tion, may be reponsible for the altered responses to sympathomimetic amines following chronic treatment with high doses of guanethidine.

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The diffusion of ions out of single capillaries

By C. C. MICHEL. *University Laboratory of Physiology, Oxford*

The distribution of autonomic nerves to the respiratory tree of the rat

By AILSA M. S. WHITE. *University Laboratory of Physiology, Oxford*

COMMUNICATIONS

Slowly-adapting cutaneous mechanoreceptors

By MARGARET R. CHAMBERS and A. IGGO. *Department of Veterinary Physiology, University of Edinburgh*

Slowly-adapting cutaneous mechanoreceptors with myelinated afferent fibres are now recognized as forming a distinct class of receptors, with characteristics that distinguish them clearly from the rapidly-adapting receptors. The latter are usually associated with hair follicles (Brown & Iggo, 1964). Much attention has been given in the last few years to the slowly-adapting units that were described to the Society earlier (Iggo, 1961; Brown, Iggo & Muir, 1963) and called 'touch corpuscles'. These units are now recognized as terminating in a characteristic structure visible on the surface of the skin in many mammals, first described histologically in 1904 by Pinkus who called them 'hair disks'. They may often be associated with long hairs—the tylotrichs of Straile (1961).

There is another slowly-adapting mechanoreceptor in hairy skin which has in the past been confused with the 'touch corpuscle' but can be distinguished from it both morphologically and physiologically. The second type of unit, which we propose to call Type II slowly-adapting mechano-

receptors, are not associated with the 'touch corpuscle'. There is no conspicuous surface feature that allows them to be recognized. Preliminary histological examination shows them to be fusiform, lightly encapsulated structures which lie in the dermis, separate from hair follicles, and are supplied by large myelinated axons.

The afferent discharge is often present in the absence of an applied stimulus, at frequencies of 3–15/sec. This is a very characteristic feature and distinguishes these units very clearly from the 'touch corpuscles' which are normally silent. A second feature is that the discharge, either background or evoked by mechanical stimulation, is regular, i.e. the inter-spike intervals at any particular frequency are fairly uniform. This is also in contrast to the 'touch corpuscles' in which the adapted discharge is usually irregular and becomes regular only during and shortly after the application of mechanical deformation. A third difference is that the Type II units are easily excited by stretching the skin, whereas the Type I units require local deformation. This difference gives to the Type I units their typical punctate sensitivity. Finally, the dynamic sensitivity of the Type II units is less. The two classes can thus be separated with certainty. During constant velocity mechanical displacement both classes exhibit an amplitude and rate of displacement sensitivity in contrast to the hair follicle receptors that are rate of displacement sensitive. They both show a weak temperature sensitivity, being excited by a fall of skin temperature.

The conduction velocities of the two types of slowly-adapting units range from 40–100 m/sec and cannot be distinguished from the Type G hair follicle afferent units but are less than Type T units.

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Blockage of direct vagal effect on acid secretion of fundic pouches by a quaternary local anaesthetic

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The direct, cholinergic, vagal stimulant effect on acid secretion has been well known for many years. The potentiating effects exerted by the vagus nerve and the intrinsic plexuses of the gastric wall on the secretory response of the parietal cell to blood-borne stimulants are not, however, so clearly understood. In the investigation of these effects it is desirable that

some means be available of reversibly blocking the local nervous elements concerned, and the quaternary derivatives of local anaesthetics appear to offer several advantages for this purpose. They have been shown to exert slow but prolonged and powerful local anaesthetic effects on nerve fibres (Nador, Herr & Petaky, 1953; Redford, 1963).

At the low pH values which can be expected in a fundic pouch, the more conventional tertiary amine anaesthetics are largely in their ionized form to which the mucosa is relatively impermeable (Schanker, 1962). Although the quaternary derivatives are fully ionized in neutral solution, Redford, Savage & Schofield (1962) have shown that they will block vagal gastrin release, when applied topically to the antral mucosa in concentrations of 1–3 %, indicating probable penetration as far as the terminal connexions to the gastrin cell. Since they are neutral salts they do not interfere with the titration of acid secretion when placed in fluid used to irrigate the pouch in the Burstall & Schofield (1953) method for collection of acid secretion. In the antrum they do not show atropine-like action since they do not block gastrin release by the direct stimulation effect of topical ACh (Redford & Schofield, 1965).

Experiments have therefore been carried out on dogs with vagally innervated fundic pouches of both Perry and Hollander types. Gastric secretion was collected by a modification of the Burstall & Schofield (1953) procedure. Vagal secretion was stimulated by teasing with food and with insulin hypoglycaemia. 3 % lignocaine benzyl chloride, added to the irrigation fluid (isotonic saline or Sorenson's glycine buffer pH 2·5) for $\frac{1}{2}$ hr before and during the period of stimulation effectively abolished the response. The same concentration of local anaesthetic did not inhibit a plateau of acid secretion at similar levels maintained by subcutaneous histamine in Heidenhain fundic pouches.

It would seem, therefore, that this anaesthetic agent penetrates the mucosa even under conditions of low pH at least as far as the terminal connexions of the vagal pathway. There does not appear to be any previous report of the blocking of direct vagal secretion in this way, and it is likely that lignocaine benzyl chloride will be a valuable agent in the investigation of nervous influences on the secretory function of the oxyntic cell.

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Criteria for the physiological perfusion of isolated rat liver

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Little information is available on the establishment of adequate criteria for the physiological function of the isolated perfused rat liver. Such criteria are necessary to enable the time of satisfactory perfusion to be prolonged by modifications in technique.

The perfusion fluid is defibrinated whole rat blood, dialysed for 48 hr at 4° C against Krebs solution, as suggested by Mayes & Felts (1966). The technique of these workers has been modified to include perfusion through the hepatic artery (as well as through the portal vein) and continuous dialysis of the perfusion fluid. These refinements enable an adequate flow through the liver sinusoids to be maintained as well as the salt balance and the blood glucose at physiological levels. Removal of liver metabolites, such as urea, is also a function of the dialysis unit.

Parameters so far examined during each perfusion include pressures and flow rates of blood entering and leaving the liver, the oxygen tension in the portal blood (usually between 80 and 100 mm Hg oxygen), and the temperature of the liver. The blood is examined for haemolysis and packed cell volume during each perfusion; the rate of haemolysis is usually 1% per hr.

Serial biochemical estimations are performed on the blood, including glucose and urea; liver homogenates are assayed for enzyme activity, in particular, microsomal glucose-6-phosphatase and both cytoplasmic and lysosomal acid phosphatase.

Histochemical demonstration of acid phosphatase activity shows that marked changes occur in the lysosomes under hyperoxic conditions at an early stage of perfusion. These early changes are indicative of cell damage leading to cell death, and are prevented by reducing the oxygen tension to physiological levels.

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The temperature sensitivity of the chloride permeability of skeletal muscle at different extracellular pH

By O. F. HUTTER and ANNE E. WARNER. *National Institute for Medical Research, Mill Hill, London, N.W. 7*

A possible active transport of noradrenaline into arterial smooth muscle cells

By J. S. GILLESPIE and D. N. H. HAMILTON. *Institute of Physiology, University of Glasgow*

Previous experiments using the Falck fluorescence technique to localize noradrenaline infused into the cat spleen have shown it to be taken up most effectively by arterial smooth muscle cells (Gillespie, Hamilton & Hosie, 1967). The green fluorescence was present inside the arterial smooth muscle cells, but the smooth muscle cells of the veins, capsule and trabeculae did not show intracellular accumulation of noradrenaline. In the present experiments, the possibility that this difference between arterial and other smooth muscle depended on access of the infused noradrenaline was investigated by incubating thin slices of spleen in noradrenaline of differing concentrations made up in the Krebs saline. In this way all the smooth muscle cells were equally exposed to noradrenaline. These *in vitro* experiments confirmed the *in vivo* experiments, namely that arterial smooth muscle took up noradrenaline, while that of the veins, trabeculae and capsule did not.

The mechanism by which noradrenaline is accumulated intracellularly was investigated *in vitro*. Phenoxybenzamine 5×10^{-5} g/ml. prevented the uptake of noradrenaline into the arterial smooth muscle; in other experiments, cooling the incubation medium to 1° C prevented uptake of noradrenaline. Phenoxybenzamine and cooling also prevented the loss of fluorescence from smooth muscle previously exposed to noradrenaline in warm Krebs solution. The half time of loss of noradrenaline from the arterial smooth muscle in slices returned to saline at 36° C is about 10 min, but even 3 hr after returning the slices to saline at 1° C, no loss of noradrenaline occurred.

The role of the membrane potential in retaining noradrenaline within the cell was investigated by using isotonic KCl as the incubation medium, which, although it caused a loss of noradrenaline from the nerve endings, did not prevent the accumulation of noradrenaline in smooth muscle.

Thus phenoxybenzamine and cooling prevent both the uptake and loss of noradrenaline from splenic arterial smooth muscle cells. These results suggest that the intracellular accumulation of noradrenaline and its subsequent extrusion may be due to an active transport mechanism and that surface receptors may be involved.

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Uptake and binding of procaine by the isolated perfused guinea-pig heart

By D. J. BOULLIN and T. J. SULLIVAN. *Department of Pharmacology and Therapeutics, St Thomas's Hospital Medical School, London, S.E.*1

We have studied the uptake and efflux of procaine hydrochloride in guinea-pig hearts and present evidence for limited cellular binding. Hearts from virgin female guinea-pigs (300–500 g) were perfused with Krebs solution by the Langendorff technique. 0·1, 1·0 or 100 μg/ml. ^{14}C-carboxyl procaine HCl (specific activity before dilution, 8·33 μC/mg) was infused at the rate of 0·6, 6 or 600 μg/min for 2, 5, 10 or 20 min. In some experiments binding was assessed by continuing perfusion for a further 15 min with procaine-free solution. For comparison, the same procedure was used with ^{14}C inulin (specific activity 3·08 μC/mg). Thereafter, tissues and solutions were assayed for total radioactivity by liquid scintillation spectrometry (Boullin, 1966). Tissue levels of procaine are expressed as μg/g after deducting the concentration in the extracellular space (0·44 ml./g).

Procaine was always detected in the heart after perfusion, tissue levels being related to the perfusion concentration. Uptake was linear with 0·1 and 1·0 μg/ml., the ratio of concentration in the heart to that in the medium (T/M) reaching 1·0 in 10 min. The rate of uptake was 2 % of the quantity infused per min. For example with 1 μg/ml., at the end of 2 min after the infusion of 12 μg of procaine, the tissue level was 0·24 μg/g.

The pattern of uptake was different with 100 μg/ml. First, uptake was not linear and, secondly, the T/M ratio was still less than 1·0 after 20 min infusion. In addition, at the end of 2 min the tissue level was 48 μg/g and the rate of uptake was 4 % per min. Subsequently the rate of uptake fell to 0·5 % per min at 10 min and 0·3 % per min at 20 min.

Efflux studies showed that procaine was lost extremely rapidly. The efflux curve showed at least two exponential components, the half times for the decline in the rate of loss being 0·9 and 1·7 min. Though the efflux of inulin followed a similar pattern, with half times of 0·5 and 1·7 min, procaine was lost at a slower rate. 47 % of the procaine present in the heart after 10 min perfusion was lost in 1 min and 90 % in 5 min. The results for inulin were 83 % in 1 min and 90 % in 2 min.

The data show that procaine taken up by the heart is not confined to the extracellular space and a small proportion is bound to cells. It is suggested that the binding is limited by rapid equilibration between uptake and release.

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The effects of hypoxia on graded exercise in man

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Four men exercised on a cycle ergometer and inspired oxygen concentrations (F_{in}, O_2) of 0·11, 0·16, 0·21, and 0·33. At each F_{in}, O_2 work was continuous and increased 200 kpm/min every 6 min until exhaustion. Severe hypoxia (F_{in}, O_2 0·11) reduced maximum oxygen intake ($\dot{V}_{O_2}$) to 65% of the normoxic value (Table 1), but at any load, $\dot{V}_{O_2}$ was independent of F_{in}, O_2. In hypoxia, heart rate (HR) and cardiac output ($\dot{Q}$) (indirect Fick CO_2 method) were increased, O_2 transport ($\dot{Q} \times$ arterial O_2 content) at any work being similar at all F_{in}, O_2; maximal HR and $\dot{Q}$ were similar at all levels of F_{in}, O_2. Ventilation ($\dot{V}_{ex}$) increased with increasing hypoxia and was related to blood lactate under all conditions ($\dot{V}_{ex} = 20{\cdot}8 + 8{\cdot}94$ La; S.D. = 15·3, $n = 91$, $R = 0{\cdot}883$), confirming the findings of Asmussen & Nielsen (1958). $\dot{V}_{ex}$ was similar at maximal loads at all F_{in}, O_2, amounting to 65–90% of the maximal voluntary sustained ventilation (Freedman, 1966). Severe hypoxia (F_{in}, O_2 0·11) increased blood lactate at any work but in maximal work lactate was lower than with 0·16 and 0·21 F_{in}, O_2. The increases in lactate with increasing work in hypoxia were highly correlated with those found in normoxia. The relative change in lactate between 4 and 6 min of any work load was unaffected by hypoxia.

TABLE 1. Average values and ranges at the highest work load attained at each inspired oxygen concentration

Inspired O_2 concn. (F_{in}, O_2)	Work (kpm/min)	Oxygen intake ($\dot{V}_{O_2}$) (l./min)	Ventilation ($\dot{V}_{ex}$) (l./min)	Cardiac output ($\dot{Q}$) (l./min)	Heart rate (HR) (/min)	Lactate (La) (mM/l.)
0·11	900 (800–1000)	2·28 (1·96–2·72)	111 (89–125)	29·8 (27·0–30·8)	176 (174–180)	8·3 (6·5–9·7)
0·16	1150 (1000–1400)	2·92 (2·58–3·58)	109 (87–143)	31·3 (30·0–32·5)	184 (175–196)	11·9 (9·7–19·9)
0·21	1200 (1000–1400)	3·06 (2·56–3·63)	106 (100–115)	28·5 (26·8–31·6)	181 (173–189)	12·2 (11·7–15·4)
0·33	1300 (1000–1600)	3·50 (2·93–3·93)	117 (70–152)	29·6 (26·7–31·3)	186 (171–202)	8·9 (5·2–10·1)

In this type of continuous, step-wise increasing exercise cardiac and ventilatory adaptations maintain a constant oxygen delivery to the muscles for a given work whatever the inspired oxygen concentration, and are the major factors which limit maximal work. Anaerobic metabolism is mainly

governed by the rate of change in these adaptations between one work load and the next; maximal work sustained for 3–6 min under hypoxic conditions does not depend on the maximal use of anaerobic processes.

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Control of the splanchno-intercostal reflex by orbital cortex in the cat

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Since the orbital gyrus in the cat receives projections from both somatic and splanchnic afferents (Korn, Wendt & Albe-Fessard, 1963; Korn & Richard, 1965), the question was raised whether this cortical area could control viscero-somatic reflexes. Thus, in cats with intact C.N.S., the influence of supra-spinal centres upon the splanchno-intercostal reflex (Downman, 1955) was tested. It had been observed that the orbital cortex exerts both facilitatory and inhibitory effects upon this spinal polysynaptic reflex arc.

In chloralosed preparations, a somatic conditioning shock applied to the trunk induces a spino-bulbo-spinal reflex (as described by Shimamura & Livingston, 1963) which activates intercostal motoneurones. When adequately timed, this activation induces a strong facilitation followed by inhibition of a subsequent submaximal splanchno-intercostal reflex discharge. This facilitation is identical in time course to the facilitation observed by Downman & Hussain (1958) in the decerebrate cat.

After single-shock stimulation or treatment by strychnine of the orbital cortex contralateral to the stimulated thoracic wall, the amplitude of spino-bulbo-spinal discharge is higher; consequently, facilitation of the splanchno-intercostal reflex is increased. Acute ablation or functional elimination of the orbital cortex produces inverse results. Different lesions of the brain indicate that cortifugal impulses, which are triggered by somatic conditioning shocks, potentiate the facilitatory effect of the spino-bulbo-spinal system at the level of the medial bulbar reticular formation.

Repetitive stimulation of the orbital cortex strongly inhibits splanchno-intercostal reflexes. This inhibitory effect is not exerted on the intercostal motoneurone itself; it is markedly reduced after destruction of bulbar reticular formation.

Orbital cortex is thus an integrative centre which can control, through the two processes of facilitation and inhibition, the size and latency of viscero-motor reflexes.

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A cardiovascular depressor reflex elicited by occlusion of the coronary sinus in the dog

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Gonzales-Serratos & Erlij (1958) and Szentivanyi & Juhasz-Nagy (1962) showed that occlusion of the coronary sinus in an anaesthetized dog or cat caused systemic hypotension and bradycardia which were abolished by section of the cervical vagosympathetic nerves. We wished to confirm the reflex nature of this response, and to investigate its mechanism.

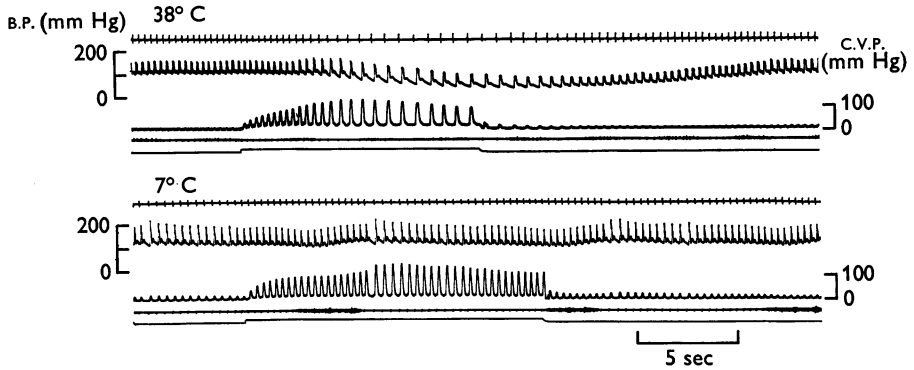

Fig. 1. The cardiovascular response to occlusion of the coronary sinus. Traces from above downwards: electrocardiogram; femoral arterial blood pressure; coronary venous pressure; diaphragmatic electromyogram; signal marker. Cervical vagi cooled to 7° C. in lower record. Coronary venous outflow occluded during signal.

A balloon cannula (Morawitz) was inserted into the mouth of the coronary sinus of open-chest dogs, anaesthetized with chloralose and urethane (70 mg/kg and 0·7 g/kg). Blood was returned to the animal via the right external jugular vein. Partial clamping of the outflow tubing after inflation of the balloon raised the coronary venous pressure (C.V.P.). Systemic arterial hypotension and bradycardia occurred in 48 of 52 occlusions (14 dogs) when the peak C.V.P. exceeded 50 mm Hg. When this peak pressure was less than 30 mm Hg, the depressor response was seen in only 2 of 51 occlusions (19 dogs). The response was abolished in three dogs

by section of the recurrent cardiac nerve and branches of the ventromedial cervical cardiac nerve (Mizeres, 1958). Recordings from efferent sympathetic nerves (ansae subclaviae) in four dogs showed a reduction of discharge during the depressor response. These changes were reversibly abolished by cooling the cervical vagi to 5–7° C.

Insertion of the cannula and inflation of the annular balloon alone (saline 0·9 %; 0·1–2·0 ml.; 7–17 mm O.D. in air), without outflow occlusion, also caused a drop in arterial blood pressure (12 of 20 experiments). However, there was either an increase or no change in the efferent sympathetic nervous discharge at the time of the hypotension, and the latter was unaffected by cervical vagotomy.

We have recorded action potentials from preparations of the above-named cardiac nerves. Afferent fibres from receptors in the epicardium and myocardium of the left ventricle show an increased discharge during occlusion of the coronary sinus.

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Secretion of chromogranin, the soluble protein from the chromaffin granules of the adrenal medulla, after splanchnic stimulation

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Observations on a histaminase of invertebrate origin, the renal appendages of *Eledone cirrhosa*

By MARGARET C. BOADLE.* *The Department of Pharmacology, Oxford, and the Marine Biological Laboratory, Plymouth*

Little is known on the occurrence of histaminase in invertebrates. On the other hand, there are many studies on the distribution of monoamine oxidase in some invertebrate phyla. In Cephalopods, many tissues are able to oxidize tryptamine, tyramine and related amines.

During a study of the enzymic activity of the tissues of *Eledone cirrhosa* it was found that histamine was oxidized by preparations of the renal

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appendages. Typical substrates of monoamine oxidase were also attacked. Liver extracts from the same species had monoamine oxidase activity, but there was no significant oxidation of histamine.

The preparation of renal appendages did not act on the two aliphatic diamines, putrescine and cadaverine. In addition, two differences between this preparation and the mammalian histaminases were noted: (1) the oxidation of histamine was not inhibited in the presence of 10^{-2} M semicarbazide, a strong inhibitor of the mammalian histaminases; (2) the preparation acted on ω-*N*-methylhistamine, a substance that is not a substrate of mammalian histaminases.

These observations are most readily interpreted by assuming that the histaminase activity is a property of a 'monoamine' oxidase present in the renal appendages. In biochemical terms, it appears that the interaction between histamine and the enzyme does not depend on the presence of pyridoxal-5-phosphate in the active centre of the enzyme (see Blaschko & Boadle, 1966).

Some time ago, Bertaccini (1961) reported that in *E. moschata* treatment with several monoamine oxidase inhibitors increased the histamine content of the optic ganglia. The presence in the species studied by Bertaccini (1961) of an enzyme similar to that found in *E. cirrhosa* could account for this finding.

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Aldosterone and angiotensin action on water absorption in the rat jejunum

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The effect of aldosterone on sodium and water absorption from everted sacs of rat jejunum has recently been described (Crocker & Munday, 1967). No immediate effect of aldosterone (up to 10 μg/ml. bathing fluid) was shown on water transport but rats placed on high sodium diets to depress endogenous aldosterone production did show an increased sodium and water transport in the sacs 28–36 hr after 5 μg aldosterone intraperitoneally (I.P.). This delayed stimulation of sodium and water transport was not a

consistent experimental result, but we observed that if it did not occur then the control values of mucosal water transfer were always significantly higher. The water transfer in aldosterone-treated rats was always constant but there was marked variation in the control (uninjected) values which often led to less marked stimulation of water transport.

These observations led to an investigation of possible endocrine factors affecting sodium and water absorption from rat jejunum.

It was established that neither the pituitary nor adrenals were involved in maintaining the high control values of mucosal water transfer.

TABLE 1. The effect of aldosterone, saline kidney extract, and angiotension on mucosal water transfer in 2 days post-operative, adrenalectomized-nephrectomized rats

Group	Mean mucosal water transfer (ml./g wet wt. of jejunum/hr)	
Control	0·70 ± 0·05 (11)	
10 μg aldosterone I.P. 28 hr before experiment	1·33 ± 0·06 (10)	< 0·001
Control	0·80 ± 0·06 (8)	
1 ml. kidney extract I.P. 20 min before experiment	1·32 ± 0·09 (8)	< 0·001
Control	0·96 ± 0·06 (5)	
With 10^{-10} g/ml. angiotensin in serosal and mucosal soln.	1·41 ± 0·09 (8)	> 0·001
Control	0·60 ± 0·03 (4)	
With 10^{-11} g/ml. angiotensin in serosal and mucosal soln.	0·87 ± 0·06 (4)	< 0·01
Control	0·73 ± 0·04 (4)	
With 10^{-10} g/ml. angiotensin serosal solution	1·25 ± 0·15 (5)	< 0·02

Control = 2-day post-operative, adrenalectomized-nephrectomized rats that are untreated.

Results expressed as means ± S.E.;

Number of animals in parentheses.

To consider a kidney effect, rats were adrenalectomized and nephrectomized and 20–24 hr later injected with 10 μg aldosterone I.P. 28 hr later the gut was removed and gave a 90% increase in water transfer over uninjected control levels. The control values were low but of the same order as obtained in earlier positive experiments.

A saline extract of rat kidneys was prepared (Gross, Brunner & Ziegler, 1965) to determine if the kidney was producing a substance that was affecting sodium and water absorption from the jejunum. One ml. of the extract was injected I.P. into 48 hr post-operative, adrenalectomized-

nephrectomized rats 20 min before removal of the gut and a 65% increase in mucosal water transfer over uninjected controls observed.

If the active principle in the extract was renin it would be expected that angiotensin might also produce an increase in water transport. Guts from adrenalectomized-nephrectomized rats were incubated in 10^{-10} g/ml. angiotensin in both serosal and mucosal solutions and gave a 47% increase in mucosal water transfer. Similar increases were observed with 10^{-11} g/ml.; and with 10^{-10} g/ml. angiotensin only in the serosal solution (Table 1).

The increased mucosal water transfer was always associated with an increased mucosal sodium flux. There was no significant change in glucose movement.

The action of aldosterone on water and sodium absorption from the jejunum cannot be explained as the result of an electrolyte adjustment by kidney, a possibility suggested by Levitan & Ingelfinger (1965).

This data suggests that the renin-angiotensin system may be affecting water and sodium absorption in the gut, the effect being a very rapid one in contrast to the long delay in the action of aldosterone reported earlier (Crocker & Munday, 1967). It could be that a membrane permeability function may be ascribed to angiotensin, with the pressor effect being obtained at higher concentrations. Both a diuretic and an antidiuretic response have been reported for angiotensin (Bock, 1963), although Leyssac, Lassen & Hess Thaysen (1961) have reported that active sodium transport is inhibited by angiotensin in isolated renal tissue.

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The uptake of plasma iron by immature red cells

By J. Fletcher* and E. R. Huehns. *Department of Clinical Haematology, University College Hospital Medical School, London W.C.*1

Dern, Monti & Glynn (1963) showed that a fraction of the plasma iron is cleared from the circulation more slowly than the rest. To explain their results they postulated a second iron binding protein distinct from transferrin. However, Hosain & Finch (1964) found no evidence for such a

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protein. An alternative explanation is that although all the plasma iron is bound to transferrin, it does not form a uniform pool. This is possible as each molecule of transferrin can carry two atoms of iron. Complexing with iron produces changes in conformation or charge of the protein and so there may be differences between molecules carrying one or two atoms of iron. Another possibility is that there are differences in the behaviour of iron attached to one of the binding sites rather than the other.

This hypothesis has been investigated by following the uptake into reticulocytes of radioactive iron, ^{59}Fe, from various preparations of plasma. In order to compare samples of plasma which have been treated in different ways the total amount of iron and transferrin must be the same in each and techniques have been devised to achieve this. The results show that the immature red cells take up iron attached to a transferrin molecule carrying two atoms before that attached to a molecule carrying one. Also iron carried on one of the binding sites is preferentially removed.

These findings suggest that the distribution of iron on transferrin leaving the bone marrow will reflect erythropoietic activity. This might be the mechanism which relates erythropoiesis to the absorption of iron in the intestine and mobilization from the reticuloendothelial system.

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Changes in the speed of mammalian fast muscle following long-term stimulation

By S. SALMONS* and G. VRBOVÁ.† *Department of Anatomy, University of Birmingham*

The motor units of mammalian slow muscle are normally activated more continuously, and at a lower frequency, than those of fast muscle (Denny-Brown, 1929; Vrbová, 1963*a*). It has been suggested that the differences between the mechanical characteristics of these muscles are largely due to the contrasting patterns of activity in their motor nerves. Thus the soleus muscle, when rendered quiescent by various operative procedures, becomes faster (Vrbová, 1963*b*). Furthermore, this increase in the speed of contraction can be prevented by electrical stimulation, and such stimulation has its maximum effect at a frequency of 10/sec, which reflects a normal pattern of discharge of tonic motoneurones (Vrbová, 1966).

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The present investigation was designed to examine the possible influence of stimulation on the time course of contraction of fast muscles. The difficulties which normally attend chronic stimulation have been overcome by the development of a totally implantable stimulator (Salmons, 1967). Stimulators were implanted in adult rabbits in an aseptic operation under pentobarbitone sodium anaesthesia. The device was sutured within the abdominal cavity with the electrode leads passing subcutaneously to one hind limb. The electrodes were fixed one on either side of the lateral popliteal nerve. Muscles supplied by this nerve were then activated at a rate of 10/sec, and the electrodes were arranged so as to produce a small but visible movement of the extremity. Stimulation was continuous over periods ranging from 1 to 6 weeks. At a terminal experiment the tibialis anterior and, in some cases, extensor digitorum longus muscles of both hind limbs were prepared for the recording of isometric contractions.

In all cases, the stimulated muscle was significantly slower than its unstimulated counterpart in the opposite hind limb. Increases of up to 140% were recorded in the time to peak of single contractions. Relaxation was correspondingly prolonged; indeed in the shorter term experiments relaxation was affected more than contraction. The fact that the tetanus: twitch ratios of all the stimulated muscles were markedly reduced suggests that a change in the time course of the active state had taken place.

Thus electrical stimulation at a frequency imitating the rate of discharge of motoneurones innervating slow muscles produces slowing of the contraction and relaxation of fast muscles. The magnitude of these changes is sufficient to account for the alterations in speed which occur following cross-innervation of slow and fast muscles (Buller, Eccles & Eccles, 1960). These results show that skeletal muscle is capable of responding adaptively to the type of activity imposed upon it.

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The innervation of the smooth muscle of the nictitating membrane of the cat

By A. C. ESTERHUIZEN, J. D. P. GRAHAM, J. D. LEVER and T. L. B. SPRIGGS. *Departments of Anatomy and of Pharmacology, University of Wales, Cardiff*

Sections of muscle treated by a formol-fluorescence technique (Spriggs, Lever, Rees & Graham, 1966) exhibit a dense plexus of beaded fluorescent fibres characteristic of adrenergic nerves. Electron microscopy reveals an abundance of unmyelinated axons, many of which contain concentrations of mitochondria and microvesicles (some with an electron-dense content) typical of terminal axoplasm. The neuromuscular interval may be as small as 200 Å but is often wider. Two to twenty-eight days after superior cervical ganglionectomy the dense fluorescent plexus indicating catecholamine-containing nerves is absent and very few axon profiles are found with the electron microscope in the denervated muscle.

Since noradrenaline is known to be selectively accumulated by adrenergic nerves, freshly excised muscle was immersed for 25 min in McEwen's solution containing $1{\cdot}2 \times 10^{-5}$ g/ml. DL-noradrenaline-7-T (81·3 μc) and then washed repeatedly for 60 min before processing the muscle for high resolution autoradiography. Characteristic silver grain deposits indicate the location of the tritium-labelled noradrenaline, and concentrations of silver grains were found over the majority of axons in the muscle. It is clearly established, therefore, that the majority of nerves in the nictitating membrane muscles are adrenergic and sympathetic in nature.

Burn & Rand (1962) have implicated acetylcholine to mediate in adrenergic transmission. We therefore investigated these established adrenergic axons for the presence of acetylcholinesterase at the electron microscope level using the acetylthiocholine technique developed by Lewis & Shute (1966). A differential count of axons in 100 μ square areas of muscle (obtained from twelve different muscle specimens from three cats) revealed means of $69{\cdot}5 \pm 7{\cdot}1$ S.E. acetylcholinesterase negative axons compared with only $0{\cdot}3 \pm 0{\cdot}2$ S.E. acetylcholinesterase positive axons.

If we make the reasonable assumption that the presence of acetylcholinesterase is indicative of a system utilizing acetylcholine as a chemical mediator, then we are unable to support the contention of Burn & Rand (1962) that a cholinergic mechanism exists in adrenergic nerve endings—at least for those adrenergic nerve endings in the cat nictitating membrane muscles.

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Effects of some anions on the mechanical threshold and electrical properties of frog twitch muscle

By C. Y. KAO* and P. R. STANFIELD.†*Physiological Laboratory, University of Cambridge*

Two micro-electrodes and a feed-back arrangement were used to hold the membrane potential constant at a point on a frog sartorius fibre. The relation between voltage and total current was studied, together with visual observations for contraction. The spike threshold was taken as the lowest potential at which inward current appeared abruptly; the mechanical threshold, that at which contraction was seen at 60 × magnification; and the threshold for delayed rectification, when the slope of the voltage-steady current (~ 100 msec) curve increased by 10% or more.

TABLE 1. Actions of anions on thresholds for spike, contraction and rectification of late outward current

Concentration used (mM)		Spike threshold (mV)	Mechanical threshold (mV)	Delayed rectification threshold (mV)	
				Isotonic	Hypertonic
Chloride	121	−58·5 ± 1·2 (10)*	−48·8 ± 0·8 (13)*	−52·0 ± 2·4 (13)*	−50·1 ± 1·5 (11)*
Bromide	115	−58·2 ± 1·6 (9)	−52·1 ± 2·1 (7)	−53·7 ± 2·3 (7)	−55·7 ± 0·9 (7)
Nitrate	115	−59·5 ± 1·6 (8)	−61·1 ± 0·7 (11)	−61·7 ± 0·7 (11)	−60·0 ± 1·2 (11)
Iodide	58	−63·1 ± 1·3 (8)	−64·6 ± 0·4 (10)	−64·2 ± 0·8 (10)	−65·3 ± 1·0 (9)
Thiocyanate	58	−59·9 ± 1·4 (11)	−67·9 ± 0·6 (10)	−69·0 ± 0·6 (10)	−69·1 ± 0·7 (10)
Methylsulphate	115	−56·7 ± 0·9 (10)	−53·1 ± 0·9 (8)	−53·4 ± 1·5 (8)	−56·0 ± 1·9 (9)

* Means ± standard error of mean, followed by number of fibres used in parentheses. Hypertonic solutions contained additional 350 mM sucrose. All experiments were performed at room temperature *ca.* 20° C. All fibres were held at −90 mV. Except those for spike thresholds, all fibres were pretreated with tetrodotoxin.

Table 1 summarizes the results obtained during March and April. In most solutions the mechanical threshold could not be determined properly unless the spike was first abolished with tetrodotoxin (0·1 μg/ml.). In nitrate and thiocyanate, where contractions normally occurred at a more negative membrane potential than did spikes, tetrodotoxin had no effect on the mechanical or the delayed rectification threshold.

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It is evident from Table 1 that the thresholds for contraction and delayed rectification are similar and that both are affected to the same extent by different anions (see Hodgkin & Horowicz, 1960; Sandow, 1965). On the other hand, the spike threshold shows no correlation with the mechanical threshold. Since the thresholds for delayed rectification are the same in hypertonic solutions, in which no visible contractions occurred, as those in isotonic solutions, the increase of outward current at the mechanical threshold cannot be attributed to some artifact caused by movement. The parallel shifts in the thresholds for contraction and rectification of the late outward current suggest either that the two processes can be affected very similarly by different agents, or that these processes are causally related.

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The effect of sodium concentration on calcium movements in giant axons of *Loligo forbesi*.

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Niedergerke (1963) established that the calcium influx in frog ventricles is markedly increased by replacing external NaCl with LiCl, choline Cl or sucrose. Table 1 summarizes experiments which show that squid axons

TABLE 1. Effect of $[Na]_o$ and $[Na]_i$ on calcium influx

$[Na]_o$ (mM)	$[Na]_i$ (mM)	Ca influx (p-mole/cm² sec)
460	70	0·15 ± 0·02 (19)
3 (Li)	70	2·1 ± 0·4 (15)
3 (dextrose)	70	2·9 ± 0·17 (5)
Prestimulated axons (3·6 or $2·4 \times 10^5$ impulses in Li or Na)		
460	30	0·17 ± 0·05 (5)
460	110	0·66 ± 0·15 (2)
3 (Li)	30	0·48 ± 0·05 (2)
3 (Li)	110	5·70 ± 0·3 (3)

The estimates of $[Na]_i$ are averaged from nine axons—five resting, two stimulated in Na and 2 in Li. Influxes are means, with S.E. and number of axons, from data on paired axons; temperature 18–22° C. Three pairs in the dextrose series were treated with 10^{-5} M ouabain, which had no effect. Axons were usually obtained from refrigerated mantles.

behave in a similar way. Influxes were determined by counting extruded axoplasm after 20 min exposure to solutions containing 11 mM-Ca labelled with ^{45}Ca (see Hodgkin & Keynes, 1957). From the upper part of the table

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† National Science Foundation Postdoctoral Fellow.

it can be seen that the average calcium influx from lithium or dextrose sea water was 15–20 times greater than that from Na sea water. The ratio in three pairs of axons exposed to identical solutions was within 0·3 of unity.

The immediate effect of changing from $[Na]_o$ to $[Li]_o$ was to increase the net entry of calcium since the calcium efflux (from axons micro-injected with ^{45}Ca), decreased if external Na was replaced by Li. This decrease in efflux was fully reversible only when calcium-free Li was used.

The lower part of the table shows the effect of changing internal sodium concentration by prestimulating axons in either lithium or sodium sea water. The results suggest that a rise in $[Na]_i$ is associated with a large increase in calcium influx.

The tentative conclusion from these experiments is that raising $[Na]_i$ or lowering $[Na]_o$ tends to make calcium ions move inward through the membrane.

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An action of chlorpromazine on spontaneous action potentials in the preganglionic superior cervical sympathetic trunk of the cat

By R. C. ELLIOTT. *Department of Pharmacology, St Bartholomew's Hospital Medical College, London, E.C.*1

Cats were anaesthetized with an ether, nitrous oxide and oxygen mixture. Action potentials were recorded from bundles teased out of the preganglionic superior cervical trunk, amplified with a narrow-band-pass preamplifier and a Solartron CX1271 amplifier. The action potentials were displayed on a Solartron oscilloscope (model CX1183) for photography, and output from the oscilloscope was fed into an audio-monitor. A counter (Advance 1 mc/S Timer Counter, Type TC 1A) measured the rate of discharge of action potentials per sec during a 10 sec period. The rate was measured during five consecutive 10 sec periods and this procedure was repeated at 5 min intervals. At 10 min intervals the oscilloscope trace was photographed for about 5 sec on moving film.

Control recordings were obtained during a 1–2 hr period, then chlorpromazine (CPZ) was injected into the femoral vein and recording continued for a further 2 hr.

CPZ in a dose of 0·2 mg/kg ($n = 3$) was followed by a small reduction in the rate of discharge. This reduction was transient and difficult to distinguish from random fluctuation. With 1 mg/kg of CPZ, a marked reduction in the rate of discharge occurred and no recovery was observed in the ensuing 2 hr period. When the average rate of discharge during the

2 hr control period was compared with the 2 hr after 1 mg/kg of CPZ the mean % reduction was 74 % (n = 5, range 42–100 %). Maximum inhibition was usually attained within 4 min of injection.

Following 0·5 mg/kg of CPZ, there was an immediate fall in the rate of discharge. In eight out of the twelve experiments, recovery to the pre-injection rates occurred during the 2 hr following the injection. When the rate of discharge immediately preceding the injection was compared with that 8 min after injection, the mean % reduction was 56 % (n = 12, range 24–84 %).

The implications of these findings were discussed.

Transmembrane potentials recorded from neurones of rabbit superior cervical ganglion

By S. D. ERULKAR and J. K. WOODWARD. *Department of Pharmacology, University of Pennsylvania, Philadelphia, Pa., U.S.A.*

Responses to preganglionic nerve stimulation have been recorded from neurones in the superior cervical ganglion of anaesthetized rabbits by means of extra- and intracellularly placed micro-electrodes. Mean values of resting membrane potentials recorded from fifty cells were 68·6 mV (range 40–105 mV). Spike amplitudes of responses from this same population of cells averaged 76·5 mV (range 40–120 mV). In comparison, antidromic stimulation of the post-ganglionic nerve (external carotid nerve) elicited spikes whose amplitudes invariably exceeded those elicited from the same unit by orthodromic stimulation. The responses of some cells to antidromic stimulation, however, consisted of a long-lasting depolarizing wave superimposed upon which were one or two spike potentials with latencies up to 70 msec. This depolarization could occur either alone or following the early antidromically elicited spike response and appeared to be synaptically generated.

Preganglionic nerve stimulation elicited one or two, or rarely three spikes in responses from different cells. When two spikes were elicited, the second spike was generated within 7–20 msec following the first spike, and the level for generation of the second spike differed from that for generation of the first spike. Direct stimulation of the cell by currents passed through the intracellularly placed micro-electrode elicited a single spike response and no repetitive firing occurred even with currents as high as 10^{-7} A. In one case, current of 10^{-8} A elicited a second spike 34 msec following the first. Repetitive stimulation of the preganglionic nerve, however, showed that the spikes could fire synchronously with stimulus rates from 30 to 50/sec and that these spikes were generated at different levels of depolariza-

tion from one another. The observed variation of the amplitude of the firing level suggests the possibility of sites for spike generation which are located remotely from the site of the recording electrode.

Finally, as the stimulus intensity was progressively increased, there occurred a progressively increasing hyperpolarization so that at intensities approximately 5 × threshold, one of the spikes in a two-spike response failed to be generated. No hyperpolarizing potentials were recorded which were not associated with a spike discharge or with previous depolarization.

Stimulation of cat aortic and carotid chemoreceptors by carboxyhaemoglobinaemia

By M. W. EDWARDS and E. MILLS. *Department of Physiology, Middlesex Hospital Medical School, London, W.*1

Duke, Green & Neil (1952) did not observe an increased impulse activity in carotid chemoreceptor afferents during carboxyhaemoglobinaemia. Recently, Paintal (1967) reported that aortic chemoreceptor activity was increased under nearly the same conditions. Since P_{a,O_2} was notmeasured, the chemoreceptor stimulation cannot be attributed solely to carboxyhaemoglobinaemia. To determine if there is such chemoreceptor stimulation it is essential that the P_{a,O_2} and arterial pressure during the period in which CO is administered should change as little as possible from control values.

Satisfactory few-fibre preparations of aortic or carotid chemoreceptor afferents were obtained in fifteen cats anaesthetized with pentobarbitone or with α-chloralose and urethane. In six experiments, animals breathed spontaneously through Müller valves. Nine animals were artificially ventilated with a Starling pump (f = 20/min; Vt = 15–20 ml./kg). Records of impulse activity and P_{a,O_2} determinations were made during control periods in which animals breathed air, 15–18 % O_2 in N_2, or both.

Spontaneously breathing animals inhaled CO (1·0 % in air) for 3–20 min. Aortic chemoreceptor activity increased in all experiments as haemoglobin CO saturation (S_{CO}) increased to 22–64 %. However, the mean arterial pressure fell 5–40 mm Hg and the P_{a,O_2} fell 2–44 torr in five of these experiments. In the one experiment in which arterial pressure and P_{a,O_2} did not fall, chemoreceptor impulse frequency was five times the air control frequency when S_{CO} was 40 %. Activity decreased when S_{CO} fell after air was substituted for CO.

Carbon monoxide (0·3–0·75 % in air) was administered to artificially ventilated cats for 4–45 min. Carotid or aortic chemoreceptor activity increased in all experiments. In four experiments, the arterial mean pressure did not change by more than 5 mm Hg and the change in P_{a,O_2}

did not exceed 4 torr. In these experiments, the maximum measured impulse frequency (9–13/sec) occurred when S_{CO} reached its highest value (18–38 %) and was 2–5 times the impulse frequency during the air control. This frequency was comparable to that produced by ventilation with 15–18 % O_2 even though the P_{a,O_2} was 16–20 torr higher during ventilation with CO.

These results demonstrate that carboxyhaemoglobinaemia stimulates both aortic and carotid chemoreceptors in the absence of significant changes in P_{a,O_2} and arterial pressure. Thus, they confirm and extend the findings of Paintal (1967). The increase in impulse activity is probably not great enough to have been detected by the methods of Duke, Green & Neil (1952). The present study helps to resolve the apparent discrepancy pointed out by Daly, Lambertsen & Schweitzer (1954) between the effects of stagnant hypoxia and anaemic hypoxia upon chemoreceptor activity.

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Effects of hypertonicity and of high potassium concentration on the rate of release of hormones from neurohypophysial glands of adult and new-born rats and birds, *in vitro*

By S. E. DICKER. *Department of Physiology, Chelsea College of Science, London, S.W.*3

It has been shown that depolarization of the fibres of the isolated pars nervosa of adult rats and birds by high K concentration *in vitro* results in an increased release of the neurohypophysial hormones (Douglas & Poisner, 1964; Dicker, 1966; Bern, 1966). This increase, however, was not observed with glands of new-born rats (Dicker, 1966). It has also been shown that when isolated glands of adult rats were incubated in a hypertonic Ringer–Locke solution, the rate of release of the hormones remained similar to that observed in control experiments (Douglas & Poisner, 1964; Lederis, 1965; Dicker, 1966).

Posterior pituitary glands of adult and new-born rats and of adult and neonate domestic fowls were incubated at 37° C, first in a $CaCl_2$ free Ringer–Locke solution (320 m-osmole/l.) and then in a $CaCl_2$ free hyper-

tonic Ringer–Locke solution containing 1·8% NaCl (540 m-osmole/l.) Incubation of glands from adult rats and birds in $CaCl_2$ free hypertonic solution produced a marked increase in the release of both oxytocic and pressor activities; this increase disappeared, however, after addition of $CaCl_2$ (2·2 mM) to the hypertonic incubating fluid. With glands of new-born rats (up to 10 days old) and new-born chicks (up to 4 weeks old), the increase of tonicity of the incubating fluid with or without $CaCl_2$ had no effect on the rate of release of neurohypophysial activities which remained comparable to that observed during control experiments.

When isolated pituitary glands of adult fowls were incubated at 37° C in Ringer–Locke solution, containing a high concentration of KCl (56 mM) and $CaCl_2$ (2·2 mM), there was an enhanced rate of release of both oxytocic and pressor activities, similar to that observed with glands from adult rats. When glands from chickens (up to 2 weeks old), however, were incubated with high KCl, no significant increase of the release of neurohypophysial activities was observed.

These results confirm and extend previous observations (Dicker, 1966; Bates & Dicker, 1967), that stimuli which normally produce an enhanced release of neurohypophysial hormones from isolated glands of adult animals have no such effect on glands of new-born animals. This appears to be true for both rats and birds. No explanation can be proposed at this stage for the protective action of $CaCl_2$ against the effects of hyperosmoticity observed with glands of adult but not with those of new-born rats or birds.

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Changes in content and intraneuronal distribution of adrenaline in the frog's heart after cutting both vagosympathetic trunks

By R. I. WOODS. *University Laboratory of Physiology, Oxford*

Under anaesthesia with MS 222 (Sandoz), the vagosympathetic trunks of frogs (*R. temporaria*) were cut 3–5 mm below the vagal ganglion. The animals were killed 1 hr to 8 days later. Air-dried whole mounts of atria, atrial septum and sinus venosus, and paraffin wax sections of freeze-dried ventricles were treated with formaldehyde gas at 75° C and examined with

* M.R.C. Scholar.

a fluorescence microscope. Adrenaline was assayed fluorimetrically by the trihydroxyindole method after chromatographic extraction of heart homogenates on alumina.

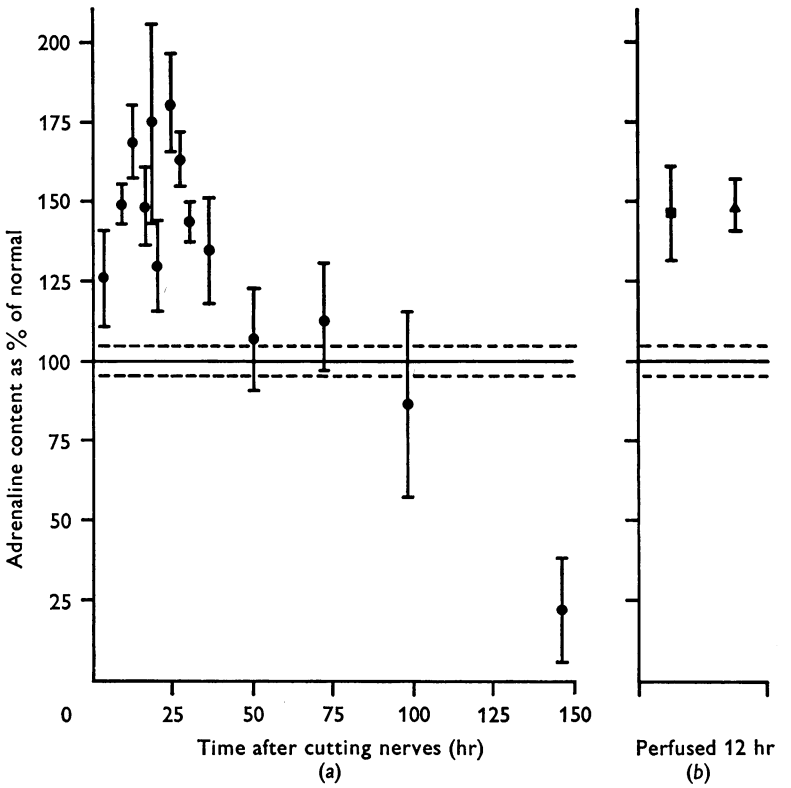

Fig. 1. (*a*) The adrenaline content of pooled hearts as a percentage of the normal, (means and S.E.); (*b*) hearts perfused with modified Ringer's solution for 12 hr, (■) isolated and (▲) in pithed frogs. S.E. of normal shown as dotted line.

Between 1 and 36 hr after cutting both vagosympathetics, there is a rise both in the intensity of nerve fibre fluorescence and in the number of fibres visible. The terminal fibres and swollen varicosities are the brightest part of the nerves during the first 3 hr following axotomy, after which the region of maximum fluorescence gradually moves up the nerve fibres into the largest bundles. In normal hearts the fluorescence is brightest in the small nerve bundles running on the muscle trabeculae and giving rise to the varicose terminal fibres, which are usually hardly visible. Loss of fluorescence and fragmentation also begins in the finest fibres, which have mainly disappeared by 12 hr. This progresses to the larger bundles which show advanced degeneration by 3 days, although some fragments are still present at 8 days.

The changes in intensity of the nerve fibre fluorescence have the same course as the changes in adrenaline content of whole hearts (Fig. 1). Hearts perfused with modified Ringer's solution for 12 hr, either isolated or in pithed frogs, show a similar increase in adrenaline content. It is concluded that the increase in adrenaline content is due to synthesis in the peripheral parts of the adrenergic neurones.

The fine structure of cat fusimotor endings

By M. N. ADAL and D. BARKER. *Department of Zoology, University of Durham*

Studies of teased silver preparations have distinguished three types of fusimotor ending in cat hind-limb spindles, the diffuse multiterminal trail ending (Barker & Ip, 1965) and two kinds of plates, Types I and II (Barker, 1966) abbreviated as p_1 and p_2 (Barker, 1967). We have observed the fine structure of p_2 plates and trail endings in spindles teased out from small pieces of de-afferentated and sympathectomized peroneous longus muscle fixed in 1 % veronal acetate buffered osmium tetroxide solution for 2 hr at 4° C. After dehydration in ethyl alcohol, the spindles were embedded in Araldite and thin sections cut, stained with lead citrate, and examined in an A.E.I. EM6B electron microscope. Electron micrograph tracings of the myoneural junctions of the two fusimotor endings are shown in Fig. 1, which includes an extrafusal motor junction for comparison. We have not yet succeeded in sectioning a p_1 plate. The essential features are as follows:

Trail endings (Fig. 1*A*). Axon terminals are applied to surface contour of muscle fibre and overlie thinly spread sole plate that lacks depressions or guttering. Junctional folds rare; unbranched and few in number when present; average depth 0·4 μ, entrance width 0·1 μ. Sections usually include several preterminal axons. Of fourteen trail-ending ramifications sectioned, nine were located on chain fibres, five on bag fibres.

p_2 plates (Fig. 1*B*). Knob-like axon terminals typically lie in shallow depressions of extensive thinly spread sole plate. Junctional folds usually present. These differ from the junctional folds of extrafusal plates in being unbranched and half the depth (average 0·5 μ), and in having entrances twice the width (average 0·1 μ). Of twenty-one p_2 plates sectioned, thirteen were located on bag fibres, eight on chain fibres.

Our observations suggest that the electron micrographs of rat fusimotor endings published by Merrilees (1960) are sections through p_2 plates, and that those published by Gruner (1961, man) and Landon (1966, rat) illustrate trail junctions.

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

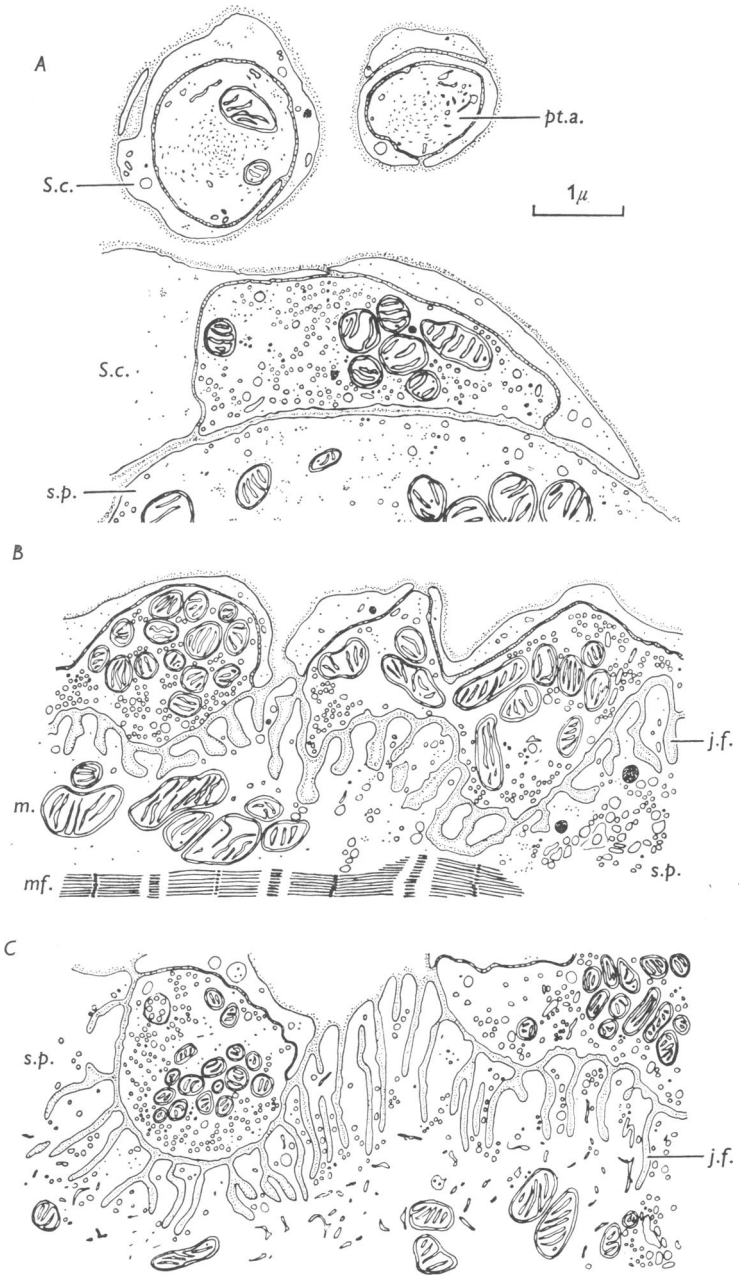

Fig. 1. Electron micrograph tracings of cat myoneural motor junctions (peroneal muscle) showing axon terminals of *A*, a trail ending (T.S.); *B*, a p_2 plate (L.S.); and *C*, an extrafusal plate (L.S.). *j. f.*, junctional fold; *m.*, mitochondrion; *m.f.*, myofibrils; *pt.a.*, preterminal axon; *S.c.*, Schwann cell; *s.p.*, sole plate.

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The distribution of myelinated fibres in peripheral nerves in the cat

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Visual responses from ipsilateral tectal units in the frog

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Gaze & Jacobson (1962*a*, *b*) found that in the frog, in addition to the retinal projection to the contralateral tectum, the nasal visual field also projected, retinotopically, to the ipsilateral tectum. The pathway for this ipsilateral projection appears to involve passage through the contralateral tectum. The receptive field characteristics of over 200 single units in this ipsilateral projection have now been studied. The major differences between responses from contralateral and ipsilateral units are:

(1) The occurrence in most ipsilateral unit responses, at all depths, of sustained discharges to standing edges in the receptive field.

(2) The complete absence, ipsilaterally, of the type of contralateral unit known as 'changing contrast detectors' (Maturana, Lettvin, McCulloch & Pitts 1960).

The field size of most ipsilateral units studied was from 5 to 8°. The response of most ipsilateral units to moving or stationary objects is remarkably similar. They respond to movement of small disks or edges from 1 to 10° in size but do not usually respond to larger objects. It may, thus, be inferred that these fields possess an inhibitory surround. The most striking feature of the ipsilateral units is their response to an edge brought into the receptive field and held there. The units show sustained firing in response to such a stimulus, the duration of the response being from several seconds to several minutes. These units also display the phenomenon of 'non-erasability', i.e. the sustained discharge is promptly inhibited by a transient step to darkness, but returns when the background is re-illuminated. A stimulus near the centre of the receptive field gives a

more sustained response than one near the periphery. The sustained discharge may be inhibited if an edge is moved in the visual field but outside the receptive field. The inhibition may be transitory or sustained and is most marked when the inhibiting edge is within 5–10° of the limit of the receptive field but has been observed when the edge was more than 90° away.

Very little evidence of directional selectivity has been found. No units that could be activated by both eyes were found.

Although all ipsilateral units tend to share the above features, they could be subdivided according to their response to background illumination. The most superficial units show no response to background changes whereas the deeper units respond to the 'on' and 'off' of background light.

A proportion of the deeper units possess distinctive properties in that they respond to large as well as small objects, show no evidence of an inhibitory surround, and respond with bursts to decremental dimming, but not decremental brightening, of the background.

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The response of dental mechanoreceptors to forces applied to the teeth

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Receptors capable of relaying information concerning the nature of forces on the teeth are located within the tooth-supporting structures, probably in the periodontal ligament, and have been classified by Pfaffmann (1939) and Ness (1954) as rapidly adapting, slowly adapting and spontaneously discharging mechanoreceptors. Although the response of these receptors to sustained forces has been investigated previously, there is no information available concerning their response to changing forces.

The activity in these mechanosensitive units was recorded in strands dissected from the inferior dental nerve in anaesthetized dogs. The lower incisor and canine teeth were stimulated using an electromagnetic force applicator (Pye-Ling vibration generator V 47) with forces varying between 50 and 200 g, applied for periods between 5 and 20 sec at linear rates varying between 25 g/sec and 2·5 kg/sec.

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Of the thirty-two mechanoreceptor units studied, five were rapidly adapting, eighteen slowly adapting, and nine discharged spontaneously. All the units showed a marked increase in the frequency of discharge when the rate of application of a standard force was increased.

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Post-tetanic increase in frequency of miniature end-plate potentials in calcium-free solutions

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In the absence of nerve impulses, miniature end-plate potentials (m.e.p.p.s) at the neuromuscular junction recur at a frequency of about 1/sec, and this frequency is known to increase after a short period of tetanic stimulation of the nerve (Liley, 1956; Brooks, 1956; Hubbard, 1963). The aim of the present experiments was to find out if the frequency of m.e.p.p.s still increased when individual nerve impulses, during the tetanus, failed to release transmitter.

In the initial experiments, frog sartorius muscles were bathed in a Ringer solution whose calcium was replaced by 2 mM-Mg. In these conditions a single impulse invades the nerve terminals but fails to release transmitter (Katz & Miledi, 1965). During tetanic stimulation (50–100/sec for 20 sec) there is cumulative facilitation and some impulses release a few packages of transmitter. To abolish this release the Mg concentration was raised to 10–20 mM; or the Ca concentration was reduced to 10^{-9} M or less, by chelation with 1 mM EGTA. In such solutions individual impulses failed to release transmitter throughout the period of stimulation, as judged by the fact that the occurrence of m.e.p.p.s shortly after the arrival of each nerve impulse was no more frequent than during the rest of the interval.

Under these conditions, the frequency of m.e.p.p.s, at some junctions, increased progressively during the tetanus and remained high for a short period after the stimulation. The ratio of m.e.p.p. frequency after the tetanus to that preceding it ranged from 1 to 5. The ratio became larger as the Ca concentrations were raised and with 1·8 mM Ca the post-tetanic increase in frequency can be 10–100 times.

An incidental observation was the finding of a decrease in the amplitude of m.e.p.p.s in the presence of a chelating agent (1 mM EDTA or EGTA).

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This reduction in size can account, at least partly, for an apparent decrease in m.e.p.p. frequency which has been reported with decalcified solutions (Elmqvist & Feldman, 1965). In our experiments, the frequency of m.e.p.p.s in some fibres gave the appearance of being reduced, probably because the lowering of amplitude caused some m.e.p.p.s to be lost in amplifier noise; but in other fibres normal m.e.p.p. frequencies were seen even after several hours with the chelating agent present.

Since a post-tetanic increase in m.e.p.p. frequency could still he obtained, although reduced, in very low Ca concentrations, it would seem that this release is not markedly dependent on external Ca, unlike the immediate release evoked by a nerve impulse. Calcium could still be responsible for the increase in m.e.p.p. frequency, if one assumes that in Ca-free solutions the nerve impulse mobilizes some Ca from bound sites in the membrane, making it available for reactions that lead to transmitter release; and that the increase in ionized Ca within the membrane decays slowly.

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Involuntary and voluntary vocal frequency modulation

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The periodicity, τ, and frequency deviation amplitude, $\delta f/f$, of involuntary vocal frequency modulation ('pitch vibrato') during tones sung at constant mean frequency depend little on the acoustic tuning of the gas-filled vocal resonators above and below the larynx (Kay, 1966).

Involuntary frequency deviation increases and periodicity decreases as sound amplitude increases. A graph of $\delta f/f$ against τ has asymptotes near $\delta f/f = \pm 0{\cdot}2\,\%$ and at τ_0, a minimal value of τ characteristic for a given subject (Fig. 1).

A singer generating his most rapid possible *voluntary* trill effects an approximately sinusoidal frequency modulation; its periodicity coincides with the subject's involuntary τ_0, though the frequency deviation is larger.

These modulation characteristics are in part determined by mechanical properties of the larynx but also in part by active physiological controls.

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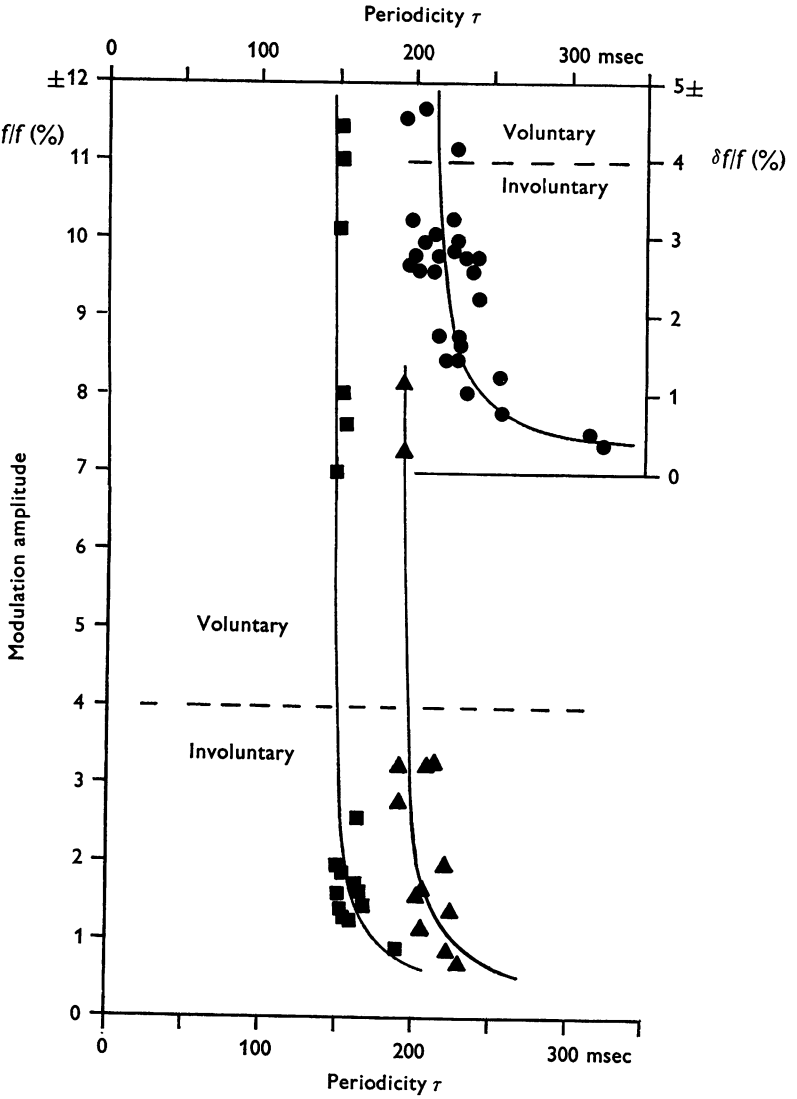

Fig. 1. Periodicity (τ) and frequency modulation amplitude ($\pm \delta f/f$) of pitch vibrato; involuntary below, voluntary above the dashed lines. Circles, untrained voice in tenor range—ordinate displaced to avoid overlap; triangles, trained tenor; squares, trained soprano.