Teaching and research apparatus made in the departmental workshop

By D. H. Brown, J. Harris, P. E. Jensen, A. Mordecai and B. J. Pitman. *Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL*

The perfusion of isolated uterine or ovarian vessels

By D. A. Sani. *Department of Physiology, University College, P.O. Box 78, Cardiff CF1 1XL*

COMMUNICATIONS

Unilateral lesions of the auditory cortex and the 'precedence effect'

By J. Cranford, I. T. Diamond, R. Ravizza and L. C. Whitfield. *Department of Psychology, Duke University, Durham, North Carolina, U.S.A. and Neurocommunications Research Unit, University of Birmingham, Birmingham 15, England*

Eight cats were trained in a Y-maze to locate trains of 1 kHz tone pips (23 msec duration at 5 per second). They were then further trained to choose the left hand when the left signal alone was on, but to choose the right when both left and right pulse trains were presented simultaneously but not coincidentally. (Two configurations were used: either the two pulse generators were unsynchronized or they were locked together so that the left preceded the right by 30 msec.) This subsequent training did not impair the performance of the animals either on the original left/right discrimination, or on transfer tests to 'virtual' signals (Wallach, Newman & Rosenzweig, 1949) which in one signal preceded the other by 5 msec.

In four of the cats an attempt was then made to remove the whole right auditory cortex from the middle suprasylvian sulcus to the rhinal fissure, while in the other four the left cortex was similarly removed. After recovery the cats with right lesions retained the L *vs.* L and R task, while the left lesion cats either failed completely to relearn it in sixty sessions or did so only with great difficulty.

As was to be expected (Neff, 1961) both sets of animals retained the simple L *vs.* R discrimination. However, this was not the case for the virtual left/virtual right signals. The left lesion animals continued to perform correctly, but whereas the right lesion animals performed correctly on virtual right they also went preferentially to the right on a virtual left signal.

A clue to this apparent asymmetry is provided by experiments (not yet complete) in reversal training, i.e. the cats are retrained to go right to a single signal on the R and left to a R and L signal. We find that the right lesion cats still respond correctly to a virtual right and the left lesion cats correctly to a virtual left. However, both groups now tend to go left when the remaining combinations arise, though the effect of retraining seems less strong than that of the original training. The results can be explained if we assume that a virtual left or virtual right signal is correctly interpreted when the cortex contralateral to the earlier component of the signal is intact. However, if this cortex is damaged the signal is ambiguous and the animal tends to the side which it has been trained to choose in the presence of a multiple source.

The results further suggest that while the loss of one cortex has no effect on the ability of cats to localize a single source it has a serious effect on their ability to deal with more complex situations.

This research was supported by The Science Research Council, the Wellcome Trust, and NIMH Grant 4849.

REFERENCES

NEFF, W. D. (1961). Neural mechanisms of auditory discrimination. In *Sensory Communication*, ed. ROSENBLITH, W. A. New York: M.I.T. Press and Wiley.

WALLACH, H., NEWMAN, E. B. & ROSENZWEIG, M. R. (1949). *Am. J. Psychol.* **62**, 315.

A comparison of stretch and vibration reflexes at the motoneurone

BY D. R. WESTBURY. *Department of Physiology, Medical School, University of Birmingham*

Until recently it has been generally thought that the afferent limb of the stretch reflex depended largely upon the primary endings of the muscle spindles. High frequency longitudinal vibration applied to the tendon of a muscle selectively excites these endings and causes a reflex contraction of the muscle in the decerebrate cat (Matthews, 1966). As vibration causes 'driving' of the primary endings at a fixed rate, occlusion can be expected between the responses to stretch and to vibration when both are excited together. Matthews (1969), recording the reflex contraction of the soleus muscle of the decerebrate cat, showed a lack of occlusion between these reflexes, and explained this result by suggesting that the secondary endings of the muscle spindles play a part in the stretch reflex.

In the experiments to be described, intracellular micro-electrodes were used to record responses from α motoneurones innervating triceps surae muscles of cats anaesthetized with pentobarbitone sodium (Nembutal). The muscles were stretched and vibrated by a servo-controlled electro-

magnetic stretcher. Other muscles in the limb were denervated. The animals were rigidly fixed.

An increase in muscle length was accompanied by an excitatory post-synaptic potential (EPSP) in motoneurones which persisted for so long as that length change was maintained. The greater the extension, the greater was the EPSP. The EPSPs showed small random fluctuations in amplitude, described as 'synaptic noise' by Granit, Kellerth & Williams (1964), representing random inputs to the motoneurone from many spindle afferents.

High-frequency vibration of the muscle group at frequencies of 30–350 Hz was associated with a maintained EPSP which fluctuated in amplitude at the frequency of vibration, due to the synchronous 'driving' of muscle spindle primary afferents caused by the vibration (Brown, Engberg & Matthews, 1967). The size of the EPSP increased as the frequency of vibration was increased, and at the same time the EPSPs became smoother. At the frequencies used, vibrations of more than 80 μ peak-to-peak caused a maximal response at the motoneurones. These amplitudes were always employed.

When a period of high-frequency vibration was superimposed upon a stretch, the responses to each appeared to sum together approximately; little or no occlusion was found between them.

This result substantiates the conclusion of Matthews (1969) that vibration and stretch reflexes do not depend entirely on the same afferents.

A grant from the Medical Research Council helped to defray the expenses of this work.

REFERENCES

Brown, M. C., Engberg, I. & Matthews, P. B. C. (1967). *J. Physiol.* **192**, 773–800.
Granit, R., Kellerth, J-O. & Williams, T. D. (1964). *J. Physiol.* **174**, 435–452.
Matthews, P. B. C. (1966). *J. Physiol.* **184**, 450–472.
Matthews, P. B. C. (1969). *J. Physiol.* **204**, 365–393.

Interneurone responses and primary afferent depolarization evoked from the medial lemniscus in the rat cuneate nucleus

By N. Davidson and Carol A. Smith. *Department of Physiology, University of Aberdeen, Aberdeen AB9 1AS*

Pyramidally evoked cortical potentials in the rat

By T. W. Stone. *Department of Physiology, University of Aberdeen, Aberdeen AB9 1AS*

Observations on the functional internal anal sphincter of the vervet monkey

By V. Rayner. *Makerere University, Kampala, Uganda*

The information which can be obtained from *in vivo* studies of the internal anal sphincter in man is naturally limited (Shepherd & Wright, 1968). We have therefore examined the innervation in the vervet monkey. The distribution of adrenergic nerves was examined by the Falck fluorescence technique, the effect of sympathomimetic and parasympathomimetic drugs on isolated strips, and finally the response of these strips to field stimulation were examined. The internal anal sphincter is not anatomically identifiable so that four serial strips 2–3 mm wide of circular smooth muscle were cut from the terminal rectum of monkeys anaesthetized with phencyclidine hydrochloride. The strips were mounted in Krebs bicarbonate saline at 37° C.

The response of the four strips to the sympathomimetic amines, noradrenaline, adrenaline, phenylephrine and isopropylnoradrenaline in concentrations of 10^{-7} and 10^{-6} g/ml. varied. The lowermost two strips were contracted by noradrenaline, adrenaline and phenylephrine, the third strip had a biphasic response to noradrenaline but were contracted by phenylephrine, while the fourth uppermost strip was relaxed by both noradrenaline and phenylephrine. Isopropylnoradrenaline relaxed all strips. These effects of noradrenaline and phenylephrine were blocked by the α blocking agents phentolamine (10^{-6} g/ml.) and phenoxybenzamine (10^{-6} g/ml.) and unaffected by the β blocking propranolol. Tetrodotoxin had no effect on these responses.

The lowermost two strips, contracting to noradrenaline, suggested a functional sphincteric region under adrenergic control and this was supported by the finding of a region of high adrenergic nerve density among the muscle cells strictly limited to this area.

The effect of nicotinic drug stimulation was studied using high concentrations of acetylcholine (5×10^{-6} to 10^{-4} g/ml.) in the presence of hyoscine (10^{-7} to 10^{-6} g/ml.). In all strips acetylcholine caused an inhibition blocked by the β blocker propranolol (2×10^{-6} g/ml.), unaffected by phenoxybenzamine and blocked by adrenergic neurone-blocking drugs (10^{-5} g/ml. bretylium, 10^{-5} g/ml. bethanidine and 5×10^{-6} g/ml. guanethidine). The inhibition was also blocked by tetrodotoxin (10^{-7} g/ml.) and hexamethonium (10^{-4} g/ml.). These results suggest an action on inhibitory adrenergic ganglion cells or inhibitory adrenergic nerve endings.

Electrical stimulation (1–20 Hz 0·5 msec 20 V) (Burnstock, Campbell, & Rand, 1966) caused inhibition of all strips followed by a rebound con-

traction in the two upper strips. This effect was blocked by tetrodotoxin but was unaffected by either blocking agents, adrenergic neurone-blocking drugs or hexamethonium.

These results are difficult to interpret. They suggest adrenergic nerves having excitatory effects, confined to the smooth muscle of an area corresponding to the anal sphincter. Adrenergic nerves activated by nicotinic stimulants have more generalized inhibitory effects also present in the sphincteric area, and finally there was a non-adrenergic non-cholinergic inhibitory innervation seen on field stimulation.

This work was supported by a grant from Makerere University Publications and Research Grants Committee.

REFERENCES

BURNSTOCK, G., CAMPBELL, G. & RAND, M. J. (1966). *J. Physiol.* **182**, 504–526.
SHEPHERD, J. J. & WRIGHT, P. G. (1968). *Am. J. dig. Dis.* **13**, 421–427.

Muscle spindle response to active muscle shortening in *Bufo marinus*

BY K. S. K. MURTHY and A. TAYLOR. *Physiology Department, King's College, London WC2R 2LS*

The quantitative understanding of the control of voluntary muscle in mammals is made difficult by the complexities of fusimotor drive. However, in amphibia intrafusal muscle fibres are innervated by branches of the axons to extrafusal fibres (Katz, 1949; Eyzaguirre, 1957; Gray, 1957). In this case it should be possible to gain meaningful information about spindle behaviour during natural active movements by studying spindle afferent discharge accompanying shortening of the muscle, against a variety of loads, brought about by ventral root stimulation.

In these experiments, on *Bufo marinus* anaesthetized with urethane, all hind-limb muscles save gastrocnemius have been denervated and the tendon prepared for easy attachment to an electro-mechanical puller or to a strain gauge via springs of various compliances. Spindle afferents were found in the 10th dorsal root.

Stimulation of fast motor fibres in the 9th and 10th ventral roots (VR) both produced contractions, but their effects on a particular afferent could differ. In Fig. 1 (*a*) single stimulation of VR 9 caused silencing during the rising part of the twitch, characteristic of extrafusal contraction. Stimulation of VR 10 (*b*) gave an initial burst, characteristic of additional intrafusal driving.

With 10/sec tetani the effect of VR 10 stimulation depended on the compliance of the load. At high compliance (*c*), there was rapid discharge during the rise and fall of contraction but silence during the plateau. At

lower compliances (*d*) and (*e*), activity persisted into the plateau, and the reflex effect of this would be to augment contraction. With higher frequencies of stimulation (*f*) and (*g*), but at the same compliance as in (*e*),

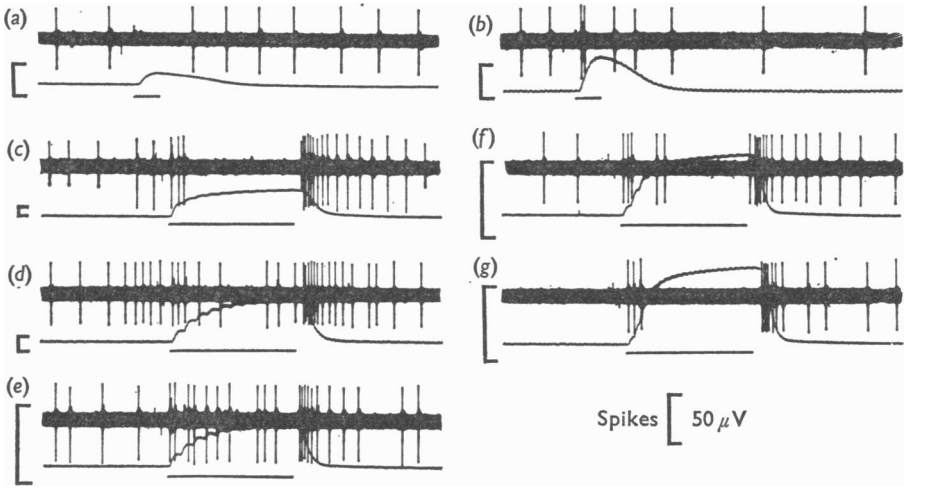


Fig. 1. Response of toad gastrocnemius muscle spindle during fast muscle contractions against spring loads. (*a*) VR 9 twitch, (*b*) VR 10 twitch, load compliance 16 μ/g wt., time calibration 100 msec. (*c*), (*d*) and (*e*) VR 10 stimulation at 10/sec for 1 sec (horizontal bar) with decreasing compliance: 32, 16 and 3·5 μ/g wt. respectively. (*f*) and (*g*) Stimulation at 15 and 20/sec, with same spring as in (*e*). Contractions are recorded as force, but the vertical bars indicate 2·0 mm shortening calculated for each spring.

the plateau discharge disappeared. The reason for this may be that the spindles are predominantly dynamic in their response under these conditions.

This work was supported by the National Fund for Research into Crippling Diseases.

We thank Dr Mary R. Davey for help with dissections.

REFERENCES

EYZAGUIRRE, C. (1957). *J. Neurophysiol.* **20**, 523–542.
GRAY, E. G. (1957). *Proc. R. Soc.* B **146**, 416–430.
KATZ, B. (1949). *J. exp. Biol.* **26**, 201–217.

Further studies on carbohydrate metabolism in infantile malnutrition

BY G. A. O. ALLEYNE, H. FLORES, P. M. TRUST and J. C. WATERLOW.
M.R.C. Tropical Metabolism Research Unit, University of the West Indies, Jamaica

It has been established that glucose utilization and the insulin response to glucose are impaired in infantile malnutrition. In the present study three approaches were used to investigate the mechanisms involved.

First, blood glucose, lactate and pyruvate were measured after a standard intravenous injection of glucose. As previous reports from this Unit have shown, blood glucose was lower in the malnourished children and there were slower rates of glucose disappearance. Blood lactate and pyruvate levels tended to be higher in the malnourished children, but in contrast with the recovered patients there was no striking rise after the glucose had been injected. These results may indicate impaired peripheral glycolysis, and therefore the glycolytic pathway in muscle was studied.

Samples of muscle were obtained from malnourished and recovered children and also from children who had never been malnourished. The muscle was homogenized, aliquots were incubated anaerobically with glucose, glucose-6-phosphate, fructose-1,6-diphosphate and phosphoenolpyruvate as substrates, and lactate production measured. There was no significant difference in lactate production by homogenates from malnourished or recovered children with any substrate, but homogenates from normal children showed relatively higher rates of lactate production from glucose-6-phosphate and fructose-1,6-diphosphate. This might indicate that there is impairment of glycolysis, perhaps at the aldolase step, in malnourished children, which persists even after clinical recovery. These findings are consistent with previous studies showing that even after apparent recovery from malnutrition glucose tolerance is still impaired (James & Coore, 1970).

Insulin sensitivity was then studied in malnourished and recovered children by measurement of glucose tolerance before and after intravenous insulin. The acceleration of glucose disappearance after insulin was slightly greater in the malnourished children.

It would seem, therefore, that the glucose intolerance of malnourished children compared with those who have recovered is the result of impaired insulin release and is not caused by any specific block at any point in the glycolytic pathway, or by insulin insensitivity.

REFERENCE

JAMES, W. P. T. & COORE, H. G. (1970). *Am. J. clin. Nutr.* **23**, 386.

The release of corticotrophin and vasopressin in the foetal sheep in response to haemorrhage

By D. Pauline Alexander,* H. G. Britton,* Mary L. Forsling,† D. A. Nixon* and J. G. Ratcliffe.† **Department of Physiology, St Mary's Hospital Medical School, London, W. 2 and †Department of Chemical Pathology, St Bartholomew's Hospital Medical College, London, E.C. 1*

Although arginine vasopressin (AVP) has been demonstrated in the sheep foetal pituitary (Vizsolyi & Perks, 1969), the mechanisms controlling its release have not been established. Moreover, there is only indirect evidence that ACTH is present and can be released in response to stress (Liggins, 1969).

This communication describes the effect of a stressful stimulus applied to the sheep foetus on the plasma concentrations of ACTH and AVP in the foetus and mother. Haemorrhage was used as it is known to be a potent stimulus in the adult of other species (Dallman & Yates, 1968; Ginsburg, 1968). Experiments were performed on fourteen ewes of the Welsh mountain breed with conceptual ages ranging between 90 and 143 days (term is approximately 147 days). The animals were anaesthetized by spinal administration of 2 ml. 20% procaine hydrochloride and the foetus exposed. Catheters were inserted into a tributary of one umbilical artery of the foetus and into a maternal dorsalis pedis artery. Sequential blood samples of 10–50 ml. depending on foetal age were taken from foetus and mother at 20–30 min intervals. Plasma ACTH was determined by radio-immunoassay, using an antiserum directed against the species common *N*-terminal portion of the molecule, after prior extraction with porous glass (Ratcliffe & Edwards, 1971); AVP was measured by bio-assay (Forsling, Jones & Lee, 1968) and, in some samples from the older foetuses, by radio-immunoassay also (Edwards, Chard, Kitau & Forsling, 1970).

Haemorrhage was associated with a rise in the foetal plasma concentrations of both hormones in all the animals. ACTH was not detectable ($< 0{\cdot}1$ ng/ml.) in the initial samples from foetuses of 107 days and less, but was present (0·1–0·42 ng/ml.) in the 130–143 day foetuses. In response to haemorrhage, however, the younger animals were capable of giving maximal ACTH levels similar to those obtained in older foetuses (1·0–2·5 ng/ml.).

Initial AVP levels (< 10–$90\,\mu$-u./ml.) were not clearly related to conceptual age. A rise in plasma concentration occurred in all foetuses in response to haemorrhage with maximal increases up to 1800 μ-u./ml. at 140 days. There was no clear correlation between foetal AVP and ACTH levels nor were the foetal levels of either hormone dependent upon those of the mother, indicating a lack of effective placental transfer.

Immunologically and biologically active ACTH, AVP and oxytocin were found in acid extracts of foetal pituitaries of all ages. Thus, the ACTH and AVP present in the foetal sheep pituitaries during the latter half of gestation can be released in response to a stressful stimulus. This may be important in the control of parturition.

The work was supported by a grant from the Medical Research Council.

REFERENCES

DALLMAN, M. F. & YATES, F. E. (1968). In *The Investigation of Hypothalamic-Pituitary-Adrenal Function. Memoirs of the Society for Endocrinology*, no. 17, pp. 39–72, eds. JAMES, V. H. T. & LANDON, J. Cambridge University Press.

EDWARDS, C. R. W., CHARD, T., KITAU, M. J. & FORSLING, M. L. (1970). *J. Endocr.* **48**, xi–xii.

FORSLING, M. L., JONES, M. M. & LEE, J. (1968). *J. Physiol.* **196**, 495–505.

GINSBURG, M. (1968). In *Neurohypophysial Hormones and Similar Polypeptides. Handbook of Experimental Pharmacology*, vol. XXIII, pp. 286–371. Berlin and Heidelberg: Springer Verlag.

LIGGINS, G. C. (1969). In *Foetal Autonomy*. Ciba Foundation Symposium, pp. 218–231. London: J. and A. Churchill.

RATCLIFFE, J. G. & EDWARDS, C. R. W. (1971). In *Radioimmunoassay Methods* (European Workshop). Edinburgh and London: E. and S. Livingstone (in the Press).

VIZSOLYI, E. & PERKS, A. M. (1969). *Nature, Lond.* **223**, 1169–1171.

The assessment of central thermoregulatory drive from hand blood flow measurements

BY Z. EVEN-PAZ, R. H. FOX and PATRICIA WOODWARD. *National Institute for Medical Research (Hampstead Laboratories), London, N. W. 3 and Department of Dermatology, Hadassah University Hospital, Jerusalem, Israel*

We are interested in the development of improved techniques for studying the mechanisms underlying temperature regulation in man. Changes in central thermoregulatory drive via the sympathetic vasoconstrictor nerves to peripheral blood vessels are usually assessed by measuring hand blood flow using venous occlusion plethysmography.

Unfortunately, hand blood flow is also influenced by vasoconstrictor stimuli of psychogenic origin, which usually appear as relatively short bursts of activity superimposed on the underlying thermoregulatory drive. It is difficult to find satisfactory objective criteria for excluding measurements affected in this way from the plethysmograph recording alone. However, the trace from a photo-electric pulsimeter does provide a sensitive indicator of psychogenic vasoconstrictor bursts (Fox, 1968). With simultaneous recording of blood flow from one hand and the pulse volume amplitude from a finger of the other hand, it is possible to exclude all blood

flow measurements that coincide with a transient reduction of pulse volume amplitude of 25 % or more and those that occur in the 5 sec period following such a reduction. The criteria given are considered adequate for the exclusion of all important psychogenic effects. This is illustrated by the record shown in Fig. 1 where our criteria would exclude flows 3, 4, 5 and 7. The mean for the 8 flows is 14·7 ml./100 ml. hand tissue.min, whereas flows 1, 2, 6 and 8 average 19·8 ml./100 ml. hand tissue.min.

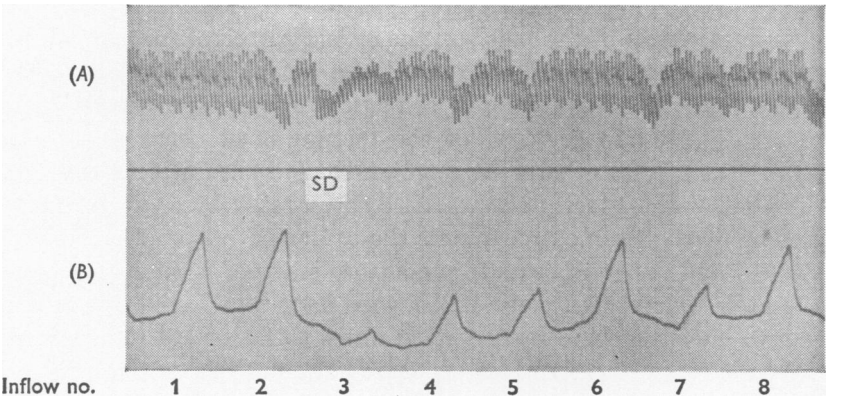


Fig. 1. Simultaneous recordings from photo-electric pulsimeter from one hand (*A*) and venous occlusion plethysmograph from the other hand (*B*). The eight inflow traces are each separated by a 15 sec interval. At SD the subject was disturbed by the approach of an observer and reacted with bursts of vasoconstriction.

The application of this technique helps to remove a source of variation in hand blood flow measurements which can otherwise make it difficult to achieve reproducible results.

REFERENCE

Fox, R. H. (1968). *Proc. R. Soc. Med.* **61**, 785–787.

The relationship of total body fat, 'fat-free mass' and total body weight in male and female human populations of varying ages

By J. V. G. A. Durnin and J. Womersley. *Institute of Physiology, University of Glasgow*

The post-metamorphic innervation by optic fibres of a virgin tectum in *Xenopus laevis*

By Joan D. Feldman,* R. M. Gaze* and M. J. Keating.*† *Neurobiology Research Unit, Department of Physiology, University of Edinburgh*

Optic fibres in *Xenopus* larvae normally reach the tectum at or shortly before developmental stage 39 of Nieuwkoop & Faber (1967), and establish connexions such that an orderly two-dimensional map across the tectal surface can be demonstrated electrophysiologically after metamorphosis. Since little is known of the mechanisms ordering retinotectal connexions, we wished to find out if the development of a normal retinotectal map was dependent on a normal temporal sequence of events; in other words, whether the initial innervation of the tectum must occur at or about stage 39. One eye was therefore removed in tadpoles at stage 29–30 so that its contralateral tectum remained uninnervated by optic fibres. Two to three months after metamorphosis the optic nerve from the remaining eye was cut and deflected so as to predispose entry of fibres into the uninnervated tectum. A small piece of Millipore filter was placed in a mid line position to encourage the regenerating fibres to innervate the ipsilateral (virgin) tectum, rather than to regenerate back to their original positions in the contralateral tectum. Several months after this operation, the retinotectal projection from the remaining eye to its ipsilateral tectum was mapped, and visual responses were also looked for on the contralateral tectum.

In all twelve animals mapped, consistent result was obtained. Retinal points projected from the remaining eye to *both* tecta such that the order predicted for a normal contralateral map (Gaze & Jacobson, 1963) was found on each tectum.

We can conclude that the time of entry of optic fibres into the optic tectum is not critical for the establishment of a normal order of retinotectal connexions in *Xenopus*. Optic fibres were not prevented by the mid line Millipore filter from regenerating to the contralateral tectum as well.

REFERENCES

Gaze, R. M. & Jacobson, M. (1963). *Proc. R. Soc.* B **157**, 420.

Nieuwkoop, P. D. & Faber, J. (1967). *Normal Table of* Xenopus laevis (*Daudin*). Amsterdam: North Holland.

* Present address: Division of Developmental Biology, National Institute for Medical Research, Mill Hill, London.

† Barry Stevens Memorial Fellow, Mental Health Research Fund.

Proximal negative response in the pigeon retina

By A. L. Holden. *Institute of Ophthalmology, Judd St, London, W.C. 1*

Burkhardt (1970) has characterized the proximal negative response (PNR) of the frog retina, and has distinguished it from the intraretinal *b*-wave. It is generated more proximally in the retina than the *b*-wave, and is ascribed to the activity of amacrine cells. The pigeon retina, like the frog retina, has a complex inner plexiform layer, rich in amacrine cell connexions. The present observations show that it too generates a proximal negative response.

The experiments were carried out on urethane anaesthetized pigeons. Intraretinal recordings were taken with KCl-filled micropipettes.

At the vitreal surface of the retina the spike discharges of ganglion cells are recorded, often riding in a transient negative-going response at 'on' and 'off'. The cells are fired by spots of light 1° in diameter: if the spot diameter exceeds 3° spike discharge is suppressed.

Advance for 20–30 μ leaves the PNR in isolation. It is maximal in response to spots of 1°, and the slow tail of the response is suppressed by spots exceeding 3° in diameter. Further advance for 150 μ passes through the PNR zone and reaches the depth of the maximum amplitude of the intraretinal *b*-wave. Unlike the PNR the *b*-wave is minimal in response to a 1° spot, and increases in amplitude as the spot diameter is increased to 5° or 10°.

Thus in the pigeon retina the PNR can be distinguished from the intraretinal *b*-wave by its depth distribution and its area dependence, and is generated in the region of the inner plexiform layer.

REFERENCE

Burkhardt, D. A. (1970). *J. Neurophysiol.* **33**, 405–420.

Adrenocortical activity in exercise

By C. T. M. Davies and J. D. Few. *M.R.C. Environmental Physiology Unit, London School of Hygiene and Tropical Medicine, London, W.C. 1*

Relatively few studies of the effects of exercise, under carefully controlled laboratory conditions, on adrenocortical function have been reported and even these are conflicting (cf. Cornil, De Coster, Copinschi & Franckson, 1965; Wenzkat, Hubl, Kirsch & Büchner, 1968).

In the present investigation we have measured the changes in plasma cortisol concentration (*F*), body temperature and energy expenditure during work of 1 hr duration on a motor-driven treadmill. Eight healthy male subjects, ages 27–42 years, were studied at various levels of exercise

up to and including maximal work. Blood was drawn from an antecubital vein at rest and at various times during exercise and recovery and later analysed for F using a modification (J. D. Few & G. C. Cashmore, unpublished) of the competitive protein-binding method of Murphy (1967). Body temperature (T_{ty}) was measured with a thermistor in the external auditory canal close to the tympanic membrane, and oxygen intake ($\dot{V}_{O_2}$) was determined by the Douglas bag method.

The results show that at the lower levels of exercise, F fell by $3{\cdot}8 \pm 2{\cdot}0$ μg/100 ml. at the 60th minute from an average resting value of $8{\cdot}8 \pm 2{\cdot}4$ μg/100 ml. and remained at this level during 60 min of recovery. At higher work loads the glucocorticoid response was more variable but a marked rise in F was only observed when energy expenditure exceeded a critical level of $\sim 60\%$ of the individual's maximum aerobic power ($\dot{V}_{O_2}$ max) which corresponded to T_{ty} of 37·5° C. Even then the change in F (ΔF) was quite small ($6{\cdot}3 \pm 3{\cdot}8$ μg/100 ml.) compared with the immediate post-exercise period when the mean ΔF rose to $11{\cdot}5 \pm 4{\cdot}7$ μg/100 ml. at the 10th min of recovery.

In two subjects 10 μCi 1,2-^{3}H-F was injected intravenously 30 min before a 60 min period of exercise corresponding to about 75% $\dot{V}_{O_2}$ max. The disappearance rate of cortisol-^{3}H (i.e. ^{3}H attached to cortisol) during the period of exercise was within the range that we have found for normal subjects at rest (half life 45–100 min). During the period of exercise there was a steady fall in the specific activity of the plasma cortisol showing that the rise in plasma cortisol observed was due to increased production of cortisol.

These findings show that in heavy exercise a small increase in cortisol production occurs. It is unlikely that this is due solely to the simultaneous rise in body temperature since it has been shown at rest that a T_{ty} of $\sim$ 38·3° C is necessary to elicit a rise in plasma cortisol (Collins, Few, Forward & Giec, 1969). The significance of the response to exercise is not at present known neither is there any evidence to show whether the stimulation of the hypothalamic-pituitary-adrenal axis is via a neural pathway or due to chemical agency.

REFERENCES

Collins, K. J., Few, J. D., Forward, T. J. & Giec, L. A. (1969). *J. Physiol.* **202**, 645–660.

Cornil, A., De Coster, A., Copinschi, G. & Franckson, J. R. M. (1965). *Acta endocr., Copenh.* **48**, 163–168.

Murphy, B. E. P. (1967). *J. clin. Endocr. Metab.* **27**, 973.

Wenzkat, P. B., Hubl, W., Kirsch, K. R. & Büchner, M. (1968). *Acta biol. med. germ.* **21**, 163–168.

A spinal autonomic reflex evoked by congestion of the mesenteric vein

By C. J. H. Andrews, W. H. H. Andrews and J. Orbach. *Department of Physiology, Royal Free Hospital School of Medicine, Hunter Street, London, W.C. 1*

Experiments were carried out on cats anaesthetized with chloralose and rabbits anaesthetized with chloralose and urethane. The abdomen was opened, usually with a longitudinal incision in the right flank. A fine catheter was passed down a small vein on the colon until its tip lay near the origin of the portal vein for the measurement of mesenteric venous pressure. Arterial pressure was recorded from a femoral artery. For eliciting the reflex, mesenteric venous pressure was raised by obstruction of the portal vein with either a polythene snare or with a small inflatable cuff. The action potentials of both distal and proximal portions of mesenteric and hepatic nerves were studied, though not all simultaneously, by placing slips of nerve under paraffin oil across stainless-steel electrodes. After amplification, action potentials were monitored on an oscilloscope and their frequency observed on a rate-meter connected to a pen recorder.

When the portal vein was obstructed there was a rapid rise in mesenteric venous pressure, with a simultaneous increase in the frequency of afferent nerve impulses in the distal portions of nerves coming from the intestine. The increase in frequency was roughly proportional to the increase of venous pressure, and was maintained as long as the pressure was increased. With a fall in pressure, the frequency of the action potentials fell rapidly. Within 0·5 sec after the beginning of the rise of mesenteric venous pressure there was a marked increase of efferent activity in the nerves to the intestine, but the increase was not maintained at its high initial value. Blood accumulated in the capacitance vessels draining into the portal vein and about 10 sec after initiation of obstruction the arterial pressure fell, and there was then an increase of firing in the hepatic efferent nerves. The afferent response in these experiments was not due to anoxia for neither they nor the increased discharge in the nerves to the intestine were induced by cutting off the arterial supply. The efferent nervous response to portal obstruction was not abolished by transection of the spinal cord at the level of C 7, by section of both vagi in the neck, or by section of hepatic nerves. Andrews & Palmer (1967) showed that congestion of the liver would give rise to afferent nerve impulses and in 1969 Beacham and Kunze demonstrated that, in cats with acute spinal transection, congestion of the renal vein would evoke additional afferent action potentials and a fall in arterial pressure. The mesenteric congestion

reflex appears to occur with a shorter delay and to have a greater effect on efferent activity.

The work was supported by a grant from the Medical Research Council.

REFERENCES

ANDREWS, W. H. H. & PALMER, J. P. (1967). *Q. Jl exp. Physiol.* **52**, 269–276.
BEACHAM, W. S. & KUNZE, D. L. (1969). *J. Physiol.* **201**, 73–85.

The effects of stimulation of the left atrial receptors on sympathetic efferent nerve fibres

BY F. KARIM,* C. KIDD, C. M. MALPUS and P. E. PENNA.† *Cardiovascular Unit, Department of Physiology, University of Leeds*

Distension of the pulmonary vein–atrial junctions in the dog (Ledsome & Linden, 1964, 1967) induces a reflex increase in heart rate; evidence was presented to indicate that the efferent pathway was entirely in the sympathetic nerves. Hakumaki (1968), however, reported that left atrial receptor activation was followed by an inhibition of activity in cardiac sympathetic fibres. The present investigation was undertaken to provide direct evidence for changes in activity in sympathetic efferent fibres following activation of left atrial receptors.

Experiments were performed on dogs lightly anaesthetized with α-chloralose. Impulse activity was recorded from 'few-fibre' preparations of right inferior cardiac sympathetic nerves, right renal sympathetic nerves and the abdominal sympathetic trunk below the origin of the renal artery. Periodic occlusion of carotid arteries was used to test the viability of the strands. The right pulmonary vein–atrial junctions were distended by small balloons (Ledsome & Linden, 1964).

In thirty-eight dogs a heart rate response to balloon distension was elicited in seventy-three tests (mean increase, + 22 beats/min; range, 3–89). This is of the same magnitude as described previously (Ledsome & Linden, 1964). Recordings were made of impulse activity in efferent sympathetic fibres after the heart rate response was reduced to less than 6 beats/min by division of the right ansa subclavia or injection of bretylium tosylate or propranolol.

In twenty-seven tests on fourteen dogs, inflation of the balloons resulted in an increase in the activity of fifteen 'few fibre' strands of cardiac sympathetic nerves (mean, + 28 %; range, 7–64). In eight strands the response was abolished when both cervical vagus nerves were cut or cooled (5° C);

* Commonwealth Scholar.
† M.R.C. Clinical Research Fellow.

in two strands it was abolished when only the right vagus nerve was cooled.

In another ten dogs, twenty-five tests on eleven strands from renal sympathetic nerves induced a decrease in impulse activity (mean, −27%; range, 9–65). In six strands this response was abolished by bilateral section or cooling (5° C) of the vagus nerves; in three strands, cooling of the right vagus nerve alone abolished the response.

Balloon inflation in another six dogs did not affect spontaneous activity in fourteen strands from the right abdominal sympathetic trunk.

Small arterial blood pressure changes associated with each balloon distension had no correlation with changes in sympathetic activity.

These results are in accordance with previous reports that left atrial receptors evoke a chronotropic response (Ledsome & Linden, 1964) unaccompanied by an inotropic effect (Furnival, Linden & Snow, 1968); and have no effect upon sympathetic supply to the hind limb (Carswell, Hainsworth & Ledsome, 1970). They also demonstrate an inhibitory effect on renal sympathetic nerve activity which has not been previously recognized as part of the response to stimulation of left atrial receptors.

This work was supported by Medical Research Council, British Heart Foundation and the Wellcome Trust.

REFERENCES

CARSWELL, F., HAINSWORTH, R. & LEDSOME, J. R. (1970). *J. Physiol.* **207**, 1–14.
FURNIVAL, C. M., LINDEN, R. J. & SNOW, H. M. (1968). *J. Physiol.* **198**, 25–26*P*.
HAKUMAKI, M. O. K. (1968). *Acta physiol. scand.* **70**, 255–256.
LEDSOME, J. R. & LINDEN, R. J. (1964). *J. Physiol.* **170**, 456–473.
LEDSOME, J. R. & LINDEN, R. J. (1967). *J. Physiol.* **193**, 121–129.

The composition of human limb segments, determined both by radiology and by dissection

BY R. E. LEVITT, R. F. MOTTRAM* and M. C. ZISKIN. *Departments of Physiology and Radiology, Temple University School of Medicine, Philadelphia, Pa. 19140, U.S.A.*

While various attempts have been made, by external measurements of limb circumferences and fat-fold thickness, and by measurements from soft-tissue X-rays, to estimate the composition of human limbs (e.g. Jones, 1970), no one has attempted a direct comparison of these estimates with values found by dissecting limb segments into their components and measuring these volumes.

* R. F. Mottram was on paid study leave from University College, Cardiff, and acknowledges the help he received from the Wellcome Trust towards his travel expenses.

We obtained amputation specimens from the pathology departments of Temple University and Philadelphia General Hospitals and froze them solid. Segments were then sawn, with cuts made vertical to their long axes. Each segment was X-rayed at 50 kV, 100 mA and 0·8 sec, at a target-film distance of 102 cm. Radiograms were taken of each segment in two planes at 90° to each other. Total limb volume, volume within the deep fascia and bone volume were each calculated by measurements taken from the X-rays, treating each part as a truncated elliptical cone. The segments were then allowed to thaw and the total volumes found by water displacement. Dissection into component parts was performed and the volumes of skin and subcutaneous tissue, muscle, bone and other deep tissues (fat, tendons, blood vessels and nerves) were also measured. The volumes thus obtained were then compared with those calculated from X-rays by the product-moment correlation method, and linear regression equations found.

A total of sixteen segments from twelve lower limb specimens were studied. Limb segment volumes measured by water displacement ranged from 180 to 1065 ml. The correlation coefficient between these and the volumes estimated from the X-rays was +0·997 and the S.E. of estimated volumes was 19·3 ml. Corresponding values for soft tissues within the deep fascia were: observed volume range 75–610 ml., $r = +979$. S.E. of estimated vol. = 33·8 ml.

A further correlation, that between all soft tissues within the deep fascia and the 'pure muscle' of this compartment, was also determined, solely from the dissection results. In this $r = +0·951$, the S.E. of estimated pure muscle = 35·1 ml., the intercept on the 'soft tissue' axis was +84 ml. and the slope of the line = +0·825.

Direct extrapolation of these results to segments of other limbs in absolute terms is obviously not possible. All the limb segments were from below the knee and all but one were removed for sequelae to arterial disease. However, these results do show that the truncated ellipse model for calculated volumes compares very well with observed limb and component volumes over regions comparable with those used in the past for circulatory and metabolic studies.

REFERENCE

JONES, P. R. M. (1970). *J. Physiol.* **207**, 1–3*P*.

Results of studies using two radiological methods in investigating the circulation of exercising human arms

By P. R. Lynch, R. F. Mottram, O. Owen and B. F. Smale. *Departments of Physiology, Radiology and Medicine, Temple University of Medicine, Philadelphia, Pa. 19140, U.S.A.*

Comparison of the work carried out by Humphreys & Lind (1963) and by Mottram (1970) on the blood flow through exercising forearm muscles suggests that the degree of extension of the elbow might affect the supply of blood to the active muscles. Extension to 150–180° reduces the strength of sustained contraction that can be maintained with a blood supply adequate to prevent fatigue and a large post-exercise hyperaemia. We therefore, in each of five volunteer subjects, performed a series of six brachial angiograms, with elbow flexed and extended, resting and sustaining 10 and 25% maximum voluntary contractions (MVC) on an isometric hand-grip ergometer (Clarke, Hellon & Lind, 1958). Conray-60 was given in 3 ml. doses with 10–15 min intervals between injections. Immediately after each dose 5–10 X-ray films were exposed at ½–1½ sec. intervals. In all subjects the films showed arterial filling, down to vessels about 1 mm in diameter. In some, venous shadows were also seen. Extending the elbow reduced the rate of arterial filling during both 10 and 25% MVC, but we detected no narrowing or kinking of major arteries around the elbow that could account for the reduction in blood flow rate.

In their studies on the site of the hyperaemia in exercising forearms Humphreys & Lind showed that, while there was an increase in blood flow in the active muscles, no change occurred in that through resting muscle. They did not study blood flow through skin, which might be expected to decrease during studies such as theirs. We employed a thermographic recorder that was in regular clinical use to study forearm skin temperatures and thus obtain an indirect measure of forearm skin blood flow. Thermograms were simultaneously obtained of the radial aspects of both forearms of twelve subjects, while one hand maintained a 10% MVC on the ergometer. The subjects had all been working in an air-conditioned building maintained at 24·5–25·5° C for some hours before the thermograms were made. All forearm skin temperatures were in the range 33–36° C. In two arms the exercising limb appeared about 1° C warmer, in four it was about 1° C cooler and in 6 there was no detectable difference in the skin temperatures.

No direct comparisons of blood flow and skin temperature have been made on this region of the body. However, the observed skin temperatures are similar to those for which Cooper, Cross, Greenfield, Hamilton & Scarborough (1949) found a good correlation between temperature and blood

flow. It is therefore concluded that the exercise studied had no major consistent effect on blood flow in the overlying skin.

R. F. M. was on paid study leave from University College, Cardiff, and acknowledges the help he received from the Wellcome Trust towards his travel expenses.

REFERENCES

CLARKE, R. S. J., HELLON, R. F. & LIND, A. R. (1958). *J. Physiol.* **143**, 454–473.
COOPER, K. E., CROSS, K. W., GREENFIELD, A. D. M., HAMILTON, D. McK. & SCARBOROUGH, H. (1949). *Clin. Sci.* **8**, 217–234.
HUMPHREYS, P. W. & LIND, A. R. (1963). *J. Physiol.* **166**, 120–135.
MOTTRAM, R. F. (1970). In *Frontiers of Fitness*, ed. SHEPHARD, R. J. Springfield, Ill.: Thomas.

A comparison of the impulse activity in single aortic baroreceptor fibres in normal and in experimental renal hypertensive rabbits

BY JENNIFER E. ANGELL JAMES. *Department of Physiology, St Bartholomew's Hospital Medical College, London EC1M 6BQ*

Previous studies in which whole nerve recordings were made in the intact animal indicate that in experimental renal hypertension the arterial baroreceptors are reset at a higher level of blood pressure (McCubbin, Green & Page, 1956; Aars, 1968). This work deals with a study of the activity of single baroreceptor fibres from the aortic arch in an isolated perfused aortic arch preparation using rabbits made hypertensive by encapsulation of one kidney combined with simultaneous contralateral nephrectomy.

In twelve animals the mean blood pressure rose from a control mean value of $79{\cdot}9 \pm$ S.E.M. 2·6 mm Hg (range 65–110) before operation to a maximum value of $162{\cdot}7 \pm 5{\cdot}5$ mm Hg (range 130–190) between 2 and 11 weeks post-operatively.

In six of these animals the baroreceptor activity was studied 7–13 weeks post-operatively, when the mean blood pressure was $156{\cdot}2 \pm 7{\cdot}7$ mm Hg (range 125–180). The aortic arch was isolated and perfused *in situ* with Krebs–Henseleit solution at a temperature of 37° C under controlled conditions as described previously (Angell James, 1968, 1969). Single or few-fibre recordings were made from the left aortic nerve.

The effects on impulse frequency of increasing the aortic arch perfusion pressure from 0 mm Hg were studied in sixty-four fibres during non-pulsatile perfusion of the aortic arch. These results were compared with similar control studies carried out on twenty-six fibres from fifteen normal rabbits (Angell James, 1968, 1969).

The mean values for the threshold pressures were higher in the hypertensive ($100{\cdot}4 \pm 5{\cdot}4$ mm Hg) than in the normal rabbits ($50{\cdot}8 \pm 6{\cdot}0$ mm Hg).

Similarly, the points of inflexion of the curves relating baroreceptor impulse frequency to aortic arch pressure were also elevated from a normal value of $116{\cdot}3 \pm 6{\cdot}8$ mm Hg to a value of $161{\cdot}0 \pm 5{\cdot}0$ mm Hg in the hypertensive animals. These curves were not only shifted to the right but were also flatter than the normal, the gradient being less in the renal hypertensive group ($0{\cdot}67 \pm 0{\cdot}1$ impulses/sec.mm Hg) than in the normal group ($1{\cdot}11 \pm 2{\cdot}2$ impulses/sec.mm Hg), the difference being highly significant ($P > 0{\cdot}001$). Thus the impulse frequency is increased less by a given rise of mean pressure in the hypertensive than in the control series.

In the hypertensive animals the impulse frequency at the elevated threshold pressure was lower than at the threshold pressure in the control series and the frequency was less at the point of inflexion.

The reason for the resetting of the baroreceptors is not fully understood. Histological studies on the aortic arch of the hypertensive rabbits used in this study revealed abnormalities in the media.

REFERENCES

AARS, H. (1968). *Acta physiol. scand.* **72**, 298–309.

ANGELL JAMES, J. E. (1968). *J. Physiol.* **198**, 51–52*P*.

ANGELL JAMES, J. E. (1969). Studies of baroreceptor discharge and vasomotor reflexes elicited from an isolated perfused aortic arch preparation. Ph.D. Thesis, University of London.

MCCUBBIN, J. W., GREEN, J. H. & PAGE, I. H. (1956). *Circulation Res.* **4**, 205–210.

Rates of absorption from tryptic hydrolysates of proteins and the corresponding acid hydrolysates or amino acid mixtures

BY R. F. CRAMPTON, S. D. GANGOLLI, D. M. MATTHEWS and P. SIMSON. *British Industrial Biological Research Association, Carshalton, Surrey and the Department of Experimental Chemical Pathology, Westminster Medical School, London, S.W.1*

The existence of a second mode of protein absorption – mucosal uptake of oligopeptides with cellular hydrolysis as distinct from intralumen hydrolysis with uptake of free amino acids – is well established (Newey & Smyth, 1959, 1960, 1962) but its quantitative importance in protein absorption is not known. The finding that amino acids are absorbed at a faster rate from certain oligopeptides than from the equivalent amino acid mixtures and that the competition for transport occurring between free amino acids is avoided when peptides are given (Matthews, Craft, Geddes, Wise & Hyde, 1968; Matthews, Lis, Cheng & Crampton, 1969) suggested a comparison of absorption rates from tryptic hydrolysates, consisting largely of peptides, and the corresponding acid hydrolysates, or,

in the case of proteins of known structure, the equivalent amino acid mixtures.

Absorption was measured by lumen disappearance from tied loops of small intestine in the rat (Matthews *et al.* 1969). 0·25 ml. of hydrolysate or amino acid mixture was introduced, containing 45 μ-moles of total α-NH_2N. After removal from the lumen, the remaining α-NH_2N was measured either directly (in the case of acid hydrolysates or amino acid mixtures) or, in the case of tryptic hydrolysates, following completion of hydrolysis with acid.

TABLE 1. Rates of absorption from complete tryptic hydrolysates of four proteins and the corresponding acid hydrolysates or amino acid mixtures

	Percentage absorption		
Protein	Tryptic hydrolysate	Acid hydrolysate	Amino acid mixture
Casein	62 ± 1·4 (15)*	46 ± 2·1 (12)	—
Bovine albumin	62 ± 1·9 (14)	33 ± 1·8 (12)	—
Lysozyme	45 ± 2·1 (6)	—	35 ± 2·6 (6)
Lactalbumin	68 ± 2·5 (5)	—	29 ± 1·7 (5)

* Mean ± S.E.M. (n).

The absorption period was 10 min. Mean loop length was 5 cm. Results have been corrected for blank values obtained by experiments in which mannitol, 300 m-mole/l., was introduced into adjacent loops in the same animals. Tryptic hydrolysis was carried out with pancreatic trypsin (Type 2, Sigma) and was taken to be complete when there was no further increase in ninhydrin-reacting material on continuing incubation.

The results (Table 1) showed that absorption from tryptic hydrolysates was up to twice as rapid as from the equivalent acid hydrolysates or amino acid mixtures. The rate of absorption from tryptic hydrolysates of casein increased progressively with the time for which hydrolysis was carried out, to the maximum value shown in Table 1.

The results suggest that oligopeptide uptake may be of major importance in the absorption of protein digestion products.

We are grateful to the British Nutrition Foundation for financial support.

REFERENCES

MATTHEWS, D. M., CRAFT, I. L., GEDDES, D. M., WISE, I. J. & HYDE, C. W. (1968). *Clin. Sci.* **35**, 415–424.

MATTHEWS, D. M., LIS, M. T., CHENG, B. & CRAMPTON, R. F. (1969). *Clin. Sci.* **37**, 751–764.

NEWEY, H. & SMYTH, D. H. (1959). *J. Physiol.* **145**, 48–56.

NEWEY, H. & SMYTH, D. H. (1960). *J. Physiol.* **152**, 367–380.

NEWEY, H. & SMYTH, D. H. (1962). *J. Physiol.* **164**, 527–551.

The entry of amino acids into the brain of the rat during the postnatal period

By Guadalupe Baños, P. M. Daniel,* S. R. Moorhouse† and O. E. Pratt.* *Department of Neuropathology, Institute of Psychiatry, de Crespigny Park, London, S.E. 5*

The rat is born somewhat immature with many of the enzymes in its brain relatively inactive until 2 or 3 weeks after birth (Ashby & Schuster, 1950; Davison & Gregson, 1962; Bayer & McMurray, 1967; Davison & Dobbing, 1968). During this period an ample supply of amino acids is needed to form new protein and those which cannot be synthesized by the cerebral cells have to be obtained from the blood. If the concentration of any amino acid in the blood is too low the immature brain may be damaged. In adult rats the entry of some twenty amino acids into the brain is effected by selective, carrier-mediated, transport processes rather than by

Table 1. The highest entry rates of radioactively labelled amino acids into the immature brain compared with the entry rates into the mature brain, expressed as a ratio. (Immature rats, under 2 weeks of age; mature rats 10 to 20 weeks of age)

Radioactively labelled amino acid	Immature / Mature
L-Serine	40–120
L-Alanine	10–60
Glycine	10–60
L-Leucine	10–35
L-Threonine	10–30
L-Tryptophane	10–15
L-Glutamic acid	11–13
L-Aspartic acid	10–12
L-Citrulline	8–10
L-Lysine	5–7
L-Methionine	5–7
L-Phenylalanine	3–5
L-Valine	3–5
L-Arginine	3–4
L-Isoleucine	3–4
L-Tyrosine	2–4
L-Histidine	2–3
L-Thyroxine	2–3
Taurine	approx. 1

* Assisted by the Research Fund of the Bethlem Royal Hospital, Medical Research Council, Nuffield Foundation, and National Fund for Research into Crippling Diseases.

† Supported by Roche Products Ltd.

diffusion; the rate of entry varies over a wide range (Baños, Daniel, Moorhouse & Pratt, 1970, 1971).

The rate of entry into the brain of most of nineteen radioactively labelled amino acids studied during the first 20 weeks of life was higher in the immature rat than in the adult; the rate for L-serine was up to 120 times higher (Table 1).

The five non-essential amino acids that have the highest ratios are all amino acids that can be synthesized by the mature brain (Cory & Rose, 1970; Shank & Aprison, 1970); our results show that they have to be taken from the blood by the immature brain.

It seems likely that the selective transport mechanisms which we have found to be present in the brain of the young adult rat are already active soon after birth and that the decrease in entry rates is related to the diminishing speed of growth of the brain, with a lessening of the need to build new protein.

REFERENCES

Ashby, W. & Schuster, E. M. (1950). *J. biol. Chem.* **184**, 109–116.
Baños, G., Daniel, P. M., Moorhouse, S. R. & Pratt, O. E. (1970). *J. Physiol.* **210**, 149*P*.
Baños, G., Daniel, P. M., Moorhouse, S. R. & Pratt, O. E. (1971). *Proc. Roy. Soc.* B (in the Press).
Bayer, S. M. & McMurray, W. C. (1967). *J. Neurochem.* **14**, 695–706.
Cory, H. T. & Rose, S. P. R. (1970). *J. Neurochem.* **17**, 1477–1484.
Davison, A. N. & Gregson, N. A. (1962). *Acta neurol. scand.* **38**, 48–49, suppl. 1.
Davison, A. N. & Dobbing, J. (1968). *The Developing Brain.* In *Applied Neurochemistry*, pp. 253–286, eds. Davison, A. N. & Dobbing, J. Oxford and Edinburgh: Blackwell Scientific Publications.
Shank, R. P. & Aprison, M. H. (1970). *J. Neurochem.* **17**, 1461–1475.

Differential changes in the 'apparent K_m' and maximum potential difference of the hexose and amino acid electrogenic transfer mechanisms of the small intestine, induced by fasting and hypothyroidism

By R. J. Levin and Gabrielle Syme. *Department of Physiology, The University, Sheffield S10 2TN, Yorks.*

The active transport of hexoses and amino acids across the small intestine generates potential differences (p.d.) that can be used as indices of the electrogenic transfer. Lineweaver–Burk transformation of the p.d./substrate concentration curves allows estimates of the 'apparent K_m' and the 'maximum p.d. developed' ($p.d._{max}$) to be obtained graphically. The effects of hypothyroidism in fed and 3-day-fasted rats on these parameters were measured using mid-jejunum of rat incubated *in vitro*.

Valine, galactose and 3-*o*-methyl glucose were the non-metabolized agents used to evoke the transfer p.d. The results are shown in Table 1. The most important observations from these data are:

(1) Fed hypothyroid rats when compared to fed controls have, for valine, a greatly increased (41 %) $p.d._{max}$ but the K_m is unchanged. This agrees well with valine transfer measured chemically (London & Segal, 1967). In the case of the hexoses, hypothyroidism did not significantly alter any parameter.

TABLE 1. The effects of hypothyroidism and fasting (3 days) on the apparent K_m and $p.d._{max}$ of the amino acid and hexose electrogenic transfer mechanisms of the rat mid-jejunum. The values (mean ± S.E.) were calculated from the Lineweaver–Burk transformation of the saturation-kinetic curves obtained for the substrate ranges of 2, 4, 8, 16 and 32 mM which were corrected for osmotic induced p.d. using mannitol (Debnam & Levin, 1970). The number of animals in each group varies between 6 and 13. The everted mid-jejunum was incubated as a sac at 37° C in bicarbonate saline gassed with 95 % O_2, 5 % CO_2. The bicarbonate saline on the serosal side initially contained 164 mM mannose as a source of hexose that could be metabolized. Differences between means were accepted as significant when $P < 0.05$ using the unpaired t test. Rats that were allowed no food for 3 days but *ad. lib.* access to water are designated 'fasted' while hypothyroidism was induced by allowing rats to drink 0·5 mM 6-propyl-2-thiouracil in their water for approximately 28 days

Experimental group	Substrate	Apparent K_m (mM)	Change (%)	$p.d._{max}$ (mV)	Change (%)
Fed control	Valine	3·9 ± 0·2	−5 %, $P > 0.4$	4·4 ± 0·2	+41 %, $P < 0.001$
Fed hypothyroid	Valine	3·7 ± 0·2		7·4 ± 0·4	
Fed control	Galactose	9·9 ± 0·8	−23 %, $P > 0.05$	14·5 ± 0·6	−15 %, $P > 0.05$
Fed hypothyroid	Galactose	7·6 ± 0·9		12·4 ± 0·9	
Fed control	3-*o*-Methyl glucose	20·4 ± 1·2	−16 %, $P > 0.3$	9·2 ± 0·6	−11 %, $P > 0.4$
Fed hypothyroid	3-*o*-Methyl glucose	17·1 ± 2·8		8·2 ± 1·0	
Fasted control	Valine	3·2 ± 0·3	−22 %, $P > 0.05$	4·6 ± 0·3	+22 %, $P < 0.05$
Fasted hypothyroid	Valine	2·5 ± 0·2		5·9 ± 0·5	
Fasted control	Galactose	6·1 ± 0·4	−38 %, $P < 0.001$	10·9 ± 0·7	−13 %, $P > 0.2$
Fasted hypothyroid	Galactose	3·8 ± 0·5		9·5 ± 0·8	
Fasted control	3-*o*-Methyl glucose	10·7 ± 1·4	+22 %, $P > 0.2$	7·8 ± 0·6	+24 %, $P < 0.05$
Fasted hypothyroid	3-*o*-Methyl glucose	13·7 ± 1·9		10·2 ± 1·0	

(2) Fasting normal rats significantly decreased the K_m for both hexoses but not for valine. The p.d.$_{max}$ was significantly decreased (25%) for galactose but not for 3-*o*-methyl glucose or valine.

(3) Fasted hypothyroid rats when compared to fasted controls showed a significant increase in the p.d.$_{max}$ for valine (22%) with no change in K_m. While the K_m for galactose was significantly decreased (38%), that for 3-*o*-methyl glucose was increased (22%), but not significantly. The p.d.$_{max}$ for galactose was unchanged but that for 3-*o*-methyl glucose was significantly increased (24%).

(4) Fasted hypothyroid rats when compared to fed hypothyroid rats had a significantly decreased K_m and p.d.$_{max}$ for valine and galactose but those for 3-*o*-methyl glucose were unaffected.

These complex, differential changes in carrier affinity (apparent K_m) and p.d.$_{max}$ for valine, galactose and 3-*o*-methyl glucose, induced by fasting and hypothyroidism suggest that specific energy sources and probably carriers must exist for each of these substrates.

REFERENCES

DEBNAM, E. & LEVIN, R. J. (1970). *J. Physiol.* **209**, 29–30*P*.
LONDON, D. R. & SEGAL, S. (1967). *Endocrinology* **80**, 623.

Permeability of mammary ducts in the lactating goat

BY J. L. LINZELL and M. PEAKER. *Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

Several workers have suggested that mammary ducts are involved in net reabsorption, but the permeability is difficult to study because lobules closely surround and open into them at frequent intervals. However, the cells lining the teat resemble those of the ducts, and an inflatable cuff was placed around the base of the teat and inflated to a pressure of 20–25 mm Hg to impede the entry of milk from the gland but not the blood flow.

When milk was left in this teat pouch for 1 hr, there was no change in volume, osmolality or in [lactose], [Na], and [Cl] and no detectable loss of ^{22}Na, ^{86}Rb, ^{36}Cl or [^{14}]Clactose. However, both [^{14}C]urea (18–30%) and [^{3}H]H_2O (66–68%) passed into blood. Permeability in the reverse direction, i.e. from blood to milk was similar. During an infusion of [^{3}H]H_2O and urea plus radioactive ions, mammary tissue became saturated (i.e. venous concentration equalled arterial) with [^{3}H]H_2O and urea within 7–10 min. However, even after 20 min no ^{24}Na, ^{42}K, ^{86}Rb or ^{36}Cl and only a little urea had passed into the milk in the pouch although [^{3}H]H_2O had reached 20–35% of the plasma level.

The ducts within mammary tissue most probably have a similar permeability to the teat duct because (i) [^{14}C]lactose introduced into the teat mixes slowly with preformed milk, but is totally recovered after as long as 16 hr, (ii) after a 25 min intra-arterial infusion of [^{3}H]H_2O, urea and radioactive ions, when plasma concentrations were constant, their distribution in various fractions of milk was unequal. The ratio of urea and ions to [^{3}H]H_2O in the first milk out, which had been previously in large ducts, was similar to that in the teat pouch, whereas in the last milk out, which could only be removed from the alveoli after an injection of oxytocin, much more urea and ions were present relative to [^{3}H]H_2O. Although alveolar milk is exposed to a larger surface area of tissue, measurement of the total quantities of indicators that had entered the milk showed that most of the radioactive ions were in the alveolar fraction, whereas urea and [^{3}H]H_2O were more evenly distributed in the various fractions. The small quantities of radioactive ions were in the alveolar fraction, whereas urea and [^{3}H]H_2O were more alveolar milk because the distribution of [^{14}C]lactose 10–20 min after injection into the teat showed that about 10% had already mixed with alveolar milk in this time.

We conclude that mammary ducts can store milk unchanged for long periods by virtue of their impermeability to its main soluble constituents.

Membrane potentials in the mammary gland of the lactating rat

By M. H. Evans, J. L. Linzell and M. Peaker. *Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge*

To decide whether the movements of ions across mammary secretory cells are active or passive, it is necessary to know the p.d. between plasma, secretory cells and milk.

Lactating rats were anaesthetized with pentobarbitone sodium. The caudal abdominal skin on one side was reflected with the mammary glands attached and pinned flat to expose the underside of the glands whose blood and nerve supply remained intact (Linzell, 1955). Polyethylene electrodes filled with agar and M-KCl were placed in a jugular vein and in one teat. The p.d. between blood and milk varied from -3 to $+2$ mV.

Intracellular recordings were made by exposing a small lobule on the surface of the gland. Glass capillary micropipettes filled with 2·7 M-KCl (resistance 10–20 MΩ) with a low tip potential were advanced into the tissue with continuous monitoring of electrode resistance and p.d. Successful impalement was indicated by sudden changes in potential usually preceded by a temporary increase in electrode resistance. If the microelectrode was withdrawn slightly immediately after penetration, it was found that stable potentials could be held for up to 20 min.

In 126 impalements in six rats the range of p.d. recorded was −11 to −60 mV with respect to blood. The mean was -34 ± 1 mV (S.E.M.). In each animal, the mean was very similar to the median and there was no evidence of more than one population. The bases of secretory cells seen from the surface of an alveolus are about $10 \times 10\ \mu$. The chances of impaling the myoepithelial cells whose processes are only about $1\ \mu$ across and which occupy only 20–30% of the surface, seems remote. Regions were found in the tissue where the p.d. was very similar to that between blood and milk in the teat and since these potentials were very stable the tip of the micro-electrode was probably in the lumen of an alveolus.

Intracellular [Na], [K] and [Cl] are higher than in milk but in the same ratio (Linzell & Peaker, 1970). Ouabain raises [Na] and [Cl] and lowers [K] and Kinura (1969) has confirmed histochemically that a Na^+- K^+-activated ATP-ase is present on the basal but not the apical cell membrane.

Milk [Na^+] and [K^+] are what would be expected if these ions are passively distributed across the apical membrane but not across the basal membrane. In the case of Cl^-, the electrical and concentration gradients across the apical membrane are in the same direction, tending to drive Cl^- out of the cell into milk. Thus an active process may maintain milk Cl^- lower than cell Cl^-.

REFERENCES

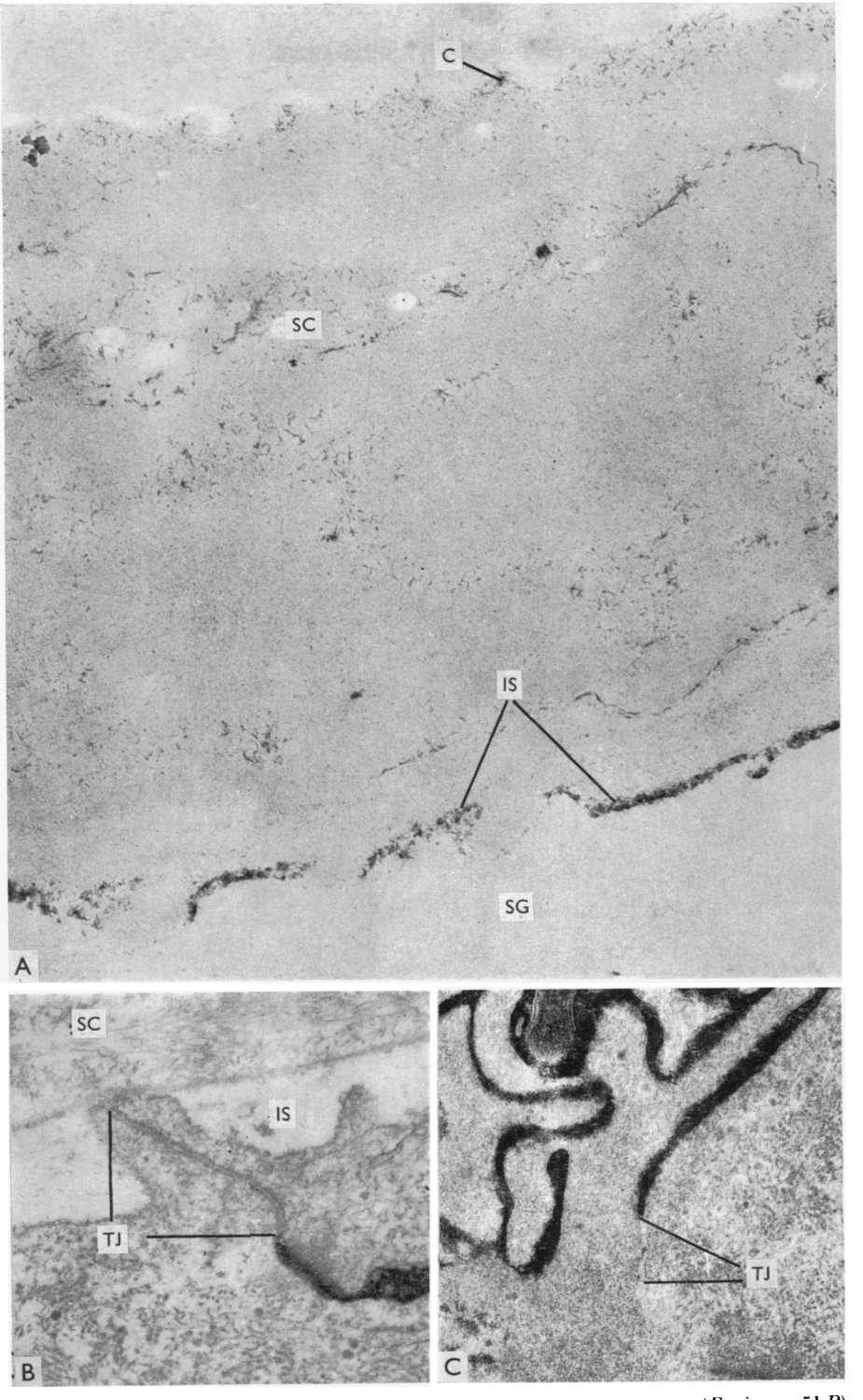
KINURA, T. (1969). *J. Jap. Obstet. Gynaec. Soc.* **21**, 301–308.
LINZELL, J. L. (1955). *J. Physiol.* **130**, 257–267.
LINZELL, J. L. & PEAKER, M. (1970). *J. Physiol.* **207**, 37–38*P*.

The site of the permeability barriers in frog skin epithelium

BY H. BRACHO, D. ERLIJ and A. MARTINEZ-PALOMO. *Departments of Physiology and Cell Biology, Centro de Investigación del I.P.N., México 14, D.F.*

To identify with the electron microscope the site of the structural barriers to diffusion in the frog skin epithelium we studied: (1) the distribution of lanthanum after exposing the external surface of the skin to Ringer which contained 0·5 mM-$LaCl_3$; (2) the localization of ruthenium red and 'colloidal' lanthanum. We tried the first method because we found that ^{140}La moves readily across the external surface of the skin into and out of a compartment that has a limited capacity and is bounded on its internal side by a barrier impermeable to lanthanum.

With this method we found electron dense precipitates within the cells of the stratum corneum and in the space between s. corneum and s. granulosum (Fig. 1*A*).



H. BRACHO, D. ERLIJ AND A. MARTINEZ-PALOMO

(*Facing p.* 51 *P*)

When ruthenium red was used it penetrated from the internal side as far as the tight junctions in the stratum granulosum (Fig. 1*B*). This marker did not penetrate the outermost surface of the skin. The results with colloidal lanthanum were similar in most cases; however, in a few instances dense precipitates were observed within the cells of the s. corneum and in the space between s. corneum and s. granulosum (Fig. 1*C*).

These observations suggest that in the frog skin there are two levels; (*a*) the stratum corneum and (*b*) the s. granulosum, at which tight junctions seal together the cells of a given layer. Of the two diffusion barriers thus formed, the first is the less selective at least as far as lanthanum is concerned.

EXPLANATION OF PLATE

Fig. 1*A*. Outer region of the frog epidermis treated from the outside with 5×10^{-4} lanthanum chloride. A fine precipitate is found along the surface coat (C) of a cornified cell, in the cytoplasm of a stratum corneum cell (SC) and in the intercellular space (IS) separating the s. corneum (SC) and the s. granulosum (SG). No precipitate is found in the s. granulosum (SG). Specimen fixed in glutaraldehyde; unstained section. × 40 000.

1*B*. A dense ruthenium red precipitate fills the intercellular space between two cells of the stratum granulosum, but is prevented from entering into the intercellular space (IS) separating this layer and the s. corneum (SC) by a tight junction (TJ). Isolated epidermis fixed in glutaraldehyde, post-fixed in osmium tetroxide with ruthenium red and embedded in Epon. × 50,000.

1*C*. Colloidal lanthanum applied from the outside forms a dense precipitate which fills the intercellular space between the s. corneum and the s. granulosum. The tracer does not permeate a tight junction (TJ) between apposing cell membranes of the s. granulosum cells. Specimen fixed in glutaraldehyde, post-fixed in osmium tetroxide with 1 % lanthum nitrate, pH 7·7, and embedded in Epon. × 50,000.

Effects of prostaglandin E_1 on dog ureter *in vitro*

By M. J. Wooster. *Physiology Department, University College, Cardiff*

Dog ureter was examined since it responds to prostaglandins of the E series by relaxation (Boyarsky, Labay & Gerber, 1966). Whole segments from the middle of the ureter were mounted in a double sucrose gap apparatus incorporating rubber membranes (König, 1962). The tissue was studied at 37 ± 1° C.

The resting potential which appeared between tissue in Krebs solution and in 2·1 % (w/v) K_2SO_4 was 49·3 ± 1·7 mV (S.E., n = 12). Action potentials were initiated by current or voltage pulses of 100 msec duration at intervals of 2 min. They took the form of a single spike of 200–400 msec duration, followed by a plateau lasting up to 3 sec. Spike amplitudes were

half to two thirds the resting potential. The spike's maximum rate of rise was 0·38–1·2 V.sec^{-1}, and the conduction velocity was 2·5–8·0 cm.sec^{-1}. Action potentials were accompanied by the development of longitudinal tensions of 100–1000 mg.

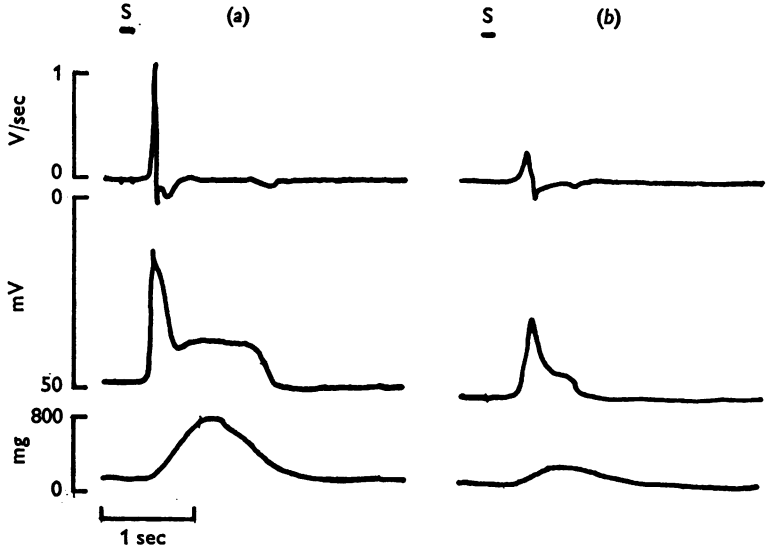


Fig. 1. Effect of PGE_1 (2×10^{-8} g/ml.) on the response of a segment of dog ureter to stimulation in the sucrose-gap apparatus. (*a*) Control response in Krebs solution. (*b*) Response 6 min after commencement of PGE_1 perfusion. Lower trace is longitudinal tension. Middle trace is membrane potential. Upper trace is first differential of the membrane potential. *S* is time of application of the stimulus (100 msec, 15 μA in each case).

Perfusion with PGE_1 ($2{\cdot}5 \times 10^{-9}$ to 1×10^{-6} g/ml.) hyperpolarized the ureter and reversibly inhibited the electrical and mechanical responses to stimulation. In 6 experiments with PGE_1 at 10^{-7} g/ml., the hyperpolarization was $5{\cdot}5 \pm 1{\cdot}7$ mV. In some cases, the ureter was rendered refractory to stimulation; but in others, PGE_1 increased the threshold stimulus required for excitation, reduced the rate of rise of the action potential and diminished the longitudinal tension response of stimulation (Fig. 1).

The ureter was sensitized to the inhibitory effects of PGE_1 by reduction of the potassium ion concentration in the Krebs solution from 5·4 to 2·0 mM, and protected when it was elevated to 10 mM. Preliminary studies suggest that the PGE_1 effect is relatively insensitive to alteration of the calcium concentration.

It is postulated that the primary response of the ureteral muscle to PGE_1 is an increase in the membrane permeability to potassium.

I am grateful to Professor D. A. van Dorp for his gift of the prostaglandin E_1 used in this study.

REFERENCES

KÖNIG, K. (1962). *Pflügers Arch. ges. Physiol.* **275**, 452–460.
BOYARSKY, S., LABAY, P. & GERBER, C. (1966). *Invest. Urol.* **4**, 9–11.

Prostaglandins, myometrial 'enhancement' and calcium

BY ELIZABETH M. EAGLING, H. LOVELL and V. R. PICKLES. *Departments of Physiology and of Mathematical Statistics and Operational Research, University College, Cardiff*

The word 'enhancement' has been used to indicate a particular kind of long-lasting potentiation of contraction responses to various spasmogens caused by prostaglandin E_1 or E_2 on the guinea-pig myometrium (Clegg, Hall & Pickles, 1966). It was postulated that enhancement might result from facilitation of intracellular movements of Ca^{2+} by the PGE, by the formation of a readily dissociable lipid-soluble Ca–PG complex (Pickles, Hall, Clegg & Sullivan, 1966).

D. A. van Dorp & I. Heertje (unpublished, elaborating on preliminary results of Pettit) have recently shown that PGE_1 forms a Ca complex with an association constant $[CaPG^+]/[Ca^{2+}].[PG^-] = 24 \pm 8$. The almost inactive $PGF_{1\beta}$ gave a constant indistinguishable from zero.

Preliminary experiments (Eleanor R. Arm, unpublished) showed that the responses of the K-depolarized guinea-pig myometrium to added Ca^{2+} were enhanced by PGE_1, but quantitative comparison with the enhancement of other spasmogens proved difficult. For the main series, non-depolarized uteri were used in a medium containing Ca 0·2, Mg 3 mM, since these gave graded and reproducible responses to added $CaCl_2$ within the range 0·1 to 5·0 mM. Phentolamine (10^{-6} g/ml.) and propranolol (10^{-6} g/ml.) were added to eliminate effects of endogenous catecholamines (Clegg, 1963).

PGE_1, 50 pg/ml., was added to the reservoir from which one horn of each uterus was perfused, while the other reservoir was left without PG. Each of the three spasmogens namely Ca^{2+}, ACh and vasopressin (Vp) was applied for 1 min to each horn at each of eight dose-levels. The reservoirs were then interchanged and the application of spasmogens was repeated. The whole procedure was repeated 24 times, the order of the 8×3 applications of spasmogens being varied each time according to a statistically predetermined plan. The results are summarized in Fig. 1.

The fact that the PGE affected the responses to three spasmogens of widely different natures in very similar ways suggests that it acts at a

point after the agonist-receptor combination, perhaps by making Ca more readily available to the intracellular compartment. The relatively low effective concentration of PGE_1 is noteworthy.

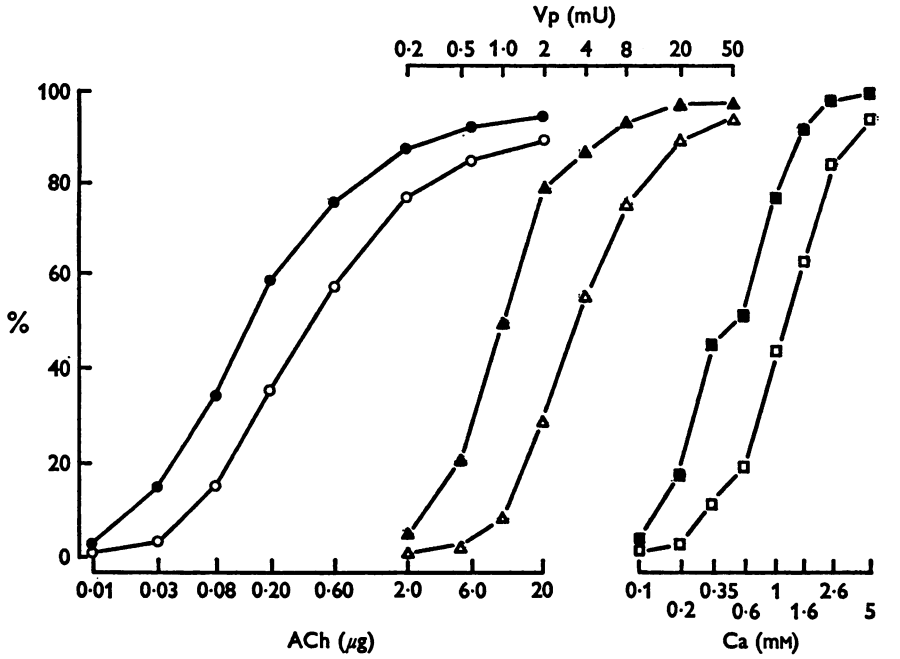


Fig. 1. Each point represents the mean of 48 responses to acetylcholine (●, ○), vasopressin (△, ▲) or calcium chloride (□, ■). Filled symbols represent responses in the presence of PGE_1 50 pg/ml., unfilled symbols the responses in its absence. The three abscissa scales show the dose-levels for the corresponding spasmogens. The ordinate scale is based on the assumption that the largest response to Ca with PGE_1 could be equated to 99·7%; this gave essentially straight lines for the Ca responses, both with and without E_1, when the points were re-plotted on log × probability paper.

We thank Professor D. A. van Dorp for the gift of the PGE_1.

REFERENCES

CLEGG, P. CATHERINE (1963). *J. Physiol.* **169**, 73–90.

CLEGG, P. CATHERINE, HALL, W. J. & PICKLES, V. R. (1966). *J. Physiol.* **183**, 123–144.

PICKLES, V. R., HALL, W. J., CLEGG, P. CATHERINE & SULLIVAN, T. J. (1966). *Mem. Soc. Endocr.* **14**, 89–102.

The efflux of ^{28}Mg from single muscle fibres

By C. C. ASHLEY and J. C. ELLORY. *Department of Zoology, University of Bristol and A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

At the present time little information is available on the movements of magnesium ions in muscle. Single muscle fibres from the barnacle *Balanus nubilus* and from the crab *Maia squinado* were dissected and cannulated as previously described (Caldwell & Walster, 1963; Ashley & Ridgway, 1970). After axial micro-injection of 0·1–0·25 μl. of *ca.* 50 mM-$MgCl_2$ (containing $^{28}MgCl_2$), pH 6·5–7·5, the efflux of the isotope was followed over a period of 2–4 hr.

The efflux following the injection attained a maximum value after about 20–25 min and then declined slowly with time (Fig. 1*a*). This maximum value for the rate constant was 0·168 (S.E. $\pm$ 0·035) $\times 10^{-4}$ sec^{-1} for *Balanus* (26 fibres) and 0·106 (S.E. $\pm$ 0·046) $\times 10^{-4}$ sec^{-1} for *Maia* (six fibres) at 20–25° C. The variation in the rate constant with time approximates to a simple exponential loss as has been previously described for sodium (Bittar, Caldwell & Lowe, 1967). This is in contrast to the behaviour of calcium (Caldwell & Lowe, 1964) and strontium (Ashley, 1967), where the rate constant shows a non-exponential behaviour suggesting a binding of the isotope internally.

Estimates of the total fibre magnesium in *Balanus* were in the range 10–18 mM/kg wet weight, although the free ionized magnesium was probably only $\lesssim$ 3–6 mM/kg wet weight. If it is assumed that the free and bound magnesium are in isotopic equilibrium, then the magnesium efflux based on the total fibre magnesium was in the range 6–12 p-mole cm^{-2}. sec^{-1}.

The rate constant was reversibly reduced by the external application of 1 mM-$LaCl_3$ or by 100 mM-$MgCl_2$ salines (Fig. 1*b*). However, 1 mM ouabain or 5 mM iodacetamide salines failed to produce a consistent inhibition of the efflux, although from considerations of the low magnesium concentration internally some energy-requiring process for extruding magnesium ions would seem necessary.

This work was supported in part by a grant from the Medical Research Council.

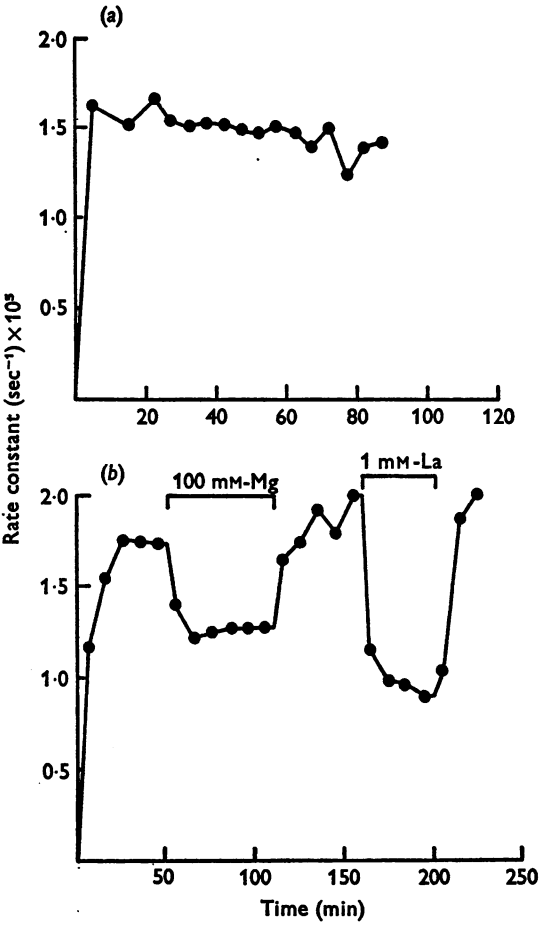


Fig. 1. The efflux of ^{28}Mg from a single barnacle muscle fibre. In (*a*) the final injected magnesium concentration after fibre dilution was estimated as about 0·7 mM. Resting potential −51 mV. In (*b*) the external magnesium concentration was increased from 23·6 mM to 100 mM for the period indicated. Subsequently, 1 mM-$LaCl_3$ saline was applied for the period indicated. Final injected magnesium concentration after fibre dilution estimated as about 0·5 mM. Resting potential −52 mV.

REFERENCES

ASHLEY, C. C. (1967). *Am. Zool.* **7**, 647–659.

ASHLEY, C. C. & RIDGWAY, E. B. (1970). *J. Physiol.* **209**, 105–130.

BITTAR, E. E., CALDWELL, P. C. & LOWE, A. G. (1967). *J. mar. biol. Ass. U.K.* **47**, 709–721.

CALDWELL, P. C. & LOWE, A. G. (1964). Cited by CALDWELL, P. C. *Proc. R. Soc.* B **160**, 512–516.

CALDWELL, P. C. & WALSTER, G. E. (1963). *J. Physiol.* **169**, 353–372.

Uptake of [^{3}H]ouabain and Na pump turnover rates in monolayer cultures of Girardi heart cells

By J. F. Lamb and D. McCall. *Institute of Physiology, University of Glasgow, W.2*

The object of these experiments was to look for evidence of an increase in pump sites on cultured cells when existing pump sites were blocked. To do so we have measured the Na and K fluxes and the [^{3}H]ouabain uptakes in Girardi cells (Girardi, Warren, Goldman & Jeffries, 1958) with a wide range of intracellular ionic concentrations.

Table 1. Collected data for Girardi cells grown for 24 hr in the ouabain concentrations shown. Na and K contents measured by ^{24}Na and ^{42}K; ouabain uptake that occurring from a 30 min exposure to a K-free 2×10^{-7} M [^{3}H]ouabain solution (Baker & Willis, 1969). The fluxes represent the net change in the Na efflux or K influx caused by the addition of 10^{-3} M ouabain. The pump turnover rate is calculated on the assumption that three molecules of Na are transported at one time. The Na and K data obtained in different cells from the ouabain uptake. Temperature 37° C.

Ouabain concentration (moles/l.)	$[Na]_i$ (m-mole/l.)	$[K]_i$ cell water	Na efflux (p-mole/cm^2 sec)	K influx	Ouabain uptake $\times 10^6$ molecules/cell		Pump turnover rate sec^{-1}
					Total	Additional	
0	19	171	14·3	8·0	2·20	0	21
10^{-8}	38	150	8·8	2·9	2·24	1·13	25
5×10^{-8}	108	74	4·5	1·8	3·82	0·80	19

New steady-state Na and K levels were produced by 24 hr treatment with ouabain or [^{3}H]ouabain [Table 1] (Lamb & McCall, 1970). Under these conditions the $[Na]_i$ rises markedly, there is a fall in the ouabain sensitive fluxes and a rise in the total [^{3}H]ouabain uptake. In normal cells the ratio of Na/K is near 3:2 as in other cells; in partially blocked cells this changes to nearly 3:1. We have assumed that for each three molecules of Na transported one ATP molecule is split.

[^{3}H]ouabain uptake during 30 min in K-free [^{3}H]ouabain was taken as a measure of the number of free pump sites (Baker & Willis, 1969) both in normal and previously [^{3}H]ouabain treated cells. Under the latter conditions this gave an additional uptake. Although we have no direct evidence that ouabain does bind specifically to the pump sites this binding is very $[K]_0$ dependent (Baker & Willis, 1970) and the $t_{\frac{1}{2}}$ of loss of the ouabain is similar in normal and partially blocked cells (12–18 hr).

Knowledge of the free pump sites and the fluxes transported by them enables a calculation of the pump turnover rate to be made. This is around 20 sec^{-1} in all conditions; a figure very similar to that found in nerve fibres (Landowne & Ritchie, 1970) but rather lower than those previously found

in R.B.C.s and other tissues (Baker & Willis, 1969). This evidence therefore supports the view that these cells are capable of increasing their number of pumping sites, and that these extra pumps have normal turnover rates.

Part of this work was supported by British Heart Foundation Grant G. 117 and a S.H.E.R.T. Grant to J.F.L.

REFERENCES

Baker, P. F. & Willis, J. S. (1969). *Biochim. biophys. Acta* **183**, 646–649.
Baker, P. F. & Willis, J. S. (1970). *Nature, Lond.* **226**, 521–525, 646–649.
Girardi, A. J., Warren, J., Goldman, C. & Jeffries, B. (1958). *Proc. Soc. exp. Biol. Med.* **98**, 18–23.
Lamb, J. F. & McCall, D. (1970). *J. Physiol.* **206**, 33–34*P*.
Landowne, D. & Ritchie, J. M. (1970). *J. Physiol.* **207**, 529–537.

Observations on the incidence and mechanism of sinus arrhythmia in man at rest

By Sheila Jennett and J. H. McKillop. *Institute of Physiology, University of Glasgow, W.* 2

The pattern of sinus arrhythmia in man has been fully described (Clynes, 1960; Davies & Neilson, 1965, 1967) but its incidence and magnitude at different ages is less well documented; also the mechanism of the fluctuation in heart rate is disputed.

Thirty-three normal male subjects were studied. In the main series of thirty, six subjects represented each of five age-groups between 5 and 70. Heart rate and respiration were continuously recorded by instantaneous rate-meter and by either pneumotachograph or strain-gauge stethograph. In three subjects brachial arterial blood pressure was also recorded, by intra-arterial cannula and transducer. All subjects were seated with the legs supported.

All subjects except one showed a measurable sinus arrhythmia. For each subject, the difference between the highest and the lowest heart rate during each of fifty undisturbed breaths was measured and the mean calculated (range 1·5–17·5 beats per min). Means for each age-group were compared by t test: there was no significant difference between the three groups under 25 (5–7, 12–14, 21–25) nor between the two older groups (41–48, 59–69), but each of the over-40 groups showed significantly smaller fluctuations than any of the under-25 groups ($P < 0{\cdot}05$). These differences were not related to differences in either respiratory frequency or mean heart rate.

The pattern of sinus arrhythmia fitted in all instances the description by the authors quoted of a biphasic change in heart rate (rise, then fall) attributable to inspiration, plus in some cases a rise attributable to

expiration. Davies & Neilson (1967) favoured a haemodynamic mechanism for the increase in heart rate: inspiration increases venous return but as expansion of the pulmonary vascular bed more than accommodates this increase, return to the left heart, left ventricular output and arterial pressure decrease; heart rate rises by a baroreceptor reflex. Seeking support for this explanation, we studied the relative time course of changes in heart rate and in arterial pressure over many respiratory cycles in each of three subjects. Arterial pressure decreased during expiration, in association with decreasing heart rate; inspiratory increase in heart rate was associated with increasing arterial pressure; the magnitude of the decrease in arterial pressure before inspiration was not significantly correlated with the rise in heart rate which followed. The suggested mechanism therefore seemed unlikely.

This work was partly supported by a grant from the Scottish Hospitals Endowments Research Trust.

REFERENCES

CLYNES, M. (1960). *J. appl. Physiol.* **15**, 863–874.
DAVIES, C. T. M. & NEILSON, J. M. M. (1965). *J. Physiol.* **176**, 1–2*P*.
DAVIES, C. T. M. & NEILSON, J. M. M. (1967). *J. appl. Physiol.* **22**, 947–955.

Studies on sinus arrhythmia

BY G. R. KELMAN and K. T. WANN. *Department of Physiology, University of Aberdeen*

When considering sinus arrhythmia, most textbooks of physiology state that heart rate increases during inspiration and decreases during expiration. In 1964 Angelone and Coulter suggested that the phasic relationship between lung volume and heart rate was not constant, but depended on the ventilatory frequency. The present study confirms this suggestion.

In six human subjects we have measured, with a Neilson beat-by-beat cardiotachometer, the heart rate response to constant-volume pulmonary ventilation at varying frequencies. The subjects breathed, via a one-way valve unit and CO_2 absorber, to and from a 6 litre spirometer containing oxygen. The volume of the system was kept constant by continually replacing the subject's metabolically consumed oxygen. Spirometer movement, and therefore pulmonary tidal volume, were recorded electrically. The subject was required to keep the spirometer volume between fixed upper and lower limits while breathing approximately sinusoidally at 4, 5, 6, $7\frac{1}{2}$, 10 or 15 breaths/min.

The amplitude of the arrhythmia (maximum heart rate minus minimum heart rate in each ventilatory cycle) varied from subject to subject; it also depended, in a given subject, on the ventilatory frequency. This amplitude

was minimal at high frequencies, and maximal in the region of 5 or 6 breaths/min. The phasic relationship between lung volume and heart rate showed less intersubject variability than did the amplitude response, but was likewise dependent on ventilatory frequency. The mean phase relationship for the 6 subjects (Fig. 1) shows that the maximal heart rate coincided

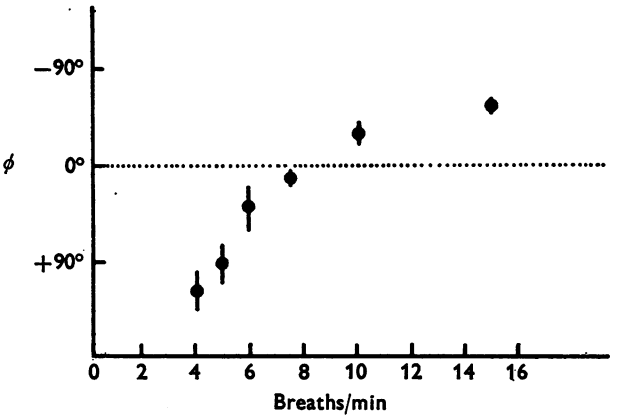


Fig. 1. Phase angle, ϕ (mean ± S.E.M.) between lung volume and heart rate as a function of ventilatory frequency in six normal human subjects. $\phi = 0$ when maximal heart rate coincides with the peak of inspiration.

with the peak of inspiration when the ventilatory frequency was about 8 breaths/min. At lower frequencies the maximal heart rate occurred before inspiration was complete; at higher ventilatory frequencies it lagged behind inspiration.

REFERENCE

ANGELONE, A. & COULTER, N. A. (1964). *J. appl. Physiol.* **19**, 479–482.

The interactions between the effects on thermoregulation of TAB vaccine injected intravenously and monoamines injected into a lateral cerebral ventricle of the Welsh Mountain Sheep

BY J. BLIGH and M. MASKREY.* *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

Bligh & Cottle (1969) produced a simple neuronal model to describe the influence of ambient temperature on the thermoregulatory effects of intraventricular noradrenaline (NA) and 5-hydroxytryptamine (5-HT) in sheep (Fig. 1*a*). As hypothalamic unit activity studies indicate a synaptic convergence of pathways from peripheral and central thermosensors (Wit &

* Wellcome Trust scholar.

Wang, 1968; Hellon, 1969) these monoamine effects might also be dependent on central temperature if they act at or beyond these points of convergence as in Fig. 1*b*. This possibility has not yet been tested directly, but as a bacterial pyrogen may act indirectly on the hypothalamic thermosensors (Eisenman, 1969; Villablanca & Myers, 1965) we have tested the effects of intraventricular monoamines 15–25 min after the onset of TAB vaccine-induced fever at an ambient temperature of 20–25° C.

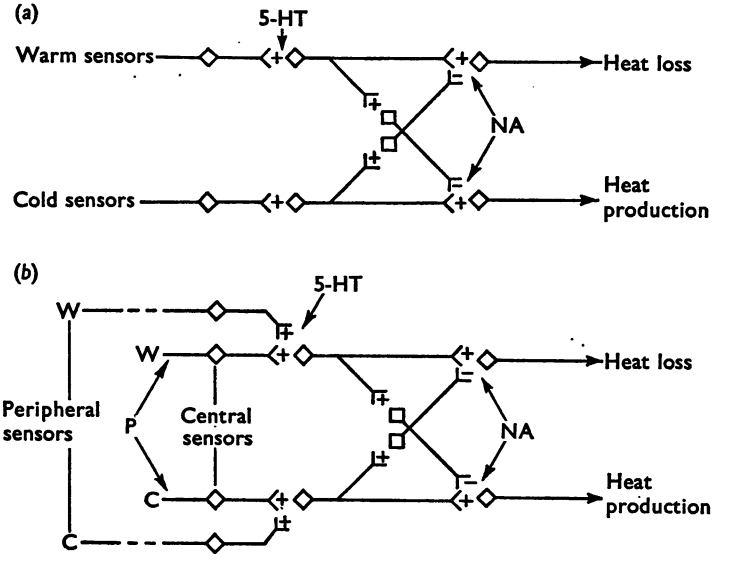


Fig. 1. (*a*) The neuronal model of Bligh & Cottle (1969) in which the input from the thermosensors is undefined. (*b*) A modified neuronal model in which the convergence of the pathways from peripheral and central thermosensors is defined. W = warmsensors; C = coldsensors; P = pyrogen.

In the Welsh Mountain wether (25–40 kg body wt.) 0·7–1·0 ml. TAB vaccine (Burroughs Wellcome) I.V. caused a fall in ear skin temperature (T_{ear}) and in respiratory frequency (*RF*), and an increase in e.m.g. activity. Rectal temperature (T_r) rose 1·1–1·3° C during 45–60 min. The intraventricular injection of 200–300 μg NA in 0·2 ml. saline during fever stopped shivering, but had no effect on T_{ear} or *RF*. The rise in T_r was slowed or halted. The intraventricular injection of 200 μg 5-HT during fever stopped shivering and caused a rise in T_{ear} and *RF*. The fever was halted and T_r started to fall.

These monoamine effects during fever fit the roles attributed to them by Bligh & Cottle (1969) and the modified model (Fig. 1*b*): NA inhibits both heat loss and heat production effectors but since heat loss was already minimized by the pyrogen, NA could inhibit only the shivering;

5-HT activates heat loss effectors and inhibits heat production effectors and thus reversed the fever syndrome.

REFERENCES

BLIGH, J. & COTTLE, W. H. (1969). *Experientia* **25**, 608–609.
EISENMAN, J. S. (1969). *Am. J. Physiol.* **216**, 330–334.
HELLON, R. F. (1969). *Experientia* **25**, 610.
VILLABLANCA, J. & MYERS, R. D. (1965). *Am. J. Physiol.* **208**, 703–707.
WIT, A. & WANG, S. C. (1968). *Am. J. Physiol.* **215**, 1151–1159.

Promotion of polyspermy in the sea-urchin egg by nicotine and its antagonism by curare

BY P. F. BAKER and R. PRESLEY. *Physiological Laboratory, Cambridge and Department of Anatomy, Cardiff*

Prevention of polyspermy in the sea-urchin eggs seems to be a two-stage process: a fast partial block followed by a slower complete block (Rothschild & Swann, 1952). The mechanism of the fast block, which reduces the rate of refertilization to less than 10% of the initial rate, is unknown; but the complete block coincides with elevation of the fertilization membrane.

In an attempt to learn more about the fast block we have re-investigated the action of nicotine, a potent polyspermy-promoting agent (Hertwig & Hertwig, 1887; Rothschild, 1953). Fig. 1 (*A* and *B*) shows that nicotine has two actions on fertilization. It increases the rate of first fertilization and also the degree of polyspermy. The polyspermy does not result solely from the increase in fertilization rate because in eggs exposed either to low concentrations of nicotine, or to high concentrations and then washed briefly in nicotine-free sea water, the initial rate of fertilization is increased without producing appreciable polyspermy. Analysis of the kinetics of fertilization shows that the fast block is impaired by nicotine, and there is a good positive correlation between the extent to which the fast block is impaired and the resulting degree of polyspermy.

The polyspermy promoting action of nicotine is blocked by curare. By itself, curare usually reduces the initial rate of fertilization, but has little effect on the natural level of polyspermy. In the presence of nicotine, curare markedly reduces the extent of polyspermy, and variably reduces the initial rate (Fig. 1*C*, *B*).

These results raise the possibility that a nicotine-like substance may play some part in normal fertilization. For instance, its production may maintain the eggs in a fertilizable state, perhaps by acting at a receptor internal to the cell membrane, and the fast block may result from inter-

ference with this system. This suggestion is given some support by the observation that both acetylcholine (10–20 mM) and eserine (1 mM) are capable of promoting polyspermy.

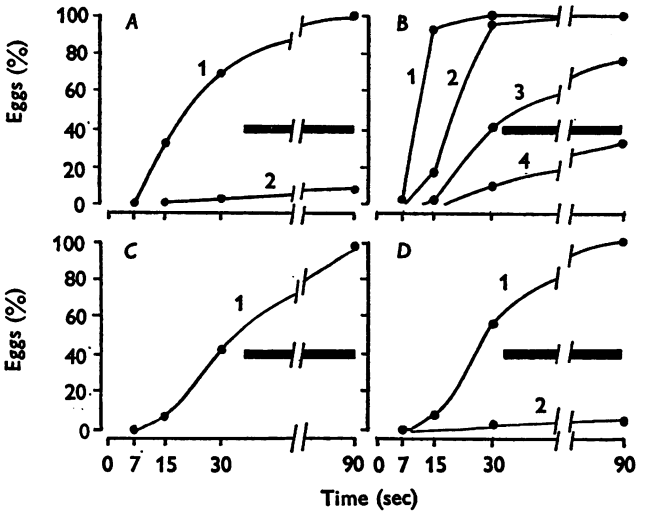


Fig. 1. Rates of uptake of male pronuclei in the same batch of eggs of *Psammechinus miliaris*. Ordinate: percentage of eggs having undergone 1, 2, 3 and 4 fertilizations; abscissa: time (sec) after addition of sperm. Points at each time determined from count on a sample of 100 eggs. Bars indicate period during which fertilization membranes were produced. Fertilization was at 15° C with a sperm density $2{\cdot}5 \times 10^7$ sperm/ml. Fertilization was stopped with KCl-sea water and the uptake of pronuclei scored as described by Presley & Baker (1970). Fig. 1*A*, control eggs. Fig. 1*B*, eggs exposed for 10 min to 25 mM nicotine in sea water. Fig. 1*C* eggs exposed for 10 min to curare (D-tubocurarine chloride, 1·5 mg/ml.) in sea water. Fig. 1*D*, eggs exposed for 10 min to curare (1·5 mg/ml.), and then for a further 10 min to sea water containing both curare and nicotine (25 mM). All solutions were of pH 8·0 and fertilization was effected in the test solution. Curves were obtained in the order *A*, *C*, *D*, *B*. It should be noted that the concentration of drugs used was very high by comparison with mammalian systems. This may reflect either a difference in sensitivity of the preparation or that the receptors are inside the cell.

REFERENCES

HERTWIG, O. & HERTWIG, R. (1887). *Jena Z. Naturw.* **20**, 120.
PRESLEY, R. & BAKER, P. F. (1970). *J. exp. Biol.* **52**, 455.
ROTHSCHILD, LORD (1953). *J. exp. Biol.* **30**, 57.
ROTHSCHILD, LORD & SWANN, M. M. (1952). *J. exp. Biol.* **29**, 469.

Preferential release of newly synthesized acetylcholine from rat cerebral cortex *in vitro*

By P. C. Molenaar, V. J. Nickolson and R. L. Polak. *Pharmacological and Biochemical Laboratories, State University of Leiden and Medical Biological Laboratory RVO-TNO, Rijswijk Z.H., The Netherlands*

Soman pretreated slices from rat brain cortex were incubated for 15 min in bicarbonate buffered Krebs solution containing glucose (10 mM), choline (0·01 mM), eserine (0·05 mM) and KCl (25 mM). This incubation was interrupted by 5 min exposure to [^{3}H]choline (6 μCi/ml., 0·01 mM). Incubation was then continued with non-labelled choline and the medium replaced every 5 min. The [^{3}H]ACh in slices and media was precipitated as the iodine-iodide complex, purified by paper electrophoresis (Potter & Murphy, 1967) and determined both by bio-assay on the leech muscle and by liquid scintillation counting. Internal standards of [^{14}C]choline were added to the samples before precipitation to correct both for losses of [^{3}H]choline and for contamination of the [^{3}H]ACh fraction with [^{3}H]choline. By comparing the bio-assayed ACh content of crude and purified samples the recovery of [^{3}H]ACh was determined.

Table 1. Radioactivities (*a*) and amounts (*b*) of ACh released from cortical slices during 5 min exposure to [^{3}H]choline (I) and subsequent 5 min incubation in non-radioactive choline (II), and corresponding values for ACh extracted from the tissue at the end of these periods. Fourth column represents specific activities (*a*/*b*) of the ACh. Means ± S.E.M.

		n	cpm [^{3}H]ACh/g tissue	ng ACh/g tissue	cpm/ng
			a	*b*	*a*/*b*
Released	I	12	61,000 ± 2,500	910 ± 47	67 ± 3
	II	8	77,000 ± 10,000	960 ± 49	80 ± 10
Extracted	I	6	145,000 ± 13,200	7,000 ± 150	21 ± 2
	II	6	181,000 ± 6,700	6,500 ± 210	28 ± 2

Table 1 shows the specific activity of the ACh released from the slices during exposure to [^{3}H]choline (I) and in the subsequent 5 min (II) to be much higher than that of the ACh extracted from the slices at the end of these 5 min periods. Thus the released [^{3}H]ACh originated from a pool of higher specific activity than the mean specific activity of the ACh in the tissue. Apparently newly synthesized ACh has a greater chance of being released than pre-formed ACh.

The amounts of ACh released in six 5 min periods following the exposure to [^{3}H]choline remained at a contant level, whereas its specific activity decreased exponentially with a half decay time of 10 min.

The release of radioactive and non-radioactive ACh was inhibited for about 70% by omitting the $CaCl_2$ from the incubation medium, but at once

restored on its re-addition. The efflux of [^{3}H]choline was not influenced by $CaCl_2$.

REFERENCE

POTTER, L. T. & MURPHY, W. (1967). *Biochem. Pharmac.* **16**, 1386–1388.

Autonomic connexions of the area postrema in the central cardiovascular response to angiotensin II

BY M. D. JOY. *Department of Medicine, St Thomas's Hospital Medical School, London, S.E. 1*

Infusions of small doses (2–32 ng/min) of angiotensin into the vertebral artery of the anaesthetized greyhound cause an increase in blood pressure, heart rate and cardiac output. This response is affected both by the withdrawal of vagal tone and an increase in sympathetic vasomotor activity (Scroop & Lowe, 1969). The site of this central action lies in the medulla (Joy & Lowe, 1970*a*) and the response itself is abolished by bilateral ablation of the areae postremae (Joy & Lowe, 1970*b*). The present study was undertaken in an attempt to elucidate the neuronal connexions involved in this central response to angiotensin in the chloralose anaesthetized (100 mg/kg) greyhound.

In one group of four dogs, unilateral ablation of the area postrema was performed on the right-hand side, while in a second group of four the same procedure was performed on the left. The blood pressure and heart rate responses to vertebral arterial infusions of angiotensin (32 ng/min) were recorded with either or both vagi blocked (by cooling) both before and after ablating one area postrema. Unilateral ablation of the area postrema did not alter the base-line blood pressure and heart rate although it reduced the response of both to infusions of angiotensin into the vertebral artery. This experiment was repeated with cooling of either the ipsilateral or the contralateral vagus nerve; in each case ablation of the area postrema further reduced both components of the remaining response to angiotensin, but did not abolish them. These results suggest that each area postrema can influence either vagus.

In order to study the role of the area postrema in the sympathetic component of the response to angiotensin, both vagi were blocked; this abolished the heart rate response, but left a significant pressor response which is mediated by adrenergic nerves (Scroop & Lowe, 1969). Unilateral ablation of the area postrema did not significantly modify this remaining pressor response although bilateral ablation abolished it. These results suggest that either area postrema can mediate the entire central sympathetic component of the response to angiotensin.

In order to determine whether these autonomic effects of angiotensin required medullary connexions with higher centres the mid-brain was transected at midcollicular level. In six dogs this procedure did not significantly modify the response to vertebral infusions of angiotensin. Following bilateral vagotomy in these animals, a pressor response remained which was abolished by subsequent treatment with bethanidine (4 mg/kg). These results suggest that neither sympathetic nor parasympathetic components of the central cardiovascular response to angiotensin are dependent upon connexions with centres above the mid-brain.

This work was supported by the Wellcome Trust and the Medical Research Council; the author thanks Professor C. B. B. Downman for helpful suggestions.

REFERENCES

Joy, M. D. & Lowe, R. D. (1970*a*). *Clin. Sci.* **39**, 327–336.
Joy, M. D. & Lowe, R. D. (1970*b*). *Clin. Sci.* **39**, 3*P*.
Scroop, G. C. & Lowe, R. D. (1969). *Clin. Sci.* **37**, 605–619.

The force–velocity characteristics of cat fast and slow-twitch skeletal muscle following cross-innervation

By A. J. Buller, C. J. C. Kean and K. W. Ranatunga. *Department of Physiology, University of Bristol, Bristol BS8 1TD*

It was demonstrated by Buller, Eccles & Eccles (1960) that by cross-innervating fast and slow-twitch muscles of the cat (i.e. joining the proximal cut end of a nerve formerly innervating a fast-twitch muscle to the distal cut end of a nerve formerly innervating slow-twitch muscle, and vice versa) the isometric twitch contraction of previously fast muscles could be made similar to those of normal or self-re-innervated slow-twitch muscles, and the twitch contractions of previously slow-twitch muscles could be altered to show some similarities to those of normal or self-re-innervated fast muscles. In a later study Buller & Lewis (1965) concluded that whereas the observed changes in the contractile behaviour of cross-innervated cat flexor hallucis longus muscles (F.H.L.) suggested alterations in both the rate of decay of active state (Hill, 1949) and the force–velocity relationship, the changes in the isometric contractions of cross-innervated cat soleus muscle suggested that in that muscle only a change in the rate of decay of active state was involved. In the same year Close (1965) using the rat soleus and extensor digitorum longus (E.D.L.) muscles clearly demonstrated that in that species cross-innervation altered the force–velocity relation not only in the fast E.D.L. muscle, but also in the soleus muscle.

More recently Buller, Mommaerts & Seraydarian (1969) have shown that

there is a marked reduction in the myosin and myofibrillar ATPase activity of cross-innervated cat F.D.L. muscles, but little or no increase in the myosin or myofibrillar ATPase activity of cross-innervated cat soleus muscle. They interpreted these observations as indicating a decrease in the maximum rate of shortening of cross-innervated fast muscle but no change in the maximum rate of shortening of cross-innervated slow-twitch muscles (see Bárány, 1967). In view of the apparent difference between the results obtained in the cat and the rat we have now compared the force velocity characteristics of cross-innervated and self-re-innervated cat muscles approximately nine months after operation.

In the cat F.H.L. muscle cross-innervation leads to a marked decrease in the maximum rate of shortening (mean: 1·34 muscle lengths/second compared with 4·02 muscle lengths/second for self-re-innervated F.H.L. muscles). In cat soleus muscles cross-innervation does not lead to any clear change in the maximum rate of shortening (mean: 2·29 muscle lengths/second compared with 2·13 muscle lengths/second for self-re-innervated soleus muscles). However, there is a difference in the shape of the force–velocity relation of cross-innervated soleus muscles (mean: a/P_0 0·308 compared with 0·195 for self-re-innervated muscles) (see Hill, 1970).

The generous help of the Muscle Dystrophy Association of America Inc. is gratefully acknowledged.

REFERENCES

BÁRÁNY, M. (1967). *J. gen. Physiol.* **50**, 197–218.
BULLER, A. J., ECCLES, J. C. & ECCLES, ROSEMARY M. (1960). *J. Physiol.* **150**, 417–439.
BULLER, A. J. & LEWIS, D. M. (1965). *J. Physiol.* **178**, 343–358.
BULLER, A. J., MOMMAERTS, W. F. H. M. & SERAYDARIAN, K. (1969). *J. Physiol.* **205**, 581–597.
CLOSE, R. (1965). *Nature, London* **206**, 831–832.
HILL, A. V. (1949). *Proc. R. Soc.* B **136**, 399–420.
HILL, A. V. (1970). *First and Last Experiments in Muscle Mechanics.* Cambridge University Press.

The effect of sympathomimetic drugs and sympathetic nerve stimulation on the activity of the rat ureter *in vivo*

BY R. J. ANCILL, D. M. JACKSON* and P. H. REDFERN. *Pharmacology Group, Bath University, Claverton Down, Bath*

Although sympathetic innervation of the ureter has been demonstrated (Ray & Neill, 1947), the nature of the influence of this innervation on ureteral activity has not been conclusively demonstrated. The results

* M.R.C. Scholar.

reported here were obtained using a method which allowed measurement of peristaltic rate without interfering with ureteral activity. In addition the activity of the ureter was made independent of urine production.

Male Sprague–Dawley rats, weighing 300–350 g, were anaesthetized with pentobarbitone (60 mg/kg, I.P.) and the trachea was cannulated. Blood pressure was recorded from the carotid artery, and the femoral vein was cannulated to allow injection of drugs. A mid-line incision was made in the abdomen, the left ureter was exposed, and the action potential of each peristaltic wave was measured, using a flexible-tipped glass microelectrode (Ancill, Jackson & Redfern, 1970). Urine production was prevented by tying off the renal artery and vein, and subsequently the ureter was perfused through a fine needle inserted through the renal parenchyma into the pelvis of the kidney. The rate of flow of the perfusate was measured photoelectrically.

Injection of isoprenaline (0·25–2·5 μg/kg), noradrenaline (0·25–3·5 μg/kg) or adrenaline (0·25–10 μg/kg) produced a decrease in peristaltic rate accompanied by a corresponding decrease in the rate of perfusion. These effects were prevented by prior injection of propranolol (1·5 mg/kg).

The sympathetic nerve supply to the ureter was stimulated through the ipsilateral greater splanchnic nerve (Carpi & Cartoni, 1968). In these experiments the renal artery and vein were not tied, but perfusion pressure was kept high so that fluctuations in the rate of urine production did not significantly affect the ureter. A square wave stimulus (8 V, 1 msec 20 c.p.s.) of 40 sec duration produced a decrease in both peristaltic rate and perfusion rate: this decrease was blocked by propranolol (1·5 mg/kg). Bretylium (10 mg/kg) prevented the effects of nerve stimulation, but not those of the exogenous catecholamines.

It is concluded that stimulation of the sympathetic nerve supply to the ureter produces inhibition of ureteral activity mediated by an action on β-adrenoceptive receptors.

REFERENCES

ANCILL, R. J., JACKSON, D. M. & REDFERN, P. H. (1970). *Br. J. Pharmac. Chemother.* **38**, 469*P*.

CARPI, A. & CARTONI, C. (1968). *Br. J. Pharmac. Chemother.* **34**, 259–266.

RAY, B. S. & NEILL, C. L. (1947). *Ann. Surg.* **126**, 709–724.

Cyclic AMP, bradykinin and sweat gland function

BY C. GORDON and V. SCHWARZ. *Department of Medical Biochemistry, University of Manchester*

It is generally accepted that the sodium concentration of mixed sweat, collected from many glands simultaneously, increases with the rate of secretion of the fluid (Schwartz & Thaysen, 1956; Cage & Dobson, 1965).

However, the first one or two samples of sweat, collected in this fashion immediately after stimulation of 3 cm^2 of skin with pilocarpine, often have a relatively high sodium concentration despite the initially low secretion rate (Sutcliffe, Style & Schwarz, 1968). To obviate the mixing of fluid from many glands, secreting at different rates and times, we developed a technique for the serial collection of sweat from a single gland.

We relied mostly on measurement of the osmolality of sweat droplets. In twenty-one samples analysed by both cryoscopic and flame photometric methods, the sodium concentrations and osmolalities showed good correlation. In several experiments we also measured the volume of each sample to enable us to calculate secretion rates.

It was possible to show that the osmolality of surface sweat falls while the secretory rate rises, both reaching a plateau some 3 min after the beginning of secretion. Substantially the same pattern is obtained after stimulation with pilocarpine or acetylcholine, applied iontophoretically, and after reflex (thermal) stimulation. The high initial osmolality is not an artifact and is not due to interference by pilocarpine or acetylcholine cations with sodium reabsorption. It is not found in children with cystic fibrosis, whose high sweat sodium concentration is pathognomonic for the disease.

The rapid change in the osmolality, characteristic of the normal sweat gland in the first few minutes following stimulation, can be prevented by pre-treating the gland with dibutyryl cyclic AMP, administered by iontophoresis. The fluid collected from such a gland has a constant osmolality, which may correspond to the plateau level eventually achieved by the untreated gland, or it may be higher in conformity with the substantial increase in secretion rate occasioned by cyclic AMP. Pre-treatment with theophylline also abolishes the rapid change and establishes an osmolality at plateau level from the first droplet that can be collected. Bradykinin similarly abolishes the normal pattern and, without greatly affecting the secretion rate, causes the osmolality of the sweat to be at or below plateau level throughout the 5 min collection period.

We conclude, tentatively, that efficient secretion of fluid and/or ductal reabsorption of sodium take a few minutes to become established and that bradykinin and cyclic AMP may be involved in the mechanism designed to achieve this end. Our studies are being extended to measurements of sodium concentrations and secretion rates.

REFERENCES

CAGE, G. W. & DOBSON, R. L. (1965). *J. clin. Invest.* **44**, 1270.
SCHWARTZ, I. L. & THAYSEN, J. A. (1956). *J. clin. Invest.* **35**, 114.
SUTCLIFFE, C. H., STYLE, P. & SCHWARZ, V. (1968). *Proc. R. Soc. Med.* **61**, 297.

An effect of stilboestrol on the constrictor response of the perfused vessels of the rabbit ear to isoprenaline

By Suhaila A. Altai and J. D. P. Graham. *Department of Pharmacology, Welsh National School of Medicine, and Department of Physiology, University College, Cardiff*

In the vascular bed of the isolated ear of the female rabbit perfused with gassed (95% O_2:5% CO_2) Krebs solution at a constant input of 2·4 ml./min by a Quickfit PT 60, Type 10 peristaltic pump, the effect of isoprenaline on vascular resistance is dose dependent. By injection 0·1 ml amounts of less than $3{\cdot}59 \times 10^{-6}$ M are dilator, $3{\cdot}59 \times 10^{-6}$ M has an ambivalent effect and $3{\cdot}59 \times 10^{-5}$ M is pressor (see Fig. 1, panel 1). If the isoprenaline is infused during 15 min, concentrations below $3{\cdot}59 \times 10^{-5}$ M are ineffective,

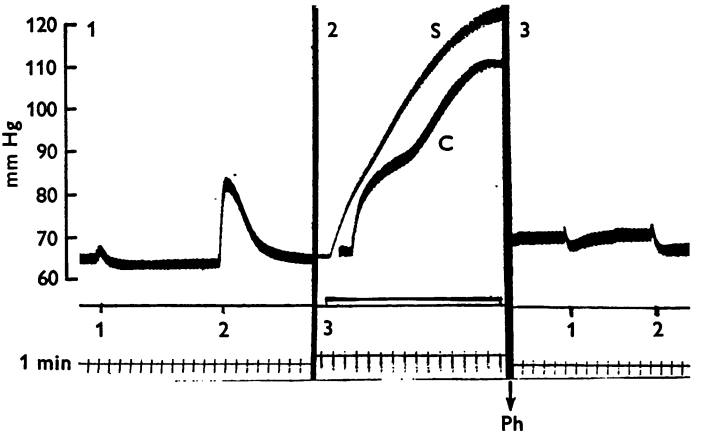


Fig. 1. Pressure in a central ear artery of a female rabbit perfused with gassed Krebs solution at 2·4 ml./min by a peristaltic pump. Time in min, injection or perfusion record, pressure record in mm Hg. Panel 1. Injections of 0·1 ml. isoprenaline $3{\cdot}59 \times 10^{-6}$ M (1) and $3{\cdot}59 \times 10^{-5}$ M (2). Panel 2. Infusion of isoprenaline 2·4 ml./min for 15 min (C) $3{\cdot}59 \times 10^{-5}$ M in an untreated rabbit and (S) $3{\cdot}59 \times 10^{-7}$ M in a rabbit given stilboestrol 1·865 m-moles kg I.M. for 3 days. Panel 3. Phentolamine $1{\cdot}325 \times 10^{-5}$ M in the perfusate abolishes the pressor response of the untreated animal to injected isoprenaline and reveals a dilator component.

but at, or above, give rise to a steep two-step rise to a plateau (see Fig. 1, panel 2C). Phentolamine $1{\cdot}325 \times 10^{-5}$ M in the perfusate abolishes these pressor responses and reveals a small but definite dilator response (see Fig. 1, panel 3). Changing the perfusate to propranolol $3{\cdot}38 \times 10^{-6}$ M abolishes the step in the pressure rise and enhances the response but does not alter the threshold. Injection of stilboestrol in oil 1·865 m-moles I.M. for 3 days not merely abolishes the step but lowers the threshold by 2 log

places (see Fig. 1, panel 2S). Phentolamine again abolishes the pressor response but no dilator component is revealed. A similar effect has been demonstrated for the action of noradrenaline on rabbit aortic strip (Bartenstone, Nasmyth & Telford, 1967).

REFERENCE

BARTENSTONE, A. J., NASMYTH, P. A. & TELFORD, J. M. (1967). *J. Physiol.* **188**, 159–176.

Oxygen dissociation curves of the blood of the rainbow trout, *Salmo gairdneri*

BY F. B. EDDY and G. M. HUGHES. *Research Unit for Comparative Animal Respiration, Bristol University, Bristol BS8 1UG.*

The oxygen dissociation curve of mammalian blood has been studied in detail, but that of the lower vertebrates has not been examined over the physiological range in any detail.

Oxygen dissociation curves of trout whole blood, which shows the Bohr and Root effects, were constructed by the method of Lenfant, Ways, Aucutt & Cruz (1969) and O_2 capacity was determined manometrically. The fish were acclimated for 3 weeks to temperatures of 6°, 15° and 20° C.

Oxygen dissociation curves at 6° C and various P_{CO_2}s are shown in Fig. 1.

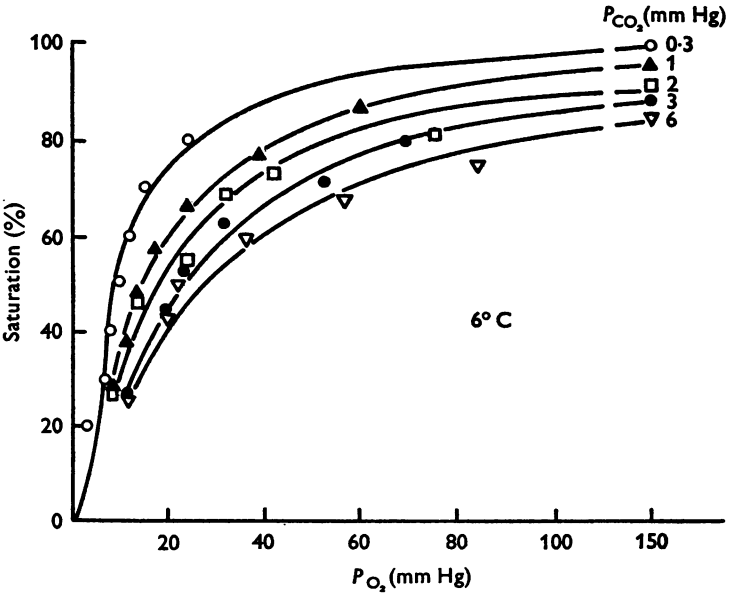


Fig. 1. Oxygen dissociation curves of the rainbow trout at 6° C and at various tensions of CO_2.

Temperature influences the blood pH of poikilotherms (Rahn, 1967), and the same trend was observed in trout; when half saturated at P_{CO_2} of 1 mm Hg the blood pH was 8·25 at 6° C and 7·60 at 20° C. The Bohr shift ($\Delta \log P_{50}/\Delta$ pH) was about $-0{\cdot}53$ for all temperatures.

Raising temperature or P_{CO_2} increased P_{50}, while increasing pH had the opposite effect. When considered quantitatively, a given increase in P_{CO_2} released more O_2 from the blood at high than at low temperatures. However, at high temperatures CO_2 produced more $[H^+]$. The ratio of O_2 released to change in $[H^+]$ was similar at all temperatures.

The value for the Bohr shift in trout resembles that found in other fish, where whole blood has been used, e.g. $-0{\cdot}47$, African lung-fish (Lenfant & Johansen, 1968), and is similar to those for many mammals (about $-0{\cdot}5$). Since the Bohr shift describes a change in blood O_2 affinity with pH, it would seem that under physiological P_{CO_2}s and temperatures this change is about the same in both trout and mammalian blood. Due to differences in buffering, a given increase in P_{CO_2} lowers pH more in trout blood than in mammalian blood.

We wish to thank the Natural Environment Research Council for their financial support.

REFERENCES

LENFANT, C. & JOHANSEN, K. (1968). *J. exp. Biol.* **49**, 437–52.
LENFANT, C., WAYS, P., AUCUTT, C. & CRUZ, Z. (1969). *Resp. Physiol.* **7**, 7–29.
RAHN, H. (1967). *Ciba Foundation Symposium, Development of the Lung*, pp. 3–23. London: Churchill.

Sodium content of rat renal medulla during water diuresis

BY H. E. F. DAVIES, MARY A. DAVIES and C. T. GOULD. *Department of Physiology, University College, Cardiff*

Water diuresis is known to reduce the renal corticomedullary gradients of osmolality and of sodium concentration. Fall in sodium concentration could theoretically be produced by decrease in tissue content of sodium or by dilution of the sodium present by increase in tissue content of water. As solute content of the medulla and papilla varies with diuresis, separation of the two processes is difficult. Gardner & Vierling (1969) found the sodium content of the papilla to increase considerably with dehydration, and also to increase slightly in water diuresis. Others have found that papillary sodium content decreased during prolonged water diuresis, and then increased after infusion of antidiuretic hormone (Atherton, Hai & Thomas, 1968; Hai & Thomas, 1969). We have followed the changes in composition of the renal medulla and papilla after a single water load, with particular regard to the changes in concentration of sodium.

Rats were given 4 ml. of water per 100 g body wt. through a tube into the stomach. 1, 2, 3 or 4 hr later animals were killed and their kidneys were quickly removed, sliced and weighed. Sodium and potassium were estimated by flame photometry. Changes in renal tissue osmolality were measured directly by the method of Appleboom, Brodsky & Scott (1965). Urines collected after administration of water had an osmolality of 194 ± (S.D.) 50 m-osmole/l. and sodium concentration 5·6 ± 3·7 mM. In the renal papilla tissue osmolality and sodium concentration both fell by 45 % and by 54 % of their control values by 1 hr, then progressively rose to about control values by 4 hr. Tissue content of potassium appeared not to change; this was used as a reference point to calculate changes in tissue content of sodium and of water. Relative to potassium, tissue content of sodium decreased by 38 %, and tissue content of water increased by 26 % at 1 hr. On this basis some 70 % of the fall in sodium concentration at 1 hr was due to loss of sodium. At 2 hr all of the fall seemed due to loss of sodium. In the inner medulla all of the fall in sodium concentration appeared due to loss of sodium.

This work was supported by a grant from the Welsh and Cardiff Hospital Boards.

REFERENCES

APPELBOOM, J. W., BRODSKY, W. A. & SCOTT, W. N. (1965). *Am. J. Physiol.* **208**, 38–45.

ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1968). *J. Physiol.* **197**, 429–443.

HAI, M. A. & THOMAS, S. (1969). *Pflügers Arch. ges. Physiol.* **310**, 297–319.

GARDNER, K. D. & VIERLING, J. M. (1969). *Am. J. Physiol.* **217**, 58–64.

Subcellular changes in ^{59}Fe distribution during iron absorption in the rat

BY A. JACOBS and M. WORWOOD. *Department of Haematology, Welsh National School of Medicine, Cardiff*

The rat has been the favoured experimental animal for studies of iron absorption from the intestine but little information is available about the subcellular distribution of this absorbed iron in the intestinal epithelial cells. The data of Brown & Rother (1963) are difficult to interpret because of the lack of information about the nature of the subcellular fractions and later investigations on the isolation of such fractions from rabbit and guinea-pig mucosa have emphasized the difficulties of working with the mucosa from rat intestine (Clark & Porteous, 1965; Hübscher, West & Brindley, 1965). In the present study we have attempted to overcome some of these difficulties in order to investigate iron transport in the small intestinal cells.

Groups of rats were given ^{59}Fe by stomach tube (5 μg iron as ferric

chloride in 0·05 N-HCl) and the whole body ^{59}Fe activity was measured in a counter consisting of a ring of eight matched Geiger Müller tubes. The animals were killed at intervals of 15 min to 18 hr after ^{59}Fe administration and the stomach and intestine were removed. ^{59}Fe in the carcass was measured in the Geiger counter. The mucosa was removed from the first 40 cm of the small intestine, homogenized, filtered through nylon mesh and separated into five subcellular fractions by differential centrifugation at forces from 400g to 105,000g. In order to characterize the fractions a number of 'marker' enzymes were assayed.

From 15 min to 4 hr after administration of the dose, ^{59}Fe was concentrated in the 105,000g sediment and the final supernatant (approximately 70% of the mucosal ^{59}Fe appeared in these two fractions). However, at 12 and 18 hr ^{59}Fe was concentrated in mitochondria. A similar mitochondrial concentration was observed after intravenous injection of plasma bound ^{59}Fe and some of the iron may therefore have been absorbed and taken up from the circulating plasma ^{59}Fe by the mucosal cells. Ferritin-^{59}Fe was assayed by precipitation from the heat-treated fractions by an antiserum to horse spleen ferritin and was found to be concentrated in the 105,000g sediment and supernatant at all times. The % of mucosal ^{59}Fe bound to ferritin increased from 31% at 15 min to 74% at 4 hr.

None of the ^{59}Fe in the supernatant was dialysable and starch-gel electrophoresis of this fraction during the early phase of iron absorption revealed two major bands of radioactivity, one corresponding to ferritin and one close to the sample origin. The absorption of ^{59}Fe was closely related to the disappearance of the latter band of ^{59}Fe and this may represent an intracellular carrier involved in the transfer of iron across the intestinal cell during the rapid phase of absorption.

We are grateful to Professor K. S. Dodgson for allowing us to use the facilities of his department. M.W. is an Anniversary Research Fellow of the Royal College of Physicians.

REFERENCES

BROWN, E. B. & ROTHER, M. L. (1963). *J. Lab. clin. Med.* **62**, 357.
CLARK, B. & PORTEOUS, J. W. (1965). *Biochem. J.* **96**, 539.
HÜBSCHER, G., WEST, G. R. & BRINDLEY, D. N. (1965). *Biochem. J.* **97**, 629.

A preliminary report on some cardiological observations made at the IXth British Commonwealth Games

BY J. FERGUSON, W. BARRY and R. C. WHITE. *Department of Physiology, Bute Medical Buildings, The University, St Andrews; Department of Physiology, University College, Cardiff, CF1 1XL; Chemical Defence Establishment, Porton Down, Salisbury.*