The advantages of incorporating a fan in such an instrument are that the bags are emptied to a constant residual volume (approximately 1 l.) and at an almost constant flow rate. This ensures that the flowmeter is always used over the same portion of its calibration curve. It is well known that the calibration of this type of flowmeter may be non-linear at low flow rates (Cox, Almeida, Robinson & Horsley, 1974).

Further developments will incorporate a calculator integrated circuit to facilitate the conversion of counts to volume, and for field use making the meter independent of mains electricity.

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COMMUNICATIONS

The influence of naloxone and normorphine on plasma corticosteroid levels in normal and stressed mice

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Immediate interest in the endogenous opiate-like substances (enkephalin and endorphin) has centred on their role in nociception. However, the multiplicity of effects of morphine suggests wider functions, for example, in the control of the hypothalamo-pituitary-adrenal axis. We describe, here, the effects of an enkephalin antagonist, naloxone, and an opiate agonist, normorphine, on plasma corticosteroid levels in stressed and unstressed mice.

Table 1. Plasma corticosteroid levels in mice (μg 100 ml.$^{-1}$); mean ± S.E. of mean (number of mice). The mice were treated as indicated and killed 30 min later, *A* without further treatment, or *B* with exposure to ether 15 min before sacrifice.

Treatment	*A*	*B*
0·9 % sodium chloride	23·3 ± 0·9 (60)	44·7 ± 2·7 (16)
Ether vapour	31·5 ± 3·2 (20)	42·8 ± 2·2 (15)
Naloxone 50 mg kg^{-1}	36·2 ± 2·4 (30)	30·9 ± 1·2 (15)
Normorphine 50 mg kg^{-1}	33·5 ± 1·1 (10)	52·7 ± 1·2 (10)

Male albino mice, 30–40 g, were allowed to acclimatise in a quiet laboratory for 2 hr prior to injection (I.P.) of 0·9 % NaCl solution, normorphine (50 mg kg^{-1}) or naloxone (50 mg kg^{-1}) or to stress by exposure to ether vapour for 1 min.

Plasma corticosteroid, measured spectrofluorimetrically (Zenker & Bernstein, 1958) was raised by ether exposure and by naloxone or normorphine, the ether effect being the most pronounced and transient. Ether exposure 15 min after naloxone treatment caused a small fall ($P = 0{\cdot}06$) in plasma corticosterone, while following normorphine treatment the rise was greater than that seen after ether alone (Table 1).

Effects of an opioid antagonist on corticosteroid levels in the absence of administered opioid have not been reported previously and indicate that enkephalins or endorphins might be involved in pathways controlling stress responses.

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Effectiveness of diazepam and its metabolite, 3-hydroxydiazepam (temazepam), for sleep during the day

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Aircrew operating world-wide east–west routes have to cope with irregular hours of duty and changes in time zones, and their work demands complex adjustments of sleep (Nicholson, 1970). The ability to create an acceptable sleep pattern is largely related to duty hours, but even with satisfactory schedules some aircrew experience difficulties, and the use of hypnotics has been considered. However, the rest periods of aircrew may not coincide with their optimum desire for sleep, and an hypnotic which is useful under normal circumstances may not be effective with an irregular pattern of rest. Indeed, many drugs have circadian activity (Reinberg & Halberg, 1971), and it is in this context that we have compared the effect of diazepam and 3-hydroxydiazepam (temazepam) on sleep during the day. Both drugs are useful hypnotics for nocturnal sleep (Nicholson & Stone, 1976).

The subjects were six healthy male volunteers aged between 19 and 28 years. The assessment of each treatment (matching placebo or dose of the drug) involved one afternoon. For the night preceding the afternoon sleep, subjects slept at home, and they were requested to refrain from napping and undue exercise during the morning, and to abstain from caffeine and alcohol on the days which involved the recordings. Seven

days separated each assessment. Three electroencephalographic, two electro-oculographic and one electromyographic channels were recorded using silver–silver chloride electrodes. The analysis of sleep stages was carried out according to the scheme of Rechtschaffen & Kales (1968). Each subject ingested 10 and 20 mg temazepam, temazepam matching placebo, 5, 10 and 15 mg diazepam and diazepam matching placebo. A double blind design was used. The subjects retired at 14.00 hr and remained in bed for 6 hr.

Total sleep time for day time sleep (mean for six subjects, 198·9 min) was increased by diazepam, but there was no change with temazepam. The total sleep time with 10 mg diazepam was 256·1 min ($P < 0.05$), and with 15 mg diazepam 297·3 min ($P < 0.01$). Sleep onset latency (mean, 22·5 min) was shortened by diazepam, but there was no change with temazepam. The mean sleep onset latencies with 5 and 10 mg diazepam were 16·2 and 15·2 min respectively ($P < 0.05$), and with 15 mg diazepam was 13·8 min ($P < 0.01$). However, awakenings to stage 0 activity were reduced by temazepam (10 and 20 mg, $P < 0.05$), and stage 1 (drowsy) sleep was reduced by both drugs ($P < 0.05$). There were also increases in stage 2 sleep with 5, 10 and 15 mg diazepam ($P < 0.05$, 0·01 and 0·001 respectively) and with 20 mg temazepam ($P < 0.01$). With 10 and 15 mg diazepam there were also increases in stage 3 ($P < 0.01$ and 0·001 respectively) and possibly in stage 4 sleep.

It would appear that, though both diazepam and temazepam are effective as hypnotics for night time sleep (Nicholson & Stone, 1976), temazepam is much less effective than diazepam for sleep during the day. Temazepam, like diazepam, reduces arousals to awake activity and drowsy sleep, but sleep onset latencies are not shortened and total sleep time is not increased. These observations suggest that circadian responsiveness may vary between drugs which have similar pharmacological effects.

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Circadian rhythms and irregular sleep schedules

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The circumglobal traveller and the shift worker are faced with similar problems in that both are expected to perform at times at variance with those appropriate to their endogenous circadian rhythms. They differ, however, in that the traveller encounters a complete set of external rhythms out of phase with his internal rhythms, whereas the night or shift worker is required to work at hours at variance alike with his endogenous rhythms and with the society about him. One might, therefore, expect adaptation to be more difficult for the shift worker, but conversely his hours of work are seldom as irregular as those of long-haul air crews. These differences can be eliminated by making the observations in an isolation unit in which subjects can alter their routines of sleep and activity in accordance with any pre-arranged design, but not be affected by the alternation of day and night and the habits of others living on a conventional nychthemeral schedule.

We have observed the rhythms of rectal temperature and urinary excretion of a subject (male, aged 28), when, over the course of 8 days, the clock was altered to mimic the time changes involved in two 8 hr westward shifts, as if he flew to Los Angeles and then on to Western Australia, and two 8 hr eastward shifts, as if he returned to England by the same route. Most of his rhythms, including temperature and urinary potassium, sodium and chloride, failed to adjust at all to these time shifts, but instead free-ran with periods of 25–26 hr. As a result, when he was again living on normal time, 8 and 9 days later, his temperature and urinary excretion of electrolytes were highest around the middle of his sleep. Only phosphate excretion gave clear evidence of any adjustment to the repeated time shifts.

By contrast, four subjects slept regularly from midnight to 04.00 and took a second 4 hr sleep irregularly at different times of day. The excretory rhythms of potassium, sodium, chloride and water of two of these four subjects maintained a periodicity close to 24 hr, as did the temperature rhythm in three of the four. Although this sleeping pattern does not parallel any of the numerous shift systems commonly worked, it does suggest that physiological rhythms could be preserved in their normal relations if at least some sleep on most nights could be taken at regular hours.

On some variable shift systems, and particularly on schedules worked by long-haul air crews, short naps of a few hours may be desired; it

appears improbable that these would disturb circadian rhythmicity if some sleep can be taken at regular hours.

The circadian rhythm of REM sleep

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In a previous investigation (Hume & Mills, 1975) five subjects slept from about midnight to 04.00 and took a second 4 hr of sleep 2 hr later every day, initially from 06.00 to 10.00 and ultimately from 20.00 to 24.00. The amount of REM in this 4 hr 'drift' sleep declined progressively as the sleep fell later in the day.

We have now repeated the experiment upon a further four subjects, with the difference that the time of the 4 hr 'drift' sleep was randomly arranged; the relationship between time of day and amount of REM was virtually identical.

It could be objected that when the 4 hr 'drift' sleep was taken later in the day it was preceded by a longer period of wakefulness; but examination of the 4 hr 'anchor' sleep, from midnight to 04.00, indicated, as in the previous study, that any effect upon REM of varying duration of prior wakefulness was trivial. By contrast in these, as in the earlier experiments, there was a strong correlation between duration of prior wakefulness and minutes of slow wave sleep (stages 3 and 4) in a 4 hr sleep, and the relationship was similar whether the 4 hr sleep was taken from midnight to 04.00, or during the day.

The relationship between amount of REM and time of day, from 08.00 to 22.00, is well fitted by linear regression but, if the values for sleep between midnight and 04.00 are included, it is also well fitted ($P < 0{\cdot}001$) by a cosine curve which, when derived from all nine subjects, defines maximum and minimum incidence at 10.36 and 22.36.

We have also examined the amount of REM in 7 hr of sleep in subjects living for a fortnight on a 'day' of 21 hr (Mills, Minors & Waterhouse, 1977). When plotted against the midpoint of sleep expressed on a conventional 24 hr clock, this also varied sinusoidally with a maximum at 10.54 and minimum at 22.54, confirming a circadian rhythm in the incidence of REM with maximum and minimum shortly before mid-day and midnight.

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Effects of water and saline on dehydration hyperthermia

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Thermoregulation is impaired by dehydration (Pitts, Johnson & Consolazio, 1944) and by a rise in plasma osmotic pressure (Nielsen, 1974). Since dehydration is associated with an increase of plasma osmolarity, dehydration hyperthermia might be explained in terms of an osmotically mediated inhibition of heat dissipation. In that case, artificially raising plasma osmotic pressure should have an adverse effect on thermoregulation even in the absence of dehydration.

To test this hypothesis the thermal responses of four male subjects to a hot environment (45° C_{db}; 32° C_{wb}) were compared under three conditions – progressive dehydration (to 95% of the initial body weight), and prevention of that dehydration by liquid replacement with either distilled water, or with 1% saline, both administered orally at body temperature. The heat exposure, which lasted for 2 hr, involved first sitting at rest for 30 min, then exercising at 75 W for 45 min on a bicycle ergometer, and finally sitting at rest for 45 min. Measurements of core (T_c) and skin temperatures were made at 2 min intervals, and of changes of intravascular volume, electrolytes, and osmolarity at 10 min intervals.

Core temperatures with dehydration and saline were not significantly different, but were higher than with water ($P < 0{\cdot}05$). Mean skin temperatures did not differ significantly between the three conditions, and no differences between the rates of body weight loss were detected.

The higher core temperatures with dehydration and saline were associated with elevated levels of plasma Na^+ and osmolarity. For the three conditions the relationship between osmolarity and T_c, and between Na^+ and T_c, could be described by parallel ($P < 0{\cdot}001$) linear regressions of positive slope.

Changes of intravascular volume differed significantly between the three conditions ($P < 0{\cdot}001$). Saline replacement reduced to 5% the 20% and 15% reductions in plasma volume observed during the final minute of exercise with dehydration and water replacement respectively. A haemoconcentration still persisted 45 min after exercise with both dehydration and water; with saline, plasma volume was increased by 5%. There was no correlation between change in plasma volume and T_c.

Thus, the prevention of dehydration by the administration of 1% saline has been shown to produce an impairment of thermoregulation comparable with that produced by dehydration alone. Since the core temperatures were so similar, and the changes in intravascular volumes so dissimilar, it seems unlikely that dehydration hyperthermia can be explained in terms

of a reduced availability of interstitial fluid at the sweat glands. The present results are, however, consistent with an osmotically or Na^+ mediated inhibition of heat dissipation.

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The cost of work in medical nursing

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Nursing is often regarded as strenuous physical work, but few measurements of the physiological cost have been made. A mean energy expenditure of 272 W was found for bedside nursing (Durnin & Passmore, 1967). However, there have been many changes in nursing organization over the past decades and a dietary survey (Fletcher, James & Coghill, 1975) found that trainee nurses had lower calorie intakes in the 1970s than the 1940s, suggesting a reduction in energy demands.

In the present study, physiological measurements were made on fourteen female nurses working on a 28-bedded medical unit of a teaching hospital (mean age 23 yr, mean weight 55 kg). Recordings of heart rate were made throughout day shifts (07.30–16.15), evening shifts (12.30–21.30) (fc_w) and during sleep (fc_s) by continuous monitoring of electrocardiograms using a modified 4-channel miniature body-borne tape recorder (Medilog, Oxford Instruments Co.). Oxygen consumption at work ($\dot{V}_{O_2,w}$) was measured concurrently for 30–40 min periods using the Miser respirometer (Eley, Goldsmith, Layman & Wright, 1975). In addition, submaximal exercise tests were performed by eight of the subjects on a friction braked bicycle ergometer and, from the results of these, $\dot{V}_{O_2\,max}$ was predicted by extrapolation of the regression line of $\dot{V}_{O_2}$ on fc to the predicted fc_{max}.

The mean levels of $\dot{V}_{O_2,w}$ and fc_w were all within recommended limits for 8 hr working. The mean $\dot{V}_{O_2}$ was $0{\cdot}44 \pm$ S.D. $0{\cdot}09$ l. min^{-1}, giving a mean energy expenditure of 147 W ranging from 111 to 194 W – comparable with light industrial work. There was suggestive evidence that junior nurses expended more energy than their more senior colleagues. The mean

relative work load [($\dot{V}_{O_2}$ at work/$\dot{V}_{O_2max}$ pred) × 100]% was 22 ± S.D. 5% and the mean fc_w was 93 ± S.D. 10 min^{-1}.

$\dot{V}_{O_2,w}$ was highest in the morning, fc_w highest in the evening. There were no significant correlations between the $\dot{V}_{O_2,w}$ and fc_w for individuals; furthermore, there was no significant correlation between the $\dot{V}_{O_2}$ and fc during particular activities. The individual calibration lines of $\dot{V}_{O_2}$ against fc from the exercise tests did not prove useful for predicting oxygen consumption at work.

We suggest that these low levels of energy expenditure were well within the physical capabilities of these nurses.

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Physiological appraisal of physical training procedures

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Physical activity of adequate intensity increases the ability of most subjects to undertake that form of exercise but the benefit is only partly transferrable to other activities (Astrand & Rodahl, 1970). The assessment procedure should take this into account, and also that for most non-athletic subjects maximal exercise is not acceptable (Cotes, 1971). Submaximal assessment is usually based on the cardiac frequency during cycling (Cotes, Berry, Burkinshaw, Davies, Hall, Jones & Knibbs, 1973; Kappagoda, Linden & Newell, 1977) but other indices, and, depending on the type of activity, other forms of test exercise may be preferable. The present study explores these possibilities.

Thirty-six male volunteers for a physical training programme, of mean age 45 yr (range 35–55 yr), were allocated at random to either jogging, stepping or mobility exercises, of 20 min duration 3 times per week for 12 weeks. Training was preceded and followed by clinical, anthropometric and physiological assessment including measurement of the ventilation, cardiac frequency and cardiac stroke output during progressive treadmill exercise up to approximately two-thirds of maximum. The subjects kept a diary of activity and recorded their cardiac frequencies be-

fore and after each training session; the mean increments (ΔfC) for jogging, stepping and mobility were respectively, 78·5, 78·2 and 29·2 min^{-1}.

Thirty-two subjects completed the study of whom two-thirds from all groups professed subjective improvement. No physiological changes were observed in the mobility group. The joggers showed an 8·4% reduction in exercise cardiac frequency standardized for O_2 consumption ($P < 0·05$). Amongst the steppers, after excluding the result for one subject who had a cold on reassessment, the exercise ventilation was reduced by 8% and the cardiac frequency by 4·7% which was materially but not significantly less than for the joggers despite similar effort (ΔfC). Both groups showed a similar increase in cardiac stroke output, which may therefore in some circumstances be the index of choice for assessing cardiovascular performance. The results raise the possibility that where subjects train using a specialized type of exercise such as stepping, this should also be used for the assessment.

We are indebted to our subjects, also Mr A. Gathergood and the *South Wales Echo*, for help with this study.

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The effects of hyperventilation on psychomotor performance

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The decrement of psychomotor performance found during hypocapnia could be mainly due to cerebral hypoxia, to carpopedal spasm or to a mixture of the two. However, most authors have studied only motor-orientated psycho-motor tasks. The experiment described here was designed to investigate the effect of hypocapnia on intellectual as well as motor performance.

The performance of nine subjects doing five different psychomotor tasks has been determined at three levels of P_{A,CO_2}. Subjects hyperventilated voluntarily at 40 l./min until P_{A,CO_2} (monitored by mass spectrometry) reached 38·5, 25·0 or 15·0 torr; CO_2 was then added to the inspirate to maintain P_{A,CO_2} steady at the selected value. After 10 min, pulmonary ventilation was reduced to 20 l./min for the rest of the exposure. The five tasks were performed in the same order three times during each exposure: once during a free-breathing control and twice during controlled P_{A,CO_2} at a

minute volume of 20 l./min. The psychomotor tasks used were motor (rotary pursuit and pegboard), intellectual (orientation of a manikin and verbal transformation) and mixed short-term memory and motor (digit recall).

There were no decrements in performance at P_{A,CO_2} levels of 38·5 and 25·0 torr. The performance of the three tasks with a motor component showed a significant decrement ($P < 0{\cdot}001$) at a P_{A,CO_2} of 15·0 torr, while there were no significant changes in the performance of the two intellectual tasks.

The motor decrements at a P_{A,CO_2} of 15 torr can be ascribed to the carpal spasm known to be present at this level. It is possible to calculate from the data of Kelman (1967) and Gottstein, Berghoff, Held, Gabriel, Textor and Zahn (1970) that the cerebral tissue oxygen tension during severe hypocapnia may not be low enough to cause changes in cerebral function (for references, see Purves, 1972); the minimum cerebral tissue oxygen tension may be comparable to that caused by breathing air at an altitude of 10,000 ft. No investigator has found decrements in the performance of pre-learned psychomotor tasks with this degree of hypoxia. Secondly, regional cerebral blood flow and therefore oxygenation can vary markedly. The lack of decrement of intellectual performance at a P_{A,CO_2} of 15 torr could therefore be due to an absence of regional cerebral hypoxia.

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The response of abdominal vagal fibres in the rat to changes in inspired oxygen concentration

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In the rat, characteristic cell groups known as 'abdominal vagal paraganglia' are found in association with the abdominal vagi and their branches. Their structure so resembles that of the carotid and aortic bodies that it has been proposed that these abdominal paraganglia might also function as arterial chemoreceptors (Hollinshead, 1941, 1946; Morgan, Pack & Howe, 1976). Some reflex (Hollinshead, 1946) and electrophysiological (Andrews, Deane, Howe & Orbach, 1972) evidence has been reported which is consistent with this suggestion. In order to test this

hypothesis further, the present study was undertaken to see whether chemoreceptor-like activity could be recorded from the abdominal vagus.

Forty-six adult male rats were used for this study. The animals were anaesthetized with sodium pentobarbitone (60 mg/kg, intraperitoneally) and the trachea was cannulated for the administration of gas mixtures. Systemic blood pressure was recorded from the carotid artery. The abdomen was opened in the mid line, the abdominal vagus cut just below the diaphragm and recordings were made from fine strands of the ventral branch of the nerve in its course along the surface of the oesophagus.

In twelve rats, spontaneously active centripetal fibres were located which responded in a graded manner to the level of oxygen in the inspired gas. Stimulus/response curves (F_{I,O_2}/impulse frequency) were constructed for these fibres. These curves closely resembled those obtained for the 'miniglomera' in the cat (Matsuura, 1973). At the end of the experiment, the animal was killed with excess anaesthetic; a prolonged after-death discharge then ensued in these vagal fibres, lasting for more than 15 min.

In five of these preparations, a discrete area was located in the nerve which responded to light mechanical stimulation (such sensitivity is known to be a feature of arterial chemoreceptors). Topical application of sodium cycanide or acetylcholine to such areas caused maximal excitation of the oxygen-sensitive fibres, thus providing a link between the present study and previous reflex investigations (Hollinshead, 1946).

The results reveal vagal fibres showing a chemoreceptor-like response to changes in inspired oxygen and certain other chemoreceptor stimulants. These observations are in keeping with the hypothesis that abdominal vagal paraganglia are arterial chemoreceptors.

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The structure and function of intra-epithelial nerve fibres of the respiratory tract in the cat

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Intra-epithelial nerve fibres have been identified by electron-microscopy in both extra- and intrapulmonary airways in a variety of species, including man (see Jeffery & Reid, 1973). There has been no previous report concerned with the ultrastructural features and concentration of these nerve fibres in the cat, an animal now much used in studies of airway reflexes and of the control of mucus secretion by the respiratory tract. The present studies were undertaken to determine the ultrastructure of these nerve fibres and their concentration at different levels of the airways of the respiratory tract, including the larynx. Furthermore, in order to obtain information regarding the function of intra-epithelial nerve fibres – i.e. whether sensory or motor – their appearance and concentration was studied following unilateral infra-nodose section of the vagus. It was assumed that cervical vagotomy would lead to the degeneration of afferent vagal fibres (whose cell bodies lie in the nodose ganglion), leaving intact the post-ganglionic parasympathetic motor fibres (whose cell bodies lie in the airway wall).

Intra-epithelial nerve fibres were found immediately above and below the vocal cords, where they were sparse, but not within the epithelium of the vocal cords themselves. Nerve fibres were identified also within the epithelium of the trachea, main carina and hilar airways, but not in small bronchi of less than 0·7 mm diameter. The greatest concentration of intra-epithelial nerve fibres was found at the carina, the majority of which lay basally within the epithelium, close to its basement membrane.

Nerve fibres were recognized as electron-lucent profiles containing neurotubules and characteristic mitochondria. Neither myelin, Schwann-cell sheath nor basement membrane were found associated with an intra-epithelial nerve fibre. Most axons were devoid of 'neurosecretory' vesicles.

Unilateral infra-nodose vagotomy and section of the superior laryngeal nerve caused a marked reduction in the number of intra-epithelial nerve fibres on the denervated side, when compared with the contralateral intact side. Nerve fibre degeneration was nearly complete by 12 days following vagotomy.

Both the ultrastructural features and degenerative changes of intra-epithelial nerve fibres suggest they subserve a sensory function in the cat.

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It is possible that they represent the airway 'irritant receptors' demonstrated in physiological studies (Mills, Sellick & Widdicombe, 1970).

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Magnitude and phase changes in heart rate variability and blood pressure during respiratory entrainment

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The role of spontaneous oscillations in the control of arterial blood pressure was previously discussed by Hyndman, Kitney & Sayers (1971). In that paper they described the hypothesis that the sino-aortic reflexes are related to a spontaneously oscillating nonlinear system which is subject to entrainment by the respiratory rhythm. The effect of changes in the respiratory frequency on arterial blood pressure and heart rate variability is the subject of this communication.

Experiments were carried out in which subjects (four) were asked to breath at constant depth and one of eight different varying frequencies. Arterial blood pressure and the e.c.g. were recorded for eight different rates and the heart rate variability signal (HRV) was computed in each case.

The results showed that over a wide range of respiratory rates both the blood pressure and the HRV exhibit clear evidence of entrainment. More detailed analysis of the blood pressure and HRV wave forms showed that the changes in blood pressure were more closely related to changes in heart rate than to respiration; for example, there were a number of cases in which the power spectrum of the HRV consisted of the fundamental and second harmonic of the respiratory rate while the blood pressure comprised only the second harmonic.

The Bode magnitude and phase plots of the two wave forms were plotted, with respiration as the reference. The magnitude plots for blood pressure had a flat response up to 0·9 rad sec^{-1} followed by a rapid fall-off, while the HRV signals had a peak at about 0·7 rad $secs^{-1}$ with a loss of entrainment above about 1 rad sec^{-1}. The two phase plots exhibited almost identically shaped responses over the range of entrainment with the exception of a clear 90° frequency independent lag in the HRV signal relative to the blood pressure. When this lag was compensated, both signals showed a steady increase in phase delay with increasing respiratory frequency.

The spectral analysis and phase plots therefore appeared to provide contradictory information because the former indicated that respiration affects blood pressure via heart rate changes, while the phase information implied that the reverse is true. The most obvious explanation is that the constant phase lag of 90° in relation to blood pressure, exhibited by the HRV waveform, is in fact a phase lead of 270°. However, this would appear to cast doubt upon the theory that respiration is reflected directly in arterial blood pressure due to its effect on the venous return to the heart.

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Thermal entrainment patterns in heart rate variability

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In a previous communication to the Society (Kitney, 1974), we described the entrainment of heart rate variability by a periodic thermal stimulus applied to one hand. In the course of our analysis of the experimental results we observed and categorized three types of entrainment: stable, metastable and unstable entrainment. These three types can be defined as follows. During stable entrainment the power spectrum of the heart rate variability (HRV – beat to beat changes in the RR interval of the e.c.g. plotted as a function of time) wave form comprises a large component at the thermal stimulus frequency which is stable in both amplitude and phase. In metastable entrainment the component is still present but its amplitude and phase can, and often do, vary. Unstable entrainment is categorized by the system exhibiting metastable entrainment followed by a complete loss of entrainment for a period.

Results obtained from experiments performed on a number of subjects have shown that during the application of the same thermal stimulus there is a continuous transition between metastable and unstable entrainment. A study of the frequency of the thermal component in the HRV wave form indicates that its frequency is not entirely stable. A consequence of this is that as the natural frequency varies so does the range of entrainment. Hence a stable stimulus frequency can sometimes lie inside and sometimes outside the range of entrainment.

The entrainment phenomenon can be described by a non-linear oscillatory system whose efferent pathway comprises a switch element and a pure time delay incorporating a linear filter (Hyndman, Kitney & Sayers, 1971).

The linear filter and the pure time delay in the context of our present work represent the characteristics of the peripheral vascular bed. The model has been used to investigate the origin of the transition between metastable and unstable entrainment. (The linear filter representation of the vascular bed was first described by Scher & Young, 1963.) The parameters of the model were systematically varied and the results showed that all the parameters except the time delay had no significant effect on the natural frequency of oscillation of the system. Conversely, even small changes in the pure time delay caused large changes in the frequency of oscillation. Our conclusion, therefore, was that the origin of the transition phenomenon was fluctuations in the time delay. In their paper, Scher & Young (1963) describe the response of the smooth muscle associated with peripheral resistance by a differential equation incorporating a time delay. It would therefore appear that the variations in the frequency of the thermal component in the HRV wave form may arise from variations in the time delay associated with the response of the peripheral smooth muscle.

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Functional residual capacity in man during altered chemical drive to respiration

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Changes in respiratory drive in response to chemical stimulation may be investigated in terms of the suction developed at the mouth ($P_{0\cdot1}$ and dP/dt max) and the rate of air flow ($\dot{v}_{0\cdot1}$) at the start of inspiration (e.g. Weiskopf & Gabel, 1974; Mathews & Howell, 1975; Whitelaw, Dorenne & Milic Emili, 1975). Via the length tension relationship of the diaphragm (Marshall, 1962; Pengelly, Alderson & Milic Emili, 1971) these indices are also affected by any concurrent change in functional residual capacity (FRC), e.g. on changing from a sitting to supine posture a mean reduction in FRC of 27% was associated with an increase in $P_{0\cdot1}$ of 13·6% (Robson, Saunders & Sen Gupta, 1977). A decrease in FRC might also be expected when the tidal volume is increased as a result of increased chemical drive to respiration.

In six healthy young adults FRC was measured by the closed-circuit helium dilution method using a respirometer (Morgan) and helium cathero-

meter (Cambridge). The reproducibility expressed as the standard deviation of a single determination was 0·29 l. and the absolute accuracy estimated for an artificial lung containing the gases used in the study was 0·3%. FRC and indices of respiratory drive were measured in duplicate under steady state conditions during breathing air, 100% O_2, 10% O_2 in N_2, 8% CO_2 in air and 7% CO_2, 10% O_2 in N_2. Compared with breathing air the latter three gas mixtures were associated with significant increases in ventilation minute volume, tidal volume and indices of drive; there was some reduction in FRC which was significant in the case of breathing 8% CO_2 in air (mean values 3·40 l. and 2·731).

Reduction in FRC during breathing CO_2 is in line with our expectations. The absence of a significant fall with hypoxia is consistent with the findings of Kellogg & Mines (1975). However, in both circumstances, an increase was observed by Garfinkel & Fitzgerald (1975). Thus more data are needed to establish unequivocally the direction and magnitude of the effects.

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Angiotensin, hypoxia, verapamil and pulmonary vessels

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In cats, angiotensin II (A II) constricts most systemic vessels but has small inconstant effects on pulmonary vessels (Barer, 1961). Interest in its function in the lung has revived because it is formed from angiotensin I in the pulmonary endothelium, increases in pulmonary effluent blood during hypoxia (Allison & Clay 1976) and is required for hypoxic pulmonary vasoconstriction in rats (Berkov, 1974). In case it might mediate hypoxic pulmonary vasoconstriction, an important regulatory mechanism, we studied the action of A II in four species.

Isolated lungs were perfused with blood at constant flow (cats, ferrets, rats), or left lower lobes were perfused at constant flow *in vivo* with venous blood (dogs, cats and ferrets anaesthetized with pentobarbitone, chloralase

and urethane respectively). Increases in pulmonary artery pressure (P_{pa}) at constant outflow pressure indicated vasoconstriction.

In vivo ferret lung vessels were totally insensitive to A II, cat lungs occasionally showed slight vasoconstriction, dog lungs slightly more, whereas all three species showed profound systemic vasoconstriction. Thus in seven cats 0·4 μg/min A II raised P_{pa} by $0·4 \pm 0·8$ (S.E.) torr ($P > 0·5$) and femoral pressure by $35 \pm 6·8$ torr ($P < 0·01$); faster rates caused large increases in left atrial pressure. Larger doses could be given to isolated lungs; cat and rat lungs showed vasoconstriction to A II but ferret lungs did not. All four species showed hypoxic vasoconstriction; this was strongest in the ferret which was insensitive to A II.

These differences in the response of the pulmonary vessels to A II and hypoxia make it unlikely that A II is a mediator of hypoxic vasoconstriction. McMurtry, Davidson, Reeves & Grover (1976) abolished hypoxic but not A II induced vasoconstriction in rat lungs with Verapamil which inhibits transmembrane calcium transport. We confirmed this in six rats and also found Verapamil to be a selective inhibitor of hypoxic vasoconstriction in six ferret lungs. Yet A II may play a supporting role as Berkov (1974) suggested. In rat lungs always, in cat lungs sometimes and in ferret lungs never, A II potentiated hypoxic vasoconstriction. Thus in five rats ventilated with nitrogen A II caused P_{pa} to rise $10·6 \pm 1·4$ torr before and $14·1 \pm 1·6$ torr after 1 μg doses of ($P < 0·01$).

Thus A II may play a role in some species in controlling pulmonary vascular reactivity.

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The projection of vagal, cardiac C-fibres to the brain stem of the cat

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Accounts of neuronal activity evoked in the brain stem by vagal C-fibres have been entirely derived from electrical stimulation of the cervical vagi (Lam & Tyler, 1952; Fussey, Kidd & Whitwam, 1973). The

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purpose of this investigation was to delineate the regions of the medulla oblongata activated by electrical stimulation of cardiac vagal branches.

Experiments were performed on anaesthetized, artificially ventilated cats in which the femoral arterial blood pressure, heart rate, end-tidal P_{CO_2}, and oesophageal temperature were monitored. Stimulating electrodes were tied to the right thoracic vagus and its branches and the nerve–electrode system was encapsulated with silicone rubber (Donoghue, Fox & Kidd, 1977). Stimulating electrodes were also placed on the right cervical vagus. The dorsal surface of the medulla was exposed and extracellular recordings of neuronal activity were made with platinum–glass micro-electrodes. The positions of electrode tracks were subsequently verified histologically.

In eighteen cats the right side of the medulla was explored within the limits of 4 mm rostral and 2 mm caudal to the obex. Recordings were made from 48 single units in which activity was evoked by stimulation of slowly conducting fibres (conduction velocity 0·5–2·5 m sec^{-1}). None of the units were spontaneously active. Using established criteria (Fussey, Kidd & Whitwam, 1970), twenty-two units were shown to be excited synaptically whilst the responses of fifteen indicated either direct orthodromic or antidromic activation; tests on the remaining eleven units were inadequate to allow differentiation. Most units were located within, or close to, the medial part of the nucleus of the tractus solitarius, between 3 mm rostral and 1 mm caudal to the obex.

In conclusion, these results show that C-fibres in the cardiac branch of the vagus project to a restricted region of the brain stem which is coincident with the sites of termination of myelinated fibres from carotid sinus and cardio-aortic receptors.

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Voltage-clamp studies in rat fast skeletal muscle

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Major advances in understanding the role of the different ions in the electrical excitation of the membrane of skeletal muscles have resulted

from voltage-clamp studies on single cells from these muscles. As the studies have been performed mainly on muscles from frog (Adrian, Chandler & Hodgkin, 1970*a*, *b*; Ildefonse & Rougier, 1972) only a little is known about mammalian skeletal muscles.

The double sucrose gap technique has been applied to rat isolated skeletal fibres. The method used allows imposition of either different constant currents or potentials on the preparation and simultaneous recording of the contraction. The cells were selected from a muscle which was found to be fast (iliacus) by a comparison between its mechanical responses, associated with action potentials, and those developed by typical slow fibres (soleus). The fibres (50–70 μm in diameter) were carefully dissected. They were 1–2 cm long with both extremities left attached to tendons.

In Ringer solution, depolarizing steps induced ionic currents which are similar to those of frog. A global inward current which inactivates and depends on $[Na]_o$ is abolished by TTX; its amplitude is maximum for +40 and +50 mV and its reversal potential is obtained between +130 and +150 mV, values which are consistent with the sodium equilibrium potential. The half-inactivation of the global Na-current occurs at +15 mV. For depolarizations between +40 and +70 mV the analysis of the inactivation of this current and the effect of low doses of TTX show that two components participate in the inward current.

There is also a delayed outward current which reaches a peak in 5–10 msec and decreases with time. At room temperature its inactivation is roughly exponential with a time constant of 100–250 msec. This delayed current is divided in two components. The first has a linear instantaneous current–voltage relation and an equilibrium potential close to the resting potential which is not modified by the holding potential. The second has an equilibrium potential +15 to +35 mV more positive than the first component. Hyperpolarizing steps induce a current that is not maintained but decreases with time and the initial current-voltage relation shows inward rectification.

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The effects of calcium on outward membrane currents in Purkinje fibres from sheep hearts

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Three main outward current systems have been identified in voltage-clamped Purkinje fibres from sheep hearts, namely (i) a time-independent current, i_{K_1}; (ii) a time-dependent K^+ current, i_{K_2}, responsible for the pacemaker activity; and (iii) a time-dependent outward current i_{x_1}, which participates in the repolarization phase of the action potential (Noble & Tsien, 1968, 1969). We have studied the effects of Ca_0 on these current systems.

Increasing Ca_0 from 2 to 8 mM produces a positive shift of about 9 mV in the activation curves of both i_{x_1} and i_{K_2}. Differences appear, though, when Ca_0 is reduced; the x_1 curve shifts $-9{\cdot}5$ mV in $0{\cdot}5$ mM-Ca_0, while the s curve (controlling i_{K_2}) shifts by only -4 mV. The difference is more striking when Mg replaces the Ca removed. In a Tyrode solution containing $0{\cdot}1$ mM-Ca and $2{\cdot}9$ mM-Mg the x_1 curve shifts -19 mV, while the s curve is unaffected. This difference may be due to different affinities for Ca of binding sites adjacent to the gating mechanisms of each channel type. Alternatively, Ca removal may have two practically opposite effects on the s curve: a surface-charge effect, causing a negative shift, and an adrenaline-like effect (Harary, Renaud, Sato & Wallace, 1976), causing a positive shift (Hauswirth, Noble & Tsien, 1968).

Calcium also has an effect on the reversal potentials of i_{x_1} and i_{K_2}. Increasing Ca_0 from 2 to 8 mM causes a 7 mV positive shift in E_{x_1} and an 8 mV positive shift in E_{K_2}. It seems likely that high Ca_0 partially inhibits the sodium pump, leading to an accumulation of K^+ in the restricted extracellular space and a consequent reduction in the potassium gradient across the membrane. The change in E_{x_1} caused by Ca occurs with a noticeably slower time course than the shift in the x_1 curve, which suggests that the pump inhibition is due to an effect of intracellular Ca.

Finally, raising Ca_0 increases i_{K_1}, an effect suggestive of a direct interaction between Ca and potassium channels (Kass & Tsien, 1976). However, we have found that small increases in K_0 also increase i_{K_1}, and that the potential-dependence of this increase closely resembles that of the Ca_0-dependent increase in i_{K_1}. The i_{K_1} channels are therefore probably not directly dependent on Ca; the effect of Ca is rather to partially inhibit the sodium pump, cause a consequent increase in K_0, and thereby increase i_{K_1}.

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In vivo [1−^{3}H]taurine efflux from rat cerebral and cerebellar cortex and cuneate nucleus

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We have studied spontaneous and K^+-evoked [1−^{3}H]taurine release from the pial surfaces of cerebellar posterior lobe, cerebral cortex forepaw

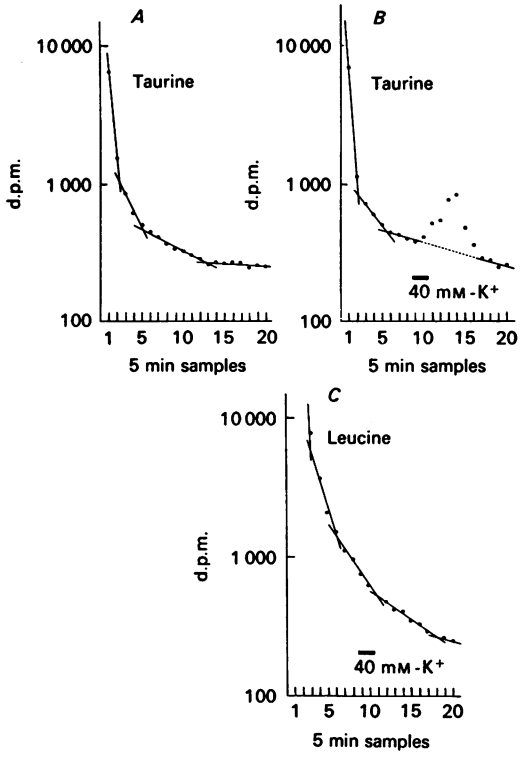

Fig. 1. [1−^{3}H]taurine and L-[G−^{3}H]leucine effluxes from the cerebellar posterior lobe. Radioactivity (d.p.m.) plotted semi-logarithmically as recovered in serial 5 min samples of superfusate. Horizontal bars (*B*, *C*) indicate superfusion with elevated (40 mM) K^+. The K^+-evoked increase in taurine efflux (*B*) is calculated by departure from the values predicted by the line of best fit for remaining points in the phase. K^+-stimulation fails to increase leucine efflux (*C*). Cuneate nucleus and cerebral cortex show similar responses.

somatosensory area and cuneate nucleus of chloralose-urethane anaesthetized rats. The superfusion and isotope-labelling techniques have been described elsewhere (Assumpção, Bernardi, Dacke & Davidson, 1976, 1977). Labelling was achieved using specific activities of 5 and 10 μc ml^{-1} respectively of [1 $-^3$H]taurine or L-[G $-^3$H]leucine.

Spontaneous efflux of both isotopes was multiphasic. A 10 min superfusion period in a slower phase with 40 mM-K^+ increased taurine, but not leucine, efflux in all three regions (Fig. 1). Mean K^+-evoked increases in taurine efflux were: cerebellum, 46% ($P < 0.001$), cuneate nucleus, 63% ($P < 0.001$), cerebral cortex, 90% ($P < 0.05$).

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Unitary activity in temporal epileptic foci of the monkey

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Epilepsy was induced in two rhesus monkeys, *Macacca mulatta*, with aluminium hydroxide cream applied, under general anaesthesia and with full aseptic precautions, to the ventral temporal cortex of one cerebral hemisphere (Nie, Upton & Ettlinger, 1973). About 12 months later electrical records were made with the animals undrugged and mildly restrained. Mass and unitary activity were simultaneously recorded from extracellular micro-electrodes in the epileptic focus and in homotopic cortex contralaterally. Interictal epileptic events were identified in the mass record and the mean frequency of unitary discharges before, during and after these events was measured.

The present preliminary report describes results from 10 units, 9 of them contralateral to the aluminium hydroxide cream. All units showed a reduction or cessation of on-going activity time-locked to both primary and secondary interictal events. The unitary quiescence started some 20 msec after the peak of the interictal event and lasted for about 0·2 sec. In addition to this period of quiescence, half the cells also showed increases in firing frequency above their resting levels. The increases occurred before or after the quiescence; in some units firing frequency was increased both before and after the quiescence. These early changes in firing

frequency often continued as a damped oscillation with a period of around 0·4 sec. Such oscillations in firing frequency continued for several seconds whereas the averaged mass potential showed deflexions which lasted for less than a second. The period of this oscillation of frequency of unitary firing was the same, however, as that of the mass record when it exhibited a spike and wave rhythm.

The changes in firing frequency associated with interictal events occurred irrespective of whether the on-going unitary activity was that characteristic of normal or of epileptic neurones (Calvin, Ojeman & Ward, 1973).

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Temperature and nervous conduction in the tortoise

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Tortoises are basking reptiles in which, under natural conditions, body temperature may rise during the day almost to mammalian levels and then fall overnight by 20 °C or so. They also sustain a continually depressed body temperature during the winter. Tortoises are therefore appropriate animals in which to examine both short- and long-term thermal influences on the nervous system.

Observations were made of conduction along the dorsal white columns of the spinal cord in decerebrate animals (*Testudo graeca*). The properties of this pathway have been described previously (Rosenberg, 1977). Short-term temperature changes were produced by controlling air temperature in the experimental enclosure (Rosenberg, 1974). Conduction velocity was measured during continuous warming or cooling; maximum rates of change of body and spinal cord temperature were $+12°$ C hr^{-1} and $-9°$ C hr^{-1} respectively. The overall range was 1–42° C.

It was found that conduction velocity is an increasing function of temperature up to 38–40° C; Q_{10} (15–25° C) = 2·5; Q_{10}(25–35° C) = 1·6 (mean values in warm acclimated animals). Cold block occurred usually below 3·5° C, exceptionally, below 1·2° C.

Long-term effects of temperature were studied by comparing warm- and cold-acclimated tortoises. All animals were warm acclimated before the start of the experiments, having been housed for several months at

ambient temperatures of 26–34° C. Cold acclimation was carried out by exposing normal and surgically prepared animals to the atmosphere of a cold-room at 5–7° C for 1–61 days. Conduction velocity was measured at the various stages of the experiment while subjecting animals to acute warming and cooling as described above. At higher acute temperatures ($\geqslant$ 25° C) conduction was faster in the warm-acclimated group than in those undergoing cold acclimation and the difference increased with the duration of acclimation period. Below 20° C there was no change in conduction velocity at a given temperature. In conclusion therefore no evidence could be found for compensatory change in the velocity of nervous transmission, during cold acclimation, such as occurs in the frog (Lagerspetz, 1974).

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Hormonal control of protein synthesis and degradation in rat skeletal muscle

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The content of proteins within a tissue is a function of both the rates of protein synthesis and degradation. Thus growth or atrophy of a tissue can occur by changes in either or both of these processes. This study concerns the changes in protein synthesis and breakdown in skeletal muscle under hormonally induced changes in growth. The average rates of protein synthesis and protein degradation were measured *in vitro* in intact isolated skeletal muscles from 60–65 g rats (Fulks, Li & Goldberg, 1975).

Hypophysectomy greatly reduces the growth rate of rats. Under this condition (4 weeks after operation) protein synthesis was reduced in both the soleus and extensor digitorum longus muscles compared with those muscles from unoperated weight matched rats.

The injection of ovine growth hormone into hypophysectomized rats, while causing growth, increased protein synthesis in both muscles but did not influence the rate of proteolysis in either muscle. Cortisone acetate injections into hypophysectomized rats decreased protein synthesis in both muscles; however, the rate of protein breakdown was not affected.

Thyroxine (T_4) or tri-iodothyronine injected into hypophysectomized

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rats in doses causing growth, accelerated both protein synthesis and degradation in the muscles. However, when larger doses of T_4 were injected, sufficient to cause a loss of weight, protein degradation was greatly increased and exceeded protein synthesis in both muscles.

Thus the lack of thyroid hormone is responsible for the decrease in protein degradation in skeletal muscle following hypophysectomy. In addition, the muscle wasting seen in hyperthyroid states may be due to accelerated protein degradation. The thyroid status of animals is known to change under a variety of physiological states, e.g. starvation, age, and may be an important factor regulating protein degradation in skeletal muscle under these conditions.

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Adrenergic motor responses to single pulse stimulation in the rat vas deferens

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The response of the vas deferens, from several species, to stimulation of its motor innervation with trains of pulses is complex, consisting of (1) an initial rapid 'twitch' which is present throughout the tissue and which is resistant to α-adrenoceptor blockers and (2) a slower, more sustained 'secondary' response which is confined mainly to the epididymal end of the tissue, is susceptible to α-adrenoceptor blockade and therefore presumed to be adrenergic (Swedin, 1971; Duncan & McGrath, 1976). Ambache, Dunk, Verney & Zar (1972) have, however, suggested that the physiological motor response corresponds to (1) and that (2) occurs only with long trains of pulses.

The present study examined whether, in rat vas deferens, a single stimulus could elicit a response exhibiting the characteristics of adrenergic transmission.

The longitudinal isometric tension was recorded from rat vasa deferentia set up as described previously *in situ* in the pithed rat (Gillespie & McGrath, 1974) or *in vitro* at 38° C in Krebs bicarbonate solution (Duncan & McGrath, 1976). Single pulses of 0·01 – 1·0 msec duration, supramaximal voltage, were applied, as appropriate, either via the pithing rod electrode at L 1–3 to stimulate the vertebral sympathetic outflow, or via field stimulation electrodes in the tissue bath.

In each case a single pulse produced a motor response with two components with respective maxima at 200–300 and 600–800 msec. In the

whole vas both *in situ* and *in vitro* the early component was normally larger in height, but if the area under the curve was considered, the late component contributed the larger fraction. In bisected vasa, in the prostatic end the early component, and in the epididymal end the late component, dominated. This distribution corresponds to the 'twitch' (early) and 'secondary' (late) components found with trains of pulses (Duncan & McGrath, 1976).

The late component was depressed by α-adrenoceptor blocking agents (phentolamine 10^{-6} M, yohimbine 6×10^{-7} M and azapetine 2×10^{-7} M) and potentiated both in height and duration by cocaine 10^{-6} M, which blocks the neuronal re-uptake of noradrenaline. In contrast the early component was not altered by these drugs.

Both *in situ* and *in vitro* temperature was critical especially for the late component where noradrenaline uptake serves to limit the response.

It is concluded that even a single stimulus produces an adrenergic motor response in the rat vas deferens but that this response may not be evident in circumstances where the early component is dominant and only the height of the response is measured.

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The effect of inosine on the recovery of isolated perfused arteries from dinitrophenol

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Lengths of isolated perfused rabbit renal or femoral arteries (de la Lande & Rand, 1965) were used to study the recovery of contractions produced by noradrenaline, after the addition of dinitrophenol to the perfusate.

After perfusion with 100 μM dinitrophenol the magnitude of the contraction was reduced by more than 90% as a result of uncoupling of oxidative phosphorylation, thereby simulating true ischaemia. The contractility recovered after the dinitrophenol had been stopped.

The rate and magnitude of recovery of contractility was compared with and without exposure to inosine during perfusion of the dinitrophenol.

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Recovery was greater and more rapid when 10 μM inosine was given than when it was absent. In five out of seven experiments when responses to noradrenaline in concentrations of 0·1–10 μg were measured it was found that the magnitude of recovery was significantly greater when inosine was given than when it was not. In a further five experiments the rate of recovery was measured. In all five experiments the artery recovered faster when inosine was given.

We conclude that inosine protects the tissue from hypoxic damage.

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Tortoise locomotion

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Tortoises (*Testudo graeca*) walking spontaneously on a horizontal surface were filmed at 24 or 32 frames/sec after equilibration at a particular ambient temperature. Cloacal temperature was measured periodically during an experiment. The films were analysed frame by frame to determine the times of onset and termination of each step. The median values of the durations of the swing and stance portions of each limb cycle and of the delays between limbs were calculated for each sequence, and investigated as functions of median cycle duration and of body temperature.

As reported for other Chelonians (Zug, 1972), the gait of *Testudo* displays the same limb sequence as the mammalian walk: left forelimb, right hind limb, right forelimb, left hind limb, with each limb 0·5 cycle out of phase with the other limb of the same girdle. No other gait was seen.

At a given temperature a change in the forward speed of locomotion was accomplished by an approximately proportionate change in the durations of all portions of the cycle, so that the phase relationships remained constant. This contrasts with the mammal, in which the phase relationships alter with walking speed (see Grillner, 1975).

Over a range of body temperatures, the stereotyped phase relationships persisted. Thus the locomotor cycle could be described by a single pattern, in which the fractions of cycle duration, averaged from 24 experiments, were as follows: forelimb swing = 0·15 (stance = 0·85), forelimb step to contralateral hind limb step = 0·14 (hind limb step to ipsilateral forelimb step = 0·36), and hind limb swing = 0·15 (stance = 0·85).

Although a range of walking speeds was seen at any given temperature, a plot of cycle frequency against temperature, using all data, revealed an

approximately linear relationship, with a slope of 0·03 sec^{-1} per degree C, and giving a Q_{10} of 2·0 between 20 and 30° C.

The tortoise is thus an attractive animal for studying the spinal pattern generator for locomotion, since the control of cycle timing, whether volitional or temperature-dependent, can be associated with the adjustment of only one parameter, the cycle duration.

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Phospholipase activity in β-bungarotoxin action

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β-Bungarotoxin (β-BuTX), isolated from *Bungarus multicinctus* venom, blocks acetylcholine release at skeletal neuromuscular junctions, without affecting the acetylcholine-sensitivity of the muscle. It has been shown recently that β-BuTX has phospholipase A_2 activity (see the references below), and it was therefore of interest to examine the relation between the phospholipase and blocking activities of the toxin.

β-BuTX was chemically modified in two ways: *p*-bromophenacyl bromide (*p*-BPB) modified one histidine residue, while iodoacetic acid (IAA) carboxymethylated methionine residue(s). Both modifications led to loss of phospholipase activity.

At frog end-plates, native toxin (1–5 μg ml^{-1}) causes a rapid fall in end-plate potential (e.p.p.) amplitude, followed by a transient increase before complete blockade (Fig. 1). At similar concentrations, *p*-BPB-treated β-BuTX had no significant effect on e.p.p. amplitude; but at 100 μg ml^{-1} there was a slow decrease to a value which remained stable for more than 2 h. IAA-treated β-BuTX had practically no effect even at 100 μg ml^{-1} (Fig. 1).

Our results suggest that β-BuTX has two actions: one responsible for a rapid decrease in transmitter release, the other, phospholipase-dependent and responsible for the transient increase in release. *p*-BPB-treated toxin, which lacks phospholipase activity, can bind to the axon membrane and reduce transmitter release, although much less efficiently than native toxin. IAA-treated toxin may not bind to the membrane (cf. van Wezel, Slotboom & De Haas, 1976) and thus be inactive.

S. A. holds a long-term E.M.B.O. Fellowship.

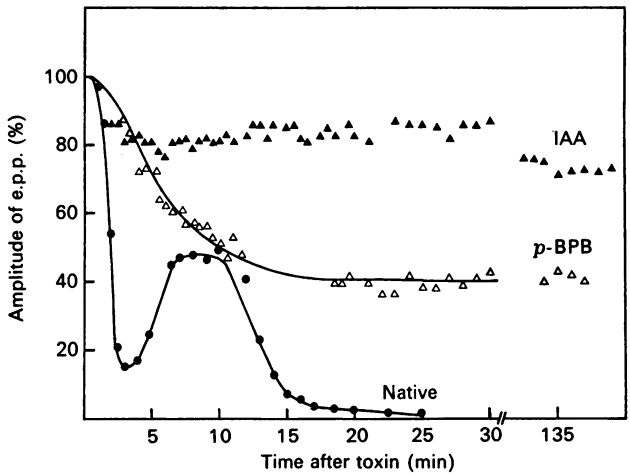

Fig. 1. Effect of native and modified β-BuTX on amplitude of intracellularly recorded end-plate potentials in curarized ($2{\cdot}5 \times 10^{-6}$ g ml^{-1}) frog sartorius. ●, native β-BuTX 5 μg ml^{-1}; △, *p*-BPB-treated β-BuTX 100 μg ml^{-1}, ▲, IAA-treated β-BuTX 100 μg ml^{-1}.

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Opiate inhibition of substance P release from the rat trigeminal nucleus, *in vitro*

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Opiates depress the activity of nociceptive neurones in laminae I and V in the dorsal horn of the cat spinal cord by a direct spinal action (LeBars, Guilbaud, Jurna & Besson, 1976). The peptide substance P may act as a transmitter released from primary sensory terminals in this region (Otsuka & Konishi, 1975) and substance P produces a specific excitation of nociceptive neurones in lamina V of the cat dorsal horn (Henry, 1976). High levels of substance P, the endogenous opiate receptor ligand enkephalin, and opiate receptor binding are found in the dorsal horn of the spinal cord (Otsuka & Konishi, 1975; Simantov & Snyder, 1977) and it has been suggested that opiate receptors may be associated presynaptically with primary afferent terminals containing substance P. The nucleus caudalis

of the trigeminal nucleus is essentially analogous to the dorsal horn of the spinal cord and also contains high levels of substance P and enkephalin. We have therefore used the trigeminal nucleus as a model to investigate the effect of opiates on the release of substance P *in vitro*.

The trigeminal nucleus from two rats (weighing 15–20 mg) was dissected from coronal slices of rat medulla oblongata under stereomicroscopic observation and chopped into $0{\cdot}2 \times 0{\cdot}2$ mm slices. The slices were transferred to a Perspex superfusion chamber and superfused with Krebs bicarbonate at 37° C containing 0·5% albumin and 30 μg/ml of the peptidase inhibitor bacitracin. Superfusate fractions (375 μl.) were collected at 1 min intervals and stored on ice before determination of substance P using a radio-immunoassay, as described previously (Jessell, Iversen & Kanazawa, 1976).

Addition of 47 mM-K^+ to the superfusing medium for 2 min produced a marked increase in the release of substance P. The peak evoked release of substance P per min represented $1{\cdot}26 \pm 0{\cdot}17$% (mean ± S.E.M., $n = 8$) of the total substance P content of the tissue, compared with a pre-stimulus spontaneous efflux of $0{\cdot}21 \pm 0{\cdot}03$% (mean ± S.E.M., $n = 8$). Addition of morphine to the superfusing medium for 7 min prior to K^+ stimulation did not affect spontaneous efflux but produced a dose-dependent inhibition of K^+-evoked substance P release; almost complete inhibition of release was observed with 10 μM morphine. Superfusion of trigeminal slices with 1 μM naloxone for 5 min prior to addition of 10 μM morphine produced an almost complete reversal of the inhibitory effects of morphine.

The opiate-induced inhibition of substance P release also exhibited a pronounced stereospecificity; more than 80% inhibition was produced with 5 μM levorphanol, whereas a tenfold higher concentration of dextrorphan did not significantly alter K^+-evoked release.

These observations suggest that opiates exert an inhibitory influence on substance P release. If substance P functions as a primary sensory transmitter associated with nociception in the spinal cord and trigeminal nucleus, the inhibition of its release from presynaptic terminals may represent a mechanism for the direct spinal analgesic actions of opiates.

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The identification of two discrete excitatory systems in the dentate gyrus of the rat

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Fibre degeneration studies have demonstrated that the molecular layer of the dentate gyrus is innervated by two major fibre systems, the fimbria and perforant path. These pathways originate in the septum and entorhinal cortex respectively. (Hjorth-Simonsen & Jeune, 1972; Mellgren & Srebro, 1973). Evidence has been published which suggests that the septo-dentate projection system is cholinergic (Storm-Mathisen, 1974; Dudar, 1975). Data including the use of glutamate diethylester (GDEE) (McLennan & Wheal, 1976) suggests that the excitatory influence of perforant path stimulation on dentate granule cells and the corresponding links in the intrinsic system within the hippocampus may be mediated by glutamate (Lømo, 1971; Crawford & Connor, 1973).

While acetylcholine (ACh) and glutamate have been implicated as neurotransmitters in the two anatomically distinct fibre systems innervating the dentate granule cell population, there have been no attempts to determine the effects of their respective antagonists on synaptically induced responses elicited from the septum or entorhinal cortex.

The responses of 72 dentate granule cells to medial septum (MS) and perforant path (PP) stimulation were examined in 27 rats anaesthetized with urethane. MS and PP stimulation evoked an orthodromic activation of granule cells which was correlated with the negative transient of the characteristic field potential elicited from each site. The effects of electrophoretic application of ACh and glutamate were examined on dentate cells identified in this manner.

The excitatory action of ACh but not that of glutamate was antagonized by atropine. GDEE blocked the excitation produced by glutamate and not ACh. The synaptically evoked excitation elicited by stimulation of MS was blocked by atropine but unaltered by GDEE, whereas the PP excitatory response was blocked by GDEE and unaltered by atropine.

The results of this study indicate that two discrete excitatory systems are present in the dentate gyrus of the rat: a cholinergic system originating in the medial septum and an excitatory amino acid mediated system originating in the entorhinal cortex.

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Convergent squint arrests the development of spatial resolving power of cells in the lateral geniculate nucleus in kittens

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Since it is well known that visual acuity of humans and cats develops during early post-natal life, we asked whether the loss of spatial resolving power of the LGN cells fed by the area centralis of the squinting eye in kittens raised with a convergent squint at the age of 3 weeks (Ikeda & Wright, 1976) was due to arrest of such development.

In twelve kittens an operation to produce convergent squint was performed at either 3, 6, 8, 10, 13 or 16 weeks of age (two kittens in each age group). When these kittens reached the age of 6–7 months, the spatial resolution of the LGN cells in layers A and A 1, which received projections from within 5° of the area centralis of the retina, were determined under nitrous oxide/halothane anaesthesia using methods previously described (Ikeda & Wright, 1976).

The loss of spatial resolving power (the highest spatial frequency of a sinusoidal grating of moderate contrast, 0·4, moved across the receptive field centre to which a cell responded with modulated firing distinguishing the dark and the light phases of the grating) of the squinting eye cells is severest in the kittens operated at 3 weeks of age. The degree of loss of spatial resolving power in the squinting eye gradually decreased in kittens operated at 6, 8 and 10 weeks. The cells fed by the squinting eye in the kittens operated at 13 weeks and 16 weeks showed no reduction in their spatial resolving power.

The spatial resolving power of the cells driven by all squinting eyes were compared with the spatial resolving power of the LGN cells obtained from normal (unoperated) kittens at different ages.

A developmental curve of spatial resolution of LGN cells obtained from four normal kittens (one kitten studied at each of 3, 5, 7 and 10 weeks) was found to fit well with a plot of spatial resolution of cells in squinting eyes against the age of operation.

These findings provide a neurophysiological counterpart for the behaviourally measured visual acuity in squinting cats (Ikeda & Jacobson, 1977).

It appears that the loss of spatial resolution (amblyopia) in the eye with convergent squint is due to arrest of development during early post-natal life, and this arrest is already apparent in LGN function.

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Convergent squint arrests the development of spatial vision in cats: behavioural evidence

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The age at onset of squint has been suspected to relate to the severity of amblyopia in esotropic patients (Newell, 1969) and in experimental esotropia (von Noorden & Dowling, 1970; Franklin, Ikeda, Jacobson & McDonald, 1976). We have now measured behaviourally the spatial vision of adult cats reared with surgically induced convergent squint in one eye from ages 3, 6, 8, 12 and 24 weeks.

Monocular visual acuity was measured in six cats with squint and three controls using a technique previously described (Jacobson, Franklin & McDonald, 1976). The acuity of the squinting eye was significantly lower than that of the non-squinting eye in cats with squint from 3, 6 and 8 weeks of age. No statistical difference in acuity between eyes was found in the 12- and 24-week-squinting cats and in the controls. The age of the squinting procedure and the difference in acuities between squinting and non-squinting eyes (expressed as a ratio) were as follows: 3 weeks – 0·31 and 0·29; 6 weeks – 0·51; 8 weeks – 0·77; 12 weeks – 0·93; and 24 weeks – 0·91.

Monocular contrast sensitivity for five spatial frequencies (0·13–2·0 c/deg.) was measured in four cats with squint and two controls. In cats with squint from 3 and 6 weeks of age, contrast thresholds at all spatial frequencies were higher in the squinting eye than in the non-squinting eye. The differences, however, were greater at higher spatial frequencies. In the 8-week-squinting cat, contrast thresholds in the squinting eye were

increased only at higher spatial frequencies. There were no significant differences in contrast sensitivity between eyes in the 24-week-squinting cat and in the controls.

The remarkable correspondence between the above results and the data on development of visual acuity in normal kittens from behavioural (Mitchell, Giffin, Wilkinson, Anderson & Smith, 1976) and evoked response (Freeman & Marg, 1975) studies warrants proposal of the hypothesis that convergent squint arrests the development of spatial vision in cats.

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Properties of type I mechanoreceptors in the rabbit hairy skin

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As part of a detailed investigation into the sensory innervation of the rabbit hairy skin, a study has been carried out on the response characteristics of type I mechanoreceptors. One of the most detailed analyses of type I receptor characteristics has been made by Iggo & Muir (1969). Our work on rabbits extends their findings.

Male rabbits (2½–3 kg) were anaesthetized with an intraperitoneal injection of a mixture of 10% urethane and 1% chloralose (6·5 ml./kg). Single-fibre recordings were made from the left sural nerve or from dorsal rootlets. In the latter case, all other leg nerves except the sural nerve were cut. All units were activated by the use of a mechanical stimulator (O'Connell, Khalafalla & Lal, 1976) which was driven from either a Devices digitimer or a ramp generator. In thirty-two cases, units were successfully recorded from dorsal rootlets having the following characteristics.

The mean static threshold of the units was a displacement of 13 μm (S.E. $\pm$ 1·2). Further displacement resulted in increased activity of the

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units up to a value of 1545 μm ($\pm$ 38·2), after which no significant change in response occurred. We were unable to detect any recognizable sustained static phase, as reported by Iggo & Muir (1969) in the cat. However, following the 'ON' response, a 'random phase' was evident. This phase which coincides with the period of sustained stimulation was characterized by an irregular discharge of impulses, not clearly related to the degree of indentation. Computation of average frequencies of this phase did, however, show some correlation with stimulus values. The units responded to velocities from 0·21 μm/sec ($\pm$ 0·02) to 10 μm/sec ($\pm$ 0·03). The frequency of the 'ON' response did not change at velocities above the latter value. Conduction velocity measurements gave a mean of 52·6 m/sec ($\pm$ 2·2) as compared with 66 m/sec ($\pm$ 1·02) reported in the rabbit by Brown & Hayden (1971).

We have found that single nerve fibres innervating type I units do not necessarily supply adjacent domes. Indeed, the innervation of domes is organized into punctate receptive areas of mean size 4·8 cm^2. The receptive areas overlap one another in a complex manner over the entire area of the leg innervated by the sural nerve. The mean number of domes innervated by a single nerve fibre is 2·7 although up to six domes per fibre can be found.

Identical results were obtained from recordings made from single fibres in the sural nerve.

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Localization and response characteristics of neurones in the caudal trigeminal nucleus responding to cooling or warming the cat face

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The nose of the cat is known to be supplied with specific warm and cold receptors (Hensel & Kenshalo, 1969). There have been brief accounts of trigeminal nucleus cells which are excited by facial cooling, but none by warming (Fruhstorfer & Hensel, 1973; Molt & Poulos, 1976). We have made a systematic survey, using tungsten electrodes, of the marginal layer between the surface of the medulla and the magnocellular part of the spinal nucleus at levels from the obex to the C 1 rootlets. Eighteen cats anaesthetized with urethane were used.

In almost every penetration single or multi-unit spontaneous activity

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was observed which could be silenced by warming the face with infra-red radiation. Opaque shields of various sizes enabled the receptive fields to be localized. In total about 180 receptive fields have been mapped with sites in the cutaneous distribution of all three divisions of the trigeminal nerve. Fields falling within the mandibular nerve distribution were found in the medial part of the marginal layer, maxillary fields predominantly in a central position, and ophthalmic fields on the lateral edge. All the fields were strictly unilateral, even in mid line areas such as tongue or nose.

Quantitative observations were made by applying step temperature changes to the receptive fields, using thermodes. The medullary units showed response characteristics similar to those exhibited by peripheral cold receptors: a phasic outburst following a step decrease in temperature, sustained firing at steady levels of temperature and a phasic pause after a step increase in temperature. Half of the neurones excited by facial cooling could also be weakly excited by brushing or pressing the face; the remainder were specifically cold-sensitive. For these specific units the mean latency to electrical stimulation of the skin was 15·2 msec (S.D. 8·3, $n = 22$).

Eleven neurones have been found which were driven by warming the face at temperatures between 30° and 45° C. Eight of these were specifically temperature-sensitive. Receptive fields were on the nose, lip, cheek, eyelid and ear.

These results show that there is a dense thermal input to the dorsal part of the spinal trigeminal nucleus and also that there is some somatotopic organization. The projection from these neurones remains to be determined.

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Is there a specific octopamine receptor in the brain of *Helix*?

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Octopamine occurs in invertebrate nervous tissue: for example, in *Aplysia*, *Homarus* and *Periplaneta* (Axelrod & Saavedra 1977) and *Helix* where it has a potent action on certain neurones (Walker, Ramage & Woodruff, 1972). The present study extends the investigation on the action of octopamine on specific *Helix aspersa* neurones. Intracellular recordings were made from neurones in the isolated snail brain using

conventional techniques. The compounds used were either applied iontophoretically or via bath addition on to the neurone under study. To determine the potencies of analogues, a standard dose of dopamine or octopamine was given and the amplitude of the response noted. After washing with snail Ringer, doses of analogue were given until a response of the same magnitude as the standard was obtained.

Experiments were performed on four different neurone types. Cell type I was inhibited by dopamine and by (−)-noradrenaline, but not by (±)-octopamine. Fluphenazine (2×10^{-7} M) or metaclopramide (4×10^{-7} M) reversibly antagonized the actions of dopamine and noradrenaline on these cells, which are thought to contain inhibitory dopamine receptors. Cell type II was excited by noradrenaline and dopamine, while octopamine was ineffective. On cell type III, dopamine, noradrenaline and octopamine each caused inhibition of firing. Phentolamine (2×10^{-7} M) reversibly blocked the action of octopamine but had no effect on dopamine, while fluphenazine (2×10^{-7} M) reversibly antagonized the dopamine inhibitory response but had no effect on octopamine inhibition. Cell type IV was excited by octopamine and inhibited by noradrenaline and dopamine.

Structure activity studies were carried out on type IV cells. (±)-Synephrine also caused excitation of these neurones and was approximately equipotent with octopamine. (−)-Phenylephrine and (−)-norphenylephrine similarly excited type IV cells, phenylephrine being about ten times less active than octopamine in this respect. Phenylethylamine, tryamine, (±)-salbutamol and (±)-phenyl-propranolamine each caused inhibition of firing of type IV cells. The excitatory action of octopamine on type IV cells was antagonized by phentolamine (2×10^{-7} M), but was not affected by fluphenazine (2×10^{-7} M). The inhibitory action of dopamine and noradrenaline on type IV cells was, however, reversibly antagonized by fluphenazine (2×10^{-7} M).

These results are interpreted as providing evidence for a specific octopamine receptor mediating excitation of neurones. The structural requirements for activity at this receptor include a hydroxyl group on the β position of the side chain and a single hydroxyl group in either the 3 or 4 positions of the benzene ring.

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Patterns of discharge from atrial receptors in the dog

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It has been suggested that 'species difference' would explain the varying results reported from investigations into the behaviour of atrial receptors (e.g. Paintal, 1972; Rao, Fahim & Gupta, 1975). In a recent study of atrial receptors in the cat, it was shown that the three patterns of discharge, Type A, Type B and Intermediate were encountered in the ratios of 2:15:8 respectively; alteration from one type to another was relatively common (Kappagoda, Linden & Mary, 1976). The present investigation explored these relationships in dogs.

Action potentials from single units were recorded from a cervical vagus in anaesthetized dogs with the chest open. Following the identification of a discharge from atrial receptors and the classification of the units into Type A, B or Intermediate, an attempt was made to alter the pattern of discharge by (i) the infusion of Dextran, (ii) haemorrhage and (iii) the administration of adrenaline. Finally the site of each receptor was located by probing with a fine glass rod and fine destruction of endocardial tissue.

Thirty units, having atrial patterns of discharge, were obtained randomly in thirty dogs. Three of these units (two with Type B and one with Type A pattern) were located outside the atria. The remaining twenty-seven units were located in the endocardium and consisted of one Type A, sixteen Type B and ten Intermediate receptors. Conversion from one type to another was achieved in the one unit of Type A, eleven Type B and eight Intermediate receptors. Therefore the ratio of Type A to Type B to Intermediate type was 1:16:10 respectively and conversion of one pattern of discharge to another was common (twenty out of twenty-seven receptors).

In a second series of experiments, only receptors with Type A pattern of discharge were studied. Out of eight units investigated, four were endocardial and conversion was achieved in all of these and of the remaining four units located outside the atrial endocardium only two could be converted. Therefore all discharges from Type A atrial receptors (i.e. endocardial) could be altered; Type A receptors from which the discharge could not be altered were found outside the atrial endocardium.

These results are similar to those reported in the study of atrial receptors in the cat. Thus in both the dog and the cat atrial receptor discharge from Type A receptors is relatively uncommon and conversion from one type to another is common. It is unlikely therefore that differences in animal

species can be used as an explanation of various results as previously suggested.

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Diffusion within parabronchial air capillaries as a limiting factor for pulmonary gas exchange in birds

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The avian lung is composed of open-ended, parallel tubes, the parabronchi. From the parabronchial lumen fine air capillaries (diameter 3–10 μm) depart in radial direction; these have intimate gas exchange contact with equally small blood capillaries. Two regimes for respiratory gas movement may be discerned within the gas phase of this lung: (1) ventilation causes *convective gas flow* through the parabronchial lumen; (2) gas transport within the air capillaries to the blood–gas barrier is accomplished by *diffusion*. The intention of this study was to estimate to what degree the diffusional resistance offered by the narrow air capillaries limits over-all gas exchange in birds.

Several models for gas transfer between air capillary gas and blood have been analysed which differ in the relative arrangement of air capillaries and blood capillaries. Results from histological investigations seem to support a model in which blood capillaries contact air capillaries along their entire length, blood flow being directed from the terminal end of the air capillary towards the parabronchial lumen. For a given set of parameters, the partial pressure profiles in air capillary gas and in blood in this countercurrent-like model are fundamentally different from those in a cocurrent-like arrangement, in which blood flow is reversed. In particular, the total drop in gas concentration over the air capillary length, resembling the effects of stratified inhomogeneities within mammalian lungs, is larger in the countercurrent-like arrangement. However, the limitation imposed on gas exchange is identical in both, which indicates that the concentration gradient in the air capillaries, which has been used earlier by Zeuthen (1942) and Hazelhoff (1951) to estimate the role of air capillary diffusion in limiting gas exchange, is only a poor measure for this effect.

Using morphometrical and physiological values for the hen and the duck it can be estimated that about 1–2% of the total resistance to O_2

and CO_2 transfer in the parabronchi is attributable to diffusion within the air capillary gas. On exercise, for example during flight, this limitation may increase to above 10% for both gases.

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Volume of gas exchanging airways in the duck

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According to anatomical estimates (Duncker, 1973) the volume of gas exchanging airways in birds, comprising the parabronchi and the air capillaries, is much smaller than the alveolar space in mammals. Since measurements of flow in the dorsomedial secondary bronchi have shown that at the end of expiration pulmonary air flow may drop to very low values (Scheid & Piiper, 1971) it is conceivable that P_{O_2} in parabronchi falls off at end-expiration thus compromising oxygenation of arterial blood. The question was put to test in experiments on ducks in which the efficacy of pulmonary O_2 transfer in terms of effective pulmonary O_2 diffusing capacity (O_2 transfer factor) was comparatively measured under conditions of steady and oscillatory ventilatory flow.

In anaesthetized domestic ducks the primary bronchi were blocked between the origin of the ventromedial and dorsomedial secondary bronchi by appropriately placed balloon catheters. The lungs were ventilated by a gas stream containing 4% CO_2 and 8–9% O_2, entering via the cannulated posterior thoracic air sacs and leaving through the tracheal cannula. P_{O_2} was measured in inflowing and outflowing gas (by mass spectrometry) and in arterial and mixed venous blood samples (by O_2 electrodes). From these values and from the O_2 uptake the effective pulmonary O_2 diffusing capacity, D, was calculated as an index for the efficacy of O_2 transfer.

When the ventilation was directed intermittently to the left or right lung, thus producing in each lung, flow for 10 sec, and no flow for 10 sec, the D value was on the average 74% of the value measured at constant flow regime. With ventilatory flow oscillating sinusoidally, simultaneously in both lungs, from zero to twice mean flow at a frequency of 10/min, the effective D was only slightly decreased.

On the basis of a model for intermittent flow, the functional volume of exchanging airways was estimated at 90 ml. (in ducks of 1·8 kg body weight). This value is about twice the anatomical estimate. Possibly the

higher effective volume is in part brought about by convective gas mixing between parabronchi and secondary bronchi as resulting from the mechanical action of the heart beat. Due to the relatively high functional volume of gas exchanging airways, the effects of physiological variations in pulmonary gas flow rate upon gas exchange efficacy at normal resting breathing rates of ducks (higher than 10/min) are expected to be small or even negligible.

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Causes and possible consequences of transcapillary diffusion potentials

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Diffusion potentials are customarily taken into consideration in relation to the cell membrane but not to capillary walls. Hilton, Hudlická & Jackson (1974) reported the 'surprising' finding that outward transcapillary diffusion of tracer amounts of radioactive phosphate ($^{32}P_i$) was decreased during muscular contractions and was increased by outward diffusion of an infused phosphate buffer. Theory predicts that the addition of a pair of oppositely charged ions with different mobilities (e.g. Na^+ and $H_2PO_4^-$) to one side of the capillary wall would cause a diffusion potential to be established across the endothelium which would disturb the electrochemical equilibrium of other ions; and this could explain the apparently anomalous effect.

Experiments have been performed to examine this prediction. Each of ten cellophane bags (pore size 24×10^{-10} m) containing 0·5 ml. of a solution (S_1) and 0·1 μCi of $NaH_2{}^{32}PO_4$ was immersed in 17 ml. of a solution (S_2) in a β-counting vial so that $^{32}P_i$ diffused out of the bags. The vials and their contents were shaken at 34° C for a set time and then each vial was opened. Its bag (with as little adherent S_2 as possible) was transferred to a second β-counting vial containing 17 ml. of water. Both vials were counted to estimate $^{32}P_i$ content outside and inside respectively. Results were expressed as the mean ratio of the final $^{32}P_i$ concentration outside the bag to that inside.

Where S_1 and S_2 were both 20 mM-NaCl, the mean value of the ratio after 50 min diffusion was $0{\cdot}04 \pm 0{\cdot}008$ (S.D.). When S_1 was sodium lactate, polyphosphate, dihydrogen phosphate and hydrogen sulphate (each containing 20 mM-Na^+) and S_2 was 20 mM-NaCl, the respective values of the ratio were $0{\cdot}065 \pm 0{\cdot}011$, $0{\cdot}086 \pm 0{\cdot}021$, $0{\cdot}105 \pm 0{\cdot}017$ and

$0{\cdot}132 \pm 0{\cdot}022$ respectively. The differences from 0·04 are significant at the 0·1% level.

The reduced, and negative, extractions of continuously infused $^{32}P_i$ during muscular contractions could therefore be explained by a diffusion potential produced by a change in composition of the interstitial fluid. It would follow that the effects of diffusion potentials may have to be taken into consideration when interpreting the significance of changes in the rates of efflux of charged molecules from contracting muscles.

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Changes in blood chemistry of the electric ray (*Torpedo marmorata*) during hypoxia

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Torpedo can survive prolonged exposure to water containing oxygen tensions of less than 10 mmHg. In addition to modifications of the ventilatory and cardiac rhythms (Hughes, 1973), significant changes in the chemical composition of the blood indicate adaptations at a biochemical level.

Fish cannulated under anaesthesia (MS 222) survive very well and remain quiescent under hypoxia. Average arterial P_{O_2} was 5 mmHg. Blood pH falls progressively (Fig. 1) and is associated with elevated levels of lactate and pyruvate; lactate/pyruvate ratio also increases. In addition, an increase in succinate (Fig. 1) strongly suggests the accumulation of multiple anaerobic end products within the tissues. This provides evidence for alternative pathways to the normal lactate pathway of glycolysis as has been found in various diving vertebrates (Hochachka, Owen, Allen & Whittow, 1975). Recovery from hypoxia is extremely rapid once ventilation has restarted; recovery of the succinate to pre-hypoxic levels is almost immediate but the fall in lactate concentration is much slower.

Some of these adaptations are probably related to the occurrence of *Torpedo* in pools of low environmental P_{O_2} at low tide.

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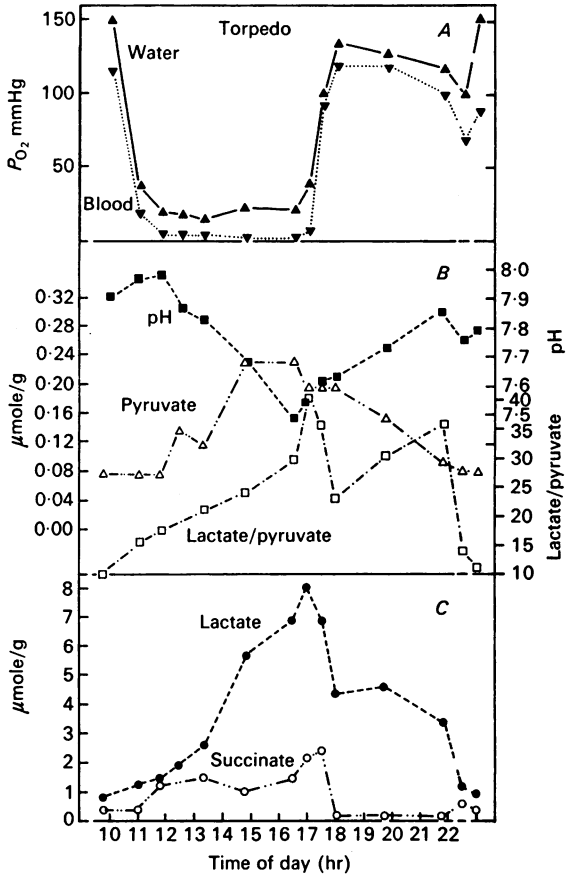

Fig. 1. *Torpedo marmorata*. Plot showing changes in blood parameters when the inspired P_{O_2} was reduced from 150 to 20 mmHg. Changes in (*A*) oxygen tension of the post-branchial blood (▼ · · · · ▼), (*B*) blood pH (■--■), pyruvate concentration (△ -·-· △), lactate/pyruvate ratio (□ -·- □), and (*C*) lactate (● - - ●) and succinate (○ -·-· ○) concentrations are shown. Hypoxia lasted from about 10.30 a.m. to 5.00 p.m. The last two points show the blood values on the two subsequent days.

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The amino acid patterns in the blood at the onset of haemorrhagic shock in the rat

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Rats under pentobarbitone sodium anaesthesia were prepared (Daniel, Pratt & Spargo, 1976, 1977) so that arterial blood and blood leaving the thigh muscles could be taken, before and after 25% of the animal's blood volume had been withdrawn over 5 min. Samples of muscle blood took 5 min to collect. One min after the 'haemorrhage' the second muscle

TABLE 1. Effect of haemorrhage on the concentration of the free amino acids in the arterial blood plasma, and the differences between their concentration in arterial blood and in venous blood draining the thigh muscles. Mean values (± S.E. of mean) from determinations in nine separate animals.

	Arterial blood concentration (μM)		Venous–arterial difference (V–A) (μM)	
Amino acid	Before	After	Before	After
Aspartate	10 ± 1·0	32 ± 4·9	+2·6 ± 1·3	−13·8 ± 4·9
Threonine	303 ± 23	397 ± 21	−4·9 ± 30	−39 ± 14
Serine	272 ± 18	342 ± 25	−7·3 ± 12·7	−67 ± 15
Glutamate	78 ± 6·4	196 ± 27	+12 ± 4·6	−77 ± 18
Glutamine	943 ± 39	1295 ± 40	+87 ± 41	−5·7 ± 36
Glycine	356 ± 45	479 ± 65	+40 ± 10	−44 ± 26
Alanine	607 ± 58	944 ± 41	+70 ± 35	−159 ± 32
Cysteine*	94 ± 17	159 ± 28	−12 ± 5·3	−42 ± 14
Valine	172 ± 5·5	291 ± 7·4	−7·0 ± 7·0	−63 ± 10
Methionine	40 ± 2·1	59 ± 3·4	+0·7 ± 1·4	−12 ± 2·3
Isoleucine	88 ± 5·7	160 ± 11	−8·0 ± 4·8	−58 ± 7·4
Leucine	134 ± 6·8	267 ± 15	−6 ± 5·8	−87 ± 16·1
Tyrosine	51 ± 4·8	105 ± 7·0	+5 ± 2·4	−48 ± 18
Phenylalanine	51 ± 2·9	108 ± 5·3	+0·8 ± 3·1	−32 ± 6·2
Histidine	86 ± 11	113 ± 8·1	+17 ± 14	−9 ± 7·0
3-Methylhistidine	11 ± 1·6	10 ± 0·5	+0·9 ± 3·1	+3·1 ± 2·9
Tryptophan	19 ± 4·0	24 ± 5·0	+16 ± 5·0	+1·1 ± 2·6
Ornithine	56 ± 4·9	73 ± 3·0	+4·4 ± 5·6	−13 ± 6·6
Lysine	364 ± 39	499 ± 47	+19 ± 17	−40 ± 14
Arginine*	140 ± 25	147 ± 18	−10 ± 29	+58 ± 26
Total	3875 ± 191	5749 ± 172	+220 ± 142	−748 ± 159

* Fewer than nine determinations.

blood sample was taken, thus the final arterial sample was 11 min after onset of the 'haemorrhage'. The effect of the 'haemorrhage' on the concentration of free amino acids in the blood and the difference between their concentrations in arterial blood and in muscle blood are shown in the Table (venous – arterial differences, V – A, are concentration in venous muscle blood minus concentration in arterial blood). Positive V – A differences denot output of amino acids from muscles: negative differences denote uptake by muscles.

The finding that amino acids which are released into the circulation as a result of rapid loss of blood are taken up by the muscles (at least temporarily) suggests that skeletal muscle acts as a regulating device to maintain the blood levels of amino acids within normal limits.

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Active and inactive renin in rabbit plasma during and after haemorrhage

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Active and inactive forms of renin have been isolated from the kidneys of several species including the rabbit (Leckie & McConnell, 1975). Renin-like enzymes have also been isolated from extra-renal sources. In this study the presence of an acid-activatable form of renin in rabbit plasma is demonstrated and the response of the circulating levels of this form of renin to haemorrhage investigated. Haemorrhage is well known to increase plasma levels of active forms of renin.

Experiments were performed on urethane anaesthetized New Zealand White rabbits of both sexes. They were subjected to either a mild (5–10% of blood volume) or severe (20–25% of blood volume) haemorrhage. There were ten animals in each group. Plasma samples, taken over a period of 2 hr during and after haemorrhage, were divided and an aliquot was dialysed to pH 3·3 over 24 hr and then dialysed back to pH 7·4. This procedure appears to activate an inactive form of renin. The remaining portion of the plasma sample was dialysed at pH 7·4 for a similar time. Plasma renin activity (PRA) was measured after dialysis by radio-immunoassay of angiotensin I generated during incubation with excess exogenous porcine renin substrate. When partially purified renal renin, which had undergone an acidification step during extraction, was added

to rabbit plasma the recovery after dialysis was 91·3% for acidified (pH 3·3) samples and 91·4% for those maintained at pH 7·4.

As predicted, samples maintained at pH 7·4 showed a progressive increase in PRA after haemorrhage to mean levels of 88% (mild) and 139% (severe) above control values. In all initial samples PRA was 15–20% higher in acidified plasma suggesting the presence of an acid-activatable form of renin. The amount of renin in this form increased after haemorrhage of either intensity but remained a constant proportion of the active form. In a further group of ten rabbits in which the renal blood vessels were ligated 30 min prior to severe haemorrhage, the PRA (pH 7·4 samples) decreased progressively, even after haemorrhage. No significant acid-activatable renin could be detected in these animals 45 min after ligation of the renal vessels. In two rabbits bilaterally nephrectomized 24 hr before haemorrhage, PRA was only 5% of normal values and no increase was observed after haemorrhage. Again no acid-activatable renin could be detected. We therefore conclude that the acid-activatable form of renin is secreted by the kidneys rather than some extra-renal source.

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The effect of α-flupenthixol on the response of carotid chemoreceptors to acetylcholine, sodium cyanide and dopamine in the cat

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Dopamine (DA) may modulate sensory activity of cat carotid chemoreceptors (Zapata, 1975; Osborne & Butler, 1975). If the theory advanced by Osborne and Butler is correct and sensory activity is indeed kept suppressed by the continuous release of DA, then block of the DA receptor should substantially increase spontaneous chemoreceptor activity and also, according to the theory, markedly reduce the response to ACh. We used α-flupenthixol, a potent inhibitor of DA in the C.N.S. (Iversen, 1975) to block the inhibitory effect of DA.

Experiments were performed on eight pentobarbitone-anaesthetized cats in which ganglio-glomerular nerves were cut, the animals were artificially ventilated and gallamine (3 mg/kg) administered. Chemoreceptor activity was recorded from the peripheral end of a sectioned sinus nerve and stimulants injected into the ipsilateral carotid artery. The results showed that α-flupenthixol (0·2 mg/kg I.A.) abolished the inhibitory effect of DA while the response to NaCN was augmented and that to ACh slightly reduced. (Fig. 1).

Providing that exogenous DA is acting at the same site(s) as endogenous DA, the results suggest that while there may be some tonic inhibition of sensory activity by DA, this is not substantial. It is also unlikely that ACh or NaCN act to any appreciable extent by inhibiting DA release.

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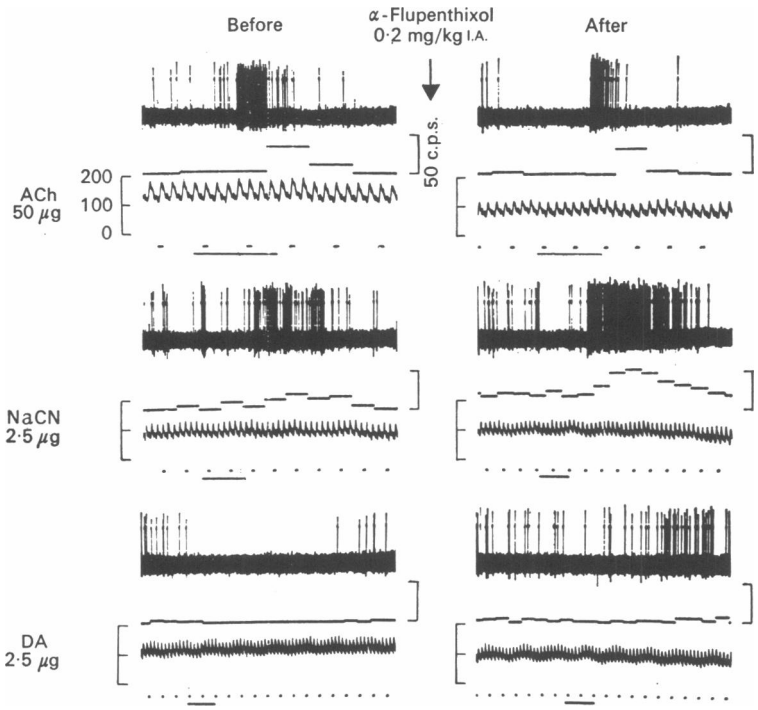

Fig. 1. Response of a chemoreceptor unit before (mean spontaneous discharge $2{\cdot}8 \pm 0{\cdot}1$ c.p.s.) and after ($2{\cdot}7 \pm 0{\cdot}1$ c.p.s.) α-flupenthixol. Panels show: action potentials; counter output; B.P.; 1 sec and injection markers.

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Personality, performance and physiological cost during vibration

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Attempts to maintain performance at a motor task during whole body vibration may be associated with the subject's belief that any extra effort will be successful. The locus of control scale (Rotter, 1966) measures a person's general belief about his ability to influence events. 'Internal' subjects, who believe that their own efforts are influential, are more likely to perform well in a skilled task (Phares, 1976). In this study the prediction was made that Internal subjects would show less performance decrement during vibration but at greater physiological cost.

Twelve male subjects each completed fourteen experimental sessions of 30 min. During the second 10 min period of twelve sessions seated subjects were exposed to whole body vibration in the G_z (longitudinal) axis. A 6 Hz sine wave was compared with a random wave form covering 0–6 Hz with 125 msec 'shocks' superimposed at regular intervals. Three acceleration levels were compared: 0·21, 0·28 and 0·35 g r.m.s. These levels are typical of cross-country vehicle movements. Mean heart rate and oxygen uptake levels were measured before, during and after vibration. Throughout the 30 min period a tracking task was performed; pseudo-random fluctuations were fed to a meter-needle and subjects had to centralize the needle by movements of a foot-pedal.

Internal subjects showed significantly smaller performance decrements under all levels of vibration ($P < 0{\cdot}002$) and the difference from External subjects was more marked during higher acceleration levels and random vibration. Heart rate results showed increases at vibration onset for all subjects and higher levels over-all for Internal subjects. During vibration the difference in heart rate level between Internal ($\bar{x} = 89$ beats/min) and External ($\bar{x} = 79$ beats/min) subjects was significant ($P < 0{\cdot}05$) and once again more marked during random and high acceleration vibration. Oxygen uptake showed small increases at vibration onset but no differences were related to locus of control (oxygen uptake before vibration $\bar{x} = 0{\cdot}31$; during vibration 0·42 L (S.T.P.D.)/min).

These results suggest that cognitive factors in personality are involved in attempts to maintain motor performance during vibration stress and that the level of performance achieved may be associated with the physiological cost incurred.

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Human response to whole-body vibration of different wave forms

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Most studies of the human physiological response to vibration have used sine-wave inputs (see review by Gierke & Clarke, 1971). However, vibration conditions encountered outside the laboratory are usually of random wave form. This difference is of importance when using laboratory studies to establish exposure limits for vibration (International Organisation for Standardisation, 1974). Therefore, the human response to sine- and random-wave inputs of the same acceleration levels was investigated in twelve young male subjects seated upright on a hydraulic vibrator.

Subjects were exposed randomly to seven 30 min experimental runs. During minutes 0–10 and 20–30 they sat at rest. During minutes 10–20, G_z (longitudinal) vibration was imposed at 0·21, 0·28 or 0·35 g r.m.s., using either a 6 Hz sine wave or a random wave. Subjects also completed a control (no vibration) run.

Oxygen uptake, pulmonary ventilation, tidal volume and respiratory and heart rates were measured during minutes 12–15 and 17–19½.

TABLE 1. Effects of sine- and random-wave vibration at different acceleration levels on oxygen uptake and heart rate in man (means ∓ 1 S.E.M. of 24 values)

Acceleration level (g r.m.s.)	Oxygen uptake (L (S.T.P.D)/min)		Heart rate (beats/min)	
	Sine wave	Random wave	Sine wave	Random wave
0.21	0·35 ∓ 0·02	0·34 ∓ 0·02	81 ∓ 2	82 ∓ 2
0·28	0·37 ∓ 0·02	0·37 ∓ 0·03	85 ∓ 3	86 ∓ 2
0·35	0·43 ∓ 0·02	0·45 ∓ 0·03	84 ∓ 3	88 ∓ 2
Control	0·32 ∓ 0·02		80 ∓ 3	

In none of the recorded variables was there a statistically significant difference between values obtained during sine- and random-wave vibration at any acceleration level.

These findings indicate that studies using only sine-wave inputs may be valuable in establishing exposure limits for human vibration of different wave forms.

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The effect of noradrenaline and angiotensin upon intestinal fluid transport *in vivo* in the rat

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Recent evidence has shown that noradrenaline stimulates electroneutral intestinal absorption *in vitro* (Brasitus, Field & Kimberg, 1977). Furthermore the hormone angiotensin stimulates electroneutral fluid absorption *in vivo* in the absence of changes in blood flow (Bolton, Munday, Parsons & York, 1975), and also releases noradrenaline both from sympathetic nerve endings and from the adrenal medulla (Peach, 1974). The following experiments were carried out to compare the effects of angiotensin and noradrenaline on intestinal fluid transport *in vivo*.

Closed sacs of rat jejunum filled with Krebs' bicarbonate saline containing either [^{3}H]inulin or [^{14}C]polyethylene glycol, to act as a non-absorbable marker, were prepared as described by Bolton *et al.* (1975). A control period of isotonic saline infusion at 1 ml. hr^{-1} (via the femoral vein) was followed by an experimental period during which saline with or without angiotensin or noradrenaline was infused at the same rate. Transmural potential difference was measured using agar/KCl electrodes attached to calomel half-cells, and blood pressure was recorded via a femoral artery cannula. All results were analysed using a paired t test.

Subpressor infusions of noradrenaline had no effect upon net fluid movement in contrast to the stimulation of fluid absorption obtained by infusing subpressor doses of angiotensin. Increasing noradrenaline infusion to 0·7 n-mole kg^{-1} min^{-1} significantly increased blood pressure and significantly reduced net transport from $1{\cdot}1 + 0{\cdot}14$ to $0{\cdot}30 \pm 0{\cdot}22$ ml. g wet wt.$^{-1}$ hr^{-1} (8). Similarly pressor doses of angiotensin also inhibited net fluid absorption. However, further increasing noradrenaline infusions to 7 n-mole kg^{-1} min^{-1} reversed the effects upon fluid movement, causing a significant stimulation from $1{\cdot}16 \pm 0{\cdot}14$ to $1{\cdot}76 \pm 0{\cdot}16$ ml. g wet wt^{-1} hr^{-1}

(8). Transmural potential difference was not affected by any dose of angiotensin whilst with noradrenaline it fell slightly but significantly from $-3{\cdot}6 \pm 0{\cdot}32$ mV to $-3{\cdot}3 \pm 0{\cdot}32$ mV with 0·7 n-mole kg^{-1} min^{-1} and to $-2{\cdot}8 \pm 0{\cdot}24$ mV at 7 n-mole kg^{-1} min^{-1}.

The results suggest that noradrenaline like angiotensin is capable of affecting intestinal fluid absorption *in vivo*.

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Site of action of acetylcholine in regulating intestinal epithelial ion transport in the rat

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Acetylcholine is known to alter ion transport in both the jejunum and colon of the rat, an action which appears to be independent of the muscle layers (Hardcastle & Eggenton, 1973; Browning, Hardcastle, Hardcastle & Sanford, 1976). Both these tissues are complex structures and the aim of this investigation was to localize the site of action of acetylcholine.

It is possible to selectively damage the intestinal villi by exposure to intraluminal hypertonic Na_2SO_4 (Roggin, Banwell, Yardley & Hendrix, 1972) and to damage the crypts using cycloheximide (Kimberg, Field, Gershon, Schooley & Henderson, 1973). The effect of these treatments on the increased potential difference *in vivo* observed in response to intravenous administration of 3 and 7 μg doses of acetylcholine was measured. in pentobarbitone-anaesthetised rats. Under control conditions 3 μg acetylcholine increased the jejunal potential difference by $2{\cdot}3 \pm 0{\cdot}3$ mV (24) and the colonic potential difference by $1{\cdot}8 \pm 0{\cdot}3$ mV (24). Seven μg acetylcholine caused changes of $3{\cdot}5 \pm 0{\cdot}3$ mV (24) and $3{\cdot}6 \pm 0{\cdot}5$ mV (24) in the jejunum and colon respectively. The transfer potential caused by 28 mM glucose was used as a measure of the functional integrity of the jejunal villi as absorption is confined to this area (Kinter & Wilson, 1965).

The lumen of the gut was exposed for 30 min to 2 M-Na_2SO_4 which was then replaced with 154 mM-NaCl. The glucose transfer potential was significantly ($P < 0{\cdot}001$) reduced from $3{\cdot}9 \pm 0{\cdot}4$ mV (8) to $1{\cdot}3 \pm 0{\cdot}3$ mV (8). Damage to the villi of the jejunum and the surface epithelium of the colon was confirmed histologically. However the response to acetylcholine was unimpaired ($P > 0{\cdot}1$).

Two hours after the administration of cycloheximide (12 mg/kg intravenously) both the jejunum and colon had a significantly ($P < 0.05$) reduced response to acetylcholine, but the glucose transfer potential in the jejunum was unchanged ($P > 0.1$). Sections of the tissues showed that extensive damage had occurred in the crypts but the villi of the jejunum and the surface epithelium of the colon appeared normal.

These changes following Na_2SO_4 and cycloheximide treatment could not be entirely attributed to alterations in tissue resistance, nor were they due to the duration of treatment.

Thus in the regulation of intestinal ion transport acetylcholine appears to act at the level of the crypts of both the jejunum and the colon.

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Inhibition of rat gastric acid secretion *in vivo* and *in vitro* by arachidonic acid and its reversal by indomethacin

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Prostaglandins (PG) of the E series are potent inhibitors of gastric acid secretion and cause mucosal vasodilation. Little is known of the effects of other components of the prostaglandin system, formed from precursor fatty acids by cyclo-oxygenase, on gastric muscosal function. We have studied the effects of arachidonic acid (AA), the precursor of the 2-series prostaglandins, and indomethacin, an inhibitor of PG synthesis, on rat gastric secretion *in vivo* and *in vitro*.

Acid secretion and gastric mucosal blood flow (MBF [^{14}C]aniline clearance) were recorded in the lumen-perfused stomach preparation of the urethane-anaesthetized rat (Main & Whittle, 1973). During the submaximal secretory response to pentagastrin (0·33 μg/kg. min I.V.), arachidonic acid (80 μg/kg. min as the sodium salt) infused intravenously for 80 min caused a significant inhibition of acid secretion and a rise in the ratio of MBF to acid secretion.

Indomethacin (20 mg/kg followed by 4 mg/kg. hr I.V. throughout the

experiment), in doses which had no significant effect on pentagastrin-stimulated secretion, partially reversed the inhibitory effect of AA. Secretion induced by dibutyryl cyclic adenosine 3,5-monophosphate (dbcAMP, 30 mg/kg I.V.) was not affected by AA.

In vitro studies were carried out on the isolated mucosa (Hearn & Main, 1975). Acid output into the mucosal solution was measured continuously by titration to pH 7 using the pH-stat method. Responses to secretogogues were obtained before and 60 min after adding drugs to the serosal solution. Arachidonic acid ($1{\cdot}6$–$3{\cdot}2 \times 10^{-5}$ M) reduced or abolished responses to histamine ($0{\cdot}6$–$2{\cdot}6 \times 10^{-5}$ M) but, like PGE_2 (Hearn & Main, 1975), had no effect on secretion induced by dbcAMP (10^{-4} M). The inhibitory effect of AA was partially or wholly blocked by indomethacin ($0{\cdot}3$–3×10^{-5} M) in concentrations which had no effect on histamine-induced secretion. Linoleic acid ($3{\cdot}2$–$6{\cdot}4 \times 10^{-5}$ M) had no significant effect on responses to histamine.

The inhibitory effect of AA on pentagastrin-stimulated acid secretion in the anaesthetized rat is similar to that observed on histamine-stimulated secretion in the conscious dog (Bieck, Oates & Adkins, 1971). Our results suggest that this effect is mediated, at least in part, by prostaglandins or other products of cyclo-oxygenase, formed either in the gastric mucosa or elsewhere. The inability of high doses of indomethacin to block completely the effect of AA may indicate that unchanged AA or metabolites formed by other enzymic pathways are also involved.

The results *in vitro* differ from those reported by Ramwell & Crane (1976) using the isolated frog mucosa in which AA potentiated histamine-stimulated secretion, an effect which was not blocked by aspirin in concentrations sufficient to block prostaglandin synthesis. The present results using indomethacin indicate that the actions of AA on the rat mucosa *in vitro* are mediated by products of cyclo-oxygenase, the net effect of which is inhibitory. The nature of these active substances has yet to be established.

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