most striking characteristics of Richard Bright's case reports is the unity of consideration for the patient with the requirements of science. While the abundant and meticulous observations serve to aid understanding of each individual and to monitor treatment, they are at the same time the material for scientific generalization, the advancement of physiology and the welfare of those the physician will never meet.

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Walking patterns of a limb after autotomy in Decapod Crustacea

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The motor output which underlies forward and backward walking has been studied in the rock lobsters *Palinurus vulgaris* and *Jasus lalandii*. During the experiments the animal is fixed, and walking is induced by passive traction of the legs against a moving treadmill belt. A reversible variable-speed motor elicits the two types of locomotion. The activity of four sets of muscles situated at the base of a walking leg has been chronically recorded: the promotor and remotor muscles of the most proximal, T-C joint, and the levator and depressor muscles of the next, C-B joint.

Decapod crustaceans possess the ability to autotomize their locomotor appendages, leaving a small proximal stump with only the four muscles described above remaining intact. After amputation this limb stump is still able to move, and the electromyographic recordings demonstrate that a forward and backward motor pattern can be elicited in it, similar to those in the other intact legs and depending upon the direction of the treadmill.

In both intact legs and leg stumps, each step of forward walking is associated with a synchronous motor discharge in the levator and promotor muscles, alternating with concurrent depressor and remotor discharges. In backward walking the relationship between muscle activities is changed: levator muscles act synchronously with the remotor while the depressors are excited with the promotor muscle. Thus the motor co-ordination is not disturbed in the stump, and it 'walks' with approximately the same period as the other legs.

Nevertheless, the relationship of the duration of a muscle burst to the duration of the step cycle is significantly altered in the stump. In intact crustacean legs, the duration of the return stroke (levation/protraction during forward walking and levation/retraction during backward walking: see Ayers & Davis, 1977), usually does not vary even though the speed or the direction of walking changes. On the other hand the power-stroke duration does depend upon the speed of walking. In an amputee, however, the return stroke duration is also variable: for example, the levator burst duration increases linearly with step duration.

The electromyographic study of amputated limb stumps, therefore, suggests that while the timing of each limb's step cycle and even the direction of walking may be centrally co-ordinated with the aid of sensory afferents from the other legs, the ratio of return-stroke/power-stroke durations is mainly under the control of proprioceptive inputs of each individual leg.

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The effects of cholinergic and anticholinergic drugs on ketamine anaesthesia in rats

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Some effects of ketamine on the C.N.S. seem to be related to a complex interaction of cholinergic and monoaminergic mechanisms (Weingarten, 1972). The influence of drugs which affect these systems has been studied in rabbits (Authier, Sindon, Chapados & Barry, 1972), cats (Hatch, 1973) and dogs (Hatch, 1974) and this study is concerned with the effects of some drugs which interact with the cholinergic system on ketamine anaesthesia in rats.

Atropine sulphate premedication (0·5 mg/kg I.P.) significantly prolonged the recovery of groups of seven rats from a single dose of ketamine (75 mg/kg I.P.) from $20{\cdot}8 \pm 0{\cdot}4$ to $25{\cdot}7 \pm 2{\cdot}1$ min (mean $\pm$ S.E.M.) whilst physostigmine sulphate premedication (0·5 mg/kg I.P.) significantly shortened the recovery time to $15{\cdot}3 \pm 1{\cdot}4$ min. The time for recovery was taken as the time between loss and regaining of the righting reflex.

Heparinized blood samples and brains were obtained from the rats at the point of recovery. The blood samples and 10% homogenates of brain in saline were centrifuged and the plasma and supernatant were stored frozen until assay for ketamine and its metabolites by gas–liquid chromotagraphy (Livingston & Waterman, 1976).

The mean concentration of ketamine in the plasma at recovery was $3{\cdot}92 \pm 0{\cdot}42$ μg/ml. in the control animals, significantly lower at $2{\cdot}71 \pm 0{\cdot}31$ μg/ml. in the atropine pretreated animals and significantly higher at $8{\cdot}70 \pm 0{\cdot}94$ μg/ml. in the physostigmine pretreated group. A similar pattern was seen for the demethylation metabolite (I) levels in plasma but the difference between the control and atropine pretreated groups was not significant. The levels of the cyclohexanone oxidation metabolite (II) showed the opposite effects with the atropine pretreated group showing a higher level than the controls, and the physostigmine pretreated group showing a significantly lower level.

The mean level of ketamine in the brains of the control animals at recovery was $19{\cdot}70 \pm 1{\cdot}92$ μg/g wet wt. and the various levels showed a similar pattern to the plasma levels, with significantly higher levels of ketamine and metabolite I in the physostigmine-treated animals, lower levels in the atropine-treated animals, and metabolite II showing the opposite effects.

Thus, the effect of atropine seems to be to prolong ketamine anaesthesia in rats despite the falling levels of ketamine and its first metabolite, whilst that of physostigmine seems to be to shorten the period despite higher circulating and brain levels.

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Dual sites for antagonism of excitatory amino acid actions on central neurones

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Magnesium ions (Evans, Francis & Watkins, 1977), α,ϵ-diaminopimelic acid (DAP) and D-α-aminoadipate (DAA) (Biscoe, Davies, Dray, Evans, Francis, Martin & Watkins, 1977) antagonize excitation of central neurones produced by either presynaptic stimulation or the application of certain excitatory amino acids, in particular, *N*-methyl-D-aspartate (NMDA). The present experiments were designed to investigate whether the three agents produce their amino acid antagonism by interactions with the same sites.

Hemisected spinal cords of frogs were mounted for measurement of ventral root polarity, and log dose–response curves were constructed for the depolarization of motoneurones produced by NMDA. The three antagonists were then introduced either singly or in pairs and NMDA doses increased to allow measurement of dose-ratios for antagonism (Gaddum, Hameed, Hathway & Stephens, 1955). Tetrodotoxin was present in the Ringer solution throughout the experiments to eliminate indirect effects.

The dose-rates obtained in three separate experiments were as follows, concentrations in parenthesis:

(*a*) Mg^{2+} (1 mM) 4·3; DAP (1·0 mM) 10·0; mixture 60.
(*b*) Mg^{2+} (1 mM) 7·8; DAA (0·25 mM) 6·6; mixture 41.
(*c*) DAP (1 mM) 7·6; DAA (0·25 mM) 5·2; mixture 15·8.

Thus the dose-ratio obtained with the mixture of DAP and DAA approximated to the sum of the dose-ratios obtained with either antagonist alone, but when either DAP or DAA was paired with Mg^{2+} the resultant dose-ratio approximated to the product of those obtained with either antagonist alone. This suggests (Ariens, Simonis & Van Rossum, 1964) that two sites are involved in the antagonism, Mg^{2+} acting at one site and the organic antagonists at the other.

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Localization of dopamine-stimulated 3,5-cyclic AMP formation in the rat preoptic anterior hypothalamus in relation to a possible thermoregulatory function for dopamine

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Dopamine may have a physiological role in thermoregulation in the rat since its injection into the preoptic anterior hypothalamus causes hypothermia, and blockade of dopamine receptors reduces the response of rats to an imposed heat load (Cox & Lee, 1977).

The location of this dopamine-sensitive area has been defined more accurately by stereotactic injection of dopamine (10 μg in 0·5 μl. in lightly restrained conscious rats) at different sites within the preoptic region and measuring fall in core temperature. The largest mean fall (1·13 $\pm$ 0·22 °C, n = 8) was obtained with co-ordinates of AP 1·8 mm, lateral 1·2 mm and depth 8·0 mm, using bregma as the reference point (Pellegrino & Cushman, 1967). Injections with their centres more than 0·4 mm either side of this active site were ineffective.

Three serial sections 0·8 mm thick were prepared from the preoptic anterior hypothalamus: one anterior, one posterior and one corresponding to the active site. The ability of these slices to synthesize 3,5-cyclic AMP was determined according to the method of Forn, Krueger & Greengard (1974). Tissue from the active site synthesized 6·94 $\pm$ 1·25 pmole.mg protein^{-1} in 10 min, which was not significantly different from the synthesis rate in the other two slices. Dopamine 20 and 100 μM increased 3,5-cyclic AMP production in the 'active slice' to 16·5 $\pm$ 1·1 and 29·3 $\pm$ 2·0 pmole.mg protein^{-1} respectively. The posterior slice was relatively unresponsive (8·5 $\pm$ 1·3 and 12·4 $\pm$ 2·6 pmole) but the anterior slice responded well (15·1 $\pm$ 3·8 and 35·3 $\pm$ 7·8). However, whereas the effect of dopamine on the 'active slice' was blocked by haloperidol (0·1 μM) but not propranolol (0·1 μM), its effect on the anterior slice was significantly reduced by both drugs. Thus the anterior slice appears to increase its production of 3,5-cyclic AMP in response to dopamine by a dual mechanism involving both dopamine receptors and β-adrenoceptors. The active slice on the other hand seems to contain a population of specific dopamine-sensitive receptors.

Therefore there appears to be within the preoptic anterior hypothalamus a population of dopamine receptors at a site coincident with the area in which an injection of dopamine causes a hypothermic response. Taken in conjunction with the earlier studies these results support the suggestion of a physiological role for dopamine in thermoregulation in the rat.

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Hyperpolarizations and increases in cyclic AMP produced by catecholamines in sympathetic ganglia are mediated by different receptors

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Greengard (1976) has proposed that the hyperpolarization observed in rabbit superior cervical ganglion on application of catecholamines is dependent on an increase in cyclic adenosine 3′,5′-monophosphate (cyclic AMP), which he attributed to α-adrenoceptor activation. However, in the rat it has been demonstrated that the elevation of cyclic AMP levels in the superior cervical ganglion by catecholamines is due to β-receptor activation (Cramer, Johnson, Hanbauer, Silberstein & Kopin, 1973).

In the present study, increases in cyclic AMP and hyperpolarizations occurring in rat sympathetic ganglia in response to catecholamines have been investigated, with the object of classifying the receptors involved. Both sets of experiments were carried out using preparations obtained as follows: superior cervical ganglia were excised under urethane anaesthesia from male Wistar rats (200–260 g), desheathed, then stored overnight at 4 °C in Krebs solution which had previously been bubbled with a 95 % O_2: 5 % CO_2 mixture.

Hyperpolarizing responses to catecholamines were recorded by the method of Brown & Marsh (1975) with the temperature maintained at 25 ± 1 °C. Application (for 60 sec) of (±)-isoprenaline (10^{-6} to 10^{-4} M) and (−)-phenylephrine (10^{-6} to 10^{-4} M) produced hyperpolarizations which were consistently antagonized by phentolamine (10^{-6} M), whereas (±)-propranolol (10^{-6} M) had little effect. This implies that the hyperpolarizations were produced by stimulation of an α-adrenoceptor. This conclusion is supported by the potency of other α-agonists in producing hyperpolarizations in this preparation (Caulfield, 1978).

Cyclic AMP production in isolated ganglia was measured after incubation with catecholamines for 15 min at 25 °C, using a modification of the Gilman protein binding assay (Gilman, 1970). Isoprenaline (10^{-8} to 10^{-6} M) produced dose-related increases in cyclic AMP whilst phenylephrine ($\leqslant 10^{-4}$ M) was without effect. The responses to isoprenaline were antagonized by propranolol (10^{-6} M), but not by phentolamine (10^{-6} M). These results are consistent with those of Cramer *et al.* (1973), indicating that catecholamines increase cyclic AMP levels in rat superior cervical ganglion by a β-receptor mechanism.

We therefore conclude that, contrary to the hypothesis of Greengard (1976), the increases in cyclic AMP and the hyperpolarizing responses produced by catecholamines in rat superior cervical ganglion are brought about by activation of receptors which are pharmacologically discrete.

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Some properties of a model of the mammalian cerebellum

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A great deal is known about the structure of the cerebellum, especially of its cortex, and recent physiological work has clarified the way in which the different types of cell interact, in particular whether they are excitatory or inhibitory to their target neurones. The complexity of the intracortical connexions makes it extremely difficult to see intuitively the implications of the organization of the cerebellum. To explore these, a number of models have been devised and their properties tested by computer simulation techniques. The present model is similar to that of Mortimer (1970) and incorporates some features of Boylls' (1975) model. It consists of an array of ninety-nine compartments each of which represents a section of cortex 300 μm square. Within each compartment the cells of each type (i.e. Purkinje, basket, stellate, Golgi and granule cells) are represented by a single element. The interconnexions of the elements within a compartment and from one compartment to another are based on those described by Palay & Chan-Palay (1974). The Purkinje cell elements project on to and inhibit underlying elements representing nuclear cells. The response of the model to a step increase (usually by 20 %) in the tonic mossy fibre input to one compartment has been studied for a range of values of the various parameters which represent the synaptic potency of each type of cell or afferent on to its target neurones. For relatively weak interactions between the neurones, the step produces an increase in activity in the Purkinje cells in the corresponding compartment and a strip of compartments aligned along the folium. The model rapidly settles to a steady state with a band of Purkinje cells showing increased activity orientated along the folium and a wider band of Purkinje cells with reduced activity on either side – a pattern not grossly dissimilar to that suggested by Eccles, Ito & Szentágothai (1967). When the strength of basket cell inhibition of Purkinje cells is increased, the initial phase of the response is similar but the model takes much longer (as much as 100 msec) to settle to a steady state which now shows bands of excited Purkinje cells running across rather than along the folium. The distribution of nuclear cell activity resulting from these two patterns of Purkinje cell activity are quite different, showing how important to cerebellar function the precise values of synaptic gains could be.

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Interneurones associated with the motor nucleus of the trigeminal nerve in the cat

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Of the various reflexes of the jaw muscles only the spindle-based stretch reflex has a monosynaptic component. Interneurones for at least some of the others are believed to lie in a specialized dorso-medial extension of the main trigeminal sensory nucleus, closely overlying the motor nucleus (Åström, 1952), called the nucleus supratrigeminalis (NST). Unit responses in this region have been reported due to jaw opening, jaw closing and tooth pressure (Jerge, 1962; Kidokoro, Kubota, Shuto & Sumino, 1968), but their characterization has previously been incomplete with regard to distinction from closely related tonically active motoneurones and first-order spindle and periodontal afferents. We have now examined the NST particularly for interneurones which could be involved in multisynaptic connexions of jaw elevator muscle spindles.

By means of extracellular micropipettes, cells have been found immediately dorsorostral to the motor nucleus giving brief, high-frequency bursts specifically in relation to minute (100 μM) fast transient stretches of jaw elevators (a powerful stimulus to spindle primaries). These cells are judged to be interneurones in that they do not give direct, short latency single spikes following stimulation of the mandibular nerve close to the skull, and so cannot be motoneurones or first-order afferents. In decerebrate preparations without anaesthesia, the interneurones are silent or spontaneously active at low frequencies, but can usually be activated by iontophoretically applied glutamate. They are little affected by large-scale slower jaw movements.

A surprising feature of their behaviour is the long latency of their burst discharge (as much as 10–12 msec) relative to the monosynaptic excitatory effect of quick stretch on the motoneurones (4·5 msec). This main effect, seen as a negative field potential in the motor nucleus, is sometimes followed by a second smaller, slower excitation, related in time to the interneurone discharge. It is possible that the interneurones described are the basis for this multisynaptic effect and hence for a multisynaptic stretch reflex.

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Bulbar raphe neurones with projections to the spinal trigeminal nucleus and the lumbar cord in the cat

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The widespread analgesia produced in freely moving cats by electrical stimulation in nucleus raphe magnus (Oliveras, Redjemi, Guilbaud & Besson, 1975) is presumably mediated by raphespinal and raphe-trigeminal neurones (West & Wolstencroft, 1977;

Lovick, West & Wolstencroft, 1977). Since the locations of the cell bodies of these two groups are similar we have investigated the possibility that some raphe neurones are branched and project to both structures.

Antidromic identification of raphespinal and raphe-trigeminal neurones was made in decerebrate cats as previously described (West & Wolstencroft, 1977; Lovick *et al.* 1977). In nucleus raphe magnus, neurones were found which could be antidromically activated both from electrodes in the lumbar cord at L1 and also from electrodes positioned in the caudal spinal trigeminal nucleus. Collision of the two antidromic spikes at time intervals shorter than the sum of their respective response latencies was taken as an indication that branching of the parent axon occurred some distance from the cell body.

Apparent conduction velocities from the trigeminal nucleus to the cell body were always considerably slower than between the spinal cord and the cell body, 1·0–6·0 m/sec and 10·6–56·6 m/sec respectively, when estimated from the latency and shortest distance between the stimulation and recording sites. This observation was unexpected. However, measurements of conduction velocities of raphespinal neurones revealed that conduction between C3 and the cell body was slower than between C3 and L1. (This phenomenon was not seen in axons conducting at speeds less than 2m/sec.)

Taken together these observations suggest that the parent axon proximal to the branching point conducts much more slowly than in the branches, possibly due to the initial unmyelinated segment. When allowance is made for this possibility, the estimated conduction velocities in the trigeminal branches become closer to those in the spinal branches.

The presence of neurones projecting to both the spinal trigeminal nucleus and the spinal cord may provide, in part, an anatomical basis for the widespread analgesia produced by stimulation in nucleus raphe magnus.

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Responses of cerebellar interpositus nuclear neurones to trigeminal inputs in the cat

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Neurones of the cerebellar interpositus nucleus (IPNs) transmit the main output of the pars intermedia of the cerebellar cortex and appear, from limb studies, to be involved primarily in controlling on-going movements (Evarts & Thach, 1969). To determine whether a population of IPNs exists, which is activated predominantly by trigeminal inputs and which could participate in jaw-movement control, we have compared responses of IPNs to trigeminal and limb afferent stimulation.

Unitary discharges were recorded in the region of the interpositus nucleus in adult cats, anaesthetized with either α-chloralose or sodium thiopentone and paralysed with gallamine triethiodide. Masseter muscular, and supraorbital, infraorbital and mental cutaneous, trigeminal branches, and the footpads of the fore- and hind limbs, ipsilateral to the recording site, were stimulated using bipolar electrodes. Stimulus parameters were adjusted to excite only large diameter myelinated fibres. Recording sites were marked by iron deposition or electrolytic lesioning.

In chloralose-anaesthetized animals, IPNs had low 'spontaneous' discharge frequencies (usually < 15/sec). Peristimulus time histograms revealed that all responsive (70/74) neurones tested received convergent inputs from trigeminal and limb nerves, but that individual IPNs differed considerably in the extent and combination of their effective stimulus sites. Fifty-two per cent of responsive IPNs were influenced by all inputs tested; the proportion responding to individual afferent sources being: masseter muscular 65%, supraorbital cutaneous 78%, infraorbital cutaneous 83%, mental cutaneous 88%, forelimb 95%, and hind limb 98%.

Responses to single volleys typically comprised three phases (cf. Armstrong, Cogdell & Harvey, 1975) sequentially (1) a burst of spikes with onset latencies of 3–17 msec for trigeminal branches, 5–20 msec for forelimb and 12–22 msec for hind limb; (2) a suppression of discharge or resumption of spontaneous firing frequency (20–100 msec latency); (3) an increase in discharge frequency (50–800 msec latency, often > 500 msec duration). However, response patterns varied in complexity from those lacking one or other phases to those in which phase 1 and/or 3 was subdivided. Similar response patterns to each effective nerve were elicited in 33% IPNs although absolute efficacies varied, trigeminal inputs being generally weaker.

In thiopentone-anaesthetized animals spontaneous discharge frequencies were substantially higher and responses were evoked in fewer units (19/35). Moreover, convergence was less evident: only 47% of responsive units exhibiting convergence from trigeminal and limb inputs and 16% from all inputs tested. Phase 1 was subdivided and phase 3 absent more frequently than in chloralose-anaesthetized preparations.

Our results indicate a more extensive convergence upon IPNs than has been described previously.

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Ovulation in the rat: duration of hypothalamic activation, and not frequency, is the critical parameter

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About 30 years have elapsed since the original demonstration that electrical stimulation of the mediobasal hypothalamus causes secretion of sufficient gonadotrophic hormone to induce ovulation (e.g. Harris, 1948). However, the relative

importance of duration of stimulation, as opposed to frequency, remains largely unknown. The present study was undertaken to provide an answer to this question.

In the first series of experiments spontaneous ovulation was blocked in eighty-eight female rats by intraperitoneal injection of sodium pentobarbitone (Nembutal, 40 mg/kg) at 13·00 h on the day of pro-oestrus. Biphasic electrical pulses (500 μA, 1 msec, 30 sec on – 30 sec off) were then applied at either 10, 25, 50 or 100 Hz, for 15, 30 or 45 min, through a bipolar coaxial platinum electrode unilaterally placed in the arcuate nucleus. The effectiveness of the stimulation was assessed by seeking and counting tubal ova the following morning. None of the eighteen rats stimulated at 25, 50 or 100 Hz for 15 min ovulated. Full ovulation was only observed when the stimulation was maintained for 45 min at a frequency $\geqslant$ 25 Hz (n = 20/20). With 30 min of stimulation approximately half the animals ovulated regardless of applied frequency (i.e. 5/9 at 10 Hz, 6/9 at 25 Hz and 4/6 at 100 Hz).

In the second series of experiments synaptosomes were prepared from median eminence tissue obtained from groups of forty female rats. The isolated nerve terminals were incubated in normal Locke solution containing Bacitracin to minimize peptidase activity. An electric field, equivalent to a potential of 4 mV across each synaptosome, was pulsed at frequencies of 5, 10, 20 and 50 Hz for 20 min periods. Samples of incubation media were taken for immunoassay of luteinizing hormone releasing hormone (LHRH) both before and after 1, 2, 5, 10 and 20 min of stimulation. Pre-stimulation values of LHRH were around 600 pg/ml. This value was not significantly elevated after 1, 2 or 5 min of stimulation at any frequency. After 10 and 20 min of stimulation, however, all four incubations contained approximately 1200 and 1500 pg/ml. LHRH respectively ($P < 0{\cdot}01$).

The data show that the duration of hypothalamic activation is more important for ovulation than frequency of stimulation. We were also unable to relate stimulation frequency to release of hormone from the hypothalamus. These findings are consistent with the observation that the adenohypophyseal response to LHRH is potentiated by prior exposure to the hormone (Aiyer, Chiappa & Fink, 1974) and maximal LH release is not achieved by a single transient increase in portal LHRH concentrations.

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Inhibitory effects of acetylcholine and dopamine on rabbit carotid chemoreceptors

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During experiments in pentobarbitone-anaesthetized rabbits we observed that intracarotid (I.C.) injection of acetylcholine (ACh, 5–250 μg) caused an immediate inhibition of respiration, whereas NaCN (1–25 μg I.C.) markedly stimulated respiration. Cutting the ipsilateral sinus nerve abolished the response to NaCN and greatly

reduced the inhibitory action of ACh. The possibility that ACh was inhibiting chemosensory activity was investigated by recording from the peripheral end of a cut sinus nerve.

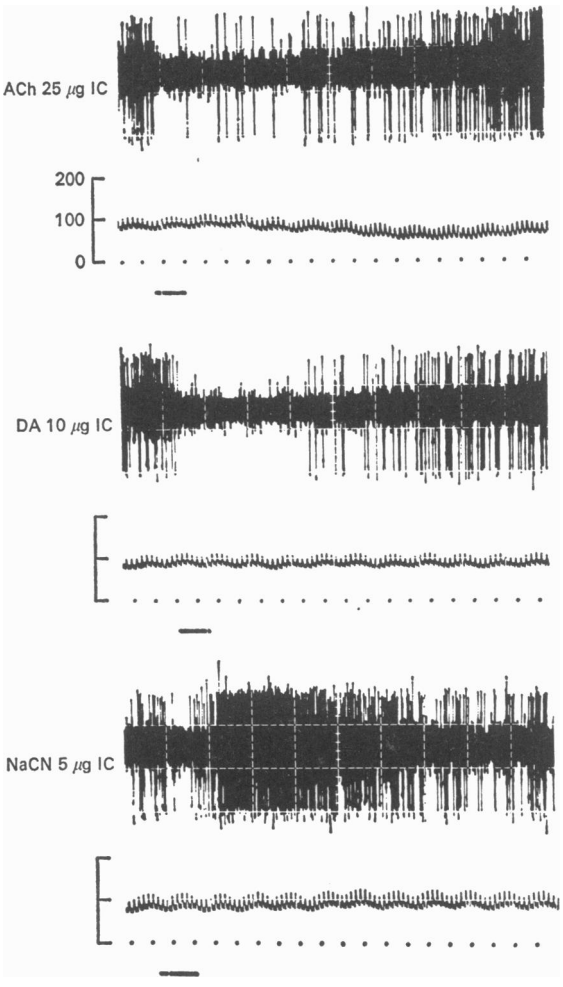

Fig. 1. Recording of chemoreceptor activity obtained from a rabbit, illustrating responses to ACh, DA and NaCN. Panels show: action potentials, B.P.; 1 sec and injection markers.

ACh (5–250 μg I.C.) caused a dose-dependent inhibition of discharge (Fig. 1). With doses greater than 100 μg the inhibition was preceded by a slight transient increase in discharge. Atropine (1–5 mg/kg I.V.) slightly reduced the response to ACh. Dopamine (DA, 10 μg I.C.) also inhibited chemoreceptor activity. To determine whether the ACh-induced inhibition was secondary to DA release, we administered the DA antagonist α-flupenthixol (0·25–0·5 mg/kg I.V.). DA-induced inhibition was abolished, whereas that caused by ACh was only slightly reduced.

In contrast to other species where ACh increases chemosensory activity, and has

been proposed as an excitatory transmitter (see review by Biscoe, 1971), our evidence shows that ACh has an inhibitory effect on rabbit chemoreceptors, implying that endogenous ACh is unlikely to be an *excitatory* transmitter in this species.

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The excitability of sinus nerve afferent terminals during the respiratory cycle

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The performance of both baroreceptor and chemoreceptor reflexes is affected by the respiratory cycle (see Haymet & McCloskey, 1975). In a recent report, Lipski, McAllen & Spyer (1977) showed that inspiratory neurones of the nucleus of the tractus solitarius (NTS) only receive an excitatory input from chemoreceptors during inspiration. These inspiratory neurones are located close to where the sinus nerve afferents, and hence chemoreceptor fibres, terminate (see, for example, Lipski, McAllen & Spyer, 1975; Jordan & Spyer, 1977). It seemed possible that the respiratory 'gating' of this input onto inspiratory cells might be due to excitability changes in chemoreceptor afferent terminals, i.e. through a presynaptic 'gate'. In the present experiments we have tested this possibility.

Experiments were carried out on chloralose-anaesthetized cats and urethane-anaesthetized rabbits which were paralysed with gallamine (Flaxedil, 3–4 mg/kg I.V.). The medulla, in the region of the NTS, was penetrated with tungsten stimulating electrodes to find areas which on stimulation would evoke antidromic activity in the sinus nerve (see Jordan & Spyer, 1977). If presynaptic mechanisms are modulating the terminals of sinus nerve afferents, then this should show itself as a change in the excitability of the terminals to antidromic excitation. Using a constant stimulating current we therefore studied the magnitude of the averaged antidromically submaximal evoked responses in the sinus nerve at different phases of the central respiratory cycle (as indicated by spontaneous activity in the phrenic nerve). Antidromically evoked potentials of various latencies (2·5–35 msec in rabbits; 2–20 msec in cats) were investigated for respiratory-related changes in excitability. In no case was a significant variation in the size of the evoked potential observed.

We conclude that central respiratory activity does not appear to modulate baroreceptor and chemoreceptor reflexes by a presynaptic action on their primary afferent fibre terminals, and this is true for both fast and slowly conducting fibres.

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Evidence suggesting that acetylcholine acts as a neurotrophic factor

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We have made a quantitative comparison of the effects of botulinum toxin (BoTx) type A treatment with those of surgical denervation on the development of TTX resistant action potentials, by recording their rate of rise in the presence of TTX 10^{-6} M in the extensor digitorum longus muscle of adult rats.

TABLE 1. Mean ± S.E.M. of maximum rates of rise of action potentials in the presence of TTX 10^{-6} M. Figures in parenthesis show number of fibres giving action potentials over total number tested. The differences between BoTx and denervated, and between BoTx and BoTx + α-NnsTx treated muscles were significant at $P < 0.001$ (Student's t test)

Procedure	Rate of rise (V/sec)
Denervated 5 days	258 ± 6 (50/50)
Denervated 8 days	183 ± 10 (24/24)
BoTx 8 days	95 ± 3 (52/70)
BoTx 6 days + denervated 2 days	268 ± 8 (25/25)
BoTx 6 days + α-NnsTx 2 days	177 ± 6 (52/52)
Denervated 6 days + α-NnsTx 2 days	180 ± 9 (22/22)

As shown in the Table, surgical denervation of 5 and 8 days' duration induced the appearance of TTX resistant action potentials in all fibres whereas BoTx did not. Similar results have been reported by Prestronk, Drachman & Griffin (1976) when they compared extrajunctional ACh sensitivity in BoTx poisoning with that following surgical denervation.

Thus some trophic influence remains in the BoTx treated nerve. The administration of α-neurotoxin from the *Naja naja siamensis* venom (α-NnsTx) to BoTx treated animals resulted in the appearance of TTX resistant action potentials in all fibres and in an increase in the rate of rise of the spike to a level comparable to that following surgical denervation (Table 1). Since the neurotoxin, like α-bungarotoxin, selectively blocks cholinergic receptors in muscle the results indicate that remaining acetylcholine release, quantal or non-quantal, from BoTx poisoned nerves has a neurotrophic action.

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The effects of potassium depolarization on the metabolism of [^{3}H]choline by rat sympathetic ganglia

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Choline uptake by cholinergic nerve terminals in sympathetic ganglia is increased by potassium depolarization (Higgins & Neal, 1977) and we have now examined the

effects of potassium depolarization on the metabolism of [^{3}H]choline in ganglia. Isolated superior cervical sympathetic ganglia were preincubated for 30 min at 37 °C in medium containing 4·7 mM-KCl (controls) or 40 mM-KCl. The ganglia were then transferred to fresh medium (4·7 mM-KCl) containing [^{3}H]choline (0·1 μM) and the incubations continued for 10 min. The radioactivity was extracted in formic acid/acetone and subjected to high voltage paper electrophoresis. The spots containing [^{3}H]choline and [^{3}H]metabolites were eluted, and the radioactivity estimated by liquid scintillation counting.

TABLE 1. Effect of potassium depolarization on the metabolism of [^{3}H]choline in sympathetic ganglia

Preincubation and incubation conditions ...	Metabolite (dpm/mg wet wt. tissue)					
	[KCl] 4·7 mM in preincubation and incubation media			[KCl] 40 mM in preincubation medium: 4·7 mM in incubation medium		
	Choline	Acetylcholine	Phosphorylated metabolites	Choline	Acetylcholine	Phosphorylated metabolites
Normal Krebs–bicarbonate Ringer	580 ± 70	560 ± 50	4600 ± 300	1300 ± 200	5400 ± 400	2300 ± 200
Krebs + 1 μM hemicholinium-3	330 ± 30	200 ± 20	4200 ± 300	330 ± 30	370 ± 40	3800 ± 400
Calcium-free Krebs medium	—	—	—	630 ± 50	1100 ± 100	4100 ± 300
Krebs + 20 mM-$MgSO_4$	—	—	—	440 ± 40	570 ± 30	4300 ± 300
Normal Krebs (chronically denervated ganglia)	510 ± 40	90 ± 10	4200 ± 200	520 ± 30	440 ± 90	4900 ± 200

Each result is the mean ± S.E. of the mean of 6–20 determinations.

Preincubation of ganglia with KCl (40 mM) increased the subsequent uptake of [^{3}H]choline by 36% ($P < 0·001$) compared with controls and [^{3}H]ACh synthesis was increased almost tenfold whilst [^{3}H]phosphorylated metabolites were halved (Table 1). These effects of potassium depolarization were greatly reduced or abolished by chronic denervation, hemicholinium-3, and by procedures which inhibit ACh release during preincubation with KCl (Ca-free and high Mg media).

The results suggest that the increase in choline uptake and ACh synthesis produced by KCl are associated with cholinergic nerve terminals and are a consequence of ACh release rather than depolarization *per se*.

A.J.H. is an M.R.C. scholar.

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Effects of exogenous ATP on contractile force and intracellular cyclic nucleotide levels in the hypodynamic frog ventricle

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We (Flitney, Lamb & Singh, 1977*a*) postulated that the inotropic effects of ATP on the hypodynamic frog ventricle are mediated through changes in cyclic nucleotide levels, and demonstrated (Flitney, Lamb & Singh, 1978) that the decline in isometric force during the development of the hypodynamic state is accompanied by a decrease in cyclic AMP and a rise in cyclic GMP. We now offer direct evidence for a close correlation between changes of twitch tension and [cyclic AMP]/[cyclic GMP] following treatment of hypodynamic ventricles with exogeneous ATP.

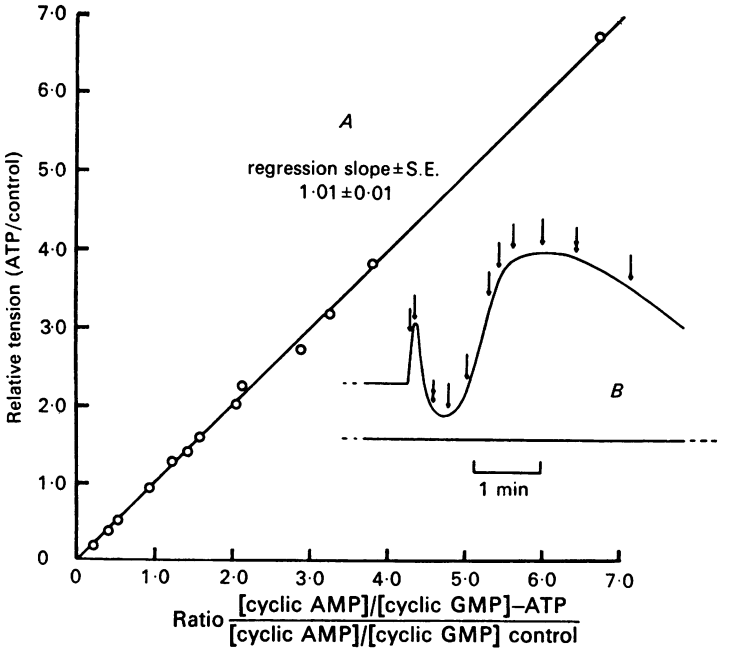

Fig. 1. Relation between relative force and cyclic nucleotide ratios (*A*) measured at various times (*B*, arrows) during the inotropic response to 10^{-3} M-ATP.

Hypodynamic ventricular strips were superfused with frog Ringer containing 10^{-3} M-ATP. Preparations were frozen at different times during the ATP response (Fig. 1*B*) and assayed for cyclic AMP, cyclic GMP and total protein. Fig. 1*A* shows the relation between twitch tension and [cyclic AMP]/[cyclic GMP]. Isometric force and cyclic nucleotide ratios are expressed as multiples of the control (hypodynamic) values. The observed linear relation (slope of regression line +S.E.: $1{\cdot}01 \pm 0{\cdot}01$; $n = 13$; $P < 0{\cdot}001$) suggests that ATP exerts its effect through changes in intracellular cyclic nucleotides and lends further support to the view (e.g. Nawrath, 1976) that these substances are important factors in determining myocardial contractility.

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Potential-dependent blockade by Ba^{2+} of resting potassium permeability of frog sartorius

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A voltage clamp was used to show that Ba^{2+} produces a blockade of the inwardly rectifying potassium permeability of frog muscle which is enhanced by hyperpolarization and relieved by depolarization. In the presence of 0·5 mM-Ba^{2+} hyperpolarization produces an inward potassium current which turns off exponentially as the blockade turns on (Fig. 1*B*). The rate of onset of the blockade is increased by increasing the hyperpolarization or $[Ba]_o$. In the absence of Ba^{2+} (Fig. 1*A*) currents show little time-dependence.

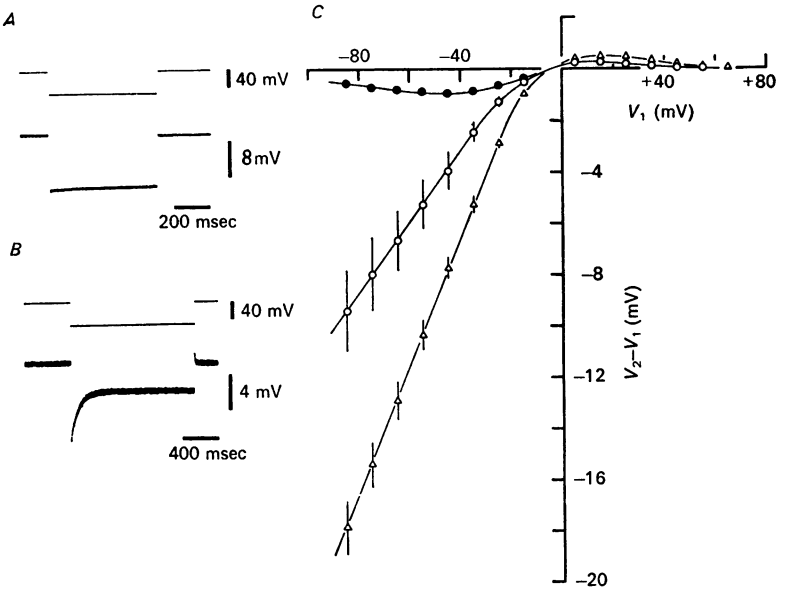

Fig. 1. *A*, voltage (upper) and current (lower) records in control solution (115 mM potassium acetate, 1·8 mM-calcium acetate, 5 mM-Tris HCl). *B*, records obtained in control solution + 0·5 mM-Ba^{2+}. *C*, mean current–voltage relations with and without Ba^{2+} (see text). A linear component obtained by extrapolating the currents for large depolarizations through the holding potential has been subtracted from the relation for each fibre.

Fig. 1*C* gives mean current–voltage relations from fibres in solutions containing no Ba^{2+} (△) and Ba^{2+} at 0·5 mM, where instantaneous currents (○) are reduced 50 %, and steady-state currents (●) are increasingly blocked by increasing hyperpolarization.

The rate of onset of the blockade in 0·5 mM-Ba^{2+} has a high Q_{10} (3·15 ± 0·08) which indicates a reaction between Ba^{2+} and a site. The potential-dependence suggests that this site may lie within the membrane. Further, since increasing $[K]_o$ reduces the effectiveness of Ba^{2+}, there appears to be competition between Ba^{2+} and K^+.

Neutral amino acid transport by rabbit ileum

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It is generally assumed that all neutral amino acids share a common carrier for transport across the rabbit terminal ileum (Preston, Schaeffer & Curran, 1974). Present experiments test this hypothesis by measuring the short-term influx of radioactive phenylalanine, leucine, alanine or glycine (1 mM) into rabbit ileum in the presence and absence of a number of non-radioactive neutral amino acids at a sodium concentration of 145 mM. The pattern of inhibition will be constant for amino acids sharing a common transport mechanism. Obtained results are shown below.

	Inhibition of uptake (%)			
Inhibiting amino acid (mM)	L-Phenyl-alanine	L-Leucine	L-Alanine	Glycine
DL-Norleucine (20)	72	81	84	88
L-Phenylalanine (5)	62	56	58	79
L-Methionine (5)	62	59	71	70
L-Leucine (5)	59	57	69	72
L-Isoleucine (5)	57	52	60	75
L-α-Amino-butyric acid (10)	53	42	65	67
L-Valine (5)	32	44	50	65
L-Homo-serine (5)	27	35	38	52
L-Glutamine (5)	17	33	19	42
L-Tyrosine (1)	7	25	11	40
L-Alanine (10)	12	34	44	76
L-Asparagine (5)	11	20	25	75
L-Threonine (5)	9	7	27	48
L-Alanine (5)	4	21	34	69
Glycine (10)	0	18	22	67
L-Serine (5)	0	8	32	61

The larger amino acids (norleucine to α-amino-butyric acid) caused similar inhibitions of all amino acids tested. Smaller amino acids (valine to serine) showed a selective decrease in their ability to inhibit the transport of phenylalanine, leucine and alanine. Increasing concentrations of phenylalanine caused complete inhibition of mediated serine transport. Increasing concentrations of serine caused a *maximal* 63% inhibition of phenylalanine transport. This is taken to be the proportion of phenylalanine carried by a broad-specificity carrier. Residual transport is via a mechanism specific for amino acids containing large apolar residues. These results do not support the hypothesis that phenylalanine entry into rabbit ileal mucosa takes place by one mechanism only (Schultz & Markscheid-Kaspi, 1971).

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Dietary restriction and miniature end-plate potentials

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Resting membrane potentials (RMPs) and spontaneous miniature end-plate potentials (m.e.p.p.s) were recorded, as described by Kelly & Roberts (1977), from rat hemidiaphragm preparations. Rats were allowed half of the weight of food eaten by control animals of the same age for 1 or 3 weeks, after which some were allowed food *ad libitum* for a further 1 or 3 weeks.

TABLE 1. Effect of dietary restriction on body weight and m.e.p.p. amplitude. Number of observations in parentheses. C = control; 1S or 3S = 1 or 3 weeks of dietary restriction; 1R or 3R = 1 or 3 weeks with food *ad lib.* after restriction

Age (days)	Treatment	Body weight (g) mean ± 1 S.E.	M.e.p.p. amplitude (mV)
30	C	103 ± 2 (5)	0·97 ± 0·06 (22)
30	1S	62 ± 1 (11)	0·63 ± 0·04 (37)
37	1S + 1R	100 ± 3 (5)	0·93 ± 0·04 (44)
44	C	205 ± 3 (5)	0·79 ± 0·06 (22)
44	3S	101 ± 2 (8)	0·49 ± 0·03 (31)
51	1S + 3R	165 ± 5 (3)	1·02 ± 0·05 (53)
51	3S + 1R	152 ± 2 (5)	0·79 ± 0·04 (52)
56	C	298 ± 5 (6)	0·67 ± 0·03 (38)
65	3S + 3R	227 ± 4 (3)	0·65 ± 0·04 (34)
110	C	435 ± 3 (3)	0·51 ± 0·03 (33)
110	1S	369 ± 19 (3)	0·56 ± 0·04 (26)
110	1S + 1R	423 ± 32 (3)	0·52 ± 0·03 (24)
110	1S + 3R	402 ± 14 (3)	0·60 ± 0·04 (36)
110	3S	259 ± 18 (3)	0·42 ± 0·03 (28)

Dietary restriction had no significant effect on RMP or m.e.p.p. frequency, but reduced m.e.p.p. amplitude by about 35 % in young rats. After animals were returned to food *ad libitum* there was an 'overshoot' of m.e.p.p. amplitude above control values. Older animals were less affected by dietary restriction. Table 1 summarizes the results of this investigation. The normal decrease in m.e.p.p. amplitude with age is significantly altered by brief periods of dietary restriction and this indicates that special care should be taken in the selection of appropriate controls when chronic experimental treatments affect the food intake of experimental animals.

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Thyroid hormones and the movement of amino acids between skeletal muscle and the blood

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Adult rats radiothyroidectomized shortly after birth or given an excess of triiodothyronine by S.C. injection, were used. Hypothyroidism reduced influx of amino acids into skeletal muscle (methods: Baños, Daniel, Moorhouse & Pratt, 1973) whereas hyperthyroidism increased it slightly (Table 1). The action of thyroid hormones was a specific transport effect since the influx of the non-metabolizable amino acid, cycloleucine, was also significantly decreased in hypothyroidism. Three days fasting caused the blood concentration of alanine to fall lower in hypothyroid than euthyroid animals and the fall was associated with a release of the essential branched chain amino acids from muscle.

TABLE 1. The effect of thyroid hormones upon the influx of some amino acids into skeletal muscle (pectoralis major). Mean values ± S.E. of mean. Number of experiments in parentheses

	Control group:	Hypothyroid group:	Hyperthyroid group:
	influx (nmole min^{-1} g^{-1} muscle) /	influx (nmole min^{-1} g^{-1} muscle) /	influx (nmole min^{-1} g^{-1} muscle) /
Amino acid	plasma amino acid (μmole $l.^{-1}$)	plasma amino acid (μmole $l.^{-1}$)	plasma amino acid (μmole $l.^{-1}$)
L-Methionine	71 ± 2 (2)	30 ± 3 (3)*	134 ± 7 (3)*
L-Tyrosine	84 ± 6 (3)	36 ± 4 (2)*	120 ± 13 (3)
L-Phenylalanine	142 ± 15 (2)	39 ± 8 (3)*	134 ± 16 (3)
L-Leucine	80 ± 5 (3)	17·8 ± 1·6 (4)*	—
L-Valine	33 ± 3 (2)	12·3 ± 2·3 (5)*	—
L-Lysine	47 ± 15 (5)	6·5 ± 0·6 (4)*	—

* Significantly different from values in control group ($P < 0.05$).

We conclude that hypothyroidism reduces the effectiveness of the way in which the muscles regulate the blood levels of gluconeogenic amino acids, especially during fasting (Daniel, Pratt & Spargo, 1977). This may have some bearing upon the muscle abnormalities associated with thyroid disorders.

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Tritiated water clearance and capillary blood flow to the epithelium of the rumen

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Indirect evidence from observations on the temporarily isolated ventral sac of the rumen of the conscious cow has suggested that the exchange of tritiated water (HTO) is largely determined by the blood flow to the epithelium (Dobson, Sellers & Thorlacius, 1971). In preliminary experiments both quantities have now been determined using the isolated ruminoreticulum of sheep anaesthetized with pentobarbitone. Through a permanent rumen cannula the organ contents were removed and epithelium rinsed. Observations were made with the lumen filled with isotonic NaCl which was stirred vigorously by gases. After HTO was injected into the lumen solution, HTO clearance was calculated using a two-compartment model from the decline in lumen concentration and the increase in plasma water concentration. Epithelial blood flow was measured from the distribution of 15 μm diameter labelled microspheres following their injection close to the aortic valve. The reticulorumen was removed post-mortem and the epithelium was dissected from the muscle prior to the estimation of the labels trapped within each capillary bed. Blood flow was calibrated from the amount of label recovered in a blood sample withdrawn at a known rate from the femoral artery. In each experiment the ruminal blood flow was altered in four stages by varying the composition of the stirring gas from pure N_2 to pure CO_2. This effectively covers the normal range of carbon dioxide in the rumen.

When expressed for 1 g dry weight of stripped epithelium, the blood flow varied over a range of 1–10 ml./min, while the clearance of HTO varied from 0·5 to 2·2 ml./min. If the clearance is apportioned between blood flow and permeability, for instance by the Renkin equation:

$$Cl = Q(1-\exp[-PA/Q]),$$

where Cl is the clearance, Q the blood flow and PA the permeability × area product. PA did not remain constant, but increased by about threefold as the blood flow increased. Since the only substantial network of capillaries in the stripped epithelial preparation lies in the submucosal layer, I conclude that a substantial part of the relation between HTO and blood flow could be due to the relation between PA and blood flow. It would seem reasonable to attribute this latter relation to changes with blood flow in the geometry of perfusion, for instance changes in the effective area available for diffusion.

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Identification of the NH_2-terminal tryptic peptide of big gastrin in hog antral mucosa

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The main forms of gastrin identified in antral mucosa and blood are peptides of seventeen (little gastrin, G17) and thirty-four (big gastrin, G34) amino acid residues. Trypsin cleaves G34 at lysine residues in positions 16 and 17 producing an NH_2-terminal peptide and a COOH-terminal heptadecapeptide identical to G17, and it has been suggested that G34 might therefore be a biosynthetic precursor of G17 (Gregory & Tracy, 1975). The plasma concentrations of G34 needed for a given rate of acid secretion are about six times those of G17, so that the factors governing the relative rates of production and secretion of G17 and G34 are of physiological importance. The biosynthesis of the gastrins is poorly understood, but if G17 was produced by intracellular cleavage of G34 then one might also expect to find equimolar quantities of an NH_2-terminal peptide.

We have examined the forms of gastrin in hog antral mucosa by radioimmunoassay and immunocytochemistry using antisera specific for different regions of G17 and G34. One antiserum (1296) was specific for the COOH-terminus of G17 and so cross-reacted with both G17 and G34 (Dockray & Walsh, 1975). A second antiserum (L33) was raised in a rabbit immunized with natural porcine G34 conjugated to bovine serum albumin; this antiserum originally contained both COOH- and NH_2-terminal specific antibodies but COOH-terminal antibodies were removed by affinity adsorption to G17 immobilized on Sepharose beads. The specificity of L33 for the NH_2-terminus of G34 was indicated by binding to ^{125}I-labelled G34 but not [^{125}I]G17. Moreover, in radioimmunoassays using [^{125}I]G34 label this antiserum cross-reacted with a component produced by trypsinization of G34 which had the characteristics of the NH_2-terminal peptide.

When boiling water extracts of antral mucosa were fractionated by gel filtration (Sephadex G50) or ion exchange chromatography (AE cellulose) over 95% of immunoreactivity (equivalent to 9·0 nmole/g) detected by 1296 was compatible with G17, and the remainder was attributable to G34. In contrast, over 95% of immunoreactivity (equivalent to 7·8 nmole/g) detected by L33 emerged in a single peak in the same position as the NH_2-terminal peptide of G34. Immunocytochemical studies of adjacent serial sections of mucosa revealed that the two antisera stained the same antral cells, suggesting a common cellular origin for G17 and the NH_2-terminal peptide. These results indicate that G34 is cleaved within G-cells to produce G17 and an NH_2-terminal peptide.

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Depressor responses to stimulation of the sacral outflow in the pithed rat

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During a study of the spinal origin of the motor (adrenergic) and inhibitory (non-adrenergic, non-cholinergic) innervation of the rat anococcygeus muscles (Gillespie & McGrath, 1973), when the vertebral outflows were stimulated at the optimal position for the anococcygeus inhibitory response, small falls in blood pressure occurred.

We have now investigated this depressor response and compared its properties with those obtained by stimulating the inhibitory pathway to the anococcygeus.

Rats were pithed under halothane anaesthesia, respired with O_2 and given pancuronium bromide (1 mg/kg, I.V.). The pithing rod was arranged to stimulate selectively different levels of the spinal outflows (1 msec pulses, supramaximal voltage). Carotid arterial pressure, heart rate and anococcygeus tension were continuously recorded (Gillespie & McGrath, 1973). Both jugular veins were cannulated, one for the constant infusion of tyramine (40 μg/min) to elevate blood pressure, the other for acute injection of other drugs.

Depressor responses were found only when stimulating in the region L5–S2 and with the arterial pressure elevated. In the absence of adrenergic blockade, stimulation at 1–5 Hz produced depressor, and at 10 Hz pressor, responses. In the presence of guanethidine (10 mg/kg) the adrenergic, pressor component was removed and stimulation at 1–100 Hz produced depressor responses with a maximum at 5 Hz and above. Responses started within 2 sec and reached a plateau after 5–10 sec. Subsequent investigation was, therefore, carried out with 10 sec periods at 5 Hz.

Depressor responses were inhibited or abolished by hexamethonium (5 mg/kg) (as were the corresponding anococcygeus inhibitory responses) suggesting that an autonomic efferent pathway with a ganglion synapse is involved.

Atropine (1 mg/kg) blocked depressor responses to acetylcholine (30 μg/kg) but had no effect on depressor nerve responses suggesting that a post-ganglionic parasympathetic cholinergic pathway is not involved.

The possibilities of β-adrenergic, histaminergic and purinergic transmission were excluded by comparing the effects of specific blocking or potentiating agents on the depressor nerve responses and on the corresponding responses to the appropriate agonists. The involvement of bradykinin could not be excluded since the antagonist employed, hesperetin (1·75 mg/kg), did not antagonize the depressor effect of bradykinin.

In conclusion, a depressor outflow with a ganglion synapse has been demonstrated which has a spinal origin and frequency–response characteristics similar to those of the anococcygeus inhibitory pathway and whose post-ganglionic transmitter remains unknown.

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Carotid body chemoreceptors in the mature sheep fetus re-examined

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Biscoe, Purves & Sampson (1969) showed that, in the mature sheep fetus, carotid body chemoreceptor activity measured in the sinus nerve was very sparse, despite a P_{a,O_2} of 20–25 mmHg. Such activity was insensitive to physiological or pharmacological stimulation. The receptors were activated only by electrical stimulation of post-ganglionic sympathetic fibres to the carotid body and temporary occlusion of the umbilical cord, which also was shown to activate these sympathetic fibres. These authors suggested that the sympathetic nerves might activate the chemoreceptors and alter their sensitivity, in particular to oxygen at the same time or shortly afterwards. We have examined this point.

Carotid body chemoreceptor activity was measured in twenty fetuses removed by hysterotomy with intact umbilical circulation from ewes, anaesthetized with pentobarbitone sodium within 5 days of natural term. Activity in the sinus nerve, sensitive to NaCN (10–100 μg), lactic acid, hypoxia, hypercapnia and sympathetic stimulation, was found in only eight fetuses. When these carotid bodies were subsequently removed and examined as superfused preparations *in vitro* (Eyzaguirre & Lewin, 1961), chemoreceptor afferent activity was found to be plentiful and was affected in exactly the same way as that from carotid bodies of adult rabbits studied in parallel.

Fetal carotid body chemoreceptor activity increased in response to umbilical cord occlusion even after the sympathetic fibres had been cut – and the responses to graded hypoxia and hypercapnia in these fetuses after birth, i.e. when the cord had been clamped and breathing started, and in naturally new-born lambs less than 72 hr old, was quantitatively similar to that seen in the adult cat (Biscoe, Bradley & Purves, 1970) whether the sympathetic fibres were intact or not. Further, simultaneous measurements of post-ganglionic sympathetic activity on one side and chemoreceptor afferent activity on the other a few minutes after birth showed the two were entirely dissociated.

The reason for the discrepancy between the present and previous results is not certain. The present results clearly indicate a change in receptor sensitivity – particularly to hypoxia – at or shortly after birth, a change which is not due to maturation or the involvement of sympathetic nerves. The possibility that this change in sensitivity is due to a change in the distribution of blood flow within the carotid body was discussed, together with ways in which this possibility might be tested.

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Tachyphylaxis of the pressor response to angiotensin II

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Bock & Gross (1964) and Stewart (1974) have proposed models of angiotensin II tachyphylaxis in which the angiotensin II receptor sites are blocked by hormone-receptor complexes. We have measured the rate of recovery of the pressor response following tachyphylaxis in the anaesthetized rat, to test whether this recovery is a first-order reaction (i.e. proportional to the number of receptor-sites inactivated).

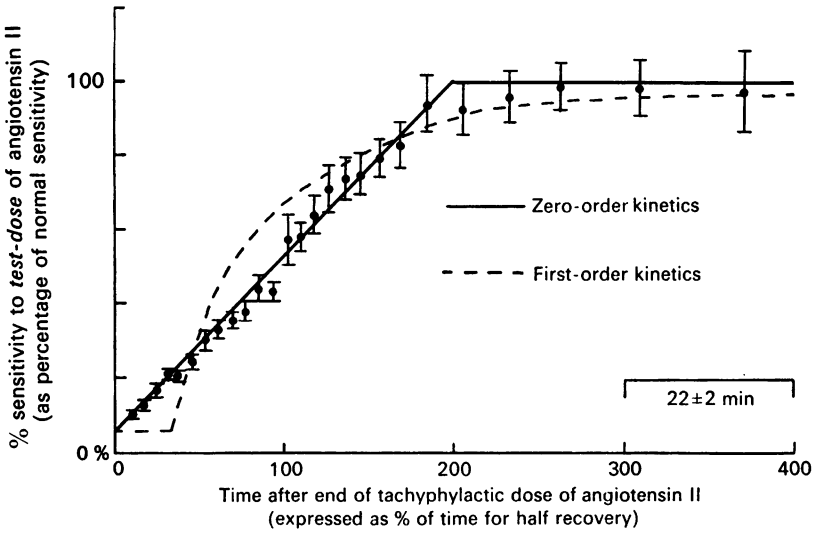

Fig. 1. Time course of recovery from angiotensin II tachyphylaxis in forty rats. Each point on the graph represents the mean ± S.D. of twenty observations.

A range of angiotensin II doses were injected in random sequence for the construction of a dose–response curve. A *test-dose* (usually 50 ng; selected to give 40 mmHg pressor response) was injected four times at intervals of 2–5 min. Angiotensin II, 2000–16000 ng.kg^{-1}.min^{-1}, was infused for 10 min to induce tachyphylaxis and the *test-dose* was then injected repeatedly for up to 60 min. The dose–response curve was then re-checked. The response to each test-dose was quantified (by reference to the dose–response curve) as the dose of angiotensin which could give an equal pressor response in the absence of tachyphylaxis (Fig. 1).

The recovery process was better described as a zero-order reaction (i.e. independent of the apparent number of receptor sites inactivated) than as a first-order reaction ($P < 0{\cdot}001$; F-test).

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Inhibition of calcium uptake by acidosis in the myocardium of the rabbit

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Acidosis in cardiac muscle reduces the deleterious effects of a period of hypoxia (Bing, Brooks & Messer, 1973) or ischaemia and delays the onset of the calcium paradox (Bielecki, 1969). A possible explanation for these phenomena is that acidosis inhibits calcium uptake. Langer & Poole-Wilson (1977) have reported inhibition of calcium efflux from the myocardium by acidosis and proposed that calcium influx was also diminished. These experiments were undertaken to measure directly the effect of an increase of CO_2 on calcium uptake.

The interventricular septum of the rabbit was perfused through its own vasculature and stimulated at 36 beats/min (Poole-Wilson & Langer, 1976). The control perfusate was maintained at 27 °C and equilibrated with a 5 % CO_2, 95 % O_2 gas mixture (pH = 7·37 ± 0·01, ± S.E. of mean). A respiratory acidosis was induced by equilibration with 30 % CO_2 (pH = 6·67 ± 0·01). The uptake of $^{47}Ca^{2+}$ was followed with a sodium iodide crystal (5 × 7 cm) placed 2 mm from the septum. The background counts were maintained constant by exclusion of all metal surfaces, return of the effluent to a reservoir 1·5 m distant and of constant volume, and separation of the septum and reservoir by 20 cm of lead. No geometrical effects were detectable. Under control conditions an initial rapid uptake of $^{47}Ca^{2+}$, presumably into the extracellular space, was followed by a slower uptake which continued for up to 5½ hr. The septum was then 80 % labelled. Increase of perfusate CO_2 after 30 or 149 min of labelling caused a reversible inhibition $^{47}Ca^{2+}$ uptake. Similar inhibition occurred if the septum was maintained quiescent. No rapid displacement of $^{47}Ca^{2+}$ from the septum was observed. During 30 min of exposure to an elevated CO_2 the increase in tissue counts was reduced by 52 % ($P < 0·001$) in comparison to the increase of counts under control conditions. The effect of an acidosis on the extracellular space was estimated from the distribution volume of ^{51}Cr-EDTA. Tissue ^{51}Cr was continuously monitored with the sodium iodide crystal. An acidosis increased the extracellular space by 8 ± 1 % ($n = 5$) within 5 min. This change could not account for the diminished uptake of $^{47}Ca^{2+}$.

It is concluded that an acidosis resulting from increase of perfusate CO_2 inhibits the uptake of calcium by the myocardium.

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Effects of renal perfusate flow on systemic blood pressure in the rat

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Occlusion of the ureter or renal vein, or altering renal perfusion pressure can induce changes in afferent renal nerve activity (Niijima, 1971). Stimulation of renal afferent nerves can in turn produce changes in systemic blood pressure (Aars & Akre, 1970; Calaresu, Stella & Zanchetti, 1976). These observations which show species variation suggest the possibility of a cardiovascular reflex involving renal baroreceptors.

Experiments have been carried out in male Wistar rats (400 g) anaesthetized with urethane. The left kidney was perfused *in vivo* via the renal artery with Krebs solution at 37 °C, the cannula being introduced via the left iliac artery. The left renal vein was also cannulated; ligation of the left spermatic and adrenal arteries and veins effectively isolated the renal vasculature from the general systemic circulation. Renal nerves and ureter were left intact. Using this preparation the effects of altering renal flow on systemic blood pressure have been examined.

In the majority of preparations, increasing renal flow produced a graded rise in systemic blood pressure with a short latency on onset. Reducing renal flow to control levels resulted in a fall in blood pressure to control values within 5–10 min. Generally, systolic pressure was increased more than diastolic pressure indicating a positive inotropic effect. Evidence for the involvement of renal stretch receptors in this response was obtained by injecting the pressor agents noradrenaline and angiotensin II into the renal perfusate. Both compounds produced a rise in renal perfusion pressure due to vasoconstriction; this resulted in a fall in systemic blood pressure. When injected during mechanically raised perfusions, these agents attenuated the rise in systemic blood pressure which is normally seen. These experiments indicate that stretch rather than pressure receptors are involved in initiating the reflex; also this type of experiment confirmed that the renal circulation was isolated, any leakage of these pressor agents into the general circulation should have elevated blood pressure. Application of lignocaine to the region of the renal afferent nerves also attenuated the response indicating the involvement of these nerves in the afferent limb of the reflex. The physiological role of this reflex is not clear; it could, however, play a role in the development of hypertension.

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Adaptation of blood gases to diurnal temperature changes in a desert lizard, *Uromastyx ancanthinurus*

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In its natural habitat the desert agamid *Uromastyx acanthinurus* is subjected to diurnal variations of its body temperature from about 38° to 25 °C. Even under

laboratory conditions the body temperature remains about 38° during day time if provision is made for appropriate behavioural temperature regulation (Götz & Albers, 1974).

The effect of the diurnal changes in temperature on the arterial pH of blood gases were observed in three unanaesthetized animals with chronically implanted catheters using continuously perfused electrodes for 24 hr; $\Delta pH/\Delta T$ ranged from $-0{\cdot}005$ to $-0{\cdot}009$. This is only half the value obtained during longer lasting temperature acclimatization or measured for blood *in vitro* (Welbers, 1976). The oxygen consumption varied corresponding to a Q_{10} from 2·26 to 4·34 during the diurnal temperature change. At 38 °C the P_{a,CO_2} was about 38 mmHg and P_{a,O_2} was about 54 mmHg. At 25 °C the P_{a,CO_2} was only 27 mmHg and P_{a,O_2} 18 mmHg.

It is concluded that the daily fluctuations of temperature affect the acid–base status of the blood in a qualitatively similar manner but quantitatively to a lesser extent than during chronic temperature acclimatization.

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Abnormal transport of horseradish peroxidase from the medial gastrocnemius muscle in dystrophic mice

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The method of retrograde transport of horseradish peroxidase (HRP) has been used to identify motoneurones innervating particular muscles (Kristensson & Olsson, 1971; Burke, Strick, Kanda, Kim & Walmsley, 1977). This communication reports the results of investigations upon the retrograde transport of HRP from the muscle of normal and dystrophic mice of the C129 ReJ strain.

The medial gastrocnemius muscle was injected bilaterally with 0·5 mg HRP in 10 μl. saline into animals with the nerves to adjacent muscles, or to all the muscles on one side, cut. After 42–48 hr the animals were perfused with phosphate buffered 4 % glutaraldehyde, pH 7·4. The spinal cord was removed after segments L1–L6 had been identified by piercing the cord on one side near the mid line and opposite the last rootlet of the appropriate dorsal roots. After 4 hr in fresh fixative the tissue was equilibrated with phosphate buffered sucrose solutions, pH 7·4, ending in 30 % sucrose. Frozen serial sections 50 μm thick were processed conventionally for the demonstration of HRP except that the sections were pre-soaked in 0·5 % $CoCl_2$ 0·1 M Tris HCl, pH 7·6 (Adams, 1977), to produce a black reaction product. The position of labelled cells was marked on drawings made from the camera lucida.

In the normal mouse, labelled motoneurones were found as a longitudinal column laterally in the ventral horn in segments L3–L4, and in addition, on the side on which

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all the nerves were intact, labelled cells were also found in a more ventral position. Between 45 and 80 labelled cells were counted in the target cell column and 60–200 labelled cells on the opposite side where all nerves were intact. There were no labelled cells where all nerves to the limb were cut. In the dystrophic mouse labelled cells were found in a similar position in the ventral horn to that in the normal mouse. Between 50 and 104 labelled cells were found on the side where adjacent muscles had been denervated but very few labelled cells, 6–22, on the side where the nerves were intact. Also, where all the nerves were cut, labelled cells were found.

It is concluded that in dystrophic mice HRP was taken up by the cut nerves due to greater spread of HRP from the labelled muscle, indicating that HRP is transported along dystrophic nerves but that uptake in the muscle is abnormal in these mice; experiments are being undertaken to test this hypothesis.

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