COMMUNICATIONS

Ovarian secretion of steroids with central depressant actions

By Margarethe Holzbauer. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

The ovary of the rat contains several steroids which have a fully saturated ring system (Holzbauer, 1969) and can be formed from progesterone by enzymes which have been demonstrated in this tissue (Wiest, 1963). Steroids of this configuration possess hypnotic activities which can be stronger than those of pentobarbitone sodium (Gyermek, Genther & Fleming, 1967).

At present the secretion of these compounds into the ovarian venous blood of the rat is being studied. The following secretion rates (μg/g ovary.min, mean $\pm$ standard error of the mean) were observed: allopregnanediol (5α-pregnane-3α,20α-diol): $0{\cdot}46 \pm 0{\cdot}08$; 20$\alpha$OH-5$\alpha$-pregnan-3-one: $0{\cdot}39 \pm 0{\cdot}047$; allopregnanolone (3$\alpha$OH-5$\alpha$-pregnan-20-one): $0{\cdot}17 \pm 0{\cdot}028$. The secretion rates of progesterone of the same rats were $0{\cdot}61 \pm 0{\cdot}13$ and those of 20-dihydroprogesterone (20αOH-pregn-4-en-3-one) $2{\cdot}85 \pm 0{\cdot}53$. These are the mean values observed in twenty-five rats during different phases of the oestrous cycle. A comparison between the secretion rates in early ($n = 8$) and in late ($n = 7$) pro-oestrus showed a significant rise in the secretion of allopregnanolone ($+190\%$, $P = 0{\cdot}01$–$0{\cdot}001$) in addition to that of progesterone ($+365\%$, $P = 0{\cdot}02$–$0{\cdot}01$) in late pro-oestrus. The secretion of 20αOH-5α-pregnan-3-one was also increased, but the difference was not statistically significant. No rise was observed for allopregnanediol and for 20-dihydroprogesterone. The changes in the ovarian secretion of these steroids during the day of pro-oestrus thus paralleled the changes in their ovarian concentrations as reported previously (Holzbauer & Mason, 1970).

These experiments show that the ovaries metabolize progesterone *in vivo* to ring saturated pregnane derivatives and secrete the metabolism products at rates similar to those of progesterone. A physiological significance in these findings might lie in the central depressant action of pregnane derivatives, especially that of pregnanolone. They may also contribute to the well-known variations in mood which occur during the menstrual cycle in humans.

REFERENCES

GYERMEK, L., GENTHER, G. & FLEMING, N. (1967). *Int. J. Neuropharmac.* **6**, 191–198.
HOLZBAUER, M. (1969). *J. Physiol.* **204**, 8–10*P*.
HOLZBAUER, M. & MASON, P. (1970). *J. Physiol.* **210**, 128–131*P*.
WIEST, W. G. (1963). *Endocrinology* **73**, 310–316.

The role of the kidney in maintaining blood pressure in the adrenalectomized rat

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Following adrenalectomy there is prolonged arterial hypotension in the rat (Imms & Jones, 1968). Catecholamine excretion is increased in these animals (Imms & Jones, 1966), suggesting an increased activity of the sympathetic nervous system in response to hypotension. Since the renin-angiotensin system is also activated after adrenalectomy (Schaechtelin, Regoli & Gross, 1963) it is possible that the kidney may contribute to the maintenance of blood pressure in these animals. This hypothesis has been investigated.

Albino rats weighing 180–250 g were adrenalectomized under ether anaesthesia and kept on normal diet with sodium supplements. Control animals were sham adrenalectomized. One or six days later all animals were anaesthetized with pentobarbitone sodium (40–60 mg/kg) and one half of each group were bilaterally nephrectomized, the remainder being sham nephrectomized. Four hours later all animals were re-anaesthetized and their arterial blood pressures recorded. Nephrectomy had little effect on the blood pressure of sham-adrenalectomized animals, but caused a marked fall in the adrenalectomized group.

Using a rat blood-pressure preparation, renal pressor activity in the plasma of adrenalectomized and sham adrenalectomized animals was compared using angiotensin (Hypertensin-Ciba) as standard. Plasma from rats adrenalectomized on the previous day had an angiotensin-like activity of 9·7 ng/ml. (range 5–15 mg/ml.), and plasma from 6-day animals had an activity of 11·3 ng/ml. (range 4–21 ng/ml.). Plasma from sham-adrenalectomized animals had an activity of less than 2·5 ng/ml.

Another possibility is that nephrectomy causes hypotension in the adrenalectomized rat as a result of metabolic changes. This was investigated by examination of the blood from control and adrenalectomized animals which had been subsequently nephrectomized. Four hours after nephrectomy the plasma sodium levels were unaffected and there was no marked acidosis. Urea and potassium levels were raised by similar amounts

in both the adrenalectomized and control groups. However, plasma potassium concentration was initially higher in the adrenalectomized group.

It would appear that a renal pressor agent, possibly the renin-angiotensin system, plays a part in maintaining arterial blood pressure after adrenalectomy.

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REFERENCES

IMMS, F. J. & JONES, M. T. (1966). *J. Physiol.* **186**, 141–142*P*.
IMMS, F. J. & JONES, M. T. (1968). *Br. J. Pharmac. Chemother.* **33**, 212*P*.
SCHAECHTELIN, G., REGOLI, D. & GROSS, F. (1963). *Am. J. Physiol.* **205**, 303–306.

A re-investigation of the sensorimotor cortical area for the hind leg in the rat

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In attempting to demonstrate the organization of the sensorimotor cortex in the rat (see Woolsey, 1958), we consistently failed to obtain a purely contralateral representation for either the short latency 'sensory' or 'motor' areas for the hind limb. In rats sufficiently deeply anaesthetized with urethane to abolish withdrawal of the hind leg to a strong pinch, electrical stimulation of the periphery has revealed that the cortical sensory representation for the hind limb is bilateral; in distinction to that for the forelimb which is entirely contralateral. The mean latencies of the cortical responses (16 rats) to stimulation of the ipsilateral and contralateral hind limbs, recorded from the centre of the short latency area, were 7·02 (± 0·35, S.D.) and 6·99 (± 0·47) msec respectively. The short latency area was ellipsoidal, approximately 2·0 mm by 1·0 mm, with its major axis aligned rostrocaudally, and its centre 1·5 mm lateral to the intersection of the coronal and sagittal sutures. The over-all area from which responses could be obtained was identical stimulating either hind leg (Fig. 1). The amplitudes of the responses from the ipsilateral limb were, however, smaller (10–50%) than those to contralateral limb stimulation.

Surface anodal stimulation of the cortex at the centre of the short latency area with single pulses (1–2 msec, 2–8 mA) gave motor movement of both hind limbs in anaesthetized rats given 60 mg/kg 1,2-dihydroxybenzene to increase central transmission (Angel, 1969). The latencies of the electromyographic responses from the ipsilateral or contralateral gastrocnemius in six rats were 7·14 (± 0·54) and 6·59 (± 0·57) msec respectively.

The amplitudes of the electromyographic responses were only slightly smaller on the ipsilateral side (85%). Bilateral muscle responses persisted after unilateral cortical ablation.

Thus one hemisphere receives information from both hind limbs and can exert an influence on the lower motorneurones controlling both hind limbs.

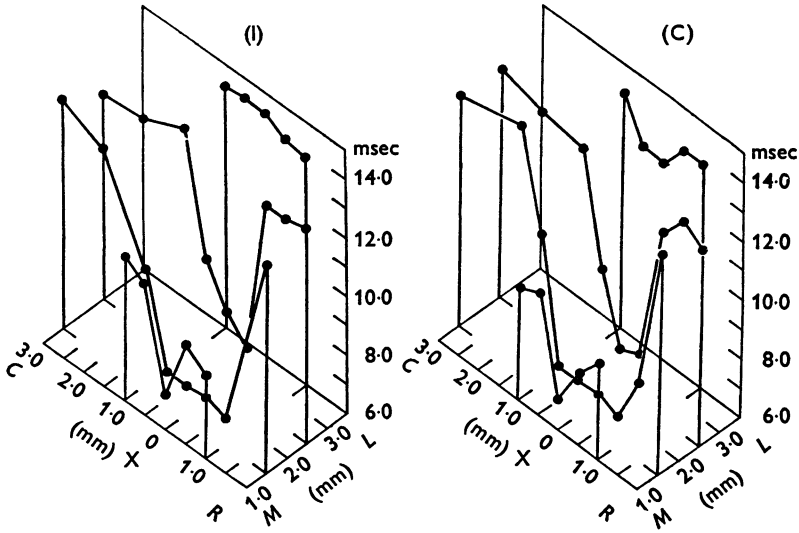

Fig. 1. Shows the mean latency of the cortical evoked response (ordinates) plotted against the position, in stereotaxic co-ordinates, of the recording electrode as a hypometric grid (*L*, *M* medio-lateral axis, *R*, *C* rostrocaudal axis). The position of the intersection of the sagittal and coronal sutures is marked with an *X*. Each point represents the mean latency of sixty consecutive responses to stimulation of the hind-limb ipsilateral (I) and contralateral (C) to the hemisphere from which the records were obtained. Stimuli were applied at a rate of 1/sec in a rat deeply anaesthetized with urethane.

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REFERENCES

ANGEL, A. (1969). *Electroenceph. clin. Neurophysiol.* **27**, 392–403.

WOOLSEY, C. N. (1958). Organization of somatic sensory and motor areas of the cerebral cortex. In *Biological and Biochemical Bases of Behaviour*, pp. 63–82, ed. HARLOW, H. F. & WOOLSEY, C. N., Madison, Wis.: University of Wisconsin Press.

How a peripheral retinal ganglion cell responds differentially to focused and defocused images

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A preliminary survey on optic nerve fibres in cats suggested that the performance of the retinal ganglion cells is critically dependent on the quality of the images at the retina. Thus, it was felt necessary, first, to analyse response characteristics within the receptive fields of these cells to focused and defocused stimuli.

Recordings from peripheral ganglion cells in cats were made intrabulbarly with tungsten micro-electrodes.

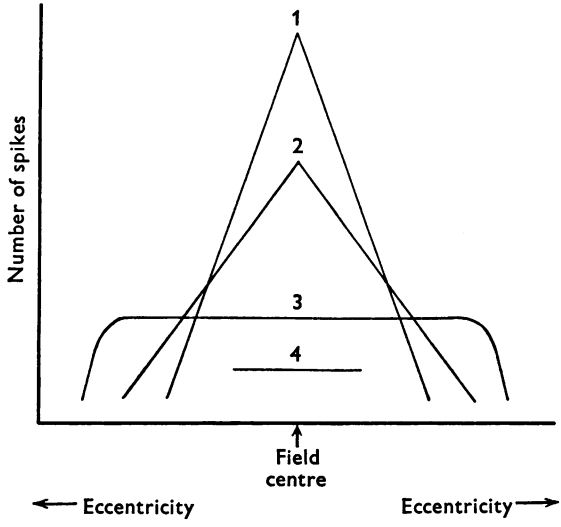

Fig. 1. Schematic representation of excitation patterns from a peripheral ganglion cell with (1) focused stimuli and progressively defocused stimuli (2), (3) and (4).

For focused stimuli, the peripheral ganglion cell fires vigorously when the centre of the receptive field is stimulated, while it's firing decreases rapidly as the eccentricity of the stimuli from the field centre increases, so that under focused conditions two stimuli separated by 15′ of arc or less give a differential response.

Under defocused stimulus conditions, however, this centre favouring mechanism is reduced until a maximum spatial error, equal to the radius

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of the field (commonly 1–5°), is possible. Under extreme defocused conditions, the cell responds more robustly to the stimulus when presented at the periphery of the receptive field, so that the average firing might even exceed that produced at the field centre. These observations are illustrated schematically in Fig. 1. Thus, the refractive state of the eye critically dictates the balance of (A) the centre favouring, and (B) the light detection, roles of the peripheral ganglion cells.

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Cerebellar initiation of discharges in sympathetic nerves

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Both Achari & Downman (1969) and Miura & Reis (1969) have shown that stimulating within a fastigial nucleus of the cerebellum causes a marked pressor response. Achari & Downman (1970) also reported other autonomic responses and showed that the changes in the heart and blood vessels were mediated by sympathetic pathways. In the present investigation it has been shown that fastigial stimulation evokes discharges in sympathetic nerves and that a pathway in the dorsolateral white column of the spinal cord is involved.

Cats were anaesthetized with either chloralose (70 mg/kg) or chloralose-urethane (35 mg and 500 mg/kg). The deep nuclei of the cerebellum were stimulated with square pulses (1·75 msec) or 50 Hz sine wave current via stereotaxically orientated electrodes of glass-coated stainless steel wire. Electrode tip positions were confirmed histologically. Stimulating a fastigial nucleus of either side with a train of two or three pulses evoked a volley discharge in the inferior cardiac, splanchnic and renal nerves. The peak amplitude of the volleys varied from 15 to 300 μV and was related to the arterial pulse, being maximum during the rising phase of the pulse. Similar discharges were evoked in the nerves by stimulating within the hypothalamus, medullary recticular substance and also in the dorsolateral white column of the spinal cord.

Continued stimulation produced a continuous discharge in all three nerves which was accompanied by rise of B.P., increased contractile force of the ventricle and sometimes tachycardia. Increased B.P. and contractile force persisted in the post-stimulation period although the nerve discharge ceased abruptly and silence was maintained for some seconds.

Using combinations of partial sectionings of the spinal cord the pathway mediating the fastigial influence on the sympathetic spinal neurones was

localized bilaterally in the dorsal half of the lateral white column, lying between the central grey matter and the dorsal cortico-spinal tract. Stimulation of the region in a cervical segment produced a pronounced discharge in the inferior cardiac and splanchnic nerves. A localized electrolytic lesion in this region, after contralateral hemisection of the cord, abolished the discharges in the nerves following fastigial, hypothalamic and medullary reticular stimulation.

REFERENCES

ACHARI, N. K. & DOWNMAN, C. B. B. (1969). *J. Physiol.* **204**, 130–131*P*.
ACHARI, N. K. & DOWNMAN, C. B. B. (1970). *J. Physiol.* **210**, 637–650.
MIURA, M. & REIS, D. J. (1969). *Brain Res.* **13**, 595–599.

Difference in sensitivity to vasoconstrictor drugs within the wall of the sheep carotid artery

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Sympathetic nerve fibres penetrate the outer $\frac{1}{2}$–$\frac{3}{4}$ of the smooth muscle of the tunica media of the sheep carotid artery (Keatinge, 1966). It seemed important to know whether the innervated and non-innervated portions of the muscle coat had different sensitivities to vasoactive hormones, and the present experiments were designed to show whether this was the case. Spiral strips cut from this artery were placed between two plates for several minutes, one maintained at a high (90° C) and the other at a low (27 or 0° C) temperature in order to kill one part of the wall by heat while leaving the other part undamaged. Strips were therefore obtained containing functioning smooth muscle either from the outer part of the media, containing nerve fibres, or from the inner nerve-free region.

Dose–response curves were determined for both types of strips in response to noradrenaline and histamine. These showed that the inner smooth muscle responded to noradrenaline in approximately 1/100 the concentration, and to histamine in approximately 1/10 the concentration required to produce comparable responses from the outer smooth muscle.

Desipramine, an inhibitor of noradrenaline uptake by adrenergic nerves (Iversen, 1967) reduced the difference in sensitivity between the two types of strip to noradrenaline. In the presence of desipramine 10^{-6} M their difference in sensitivity to noradrenaline became similar to their tenfold difference in sensitivity to histamine.

The results indicate a large difference in sensitivity of the inner and outer parts of the artery wall to noradrenaline which is partly due to uptake of noradrenaline by nerve fibres in the outer part of the wall, and

partly due to differences in sensitivity of the smooth muscle cells of the two regions to noradrenaline.

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REFERENCES

IVERSEN, L. L. (1967). *The Uptake and Storage of Noradrenaline in Sympathetic Nerves.* Cambridge University Press.
KEATINGE, W. R. (1966). *J. Physiol.* **185**, 701–715.

Fever produced in rabbits and cats by prostaglandin E_1 injected into the cerebral ventricles

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Milton & Wendlandt (1970) showed that prostaglandin E_1 (PGE_1) injected into the third ventricle of unanaesthetized cats caused an immediate rise in rectal temperature associated with vigorous shivering and piloerection. The threshold dose (about 10 ng) was considerably lower than that of 5-HT or of any other substance known to raise temperature in cats on intraventricular injection. As they pointed out, this observation is of interest not only because of the low threshold but also because of the possibility that PGE_1 is a natural constituent of c.s.f. They were able to extract a prostaglandin-like substance from c.s.f. of a cat with raised temperature. Previously such a substance had been found in the effluent from the perfused cerebral ventricles of anaesthetized cats (Feldberg & Myers, 1966).

Whenever a substance injected intraventricularly into cats raises temperature, the question arises of whether it will have the same effect in rabbits because of the known species differences regarding temperature responses to monoamines. In cats, catecholamines lower and 5-HT raises temperature, whereas in rabbits the catecholamines raise and 5-HT lowers temperature on intraventricular injection. The effect of PGE_1, however, appears to be species independent because PGE_1 was found to raise temperature in rabbits as well as in cats.

In unanaesthetized cats and rabbits with chronically implanted Collison cannulae into the left lateral cerebral ventricle, PGE_1 was injected in a volume of 0·2 ml. The injections produced hyperthermia. The threshold dose was between 10 and 20 ng in both species. These injections raised temperature 0·1–0·3° C; injections of 50–100 ng caused rises of between 0·8 and 2° C. Temperature began to rise within 1 or 2 min of the injection and

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continued to rise for at least 30–60 min. In cats vigorous shivering, and in rabbits skin vasoconstriction with increased muscle tone appeared to be the main cause of the hyperthermia. Hyperthermia was obtained with intraventricular PGE_1 (10 to 100 ng) also in the rat, a species which responds differently again from cats and rabbits to the monoamines.

If it should turn out to be true that PGE_1 is a natural constituent of the anterior hypothalamus it may be a mediator common to various hyperthermias. Pyrogen may act by release of PGE_1, a possibility pointed out by Milton & Wendlandt. On the other hand, recent results suggest that pyrogen fever is a sodium fever and that pyrogen acts by 'removal of the calcium brake' in the anterior hypothalamus (Feldberg, Myers & Veale, 1970; Feldberg & Saxena, 1970; Myers & Veale, 1970). These views are not in contradiction to each other. It is only necessary to attribute this action, i.e. the removal of the calcium brake, to the PGE_1 released by the pyrogen.

PGE_1 was kindly supplied by Dr A. S. Milton.

REFERENCES

FELDBERG, W. & MYERS, R. D. (1966). *J. Physiol.* **184**, 837–855.
FELDBERG, W., MYERS, R. D. & VEALE, W. L. (1970). *J. Physiol.* **207**, 403–416.
FELDBERG, W. & SAXENA, P. N. (1970). *J. Physiol.* **207**, 52–53*P*.
MILTON, A. S. & WENDLANDT, SABINE. (1970). *J. Physiol.* **207**, 76–77*P*.
MYERS, R. D. & VEALE, W. L. (1970). *Science, N.Y.* **170**, 95–97.

Growth and development of a sympathetic ganglion: maturation of transmitter enzymes and synapse formation in the mouse superior cervical ganglion

BY I. B. BLACK, F. E. BLOOM, I. A. HENDRY and L. L. IVERSEN. *Department of Pharmacology, University of Cambridge*

Choline acetyl transferase and tyrosine hydroxylase activities and total protein were estimated in aliquots of homogenized ganglia pairs (left and right mouse superior cervical ganglia) using sensitive micro-radiochemical and colorimetric assay procedures. The results obtained with ganglia from mice of different ages showed that there were highly significant increases in total ganglion protein, choline acetyl transferase, and tyrosine hydroxylase during the first 3 weeks after birth. There was a 34-fold increase in the total choline acetyl transferase activity during development, the enzyme increasing progressively from birth and reaching adult levels by 25 days. Tyrosine hydroxylase increased by six-fold between birth and adulthood, with the most rapid increase occurring between 7 and 13 days, by which time the enzyme had reached adult levels. Total ganglion protein showed a

gradual threefold increase throughout development; there were, thus, marked increases in the specific activities of the two transmitter enzymes during the postnatal period.

In an attempt to correlate these biochemical findings with the morphological changes occurring in the developing ganglion, estimates were made of ganglion volume and total numbers of synaptic junctions, using light microscope and electron microscope techniques (Bloom & Aghajanian, 1968). There was approximately a 500-fold increase in the total number of ganglionic synaptic junctions during the first 8 days after birth, with only a small increase between 8 and 30 days. Total ganglion volume, on the other hand, estimated by light microscope examination of serial sections of the same fixed tissues, increased progressively between 1 and 30 days, with a 70-fold rise during this period.

Thus, the developmental rise in choline acetyl transferase activity occurs both during and after the early period in which the majority of ganglionic synapses are formed. The increase in the activity of this presynaptic enzyme, therefore, does not simply reflect the invasion of the ganglion by preganglionic cholinergic fibres and subsequent synapse formation, but may also be associated with the functional maturation of these synapses. The elevation of tyrosine hydroxylase activity, however, occurs subsequent to the early phase of synapse formation, indicating that the development of this enzyme may depend on the innervation of the post-synaptic cells.

To investigate this possibility, superior cervical ganglia were unilaterally decentralized in 7-day-old mice by surgical transection of the preganglionic nerve trunk. The contralateral intact ganglion served as a control. At 21 days of age, ipsilateral ptosis and decreased ganglion choline acetyl transferase indicated the efficacy of this procedure. The decentralized ganglia contained significantly lower tyrosine hydroxylase activity than the contralateral control ganglia (30% of control values). These preliminary findings thus suggest that the preganglionic innervation of adrenergic neurones in the sympathetic ganglion may influence the maturation of these neurones.

REFERENCE

BLOOM, F. E. & AGHAJANIAN, G. K. (1968). *J. Ultrastruct. Res.* **22**, 361–375.

The stimulation of sheep monocyte mitosis *in vitro* by autologous plasma taken after implanting a Teflon chamber into the sheep

BY B. GREENWOOD. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

Sheep blood monocytes will grow and mitose when cultured in autologous plasma upon glass coverslips (Greenwood, 1969). During the growth

phase the monocyte assumes the so-called macrophage form. Mitosis, which occurs after a few days in culture, gives rise to two daughter cells of macrophage appearance, far larger than blood monocytes *in vivo*. Mitotic indices are always low, reaching a peak of about 0·8% after 8–10 days in culture.

During experiments in which Teflon chambers were being implanted subcutaneously into the shoulder region of sheep in order to collect wound fluid (Greenwood, 1970), monocytes from the blood of these sheep, taken within a week of operation, showed more than the expected number of mitoses in culture. During this period the wound fluid from the Teflon chamber contained few monocytes, and none was seen in mitosis.

It was not clear if these numerous mitoses in culture indicated a fresh monocyte population with an increased mitotic potential, or the presence in the plasma of some mitogenic component.

By culturing pre- and post-operative blood monocytes with both pre- and post-operative autologous plasmas, it was possible to show that a mitogenic stimulus lay in the post-operative plasma.

In order to study the mitogenic potency of the plasma a control sample was taken before operation, and samples were taken on each day of the first post-operative week. All these samples were stored at −20° C. Some weeks after operation blood was taken from the sheep and a series of monocyte cultures set up. These were grouped, and each group had, as its medium, the pre-operative plasma or that of one post-operative day. In this way all the plasmas were tested against similar populations of monocytes.

These experiments showed that, on post-operative days 1 and 2, and sometimes 3, the plasma was usually toxic to cultured monocytes, often killing them. The mitogenic effect became prominent on days 4 and 5, and sometimes as early as day 3. On days 6 and 7 the mitotic indices were of levels comparable with those of cells in the control plasma.

It is not clear whether the mitogenic effect is due to an increase in some stimulating substance, or to a decrease in an inhibitor of mitosis, such as a chalone (Bullough, 1962). A chalone has been shown to exist for granulocytes (Rytömaa & Kiviniemi, 1968), but its action is upon the immature precursors in the marrow. The mitoses described here occurred in cells which would normally be regarded as fully mature, and only after the monocyte to macrophage morphological change had taken place.

REFERENCES

Bullough, W. S. (1962). *Biol. Rev.* **37**, 307–342.
Greenwood, B. (1969). *J. Physiol.* **202**, 92–93*P*.
Greenwood, B. (1970). *J. Physiol.* **210**, 118–119*P*.
Rytömaa, T. & Kiviniemi, K. (1968). *Cell Tissue Kinet.* **1**, 329–340.

The sites of salicylate-induced antipyresis in the central nervous system of the rabbit

BY W. I. CRANSTON and M. D. RAWLINS. *Department of Medicine, St Thomas's Hospital Medical School, London, S.E. 1*

It has previously been demonstrated that at least part of the antipyretic action of salicylate is mediated through a direct action within the central nervous system (Cranston, Luff, Rawlins & Rosendorff, 1970). In order to determine the site of this central action, local injections of sodium salicylate have been given into various areas of the brains of febrile and afebrile rabbits.

Experiments were performed on lightly restrained, conscious rabbits to whose skulls Monnier & Gangloff (1961) headplates had been previously affixed under general anaesthesia. Rectal temperature was measured with a thermistor inserted 8–10 cm into the rectum; recordings were made every minute. In fifty-one experiments, a steady state of fever was induced by an intravenous priming injection followed by a sustaining infusion of homologous endogenous pyrogen (Cranston *et al.* 1970). Four hours after the start of the pyrogen infusion, bilateral (10 μl.) injections of 6–30 μg sodium salicylate in artificial c.s.f. were made into various areas of the brain using the co-ordinates of Monnier & Gangloff (1961). At the end of each experiment 10 μl. indian ink were injected to permit subsequent location of the injection site.

Changes in rectal temperature following micro-injections were measured by planimetry, and antipyresis was considered to have occurred if the response to salicylate exceeded the range of the responses to injections of artificial c.s.f. alone. Consistent antipyretic responses to salicylate injections were observed in the preoptic hypothalamus and the periaqueductal grey matter of the mid-brain. In seventeen afebrile rabbits, no antipyretic responses were observed following the micro-injection of salicylate in either of these areas.

Endogenous pyrogen has been shown to exert its pyrogenic action in the same areas of the brain. The present findings are compatible with the hypothesis that salicylates produce antipyresis by competitively antagonizing the effects of endogenous pyrogen in specific areas of the central nervous system.

We are grateful to the Medical Research Council for the loan of apparatus.

REFERENCES

CRANSTON, W. I., LUFF, R. H., RAWLINS, M. D. & ROSENDORFF, C. (1970). *J. Physiol.* **208**, 251–259.

MONNIER, M. & GANGLOFF, H. (1961). *Atlas for Stereotoxic Brain Research on the Conscious Rabbit*, vol. I. Amsterdam: Elsevier.

An analysis of the pyrogen-induced inhibition of gastric motility in sheep

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Pyrogen injected into conscious goats induces an inhibition of gastric (reticulo-ruminal) movements in association with the pyrexia (van Miert, 1968). The mechanisms underlying this inhibition are being investigated in halothane-anaesthetized sheep with gastric movements present. A test dose of pyrogen (*Escherichia coli* lipopolysaccharide) injected intravenously (0·2–1·0 μg/kg body weight) gives a characteristic response; namely a latent period of 10–13 min is followed by a sudden and total stasis of the reticulo-rumen for 20–40 min. Contractions reappear and gradually return to normal during the ensuing 20 min or more.

This test dose response is unaffected (i) by complete transection of the brain stem at levels either > 10 mm rostral or > 5 mm caudal to the obex (thereby leaving the gastric centres and their vagal connexions intact–Harding & Leek, 1970), (ii) by section of the major splanchnic nerves, removal of the adrenal glands or prior injection of adrenergic blocking agents, and (iii) by removal of the abomasum.

Single-fibre techniques are used to record from gastric vagal nerve fibres transmitting (i) afferent impulses from in series reticulo-ruminal tension receptors to the gastric centres (Leek, 1969), and (ii) efferent impulses from the gastric centres to the reticulo-ruminal musculature (Iggo & Leek, 1967). At the onset of the stasis (0–8 min) there is little or no afferent activity and no efferent activity. Experimentally induced tension receptor activity elicits a few gastric movements during this initial period only. After about 8 min of stasis, afferent (tension receptor) activity becomes normal or supranormal but efferent activity remains absent until the reappearance of gastric movements. Contractions of the reticulo-rumen may be evoked directly but not reflexly at any time during the stasis by stimulating motor fibres in a cervical vagus nerve electrically. No change in the tension receptor sensitivity seems to be involved, as the response to tension changes imposed by respiratory movements remains unaltered throughout.

Our tentative conclusions are that the pyrogen-induced inhibition of gastric movements is the reflex consequence of an initial relaxation of reticulo-ruminal muscle (i.e. fewer and weaker intrinsic movements) and of a subsequent, prolonged depression of the gastric centres in the medulla oblongata. The inhibition is not due primarily to raised sympathetic/

adrenal activity, to the reflex consequences of reduced abomasal acid secretion or to variations in the activity of central nervous connexions with regions higher or lower in the brain stem than the gastric centres.

This investigation was carried out in Edinburgh on a grant kindly provided by the Agricultural Research Council.

REFERENCES

HARDING, R. & LEEK, B. F. (1970). *J. Physiol.* **209**, 42-43*P*.
IGGO, A. & LEEK, B. F. (1967). *J. Physiol.* **191**, 177–204.
LEEK, B. F. (1969). *J. Physiol.* **202**, 585–609.
VAN MIERT, A. S. J. P. A. M. (1968). *Vet. Rec.* **82**, 632–634.

Convulsive effects of bicuculline in photosensitive baboons (*Papio papio*) and rhesus monkeys (*Macaca mulatta*)

BY B. S. MELDRUM. *M.R.C. Neuropsychiatry Unit, Carshalton, Surrey*

In baboons from the Casamance region of Senegal stroboscopic stimulation at about 25 flashes/sec (ILS) commonly induces intermittent myoclonus and electroencephalographic (e.e.g.) signs of epilepsy ('spikes' and 'spikes and waves'). In a minority of these animals the myoclonus becomes sustained and leads to a tonic-clonic seizure (Killam, Killam & Naquet, 1967). Pyridoxine antagonists, which reduce the activity of glutamic acid decarboxylase and hence the rate of formation of γ-aminobutyric acid (Holtz & Palm, 1964), in subconvulsive doses, produce a marked enhancement of myoclonic and e.e.g. responses to ILS (Meldrum, Balzano, Gadea & Naquet, 1970; Meldrum, 1971).

Bicuculline, a convulsant isoquinoline alkaloid, has been shown by Curtis, Duggan, Felix & Johnston (1970) to block the inhibitory action of GABA on cortical neurones.

In eight adolescent baboons (weights 3–7 kg) and six adult monkeys (weights 4–6 kg) skull electrodes were chronically implanted to permit the extradural recording of electrical activity from the frontal, rolandic and occipital cortex. Subsequently, at approximately weekly intervals, the animals were placed in a primate chair and given a rapid intravenous injection of a solution of bicuculline (0·1–0·6 mg/kg). Motor and e.e.g. responses to ILS were observed before and at intervals after the injection.

The dose of bicuculline just adequate to induce a seizure varied from 0·1–0·4 mg/kg. In the baboons sensitivity to bicuculline correlated with initial responsiveness to ILS.

In most animals subconvulsive doses did not augment motor or e.e.g. responses to ILS in the following 90 min. In one monkey and one baboon transient generalized myoclonus, with fronto-rolandic spikes and waves,

appeared 5–20 sec after the injection. Both these animals showed slightly enhanced responses to ILS 1–5 min later.

Seizures induced by bicuculline (0·1–0·6 mg/kg) began 2–7 sec after the injection with a cry, irregular jerks, and cortical spikes and waves. The latter either began synchronously throughout the cortex or appeared < 1 sec sooner in the frontal cortex. Seizures continued for 2–300 min with rapidly alternating tonic and clonic phases or prolonged rhythmic myoclonic activity with generalized, symmetrical spikes and waves. Seizure activity was sometimes sustained without interruption for more than 50 min, suggesting that any post-ictal silence following a brief seizure is unlikely to be due to depletion of energy reserves or transmitter substances.

The effects of bicuculline differ from those of the pyridoxine antagonists studied previously (4-deoxypyridoxine, thiosemicarbazide and isoniazid) in that subconvulsive doses produce a less marked enhancement of photosensitivity, the latency to seizures is extremely short, the seizures do not usually originate in the occipital cortex, and seizure activity is sustained without intervening periods of more normal e.e.g. activity.

REFERENCES

CURTIS, D. R., DUGGAN, A. W., FELIX, D. & JOHNSTON, G. A. R. (1970). *Nature, Lond.* **226**, 1222–1224.

HOLTZ, P. & PALM, D. (1964). *Pharmac. Rev.* **16**, 113–178.

KILLAM, K. F., KILLAM, E. K. & NAQUET, R. (1967). *Electroenceph. clin. Neurophysiol.* **22**, 497–513.

MELDRUM, B. S. (1971). *Br. J. Pharmac.* (in the Press).

MELDRUM, B. S., BALZANO, E., GADEA, M. & NAQUET, R. (1970). *Electroenceph. clin. Neurophysiol.* **29**, 333–347.

The importance of sodium and potassium ions for the active transport of glucose from the lumen of isolated loops of hamster small intestine *in vitro*

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It has been claimed by several authors (Riklis & Quastel, 1958; Parsons & Wingate, 1961; Crane, 1962; Csaky, 1963) that the presence of Na^+ is essential for the absorption of glucose from the small intestine, but that the presence of K^+ is not (Riklis & Quastel, 1958; Bihler & Crane, 1962).

Preliminary experiments carried out by us, however, suggested that a reduction in Na^+ concentration might not be as detrimental to glucose transport as hitherto believed. In the present investigation, loops of

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hamster small intestine were suspended in an isolated organ bath and the lumen was perfused with the help of a peristaltic pump. The composition of the solutions used expressed in mM/l. was:

(1) Mucosal: NaCl 117·4, KCl 5·28, KH_2PO_4 3·47, $NaHCO_3$ 26·16, $CaCl_2$ 0·0276, $MgSO_4$ 0·0065, $MgCl_2$ 0·0065, glucose 12·8.

(2) Serosal: NaCl 111·7, KCl 5·28, KH_2PO_4 3·47, $NaHCO_3$ 26·16, $CaCl_2$ 2·76, $MgSO_4$ 0·65, $MgCl_2$ 0·65, glucose 12·8.

As the concentration of glucose was the same in the mucosal and in the serosal fluids only active (uphill) transport was studied. In order to test the importance of Na^+ and K^+ these were removed from the mucosal fluid and isosmolarity preserved by making up the deficit with mannitol. With the normal Na^+ and K^+ concentration, which is approximately that of normal human ileal juice, just over 1·0 mg of glucose was lost from the lumen per cm intestine per hour and about 0·8 mg was gained on the serosal side per cm intestine per hour. Complete removal of Na^+ from the mucosal perfusion fluid reduced glucose loss by 33%, but this was not significant at the 5% level. On the serosal side no change in gain of glucose could be detected. Removal of K^+ from the mucosal fluid caused a similar reduction of mucosal glucose loss (34% ± 17%) and this again was not significant at the 0·05 level. There was, however, a significant depression of serosal gain (65·5% ± 31%; P = 0·05–0·02).

Although the individual removal of Na^+ and K^+ did not significantly depress the mucosal loss of glucose, the simultaneous removal of both cations did significantly depress mucosal loss (69% ± 16·5%; $P < 0·001$) as well as serosal gain (82% ± 27%; P = 0·01–0·001).

It thus appears that, contrary to the opinion held hitherto, Na^+ is not essential for the active transport of glucose across the small intestine provided that the mucosal fluid contains an adequate amount of K^+, as mucosal loss and serosal gain do not differ significantly from control values in its absence. K^+ too, in the presence of an adequate amount of Na^+, is not essential for glucose absorption as far as removal from the mucosal fluid is concerned, although in its absence from the mucosal fluid serosal gain of glucose is significantly depressed.

REFERENCES

BIHLER, I. & CRANE, R. K. (1962). *Biochim. biophys. Acta* **59**, 78.
CRANE, R. K. (1962). *Fedn Proc.* **21**, 891–895.
CSAKY, T. Z. (1963). *Fedn Proc.* **22**, 3–7.
PARSONS, D. S. & WINGATE, D. L. (1961). *Biochim. biophys. Acta* **46**, 170–183.
RIKLIS, E. & QUASTEL, J. H. (1958). *Can. J. Biochem. Physiol.* **36**, 347–363.

The effect of insulin on brown adipose tissue *in vivo*

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In these experiments the effects of insulin infusion on the metabolism of brown adipose tissue *in vivo* was investigated. The rabbits, aged 9 days, had been left unfed for 48 hr prior to investigation. The details of the technique used to collect blood samples and to measure the rate of blood flow from brown adipose tissue have been reported (Hardman & Hull, 1970). The arteriovenous differences across brown adipose tissue of glucose, free fatty acids and glycerol were measured before and at the tenth minute of an I.V. infusion of bovine insulin (10000 μ-u./kg min or 100 μ-u./kg min). The results using the two doses of insulin were similar and are shown together in Table 1.

Table 1. Arterial concentrations and arteriovenous differences of glucose, free fatty acids and glycerol across, and venous outflows from, the cervical brown adipose tissue of sixteen young rabbits, before and at the tenth minute of an infusion of insulin. The results are given as means ± S.E. of mean

	Initial		After insulin infusion	
	Arterial concentration	Arteriovenous difference	Arterial concentration	Arteriovenous difference
Blood glucose (mg/100 ml.)	141 ± 5·5	−3·1 ± 1·4	152 ± 5·3	−6·7 ± 0·9
Plasma free fatty acids (m-equiv/l.)	0·41 ± 0·05	+0·35 ± 0·05	0·35 ± 0·03	+0·17 ± 0·04
Plasma glycerol (mM/l.)	0·18 ± 0·01	+0·08 ± 0·01	0·17 ± 0·02	+0·10 ± 0·02
Blood flow (ml./min)	0·67 ± 0·06		0·56 ± 0·05	

Insulin reduced the arteriovenous difference of free fatty acids ($P > 0{\cdot}01$) and increased the arteriovenous difference of glucose ($P > 0{\cdot}05$). The rate of triglyceride hydrolysis, as judged by the rate of glycerol release, remained unchanged. The results show that insulin has a marked and immediate effect on the rate of fatty acid release from brown adipose tissue of starved young rabbits.

REFERENCE

Hardman, M. J. & Hull, D. (1970). *J. Physiol.* **206**, 263–274.

The bronchodilator action of carbon dioxide

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CO_2 causes bronchoconstriction in intact dogs with intact vagi (Nadel & Widdicombe, 1962; Green & Widdicombe, 1966). In isolated cat lungs bronchodilatation may occur (Nisell, 1950). After pulmonary artery occlusion, bronchoconstriction occurs which is attributed to local hypocapnia since it is reversed by ventilation with CO_2 (Severinghaus, Swenson, Finley, Lategola & Williams, 1961). We have demonstrated a bronchodilator action of CO_2 when bronchial tone is high from several causes.

In anaesthetized open-chest cats or dogs ventilated with positive pressure, we measured intratracheal or intrabronchial pressure (P), air-flow with a pneumotachograph ($\dot{V}$) and tidal volume (V) by integrating the flow trace. Dynamic compliance (DC) was measured as V/P at zero airflow, total lung resistance (TLR) as the slope of the P/$\dot{V}$ loops (subtractor method, Mead & Whittenburger, 1953). Tests were made on both lungs or a separately ventilated lobe; a few were made on isolated perfused lungs.

Occlusion of the left lower lobe pulmonary artery caused increases in lobar TLR, decreases in DC (thirty cats). Ventilation of the lobe with increasing CO_2 concentrations (1–15%) progressively but never wholly reversed these changes. When the lobe was ventilated beforehand with CO_2, arterial occlusion had no effect.

Infusions of 5-hydroxytryptamine increased TLR, decreased DC. Ventilation with increasing CO_2 concentrations progressively reversed these changes (1–9% to both lungs in thirteen cats and three dogs, 1–15% to one lobe in 9 cats). Arterial P_{CO_2} rose from 22 to 66 torr. In lobar experiments the changes were reversed by perfusing the lobe with hypercapnic blood while ventilating with CO_2-free gas.

CO_2 dilatation during nervous bronchoconstriction was less clear-cut; it was definite during acetylcholine bronchoconstriction. Vagal stimulation after eserine caused intense bronchoconstriction which was partly reversed by CO_2 in six out of seven cats; on withdrawing CO_2 the previous state was restored in only two cats. It was difficult to produce stable bronchoconstriction with nerve stimulation. Infusions of acetylcholine caused increases in TLR and decreases in DC which were reversibly reduced by CO_2 (four isolated cat lungs, four intact cats, one intact dog).

CO_2 reduced post mortem bronchoconstriction for about 0·5 hr after death in nine out of ten cats.

The effects of CO_2 during 5-HT and arterial occlusion were present after autonomic blockade (bretylium and atropine, four cats).

Thus CO_2 causes bronchodilatation through a local mechanism when

bronchial tone is high over a range of tensions found in health and disease. It appears to play a role in regulating local ventilation after pulmonary artery occlusion. By contrast P_{O_2} appeared to be the major factor regulating local blood flow after bronchial occlusion (Barer, Howard, McCurrie & Shaw, 1969).

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REFERENCES

BARER, G. R., HOWARD, P., MCCURRIE, J. R. & SHAW, J. W. (1969). *Circulation Res.* **25**, 747–764.
GREEN, M. & WIDDICOMBE, J. G. (1966). *J. Physiol.* **186**, 363–381.
MEAD, J. & WHITTENBURGER, J. L. (1953). *J. appl. Physiol.* **5**, 779–796.
NADEL, J. A. & WIDDICOMBE, J. G. (1962). *J. Physiol.* **163**, 13–33.
NISELL, O. (1950). *Acta physiol. scand.* **20**, suppl. 73.
SEVERINGHAUS, J. W., SWENSON, E. W., FINLEY, T. N., LATEGOLA, M. T. & WILLIAMS, J. (1961). *J. appl. Physiol.* **16**, 53–60.

Pulmonary vasodilator and vasoconstrictor actions of histamine

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Histamine is known to cause pulmonary vasoconstriction but a dilator action was demonstrated under conditions of high vascular tone in the foetal lung (Dawes & Mott, 1962). Hauge (1968*a*) provided evidence that histamine might mediate the pulmonary vasoconstrictor action of hypoxia in the rat. Conditions under which histamine caused pulmonary vasoconstriction or dilatation were studied in rats and cats.

Fourteen rat lungs were perfused at constant flow with homologous blood at 37° C by a modification of Hauge's method (1968*b*). In the control state (ventilation with $O_2 + 5\%$ CO_2), histamine (25 μg–1 mg) had no effect or caused a trivial fall in pulmonary artery pressure (P_{Pa}); vasoconstriction was never observed. When pulmonary vascular tone was raised during hypoxia (ventilation with $N_2 + 5\%$ CO_2), histamine (10–500 μg) always caused a fall in P_{Pa}. Pressure/flow relationships were measured by the vertical column method (Nichol, Girling, Jarrard, Claxton & Burton, 1951); Fig. 1 shows the results from a typical experiment. Hypoxia caused an increase in pulmonary vascular resistance; increasing doses of histamine moved the curve progressively nearer to the control. In six anaesthetized, open-chest cats, the left lower lobe was perfused at a constant rate with arterial blood. In the control state during ventilation with 100% O_2, infusions (0·79–3·93 μg/min) or single doses (1–10 μg) of histamine caused a rise in P_{Pa}. When the pulmonary vascular tone was raised by hypoxia, hypercapnia or 5-hydroxytryptamine infusion, histamine infusions or

single doses caused a fall in P_{Pa}. The effect was dose-independent; the same dose caused both vasoconstriction and vasodilatation according to the pulmonary vascular tone.

Thus pulmonary vasodilatation was demonstrated in two species when vascular tone was high. There was no evidence as to whether dilatation and constriction affect the same or different vessels.

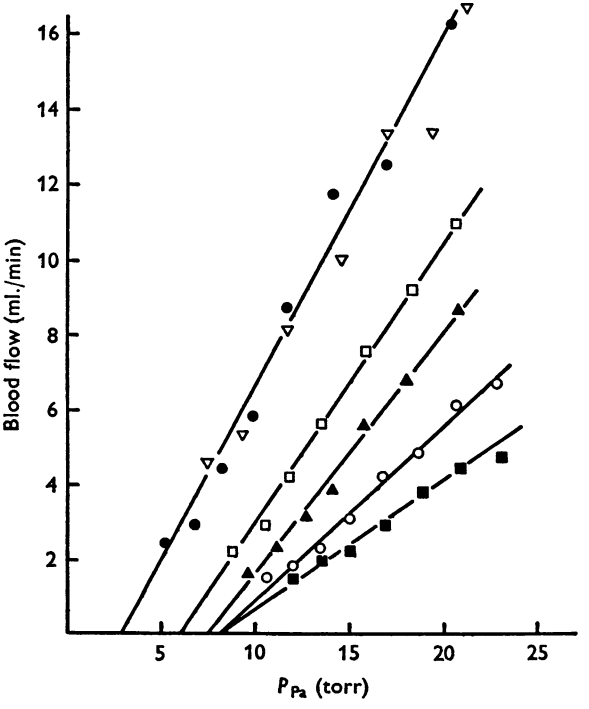

Fig. 1. Pressure/flow diagrams illustrating the vasodilator action of histamine in the isolated rat lung when vascular tone is raised during hypoxia. ●, Control, ventilation with $O_2+5\%$ CO_2; ■, hypoxia, ventilation with $N_2+5\%$ CO_2; ○, hypoxia, after 25 μg histamine; ▲, hypoxia, after 100 μg histamine; □, hypoxia, after 200 μg histamine; ▽, control, ventilation with $O_2+5\%$ CO_2. Regression lines are included.

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REFERENCES

DAWES, G. S. & MOTT, J. C. (1962). *J. Physiol.* **164**, 465–477.
HAUGE, A. (1968*a*). *Circulation Res.* **22**, 371–383.
HAUGE, A. (1968*b*). *Acta physiol. scand.* **72**, 33–44.
NICHOL, J. T., GIRLING, F., JARRARD, W., CLAXTON, E. B. & BURTON, A. C. (1951). *Am. J. Physiol.* **164**, 330–344.

Effects of changes in P_{CO_2} and pH on bronchoconstriction in the dog

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In the intact dog, hypercapnia causes bronchoconstriction which is mediated through the vagi (Green & Widdicombe, 1966). On the denervated bronchus, however, the effect of raised CO_2 is probably to cause bronchodilatation (Nisell, 1950), but this is less certain, and has therefore been examined in the present study.

In the first series of experiments fourteen dogs were anaesthetized with chloralose and urethane, paralysed with succinyl choline and artificially ventilated through a tracheostomy. Airflow was measured with a pneumotachograph, tidal CO_2 was monitored continuously and total pulmonary viscous resistance (R_L) was measured, after insertion of a pleural catheter, by the subtractor method (Mead & Whittenberger, 1953). Bilateral cervical vagal section was performed and bronchoconstriction was then induced by electrical stimulation of the peripheral ends of both vagi, by infusion of acetylcholine and by infusion of serotonin.

When the dogs were ventilated with 10% CO_2 in air instead of air, the bronchoconstrictor response to continuous infusion of serotonin was decreased (mean fall in R_L = 42%; $P < 0{\cdot}001$), but the responses to stimulation of the vagi (mean rise in R_L = 2·5%) and infusion of acetylcholine (mean fall in R_L = 8%) were not significantly changed. The action of CO_2 in reducing bronchoconstriction due to serotonin did not appear to be sympathetically mediated since it was unaltered after administration of propranolol (0·5 mg/kg), but may have been due to a change in pH since infusion of dilute hydrochloric acid also reduced serotonin-induced bronchoconstriction in seven dogs (mean fall in R_L = 26%).

The latter hypothesis was tested in a second series of experiments using isolated segments of canine bronchi suspended in Krebs–Ringer solution. The isometric contractile responses of these bronchial segments to acetylcholine (1·0–10 μg/ml.) were unaffected when the CO_2 concentration in the tissue-bath was increased from 5 to 10% CO_2, but the responses to serotonin (0·2–0·8 μg/ml.) were reduced in the presence of 10% CO_2 (mean decrease in active tension = 28%; $P < 0{\cdot}001$). When the same change in pH in the tissue-bath fluid was caused by addition of hydrochloric acid, serotonin-induced contraction was reduced by a similar amount (mean decrease in active tension = 23%; $P < 0{\cdot}001$).

It is concluded that CO_2 can cause relaxation of the pharmacologically constricted bronchus, probably through a fall in pH, but that it may act on specific bronchoconstrictor agents rather than on the contractile mechanism of bronchial smooth muscle.

REFERENCES

GREEN, M. & WIDDICOMBE, J. G. (1966). *J. Physiol.* **186**, 363–381.
MEAD, J. & WHITTENBERGER, J. L. (1953). *J. appl. Physiol.* **5**, 779–796.
NISELL, O. L. (1950). *Acta physiol. scand.* **20**, suppl. 73, 1–62.

EMG activity in tench (*Tinca tinca*, L.) gill lamellae and its association with coughing

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Normal respiration in the tench consists of a unidirectional flow of water over the gills created by movements of the mouth and operculum (Hughes & Shelton, 1958). At intervals the flow of water may be reversed and Kuiper (1907) called this coughing. Hofdijk–Enklaar (1951) observed that the gill filaments adducted during coughing, and Bijtel (1951) postulated that the gill lamellar muscles were only active during coughing.

In the present investigation experiments were conducted on tench decerebrated or anaesthetized with Nembutal (30 mg/kg I.P.). In both decerebrate and anaesthetized fish bursts of gill lamellar electromyographic (EMG) activity concurrent with respiratory movements were observed. Small amplitude bursts occurred in phase with opercular adduction and bursts often of larger amplitude and of shorter duration occurred in phase with opercular abduction. The larger amplitude bursts and the associated opercular abduction sometimes occurred with every respiration but more often every two to six respirations. A similar pattern of activity was observed in the central end of the post-trematic branch of IX (posterior to the first internal branchial cleft) but no such efferent activity was detected in the pretrematic branch of X (anterior to second internal branchial cleft). Section of the post-trematic nerve abolished the EMG activity in the corresponding gill.

Stroking the gills caused an increase in the EMG and efferent nerve activity in both anaesthetized and decerebrate fish. In the latter it was found that stroking a gill lightly produced an increase in the EMG activity of that gill alone; stronger stimulation produced an increase in the EMG activity of all gills and yet stronger stimulation also elicited a cough. After section of the post-trematic nerve to the first gill, light stroking still produced an increase in its efferent nerve activity. When the pretrematic nerve alone was sectioned stroking the posterior hemibranch of the gill no longer elicited the reflex, but it could still be elicited by stroking the anterior hemibranch. Section of both these nerves abolished it. Recordings

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of afferent activity in these nerves showed that they both carried impulses from gill touch receptors. In addition, the post-trematic nerve carried impulses from gill raker and pharyngeal touch receptors.

In decerebrate but not in anaesthetized fishes a cough could be elicited by placing a pellet of rabbit food or cotton wool in the mouth or by gently prodding the inside of the mouth with forceps. This cough was associated with an increase in the EMG activity in the gills. Coughing induced in this way and the associated increase in gill EMG activity were abolished by bilateral, but not unilateral, section of the Vth and VIIth nerves. After section of these nerves an increase in gill EMG activity could still be caused by stroking the gills.

REFERENCES

BIJTEL, J. H. (1951). *Arch. néerl. Zool.* **8**, 267–288.
HOFDIJK-ENKLAAR (1951). Quoted by Bijtel above.
HUGHES, G. M. & SHELTON, G. (1958). *J. exp. Biol.* **35**, 807–823.
KUIPER, T. (1907). *Pflügers Arch. ges. Physiol.* **117**, 1–107.

Inhibition of gastrin-induced gastric secretion by atropine in innervated and denervated pouches in the conscious rat

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In attempts to understand the mode of action of gastrin, the influence of atropine on various effects exerted by gastrin on secretory cells and smooth muscle has been studied. The pattern of atropine effects thereby observed appears equivocal, displaying species differences and dependence on the state of anaesthesia.

Gastrin-induced acid secretion is strongly inhibited by atropine in man and the conscious dog (Gregory & Tracy, 1961). In the anaesthetized cat, by contrast, atropine has not been found to inhibit acid secretion evoked by gastrin extracts of various degree of purity (Edkins, 1906; Komarov, 1942; Blair, Harper, Lake & Reed, 1961). On the other hand, in the conscious cat, atropine does inhibit acid secretion excited by gastrin extracts or gastrin pentapeptide (Emås, 1968; Borg & Emås, 1970). In the anaesthetized rat, atropine is reported not to suppress gastrin-induced acid secretion (Bennett & Hogbin, 1968).

In the present study, female rats, weighing about 200 g, were provided with denervated or innervated pouches as previously described (Svensson, 1970). During the tests the rats were awake or fallen asleep and kept in a Bollman cage. Gastric juice was collected in 30 min portions employing a perfusing arrangement. Acid secretion was determined by titration against 0·1 N-NaOH with phenol red as indicator.

In a series of experiments, hog gastrin II was infused intravenously for 6 hr at a dose of 0·3 μg/hr in the Heidenhain pouch rats and 0·08 μg/hr in the Pavlov pouch rats. The dosages employed were so chosen as to give submaximum acid secretory responses which were maintained for the period of observation. In the experiments with atropine, gastrin was similarly infused. At 2 hr after the onset of gastrin infusion, atropine sulphate, 100 μg/kg, was injected intravenously. As a result, acid secretion was inhibited by about 50 % for 2 hr in both types of stomach preparations. Comparable rates of acid secretion in the Heidenhain pouch, evoked by infusion of methacholine, were nearly completely inhibited by the same dose of atropine.

From the above results it is evident that gastrin-induced acid secretion is inhibited by atropine in the conscious rat in conformity with results obtained in man, conscious dogs and cats. The mechanism by which atropine exerts the inhibitory action is unknown. Whether the inhibition is due to obviating a background cholinergic activity, which in the Heidenhain pouch is apt to be low, or to atropine interfering at an excitable site common to gastrin and vagal influence (Rosengren & Svensson, 1969) remains an open proposition.

Hog gastrin II was donated by Professor R. A. Gregory.

REFERENCES

BENNETT, A. & HOGBIN, B. M. (1968). *J. Physiol.* **198**, 91–92*P*.
BLAIR, E. L., HARPER, A. A., LAKE, H. J. & REED, J. D. (1961). *J. Physiol.* **159**, 72–73*P*.
BORG, I. & EMÅS, S. (1970). *Scand. J. Gastroenterol.* **5**, 369–374.
EDKINS, J. S. (1906). *J. Physiol.* **34**, 133–144.
EMÅS, S. (1968). *Am. J. Dig. Dis.* **13**, 572–578.
GREGORY, R. A. & TRACEY, H. J. (1961). *J. Physiol.* **156**, 523–543.
KOMAROV, S. A. (1942). *Revue can. Biol.* **1**, 377–401.
ROSENGREN, E. & SVENSSON, S. E. (1969). *J. Physiol.* **205**, 275–288.
SVENSSON, S. E. (1970). *J. Physiol.* **207**, 329–350.

Effects of swelling upon the respiration and K content of kidney slices incubated in dilute saline media

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When the hydration of mammalian tissues increases during incubation in hypotonic saline media, this swelling is accompanied by decreases in the respiration (Robinson, 1950) and K content (Rixon & Stevenson, 1957) of

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the tissue. This investigation is concerned with the relative parts played by changes in the concentrations of external ions and, as suggested by the findings of Little & Robinson (1967), by increases in tissue water content in producing such effects in kidney slices.

TABLE 1. Effects of swelling upon K content and oxygen uptake of rat renal cortex slices incubated at 38° C for 1 hr

	Water content (kg/kg dry wt.)	K content (m-equiv/kg dry wt.)	Oxygen uptake (μl./mg wet wt./hr)
Control medium	2·81 ± 0·09	231 ± 10	4·1 ± 0·2
Low-NaCl medium	8·96 ± 0·30	90 ± 10	0·6 ± 0·2
Low-NaCl medium with PEG	2·85 ± 0·08	165 ± 5	3·0 ± 0·2

Each value is the mean ± S.D. from twelve experiments.

Slices of renal cortex from adult male rats were incubated either in a balanced saline medium, similar in composition to medium '*A* 1' of Robinson (1949) and containing NaCl as principal solute, or in hypotonic media with reduced concentrations of NaCl. Table 1 shows that slices allowed to swell in the virtual absence of external NaCl consumed oxygen at rates which were about 15% of those of control slices incubated in the NaCl-rich medium, and retained about 40% as much K. If this swelling were prevented by adding polyethylene glycol (PEG) of average mol. wt. 6000 to the medium, the oxygen uptake and K content of the tissue were reduced but only to about 70% of the corresponding values found for the controls. When slices were incubated with higher concentrations of NaCl in the medium, less extreme degrees of swelling appeared to be associated with smaller reductions in the respiration and K content of the tissue.

These results suggest that tissue water content is as important as the concentration of external Na and Cl in determining the respiration and K content of kidney slices in hypotonic saline media.

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REFERENCES

LITTLE, J. R. & ROBINSON, J. R. (1967). *J. Physiol.* **191**, 91–106.
RIXON, R. H. & STEVENSON, J. A. F. (1957). *Q. Jl exp Physiol.* **42**, 346–356.
ROBINSON, J. R. (1949). *Biochem. J.* **45**, 68–74.
ROBINSON, J. R. (1950). *Proc. R. Soc.* B **137**, 378–402.

Sodium and urea concentrations in renal papillary fluid of rats, with dehydration and vasopressin (Pitressin) administration

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Past methods for the investigation of changes in the renal medulla, during dehydration or the administration of antidiuretic hormone, have included the measurement of the melting-point of frozen tissue slices (e.g. Bray, 1960), and chemical analyses of tissue slices (e.g. Valtin, 1966).

Table 1. Concentrations of sodium and urea in m-mole/l. (means ± S.D.)

		Heterozygous rats	
		Untreated	48 hr dehydration
Sodium	Papilla	287·7 ± 61·2	485·6 ± 74·0
	Urine	201·0 ± 27·8	298·2 ± 73·0*
Urea	Papilla	379·7 ± 123·0	810·0 ± 126·6
	Urine	533·4 ± 210·8	1469·0 ± 301·0*
No. of animals		9	8

		Homozygous Brattleboro' (DI) rats		
		Untreated	+ Pitressin	14 hr dehydration
Sodium	Papilla	169·0 ± 46·2	230·8 ± 34·0	178·5 ± 22·7
	Urine	24·6 ± 4·8	169·6 ± 49·5	22·0 ± 6·6†
Urea	Papilla	71·7 ± 13·5	347·1 ± 158·8	283·7 ± 102·6
	Urine	74·7 ± 10·3	495·1 ± 192·5	414·3 ± 77·0†
No. of animals		8	8	7

* Urine sodiums and ureas estimated from a previous graph of urine solute concentrations against time during 72 hr dehydration of the same eight rats.

† Urines from bladder at death. Urines from other groups collected in metabolism cages.

A method recently demonstrated (Lee, Lewis & Williams, 1971), which employs centrifugation of the isolated papilla, was shown to give a representative sample of papillary interstitial fluid. Using this method, the sodium and urea concentrations of papillary fluid have been measured in two groups of 'normal' rats (heterozygous Brattleboro'), untreated and dehydrated, and in three groups of rats with hereditary hypothalamic diabetes insipidus (homozygous Brattleboro' DI), untreated, dehydrated or given exogenous vasopressin (1 i.u./day for 7 days, subcutaneous Pitressin

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Tannate in Oil). The changes in sodium and urea concentrations were also measured in urine samples. Results are given in Table 1.

Under all conditions sodium concentrations were significantly higher ($P < 0{\cdot}05$, Student t-test) in the papillary fluid compared with the corresponding urine, whereas urea concentrations were always lower, often significantly. Following the administration of Pitressin Tannate in Oil to DI rats both sodium ($P < 0{\cdot}01$) and urea ($P < 0{\cdot}001$) concentrations rose significantly in papillary fluid and urine; a similar result was obtained by dehydration of normal rats. However, with dehydration alone of DI rats only the urea concentration rose significantly ($P < 0{\cdot}001$ in both papillary fluid and in urine).

To account for the changes observed we consider the possibility that the action of antidiuretic hormone may involve factors other than the increase in permeability to water of the papillary collecting ducts.

We should like to express our thanks to Professor H. Valtin for supplying the Brattleboro' rats, and Parke Davis and Co. for the Pitressin Tannate in Oil.

REFERENCES

Bray, G. A. (1960). *Am. J. Physiol.* **199**, 915–918.
Lee, J., Lewis, A. F. & Williams, P. G. (1971). *J. Physiol.* **214**, 9–11*P*.
Valtin, H. (1966). *J. clin. Invest.* **45**, 337–345.

The rapid effects of lysine-vasopressin clearance on renal tissue composition in the conscious water-diuretic rat

By J. C. Atherton, Jeanne A. Evans, R. Green and S. Thomas. *Department of Physiology, University of Manchester*

Continuous infusion of lysine-vasopressin in the conscious rat results in progressive repletion of the corticomedullary solute concentration gradients after previous dissipation by sustained water diuresis (Hai & Thomas, 1969). Whether the reduction in medullary osmolality induced by water loading is entirely dependent on suppression of endogenous antidiuretic hormone (ADH) release is unknown, since infusions of isotonic saline (Atherton, Green & Thomas, 1970) and hypertonic mannitol (Atherton, Hai & Thomas, 1968) also cause profound reductions in medullary solute concentrations.

In the present experiments on conscious, water-loaded rats, ADH (60 μ-u. lysine-vasopressin/min 100 g body wt.) was given by continuous intravenous infusion until a steady-state existed with respect to both urinary and renal tissue composition: the ADH infusion was then stopped. In order to avoid changes in body fluid volume, a constant fluid load was

maintained during and after the period of ADH infusion. The following changes were observed during the latter period:

(*a*) Urinary flow increased and osmolality decreased rapidly (from 1247 ± 76 (S.E.M.) μ-osmole/g H_{20} to 166 ± 20 μ-osmole/g H_{20} at 45 min).

(*b*) Papillary sodium and urea concentrations also decreased rapidly, reaching low values characteristic of sustained water diuresis within 45 min. These changes were compounded of decreases in papillary sodium and urea contents, and an increase in papillary water content.

It is concluded that the diuresis caused by rapid plasma clearance of neurohypophysial hormones is attributable not only to decreased nephron permeability to water but also to a decrease in the osmotic force responsible for water reabsorption from the collecting duct; and that the changes in medullary composition result from direct intrarenal effects, independent of changes in body fluid volume.

REFERENCES

ATHERTON, J. C., GREEN, R. & THOMAS, S. (1970). *J. Physiol.* **210**, 45–71.
ATHERTON, J. C., HAI, M. A. & THOMAS, S. (1968). *J. Physiol.* **197**, 411–428.
HAI, M. A. & THOMAS, S. (1969). *Pflügers Arch. ges. Physiol.* **310**, 297–319.

Changes in renal function associated with the development of resistance of the renal vasculature to the arterial infusion of angiotensin

BY G. L. KLEIN, IVOR H. MILLS and R. J. WILSON. *Department of Investigative Medicine, Addenbrooke's Hospital, Cambridge*

Angiotensin was infused into the left renal artery of dogs to facilitate separation of the renal response to systemic effects and the direct effect on the kidney. Infusions were for 40 min in doses of 0·01, 0·1, 1·0 or 10 μg min^{-1}.

At the lowest dose there was no significant change in G.F.R. (C_{IN}) on either side but a significant fall in sodium excretion on the infused side ($P < 0{\cdot}05$) but not on the contralateral side. At higher dose levels there was a fall in G.F.R., C_{PAH}, urine volume and sodium and potassium excretion in the first 10 min in all dogs except three infused at 10 μg min^{-1}. Only these three dogs had a fall in urine osmolality at this stage (mean fall $\pm$ S.E.M. $= 304 \pm 34$ mOsm kg^{-1}: $P < 0{\cdot}001$). In the second and subsequent 10 min periods the G.F.R. recovered in all experiments but the sodium excretion did not reach control levels except in the 10 μg min^{-1} infusion experiments. Whereas in the lowest two doses of infusion the sodium excretion continued to fall throughout the 40 min, in the 1·0 μg min^{-1} infusions the mean sodium excretion began rising after the first

10 min. This was associated with a progressive fall in urine osmolality (mean fall in fourth experimental period $\pm$ S.E.M. = 155 ± 50; P, on paired values, $< 0{\cdot}02$). At the same time the urine osmolality from the uninfused kidney showed no significant change from its initial high control level of 1020 ± 124 (S.E.M.). This indicates that the change in urine osmolality is due to an intrarenal mechanism and not to a change in circulating A.D.H.

When angiotensin was infused at 10 μg min^{-1} there was a progressive fall in urinary osmolality after the first 10 min on both left and right sides. On the left it fell from a mean ($\pm$ S.E.M.) of 920 ± 107 mOsm kg^{-1} to 391 ± 55 ($P < 0{\cdot}001$); on the right side from 1061 ± 136 to 589 ± 83 ($P < 0{\cdot}005$). After the infusion at either this dose or 1·0 μg min^{-1} was stopped, neither side had a significant rise in urinary osmolality during the next 20 min. By contrast, the elevated sodium excretion with the 10 μg min^{-1} infusion dropped significantly on the left side during the first 20 min of recovery ($P < 0{\cdot}01$).

During the infusions, whenever the rate of sodium excretion by the kidney was rising there was an associated fall in urine osmolality. However, since in the post-infusion recovery stage the changes in sodium and water excretion are out of phase, one explanation for the changes would be that the mechanism which enables the kidney to develop vascular resistance to angiotensin is itself responsible for the changes in urinary osmolality.

The occurrence of some amino acids in, and their release from, isolated supra oral sphincter preparations of the sea anemone *Actinia equina*

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

Glutamic acid produces a strong inhibition of the contractile response of the isolated supra oral sphincter preparation of the sea anemone, *Actinia equina*, to electrical stimulation. This action seems to be exerted on a glutamate receptor with high specificity (Carlyle, 1970, 1971). These observations suggested that glutamic acid, or a chemically related substance, might have some transmitter function in the supra oral sphincter.

Sphincters have been homogenized, the protein precipitated and the amino acids in the supernatant separated and estimated by thin-layer chromatography, gas-liquid chromatography (Islam & Darbre, 1969) and analytical ion exchange chromatography on a Technichon Auto Analyser using the method of Nunn & Vega (1968). Glutamic acid is found in the supra oral sphincter together with sixty-six to seventy other ninhydrin

positive materials of which thirty-six have been identified and estimated (Table 1.) It seems that γ-amino-butyric acid is absent, though the evidence is, as yet, inconclusive on this point.

TABLE 1. Concentrations of some free amino acids and related substances (in n-mol ± S.E./g) in the supra oral sphincter of *Actinia equina*. Values are the mean of seven experiments

Cysteic acid	82 ± 16	Cystine	372 ± 54
Taurine	47 ± 9	Valine	87 ± 17
Aspartic acid	23 ± 7	Methionine	128 ± 24
Hydroxyproline	38 ± 11	Isoleucine	Trace
Threonine	470 ± 44	Leucine	100 ± 14
Serine	370 ± 62	Norleucine	347 ± 38
Asparagine	154 ± 28	Tyrosine	174 ± 32
Glutamic acid	1590 ± 370	Dopa	17 ± 4
Glutamine	14 ± 6	Phenylalanine	148 ± 16
Sarcosine	Trace	Ethanolamine	80 ± 7
Proline	40 ± 8	Tryptophan	872 ± 84
Glycine	380 ± 47	Ornithine	87 ± 23
Alanine	310 ± 38	Lysine	272 ± 103
Citrulline	16 ± 7	Histidine	107 ± 42
α-Amino-*n*-butyric acid	12 ± 4	3-Methyl histidine	Trace
α-Amino-*iso*-butyric acid	42 ± 8	Arginine	122 ± 34
β-Amino-*iso*-butyric acid	Trace	Homoarginine	Trace
β-Alanine	727 ± 102	Cysteine	37 ± 9

When suspended in sea water the sphincter releases glutamic acid (0·7–2·1 n-mol/g.min) and other substances. Electrical stimulation increases the output of glutamic acid (2·1–9·8 n-mol/g.min). However, this release is accompanied by the release of other amino acids and ninhydrin positive substances so that it is too early to suggest that glutamic acid has a transmitter function in this preparation.

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REFERENCES

CARLYLE, R. F. (1970). *J. Physiol.* **208**, 67–68*P*.
CARLYLE, R. F. (1971). *J. Physiol.* **212**, 32 & 33*P*.
ISLAM, A. & DARBRE, A. (1969). *J. Chromat.* **43**, 11–24.
NUNN, P. & VEGA, A. (1968). *Sixth Colloquium on Amino Acid Analysis*, pp. 80–86. Geneva: Technicon International Division S.A.

The effect of carabachol on the efflux of sodium from smooth muscle

By A. F. Brading. *Department of Pharmacology, University of Oxford*

Cholinergic drugs depolarize intestinal smooth muscle (Kuriyama, 1970). One mechanism which may be involved is an increase in membrane permeability to sodium. The experiments reported here were designed to test this possibility. Guinea-pig taenia coli muscle was used, and the effect of application of carbachol ($5{\cdot}5 \times 10^{-5}$ M) was studied on the washout of ^{24}Na from the tissue.

In normal Krebs solution, this concentration of carbachol had no effect on the Na efflux, although it produced about 100-fold increase in the rate of K loss (Burgen & Spero, 1968). The apparent lack of effect of carbachol on the Na efflux could be due to the difficulty of observing an increased efflux, since Na exchanges extremely rapidly in this tissue, and the ionic concentration gradient is unfavourable.

To improve the situation, several methods of increasing the $[Na]_i$ were employed. These were: exposure to (*a*) cold (3° C), (*b*) Ca^{2+} free, Mg^{2+} free Krebs solution, (*c*) ouabain $1{\cdot}7 \times 10^{-6}$ M, and (*d*) K^+ free solution. The tissues were loaded in these solutions until the $[Na]_i$ approached $[Na]_o$ (total tissue sodium increasing by 1·8–2·2-fold). Under these conditions the efflux of sodium can be followed accurately for at least 2 hr.

Carbachol had no effect on the Na efflux at 3° C, or in Ca^{2+} free, Mg^{2+}-free Krebs solution. Further, it had no effect on the Na^+ efflux from tissues preloaded in Ca-free Mg-free solution and washed out in normal Krebs (these tissues respond mechanically to carbachol). After 4 hr exposure to ouabain, carbachol evokes a small contraction, but there was little increase in the Na^+ efflux. Only after 4 hr exposure to K^+-free Krebs solution was an increase in rate of Na^+ loss consistently observed. The maximum increase was from 0·035 to 0·055 min^{-1}, an increase of only 1·57-fold.

Using the constant field assumptions (Hodgkin & Katz, 1949), depolarization of the cells should increase the rate of Na loss even if no change in P_{Na} occurs. The increase in Na efflux produced by carbachol in K^+-free solution could be explained by a depolarization of about 15 mV, with no change in P_{Na}, or by a 57 % increase in P_{Na} with no depolarization, or by an appropriate combination of these effects. In tissue with high $[Na]_i$ where no effect of carbachol on Na efflux was observed, it might be concluded that the drug had no effect on either membrane potential or P_{Na}, but when a mechanical response occurs, this explanation seems rather improbable. These results could also be explained if carbachol increases intracellular binding of sodium, thus reducing the free sodium concentration.

If at the same time the intracellular free potassium concentration is increased this could account for the surprisingly large increase in potassium efflux seen in response to carbachol in normal solution.

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REFERENCES

BURGEN, A. S. V. & SPERO, L. (1968). *Br. J. Pharmac.* **34**, 99–115.
HODGKIN, A. L. & KATZ, B. (1949). *J. Physiol.* **108**, 37–77.
KURIYAMA, H. (1970). *Smooth Muscle.* Ed. BÜLBRING, E., BRADING, A., JONES, A. W. & TOMITA, T. London: Edward Arnold.

The measurement of blood flow by indicator-dilution methods

BY HILARY BATE and JOHN A. SIRS. *Physics Department, The Medical College of St Bartholomew's Hospital, London, E.C. 1 and Department of Biophysics, St Mary's Hospital Medical School, London, W. 2*

In a preceding communication (Bate, Rowlands, Sirs & Thomas, 1969) we suggested that the differences between indicator–dilution curves, obtained when two indicators are injected simultaneously, may be due to the effect of molecular diffusion superimposed on the mass flow. Further investigation of this problem has revealed that indicator dilution methods for measuring blood flow (Zierler, 1962) are only valid when the indicator is uniformly distributed across the cross-section at the observation point. While this may occur with slow flow rates, when the diffusion of the indicator is the dominant factor, in general with *in vivo* measurements this is not the case. An experimental investigation has been made by measuring the flow rate in a glass tube directly and by the indicator-dilution method using radioactive iodide [^{131}I]. At a true measured flow rate of $97{\cdot}8 \times 10^{-3}$ cm^3 s^{-1}, the indicator–dilution method gave $83{\cdot}4 \times 10^{-3}$ cm^3 s^{-1}. By interposing a mixing chamber in the flow system, just prior to the scintillation detector, to provide uniform distribution of the iodide across the tube, the two methods of measurement agreed within the experimental error of $\pm 4\%$.

REFERENCES

BATE, H., ROWLANDS, S., SIRS, J. A. & THOMAS, H. W. (1969). *J. Physiol.* **202**, 38–39*P*.
ZIERLER, K. L. (1962). In *Handbook of Physiology*, Section 2: Circulation, vol. I, pp. 585–615, ed. HAMILTON, W. F. Washington, D.C.: American Physiological Society.

Is there a T-system in frog cardiac muscle cells?

By R. A. Chapman. *Department of Physiology, University of Leicester.*

Electron microscopic investigation of the structure of frog ventricle has failed to reveal a system of transverse tubules (T-system) in these muscle cells (Staley & Benson, 1968). Similarly, the ultrastructure of frog auricular trabeculae shows little evidence of a T-system. The consequences of these observations have been tested on isolated auricular trabeculae by recording with intracellular micro-electrodes the time courses of the change in membrane potential when the [K] in the bathing medium is rapidly altered, using the method of Chapman & Tunstall (1969), and by exposing the muscle to Ringer made hypertonic with glycerol, a procedure known to disrupt the T-system in other muscles (Howell, 1969).

The change in membrane potential on increasing $[K]_o$ is more rapid than that associated with a reduction of $[K]_o$. However, when the approximately logarithmic relationship between the $[K]_o$ and the membrane potential is taken into account the half times of the changes in membrane potential are not significantly different (increasing $[K]_o$ $t_{\frac{1}{2}}$ 3·37 sec, S.E. 0·28; decreasing $[K]_o$ $t_{\frac{1}{2}}$ 3·12 sec, S.E. 0·38; 27 cells from five preparations).

Exposure of auricular trabeculae to Ringer + 400 mM glycerol leads to a rapid reduction of the twitch response due to a shortening of the time to the peak of the twitch. The peak time then lengthens and the twitch tension increases for up to 1 min, after which there is a slow decline of the twitch not accompanied by a change in the peak time, which continues for up to 20 min. Removal of the glycerol causes the twitches to first shorten and then lengthen, so that the amplitude of the twitch first falls below and then rises above the level in glycerol-Ringer, and a small phasic contracture may develop. The twitch tension and the contractures evoked by K-rich solutions (high-K) then fall until they are stabilized at the pre-glycerol level after about 30 min.

When the bathing fluid is made hypertonic with a non-permeating molecule, such as sucrose, the twitch response and contracture are almost immediately abolished ($t_{\frac{1}{2}} \simeq 3$ sec). Return to normal tonicity after a few minutes results in the rapid reappearance of twitch and high-K contractures. Exposure to Ringer + 400 mM sucrose for periods over 10 min has more profound effects. Return to normal tonicity Ringer then results in the development of a large, often long lasting contracture. The twitch response returns as this contracture subsides and can take up to 1 hr to return to the pre-sucrose level in normal Ringer solution.

These results, on the effect of pre-treatment with hypertonic glycerol Ringer and on the rate of change in membrane potential associated with suddenly altering the $[K]_o$, suggest that there is little or no T-system present

in frog cardiac muscle cells. These findings therefore support the conclusions drawn from studies of the ultrastructure of frog heart.

REFERENCES

CHAPMAN, R. A. & TUNSTALL, J. (1969). *J. Physiol.* **201**, 9–11*P*.
HOWELL, J. N. (1969). *J. Physiol.* **201**, 515–533.
STALEY, N. A. & BENSON, E. S. (1968). *J. cell Biol.* **38**, 99–114.

Some effects on vestibulo-spinal outflow at lumbosacral levels from neck muscle afferents

BY V. C. ABRAHAMS. *Department of Physiology, Queen's University at Kingston, Ontario, Canada*

In the decerebrate cat a motor discharge is initiated at all levels of the spinal cord by stimulation of the vestibular nerve (Gernandt, Iranyi & Livingston, 1959). Head ventroflexion strongly inhibits the vestibular induced discharge at cervical levels but only weakly affects discharge at lumbosacral levels (Gernandt & Gilman, 1959). This inhibiting effect of head position was attributed to the activation of receptors in the dorsal muscles of the neck. Descending effects from afferents from the dorsal muscles of the neck on the lumbosacral cord were found to be powerful in the cat anaesthetized with chloralose, but absent in the spinal or decerebrate cat (Abrahams & Falchetto, 1969; Abrahams, 1970*a*). Presumably, this is because descending spinal effects from neck muscle afferents are dependent on the integrity of supratentorial structures. In particular they are dependent on the integrity of regions of the cerebral cortex in the anterior pole of the suprasylvian gyrus which receive input from neck muscle proprioceptors (Landgren & Sylfvenius, 1968; Abrahams, 1970*a*).

It now seems apparent that in common with other descending effects from neck muscle nerves, supratentorial structures are necessary for neck muscle afferents to affect vestibulo-spinal output at lumbosacral levels. In the chloralose-anaesthetized cat, stimulation of the cut central end of the nerve to the neck muscle, biventer cervicis, inhibits vestibulo-spinal output in L7 and S1 ventral roots for 200–400 msec. Stimulation of the vestibular nerve in the chloralose-anaesthetized cat also leads to a cycle of presynaptic and post-synaptic excitability changes in the lumbosacral cord resembling those that follow stimulation of skin and muscle nerves entering the cervical cord (Alvord & Fuortes, 1954; Abrahams, 1970*b*). Decerebration abolishes the ability of neck muscle nerves to affect lumbosacral outflow and reduces or abolishes the effects of vestibular nerve stimulation on lumbosacral excitability.

Neck muscle effects on vestibulo-spinal outflow are unlikely to be dependent on the same cortical areas that are involved in other descending

effects from neck muscles (Abrahams, 1970*a*). In six out of eight experiments the prolonged inhibition of vestibulo-spinal outflow which follows neck muscle nerve stimulation not only survived excision of the contralateral anterior pole of the suprasylvian gyrus, but also survived excision of the neighbouring region of the ectosylvian gyrus which receives input from vestibular nerves (Walzl & Mountcastle, 1949; Landgren, Silfvenius & Wolsk, 1967*b*). It is concluded that neck muscle afferents can affect vestibulo-spinal output at lumbosacral as well as at cervical levels. For these effects to be readily demonstrated, supratentorial structures are necessary.

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REFERENCES

ABRAHAMS, V. C. (1970*a*). *J. Physiol.* **209**, 45–56.
ABRAHAMS, V. C. (1970*b*). *J. Physiol.* **210**, 175–176*P*.
ABRAHAMS, V. C. & FALCHETTO, S. (1969). *J. Physiol.* **203**, 435–447.
ALVORD, E. C. & FUORTES, M. G. F. (1954). *Am. J. Physiol.* **176**, 253–261.
GERNANDT, B. E. & GILMAN, S. (1959). *Expl Neurol.* **1**, 274–304.
GERNANDT, B. E., IRANYI, M. & LIVINGSTON, R. B. (1959). *Expl Neurol.* **1**, 248–273.
LANDGREN, S. & SILFVENIUS, H. (1968). *Acta physiol. scand.* **74**, 340–347.
LANDGREN, S., SILFVENIUS, H. & WOLSK, D. (1967). *J. Physiol.* **191**, 561–573.
WALZL, E. M. & MOUNTCASTLE, V. B. (1949). *Am. J. Physiol.* **159**, 595.

Conduction block after a pneumatic tourniquet

BY G. DANTA, T. J. FOWLER and R. W. GILLIATT. *Institute of Neurology, Queen Square, London, W.C. 1*

The peripheral nerve conduction block which may follow the application of a tourniquet (Denny-Brown & Brenner, 1944) has been studied in sexually mature female baboons (*Papio papio*). Under barbiturate anaesthesia a rubber cuff 10×5 cm in a specially reinforced sleeve (Accoson) was inflated around the knee for periods of 1–3 hr, the cuff pressure being maintained at approximately 1000 mm Hg. In order to test conduction through the site of nerve compression, the motor fibres to a small foot muscle (abductor hallucis brevis) were stimulated above and below the level of the cuff, and the muscle response recorded through subcutaneous belly-tendon electrodes. Nine nerves were studied in eight animals. Repeated examinations were made under anaesthesia, commencing 1–6 days after application of the tourniquet and continuing for up to 6 months.

In the first week after making the lesion, the muscle response to nerve stimulation in the thigh was abolished in two animals and reduced in amplitude in the remainder. In Fig. 1 the amplitude of the muscle response to nerve stimulation in the thigh is shown as a percentage of that obtained

by stimulation at the ankle in the same experiment. The duration of the tourniquet in minutes is also shown. It can be seen that, although there was considerable individual variation, a long tourniquet time usually resulted in a more severe and persistent conduction block than a short one. In the two most severely affected animals 6–8 weeks elapsed before significant recovery occurred.

Maximal motor conduction velocity through the compressed region was reduced, as described by Mayer & Denny-Brown (1964) in cats. In some of our animals this reduction was seen as early as the first day after the lesion was made. Maximal velocity gradually increased during the recovery period but in the two most severely affected animals it was still abnormal after 4–6 months.

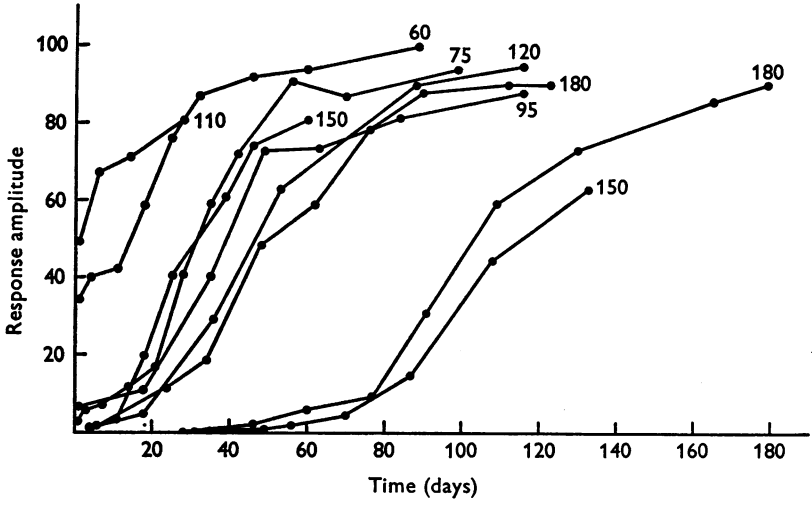

Fig. 1. Recovery after conduction block produced by a tourniquet in nine nerves. Muscle action potentials recorded from abductor hallucis brevis with motor nerve stimulation in thigh and at ankle; cuff applied at knee. On vertical scale, amplitude of muscle response to proximal stimulation as percentage of response to distal stimulation. Horizontal scale, time in days after tourniquet. The duration of the tourniquet in minutes is shown for each nerve.

Little damage to nerve fibres below the level of the cuff occurred. With below-knee or ankle stimulation, the muscle response from abductor hallucis brevis was only slightly reduced in amplitude after the tourniquet; conduction velocity between knee and ankle did not show a significant change.

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REFERENCES

DENNY-BROWN, D. & BRENNER, C. (1944). *Archs Neurol. Psychiat., Chicago* **51**, 1–26.
MAYER, R. F. & DENNY-BROWN, D. (1964). *Neurology, Minneap.* **14**, 714–726.

The effect of intraventricular noradrenaline, and acetylcholine, on plasma unesterified fatty acids in the sheep

BY D. P. CLOUGH and G. E. THOMPSON. *Department of Physiology, Hannah Dairy Research Institute, Ayr, Scotland*

Administration of noradrenaline (3 μg/kg) into a lateral ventricle of the brain of oxen when in a cold environment, results in inhibition of fat mobilization and a fall in plasma unesterified fatty acid (U.F.A.) concentration (Clough & Thompson, 1970). The present experiment was undertaken to confirm this effect of noradrenaline in sheep, and to study the effect of intraventricular acetylcholine on plasma U.F.A. concentration.

Four Finnish Landrace × Dorset Horn sheep, each with a lateral ventricle of the brain and a jugular vein cannulated, were given intraventricular noradrenaline (3 μg/kg) when in an environment of $-15°$ C, and intraventricular acetylcholine (5 μg/kg) with eserine (5 μg/kg) when in an environment of $+5°$ C, and blood samples (20 ml.) were taken from the jugular cannula. The lipids were extracted from the plasma of each blood sample and the U.F.A. separated from other lipid classes by thin layer chromatography. The methyl ester derivatives of the fatty acids were then separated by gas-liquid chromatography and quantitated by comparison with a heptadecanoic acid internal standard.

Between 10 and 30 min after a control injection of sterile water into the lateral ventricle of the sheep at $-15°$ C, plasma U.F.A. concentration averaged 26·8 mg/100 ml. Between 10 and 20 min after noradrenaline injection, the total U.F.A. fell by $10·9 \pm 2·13$ (S.E.M.) mg/100 ml. ($P < 0·02$). The decrease in total plasma U.F.A. resulted from decreases in all the major individual fatty acids, but oleic acid decreased more than the others.

Between 15 and 45 min after a control injection of sterile water into the lateral ventricle of the sheep at $+5°$ C, plasma U.F.A. concentration averaged 12·0 mg/100 ml. Between 15 and 30 min after injection of acetylcholine with eserine, the total U.F.A. increased by $16·0 \pm 4·94$ mg/100 ml. ($P < 0·05$). The increase in total plasma U.F.A. resulted from increases in all the major individual fatty acids, but oleic acid increased more than the others. Acetylcholine with eserine had the same effect on plasma U.F.A. concentration when injected into two sheep in an environment of $+30°$ C.

These results indicate that, injected into the brain of the sheep, acetylcholine and noradrenaline respectively result in fat mobilization and inhibition of fat mobilization.

REFERENCE

CLOUGH, D. P. & THOMPSON, G. E. (1970). *J. Physiol.* **208**, 85–86*P*.

Deformation of the lung by its own weight

BY FRANK L. MATTHEWS and JOHN B. WEST. *University of California, San Diego, La Jolla, California and Imperial College, London, S.W. 7*

The lung is easily deformed by small pressures and there is now abundant evidence that it is distorted by its own weight. We have analysed the distribution of gravity-induced stress, strain and surface pressure in a theoretical model using the technique of finite elements. These predictions have been compared with histologic measurements on dog lungs fixed *in situ* by freezing.

The results show that in the upright lung, the alveoli decrease in size from apex to base. The stresses are greatest at the apex of the upper lobe. As the lung is inflated from very low volumes to total lung capacity, the volume of the alveoli at the apex and the stresses in this region first decrease then increase. This behaviour can be explained by the increasing rigidity of the expanded lung which enables it to resist distortion by its weight. At functional residual capacity, the stress at the apex is near its minimum. The differences in intrapleural pressure down the lung are volume dependent increasing strikingly at very low volumes. In the inverted lung, the regional differences in stress, strain and surface pressures are less marked because of the shape of the chest.

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Electrical measurement of pulmonary oedema with a focusing conductivity bridge

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Lung resistivity, strongly influenced by water content, can be effectively measured through the chest wall provided chest wall current is shielded from the measuring electrode. Adapted from the guard ring principle of Graham (1965) and Cooley & Longini's (1968) single follower guard ring method, the device schematically shown in Fig. 1 consists of a 5 cm diam.

'detector' electrode surrounded in four quadrants by guard electrodes (only two are illustrated), each with its own skin potential detector and operational amplifier servo-nulling the lateral gradient on the skin surface. Measuring electrode current is thus largely normal to the surface and is primarily dependent upon the conductivity of the ipsilateral underlying

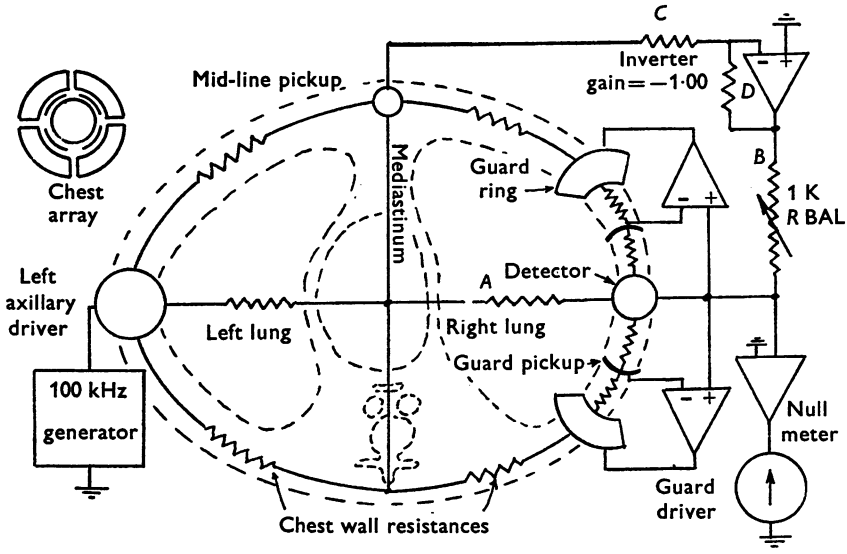

Fig. 1. A schematic circuit of the resistances of lung and chest wall in a mid-thoracic transection, with the method of cancelling chest wall current in a detector electrode by using four quadrant guard rings (two shown) operationally driven to null the lateral gradient of skin potential. When the bridge is balanced to null the potential on the detector electrode, unilateral lung resistance A = calibrated resistance B.

lung. The unilateral lung resistance, A, is measured by detecting body mid-line potential, inverting it with gain = $-1{\cdot}000$ (resistance of C = D) to drive the balancing resistor B. Capacity and resistance are separately manually balanced by shifting null detector phase sensitivity. The four skin servo-guard amplifiers which 'focus' the electrode are mounted in the chest electrode array, and the entire device weighs 1·5 kg when mains operated.

Pulmonary oedema, induced by fluid overload in fourteen dogs, was quantified by a double indicator dilution method (Chinard, Enns & Nolan, 1962) using tritium and dye or heat and conductivity after I.V. injection of room-temperature 3 % NaCl detected from an aortic blood temperature and conductivity sensing catheter. Chest conductivity increased 0·21 % for each 1 % rise in lung water, with a detection threshold of about 4 % change in

lung water. While acute occlusion of the main pulmonary artery had no immediate effect on conductivity, haemorrhagic shock slowly doubled resistance. In normal men, resistance varied from 350 to 700 ohms at FRC (functional residual capacity), which is in reasonable agreement with calculated resistivity of the cylinder of lung under the measuring electrode. Day-to-day repeatability was ± 5 %. Resistance varied two-fold over the full range of lung volume.

REFERENCES

CHINARD, F. P., ENNS, T. & NOLAN, M. F. (1962). *J. appl. Physiol.* **17**, 179–183.
COOLEY, W. L. & LONGINI, R. L. (1968). *J. appl. Physiol.* **24**, 429–432.
GRAHAM, M. (1965). *IEEE Trans. bio-med. Engng.* **125**, 197–198.

Bradykinin in forearm venous blood during sweating

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Fox & Hilton (1958) showed the presence of a plasma-kinin forming enzyme in human sweat, with increased amounts of bradykinin in the perfusate of the subcutaneous space in the forearm during sweating. They considered that vasodilatation in the forearm during body heating was the result of bradykinin formation. Allwood & Lewis (1964) were unable to find an increase in bradykinin in venous blood samples withdrawn during forearm vasodilatation produced by various means, including body heating, compared with that in control samples. Carretero, Nasjletti & Fasciolo (1965) also found no significant difference in bradykinin in blood samples withdrawn from the antecubital vein during thermal vasodilatation.

In the present experiments blood samples withdrawn from superficial forearm veins during more intensive stimulation of sweat glands have been assayed for bradykinin by the method of Allwood & Lewis (1964). In fifteen experiments on ten healthy male subjects, immersion up to the chin in hot baths at 40–41° C for 30–50 min produced no significant difference in bradykinin content in effluent venous blood, although there was a mean rise in oral temperature of 1·3° C and a loss in weight due to sweating of 520 g.

In six healthy subjects given insulin I.V. there was no increase in bradykinin in venous blood samples withdrawn during sweating during insulin hypoglycaemia, at a time when forearm blood flow, measured by venous occlusion plethysmography, was approximately doubled.

In three other subjects there was no increase in bradykinin in venous blood samples withdrawn during the 'cold sweat' following vaso-vagal syncope.

REFERENCES

ALLWOOD, M. J. & LEWIS, G. P. (1964). *J. Physiol.* **170**, 571–581.
CARRETERO, O., NASJLETTI, A. & FASCIOLO, J. C. (1965). *Experientia* **21**, 141–142.
FOX, R. H. & HILTON, S. M. (1958). *J. Physiol.* **142**, 219–232.

Decreased calcium binding by cardiac relaxing system isolated from reserpine-treated guinea-pigs

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