(*b*) the opportunity of making original observations and organizing group experiments,

(*c*) the basic information gained about cardiovascular pharmacology and physiology.

COMMUNICATIONS

Effects of lowered temperature on the responses of the smooth muscle of the isolated, blood-perfused dog's spleen to sympathetic nerve stimulation

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Differences have been observed in the responses of the capsular and vascular smooth muscle of the dog's spleen to various stimuli. Low-frequency sympathetic nerve stimulation (1–3 Hz) is accompanied by almost maximal contraction of the splenic capsule, whilst changes in splenic vascular resistance are minimal and not sustained (Davies, Gamble & Withrington, 1968*a*). Higher frequencies of stimulation (3–10 Hz) are required to elicit increases in splenic vascular resistance. This pattern is mimicked by close arterial infusions of adrenaline and noradrenaline (Davies, Gamble & Withrington, 1968*b*). In the present series of experiments further differences were observed in the responses of the two types of smooth muscle to sympathetic nerve stimulation when the temperature was lowered.

The spleen of one dog was isolated, placed in a plethysmograph and perfused with blood from a femoral artery of a second, donor dog. Perfusion pressure, mean splenic arterial blood flow and changes in spleen volume were recorded. Splenic venous blood temperature was also continuously recorded by means of a thermistor. The temperature of the spleen was lowered by cooling the arterial blood by passage through water-cooled coils, and by lowering the temperature of the liquid paraffin in the plethysmograph.

The splenic vascular resistance increased as the temperature was lowered and, on rewarming the spleen, the vascular resistance decreased again, usually to a level lower than during the precooling stage.

The splenic sympathetic nerves were stimulated (50 V, 0·5 msec) at five frequencies (0·1, 0·5, 1·0, 2·0, 3·0 Hz) within the physiological range to obtain plateau responses. In most experiments the test stimuli were applied at 37°, 27° and again at 37° C. The effect of this temperature reduction on the smooth muscle responses to sympathetic nerve stimulation was to decrease the capsular contraction and to increase the vaso-

constrictor response at all five frequencies. For example in six experiments the mean increase in splenic vascular resistance to 1 Hz at 37° C was 33%, at 27° C 235% and on rewarming to 37° C 32%; the corresponding mean capsular contractions were 38·5, 19·7 and 34·0 ml. At the stimulation frequency of 3 Hz the mean changes in splenic vascular resistance were 620, 1485 and 465%, whilst the mean capsular contractions were 40·5, 23·5 and 43·4 ml at 37°, 27° and 37° C respectively. The frequencies of nerve stimulation (0·1, 0·5 Hz) which at 37° C had no effect on splenic vascular resistance induced marked vasoconstriction at a spleen temperature of 27° C.

These differential changes in smooth muscle responses, produced by cooling the spleen, could arise from alterations in the sensitivity of the smooth muscle cells to the transmitter noradrenaline or to changes in the release, inactivation and nature of the transmitter at the two sites.

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Effect of gastric peptides on acid and pepsin secretion in the frog *Discoglossus pictus*

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Discoglossus pictus Otth, a frog of the family Discoglossidae, is mainly distributed in the Western Mediterranean. In common with other amphibians the gastric mucosa contains cellular components that secrete both acid and pepsin, unlike mammalian gastric mucosa which contains separate acid and pepsin-secreting cells. Mammalian gastric stimulants, gastrin and histamine, are effective in promoting gastric secretion both *in vivo* and *in vitro*. The effect of pentagastrin, caerulein, and a crude skin extract of *D. pictus* have been investigated in intact frog preparations by the method described by Morrissey & So (1970). Pentagastrin is a synthetic pentapeptide and caerulein is a skin peptide from the Australian frog, *Hyla caerulea*. These two molecules possess the same terminal tetrapeptide amino acid sequence as human gastrin. Ten experiments were carried out on each stimulant and its controls. The perfusate from the frogs' stomach was tested for peptic activity by the method of Anson (1938). Changes in acid secretion were expressed as μ-equiv H^+ secreted per 15 min. Saline blanks showed that the hydrogen-ion concentration in the stomach per-

fusate did not increase significantly over a 3 hr period indicating a low level of spontaneous secretion. Pentagastrin (2 μg) gave a marked response, with a mean peak acid output of $105{\cdot}50 \pm 4{\cdot}01$ (S.D.) μ-equiv H^+ per 15 min. Caerulein (2 μg) gave only a small response, the mean peak output being $13{\cdot}66 \pm 1{\cdot}10$ (S.D.) μ-equiv H^+ per 15 min. The *Discoglossus pictus* skin extract results fell within the range of the saline blanks as far as acid secretion was concerned. The peptic response showed an inverse relationship to the acid response. Thus pentagastrin (2 μg) showed only a slight activity, the mean peak response being $1{\cdot}58 \pm 0{\cdot}52$ (S.D.) pepsin units (P.U.) $\times 10^4$, whereas caerulein (2 μg) elicted a large mean peak response of $4{\cdot}75 \pm 0{\cdot}51$ (S.D.) P.U. $\times 10^4$. The mean peak result for the skin extract fell between these two values, being $2{\cdot}50 \pm 1{\cdot}33$ (S.D.) P.U. $\times 10^4$. These pepsin secretory responses would indicate that the skin extract displays properties similar to caerulein, an amphibian skin peptide.

When caerulein is made up in ethanol the peptic response is similar to caerulein in saline but the response is extended over a period of 2 hr with a mean peak response of $4{\cdot}14 \pm 2{\cdot}45$ (S.D.) P.U. $\times 10^4$.

The inverse pattern of response whereby pentagastrin mainly affects the acid secretion and caerulein the peptic secretion at a given dose is being further investigated. It has been shown by Morrissey (1970) that the threshold dose for pepsin secretion in bullfrog gastric mucosa in response to gastrin stimulation is ten times higher than for acid secretion, reflecting a marked difference in the pepsin- and acid-releasing mechanisms.

The author wishes to thank the Medical Research Council for financial assistance towards laboratory expenses. Professor W. H. Bannister kindly provided facilities in the Department of Physiology and Biochemistry, Royal University of Malta. Professor V. Erspamer provided a sample of caerulein and Dr Fitzgerald of I.C.I. Pharmaceuticals a supply of pentagastrin, both of which are gratefully acknowledged.

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Resistance to shortening at the I-filament length in frog muscle fibres

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When a frog muscle is placed in a hypertonic solution the isometric resting tension is increased, apparently because of cross-bridge activity (Hill, 1968). It seemed likely that an isolated fibre would shorten in a hypertonic solution if the ends were left free; the reverse effect—of elongation in a hypotonic medium—has already been noted (Blinks, 1965).

Fig. 1 shows the results of an experiment in which the sarcomere length was measured at 24 points along a 0·7 mm length of a single fibre in solutions of increasing tonicity. There appear to be two plateaus (the first at 2·0 μ and the second at 1·93 μ approximately) at which shortening is impeded until the tonicity of the medium, presumably reflected in the force tending to shorten the fibre, is sufficiently high. However, these effects were variable. Some fibres shortened below 2·0 μ in mildly hypertonic solutions while a few fibres persisted at 2·0 μ up to very high tonicities. Most fibres shortened when the osmotic strength reached about twice that of Ringer, either 'sticking' at 1·93 μ with a marked reduction in the scatter of sarcomere lengths or undergoing a prolonged slow contracture to much shorter sarcomere lengths.

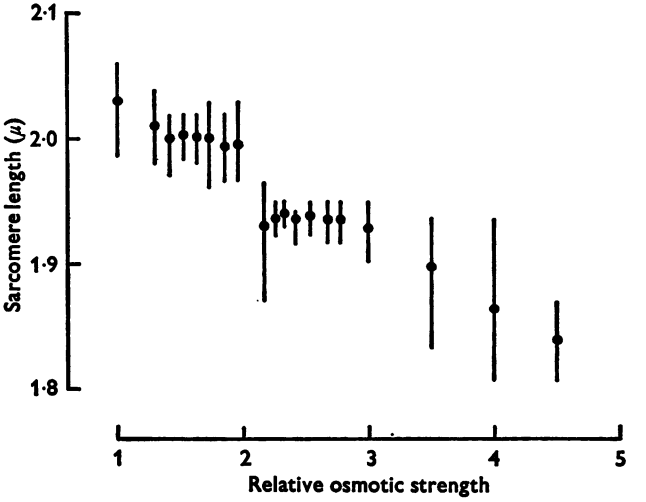

Fig. 1. Sarcomere lengths measured on an isolated fibre in solutions of varying tonicity. Vertical lines denote ranges of sarcomere length for 24 measurements in each solution. Circles are mean values. Osmotic strength is given relative to normal Ringer solution. Solutions were made hypertonic by adding NaCl to Ringer solution.

The range of sarcomere lengths for the second plateau was usually 1·92–1·95 μ with a mean for the results from 5 fibres of 1·936 μ (standard deviation $\pm$ 0·009). This value agrees well with the most recent X-ray and electron microscope determination of the I-filament length of 1·92 $\pm$ 0·01 μ by H. E. Huxley and S. Page (personal communication). The second plateau suggests the existence of resistance to shortening when the ends of the thin filaments meet. The first plateau may be due to interaction between the ends of the thin filaments and the M-line. The resistance to

shortening may account for a substantial proportion of the drop in isometric tetanic tension between about 2·0 and 1·7 μ (Gordon, Huxley & Julian, 1966).

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Voltage-dependent oscillations of the membrane potential (slow waves) produced by acetylcholine or carbachol in intestinal smooth muscle

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The action of acetylcholine on the membranes of nerve and muscle in situations hitherto described is adequately explained as a simple opening of ion channels which is independent of the membrane potential (see review by Ginsborg, 1967). It is uncertain at present whether such a simple mode of action is adequate to explain the effects of acetylcholine on the cells of the longitudinal muscle of the guinea-pig ileum.

A longitudinal strip of ileal wall was introduced into apparatus which enabled current to be applied extracellularly if desired (Abe & Tomita, 1968). Intracellular recording was made in the extrapolar region from cells of the longitudinal muscle layer with potassium chloride-filled glass microelectrodes of 30–100 MΩ resistance.

In isotonic solution the membrane spontaneously discharged bursts of action potentials. Acetylcholine (10^{-8} g/ml.) depolarized slightly and produced oscillations of the membrane potential (slow waves) over 40 mV in size, which had a period of oscillation of about 1 sec.

In hypertonic solution, which abolished the spontaneous activity of the membrane, acetylcholine and carbachol produced identical responses. Typically these began with a depolarization of a few millivolts which was sufficient to initiate action potential discharge; this part of the response could be mimicked by depolarizing the membrane slightly with outward current. Slow waves generally appeared at a slightly lower membrane potential than action potentials and they were smaller (about 20 mV) and their duration was greater (about 2·5 sec) than in isotonic solution. Slow waves persisted long after action potentials had disappeared and they were not seen in the absence of muscarinic stimulant if the same cell was depolarized by passing current or by increasing the external potassium concentration. The slow waves evoked by acetylcholine or carbachol do not seem to involve nerves, as mechanical denervation of the muscle (Paton & Zar, 1968), alone or in combination with tetrodotoxin (10^{-7} g/ml.), had no effect on them.

When the membrane potential was changed by passing current, it was found that slow waves were abolished during strong hyperpolarization. There was a critical membrane potential or threshold at which slow waves appeared and below this their rate of rise was proportional to the level of the membrane potential. At membrane potentials less than about 15 mV, slow waves were absent. During a slow wave, parallel changes occurred in membrane resistance and in the membrane potential. Altering the external chloride or potassium concentrations produced relatively minor changes in the slow waves, but reduction of the external sodium concentration rapidly abolished them. These results could be explained if the increase in ion permeability produced by carbachol or acetylcholine modified the behaviour of voltage-sensitive ion channels, or if these agents affected the latter directly.

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Phenoxybenzamine and body temperature

BY W. FELDBERG and P. N. SAXENA.* *National Institute for Medical Research, Mill Hill, London, N.W. 7*

In unanaesthetized rabbits and cats the alpha-adrenergic blocking agent phenoxybenzamine hydrochloride, injected into a cannulated lateral cerebral ventricle, produced opposite effects on body temperature: a fall in rabbits and a rise in cats.

In rabbits an intraventricular injection of 20–50 μg produced a fall of 1–2° C and the maximal fall was reached in 1·5–2 hr. These doses, and even larger ones (100 and 200 μg) did not affect temperature when injected intravenously. When the beta-adrenergic blocking agent propranolol was injected intraventricularly (200–500 μg) temperature was not affected.

In cats an intraventricular injection of 150–200 μg of phenoxybenzamine produced a gradual rise in temperature which continued for hours, and temperature then remained high for at least 24 hr.

Noradrenaline is known to raise temperature in rabbits and to lower it in cats when acting on the anterior hypothalamus. If the temperature effects obtained in these two species with phenoxybenzamine resulted entirely from blocking the action of noradrenaline on the anterior hypothalamus, they would suggest that noradrenaline is continuously released from

* Wellcome Trust Fellow.

adrenergic neurones ending on this part of the brain, and the effects should then no longer occur when the hypothalamus has been depleted of its catecholamines. This was shown to be the case in rabbits in which depletion was achieved by reserpine.

Cooper, Cranston & Honour (1967) found that in rabbits, an intraventricular injection of reserpine (0·35–0·75 mg) caused catecholamine depletion in the hypothalamus. We have now found that after a single injection of 0·75 mg reserpine phosphate into a lateral ventricle, or after several such injections, given at 24 hr intervals, phenoxybenzamine no longer lowered body temperature when injected intraventricularly. It took over a week—the time probably required to restore the catecholamines in the hypothalamus—before the full hypothermic response was again obtained with the phenoxybenzamine injections.

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Evidence concerning the effects of endogenous noradrenaline upon body temperature in cats and rabbits

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Noradrenaline is found in relatively high concentrations in the hypothalamus of several species of animals and it has been suggested that this substance is concerned in thermoregulation, possibly acting as a central transmitter. In the cat, injection of noradrenaline into the lateral cerebral ventricle, in a dose of 50 μg, causes a fall of body temperature lasting 2–3 hr (Feldberg & Myers, 1964). In contrast, intraventricular injections of 10 μg noradrenaline in the rabbit causes a rise of body temperature (Cooper, Cranston & Honour, 1965). These doses are enormous in comparison with the total amount of noradrenaline in the hypothalamus, and there is little evidence that endogenous noradrenaline can influence body temperature.

Iminodibenzyl antidepressant drugs, such as imipramine and N-desmethylimipramine, inhibit the uptake of released noradrenaline by the pre-synaptic membrane (Axelrod, Whitby & Hertting, 1961). If noradrenaline acts as a transmitter, the local effect of these drugs should be to raise the monoamine concentration at the synapse, and thus produce temperature changes similar to those caused by intraventricular injections of noradrenaline; the direction of temperature change should be opposite in cats

and rabbits. Any such changes should be less marked if imipramine is given to an animal whose endogenous stores of noradrenaline have been depleted.

Intraventricular injections of desmethylimipramine (625 μg) caused a temperature rise of 1·06, S.E. $\pm$ 0·36° C in six conscious rabbits; the temperature rose over 1½ hr and remained stable for the succeeding 2½ hr. In four cats, similar injections of imipramine (625 μg) caused a fall of rectal temperature of 1·7, S.E. $\pm$ 0·16° C, 1 hr after the injection. Temperatures returned to the control level 2½–3 hr after the injections.

In both species, six intraventricular injections of 10–20 mg of α-methyl-paratyrosine (methyl ester hydrochloride) were given at hourly intervals. Next day, intraventricular injections of imipramine or desipramine were given, as before, and the temperature changes were much diminished.

In cats, depletion of endogenous noradrenaline caused only a slight reduction of the temperature response to injected noradrenaline. This implies that the diminished response of the depleted animal to imipramine is not accounted for by a non-specific action of α-methyl-paratyrosine.

These observations show that endogenous noradrenaline can exert an effect upon body temperature, and are compatible with the hypothesis that noradrenaline may act as a central transmitter in thermoregulation.

Impramine and desmethylimipramine were kindly supplied by Dr Cyril Maxwell of Geigy (U.K.) Ltd., Macclesfield and one of us (R.H.L.) is supported by the National Fund for Research into Crippling Diseases. Equipment was loaned by the Medical Research Council.

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Effect of amino acids on the hydrolysis of dipeptides by rat small intestine

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Newey & Smyth (1960) showed that the terminal stage of dipeptide digestion may take place intracellularly, and this means that the intracellular events involve both peptide hydrolysis and amino acid transfer. In attempting to analyse the transfer process use is frequently made of other amino acids which may compete for the mechanism. It might be important, however, to consider also the effect of amino acids on the peptidases as earlier work (Grassman, Klenk & Peters-Mayr, 1935; Nishi, 1960) has indicated possible effects of amino acid on peptidases in other

tissues. Except for a brief report by Newey & Smyth (1962) that L-methionine inhibits intestinal hydrolysis of glycyl-glycine no information is available on this aspect of intestinal peptidases. The present study is concerned with the effect of a number of amino acids on the hydrolysis of glycyl-glycine, glycyl-L-leucine and L-leucyl-glycine by rat intestinal homogenate.

White rats of the Sheffield strain were anaesthetized with Nembutal. After removal of the intestine, the middle fifth of the combined jejunum and ileum, weighing about 1·3 g, was homogenized in 32·5 ml. of bicarbonate saline (Krebs & Henseleit, 1932). Aliquots of the homogenates were taken and diluted 1 in 4, 1 in 20 and 1 in 80 respectively for the study of hydrolysis of glycyl-glycine, L-leucyl-glycine and glycyl-L-leucine. Dipeptides and amino acids being tested were present in an initial concentration of 15 mM. Homogenates were incubated at 38° C for 15 min, and the amounts of glycine liberated were estimated.

It was found that a number of amino acids caused inhibition of hydrolysis of both glycyl-glycine and of glycyl-L-leucine, the most effective being L-methionine, L-leucine and L-histidine. In contrast, most of these amino acids (except L-histidine) did not inhibit hydrolysis of L-leucyl-glycine and some even caused stimulation. The difference was most marked with L-methionine, which caused 47 % inhibition of hydrolysis of glycyl-L-leucine but 28 % stimulation of hydrolysis of L-leucyl-glycine. Preliminary experiments with everted sacs showed corresponding patterns of effects on the peptidase activity as was obtained with the homogenates.

The results show that in considering the details of final digestion of dipeptides, whether in the intestinal lumen or in the columnar cell, consideration must be given to the effects of amino acids which may be present either from liberation from the dipeptide or from other sources.

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The effect of cervical cord transection on cardiovascular responses mediated by the vagus nerves

By M. D. Joy, Helen Lavery, R. D. Lowe and G. C. Scroop.* *Department of Medicine, St Thomas's Hospital Medical School, London, S.E. 1*

Transection of the spinal cord in the mid-cervical region has been used as a method for distinguishing between those cardiovascular responses which are due to stimulation of central sympathetic structures and those which are mediated by the vagus nerves since this procedure interrupts sympathetic transmission completely while retaining the vagus and its central connexions intact. However, experiments in chloralose-anaesthetized greyhounds indicate that this procedure, while abolishing sympathetically mediated responses, can also inhibit those responses which are mediated in part or predominantly by the vagus nerves to the heart.

Two different methods were used to elicit cardiovascular responses which receive a contribution from withdrawal of cardiac vagal tone. Bilateral occlusion of the common carotid arteries causes an increase of blood pressure and tachycardia which appears to be partially mediated by the vagus nerves because the changes of heart rate are too rapid to be due entirely to changes of sympathetic tone (Warner & Russell, 1969). Vertebral artery infusions of angiotensin (1 ng/kg.min) in the chloralose-anaesthetized greyhound cause increases of blood pressure and heart rate which are mediated principally by withdrawal of cardiac vagal tone (Scroop & Lowe, 1968, 1969).

Sympathetic transmission was interrupted either by cervical cord transection or by systemic administration of the adrenergic neurone blocking drug, bethanidine (4 mg/kg). The immediate effect of cervical cord transection was to abolish the response to intravertebral angiotensin and greatly reduce or abolish that to carotid artery occlusion. After the elapse of 2–4 hr the response to intravertebral angiotensin returned almost to control levels, while that to carotid artery occlusion remained substantially reduced. Subsequent vagotomy abolished the responses which remained indicating that they were mediated entirely by the vagus. In contrast to the above results, sympathetic blockade with bethanidine had no significant effect on the response to intravertebral angiotensin, and although the response to carotid artery occlusion was greatly reduced by this treatment, the reduction was significantly less than that produced by cervical cord transection. The much smaller effect of bethanidine on these responses could not have been due to any inadequacy of sympathetic blockade since the residual responses, although larger than those remaining after cord section, were, nevertheless, abolished by vagotomy.

* G. C. Scroop is a C. J. Martin Travelling Fellow of the National Health and Medical Research Council of Australia.

It is concluded that substantial pressor effects can be mediated by the vagus nerves in the greyhound and that although centrally mediated sympathetic effects in the cardiovascular system can be prevented to an equal degree, either by cervical cord transection or by bethanidine, the procedure of cervical cord transection can, in addition, inhibit responses mediated by the parasympathetic nervous system. For this reason, abolition of a particular cardiovascular response by cervical cord transection cannot be construed as unequivocal evidence that it is mediated by the sympathetic nervous system alone.

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Properties of motor units in normal and partly denervated human muscles

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The isometric twitches of single motor units in the extensor hallucis brevis (EHB) muscle have been recorded following threshold stimulation of the deep peroneal nerve. The contraction times of 122 units in EHB muscles of healthy adults were found to differ considerably, ranging from 35 to 98 msec; the corresponding values for the whole EHB muscles varied from 50 to 78 msec (mean 62·6, S.D. $\pm$ 6·9 msec). The twitch tensions of the single units ranged from 2 to 14 g with a mean of $5{\cdot}5 \pm 2{\cdot}2$ g; since the mean tension for the whole EHB muscle was 313 g, this last result suggested that the muscle contained approximately 56 motor units. It was estimated that, due to the oblique insertion of the EHB tendon on to the base of the proximal phalanx of the great toe, the actual tensions developed by the muscle would have been roughly 3·5 times the recorded values given above.

Patients were then studied in whom EHB muscles had probably been partly denervated for longer than 6 months. The twitch contraction times of 31 surviving motor units ranged from 46 to 125 msec and in ten out of twenty-one patients the whole muscle twitches were abnormally slow. The recorded twitch tensions of the remaining units were often larger than normal (up to 62 g; mean $16{\cdot}3 \pm 16{\cdot}7$ g). In addition the amplitudes of the evoked motor unit action potentials, recorded with a strip of silver foil over the end-plate zone of the muscle, were also increased in partly denervated muscles.

* British Council Scholar.

The enhanced mechanical and electrical responses of the motor units surviving in partly denervated muscles suggested that either their muscle fibres had hypertrophied or that annexation of denervated fibres had occurred, or that both events had taken place. Irrespective of the nature of the mechanism involved, the compensatory process was so effective that the twitch tensions of most partly denervated muscles remained within the normal range until fewer than 10% of axons were left.

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Urine temperature measurement in human circadian rhythm studies

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The idea of using voided urine to assess the circadian rhythm of body core temperature is not new. Indeed a figure illustrating this periodicity appeared in a physiology book nearly 50 years ago (Cathcart, Paton & Pembrey, 1922). However, the method seems to have been neglected since standard texts on temperature measurement contain no reference to the method (see, e.g. Herzfeld, 1962) and most long-term field studies of circadian rhythms use the oral temperature (see, e.g. Halberg *et al.* 1969), rectal temperature being relatively inconvenient in the ambulent subject.

In the present study seventy-three synoptic urine and oral temperature observations were made on one adult male subject over thirteen normal laboratory workdays. Both measurements were made with relatively accurate maximum recording mercury clinical thermometers designed, in fact, to follow the phases of the female menstrual cycle for the purpose of 'safe period' contraception (Ovulindex thermometer by Linacre Laboratories). After hand-shaking down the mercury, one was placed under the tongue (5 min) and the other was placed a few millimetres from the urinary meatus and urine passed on it. In practice urine volumes of under 100 ml. were insufficient to achieve an end point and these readings were rejected. With greater volumes one was able to see that the mercury, after a latent period of a few seconds, rose rapidly to a final reading. The whole operation, including recording, was over in about 20 sec.

The results of this study showed that the urine temperature level was 0·33° C higher (36·87° C) than the oral, the circadian amplitude was greater (over 50%), yet the standard errors were less.

The effectiveness of the urine temperature method in revealing circadian changes is probably due to the relative insulation of the bladder urine from

the external and internal environment and the resistance of a volume of urine in the bladder to short-term temperature changes (e.g. due to exercise). Apart from these advantages the urine temperature method is carried out much more rapidly. Five minutes are necessary with an oral measurement (so that sublingual temperature may equilibrate to a constant level after the mouth is closed).

The immediate objective in carrying out this pilot survey was to find a workable method of studying the circadian rhythm of human temperature in three subjects attempting to ski from northern Canada to the Pole and living in a very small mountain tent (Simpson, 1970). Initial calculations on the 45 days of pooled data with a least squares spectral analysis indicate a significant circadian rhythm with a level 0·19° C (36·68° C) lower than the laboratory result. Such a small lowering argues for relatively little interference from the environmental temperature—at times as low as −50° C.

This field study using the urine temperature method indicates it is useful not only on laboratory studies but also in difficult field conditions.

The author is grateful to the Medical Research Council for financial support and to Dr Franz Halberg for the statistical analyses.

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A man with too long a day

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It is known (Aschoff & Wever, 1962; Siffre, 1963; Mills, 1964, 1967; Siffre, Reinberg, Halberg, Ghata, Perdriel & Slind, 1966; Colin, Timbal, Boutelier, Houdas & Siffre, 1968) that human subjects in isolation follow an activity cycle somewhat longer than 24 hr, although most of us have little difficulty in conforming to the 24 hr period of light and darkness and of the activities of those around us.

We encountered recently a subject who claimed that he was unable to live on a day of 24 hr duration, going to bed and waking later each successive day until the asynchrony between his habits and those of his

associates compelled him to revert abruptly to normal timing; observations on his sleeping habits confirmed this.

He was then confined in an isolation unit, without a timepiece, and his habits were recorded by a remote signalling device; he there followed an activity cycle of 26 hr. After 5 days a clock, which he knew could be adjusted to gain or lose several hours a day, was started, and he was asked to try to conform his habits to the time recorded on the clock; unknown to the subject, this clock was running at a normal rate, though its absolute

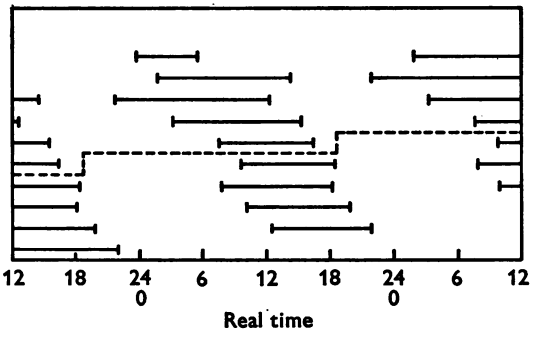

Fig. 1. Activity of subject in isolation. The bars indicate time in bed and the spaces ambulance. Each line represents one whole day, together with the previous and the following 12 hr. Succeeding days are plotted from above downwards. The dotted line indicates the time when the clock was started.

time was in error since it was started at the time which he believed it to be. He was still unable to conform his habits to a 24 hr cycle, just as when living in nychthemeral surroundings (Fig. 1). Measurements of his plasma 11-hydroxycorticosteroids, body temperature and excretion of sodium, chloride, potassium and steroid indicated that these followed a rhythm in accordance with his activity cycle.

Our thanks are due to the Nuffield Foundation for a grant which permitted the construction of the Isolation Unit and to the Medical Research Council for an equipment and expenses grant.

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The action of glutamic acid and some derivatives on isolated supra oral sphincter preparations of the sea anemone *Actinia equina*

By R. F. Carlyle. *Department of Pharmacology, King's College, W.C.2*

Of the common amino acids only glutamic acid inhibits the contractile response to electrical stimulation of isolated rings of supra oral sphincter from the anemone, *Actinia equina* (Carlyle, 1970). The possibility exists that deamination of glutamic acid might produce sufficient ammonia to cause the observed depression of contraction. Ammonia in concentrations higher than 10^{-5} M certainly produces an abolition of the responses to electrical stimulation, but this depression, unlike that of glutamic acid, is only reversed by prolonged washing (2–3 hr). The level of ammonia in isolated preparations has been measured by a modification of the phenate-hypochlorite reaction (Kaplan, 1969). In twelve control experiments the level of ammonia in supra oral sphincters was found to be $8{\cdot}95 \pm 1{\cdot}1$ S.E. μg/g. In eight experiments where the contractile response had been abolished by glutamic acid (5×10^{-3} M) the level of ammonia fell to $6{\cdot}61 \pm 0{\cdot}63$ S.E. μg/g. It therefore seems unlikely that glutamic acid acts by freeing ammonia.

Further observations of the actions of derivatives of glutamic acid show that the inhibitory effect is produced by only a very limited number of derivatives. The results are summarized in Table 1 (see opposite page), from which it is obvious that the glutamate receptor in this preparation shows an unusually high degree of structural specificity.

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Noradrenaline release in an isolated, adrenergically innervated muscular artery

By C. Bell and Marthe Vogt. *Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

TABLE 1. Structure-activity relationships of derivatives of L-glutamic acid

The activity of L-glutamic acid is taken as unity. Approximate relative activities are expressed as: < 0·1, (+); 0·1–0·5, (++); 0·5–1, (+++); 1–5, (++++); 5–10, (+++++).

Structural feature	Compound	Structure	Relative activity
1. Distance between amino group and carboxyl group	DL-Aspartic acid	$HO_2C.CH_2.CH(NH_2).CO_2H$	0
	DL-Glutamic acid	$HO_2C.[CH_2]_2.CH(NH_2).CO_2H$	+++
	DL-α-Amino adipic acid	$HO_2C.[CH_2]_3.CH(NH_2).CO_2H$	0
	DL-α-Amino pimelic acid	$HO_2C.[CH_2]_4.CH(NH_2).CO_2H$	+++
	L-α-Amino pimelic acid	—	+++
	DL-α-Amino suberic acid	$HO_2C.[CH_2]_5.CH(NH_2).CO_2H$	0
	DL-α-Amino sebacic acid	$HO_2C.[CH_2]_7.CH(NH_2).CO_2H$	0
2. Optical configuration	L-Glutamic acid	—	+++
	D-Glutamic acid	—	+++
3. Variation of the ω-acidic group	DL-Cysteic acid	$HO_3S.CH_2.CH(NH_2).CO_2H$	0
	DL-Homocysteic acid	$HO_3S.[CH_2]_2.CH(NH_2).CO_2H$	+++++
4. Relative position of amino group	β-Amino glutaric acid	$HO_2C.CH_2.CH(NH_2).CH_2.CO_2H$	0
5. Substitution in the carbon chain	DL-β-Hydroxy glutamic acid	$HO_2C.CH_2.CH(OH).CH(NH_2).CO_2H$	0
	DL-γ-Hydroxy glutamic acid	$HO_2C.CH(OH).CH_2.CH(NH_2).CO_2H$	+
	L-γ-Hydroxy glutamic acid	—	+
	DL-γ-Methylene glutamic acid	$HO_2C.CH{=}CH.CH(NH_2).CO_2H$	+++
	DL-2-6-Diamino pimelic acid	$HO_2C.CH(NH_2).[CH_2]_3.CH(NH_2).CO_2H$	0
	L-γ-Hydroxy γ-methyl glutamic acid	$HO_2C.C[(OH)(CH_3)].CH_2.CH(NH_2).CO_2H$	0
	Erythro-β-chloro-L-glutamic acid	$HO_2C.CH_2.CH(Cl).CH(NH_2)CO_2H$	++
	Threo-β-chloro-L-glutamic acid	—	++
6. Modification of amino group	*N*-Methyl-DL-glutamic acid	$HO_2C.[CH_2]_2.CH(NH.CH_3).CO_2H$	0
	NN-Dimethyl-DL-glutamic acid	$HO_2C.[CH_2]_2.CH[N.(CH_3)_2].CO_2H$	0
	α-Glutarobetaine	$HO_2C.(CH_2)_2.CH[N^+(CH_3)_3].CO_2$	0
	N-Acetyl-DL-glutamic acid	$HO_2C.[CH_2]_2.CH(NH.OC.CH_3).CO_2H$	0
7. Replacement of amino group	Glutaric acid	$HO_2C.[CH_2]_3.CO_2H$	0
	α-Ketoglutaric acid	$HO_2C.[CH_2]_2.C{=}O.CO_2H$	0
	β-Ketoglutaric acid	$HO_2C.CH_2.C{=}O.CH_2.CO_2H$	0
	Tartronic acid	$HO_2C.CH(OH).CO_2H$	0
	D-Malic acid	$HO_2C.CH_2.CH(OH).CO_2H$	0
	L-Malic acid	—	0
8. Ring closure	Pyroglutamic acid	$HO_2C.CH.[CH_2]_2.C{=}O.NH$	0
9. ω-Amide link	DL-Asparagine	$HO_2C.CH(NH_2).CH_2CONH_2$	0
	DL-Glutamine	$HO_2C.CH(NH_2).[CH_2]_2CONH_2$	+
	L-Glutamine	—	+
α-Amide link	DL-Isoglutamine	$HO_2C.[CH_2]_2.CH(NH_2).CO.NH_2$	0

b

Action of crotoxin and crotactin from the venom of *Crotalus durissus terrificus* (South American rattlesnake) on the frog neuromuscular junction

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Crotoxin, isolated in crystalline form from the venom of *Crotalus durissus terrificus* (South American rattlesnake), is toxic and possesses phospholipase A and hyaluronidase activity (Slotta & Fraenkel-Conrat, 1938; Slotta, 1955). When injected into cats and dogs, neuromuscular block was produced (Brazil, 1966). Neumann & Habermann (1955) and Habermann (1957) prepared from crotoxin by ion-exchange chromatography a toxic substance, crotactin, and non-toxic phospholipase A.

The effects of crotoxin and crotactin on the frog neuromuscular junction were investigated using the sartorius muscle–sciatic nerve preparation from *Rana temporaria*. Both substances completely blocked contraction elicited by nervous stimulation though spontaneous contractions usually preceded complete blockade.

Neither crotoxin nor crotactin altered the muscle membrane potential. In a study of the effect on spontaneous miniature end-plate potentials (m.e.p.p.s), tetrodotoxin was added prior to crotoxin to prevent spontaneous contractions arising (Fig. 1). A reduction of m.e.p.p. frequency always preceded the onset of 'large' potentials (mostly 1–5 mV) which were followed by an explosive burst of m.e.p.p.s. The effect of crotoxin and crotactin on the quantal content of transmitter released by an impulse was determined by recording m.e.p.p.s and evoked e.p.p.s in the presence of Ringer containing Ca 0·9 mM, Mg 6 mM, neostigmine 10^{-6} g/ml. Both neurotoxins produced a rapid fall in transmitter output though a small increase occurred prior to block. Frequency and amplitude changes in spontaneous potentials occurred as previously. If the preparation was then perfused with normal Ringer–neostigmine solution, e.p.p.s could again be recorded for a period. Crotoxin reduced to a small extent the sensitivity of the end-plate region to carbachol.

As crotoxin and crotactin give similar responses, the neurotoxic effect is not due to phospholipase A. The active component appears to act on the presynaptic terminals, and, in respect to an explosive burst of m.e.p.p.s, resembles the action of black widow spider venom (Longenecker, Hurlbut, Mauro & Clark, 1970).

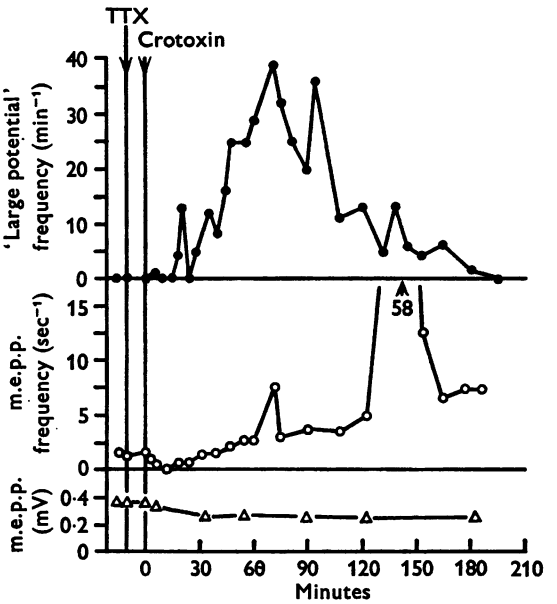

Fig. 1. The effect of crotoxin 10^{-5} g/ml. on the frequency of m.e.p.p.s and spontaneous 'large' potentials and on the amplitude of m.e.p.p.s in the presence of tetrodotoxin (TTX) 4×10^{-7} g/ml., neostigmine 10^{-6} g/ml. 'Large' spontaneous potentials (●) were arbitrarily defined as of amplitudes $\geqslant$ 1 mV (> 3 quanta) and were not included in the calculation of m.e.p.p. amplitude (△) or frequency (○).

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The lack of an electrical threshold discrimination between group Ia and group Ib fibres in the nerve to the cat peroneus longus muscle

BY J. J. B. JACK and CATHERINE R. MACLENNAN.* *University Laboratory of Physiology, Oxford*

Bradley & Eccles (1953) first correlated fast and slow components of the group I compound action potential in thigh muscle nerves with activity in

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spindle (Ia) and tendon organ (Ib) afferents respectively. Their conclusions were extended, by Eccles, Eccles & Lundberg (1957), to afferents in leg muscle nerves, where the presence of two such components is less often observed. However, studies on receptor-identified fibres in the medial gastrocnemius (Sumner, 1961; C. M. L. Coppin, unpublished observations), semitendinosus (Coppin, Jack & McIntyre, 1969) and soleus (Coppin, Jack & MacLennan, 1970) muscle nerves have shown that Ia afferents have conduction velocities and thresholds distributed throughout the group I range. In these three muscle nerves, nevertheless, tendon organ fibres have been found predominantly in the less excitable part of the group I range; for example, an electrical stimulus exciting 50% of the Ia fibres has been found to excite the following percentages of Ib fibres: none in semitendinosus, 3% in soleus and 10 to 15% in medial gastrocnemius. We now present results for a muscle nerve in which the threshold separation between Ia and Ib fibres is negligible.

The properties of 236 afferent fibres from the peroneus longus muscle were investigated, using similar techniques to those of Coppin *et al.* (1969). 172 of these fibres were identified as arising from muscle spindles and 60 from tendon organs. As in Coppin *et al.* (1970), the distinction between group I and group II has been drawn at a normalized conduction velocity of 0·65: this corresponds to a mean conduction velocity of 72·8 m/sec, with the range of maximum conduction velocity (measured from the peroneus longus compound action potential) being from 92·0 to 127·5 m/sec. On this basis, 113 of the spindle afferents were classified as group I. The conduction velocities of both Ia and Ib afferents were distributed throughout the group I range and no significant difference was found between the mean conduction velocities for these two afferent fibre types.

The pooling of single fibre threshold measurements showed that there was no substantial difference between group I spindle and tendon organ afferents in their recruitment with increasing stimulus strength. A shock exciting 50% of the Ia afferents also excited over 40% of the tendon organ fibres: for this muscle nerve, therefore, graded electrical stimulation cannot be used to distinguish between the central effects of group I spindle and tendon organ afferent fibres.

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The force velocity and 'series elastic component' characteristics of cat fast-twitch and slow-twitch skeletal muscle

BY A. J. BULLER, C. J. C. KEAN and K. W. RANATUNGA. *Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD*

The effect of antidiuretic hormone on the rate of sweat production in man

BY JUDITH A. ALLEN and I. C. RODDIE. *Department of Physiology, The Queen's University of Belfast*

Antidiuretic hormone increases transport of water through frog skin. It also plays an important part in the water balance of man by promoting reabsorption of water through the epithelium of the distal tubules and collecting ducts of the kidney. A similar action on the epithelium of the sweat gland tubules might be expected to decrease the rate of sweating.

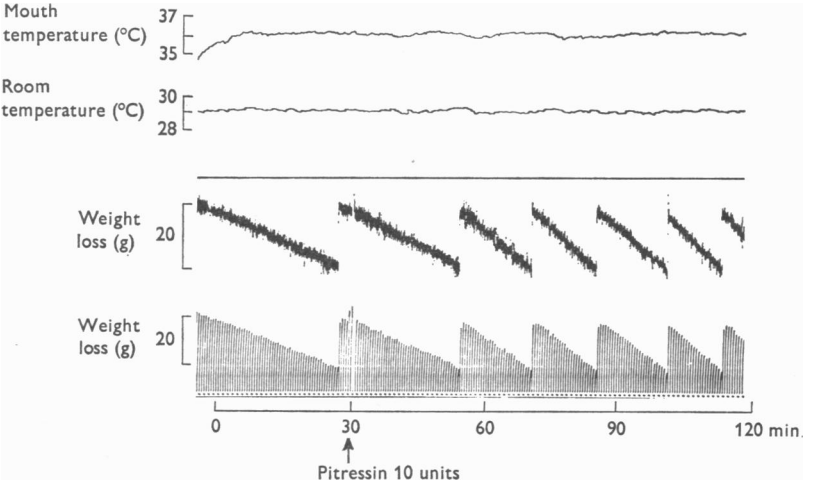

Fig. 1. The effect of a subcutaneous injection of 10 units of Pitressin on the rate of weight loss at 29° C. The top two traces represent mouth and environmental temperatures. The slopes in the third record show the rate of loss of body weight of the subject. The bottom trace is the integral every 30 sec of the area under the weight loss slope.

When sweating was measured from small areas of skin or by single measurements of body weight, ADH was found to increase (Ladell, 1948), decrease (Hankiss, 1959; Fasciolo, Totel & Johnson, 1969) or not affect (Pearcy, Robinson, Miller, Thomas & De Brota, 1956; Ladell & Whitcher, 1960) the rate of sweating.

In the present experiments water loss by insensible perspiration and

sweating was estimated in five subjects at three different environmental temperatures from continuous records of total body weight.

Fig. 1 shows a typical experiment at 29° C. After a 30 min control period a subcutaneous injection of ADH (Pitressin, 10 units) was given. The weight loss slopes became steeper indicating an increased rate of weight loss. For five subjects the mean resting rate of weight loss was 2·5 g/m². 5 min (S.E. ± 0·3). Thirty minutes after the injection of Pitressin the rate had risen significantly at the 2% level to 3·9 g/m². 5 min (± 0·4).

At 18° C the mean resting rate of weight loss was 1·4 g/m². 5 min (± 0·1). Pitressin did not alter this significantly.

At 37° C Pitressin raised the rate of weight loss from a control level of 6·2 g/m². 5 min (± 0·5) to 8·0 (± 0·3). This rise was significant ($P < 0·05$).

The results indicate that antidiuretic hormone does not decrease the rate of sweating in man.

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Ankle clonus—an autonomous central pace-maker?

BY E. G. WALSH. *Department of Physiology, University of Edinburgh*

Observations have been made on four patients with spinal cord lesions. Forces provided by a printed motor (type G16M4) have been applied to the foot and the motion recorded (cf. Walsh, 1969; 1970*a*, *b*, *c*). The axis of the ankle has been vertical. For sustained clonus it was necessary to provide a steady force in a dorsiflexing direction. Observations relevant to the origin of the rhythmicity are:

1. The amplitude of the clonus can be altered over a wide range by using different amounts of the steady biasing force. There is, however, little or no alteration in the rate of the oscillations.

2. If the steady force is withdrawn the motion at the ankle ceases forthwith yet the rhythmic electromyographic discharges from the calf muscles may persist for a number of beats.

3. The mechanical properties of the system may be changed by adding inertia. By this means the amplitude of the clonus may be reduced substantially without changing its rate.

4. If, in addition to the steady force, a rhythmic component is added the amplitude of the oscillation varies. The envelope of the excursions corresponds to the production of beats, when the biological rate differs a little

from that of the applied oscillation (Fig. 1). An instantaneous record of the phase of the motion with respect to the applied sinusoid has been obtained in one patient. As the amplitude of the motion varied the phase vector was seen to rotate through the anticipated 2π rad.

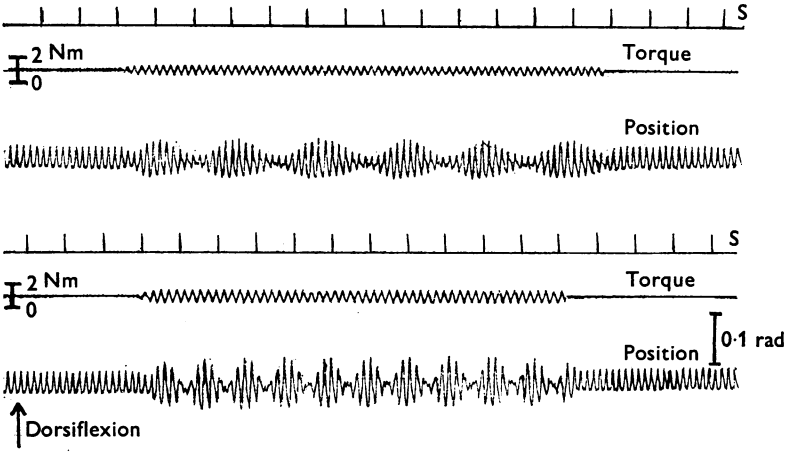

Fig. 1. Interference between clonus at 6 Hz and a rhythmic force at 5½ Hz (upper) and 5 Hz (lower). Beat frequencies, ½ Hz and 1 Hz respectively correspond to rate difference between clonus and applied torque. (J.B. ♂aet 23 Traumatic Paraplegia 4 years. R. ankle.)

The current assumption that clonus is the result of self-excitation of hyperactive stretch reflexes is not supported. The findings point to the existence of a spinal pace-maker and indicate that the rhythm is centrally tuned.

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Oxygen uptake and active transport of sodium by the foetal gastric mucosa

By G. H. Wright.* *Cardiovascular Research Institute, University of California Medical Center, San Francisco, California 94122, U.S.A.*

The gastric mucosa of the rabbit foetus is constituted predominantly of non-differentiated cells (Menzies, 1958) which perform an active transport of sodium ions from mucosa to serosa (Wright, 1962). During the last 7 days of gestation, a small number of oxyntic cells appear and concurrent with this is a small secretion of hydrochloric acid into the mucosal solution.

The present study is concerned with the relationship between active sodium transport and oxygen uptake by the foetal gastric mucosa. Stomachs were removed from foetuses of New Zealand White rabbits at 28 days gestation age and, after excision, were mounted as membranes between a pair of special glass chambers for short-circuit current measurement. A Clarke-type oxygen electrode was sealed into each chamber and used to measure the fall in partial pressure of O_2 of the solutions bathing the membrane: the volume of the solutions was known and hence the rate of O_2 uptake was calculated.

Krebs Ringer, buffered with bicarbonate, was used on the serosal side and 154 mM-NaCl or choline chloride was placed on the mucosal side. The experiments were carried out at 35° C and the P_{O_2} of the solutions was about 600 mm Hg at the commencement of the experiments and was not allowed to fall below 400 mm Hg.

Both short-circuit current and O_2 uptake were greater when Na was present on the mucosal side than when choline replaced Na on that side. Previous work by Mason & Wright (1967) has shown that the increase in short-circuit current under these conditions is equal to the rate of net sodium transport from mucosa to serosa.

The quotient $\Delta I_{Na}/\Delta q_{O_2}$, representing the increase in oxygen utilization, was calculated for each experiment and the mean of several runs was obtained. The mean quotient obtained from 23 preparations was 3·35 equiv. Na transported per mole of O_2 used (S.E. = $\pm$0·39).

This quotient may be considered significantly close to 4 and is thus low compared with a value of 18 which is found for similar epithelial sodium transport systems, e.g. frog skin (Zerahn, 1956). However, it should be remembered that the Na^+ transporting cells of the foetal gastric mucosa are non-differentiated and many are destined to become oxyntic cells. It is well established that oxyntic cells have a H^+ transport: O_2 uptake quotient of 4 (Bannister, 1965). It may be possible that this transport system is

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present in a rudimentary form in the non-differentiated cells where it is transporting Na^+ rather than H^+.

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The effects of ions on the transuterine endometrial potential difference

BY CHRISTINE KYRIAKIDES and R. J. LEVIN. *Department of Physiology, University of Sheffield, S10 2TN*

Levin & Edwards (1968) recorded, both *in vivo* and *in vitro*, an electrical potential difference (p.d.) across rat uterus that varied with the oestrous cycle and was generated by the endometrium. This communication describes the effects of tannic acid and of ion changes on the transuterine endometrial p.d. measured across oestrous or pro-oestrous uteri incubated *in vitro*.

In vivo measurements at pro-oestrus invariably gave high p.d. at either end of the uterus but at oestrus, while high values were always obtained at the cephalic end, occasional low p.d. were obtained at the caudal end. *In vitro*, however, oestrus and pro-oestrus p.d. were similar.

Addition of 1–20 mM tannic acid to the endometrial fluid caused a rapid, irreversible fall in p.d. but no change occurred when it was added to the serosal fluid, confirming the endometrium's role in generating the p.d.

The effects of changing the ionic composition of the fluids bathing the endometrium and serosa on the p.d. can be summarized thus:

(1) Reducing the [NaCl] of the endometrial fluid using mannitol as replacement solute caused the p.d. to increase significantly, the serosa becoming more positive to the endometrium. Reducing the [NaCl] in the serosal fluid with mannitol caused only slight decreases in p.d. at oestrus and slight increases at pro-oestrus.

(2) Reducing the [NaCl] of the endometrial fluid with Tris chloride had no effect on the p.d. nor did reduction of the [Cl'] using sulphate or nitrate as the replacement ions.

(3) Reducing the [Na^+] in the endometrial fluid with potassium caused no significant change but when the [Na^+] was replaced by potassium in the

serosal fluid, a precipitous fall in the p.d. occurred when the [K^+] became greater than 20 m-equiv/l. At 123 m-equiv K^+/l. the polarity of the p.d. reversed.

The increased p.d. observed with mannitol replacement of the NaCl in (1) is probably caused by changes in the conductivity of the endometrium concomitant with the lowered ionic strength of the buffer as similar reductions in the [Na^+] or [Cl'] of the endometrial fluid in (2) with other solutes did not affect the p.d. The results from (2) and (3) indicate that the endometrium's luminal and serosal membranes have different ionic permeabilities. The luminal membranes do not appear sensitive to changes in [Na^+], [K^+], or [Cl'] but the serosal membranes, while little affected by changes in [Na^+] or [Cl'], are very sensitive to increases in [K^+]. Although the log [K^+]/p.d. relationship was not linear like that of a potassium electrode, it appears that potassium movement across the serosal membrane plays some part in the mechanisms that generate the transuterine endometrial p.d.

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The contribution of small and large vesicles to noradrenaline release

BY MARIANNE FILLENZ and P. R. C. HOWE.* *University Laboratory of Physiology, Oxford*

Sympathetic nerve terminals contain noradrenaline in three compartments: the cytoplasm, and small and large dense-cored vesicles (Bisby & Fillenz, 1970). We have carried out experiments to determine their relative contribution to impulse-evoked release of noradrenaline. The α-adrenergic blocking agent phenoxybenzamine has been used to increase reflex stimulation of sympathetic nerve activity (Dontas & Nickerson, 1957). Phenoxybenzamine also blocks catecholamine uptake (Brown, 1965; Gillespie, Hamilton & Hosie, 1970). We have used the reduction in noradrenaline stores as a measure of transmitter release. This is an underestimate since it ignores the accelerated synthesis demonstrated by Dairman *et al.* (1968).

Phenoxybenzamine 25 mg/kg was given to rats by intraperitoneal or intravenous injection. Hearts and spleens were examined by the fluorescence method of Hillarp and Falck.

Noradrenaline content of whole organs and subcellular fractions was assayed by the trihydroxyindole method (Blakeley, Dearnaley & Harrison, 1970) and protein by Lowrie's method (1951).

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In phenoxybenzamine treated rats fluorescence microscopy showed virtual disappearance of nerve terminals in heart muscle and splenic smooth muscle and a marked reduction in fluorescence intensity of perivascular nerve terminals. Phenoxybenzamine reduced the noradrenaline content of heart and spleen to 30% of normal, 6 hr after intraperitoneal and 2 hr after intravenous administration.

Differential centrifugation always showed a greater reduction in the particle-bound noradrenaline (measured as the noradrenaline per mg of protein of the microsomal pellet) than in the noradrenaline content of the whole organ. However, there was also a reduction in the noradrenaline content of the final supernatant, which represents cytoplasmic noradrenaline as well as vesicular noradrenaline released during homogenization. There was therefore only a small reduction in particle-bound noradrenaline expressed as a percentage of the total.

In order to determine whether both small and large noradrenaline-storage vesicles contribute to release, we layered resuspended microsomal pellets from hearts of normal and phenoxybenzamine treated rats on to linear sucrose density-gradients.

The distribution of noradrenaline throughout the gradient was examined after centrifugation.

Two clear-cut results emerged from these experiments: (i) there was a reduction in the noradrenaline concentration of the low density fractions, representing small dense-cored vesicles, and also of the high density fractions, representing large dense-cored vesicles; (ii) the reduction in the low-density fractions was always greater than in the high-density fractions. We conclude from these experiments that both small and large dense-cored vesicles contribute to transmitter release, but the contribution of the former is greater than that of the latter.

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The effect on intertectal neuronal connexions of rearing *Xenopus laevis* in total darkness

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In anurans the visual projection from an eye to its ipsilateral tectum involves at least two stages. The first stage consists of a direct retinal projection to the contralateral optic tectum, while the second stage involves an intertectal linkage from the contralateral to the ipsilateral tectum (Gaze & Jacobson, 1963; Keating & Gaze, 1970).

The mechanisms ordering retinotectal connexions in the direct contralateral projection are operative before the onset of visual function (Sperry, 1951, 1963). Gaze, Keating, Székely & Beazley (1970) have suggested that the precise pattern of connexions in the intertectal system, on the other hand, is determined by factors related to visual function. They proposed that the formation of the intertectal connexions was controlled by a functional interaction, at the tectal level, between the two direct contralateral projections of binocular visual space. These authors argued that the mechanisms controlling this selective linkage involved the joining of the two areas, one on each tectum, that are simultaneously receiving similar spatiotemporal patterns of excitation because they are both excited from the same point in binocular visual space.

To test this hypothesis animals were reared in total darkness from stage 58 (Nieuwkoop & Faber, 1967), which is prior both to the appearance of a binocular portion of the visual field and to the onset of tectal responses to stimulation of the ipsilateral eye (Beazley, Keating & Gaze, 1970). Animals were kept in total darkness until the terminal recording experiment 1 year later and should thus have had no opportunity for any tectal interaction of the visual inputs from binocular visual space. The pattern of intertectal connexions should, therefore, be abnormal. The contralateral retinotectal projection, however, is determined by pre-functional factors and should not, therefore, be greatly affected by visual deprivation.

Electrophysiological mapping of the visual projections in the adult dark-reared animals showed that the contralateral retinotectal projection was essentially normal. The ipsilateral retinotectal projection, which reflects the state of the intertectal connexions, showed approximations to the normal pattern in that the projection arose from the appropriate part of the visual field and was correctly orientated along the medio-lateral axis of the tectum. Even in the distribution of field positions projecting along the rostro-caudal tectal axis there were signs of the correct order. Individual

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responses arose, however, from much wider areas of the visual field than normal and, in addition, the maps frequently showed considerable confusion of field positions.

We interpret these results as indicating that the pre-functional growth process produces an approximation to the normal pattern of intertectal connexions but that the final refining of this approximate pattern requires binocular visual function.

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