differences have been observed between the ionic composition of the perfusion fluid and that of the pericardial like fluid formed on the surface of the heart.

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Histochemistry of developing muscle in rabbit and cat

BY ANTHEA ROWLERSON. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH*

The influence of insulin on mixed protein synthetic rate in the sheep placenta *in utero*

BY SYLVIA CHRYSTIE, JANE HORN, D. NOAKES, M. STERN and MAUREEN YOUNG. *Department of Gynaecology, St Thomas's Hospital, London SE1 7EH*

COMMUNICATIONS

The effect of vascular perfusion of the choroid plexus on the secretion of cerebrospinal fluid

BY R. DEANE and M. B. SEGAL. *Sherrington School of Physiology, St Thomas's Hospital Medical School, London SE1 7EH*

Many epithelia have a complex microcirculation yet the role of this structure and the effect of vascular perfusion has largely been ignored in the study of secretion by these tissues. In a previous communication we have shown that the transport of sugars across the isolated perfused choroid plexus of the sheep was sensitive to the rate of vascular perfusion (Deane & Segal, 1978). A similar finding has been observed in the perfused frog small intestine (Boyd & Parsons, 1978). The effect of perfusion on the rate of c.s.f. secretion and on ion transport has been investigated.

A reduction in the rate of vascular perfusion from $0{\cdot}63 \pm 0{\cdot}08$ to $0{\cdot}25 \pm 0{\cdot}04$ ml./min ($n = 5$) caused a decrease in the secretion of c.s.f. from $21{\cdot}37 \pm 2{\cdot}69$ to $9{\cdot}15 \pm 1{\cdot}51$ μl./min. The rate of sodium and chloride transport from blood to c.s.f. was also reduced; $5{\cdot}88 \pm 1{\cdot}71$ μequiv Na^+/min to $3{\cdot}74 \pm 0{\cdot}061$ μequiv Na^+/min and $7{\cdot}02 \pm 1{\cdot}03$ μequiv Cl^-/min to $4{\cdot}65 \pm 0{\cdot}52$ μequiv Cl/min. All values expressed as mean $\pm$ S.E.M.

The degree of inhibition of c.s.f. secretion by Diamox (acetazoleamide, 10^{-7} M) was shown to be sensitive to the rate of vascular perfusion, the inhibition being increased at the lower rate of perfusion.

Vascular perfusion would thus appear to increase the rate of secretion of c.s.f. by the choroid plexus and to increase both the transport of ions and sugars. A possible explanation of these observations could be that vascular perfusion causes hydrostatic

ultrafiltration through leaky pathways in the epithelium. This explanation is not supported by arteriovenous extraction studies in which the extraction of electrolytes is decreased by increased rates of vascular perfusion.

Vascular perfusion must therefore be an important part of the *in vivo* secretory process and must be included in future models of the secretory mechanism.

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The intimal surface of the rabbit aorta

BY E. SVENDSEN and A. R. TINDALL. *Institute of Medical Biology, University of Tromsø, Norway*

We have been interested in the question of the existence or otherwise of the folds on the intimal surface of the rabbit aorta at normal distensions. We have used the two-component setting substance Silgel 504 (Wacker-Chemie GMBH, Munich, West Germany) to take impressions of the aorta. Since Silgel is relatively viscous, it was not possible to measure the filling pressure with a blood pressure transducer, but it was the distension as a result of the pressure that was important. We monitored therefore the diameter of the aorta *in somno* (under anaesthesia) and during the filling process.

Since opening the thorax was precluded by the demands of the experiments, we monitored the distension of the abdominal aorta at about 5 cm anterior to the iliac bifurcation. The diameter was measured by means of a small rod (weighing 0·5 g) resting on the aorta and actuating a small mirror. The movement of the mirror was magnified by an optical lever.

After the measuring apparatus was in place, and a carotid and a femoral artery were cannulated, 1000 units of heparin were given. This was followed by an overdose of Nembutal. When the animal was nearly or quite dead, a saline suspension of Sephadex spheres (40–80 μm) was injected via the carotid artery. The spheres soon blocked all arteries branching from the aorta. It is important not to inject the spheres too early. They block the arteries very quickly and under normal anaesthesia the blood pressure rapidly reaches abnormally high levels.

Subsequently, freshly prepared Silgel solution was injected into the aorta, the cannula in the femoral artery being open and allowing excess saline and Silgel to escape. The Silgel solution also contained spheres, and when it was seen coming from the femoral artery cannula, this was closed and the injection pressure gradually increased up to either diastolic or systolic value. This pressure was maintained until the Silgel set, in approximately 1–1½ h. The preparation was left overnight, and next morning the distension was again checked before the cast was removed. The experiments reported here have been performed taking care not to allow the distension to become greater than a known value at any time during the course of the procedures.

We find that the surface corrugations are clear at less than diastolic distension and become progressively less distinct as distension is increased. At distensions above systolic pressures the surface is best described as finely rugose. On side branches the folds are often more regular and lie straight and parallel with each other.

These features of the intima may have significance in the flow of blood and the formation of atherotic plaques.

Casts and illustrations were available for inspection.

What is the fate of exogenous prostaglandin endoperoxide, PGH_2, in isolated lung?

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Arachidonic acid (AA) is metabolized on passage through the pulmonary circulation of isolated guinea-pig lungs to products which include prostaglandins (PGs) and thromboxanes (Tx) (Hamberg & Samuelsson, 1974). The first step in this metabolic pathway is formation of the endoperoxides PGG_2 and H_2. The PGs and TxA_2 are formed from these common endoperoxide intermediates. We have therefore investigated the metabolism of exogenous PGH_2 in the pulmonary circulation of isolated lungs.

Guinea-pig isolated lungs were perfused (8 ml./min) via the pulmonary artery with Krebs solution. The lung effluent superfused bioassay tissues including rat stomach strip, rabbit aorta and rat colon, with a second set of tissues, rat stomach strip and rat colon, separated by a coil giving a delay of 75 sec. Arachidonic acid (1–5 μg) injected through the pulmonary circulation caused a contraction of all the tissues in the first set and a relaxation of the second rat colon, i.e. after the delay. We interpret this as showing conversion of AA to TxA_2 – contracting the tissues in the first bank – and to PGI_2 – relaxing the second rat colon after TxA_2 had mostly degraded in the delay coil, in agreement with Alabaster & Hawkeswood (1978).

Metabolism of PGH_2 was studied by injecting 100–450 ng (the amounts equivalent to 1–3 μg AA) into the pulmonary circulation. In nine experiments less than 20% of the activity of PGH_2, as measured by contractions of the rabbit aorta, survived passage through the lung. Since the rabbit aorta is more responsive to TxA_2, we also deduce that even less PGH_2 was transformed to TxA_2. There was never a relaxation of the second rat colon, i.e. less than 50 ng PGI_2-like activity was formed. Overall, PGH_2 was extensively inactivated and its metabolism differed qualitatively and quantitatively from that of an equivalent amount of AA. We have observed similar inactivation of PGH_2 in rat isolated lung (3 expts.) and in two samples of human lung.

We conclude that exogenous PGH_2 is not converted to PGI_2 as rapidly and completely by intact endothelial cells as has been proposed (Moncada, Gryglewski, Bunting & Vane, 1976). The difference between metabolism of AA and PGH_2 might be explained if PGG_2 were the better substrate for subsequent enzymic transformations.

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Carotid baroreceptors and the hind-limb vascular capacitance

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Changes in carotid sinus pressure cause significant changes in abdominal vascular capacitance (Hainsworth & Karim, 1976). However, although responses of individual veins in the hind limb have been studied (e.g. Hainsworth, Karim & Stoker, 1975), the overall capacitance response in the hind limb is not known.

Dogs were anaesthetized with chloralose (0·1 g/kg) and artificially ventilated. Both carotid sinuses were vascularly isolated and perfused with blood. The left hind limb was vascularly isolated (Hainsworth *et al.* 1975). The femoral artery and the central end of a superficial metatarsal vein were perfused at constant flows with blood from a membrane oxygenator. Blood from the femoral vein drained into a reservoir and was returned to the oxygenator. Responses of arterial and venous resistance were determined by measuring changes in their perfusion pressures. Capacitance responses of the limb were determined by integrating changes in the outflow from the femoral vein using an electromagnetic flowmeter and an on-line computer system (Hainsworth, Karim, McGregor & Wood, 1979).

In thirty-five tests in seven dogs increasing carotid sinus pressure from 8 to 27 kPa resulted in decreases in femoral arterial pressure from 18·5 kPa (mean; S.E. $\pm$ 0·53) to 12·4 $\pm$ 0·4 kPa representing a 35·2 % change in resistance. There was no significant change in perfusion pressure in the superficial vein. The capacitance of the limb showed a small decrease (average change $-3{\cdot}4 \pm 0{\cdot}2$ ml.). However, this response is thought to be a secondary effect of the arterial vasodilation since similar changes occurred when arterial resistance was decreased by an infusion of adenosine which does not affect, at least, the superficial vein (Cotterrell & Karim, 1979). Further, during the increase in venous outflow there was a decrease in haematocrit of 1·2 $\pm$ 0·2 %, suggesting that some of the volume change is due to absorption of tissue fluid.

The results of the present investigation thus showed that although changes in carotid sinus pressure result in large changes in arterial resistance in an isolated perfused hind limb, there was no significant change in tone of a perfused superficial vein, and no change in vascular capacitance.

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Vascular smooth muscle ultrastructure in hypoxia

By A. B. Ebeigbe*, H. Y. Elder and Sheila Jennett. *Institute of Physiology, University of Glasgow, G12 8QQ*

A correlation between oxygen tension of the medium and contractility of isolated vascular smooth muscle (VSM) is well established, but the precise mechanism of action is still unclear. The decrease in mechanical activity might be associated with ultrastructural changes, and ultrastructural differences between VSM from different sites might be related to differences in latency and extent of response (Ebeigbe & Jennett, 1978).

We have investigated ultrastructural characteristics of calcium-containing compartments (mitochondria, sarcoplasmic reticulum (SR), plasma membrane) and cell-to-cell contacts, in normal and hypoxic rat portal vein.

Portal vein strips were suspended in Krebs solution and isotonic spontaneous contractions recorded; control normoxic strips were bubbled throughout with 16% oxygen, 5% CO_2; others were made hypoxic for 30 min ($P_{O_2} < 14$ mmHg) by switching to 95% nitrogen, 5% CO_2; pH and P_{CO_2} were constant. Hypoxia resulted in loss of spontaneous contractions.

Twelve rats were used for six normoxic and six hypoxic strips for electron microscopy. Strips were perfuse-fixed in the organ bath, in buffered glutaraldehyde, and post-fixed in OsO_4. The plane of section was transverse to the long axis of the vessel and the muscle fibres. Quantitative estimations were carried out using computer-linked planimetry (Biddlecombe, Dempster, Elder, Kerr, Moss, Peachey & Spurway, 1977) for whole cell and mitochondrial size ratios and for surface vesicles (SV) and cell-to-cell contacts expressed as number per unit membrane length. Normoxic and hypoxic series were compared by Student's t test.

Hypoxic cells showed: increased mitochondrial size (normoxic:hypoxic, 1:1·5; $P < 0{\cdot}001$); increased number of SV (normoxic: hypoxic 1: 2·05; $P < 0{\cdot}001$); fewer cell-to-cell contacts (1 per 23 μm of linear cell membrane in normoxic samples and 1 per 85 μm in hypoxic samples; $P < 0{\cdot}001$; $n = 6$ hypoxic and 6 normoxic strips for each comparison). There was complete absence of mitochondrial granules; a mean of 0·4 granules per mitochondrion was present in normoxic VSM (preliminary X-ray microanalysis in OsO_4/pryoantimonate-fixed normoxic VSM shows Ca^{2+} in these granules). Associations between SR, SV and mitochondria were more often observed in hypoxic specimens.

Reduction in cell-to-cell contacts suggests impaired intercellular communication. Popescu (1977) has proposed that SV may extrude calcium from VSM; the increase in SV number we observed might be related to increased calcium extrusion. Loss of mitochondrial granules may be due to redistribution of calcium.

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Autonomic nervous activity and myocardial necrosis after DC shocks from cardiac defibrillators

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High-energy d.c. shocks from cardiac defibrillators can cause cardiac necrosis (Dahl, Ewy, Warner & Thomas, 1974; Allen, Pantridge & Patton, 1978). The mechanism of this myocardial damage is not clear.

When 400 J of energy were discharged from the capacitor of a defibrillator (Hewlett-Packard 702-2A; pulse duration 5 msec) across the closed chest of anaesthetized dogs (pentobarbitone 30 mg/kg I.V.) there was a large peak potential difference across the chest and peak transthoracic current. A thermistor catheter (Edwards Laboratories) in the cornonary sinus recorded a peak increase in blood temperature of $0{\cdot}07 \pm 0{\cdot}02$ °C at 20–40 sec after one shock of 400 J (mean ± S.E.M.; 4 dogs). Propranolol (0·4 mg/kg I.V.) reduced this late rise in blood temperature.

Damaged myocardium after d.c. shocks showed disruption of normal sarcomeres. Myofibrils were clumped in irregular bands. Similar contraction bands occur in hearts treated with excessive doses of catecholamines (Reichenbach & Benditt, 1970).

However, pre-treatment with propranolol failed to prevent cardiac necrosis after ten shocks of 400 J at intervals of 0·5 min. One group of six dogs received propranolol (0·4 mg/kg I.V.), 5 min before the shocks, and the other 0·9 % NaCl solution (10 ml.). Three days later $21{\cdot}8 \pm 8{\cdot}8$ g of grossly damaged myocardium were found in the hearts of the four surviving dogs which had received propranolol, and $30{\cdot}1 \pm 10{\cdot}6$ g in the five surviving saline-treated animals ($P > 0{\cdot}05$). β-adrenoceptor blockade with propranolol can reduce the extent of necrosis after occlusion of a major canine coronary artery (Kloner, Fishbein, Cotran, Braunwald & Maroko, 1977).

In a further set of experiments the effects of pre-treatment with reserpine (0·25 mg/kg I.V., 1 day before the shocks) and atropine (0·2 mg/kg I.V., 5 min before the shocks) were compared with those of atropine alone (0·2 mg/kg I.V., 5 min before the shocks), or NaCl alone (10 ml. of 0·9 % solution I.V., 5 min before the shocks). Three days later there was no significant difference between the weights of grossly damaged myocardium ($19{\cdot}8 \pm 4{\cdot}1$ g, reserpine and atropine; $19{\cdot}6 \pm 5{\cdot}0$ g, atropine alone; $13{\cdot}0 \pm 4{\cdot}8$ g, saline-treated; five survivors in each group).

While d.c. shocks from cardiac defibrillators may release catecholamines in the myocardium, neither blockade of cardiac β-adrenoceptors or cholinoceptors, nor depletion of myocardial catecholamines can prevent extensive myocardial damage after such shocks.

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Requirement of the adrenergic nervous system for conservation of sodium by the rabbit kidney

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There is a delay in adaptation of renal sodium excretion ($U_{Na}V$) to reduced sodium intake in patients with generalized failure of the autonomic nervous system (Wilcox, Aminoff & Slater, 1977). We have produced an animal model of the human disease to investigate the role of the nervous system in sodium conservation.

Rabbits were housed in metabolic cages. Daily 'sodium balances' were calculated as the ratio between $U_{Na}V$ and dietary sodium intake (Na_{in}). Faecal losses of sodium were ignored. Dietary sodium content was reduced from 177 to 8 μmole g^{-1} and 'balances' studied for the next 3 days. In untreated rabbits, the ratio $U_{Na}V/Na_{in}$ on the day preceding sodium restriction was $0{\cdot}99 \pm 0{\cdot}05$ and during sodium restriction it was: day 1, $7{\cdot}54 \pm 1{\cdot}23$; day 2, $2{\cdot}07 \pm 0{\cdot}38$; day 3, $1{\cdot}07 \pm 0{\cdot}25$ (mean $\pm$ S.E.M., $n = 27$).

The role of the renal nerves was investigated in six rabbits. Under thiopentone sodium anaesthesia both kidneys were dissected free of fat, the nerves were severed, and renal arteries, veins and ureters cleared of adventitious tissue for a length of $\geqslant 0{\cdot}5$ cm. This cleared area was painted with 5% phenol in ethanol. Between 3 and 7 weeks after operation the rabbits' responses to sodium deprivation were studied thrice. $U_{Na}V/Na_{in}$ was increased on each of the 3 days of restricted sodium intake. The increase was significant as assessed by analysis of variance (variance ratio (F) = 5·81, 1 degree of freedom, $P < 0{\cdot}05$). In the four sham-denervated control rabbits this change was not observed ($F = 0{\cdot}53$, $P \simeq 0{\cdot}5$).

A reversible model of sympathetic failure was investigated by treating seven rabbits with either guanethidine (12 mg kg^{-1} every 12 h, I.P.) for $\geqslant 5$ days or reserpine (0·05 mg kg^{-1} day^{-1}, I.V.) for $\geqslant$ 6–8 weeks. These doses sufficed to increase 2–5 times the dose of tyramine needed to produce a given pressor response. On restricting sodium intake in these rabbits, $U_{Na}V/Na_{in}$ was greater than when they received no drugs, both with guanethidine ($F = 34{\cdot}62$, $P < 0{\cdot}001$) and reserpine ($F = 15{\cdot}30$, $P < 0{\cdot}001$), particularly on day 2. The effect was reversed within 3 weeks of stopping the drugs.

However, in some rabbits both drugs reduced food intake which itself impaired sodium conservation. Nevertheless, excess sodium excretion was inversely related to deficiency in food intake (day 2: $r = -0{\cdot}57$, $P < 0{\cdot}005$, $n = 25$), and thus the increase in $U_{Na}V/Na_{in}$ in rabbits receiving drugs cannot be entirely attributed to reduced food intake. The excess $U_{Na}V$ on day 2 in rabbits who were not starved was $1{\cdot}13 \pm 0{\cdot}57$ m-mole with guanethidine and $1{\cdot}69 \pm 0{\cdot}61$ m-mole with reserpine.

Thus different experimental circumstances sharing the common element of inhibition of the function of the renal adrenergic nervous system retard the rabbit's ability to conserve sodium during adaptation to restricted sodium intake.

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Pulmonary capillary luminal surface carbonic anhydrase

By M. A. Hanson, P. C. G. Nye and R. W. Torrance. *University Laboratory of Physiology, Parks Road, Oxford OX1 3PT*

Crandall now accepts that carbonic anhydrase, fixed to lung tissue, acts upon blood plasma as it traverses the pulmonary capillaries (Crandall & O'Brasky, 1978). We suggest that this carbonic anhydrase is on the luminal surface of the capillary endothelium.

We found in cats under pentobarbitone that Tris (2 ml. 150 mM) injected suddenly into the right atrium transiently lowered $F_{ET}CO_2$, presumably by drawing CO_2 from the alveoli and fixing it in Tris $H^+HCO_3^-$. The fall in $F_{ET}CO_2$ was increased by adding carbonic anhydrase (100 units/ml.) to the injectate and was reduced by prior intravenous acetazolamide (50 mg/kg). Benzolamide (2 mg/kg I.V.), a carbonic anhydrase inhibitor which penetrates cells more slowly than acetazolamide, also reduced the effect of tris on $F_{ET}CO_2$ but the effect was restored if carbonic anhydrase (*ca.* 500 units/ml.) was added to the Tris injection. After acetazolamide, the concentration of carbonic anhydrase needed for restoration increased with the dose of acetazolamide.

Even if benzolamide or acetazolamide was added to the injectate (20 μg/ml.) it reduced the effect of Tris and also of bovine albumin (20%, alkalinized) on $F_{ET}CO_2$ as much as did the drug given intravenously.

Further, in isolated cat lungs perfused with low P_{CO_2} saline and ventilated with 5% CO_2 in O_2, tris or albumin lowered $F_{ET}CO_2$. Benzolamide or acetazolamide added to these injections reduced their effect.

Our observations with intravenous doses of carbonic anhydrase inhibitors do no more than support the conclusion already reached that plasma is acted upon by carbonic anhydrase fixed to lung tissue. This carbonic anhydrase could be (1) on the luminal surface of capillary endothelial cells thus acting directly on plasma, (2) on their albuminal surface or deeper thus acting extracellularly outside the vessels or (3) within capillary endothelial cells, there forming H_2CO_3 which diffuses into the plasma through their membranes.

Benzolamide penetrates cells slowly. Also, proceeding from Chinard (1969), neither benzolamide nor the buffers should significantly leave the vessels during their first transit. Benzolamide must, however, inhibit carbonic anhydrase in its first transit if it can reduce the action of Tris or albumin injected with it. This suggests that the carbonic anhydrase is on the luminal surface of the capillaries and so acts directly on plasma.

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The effects of 1-sar-8-ala-angiotensin II (saralasin) on the renal responses to a high dose of angiotensin II in rats

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It is well established that low doses of angiotensin II cause sustained reductions in the urinary excretion of sodium and water, while high doses have a biphasic action (i.e. an intial retention of sodium and water, followed by natriuresis and diuresis; Barraclough, Jones & Marsden, 1967). We have investigated the renal actions of a high dose of angiotensin II (700 ng kg^{-1} min^{-1}) in the presence and absence of saralasin (5 μg kg^{-1} min^{-1}), which is a competitive antagonist of some of the actions of angiotensin, including the renal vasoconstrictor actions (see Kimbrough, Vaughan, Carey & Ayers, 1977). The experimental results below are quoted as the mean $\pm$ S.E. of the mean, and were analysed by paired Student's t test.

In eleven female rats angiotensin infusion caused initial significant reductions in urine flow (-37 ± 16 μl. min^{-1}; $P < 0.01$) and $U_{Na}V$ (-5.5 ± 2.2 μmole min^{-1}; $P < 0.05$); this lasted 5 min. In the subsequent 10 min of the angiotensin II infusion the urine flow rose significantly above the pre-angiotensin II level ($+96 \pm 31$ μl. min^{-1}; $P < 0.02$), as did $U_{Na}V$ ($+16.2 \pm 2.2$ μmole min^{-1}; $P < 0.01$).

In eight similar rats given a continuous background infusion of saralasin, angiotensin II did not cause an initial (5 min) reduction in urine flow (the change from the pre-angiotensin II value was -2 ± 10 μl. min^{-1}; NS) nor in $U_{Na}V$ (0 ± 1.4 μmole min^{-1}; NS). Thus saralasin abolished the sodium and water retaining actions of angiotensin II. In the subsequent 10 min period of angiotensin II infusion the urinary responses were similar to those in animals not given saralasin: the urine flow rose significantly above the pre-angiotensin II level ($+70 \pm 21$ μl. min^{-1}; $P < 0.02$) as did $U_{Na}V$ ($+10.3 \pm 3.0$ μmole min^{-1}; $P < 0.02$). Thus saralasin did not abolish the diuretic and natriuretic actions of angiotensin II.

The angiotensin II infusion caused a significant pressor response in untreated animals ($+43 \pm 5$ mmHg; $P < 0.001$), but the response was much much smaller ($+9 \pm 4$ mmHg; NS) in saralasin-treated rats. Thus saralasin significantly inhibited the pressor response to angiotensin II ($P < 0.001$; unpaired t test).

After the infusion of angiotensin II was stopped, retention of sodium and of water were again observed; these responses were also abolished by saralasin treatment.

It is inferred that (1) the two phases of angiotensin action are due to two independent renal responses; (2) these two responses are mediated by different renal receptors; (3) the initial antinatriuretic response may depend on haemodynamic changes; (4) the natriuretic phase is not dependent on the pressor response to angiotensin II.

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Exposure to oxygen inhibits metabolism of vasoactive hormones in rat isolated lung

By Y. S. Bakhle, J. Hartiala and H. Toivonen. *Department of Pharmacology, Royal College of Surgeons, London WC2A 3PN, and Department of Physiology, University of Turku, Turku, Finland*

Exposure of rats to high concentrations of oxygen causes, among other effects, diminished pulmonary metabolism of 5-hydroxytryptamine (5-HT; Block & Fisher, 1977) and prostaglandin E_2 (PGE_2; Klein, Fisher, Soltoff & Colburn, 1978). Both these substrates are metabolized by intracellular enzymes. We have investigated the effect of oxygen exposure on the activity of the membrane-bound angiotensin converting enzyme in lung towards angiotensin I and bradykinin. To make comparison with earlier work easier, we also studied 5-HT and PGE_2 metabolism.

Male rats (250–310 g) were exposed to 95% O_2 at 1 ata for times up to 60 hr (Välimäki, Kivisaari & Niinikoski, 1974); 'sham' exposure was to air under the same conditions and control rats were untreated. Immediately after exposure the lungs were removed and perfused with Krebs solution via the pulmonary circulation. Metabolism of angiotensin I, bradykinin and PGE_2 was measured by bioassay and that of 5-HT by a radiochemical method using [5-^{14}C]hydroxytryptamine.

Sham exposure of rats up to 60 hr did not affect the metabolism of any of the four substrates and neither did oxygen exposure for 24 hr. However, by 36 hr, PGE_2 metabolism was decreased, i.e. survival was increased, rising to a maximum at 48 hr (23 ± 3%; control 8 ± 1%, $n = 6$). Angiotensin conversion was next affected, falling after 48 hr and 60 hr to 16 ± 2% from a control value of 24 ± 2%. Bradykinin and 5-HT metabolism were not decreased until after 60 hr oxygen exposure.

The diminished conversion of angiotensin I suggests that oxygen exposure affects the plasma membrane of endothelial cells. Our experiments are in general agreement with earlier work but, whereas Block & Fisher reported a reduction in 5-HT metabolism after 18 hr, we observed no effect until after 60 hr exposure. Furthermore, in our experiments, the early effects on PGE_2 metabolism suggest an early effect of oxygen on non-endothelial cells, whereas Kistler, Caldwell & Weibel (1967) showed endothelial cells to be affected first. These discrepancies reinforce the view (Clark & Lambertsen, 1971) that although there is good agreement that exposure to high concentrations of oxygen damages lungs, the details of that damage vary considerably between different experimental situations.

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Metabolism of arachidonic acid and its endoperoxide prostaglandin H_2 in isolated lungs from guinea-pigs and rabbits

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Arachidonic acid (AA) perfused through the pulmonary circulation of guinea-pig isolated lungs is metabolized to a mixture of myotropic substances consisting principally of thromboxane A_2 (TxA_2) and prostaglandin I_2 (PGI_2) (Alabaster & Hawkeswood, 1978*a*; Boot, Cockerill, Dawson, Mallen & Osborne, 1977). The immediate precursor of both TxA_2 and PGI_2 is the endoperoxide PGH_2 and microsomal preparations of rabbit lung convert PGH_2 to TxA_2 and 6-keto $PGF_1\alpha$, the stable metabolite of PGI_2 (Sun, Chapman & McGuire, 1977).

The metabolism of AA and PGH_2 in Krebs-perfused isolated lungs from the guinea-pig and rabbit has been compared, measuring the myotropic products in lung perfusate by bioassay (Vane, 1964) incorporating a delay circuit (Alabaster & Hawkeswood, 1978*b*). The following assay tissues were used: rabbit aorta, rabbit mesenteric artery, rat stomach strip, bovine coronary artery and rat colon.

In guinea-pig lung, AA (2·5 μg) was metabolized principally to TxA_2 (104 ± 15 ng, $n = 8$) and to PGI_2 (60 ± 5 ng, $n = 7$). A small amount (10–20 ng) of PGE_2 was also produced but no PGH_2 was detected (lower limit of sensitivity of assay was 16–20 ng). The amounts of the products were dose-related over the AA range 1–10 μg. In addition, PGH_2 (30–70 ng) was detected in three out of five lungs after AA 10 μg. Rabbit lung also converted AA (2·5–20 μg) to TxA_2, PGI_2 and PGE_2 in similar relative proportions but the amounts were some 15–25% of those in guinea-pig lung.

The endoperoxide PGH_2 is rapidly and specifically converted to PGI_2 by microsomal preparations of endothelial cells (Moncada, Herman, Higgs & Vane, 1977). However, PGH_2 (200–400 ng) in guinea-pig lungs was converted to TxA_2 (20–40 ng) but was not converted to any detectable PGI_2. In five lungs, injections of 800 ng PGH_2 produced TxA_2 101 ± 13 ng, PGI_2 10–16 ng (in three out of five lungs) and small amounts of PGE_2 (20–30 ng) and surviving PGH_2 (10–25 ng). In contrast, PGH_2 (200–800 ng) was not metabolized to any detectable TxA_2 or PGI_2 in rabbit lungs and only unchanged PGH_2 and some PGE_2 was detected, which together accounted for only 6–8% of the PGH_2 injected.

It seems unlikely therefore that the lungs of guinea-pig and rabbit have an important role in the conversion of PGH_2, produced for example by aggregating platelets, into the potent anti-aggregatory and vasodilator PGI_2. However, the lung may well produce PGI_2 from either endogenous or exogenous AA for release into the systemic circulation.

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Cerebral lysosomal enzyme levels and the immune response to infection with avirulent Semliki forest virus in *nu/nu* (athymic) and conventional mice

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The pathological changes which develop in the central nervous system of conventional (Swiss/A_2G) mice, or mice heterozygous for the *nu* gene, after infection with an avirulent strain of Semliki forest virus (SFV A7[74]) include meningitis, perivascular inflammation, microcystic changes, astrocytosis and demyelination. To date, infection of homozygous *nu/nu* (athymic) mice bred in our Animal House, does not result (apart from the microsystic changes) in the production of pathological changes. The pathology might therefore be attributed to an immunologically mediated reaction, probably dependant on the presence of immunocompetent T-lymphocytes (Jagelman, Suckling & Webb, 1978).

Previous work in conventional mice has suggested that rises in the activities of certain lysosomal enzymes in brain may reflect the development of some aspects of the intracerebral immune response. These increases in activity are not due to high virus titres in the brain itself because they do not occur until levels of brain virus have begun to fall as the intracerebral immune reaction develops (Suckling, Webb, Chew-Lim & Oaten, 1976). Additional evidence for this suggestion would be provided by following the activity of a representative lysosomal enzyme in the brains of *nu/nu* mice infected with SFV A7(74) where the conventional intracerebral immune response-mediated pathology is absent.

The activity of *N*-acetyl-β-D-glucosaminidase in uninoculated groups of *nu/nu*, *nu/+* and conventional Swiss/A_2G mice did not vary significantly between the groups and an overall mean was $13{\cdot}6 \pm 0{\cdot}6$ mg *p*-nitrophenol liberated per g wet weight per hour ($n = 15$). In infected *nu/nu* mice *N*-acetyl-β-D-glucosaminidase activity was $14{\cdot}3 \pm 0{\cdot}2$ (4) on day 5 post-inoculation and insignificantly increased ($P > 0{\cdot}05$) to $14{\cdot}8 \pm 0{\cdot}7$ (8) by day 10 post-inoculation in *nu/nu* mice. Corresponding values for *nu/+* mice were $13{\cdot}4 \pm 0{\cdot}3$ (5) and $16{\cdot}8 \pm 0{\cdot}9$ (9) which represents an increase of 25%, significant at the 0·01% level. In conventional mice an increase of 30% was observed again significant at the 0·01% level.

We therefore confirm that the increase in *N*-acetyl-β-D-glucosaminidase activity may reflect the development of the intracerebral immune response.

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The glycoprotein from human gastric mucus gel and its breakdown by pepsin

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Gastric mucus gel is considered to protect the mucosa by forming on its surface an unstirred layer, in which bicarbonate ions secreted by the mucosa neutralize the HCl diffusing in from the lumen (Hollander, 1954). Mucus is a glycoprotein gel and to understand its structure and function it is important to isolate from it the undegraded glycoprotein in contrast to the degraded material found in the lumen (Allen, 1978).

We have isolated the undegraded glycoprotein from human gastric mucus gel obtained from gastric resection specimens. The mucus gel was solubilized by homogenization in a neutral solution to inactivate pepsin. The glycoprotein was purified from free, non-covalently bound protein and nucleic acid by density gradient centrifugation in a CsCl gradient (Starkey, Snary & Allen, 1974). On ultracentrifuge analysis the glycoprotein sedimented as a single peak with a molecular weight of 2×10^6. On gel-filtration analysis, using Sepharose 2B, this glycoprotein was completely excluded.

The glycoprotein, after proteolytic digestion with pepsin, papain or Pronase, or chemical reduction by mercaptoethanol, eluted as a single included peak on Sepharose 2B. In each case an identical elution profile was obtained to the corresponding proteolytically digested or reduced glycoprotein of known molecular weight, 500,000, from pig gastric mucus.

These results demonstrate that the glycoprotein (mol. wt. 2×10^6) in human gastric mucus has a polymeric structure of glycoprotein subunits (mol. wt. about 500,000). These subunits are joined by disulphide bridges located on regions of the non-glycosylated protein core which are hydrolysed by pepsin and other proteolytic enzymes. From these findings it is clear that results obtained in pig gastric mucus (see Allen, 1978) are directly applicable to man and thus there is a direct relationship between the breakdown, by proteolysis or chemical reduction, of the undegraded glycoprotein to subunits and the solubilization of the mucus gel and loss of the viscous properties of its glycoprotein constituents.

It follows that on the mucosal surface there is a dynamic balance between production of new gel and its erosion by pepsin, the undegraded glycoprotein and the glycoprotein subunits being characteristic of the former and latter respectively. In pathological conditions this dynamic balance may be upset and the ratio of degraded to undegraded glycoprotein increased.

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Hepatocellular extraction and retention of fructose in conscious sheep

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In man, fructose has a much shorter biological half-life than D-glucose (Smith, Ettinger & Seligson, 1953; Conard, 1955). Although the hepatic first-pass extraction of an infused trace of D-glucose is three times that of fructose, the former rapidly leaves the liver cells whilst the latter does not (Hooper & Short, 1977). This observation in conscious sheep provides evidence of hepatic retention of fructose but not of D-glucose. However, the observations were made in the presence of 2·4 mM blood D-glucose, and only tracer amounts of fructose. Experiments have now been performed in which the hepatic extraction and retention of fructose was examined in sheep with elevated plasma fructose concentrations.

Six experiments were performed on three sheep with chronic, indwelling portal venous (PV) and hepatic venous (HV) catheters. A continuous infusion, double-indicator dilution technique (using [^{3}H]fructose with [^{14}C]sucrose as the extracellular reference, Hooper & Short, 1977) was used to determine the fructose extraction during an initial control run (tracer fructose only) and an experimental run, 20 min later, in which PV fructose concentrations of 1·6–18·5 mM were achieved by infusion of unlabelled D-fructose. Mean hepatic fructose extraction ($\bar{E}_{fructose}$) was calculated for the first 5 sec after the initial appearance of the [^{14}C]sucrose reference in the HV. Efflux was monitored from 5 to 18 sec after initial appearance, using the regression of extraction with time.

During the control run the $\bar{E}_{fructose}$ was 21·3 ± 3·5 % (S.D., n = 6) and the regression of extraction with time was negligible, indicating that there was no fructose efflux. With elevated PV fructose concentrations the $\bar{E}_{fructose}$ remained unchanged (21·3 ± 9·9 %, S.D., n = 6). A significant efflux of fructose was only observed in three of the six experimental runs, the mean regression coefficient being $-0{\cdot}70 \pm 0{\cdot}40$ % sec^{-1} (S.D., n = 3). The magnitude of the efflux did not appear to be related to the fructose concentration.

The observation that increasing the PV fructose concentration does not affect the hepatic fructose extraction is contrary to the results obtained in earlier preliminary experiments (Hooper & Short, 1977). Together with the observation that fructose efflux was not consistently reduced, this implies hepatic retention of fructose greatly in excess of D-glucose. This provides a basis for rapid equilibration of D-glucose between liver cells and blood whilst also providing for the more rapid disappearance of fructose from the circulation.

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Effects of sodium cholate infusions on bile flow in guinea-pigs

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Bile salts are believed to stimulate bile flow by creating an osmotic gradient within the biliary canaliculi (Sperber, 1959). Bile salts differ in their effectiveness as choleretics, and various explanations have been proposed including differences in micellar aggregation and effects on independent secretory mechanisms (Erlinger & Dhumeaux, 1974).

Guinea-pigs (350–460 g) were anaesthetized with urethane (7·5 ml./kg. body weight I.P. of a 25% solution; two-thirds of this as an initial dose). Bicarbonate-saline ($NaHCO_3$ 120 mM; NaCl 30 mM) was infused continuously via the right jugular vein at the rate of 0·18 ml./min. Bile salts were infused for one hour at rates ranging from 0·45–0·90 μmole/min. Bile was collected by cannulation of the bile duct. The cystic duct was ligated. Bile salts in bile were separated by thin-layer chromatography (Panvelliwalla, Lewis, Wootton & Tabaqchali, 1970) into four fractions: cholate, glycochenodeoxycholate, glycocholate, and all the taurine conjugates. Each fraction was eluted and assayed with steroid dehydrogenase.

The increase in bile flow in μl./min (y) produced by infusions of sodium cholate could be linearly related to the increase in the rate of secretion of bile salts in bile in μmole/min (x): $y = 20{\cdot}81x + 7{\cdot}07$ ($n = 7$; $r = 0{\cdot}83$). The slope of this line does not differ significantly from that obtained for infusions of sodium taurocholate: $y = 20{\cdot}32x + 1{\cdot}12$ ($n = 8$; $r = 0{\cdot}96$). The vertical distance between the two lines (6·22 μl./min) differs significantly from zero ($P < 0{\cdot}002$). In the experiments with sodium cholate only 10% of the bile salt in bile appeared as unchanged sodium cholate. The remainder was conjugated with glycine and taurine. The ratio of glycine to taurine conjugation was 0·3 at the lower rates of bile salt infusion rising to 1·0 at the higher rates. Simultaneous infusion of taurine together with sodium cholate (0·45–0·9 μmole/min) after a priming dose of taurine of 30 μmole ($n = 9$) increased the proportion of taurine conjugated bile salts in bile to 75% but did not reduce the vertical distance between the regression lines (6·22 μl./min; $P < 0{\cdot}001$), the slope of the line again not differing significantly from that for sodium taurocholate ($y = 16{\cdot}01x + 9{\cdot}44$; $r = 0{\cdot}63$). As sodium cholate appeared in bile mainly as sodium taurocholate the additional choleretic effect may be due to an effect of sodium cholate on other secretory mechanisms.

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Reduced liver glycogen depletion in fasted genetically obese (Zucker) rats

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A strikingly high liver glycogen concentration (approximately 6%, w/w) was reported (Lemonnier, 1971) in 2½-month-old obese rats of the Zucker strain following an 18 hr fast, while in lean litter-mates the glycogen levels fell to less than 1%.

Twelve rats were used in a 2 × 2 factorial design (see Table 1) to investigate this phenomenon. At between 14.00 and 15.00 hr on the day of experiment a lethal dose (60 mg) of pentobarbitone sodium was injected intraperitoneally. As each animal became deeply anaesthetized the liver was removed and frozen. Three or four replicate assays were performed on weighed samples of each liver, using a spectrophotometric modification of a microassay for glycogen (Lust *et al.* 1975).

TABLE 1. Liver glycogen concentration, % wet weight in fed or fasted lean or obese rats of the Zucker strain. One rat (*) was female

	Obese			Lean		
	Body wt (g)	Age (day)	Liver glycogen, % wet weight (mean ± S E.M.)	Body wt (g)	Age (day)	Liver glycogen, % wet weight (mean ± S.E.M.)
Fasted (24 hr)	473	138	1·50 ± 0·02	293	138	0 (−0·01 ± 0·13)
	495	118	1·19 ± 0·11	329	118	0 (−0·10 ± 0·07)
	545	124	1·93 ± 0·06	266	124	0·26 ± 0·14
		Mean	1·54 ± 0·21		Mean	0·09 ± 0·09
Fed	580	101	3·44 ± 0·12	340	101	5·17 ± 0·07
	480*	108	3·87 ± 0·08	342	108	8·05 ± 0·23
	575	120	3·63 ± 0·03	342	120	4·68 ± 0·18
		Mean	3·65 ± 0·13		Mean	5·97 ± 1·05

The results are shown in Table 1. Mean values for each liver were calculated, and group mean values calculated from these all differed significantly from each other (*t* test for two independent samples). Analysis of variance revealed a strong interaction between the effects of obesity and of fasting on liver glycogen levels ($F = 67{\cdot}1$, D.F. 1, 8, $P < 0{\cdot}001$).

Quantitatively, these results differ from those published previously (Lemonnier, 1971). Nonetheless, liver glycogen reserves were exhausted relatively slowly by the fasting obese rats, which would be consistent with other evidence implying that gluconeogenesis may be particularly active in these animals (Wade, 1979).

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An unpublished manuscript of Francis Glisson (1662) on the relation of the Harveian circulatory theory to medicine

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Francis Glisson (1597–1677), Regius Professor of Physick in Cambridge from 1636, a founding Fellow of the Royal Society, and thrice President of the College of Physicians, is well known for his study of rickets and of the anatomy of the liver, and included in his contributions to physiology is the notion of irritability. His writings richly exhibit humanistic scholarship. In 1662 Glisson examined for the M.D. degree Dr R. Spratlinge, who presented a thesis that 'The doctrine of the circulation of the blood in no way changes the ancient *methodus medendi*.' The record of this is on three folios of Glisson's somewhat conservative handwriting (Glisson, 1662). Three sides have disconnected notes, presumably made while the thesis was being presented, and three have a single passage of continuous prose setting out Glisson's opinion.

Galen's *methodus medendi* is a logical scheme, with associated physiology (in the word's modern sense), providing a theoretical framework for medicine. The circulatory theory was seen to challenge this *methodus* (Frank, 1971; Whitteridge, 1971). In this MS Glisson first argues that the *methodus* is not changed by the circulatory doctrine, which simply elucidates the Galenic 'vital' faculty. He next abandons this position since there are exceptions to the equivalence, namely the indications for therapeutic blood-letting, which must now be different. However, the medicine of particular cases rests not on the *methodus* but on that *experientia* (disciplined experience) on which the *materia medica* is based, while the *methodus* stands as a permanent theoretical system. Thus Glisson accepts the thesis on the ground of a divorce between theory and practice.

We find therefore a scholar and experimental philosopher, as Glisson was, supporting empirical medicine. However, the theoretical disarray of medicine after Harvey can be exemplified from other sources also.

This communication was illustrated by photo-copies, a transcription and a translation.

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The electrical potential across the ovine nasal mucosa

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The main function of the respiratory epithelium of the nasal cavity is to secrete fluid to humidify the inspired air.

As the first step in an investigation of the process of secretion we have investigated the electrical properties of this tissue.

Sheep were killed under thiopentone sodium anaesthesia and the nasal cavity opened. Pieces of mucosa were freed from the underlying structures by blunt dissection and mounted in conventional Ussing chambers.

Upon setting up the tissue a small potential of $1{\cdot}07 \pm 0{\cdot}78$ mV ($n = 19$) was recorded between the two surfaces, the mucosal side being positive. Mucosa covering the ethmoid bone gave higher potentials than that from other regions but this difference did not correlate with the resistance of the tissues which varied between 40 and 100 Ω cm^2 with an average value of 70 ± 11 Ω cm^2 ($n = 19$).

The potential was reduced when sodium in the bathing medium was replaced with choline but was not affected by 10^{-2} M ouabain. This suggested that the potential might not be due to transport as we had assumed but was either a Donnan potential due to secreted mucus or a receptor potential from the olfactory sensory endings. To test the first possibility mucus was dialysed against saline and the potential across the dialysis membrane measured. The result agreed with that found by Balfre (1979) in the chicken, that the mucus side was a fraction of a millivolt negative with respect to the saline. The receptor potential is also reported as being negative at the mucosal surface (Ottoson, 1959) so neither of these possibilities will account for our observation.

We are therefore presently investigating the effect of ion replacement and metabolic and transport inhibitors on the transepithelial ion fluxes.

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Monosynaptic excitation of bulbospinal respiratory neurones by chemoreceptor afferents in the carotid sinus nerve

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The carotid sinus nerves of cats anaesthetized with pentobarbitone and paralysed with gallamine were freed from surrounding tissue, laid in continuity over a platinum wire electrode and covered in Vaseline. Baroreceptor and chemoreceptor discharges appeared as diphasic unitary spikes. With other arterial branches tied, occlusion of the common carotid artery led to a fall in intrasinusal pressure, abolition of baroreceptor discharges and the appearance of abundant chemoreceptor discharges with spikes of longer duration than the baroreceptor spikes.

Simultaneous recordings were also made from single respiratory neurones via glass micro-electrodes in the ipsilateral nucleus of the solitary tract and from the phrenic nerve (Fig. 1*A*). Neurones were identified as bulbospinal by antidromic invasion following stimulation of the spinal cord (Fig. 1*B*).

Cross-correlation histograms were constructed between the discharges of chemoreceptors and respiratory bulbospinal neurones. When narrow ranges of chemo-

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receptor spike amplitude were used, some ranges (arrows in Fig. 1*A*) gave repeatable peaks in the histograms (Fig. 1*C*, *D*). The increased probability of firing restricted to one bin (0·6 msec) demonstrates monosynaptic excitation of the bulbospinal neurone from a chemoreceptor afferent with a spike within that amplitude range.

Experiments are in progress using, for the cross-correlation, discharges of single chemoreceptor afferents recorded in the petrosal ganglion.

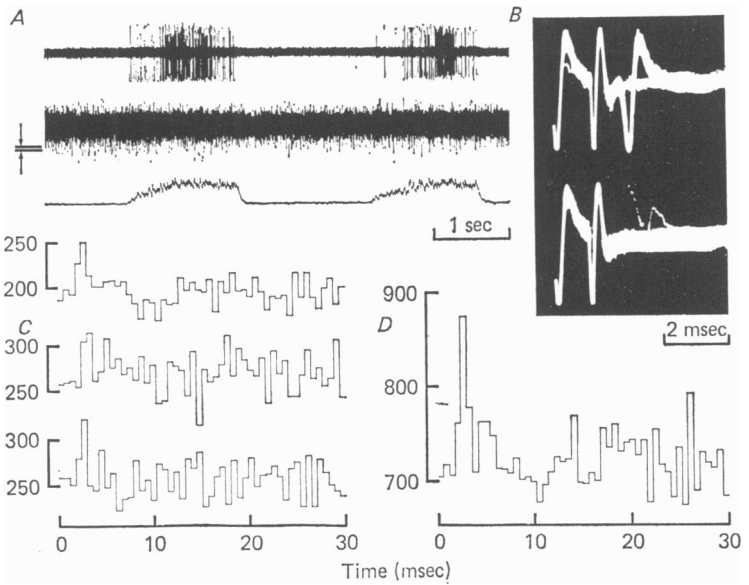

Fig. 1. *A*, top, bulbospinal neurone; middle, chemoreceptor discharges; bottom, integrated phrenic activity. *B*, collision test for bulbospinal neurone. Independent (*C*) and summed (*D*) cross-correlation histograms for data illustrated in *A* (chemoreceptor discharges as triggers).

Dopamine and homovanillic acid concentrations in the post-mortem brain in schizophrenia

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Concentrations of dopamine and homovanillic acid (HVA) have been assessed by radiometric and gas chromatographic methods in caudate nucleus, putamen and nucleus accumbens, in post-mortem brains from patients with schizophrenia and controls. Diagnosis was established by application of Present State Examination (Wing, Cooper & Sartorius, 1974) criteria to case notes. The findings in series of brains collected at two centres (Cambridge and the C.R.C.) were compared.

Dopamine concentrations were significantly increased in caudate nucleus ($P < 0·05$) in the C.R.C. series and in nucleus accumbens ($P < 0·005$) in the Cambridge series. Homovanillic acid concentrations were significantly increased in putamen in the Cambridge series and were significantly ($P < 0·02$) decreased in caudate nucleus in the C.R.C. series. Calculation of HVA/dopamine ratios (a possible index of

dopamine turnover) revealed a significant increase in this index in nucleus accumbens of both schizophrenic and controls by comparison with other areas in the C.R.C. series. A significant decrease in dopamine turnover assessed as HVA/dopamine ratios in caudate nucleus of schizophrenics in the C.R.C. series was not replicated in the Cambridge series of brains.

TABLE 1

	Caudate	Putamen	N. Accumbens
	C.R.C.		
Dopamine (μg/g)			
Controls	1·6 ± 0·3 (19)*	2·0 ± 0·4 (19)	0·9 ± 0·3 (18)
Schizophrenics	2·5 ± 0·3 (18)	2·2 ± 0·3 (18)	0·7 ± 0·1 (16)
HVA (μg/g)			
Controls	5·4 ± 0·3 (19)**	4·7 ± 0·5 (19)	4·7 ± 0·5 (18)
Schizophrenics	3·8 ± 0·4 (18)	5·9 ± 0·6 (18)	5·5 ± 0·5 (16)
HVA/DA ratio			
Controls	4·7 ± 0·7 (19)**	4·0 ± 0·8 (19)	11·0 ± 2·0 (18)
Schizophrenics	1·9 ± 0·4 (18)	3·8 ± 0·7 (18)	11·9 ± 2·2 (16)
	Cambridge		
Dopamine (μg/g)			
Controls	1·7 ± 0·2 (22)	2·4 ± 0·2 (18)	1·4 ± 0·1 (23)***
Schizophrenics	2·0 ± 0·2 (24)	2·5 ± 0·3 (17)	2·0 ± 0·1 (23)
HVA (μg/g)			
Controls	4·3 ± 0·4 (25)	7·4 ± 0·6 (20)**	4·4 ± 0·4 (24)
Schizophrenics	5·6 ± 0·8 (28)	10·3 ± 0·9 (19)	4·9 ± 0·6 (24)
HVA/DA ratio			
Controls	2·9 ± 0·4 (22)	3·7 ± 0·4 (18)	3·5 ± 0·4 (23)
Schizophrenics	3·0 ± 0·5 (24)	4·5 ± 0·5 (17)	2·7 ± 0·3 (23)

* $P < 0.05$ for t test versus controls; ** $P < 0.02$ for t test versus controls; *** $P < 0.005$ for t test versus controls; values $\pm$ = S.E.M.

The findings suggest that although dopamine concentrations may be somewhat increased in some areas of schizophrenic brain, dopamine turnover, assessed by concentrations of homovanillic acid or HVA/dopamine ratios, is not consistently increased.

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The mechanism of potassium dispersal in brain tissue

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When a local build-up of extracellular potassium occurs in neural tissue, depolarization of nerve and glial cells may cause currents to flow through the cells in such a manner as will assist the dispersal of potassium (the spatial buffer mechanism: Kuffler, Nicholls & Orkand, 1966). The fact that such regions commonly develop an extracellular negativity with respect to the rest of the tissue (see Somjen, 1979, for review) supports this idea, but it has never been clear whether the contribution

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made to K^+ dispersal is significant compared with extracellular diffusion. In the present study the extracellular negativity is examined quantitatively both in isolated pieces of frog brain *in vitro* and in the neocortex and cerebellum of anaesthetized rats. The results suggest that the spatial buffer mechanism is in some circumstances the principal means for K^+ dispersal.

A closed chamber on the brain surface (0·5–3 mm diameter) is perfused with artificial cerebrospinal fluid so that the surface K^+ concentration can be changed rapidly. The resulting changes of potential are measured with bridge electrodes between the interior of the chamber and the bath or pool of fluid (of fixed composition) outside the chamber. Raising the K^+ concentration makes the chamber negative, by approximately 0·5 mV/mM for small concentration changes. The initial rise is more rapid in the frog (2–3 sec for 50 % rise) than in the rat (20–40 sec). The potential changes in the *in vitro* preparation are reduced to 10 % by formalin fixation but are little affected by ouabain (10^{-4} M). The size and time course of the effects of changes from base-line levels (2·5–3 mM) to values over the range 0–20 mM can be fitted by simultaneous numerical solutions of the diffusion and cable equations implicit in the spatial buffer hypothesis. The data requires parameters such that over distances greater than a certain space constant (*ca.* 200 μm (rat) or 30 μm (frog)) about 80 % of the K^+ moving through the tissue is moving through cells rather than through extracellular space. This is a similar conclusion to that previously reached (in experiments on rats) for a flux of K^+ caused by current through the tissue rather than by a concentration gradient (Gardner-Medwin, 1977, 1978; Gardner-Medwin & Nicholson, 1978). Whether or not the underlying mechanism is essential for normal neural functioning, it probably helps to reduce the build-up of K^+ round active neural tissue until such time as it is restored by active transport to its cells of origin.

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Responses of muscle and joint afferents recorded from the Gasserian ganglion of rabbits

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Recordings were made from neurones in the mandibular area of the Gasserian ganglion in rabbits which were anaesthetized with halothane and N_2O. The skull was fixed in a frame and the root of the left zygomatic arch was exposed and a pin inserted into its upper surface. Medial and lateral bur cuts were made to detach the temporo-mandibular joint socket from the skull while leaving the articular capsule intact. The left cerebral hemisphere was removed to allow recordings to be made with tungsten micro-electrodes from the left Gasserian ganglion. Stimulating electrodes were implanted into the masticatory area of the right cerebral cortex. Jaw

movements were recorded as the changing rectangular co-ordinates of spots of light projected on to photodiode position transducers from a small lamp attached to the mandibular symphisis. Of the 1500 units that fired in phase with active or passive jaw movements, the vast majority also responded to local stimulation of hairs, skin, mucosa or teeth.

Eleven units were identified as temporomandibular joint receptors by tilting the detached socket while the jaws were fixed together to prevent stimulation of other receptors. The pin attached to the socket was then clamped to the frame and each unit's response recorded during passive jaw movements and, when possible, during cortically evoked masticatory movements. In lagomorphs, as in man, the mandibular condyle moves in the horizontal plane and rotates about horizontal and vertical axes during mastication. Consistent with this, the joint receptors were generally excited by a combination of rotation and horizontal displacement of the jaw and with each there was a correlation between firing frequency and chin displacement through some part of the range of possible movements. Five units were most active during depression of the mandible, when the condyle glided forward and rotated. The remainder were activated by moving the mandible in the horizontal plane and this was usually coupled with horizontal rotation of the condyle. All were unidirectional and, with one exception, no impulses were generated when the jaw was in the resting position. Six were rapidly adapting.

Twelve units were identified as muscle afferents by local mechanical and electrical stimulation of the jaw closing muscles: 7 appeared to be from spindles and 5 from Golgi tendon organs. During masticatory movements, the spindles discharged most rapidly during jaw opening while the tendon organs were active during closure.

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Evidence for a transcortical cutaneous reflex response in man

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Modest electrical stimulation of the digital nerves of the index finger produces marked changes in the surface e.m.g. signal recorded during steady voluntary contraction of the 1st dorsal interosseous muscle. As found previously using single motor unit recording (Stephens & Usherwood, 1976), the reflex response is typically triphasic consisting of an increase in e.m.g. activity (E_1) at a latency similar to the tendon jerk, followed by a decrease (I_1), which in turn is followed by a larger increase (E_2) some 15–20 msec after the onset of E_1. Studies in patients have now revealed changes in the E_2 response rather similar to those reported previously for the long latency excitatory response which follows muscle stretch and which is often referred to as an example of a long loop perhaps transcortical reflex (Marsden, Merton, Morton & Adam, 1977*a*, *b*).

Patients were required to maintain a force of contraction such that the electrical activity recorded over the surface of 1st dorsal interosseous remained at approximately 20% of that associated with a maximum voluntary effort. At the same time

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single electric shocks were delivered to the digital nerves of the index finger at 3 s^{-1} via ring electrodes with a stimulus strength twice threshold for perception. The surface recorded e.m.g. signal was rectified and averaged for 512 or 1024 sweeps time locked to each stimulus. In patients with well documented lesions affecting either the dorsal columns, motor cortex or descending motor pathways the E_2 phase of the cutaneous reflex was either delayed or absent. No such changes were seen in patients with lesions affecting the frontal lobe, occipital lobe, cerebellum or spinothalamic pathways.

In normal subjects a triphasic e.m.g. response can also be recorded from extensor digitorum brevis following twice threshold stimulation of the digital nerves of the second toe. As in the hand, the latency of the first increase in e.m.g. (E_1) is similar to that of the tendon jerk or F + M time for this muscle. The difference between this latency and the beginning of the second increase in e.m.g. activity (E_2) is, however, some 10 msec longer in the foot than in the hand. This result and the results obtained in patients are most easily explained on the basis that the second excitatory component of the cutaneous reflex is a transcortical response involving the sensorimotor cortex.

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Sensory innervation of cat hind-limb muscle spindles

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Reconstructions of primary and secondary endings from serial 1 μm transverse sections of tenuissimus spindles revealed differences in the disposition of terminals on bag_1 (b_1) and bag_2 (b_2) fibres, which can be recognized in teased, silver preparations. We were therefore able to examine such preparations of spindles from various hind-limb muscles and ascertain the distribution of primary and secondary afferents to b_1, b_2 and chain (c) muscle fibres.

Among 245 primary afferents, 204 had a b_1b_2c distribution, 30 b_2c, 4 b_1c, 6 b_1c, and 1 b_1b_2. Restriction to two fibre types occurred in double primary endings (ten in sample) and in tandem linkages in which the b_2 fibre was continuous from one capsule, where it was accompanied by b_1 and c fibres, to another, in which it was accompanied by c fibres only (usually two) and usually lacked a nuclear bag. The diameter range of the b_2c primary afferents supplying these one-bag-fibre spindles was 2–9 μm (peak 4 μm). Most (82 %) fell within the same diameter range as the b_1b_2c primary afferents (4–12 μm, peak 6 μm); they would thus overlap in conduction velocity, but would presumably lack dynamic sensitivity. The proportion of such afferents supplying peroneus brevis was 25·0 % (9 of 36), tenuissimus 5·4 % (4 of 74), and superficial lumbrical 3·1 % (1 of 32).

Spindles with b_1,b_2, and c fibres may sometimes have more than one b_1 fibre, rarely more than one b_2. Among 204 b_1b_2c primary afferents, 24 were distributed to spindles with two b_1 fibres, 2 to spindles with three b_1 fibres, and 1 to a spindle with two bag

fibres of each type. The proportion of spindles with more than one b_1 fibre was 26·7 % in superficial lumbrical (8 of 30), 11·5 % in peroneus brevis (3 of 26), and 6·3 % in tenuissimus (4 of 64).

The distribution of 197 first-order branches of 88 b_1b_2c primary afferents was 'segregated' in 67·6 % (i.e. the branches exclusively supplied either b_1, or b_2 and c, or c fibres), and 'mixed' in 32·4 %, as follows (%): b_1, 23·9; b_2, 4·1; c, 11·7; b_2c, 27·9; b_1b_2c, 16·2; b_1c, 13·2; b_1b_2, 3·0. Mixed distributions occurred more frequently among 22 superficial lumbrical afferents (77·2 %) than among 29 tenuissimus afferents (38 %).

Most secondary afferents terminated on all three muscle-fibre types as endings located in the S_1 position. Of 273 secondary afferents, 73·3 % had a b_1b_2c distribution, 14·0 % b_2c, 8·0 % b_1c, and 4·7 % c. The diameter range of 193 b_1b_2c secondary afferents was 1–7 μm (peak 3 μm); 40·9 % fell within the same diameter range as the b_1b_2c primary afferents. The maximum area of innervation on b_1 fibres in b_1b_2c secondaries was typically about half that supplied to b_2 fibres.

Long latency stretch reflex of the human thumb can be reversed if the task is changed

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When a subject performing a simple manual tracking task meets with an unexpected perturbation, short- and long-latency automatic electromyographic (e.m.g.) responses are elicited (Marsden, Merton & Morton, 1976). When the prime-moving muscle is stretched, there is an automatic increase in agonist e.m.g. activity, and when the muscle is unloaded there is a decrease. We now have investigated the effect of distant perturbations on the activity of a prime mover. Our subjects executed a tracking task by flexion of the right thumb against a force transducer. The semi-pronated right forearm pointing vertically downwards was attached to a torque motor in front of the subject via a short chain wrapped around the wrist. In randomly selected trials the motor pulled on the wrist, thereby lifting the thumb away from the force transducer, consequently unloading the thumb flexor muscles. This caused an increase in the e.m.g. of flexor pollicis longus about 50 msec later, not the decrease expected from the muscle shortening. This automatic response tended to restore the thumb pad back to the force transducer and overcame the usual silencing of the muscle produced by directly unloading flexor pollicis longus. The same response could be elicited from flexor digitorum profundus during finger flexion; it was not affected by motor point anaesthesia of the latter muscle sufficient to markedly reduce its response to direct muscle stretch, suggesting that it was not caused by direct activation of that muscle's spindles. The response persisted after the skin of the wrist under the chain had been anaesthetized, indicating that it was not a cutaneous reflex. However, the response disappeared when movement of the upper arm was restricted, suggesting that the effective stimulus was a perturbation of proximal muscles or joints. The response was unaffected by thumb anaesthesia, but was not elicited in most subjects when the force transducer was held free in the hand rather

than being fixed to some external object. In this case displacement of the wrist caused no change in the relationship of the thumb to the force transducer and the usual response would have been inappropriate.

We conclude that local muscle reflexes can be over-ridden by other responses elicited by distant perturbations. The muscle's response appears to depend on the exact function that it is performing at the time.

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Asynchronous motor unit activities and tremor

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Mainly because of the regularity of the tremor oscillation, muscle tremor is widely thought to imply a strong synchronization between motor unit activities. However, experimental investigations have shown no sychronization in several cases (e.g. Taylor, 1962) and limited synchronization, characterizing essentially uncorrelated processes, in others (Dietz, Bischofberger, Wita & Freund, 1976).

We have therefore used uncorrelated renewal spike trains to drive the active motor units in a computer model of skeletal muscle in which the muscle force is produced as the sum of the contributions of the individual units. The model (*J. Biomed. Eng.*, in the Press) is based on the findings of Milner-Brown, Stein & Yemm (1973): ignoring minor details, at any level of contraction, the majority of active motor units in the model are small in size and fire at relatively high rates. The muscle-force wave forms produced using this model were very similar to tremor records – they contained a fairly regular oscillation. The observed regularity of the simulated force oscillation is particularly significant at low levels of contraction where there are no major differences in the sizes of active motor units. Since its frequency is very near to the firing rates of the (quite a few) recently recruited, relatively large, units, the force oscillation has to be the result of the combined activities of these units. The activities of large units, firing near their recruiting rate, has been proposed in the past as the basis for tremor; but it was never clearly specified how the combination of quite a few of them could, in the absence of strong synchronization, produce the regular tremor oscillation. However, the regular oscillation is to be expected on theoretical grounds since the frequency content of uncorrelated processes is preserved during summation. Indeed, it can be shown that the spectrum of the process resulting from the superposition of a number of identically distributed renewal point processes, which are uncorrelated, differs from that corresponding to perfectly synchronized ones by only a scaling factor.

It thus appears that the significance, in the tremor case, of the 'chance synchronization' proposed by Taylor (1962) for the grouping of action potentials, has not been properly appreciated by other workers: asynchronous motor unit activities are widely thought to account for the relatively smooth contraction of muscle; our simulation results and theoretical analysis strongly suggest that they can also account for tremor.

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Responses of human muscle spindle afferents to the sudden onset of load at the beginning of a position holding task

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The activity of muscle spindle afferents from finger extensor muscles was recorded in the left radial nerve in man, using percutaneously inserted tungsten needle electrodes (Vallbo & Hagbarth, 1968). Loads of approximately constant torque (0·01–0·25 N m) were generated by a servo motor which was firmly connected to the particular finger the passive flexion of which most effectively excited the afferent unit. Initially the metacarpophalangeal joint of the finger was held in a fixed position by the motor. The subject was then relaxed but was instructed to maintain this position in the face of loading. Visual feedback on any deviation of the finger from the desired position was presented on an oscilloscope. Movement at the metacarpophalangeal joint was monitored with the angular position transducer of the motor. The sudden application of approximately isotonic loads caused initial finger flexion with forced lengthening of the receptor bearing muscle. Frequently the subjects then responded with oscillatory movements of decreasing amplitude and frequency (1–4 cycles over a period of 1 sec) in the course of restoring the desired finger position. The afferents often responded to such adjustments with a series of impulse bursts. The first burst was normally small (2–4 impulses) and coincided with the initial stretch. Subsequent bursts were often larger. During faster oscillatory movements (usually during the first half of the sequence) the burst still tended to occur during the lengthening phase. During the slower cycles (often towards the end of the adustment movement) the bursts occurred both during the trough between the shortening and lengthening phase and during the early lengthening phase of the movement. In the extreme, afferent bursts could be restricted to the shortening phase, whilst there was silence during the lengthening. During the steady contraction, when initial finger position had been restored, the mean rate of discharge was raised above the level of the control response before loading. This and the occurrence of firing during muscle shortening suggests that the extrafusal contraction during the adjustment and position holding was accompanied by fusimotor activation. Thus any contribution the afferent responses provide to the performance of this corrective task depends upon the complex interaction of fusimotor excitation and muscle stretch.

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Different behaviour of fast and slow efferents to masseter muscle in reflex movements in the cat

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Two populations of motor fibres have been identified in recordings from central cut ends of intra-muscular nerve filaments of the masseter muscle. Some had high conduction velocities (35–100 m/sec), marked sensitivity to muscle stretch, low spontaneous discharge and little response to light skin stimulation. They were presumed to be alpha motor axons.

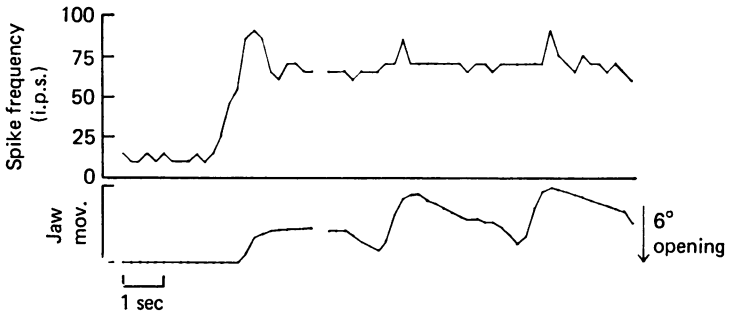

Fig. 1. Action of a presumed fusimotor fibre of masseter nerve during reflex jaw movements.

Others showed marked sensitivity to skin stimulation and little or no response to muscle stretch. Most had conduction velocities less than 35 m/sec and higher levels of spontaneous discharge. They were thought likely to be fusimotor axons.

During reflex jaw movements under light pentobarbitone anaesthesia the firing of presumed alphas was modulated in parallel with masseter e.m.g. In contrast, presumed fusimotors in some cases showed the pattern of firing seen in Fig. 1. Note that the firing does not fluctuate significantly with the movements but rises with their onset and is then maintained high throughout.

Thus for such movements, close 'α–γ co-activation' is not to be seen, but rather a degree of independence, and this is consistent with the results of spindle afferent recordings in conscious, naturally behaving animals (Cody, Harrison & Taylor, 1975).

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Possible evidence for synchronisation amongst fusimotor neurones

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It is known that an individual gamma efferent innervates many muscle spindles and that it produces the same effect (static or dynamic) on all the spindle primary endings it is associated with (Emonet-Denand, Laporte, Matthews & Petit, 1977). If one were to record from a spontaneously discharging gamma efferent, without damaging its peripheral connexion, it should be possible to determine its peripheral action by studying the multi-unit activity in dorsal root filaments with the use of the technique of spike-triggered averaging.

Cats under light anaesthesia (halothane or sodium pentobarbitone), to ensure spontaneous fusimotor activity, were used in acute experiments. No peripheral nerves were cut in order to obtain maximum possible afferent input. Muscle nerves in the left hind limb were exposed for stimulation and identification of efferents and afferents to those muscles.

After a lumbar laminectomy, multi-unit activity of afferents in a filament of L7 dorsal root was monitored, and averaged after processing by a ratemeter-integrator (time constant 10 msec). In the initial experiments the dorsal root recording was done in continuity but in later experiments roots were cut proximally to confirm that the recorded effects were of peripheral origin. The L7 ventral root filaments were teased in continuity till spontaneously discharging units were obtained. Gamma efferents were identified with regard to conduction velocity (20–40 m/sec) and destination by stimulation of various muscle nerves. The activity from the gamma efferent was then used to trigger the averager.

Two types of averaged neurograms have so far been observed. One occurs at a latency of 8–10 msec, rises and reaches a maximum in 15 msec. The other occurs at a latency of 15 msec and reaches a peak in 50 msec. Explanation of the temporal features of these averaged neurograms must await further refinement in the recording techniques; however, some interesting features have emerged.

The neurograms were still obtained after cutting the ventral root filament distally, but disappeared after cutting the entire L7 ventral root. The neurograms were most easily obtained in cats which showed considerable spontaneous efferent activity.

These results suggest that many gamma efferents of the same functional type may fire in near synchrony to ensure reinforcement of their peripheral effects. Taken together with the observation that the spindle primary endings situated in close vicinity show a strong correlation in their discharge patterns (Windhorst & Meyer-Lohmann, 1977), a grouping of fusimotor activity may have significance for peripheral motor control.

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The course of post-ganglionic sympathetic fibres to the face and jaws in the cat

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It is commonly assumed that the post-ganglionic sympathetic fibres to the face and jaws are distributed exclusively with branches of the external carotid artery. However, Langley (1900) stated that sympathetic fibres were distributed with the branches of the trigeminal nerve (V). Also, Ogilvie (1969) showed that vasoconstriction in cat tooth pulp produced by cervical sympathetic stimulation was blocked by section of the mandibular division of V at the foramen ovale. In cats anaesthetized with either pentobarbitone sodium or a mixture of alphaxalone and alphadolone acetate, we have established the presence of sympathetic fibres in the inferior alveolar and infraorbital nerves and the pulps of canine teeth by recording compound action potentials from these structures during ipsilateral cervical sympathetic stimulation (10 V, 1·0 msec, 1/sec).

The compound action potentials recorded from the inferior alveolar nerve near the mandibular foramen had latencies between 25 and 88 msec and durations of approximately 150 msec. This response was not affected by section of the sensory and motor roots of V but was either completely or nearly completely abolished by intracranial section of the mandibular division of V at the foramen ovale. Section of the internal carotid plexus in the roof of the tympanic bulla always blocked the response and section of the contents of the foramen lacerum blocked it either completely or almost completely. When the inferior alveolar nerve was stimulated in paralysed, artificially ventilated animals, no response was recorded from the cervical sympathetic trunk. These results show that the responses were not reflex in origin or due to stimulation of afferent nerves travelling with the sympathetics. They establish that post-ganglionic sympathetic fibres are present in the inferior alveolar nerve and that most travel from the superior cervical ganglion via the internal carotid plexus and the trigeminal ganglion. A few fibres appear to cross the base of the skull from the internal carotid plexus to join the mandibular division of V without entering the cranial cavity. By moving the recording or stimulating electrodes, the conduction velocities of the fastest pre- and post-ganglionic fibres were estimated. The results for pre-ganglionic fibres were 6·1, 8·2 and 9·4 m/sec, and for post-ganglionic 0·9 and 1·3 m/sec.

Similar results were obtained for the course of sympathetic fibres in the infraorbital nerve. Very small compound action potentials with latencies from 127 to 194 msec could be resolved by averaging the records from 6 of the 26 teeth examined. The responses were abolished by sectioning the internal carotid plexus or the appropriate division of V.

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Rate of reinnervation of extensor digitorum longus muscle in dystrophic mice

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The extent of functional reinnervation of extensor digitorum longus muscle (EDL) was tested in young (4- to 6-week) and old (4- to 6-month) dystrophic mice of the strain C57Bl/6J dy^{2J}/dy^{2J} and their phenotypically normal litter-mates. Denervation was performed under barbiturate anaesthesia by cutting the lateral popliteal nerve at its point of entry into EDL. The degree of reinnervation was expressed as a reinnervation ratio (R.I.R.), where R.I.R. = functional innervation ratio of denervated EDL divided by that of contralateral innervated EDL. Functional innervation ratio is calculated as twitch tension evoked by nerve stimulation divided by that produced by direct stimulation of the muscle. R.I.R. was determined 2, 4 or 6 weeks after nerve section in the younger groups and after 4, 8 or 12 weeks in the older mice. At the appropriate time after denervation the mice were anaesthetized with barbiturate, and EDL muscles of both legs prepared for tension recording. The experiments were carried out with the entire hind limbs immersed in phosphate, bicarbonate buffered Ringer solution at room temperature (20–22 °C).

In both young and old groups the degree of reinnervation attained in dy^{2J} mice was lower at all times than in control animals. Essentially complete reinnervation was seen after 6 weeks in young controls (R.I.R. = $0{\cdot}97 \pm 0{\cdot}12$) whereas in young dy^{2J} mice R.I.R. = $0{\cdot}65 \pm 0{\cdot}11$ (mean ± S.E.M.). A similar failure to reinnervate was observed in the older group of dystrophic mice in which R.I.R. at 12 weeks was $0{\cdot}43 \pm 0{\cdot}10$. The comparable control group value was $0{\cdot}83 \pm 0{\cdot}09$. Following denervation in the young mice there was a significant slowing of the twitch, both the time to peak tension and the time to relax to half peak tension being prolonged. In addition muscle weight decreased and twitch tension normalized per g wet wt. of muscle increased. However, the recovery of all these parameters was delayed in the dy^{2J} mice, reflecting the abnormal rate of reinnervation. In the older dystrophic mice less change was observed in the times to peak tension and half relaxation and no recovery was seen in either of these properties during the 12 weeks allowed for reinnervation. Normalized twitch tension increased to about twice that of the innervated leg and also remained elevated throughout the 12-week period.

These results suggest that reinnervation is impaired in dy^{2J} mice, particularly in those older mice exhibiting severe signs of dystrophy.

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Nasal vasoconstriction induced by electrical stimulation of the cat hypothalamus

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A 'nasal centre' has been proposed to be represented in the human hypothalamus which regulates the cyclic changes in nasal resistance to airflow by influencing autonomic tone (Stoksted, 1953). So far the only experimental evidence supporting this hypothesis is a single observation that electrical stimulation of the cat hypothalamus causes nasal vasoconstriction (Malcomson, 1959).

In the present study cats were anaesthetized with α-chlorolose and a neuromuscular relaxant was administered as required. A co-axial stimulating electrode was inserted into the hypothalamus by means of a stereotaxic apparatus. The results of preliminary experiments have shown that bilateral nasal vasoconstrictor responses may be readily induced by electrical stimulation in various areas of the hypothalamus. The active areas broadly overlapped the areas previously described as causing pupillodilation and termed 'the dynamogenic zone' (Hess, 1954), and were similar to the 'defence area' (Abrahams, Hilton & Zbrozyna, 1960). The nasal vasoconstrictor responses were often accompanied by contractions of the nictitating membrane and changes in arterial blood pressure, although in some areas nasal responses alone were found. Any influence of the adrenal glands on the responses was eliminated by routine bilateral adrenalectomy. The nasal vasoconstrictor response elicited by electrical stimulation of the hypothalamus was greatly reduced after bilateral section of the sympathetic trunks in the neck, indicating that the response was mediated through the sympathetic nervous system.

Only vasoconstrictor responses were elicited on stimulation of the hypothalamus and at present no evidence of any parasympathetic representation has been found.

The very regular nature of the cyclic changes in nasal resistance observed in man leads one to conclude that some area of the central nervous system controls the reciprocal activation of autonomic outflow to the nasal mucosa with clocklike regularity (Eccles, 1978). However, anatomical localization of this area has not yet been achieved.

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The early metabolic response of lambs to small doses of 3,3',5-triiodothyronine

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A small dose (5 μg/kg) of 3,3',5-triiodothyronine (T_3) gives a significant increase in $\dot{V}_{O_2}$ in the rat after a delay of only 1 or 2 hr (Moore, Moore & Moore, 1976, 1978). The rabbit may behave similarly (Andrews, Lynch & McGahan, 1978). We now report responses of the new-born (37–180 hr post-partum) lamb. $\dot{V}_{O_2}$ was measured by an open-circuit method in a small climatic chamber at T_a 25 °C. After 1 hr 1·0 ml.

Table 1. Mean hourly $\dot{V}_{O_2}$ (ml./kg min^{-1}) ± S.D. of lambs before and after injection with saline or T_3

	Pre-injection	0–60 min	60–120 min	120–180 min
Saline	21·19 ± 2·33	21·40 ± 3·37	22·11 ± 2·48	22·40 ± 2·91
T_3 1 μg/kg	18·15 ± 3·33	19·16 ± 1·81	21·32 ± 2·02*	22·52 ± 1·52**
T_3 5 μg/kg	19·64 ± 3·78	19·12 ± 2·65	21·87 ± 3·02	22·52 ± 3·17

n = 6 in each category.
* $P < 0·05$.
** $P < 0·01$.

S.C. injection of solvent (alkalinized physiological saline) or 1 μg T_3/kg or 5 μg T_3/kg was given to each of six animals and $\dot{V}_{O_2}$ measured continuously. The mean $\dot{V}_{O_2}$ values, Table 1, show that at the lower dose of T_3 there was a significant increase in $\dot{V}_{O_2}$ after 2 and 3 hr. Means over 20 min periods show a rise in $\dot{V}_{O_2}$ significant ($P < 0·05$) in the 60–80 min period after injection. There was no significant change in the control group or the group receiving the higher dose of T_3 (5 μg/kg).

Thus in the new-born lamb small changes in output of thyroid hormone by the thyroid gland could act in the short-term regulation of heat production.

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The messenger role of cyclic GMP and calcium in the exocrine pancreas

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The secretion of proteases from isolated pancreatic lobules in response to acetylcholine (ACh), cholecystokinin and, more recently, substance P is preceded by a rapid and transient rise in intracellular cyclic 3',5'-guanosine monophosphate (cyclic GMP) (Albano, Bhoola & Harvey, 1976, 1978). Calcium ions are intimately linked to the process of stimulus-secretion coupling (Douglas, 1968), with trans-

location of intracellular calcium following acinar cell membrane depolarization in response to acetylcholine (Peterson & Ueda, 1976).

In the present study we have examined the importance of calcium ions for the ACh and substance P evoked increases in cyclic GMP. Incubation of pancreatic lobules in calcium-free Krebs buffer containing 0·1 mM EGTA, for 15 min prior to addition of ACh or substance P, resulted in a diminution of the increase in cyclic GMP accumulation from eight-fold to four-fold in response to substance P and a failure to respond to ACh. Increasing the external calcium from 1·0 to 10 mM produced a 100% increase in enzyme release but failed to alter basal intracellular levels of cyclic GMP over a time course of 0·5–30 min, whereas ACh and substance P in the same experiments significantly increased this nucleotide. A similar increase in intracellular calcium can be achieved with the divalent cation ionophore, A23187 (Ely Lilly). No change in cyclic GMP levels in response to the ionophore was observed in experiments on pancreatic lobules (incubated in Krebs buffer containing 0·1 mM-3-isobutyl 1-methyl xanthine or 2 mM theophilline, but no bovine serum albumin) with time (0·5–30 min) and dose dependency studies (2·5, 5·0 and 10 μg/ml.). High extracellular potassium is also considered to increase intracellular calcium. Pancreatic lobules were preincubated in high potassium Krebs buffer (75 mM-KCl or 70 mM-CH_3SO_4K with 5 mM-KCl) for 20 min. The depolarized lobules gave no further rise in the increase in cyclic GMP produced by ACh and substance P.

The complete dependency of the ACh-mediated rise in cyclic GMP, but not substance P, on extracellular calcium suggests a primary requirement of calcium in the activation process of the muscarinic receptor. Our results are in accord with the view that activation of membrane-bound guanylate cyclase by transmitter substances may occur independently of calcium and supports a second messenger role for cyclic GMP in the exocrine pancreas.

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The effect of pretreatment with intraventricular 6-hydroxydopamine on hypothalamo–pituitary–adrenocortical function in the rat

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Noradrenaline has been shown to play a role as an inhibitory neurotransmitter in the control of ACTH secretion (Ganong, 1972), although there may also be an excitatory component (Krieger & Krieger, 1970). The inhibitory effect is mediated via an alpha-receptor mechanism (Scapagnini & Preziosi, 1973; Jones, Hillhouse & Burden, 1976). In the present study adrenocortical function has been investigated in

rats receiving 6-hydroxydopamine (6-OHDA), a drug which causes destruction of the axons of catecholaminergic neurones.

Female Wistar rats weighing 150 g were anaesthetized with ether and 6-OHDA (250 μg, free base) was injected into the lateral ventricle on two occasions 24 hr apart. The 6-OHDA was dissolved in 0·9% (w/v) saline containing ascorbate (1 mg/ml.) and 20 μl. were injected.

The pretreatment with 6-OHDA caused an 80% depletion of noradrenaline content of the hypothalamus measured 4 or 14 days later. The stress response in rats anaesthetized with pentobarbitone (50 mg/kg I.P.) measured by corticosterone production *in vitro* was found to be reduced in response to a low-intensity stress of cutting the skin when animals treated with 6-OHDA are compared with controls. This applied equally to animals pretreated with 6-OHDA 4 or 14 days previously. However, when the stress was the rather more severe operation of sham bilateral adrenalectomy the magnitude of the response was the same in both the vehicle- and 6-OHDA-injected animals.

The release of corticotrophin releasing factor (CRF) is stimulated from the hypothalamus *in vitro* when acetylcholine is added to the incubating medium and this release is inhibited by noradrenaline (Jones *et al.* 1976). Hypothalami were therefore removed 4 and 14 days after treatment with 6-OHDA and incubated *in vitro*. Their responsiveness to a dose of 3 pg/ml. acetylcholine was the same in both vehicle and 6-OHDA-treated animals. However, the threshold inhibitory dose of noradrenaline was reduced from 1 ng/ml. to 100 pg/ml. in animals pretreated 14 days earlier, but not in the 4-day treated animals.

It is possible therefore that the reduced responsiveness to the stress of cutting the skin in 14-day animals is due to the development of super-sensitivity to the inhibitory action of noradrenaline from the remaining noradrenergic fibres. This explanation cannot hold for animals treated for 4 days, and a more likely explanation is the existence of an excitatory noradrenergic pathway to CRF-ACTH secretion and that the destruction of this putative pathway is responsible for the changes in stress-induced responsiveness.

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Factors affecting insulin release in the sheep fetus

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Although insulin release *in utero* has been studied in detail in the sheep fetus (see Bassett & Jones, 1976), little is known about the factors influencing fetal β-cell reactivity. In the present experiments, the effects of metabolite concentrations and adrenaline infusion on the β-cell response to arginine and glucose have been examined in the chronically catheterized sheep fetus.

Intravenous catheters were inserted into thirty-two Welsh Mountain ewes under

halothane anaesthesia between 116 and 136 days gestation. Intravenous infusions of either L-arginine (200 mg in 10 ml. 0·9 % saline) or glucose (1 g as a 50 % solution) were given over 5 min to each fetus at least 4 days post-operatively, to elicit insulin release (Fowden, 1977). These test doses were given alone or during intravenous infusions of adrenaline or amino acids. Adrenaline infusion (1 μg/min for 2–2½ hr) was begun after an initial dose of 5 μg and the arginine or glucose was given 30 min later. The amino acid mixture (Vanim N) was infused at a rate of 13·3 mg/min for 1 hr and then at 5·3 mg/min for 90 min before and 30 min after the glucose infusion. A solution of similar ionic composition to the amino acid solution was infused as a control procedure.

There was a significant correlation between the rise in the plasma insulin concentration in response to glucose and the basal fetal plasma glucose concentration in 12 fetuses ($r = 0{\cdot}86$, $P < 0{\cdot}01$), whereas arginine stimulated insulin release was unrelated to any of the basal metabolite concentrations. The β-cell responses in both arginine (three experiments) and glucose (four experiments) were abolished by adrenaline infusion.

The β-cell response to glucose was potentiated and occurred more rapidly during amino acid infusion. In four fetuses the maximum increment in the insulin concentration in response to glucose was $61{\cdot}6 \pm 14{\cdot}4$ μu/ml. during amino acid infusion which was significantly greater than the value of $13{\cdot}8 \pm 2{\cdot}8$ μu/ml. observed in the control experiments ($P < 0{\cdot}02$). During amino acid infusion the plasma α-amino nitrogen concentration rose by 29 ± 5 % from an initial value of $5{\cdot}2 \pm 0{\cdot}6$ mg %. Amino acid infusion alone did not alter the insulin concentration.

The results suggest that the high endogenous concentration of amino acids in the fetus compared to the mother may not directly affect fetal insulin secretion but may potentiate glucose stimulated insulin release. Such an effect might explain, in part, the apparent enhanced β-cell reactivity to glucose in the fetal sheep compared to that of the mother (Shelley, 1973).

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Entrainment of oviposition in Japanese quail using ahemeral light–dark cycles

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Ahemeral (non 24 hr) light–dark (L–D) cycles have previously been used to entrain oviposition in the fowl for periods of about 21 days, with data collection commencing after 7 days of this treatment (Bhatti & Morris, 1978).

Japanese quail have been similarly entrained to 28 hr (16⅓ hr L, 11⅔ hr D) cycles using techniques described by Dacke (1977). The results (Fig. 1) indicate that individual birds require from 6 to 18 such cycles (1–3 weeks) for complete entrainment

of oviposition. It is therefore considered that experiments involving entrainment periods of less than 3 weeks should be treated cautiously since ovipository data from individual birds, previously interpreted as 'free running', may simply reflect the longer period of adaptation.

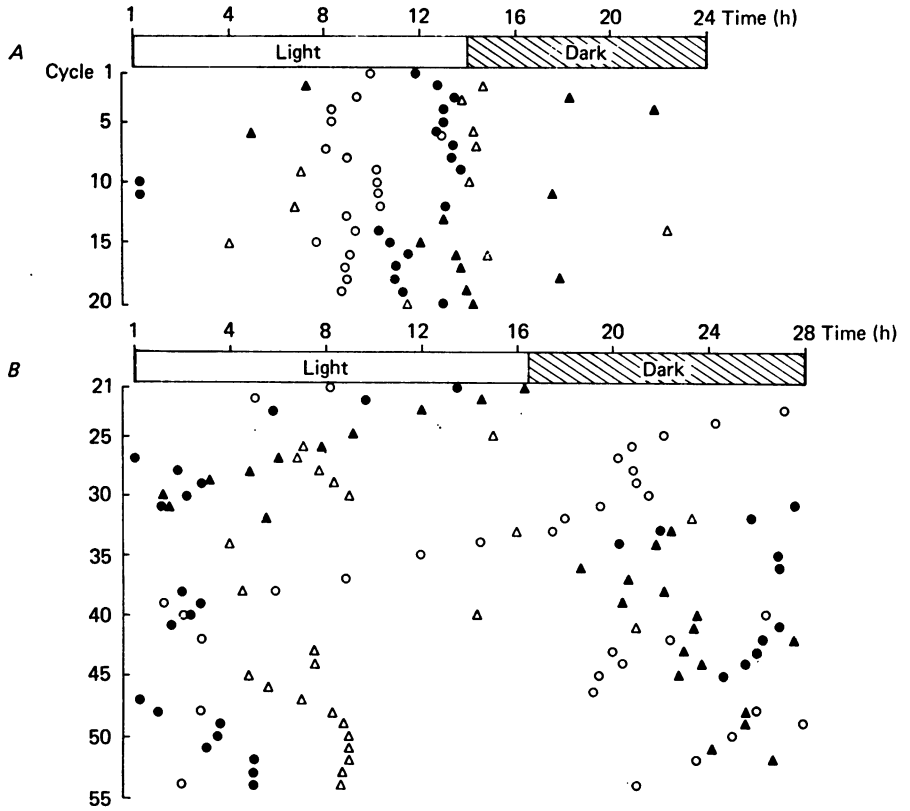

Fig. 1. Oviposition patterns for four individual quail hens (indicated by ●, ○, ▲ and △) are shown. On a 24 hr (14 hr L, 10 hr D) cycle (*A*), oviposition occurs predominantly in the latter portion of the light period. Following transfer to a 28 hr (16⅔ hr L, 11⅓ hr D) cycle (*B*), individual hens show variable periods of adaptation. By cycle 39 (18 ahemeral cycles) all birds appear to be entrained to the ahemeral cycle with oviposition undergoing substantial phase shifts.

In a second experiment four hens, previously entrained to the 28 hr cycle for 3 months, were readapted to the 24 hr cycle. In this case all birds showed complete entrainment to the 24 hr cycle within 6 days.

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Primary afferent depolarization evoked from stimulation of brain stem raphe nuclei in the rat

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A sucrose-gap apparatus has been designed (Roberts & Wallis, 1979) for the *in vivo* study of primary afferent depolarization (PAD) in the spinal cord. This technique allows a continuous record to be taken of the membrane potential of primary afferents entering the dorsal horn of the spinal cord. The effects of drugs administered intravenously or intra-arterially have been investigated on depolarizations resulting from (i) stimulation of an adjacent dorsal root and (ii) stimulation of mid-brain and brain stem raphe nuclei.

Male Wistar rats were anaesthetized with halothane, a laminectomy was performed and the dorsal roots of L5 and L6 were cut distally. The L5 root was drawn through holes in two rubber membranes approximately 6–8 mm apart and isotonic sucrose solution was perfused between these membranes. The cut end of the root lay in isotonic KCl solution. The mean potential recorded between the KCl and the indifferent electrode was $56{\cdot}9 \pm 3{\cdot}4$ mV ($n = 20$). Stimulation of the L6 dorsal root evoked a depolarizing potential of $0{\cdot}96 \pm 0{\cdot}18$ mV ($n = 11$). Depolarizing potentials were also evoked by stimulation of nucleus raphe dorsalis, raphe medianus and raphe magnus. The threshold of stimulation to evoke depolarization of primary afferents was lowest for raphe magnus. Low-intensity stimulation of raphe magnus ($\simeq 70\ \mu$A) evoked a response with a latency of 46 ± 8 msec, possibly indicating a conduction velocity of 1·7 m sec^{-1}.

The length constants for the depolarizations evoked from raphe magnus and dorsal root stimulation were similar indicating that raphe magnus and dorsal root depolarized fibres of comparable diameter. It is interesting, however, to note that simultaneously evoked PADs from raphe magnus and dorsal root do not summate.

Intravenous injections of high doses of 5-hydroxytryptamine antagonists cinanserin (up to 28 mg kg^{-1}), methergoline (up to 10 mg kg^{-1}) and methysergide (up to 7 mg kg^{-1}) had no inhibitory effect on either raphe or dorsal root evoked potentials. However bicuculline (2 mg kg^{-1}), administered intravenously, selectively antagonised the dorsal root evoked potential.

Proudfit & Anderson (1974) have reported long-latency dorsal-root potentials in the cat in response to brain stem raphe stimulation but contrary to the experiments reported here these potentials were shown to be antagonized by cinanserin and methysergide.

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Cell bodies of aortic nerve efferent fibres in the rabbit and cat

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Electrophysiological experiments have demonstrated the existence of efferent activity in the aortic nerve of the cat. Supranodose section of the vagus abolishes such activity, suggesting that the efferent cell bodies lie within the central nervous system (Neil & O'Regan, 1971); however, their precise location is not known. Our objective was to locate the cell bodies of aortic nerve efferent fibres using the retrograde intra-axonal transport of horseradish peroxidase (HRP) as a neuronal marker.

In ten rabbits and five cats anaesthetized with pentobarbitone sodium (40 mg/kg) the cut central end of an aortic nerve was placed in 2–3 μl. drop of 30% HRP (Sigma, type VI) in saline for 8–24 h. The animal was then killed and the brain stem sectioned and processed for the detection of HRP-labelled cell bodies using the *p*-phenylenediamine dihydrochloride/pyrocatechol method (Hanker, Yates, Metz & Rustioni, 1977). Specific details of the techniques have been described (Kidd & McWilliam, 1979).

No labelled cell bodies were found despite an extensive search of the brain stem from 5·2 mm rostral to 3·9 mm caudal to the obex in the rabbits and from 10·8 mm rostral to 16·4 mm caudal to the obex in the cats.

In one rabbit and four cats, HRP was applied to the cervical vagal nerve. In these animals HRP-labelled cell bodies were found ipsilaterally in the dorsal motor vagal nucleus and the nucleus ambiguus.

We conclude that the cell bodies of aortic nerve efferent fibres in the rabbit and cat are not located in the brain stem. This confirms the report of Garcia, Jordan & Spyer (1979) in the rabbit and is compatible with a study by Kalia & Davies (1978), who were unable to locate the cell bodies of carotid sinus nerve efferent fibres in the cat. It is possible that the cell bodies may be located peripherally.

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Stability of retinal positional specificity in disarranged embryonic eyes in *Xenopus*

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In *Xenopus* embryos of stage 32 we have formed eyes made up of two right ventral halves ($V_R V_R$), a right temporal and a left nasal half ($T_R N_L$) and a right nasal and a left temporal half ($N_R T_L$). Such eyes therefore possess reversed polarity along the naso-temporal axis ($V_R V_R$) or along the dorso-ventral axis ($T_R N_L$; $N_R T_L$). The visuotectal map from one such animal ($T_R N_L$), recorded at stage 57 is shown in Fig. 1.

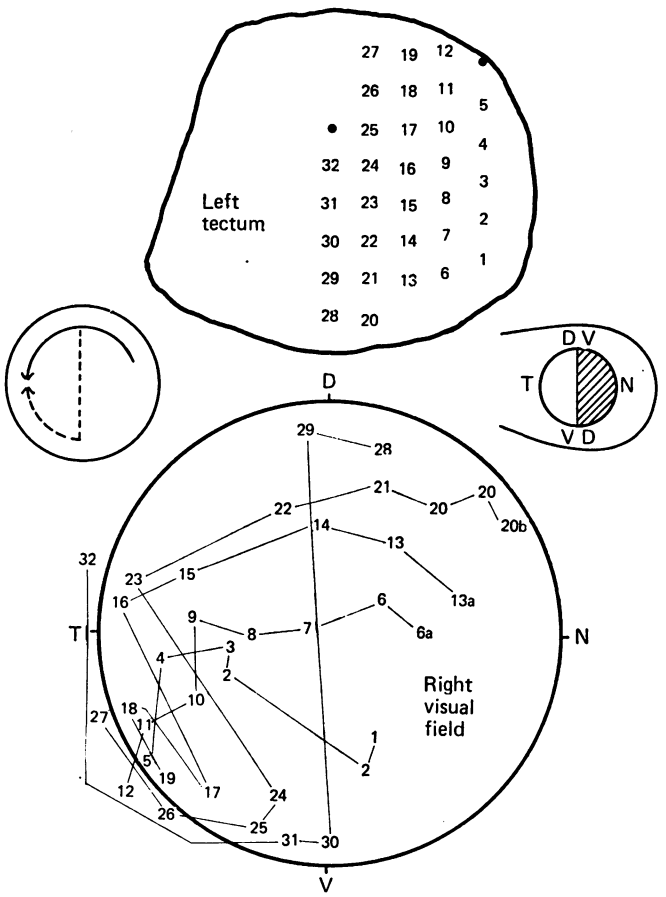

Fig. 1. Visuotectal projection from a $T_R N_L$ eye. Corresponding numbers on tectum and field represent recording and stimulating positions. a, b: field positions from deeper in tectum. Filled circles on tectum indicate no response. Right inset shows nature of operation. Left inset summarizes a normal projection (solid arrow) and the D–V inverted projection from transplanted N_L retina (dashed arrow). N, D, T, V: Nasal, dorsal, temporal, ventral.

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Retinal fragments disarranged in this way tend to maintain their original programmes for the development of the positional specificities, which may be used in the establishment of the retinotopic map. Of animals successfully recorded, three out of three V_R V_R eyes, five out of eight T_R N_L eyes and four out of six N_R T_L eyes showed this behaviour. Two of the animals behaving otherwise showed essentially normal maps and three were uninterpretable.

These results are evidence for considerable stability of the retinal developmental programme, even in the face of gross retinal disarrangement.

The 'shrinkage' of LGN cells, amblyopia and cortical ocular dominance

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In Nissl stained preparations of the lateral geniculate nucleus (LGN) of cats with monocularly induced convergent squint, the cells in those layers receiving an input from the squinting eye showed a 'shrinkage' associated with a loss of contrast sensitivity and spatial resolving power – amblyopia (Einon, Ikeda & Tremain, 1978). The amblyopia of the LGN cells has now been shown to reflect the amblyopia of retinal ganglion cells in the squinting eye (Ikeda & Tremain, 1979). The reason for the cell 'shrinkage' in the LGN is not clear.

To investigate this problem we have measured the perykaryal size of LGN cells in cats with convergent or divergent squint and also in cats atropinised in one eye from the age of 3 weeks (Ikeda & Tremain, 1978). We asked the question whether 'shrinkage' is correlated with changes in the proportion of visual cortical cells in area 17 driven by the affected eye, or with the proportion of binocularly driven cells.

In all cats, except controls, there was a marked loss of binocularly driven cortical cells, but amblyopia of the LGN cells was found only in cats whose squinting or atropinized eye had lost the ability to fixate or accommodate. The degree of 'shrinkage' was correlated with both the degree of amblyopia found in the LGN cells and with the proportion of the cortical cells driven from the affected eye compared with those driven by the normal eye. The shrinkage was not correlated with changes in the proportion of binocularly driven cortical cells.

We suggest that the weaker input from amblyopic retinal ganglion cells does not provide the input necessary for the normal development of the LGN cells thus leading to 'shrinkage'. In turn, the inadequate development of the LGN cells leads to a deficiency in the normal development of their terminals in the visual cortex, and this allows the terminals from the normal eye to dominate the responses of visual cortical cells.

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