COMMUNICATIONS

Stimulation and inhibition of acid secretion from the rat isolated gastric mucosa

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Studies on the rat *in vivo* suggest that prostaglandins may have a local role in the regulation of acid secretion in the gastric mucosa (Main & Whittle, 1975). To test this hypothesis and to investigate the mode of action of anti-secretory drugs we have developed an isolated rat gastric mucosal preparation which responds to secretogogues.

Fed rats weighing 50–100 g were anaesthetized with pentobarbitone (S.C.) The muscle overlying the non-antral glandular region of the stomach was removed by dissection following its separation from the mucosa by a technique described by Forte, Forte & Machen (1975) for the piglet. A piece of mucosa was removed, mounted over a 1 cm^2 opening on a polyethylene vessel and placed in an organ bath at 36° C containing 35 ml. Krebs solution (Sernka & Hogben, 1969) which bathed the serosal surface and was gassed vigorously with 95% O_2 and 5% CO_2. The mucosal surface was perfused (0·5 ml./min) with unbuffered solution gassed with oxygen. The volume of solution on the mucosal side was 1·5 ml. Acid secretion was measured continuously by means of a dual micro-electrode in the mucosal solution connected via an anti-log unit to a pen-recorder.

Spontaneous secretion reaching an early peak and declining to steady low levels within 90–120 min was usually observed. All preparations gave maintained, dose-related responses to histamine (10^{-6} to 10^{-4} M serosally) which were readily reversed on washing. Dose–response curves to pentagastrin (10^{-8} to 10^{-6} M) were less steep and had a lower maximum than with histamine. Some preparations did not respond even to the high concentrations of pentagastrin. Carbachol (10^{-8} to 10^{-6} M) and acetylcholine (10^{-7} to 10^{-5} M) were capable of stimulating high rates of secretion, though tachyphylaxis was sometimes observed. Dibutyryl cyclic adenosine 3′,5′-monophosphate (dbcAMP) ($0·1–2·5 \times 10^{-4}$ M) induced high rates of secretion (up to 10 μequiv/cm^2 hr^{-1}) which were maintained for several hours. Thus the isolated rat mucosa, like the isolated rat whole stomach preparation (Parsons, 1975), responds to a variety of secretogogues and differs from the piglet mucosa which responds only to histamine.

Metiamide ($10^{-7}–3 \times 10^{-5}$ M), a histamine H_2-receptor antagonist, inhibited the secretory responses to histamine but not to dbcAMP. Prostaglandin E_2 ($0·5–2 \times 10^{-6}$ M) abolished the responses to histamine,

pentagastrin, carbachol and acetylcholine but even in higher doses (10^{-4} M) did not alter the responses to dbcAMP. The latter result, together with evidence from the amphibian mucosa *in vitro* (Way & Durbin, 1969) and the rat *in vivo* (Main & Whittle, 1974), supports the hypothesis that prostaglandins act directly on the mucosa at a stage in the secretory process prior to the involvement of cyclic AMP.

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The effects of pentagastrin on gastric (abomasal) motility in the unweaned calf

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Gastric emptying was shown in man by Hunt & Ramsbottom (1967) to be delayed by intravenous infusion of porcine gastrin II and by Dozois & Kelly (1971) in the dog by intravenous pentagastrin infusion.

We tested the effects of intravenous pentagastrin using conscious unweaned calves when gastric (abomasal) emptying was under optimal stimulation by constant duodenal infusion of water or sodium bicarbonate and the gastric efflux diverted from the duodenum (Bell & Mostaghni, 1975). During this experimental procedure, gastric e.m.g.s were recorded simultaneously from the body and antrum (Bell & Grivel, 1975).

Continuous intravenous infusion of pentagastrin, 0·01–0·03 $\mu g\ kg^{-1}\ min^{-1}$, always produced reductions in the total volume of test meal evacuated and e.m.g. activity from all regions of the stomach was reduced. The onset of reduced activity occurred within 120–180 sec of the start of pentagastrin infusion, the effect being greatest with the larger amounts of pentagastrin, and normal e.m.g.s recurred within 120 sec of cessation of infusion.

Single intravenous injections of pentagastrin abolished both gastric emptying and all e.m.g. activity except for persistence of the basic electrical rhythm in the antrum (Fig. 1). A dose relationship was estab-

lished with the first detectable response at 0·025 μg kg^{-1} and maximal effects at 10 μg kg^{-1}.

Hunt & Ramsbottom (1967) suggested retardation of emptying of gastric test meals by gastrin in man might be associated with contraction of the duodenum. Dozois & Kelly (1971) produced evidence that weaker

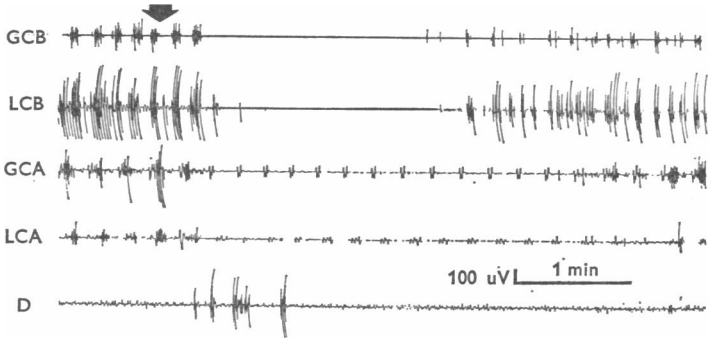

Fig. 1. E.m.g. recorded from stomach (abomasum) and duodenum of unweaned calf. Electrode placement: GCB, greater curvature body (fundus); LCB, lesser curvature body; GCA, greater curvature antrum; LCA, lesser curvature antrum; D, duodenum, 10 cm aboral to pylorus. At the arrow, 10 μg kg^{-1} pentagastrin in 5 ml. saline injected rapidly into the jugular vein. Coincidentally with the abolition of the e.m.g. gastric emptying ceased.

contractions of the antral pump slow gastric emptying. Our results indicate that pentagastrin reduces gastric emptying by inhibiting gastric smooth muscle of both body and antrum either directly or through some local intramural nervous mechanism.

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Electrical vagal-induced gastric antral contractions and gastric emptying of a liquid test meal in anaesthetized cats

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We recently reported the characteristics of antral contractions evoked by electrical stimulation of the vagus in cats (Brooks & Carr, 1975). Others have reported that pentagastrin increased contractile activity in the antrum but delayed gastric emptying (Cooke, Chvasta & Weisbrodt, 1972). In the current experiments we have studied the effect of electrical stimulation of the peripheral end of the cut dorsal trunk of vagus on gastric emptying of 0·9% saline containing 30 p.p.m. of phenol red (George, 1968). Five cats were anaesthetized with chloralose, equipped with a gastric fistula and with a strain-gauge which was sewn to the serosa of the antrum. A balloon was placed in the cardia via the oesophagus which was ligated around the tube to the balloon. Gastric emptying was determined by collecting the effluent from a tube introduced into the duodenum 6 cm distal to the pylorus. Drainage volumes were noted each minute. The stomach was emptied at 30 min and washed out with 25 ml. of 0·9% saline. Phenol red concentrations were determined spectrometrically and the pH of samples checked electrometrically. The test meal was 50 ml. in 22 experiments and 25 ml. in 13.

After the 25 ml. meal there was a statistically significant increase in gastric emptying during electrical stimulation over a period of 30 min (24·7 ml. $\pm$ 1·5 (S.E.M.) compared to 15·5 $\pm$ 2·6). The amount of phenol red emptied increased from 0·360 $\pm$ 0·063 mg to 0·529 $\pm$ 0·031 mg. The volume of liquid remaining in the stomach at 30 min fell from 10·3 $\pm$ 2·5 ml. to 3·5 $\pm$ 0·8, while the amount of phenol red remaining fell from 0·323 $\pm$ 0·052 to 0·091 $\pm$ 0·027 mg. Similar changes occurred after the 50 ml. test meal but only the increase in phenol red emptying (0·922 $\pm$ 0·041 increasing to 1·226 $\pm$ 0·061 mg) and the decrease in residual volume in the stomach (13·7 $\pm$ 2·1 ml. falling to 4·6 $\pm$ 1·1) were statistically significant.

The differences between control and stimulated experiments in cumulative volumes leaving the stomach were statistically significant after the second 5 min interval for the 25 ml. meal and after the fourth 5 min. interval for the 50 ml. meal. Emptying continued up to 30 min. after the 50 ml. meal in all experiments but the volume leaving the stomach increased less than 1 ml. after the first 15 min with the 25 ml. meal without stimulation.

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After the larger but not the smaller meal antral contractions appeared and continued without vagal stimulation for 4–5 min. Antral contractions (up to 10 g of force) were produced by electrical stimulation of the vagus. In both cases there was increased gastric emptying. In addition, the pH of the duodenal effluent fell as low as 1·8 without inhibiting vagally induced contractions.

We conclude that vagally induced antral contractions accelerate the gastric emptying of an isotonic liquid test meal. This is achieved primarily by increasing the second phase of emptying that follows after the first 5 min of rapid emptying, so that at 30 min emptying during vagal stimulation is virtually complete after a 25 ml. meal. Our results confirm the qualitative observations of gastric emptying noted fluoroscopically in dogs during vagal stimulation (M'Crea, M'Swiney & Stopford, 1925).

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Reflex inhibition of parotid salivary secretion from the portal venous system of sheep

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Secretion from the parotid salivary glands of conscious, anaesthetized and decerebrate sheep declines when hyperosmolal solutions are injected or infused intravenously, particularly when such solutions are administered into the portal vein or tributaries of it (Carr & Titchen, 1972). The present experiments were undertaken to assess the contribution of afferent fibres in abdominal branches of the vagus nerves, and in the splanchnic nerves, to this salivary reduction.

In nine sheep, in which anaesthesia was maintained during dissections with ether and during the period of experimental observations with chloralose (60 mg kg^{-1} I.V.), parotid salivary secretion was enhanced by rhythmic (12–18 min^{-1}) distension of an elongated balloon located in the thoracic portion of the oesophagus. Loose ligatures were placed around the dorsal and ventral branches of the vagi in the abdomen so these could be cut in the course of experiments. Similar ligatures were placed around the major divisions of the splanchnic nerves.

Parotid salivary secretion evoked by the oesophageal distension increased to a peak after 30–90 sec and then declined steadily, over 30–40 min, to near pre-stimulation levels. Hyperosmolal solutions of saline (1200–1500 m-osmoles kg^{-1}) injected intravenously in volumes of 10–40 ml., at rates of 0·4–0·8 ml. sec^{-1}, reduced stimulated parotid flow by up to 40% within 30 sec of the start of injections. At the slower rates particularly, the salivary reductions with injections into tributaries of the portal vein were greater than with identical injections into a femoral vein or the caudal vena cava. Routinely the injection rate was reduced until no effect was obtained when the systemic route was used but a reduction of 20–25% was still observed with the portal route.

This residual fall in salivary secretion caused by injections into the portal circulation was less after the abdominal vagi were cut. In two experiments, section of the ventral branch abolished the effect and in six experiments diminished it to 5–15%. In one of these latter experiments section of the dorsal branch of the vagus caused an additional diminution from 15 to 5%. Greater inhibition of salivary secretion could be restored after the vagal section, in all experiments, by injection of the hyperosmolal saline at faster rates or in larger volumes. Under these conditions a further loss of the inhibition followed bilateral section of the splanchnic nerves. In no experiment, however, was it possible, by a combination of vagotomy and splanchnotomy, to abolish inhibitory responses of the parotid glands to these higher rates or volumes by hyperosmolal saline.

These results suggest that afferent fibres which innervate the portal vasculature or abdominal structures supplied by it and which exert an inhibitory effect on parotid salivary secretion, course in abdominal branches of the vagus nerves. Other inhibitory afferent fibres run in the splanchnic nerves.

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The measurement of cerebral blood flow in the rat by ^{85}krypton washout

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The radioactive inert gas washout technique gives accurate measurements of compartmental cerebral blood flows and allows repeated measurements in each animal. It has been extensively used in man and other

species (Purves, 1972). Since little is known of cerebral blood flow in the rat, the present study was undertaken.

A retrograde cannulation of the external carotid artery was carried out in Nembutal anaesthetized rats, so that ^{85}Kr in 0·2 ml. isotonic saline could be injected into the blood flowing from the common carotid to the internal carotid artery. The resulting washout curves were recorded with an external scintillation detector placed over the head, and were analysed by a computer-assisted peeling method and also by the initial slope method. Autoradiography was used to confirm this compartmental analysis; animals were killed at various time intervals after ^{85}Kr injection, the heads were removed, frozen, sectioned sagitally 3 mm from the mid line, and held close to X-ray film to indicate the presence of ^{85}Kr in the tissue. Correct positioning of the cannula was confirmed in each animal by the injection of Evans Blue solution, giving staining of the brain and some extracerebral tissue on the ipsilateral side.

TABLE 1. Cerebral blood flow in the rat, measured by ^{85}Kr, washout, and the effect of varying the arterial blood–gas composition.

The initial slope blood flow is from an approximate method for calculating grey-matter flow. F_g (grey matter flow) and F_w (white matter flow) were also calculated by the peeling method. The blood–brain partition coefficients for ^{85}Kr were taken as 1·3 for white matter and 0·95 for grey matter (Haggendal & Nilsson, 1966). Mean arterial blood pressure was measured from the external carotid artery cannula

	Control (breathing air) mean ± S.E.M.	Experimental (breathing 95% O_2/5% CO_2), mean ± S.E.M.	*n*	Statistical evaluation by Wilcoxon signed-rank test
P_{a,O_2} (mmHg)	75 ± 6	359 ± 32	8	$P < 0.01$
P_{a,CO_2} (mmHg)	40 ± 1	56 ± 4	8	$P < 0.01$
Mean arterial blood pressure (mmHg)	74 ± 10	84 ± 7	6	N.S.
Initial slope blood flow (ml. min^{-1} 100 g tissue^{-1})	71 ± 11	106 ± 9	8	$P < 0.05$
F_g ml. min^{-1} 100 g tissue^{-1}	89 ± 19	156 ± 26	6	$P < 0.05$
F_w ml. min^{-1} 100 g tissue^{-1}	25 ± 3	44 ± 13	6	N.S.

The blood flow values and the compartmental definition so obtained agree well with those from other species (Purves, 1972) and with the data available for the rat (Nilsson, 1974). These data also clearly show that the initial slope calculation underestimates the grey-matter blood flow (see Table 1). Giving the animals 95%, O_2/5% CO_2 to breathe caused a marked, statistically significant increase in grey matter blood flow, in the absence

of significant alteration in mean arterial blood pressure. This finding is consistent with results reported for other species, and also indicates that the cerebral vasculature is capable of dilatation in response to appropriate stimuli.

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Antidromic dilatation in skeletal muscle

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It has long been known that a substantial vasodilatation can be produced in skin by antidromic stimulation of its sensory nerve supply. There is some question, however, whether a similar response occurs in skeletal muscle. Bayliss (1900) reported that antidromic stimulation of dorsal roots produced a small but significant increase in the volume of the dog hind limb, skinned and ligated at the ankle, and concluded that this would be associated with an increase in muscle blood flow. On the other hand, Celander & Folkow (1954), when recording venous outflow from the skinned hind limb of the cat, were unable to demonstrate any change in muscle flow on antidromic stimulation of the sensory nerves.

The present experiments were performed on cats anaesthetized with chloralose, the venous outflow being recorded from the gastrocnemius muscle. Laminectomy was performed from L6 to S1, the dorsal roots cut close to the cord and their peripheral ends placed on silver-wire electrodes. Rectangular pulses of 0·1–0·5 msec were applied, at intensities of 0–15 V and frequencies of 0·5–20 Hz, for 15–20 sec.

Antidromic stimulation of the dorsal root of L7 regularly increased the flow through the gastrocnemius muscle by 50 %: stimulation of L6 and S1 produced smaller flow increases. The latency of these responses was comparatively long, being 10–15 sec from the onset of stimulation. The dilatations were never associated with muscular contraction and were unaffected by section of the ventral roots L6–S1 or by cord transection at L6. They can only have been due to antidromic stimulation of sensory nerve fibres.

The most effective stimulus parameters for dilatation were pulses of 8–10 V and 0·3 msec duration at frequencies of 10–12 Hz. Stimulation at lower intensities or with shorter pulse width had no effect but, when both were supramaximal, frequencies as low as 4 Hz could elicit a dilatation. These results suggest that the response is due to activation of the smaller unmyelinated afferent fibres.

Various substances have been proposed as mediators of antidromic vasodilatation, although the evidence is inconclusive. The present study has provided evidence that prostaglandins may be involved. Indomethacin injected close arterially (80–160 μg) or intraperitoneally (2 mg/kg) had no effect on blood pressure but produced a 70–100% reduction of the dilator response; at the same time, dilator responses to close arterial injections of ACh (0·1–1 μg) were unchanged. Acetylsalicylic acid injected intraperitoneally (25 mg/kg) was as effective as indomethacin.

Thus, a substantial vasodilatation can be produced in skeletal muscle by antidromic stimulation of the smaller unmyelinated fibres. According to our results, this dilatation could be due to prostaglandins, released from nerve endings or other cellular elements within the muscle.

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Total and capillary blood flow through the testes of anaesthetized rams

BY H. P. GODINHO and B. P. SETCHELL. *A.R.C. Institute of Animal Physiology, Babraham, Cambridge*

Capillary blood flow through the testes of four rams anaesthetized with pentobarbitone sodium was determined by infusing [^{24}Na]Cl (12–18 μCi in 2·5 ml. 0·9% NaCl, 0·25 ml./min) into a catheter in the testicular artery on the surface of the testis. The radioactivity in the testis was measured with a directional scintillation counter (Labgear, Cambridge Type D 4133) and 1 min blood samples were withdrawn continuously from catheters in the internal spermatic vein and the abdominal aorta. At the end of the 10 min infusion, the sampling of blood and measurement of radioactivity in the testis was continued for a further 15 min. Saturation and desaturation of the tissue, determined directly by the scintillation counter and indirectly from the venous–arterial concentration of ^{24}Na were simple exponentials. Capillary blood flow could be calculated from the formula

$$F = \lambda \frac{\ln 2}{t_{\frac{1}{2}}},$$

where F = plasma flow in ml. min^{-1} g^{1}, λ = partition coefficient of ^{24}Na between testis and blood plasma (0·18 as determined in preliminary experiments), $t_{\frac{1}{2}}$ = half time of saturation or desaturation of ^{24}Na in the testis.

Blood flow was then calculated by multiplying plasma flow by $100/100-H$, where H is the haematocrit.

Total blood flow was calculated from the known infusion rate of ^{24}Na and the concentration of ^{24}Na in the plasma from the internal spermatic vein (less the concentration in arterial plasma) at equilibrium, which was usually reached after about 8 min infusion. This gave a plasma flow in ml. min^{-1} which could be converted to blood flow as described above. Because of the anatomy of the vascular supply to the testis, it was possble to make a reasonably precise estimate of the mass of tissue drained at the point of sampling, and therefore to convert the flow in ml. min^{-1} to flow in ml. min^{-1} g for comparison with the measurements of capillary flow.

Capillary blood flow through the testis was $0{\cdot}120 \pm 0{\cdot}019$ ml. min^{-1} g^1 (4 observations) determined by saturation and $0{\cdot}099 \pm 0{\cdot}008$ ml. min^{-1} g^1 determined by desaturation of the tissue, measured directly, $0{\cdot}128 \pm 0{\cdot}011$ ml. min^{-1} g^1 determined by saturation and $0{\cdot}120 \pm 0{\cdot}019$ ml. min^{-1} g^1 determined by desaturation of the tissue estimated from the venous–arterial concentration difference of ^{24}Na. Total blood flow in these experiments was $0{\cdot}18 \pm 0{\cdot}016$ ml. min^{-1} g^1. Local heating of the testis for between 90 and 120 min to between 40 and 42° C appeared to have no effect on either capillary or total blood flow. No anatomical basis has yet been found for the considerable difference between total and capillary blood flow.

H. P. Godinho was a British Council Fellow on leave from the Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

Regional renal blood flows in the hypothermic rat and dog

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Lowering the body temperature of homoiotherms to 25–27° C induces renal vasoconstriction (Chapman, Munday & Withey, 1975). Separate experiments in rats and dogs show that this vasoconstriction occurs predominantly in inner cortical and medullary regions of the kidney.

Animals, anaesthetized with sodium pentobarbitone, were cooled by placing crushed ice over the abdomen and thorax. Dogs were cooled to 27° C and held at this temperature for 2 hr. Rats were cooled and held at 25° C for 30 min. Dogs received a 15 ml. hr^{-1} kg^{-1} isotonic saline infusion; rats did not.

Regional renal blood flows were measured by the ^{86}Rb-uptake method (Sapirstein, 1958) which is known to give reliable blood flow measurements in hypothermia (R. A. Willson, B. J. Chapman & K. A. Munday, to be published). The animals were killed 20–90 sec after I.V. injection of [^{86}Rb]Cl in isotonic saline and the kidneys removed within 20 sec to acetone/dry ice.

The distribution of ^{86}Rb in kidney slices was assessed by autoradiography and by dissecting into regions and scintillation counting. The small size of the rat kidney prevented reliable separation of the outer and inner cortex, but the autoradiographs indicate that inner cortical and outer medullary blood flows were similar.

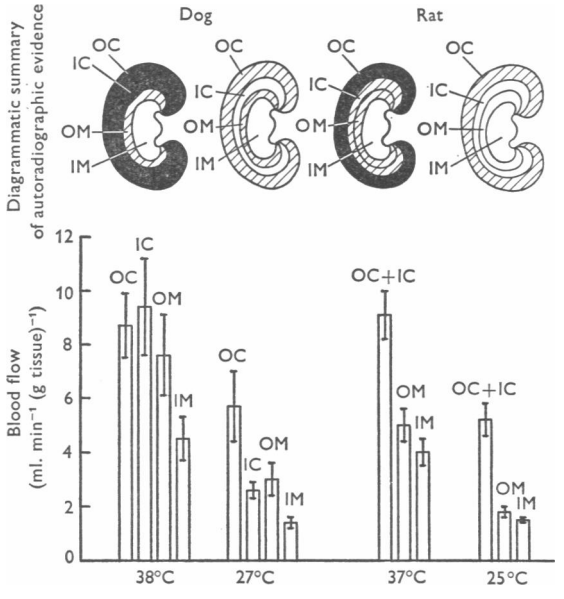

Fig. 1. The effect of hypothermia on regional blood flows in the kidneys of dog and rat, measured by ^{86}Rb uptake. OC, outer cortex; IC, inner cortex; OM, outer medulla; IM, inner medulla. In the autoradiographs, black = greatest, white = lowest, hatched = intermediate [^{86}Rb] contents.

The outer cortical blood flow was reduced by 34% in dogs and by approximately 43% in rats (Fig. 1). Inner cortical and inner medullary regions in both species, and outer medulla in the rat showed blood flow reductions of 74–82%. The autoradiograms show that there was an outer medullary region in which blood flow was not markedly reduced in the dog; this may be associated with the longer period of hypothermia, the rapid saline infusion (and elevated urine flow) or species difference.

These findings may help explain the diuresis, the enhanced medullary tonicity and the capacity for blood flow autoregulation of hypothermic kidneys.

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Blood flows in the lower leg during recovery from fractures of the tibia and fibula

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During the immobilization of a limb following fracture, atrophy of skeletal muscle occurs, and when activity recommences there is a reduction in the aerobic working capacity of limb (Davies & Sargeant, 1975). It was therefore of considerable interest to examine the blood flow through the injured limb.

The subjects were seven young servicemen who were undergoing a full-time course of treatment at the Joint Services Medical Rehabilitation Unit. They had suffered unilateral fractures of the shaft of the tibia and fibula 187 (range 101–295) days prior to the investigation, and the injured limb had been immobilized in plaster of Paris until 33 (range 15–90) days prior to the investigation.

Blood flow was measured simultaneously in the two calves using venous occlusion plethysmographs filled with water at $34 \pm 1°$ C (Barcroft & Swan, 1953). The temperature of the laboratory was $22 \pm 2°$ C. The collecting cuffs were placed around the thigh immediately above the patellae, and cuffs around the ankles were inflated to 200 mmHg to prevent flow to the feet. The volume of the leg enclosed in the plethysmograph was measured by water displacement.

A total of twelve determinations were performed on the seven subjects. The flow (mean $\pm$ S.D.) through the calf of the injured leg ($5{\cdot}47 \pm 1{\cdot}14$ ml. 100 ml.$^{-1}$ min^{-1}) was significantly higher ($P < 0{\cdot}05$) than on the uninjured side ($3{\cdot}12 \pm 0{\cdot}30$ ml. 100 ml.$^{-1}$ min^{-1}).

To see whether the vessels of the injured limb retained the ability to dilate in response to the accumulation of metabolites, the circulation to each limb was occluded for 10 min by a sphygmomanometer cuff placed around the thigh and inflated to 200 mmHg. The reactive hyperaemia on the injured side was equal to or exceeded that on the uninjured side. Since the local responses to occlusion of the circulation and to dynamic exercise may be similar (Barcroft, 1972), it appears likely that the vessels dilate normally during exercise in an injured limb.

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The behaviour of isolated cell nuclei when divalent cations are removed

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A necessary stage in the procedure for the purification of nuclear envelope is the production of swollen and disrupted nuclei (Harris & Milne, 1974). Experiments have been performed using isolated chicken erythrocyte and pig liver nuclei in an attempt to gain some understanding of the involvement of divalent cations in this swelling.

The amount of swelling of isolated nuclei has been determined by measuring the volume of samples of centrifugally pelleted nuclei (3000 rev./min for 10 min) on treating the nuclei with decreasing concentrations of magnesium and calcium. The state of integrity of the nuclei and the degree of chromatin condensation has been followed by electron microscopy. Equal quantities of nuclei were suspended in 10 mM Tris-HCl (pH 7·4) containing magnesium or calcium chloride (0–10 mM) and also 250 mM sucrose in one series of experiments. The nuclei were centrifugally pelleted and resuspended two times in solutions of varying divalent ion concentration, with a period of 5 min for equilibration before centrifugation. After the second centrifugation the volume of the pelleted nuclei was determined directly from the graduations on the centrifuge tube.

On reducing the concentrations of magnesium chloride over the range 1·0–0·01 mM, the volume of the pelleted nuclei increases approximately four times, and remains constant with any further reduction. Below a concentration of 0·5 mM magnesium the pellet of nuclei becomes very gelatinous. A linear relationship is obtained on plotting the pellet volume against log magnesium concentration over the concentration range 1·0–0·01 mM. The presence of 250 mM sucrose does not significantly alter the degree of nuclear swelling or the range of magnesium concentration where it occurs. The behaviour of nuclei in the presence of calcium is very similar to magnesium, but the swelling does not commence until a 0·5 mM concentration has been reached and the slope of the relationship between pellet volume and log ion concentration is increased. No detectable difference is found between the two types of nuclei, even though the chicken erythrocyte nuclei are metabolically inactive with respect to RNA synthesis.

In the electron microscope a slight initial swelling of the nuclei is observed to occur as the magnesium concentration is lowered beneath 1 mM. This is followed by the release of dispersed chromatin at lower concentrations, which binds the nuclei together. Thus, the fourfold increase in nuclear pellet volume under the conditions described above is due to the

dispersion and escape of the chromatin rather than a reversible swelling of intact nuclei.

The importance of the presence of divalent cations rather than the maintenance of isotonicity for the preservation of nuclear integrity is emphasized by the results of the experiment performed in the presence of sucrose. The nuclear envelopes are not destroyed when the chromatin is released (Harris, 1974), but are trapped within the swollen gel. Solubilization of the gelatinous chromatin can be produced by incubation with deoxyribonuclease, thus allowing the nuclear envelope to be isolated centrifugally (Harris & Milne, 1974).

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Action of lithium on the responses of the rat superior mesenteric vascular bed to noradrenaline and prolactin

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The superior mesenteric vascular bed of the rat was dissected out and perfused as previously described (Manku, Nassar & Horrobin, 1973). If perfusion rate is kept constant, injection of noradrenaline into the cannula produces a transient rise in pressure. Ovine prolactin in a concentration of 50 ng/ml. in the perfusate potentiates the response to noradrenaline while a concentration of 500 ng/ml., after a brief initial potentiation, produces marked inhibition (Manku *et al.* 1973). It has been suggested that lithium may be able to inhibit some of the actions of prolactin (Horrobin, 1974). Lithium in a concentration of 2 mM (the upper limit of the therapeutic concentration in human plasma) had no significant effect on the responses of the preparation to noradrenaline in the absence of prolactin. When added in the presence of prolactin this same concentration of lithium could reverse the effects of both the high and low concentrations of the hormone (Fig. 1).

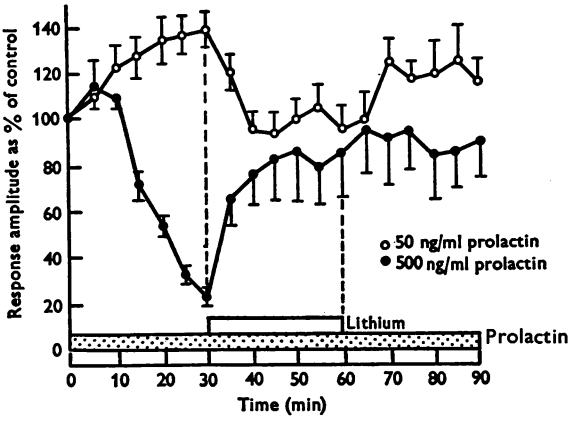

Fig. 1. The effects of prolactin and lithium on the pressor responses of the rat superior mesenteric vascular bed to noradrenaline. Each prolactin concentration was perfused through eight preparations. Lithium in a concentration of 2 mM was added to the perfusate during the period indicated. Results are expressed as percentages of the mean amplitude of three test responses obtained before prolactin was added to the perfusate. The bars indicate S.E.M.

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Specificity of nuciferine as an antagonist of amino acid and synaptically evoked activity in cells of the feline thalamus

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In a previous communication to the Society, Hind & Kelly (1975) drew attention to the ease with which iontophoretically applied nuciferine (L-5,6-dimethoxyaporphine) antagonized glutamate-evoked firing in the cuneate nucleus. In addition, nuciferine blocked orthodromic responses without altering those evoked antidromically. These findings have now been extended by testing the specificity of nuciferine on thalamo-cortical relay neurones which, unlike the neurones of cuneate nucleus, can be excited readily by acetylcholine.

Acetylcholine and glutamate were applied alternately from five-barrel glass micropipettes placed stereotaxically in the centro-basal complex of the thalamus of cats paralysed with gallamine triethiodide and anaesthetized with an oxygen–nitrous oxide–Halothane mixture. The micropipette

was used to record extracellularly from thalamo-cortical relay cells identified by antidromic stimulation of the ipsilateral pericruciate cortex.

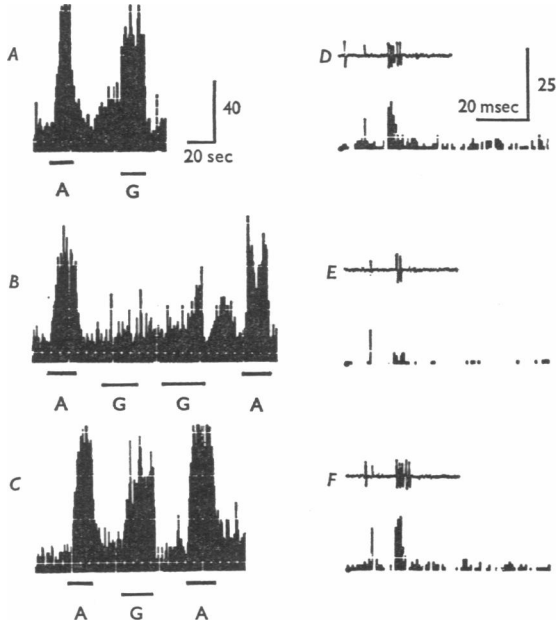

Fig. 1. Specificity of the effect of iontophoretically applied nuciferine on the responses of a neurone on the ventrobasal thalamus. (*A–C*), firing-rate histograms photographed from the display of a PDP 12 computer to show the response to iontophoretic applications of acetylcholine (A, 50 nA) and glutamate (G, 65 nA) before (*A*), 3 min after the onset of a 80 nA applications of nuciferine (*B*) and 4·5 min after the end of the application (*C*). The histogram was prepared at a resolution of 0·5 sec. (*D–F*), post-stimulus latency histograms (20 sweeps; bin width = 1 msec) and single-sweep oscilloscope traces to show the effect of another application of nuciferine (80 nA) on the response of the same neurone to electrical stimuli applied to the contralateral median nerve (2·5 V, 0·2 msec, 0·5 Hz); before (*D*), 3 min after the onset of the application (*E*) and 6·5 min after the end of the application (*F*).

Fig. 1 shows a typical experiment in which nuciferine (positive current, 20 mM soln., pH 3·5–4·0) abolished the responses to glutamic acid without reducing the excitation evoked by acetylcholine. During a subsequent application of nuciferine the spontaneous activity was reduced threefold and the orthodromic response to stimulation of the contralateral median nerve was virtually abolished. If the specificity of a glutamic antagonist can be defined in terms of its ability to abolish the response to glutamate without reducing the excitatory response to an equipotent application of acetylcholine, the action of nuciferine proved to be specific in 26 of the

39 cells tested. On seven cells, however, the reduction in glutamate firing was accompanied by a similar change in the response to acetylcholine. In keeping with the view that the somato-sensory pathway to the thalamus is unlikely to be cholinergic (Phillis, 1971), the specific action of nuciferine on glutamate activity was often accompanied by a reduction in both spontaneous and synaptically evoked activity.

We are indebted to Dr E. A. Kimes of Smith Klein & French Laboratories, Philadelphia, for supplies of l-5,6-dimethoxyaporphine, SK & F. L-8419. Y.B.-A. is an associate of the CNRS, France.

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Morphine hyperthermia, prostaglandin synthetase inhibitors and naloxone

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It is well known that morphine produces hyperthermia when administered to the conscious cat. In 1974 Collier, McDonald-Gibson & Saeed suggested that morphine stimulated the enzyme prostaglandin synthetase and that this was the mechanism by which morphine produced hyperthermia since the prostaglandins of the E series are known to increase body temperature when injected into the C.N.S. (Milton & Wendlandt, 1970). In order to test the hypothesis of Collier *et al.* morphine hyperthermia has been produced in the conscious cat by both intravenous injection and injection directly into the C.N.S., and the effects of prostaglandin synthetase inhibitors on the resulting hyperthermia studied. In addition, the effect of the morphine antagonist naloxone has been investigated.

Morphine I.V. in doses of between 0·13 and 0·65 mmol/kg produced intense pupillary dilatation, salivation and increased motor activity. Body temperature began to rise within minutes of the administration of the morphine and remained elevated for many hours. 4-Acetamidophenol (paracetamol) 0·33 mmol/kg or indomethacin 5·5 μmol/kg administered I.P. 30 min before the morphine injection was without effect on any of the symptoms, and similarly when administered at the height of the sustained temperature rise failed to reduce deep body temperature. In contrast I.V. naloxone (0·065 mmol/kg) completely prevented the pupillary dilatation and the increased motor activity produced by 0·65 mmol morphine I.V. and there was no rise in deep body temperature. When naloxone was

administered during the sustained hyperthermia, the increased motor activity stopped immediately and deep body temperature rapidly fell to normal. Naloxone itself had no effect on deep body temperature, though it did produce salivation.

Morphine (16 μmol) was injected into the thermoregulatory area of the anterior hypothalamus; it was without any immediate effect on deep body temperature, though in some animals pupillary dilatation and shivering were observed after about an hour and deep body temperature rose. If the morphine had access to the cerebroventricular system intense shivering was observed accompanied by a rise in deep body temperature. The shivering and rise in deep body temperature were not affected either by 4-acetamidophenol or indomethacin given I.P. In contrast to the effect of morphine, prostaglandins of the E series produce immediate shivering and a rise in deep body temperature when applied to the anterior hypothalamus, and both prostaglandin hyperthermia and pyrogen fever are associated with sedation.

These results therefore suggest that the hyperthermia produced by morphine in the conscious cat is not due to stimulation of the enzyme prostaglandin synthetase with subsequent release of a prostaglandin of the E series, but is due to increased motor activity as a result of stimulation of morphine receptors which are blocked by the morphine antagonist naloxone.

Naloxone hydrochloride was kindly supplied by Endo Laboratories Inc., Garden City, New York.

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The lack of relationship between the concentration of lithium and the inhibition of the action of vasopressin by lithium in the rat

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Harris & Jenner (1972) have shown that the intravenous infusion of lithium will inhibit the response to vasopressin in a rat bioassay preparation (Bisset, 1962). The inhibition can be shown within 10 min and it is quickly reversed on return to a lithium-free infusion, despite the fact that relatively high serum and whole body levels of lithium persist. Since Torp-Pedersen & Thorn (1973) failed to show any recovery of the response to vasopressin after lithium, this study was designed to look again at the

relationship between lithium concentration and the inhibition of the antidiuretic response to vasopressin.

Male rats (Wistar CFY (0·12–0·15 kg)) were anaesthetized with ethanol contained in a modified Czaczkes, Kleeman & Koenig (1964) infusion, given at 0·2 ml./min (see Harris & Jenner, 1972). When the animals showed a constant antidiuretic response to Pitressin (40 μ-u.), lithium was substituted for equimolar amounts of sodium in the infusion, and the response to Pitressin during lithium infusion was compared to the normal response.

An infusion of 12·8 m-equiv/l. lithium produced a fairly constant inhibition of the response to Pitressin for any particular animal for several hours and this inhibition was always reversible in all animals studied. However, measurement of the serum concentration of lithium showed that after 60 min of 12·8 m-equiv/l. lithium infusion the serum level had risen to 0·6–1·0 m-equiv/l. by which time the maximal inhibition of the Pitressin response was well established. When a lithium-free solution was then infused for 60 min, the serum lithium remained at 0·6–1·0 m-equiv/l. although the responses to Pitressin returned to normal. Renal cortex and papilla levels of lithium also showed no relation to the inhibition of Pitressin and the gradient of lithium concentration for papilla to cortex remained constant throughout the experiments.

Although the inhibition of the response to Pitressin remains steady for hours in any one animal, increasing the rate of lithium infusion by increasing the concentration of lithium in the infusion increased the inhibition, and similarly decreasing the rate of lithium infusion decreased the inhibition of the response to Pitressin. A large single injection of lithium did not produce an inhibition of Pitressin when given 10–20 min after the injection.

While these results are difficult to explain, they support Harris & Jenner (1972) in showing that the inhibition of Pitressin is not related to the serum, kidney or urine concentration of lithium, and they also show that the rate of administering lithium to the animal seems to be more important than the amount of lithium in the body in determining the inhibitory effect of lithium on the antidiuretic effect of vasopressin.

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The effects of prolactin on renal excretion, and on voluntary intake of water and sodium in rabbits

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Prolactin caused renal retention of water and ions in man (Horrobin, Burstyn, Lloyd, Durkin, Lipton & Muiruri, 1970), sheep (Burstyn, Horrobin & Manku, 1972) and rabbits (Burstyn, McKillop & Lloyd, 1974). The human volunteers also reported sensations of thirst and salt craving, apparently caused by prolactin (Horrobin *et al.* 1970). It is this subjective observation which we wished to test.

Seven rabbits in metabolism cages were allowed free access to food, water, and 1% saline solution. Daily consumption of each of these was measured. The faecal and urinary output was collected and analysed. The 5-day experimental period, during which the animals were each given 100 i.u. ovine prolactin (Ferring) i.m. twice daily, was preceded by 7 days and followed by 9 days of control observations.

The results show that prolactin caused renal retention of both sodium and water ($P < 0{\cdot}01$), but with no compensatory reduction of either saline or water intake, though the involuntary sodium intake (in food) was slightly reduced through some loss of appetite.

Prolactin caused each rabbit to accumulate an average of 37 ml./day of water and 12·3 m-equiv/day of sodium during the 5-day period of treatment. This fluid gain is hypertonic in that 12·3 m-equiv of sodium should have been accompanied by 82 ml. water if isotonicity was to be preserved.

We believe these results indicate that prolactin has actions on both volume and osmoregulatory systems, causing the accumulation of a quantity of hypertonic fluid without allowing the animal to compensate by reducing intake. There was no evidence for an increased thirst or salt craving during prolactin treatment.

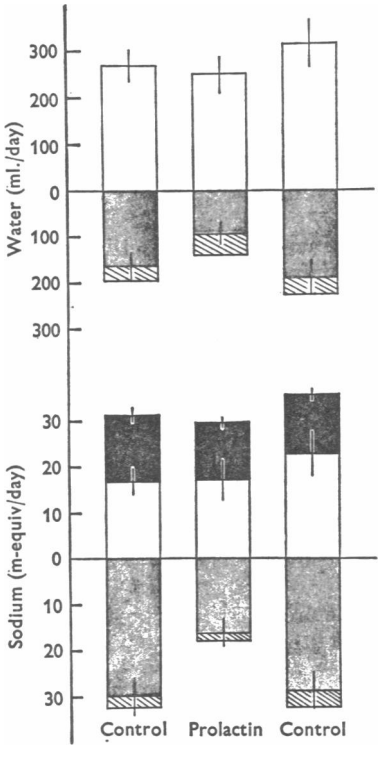

Fig. 1. Comparison between the intake and excretion of water, and sodium before, during and after treatment with prolactin. Diagonal lines, faecal excretion; grey stippling, urinary excretion; white, voluntary intake (as water and saline); black, involuntary intake (as food). Each column represents the mean of seven animals ± S.E.M.

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The failure of peripheral renin-angiotensin activation to induce sodium appetite

BY J. T. FITZSIMONS and J. B. WIRTH. *The Physiological Laboratory, Cambridge*

The hypothesis that release of renin from the kidney causes increased sodium appetite was tested using adrenalectomized rats. The adrenals were removed at least 3 weeks before testing and the rats were maintained

on laboratory diet, distilled water, and 2·7 % NaCl. Results are shown in Table 1.

Removal of circulating renin through bilateral nephrectomy nearly abolished sodium intake. The decreased intake was not attributable to sodium retention resulting from anuria, since sodium retention was less in nephrectomized rats than in controls (0·25 ± 0·11 *vs.* 1·15 ± 0·31 m-mole Na/100 g body weight at 6 hr, $P < 0·05$). The loss of sodium appetite

TABLE 1. Effect of peripheral renin-angiotensin activation on NaCl and water intakes in two-bottle choice tests of adrenalectomized (except group 6) rats.

(Na-deplete rats were deprived of NaCl solution overnight. Operative procedures were under ether with no delay before testing. Rats were injected 15 min before testing. Amounts of renin are in Goldblatt units. Intakes are shown as mean ± S.E. of mean in ml./100 g body weight. Asterisks indicate significance levels compared with controls in each Group (Student's *t* test): *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$.)

Group	Na balance	Procedure	Duration of test (hr)	*N*	Water intake	2·7 % NaCl intake
1	Deplete	Bilateral nephrectomy	6	6	0·98 ± 0·46**	0·56 ± 0·22*
		Bilateral nephrectomy + renin 10 u. I.P.		4	0·48 ± 0·48*	0·28 ± 0·28**
		Bilateral ureteric ligation		6	4·94 ± 0·75	0·67 ± 0·19*
		Unilateral ureteric ligation		6	2·73 ± 1·25	3·22 ± 0·85
		Sham-operated control		7	4·52 ± 0·91	3·54 ± 1·02
2	Deplete	Isoprenaline 100 μg/kg S.C.	3	6	3·67 ± 0·78	3·63 ± 0·50
		Phentolamine 10 mg/kg S.C.		7	3·09 ± 0·50	1·50 ± 0·50**
		Isotonic saline control 0·25 ml. %		8	3·08 ± 0·67	4·49 ± 0·50
3	Replete	Isoprenaline 100 μg/kg S.C.	3	6	3·77 ± 0·80***	0·68 ± 0·32
		Isotonic saline control 0·25 ml. %		8	0·13 ± 0·07	0·48 ± 0·25
4	Deplete	Renin 10 u. I.P.	6	6	3·90 ± 0·90	4·80 ± 0·54
		Isotonic saline control 2 ml. I.P.		6	3·80 ± 0·96	4·03 ± 0·62
5	Replete	Renin 10 u. I.P.	6	11	0·75 ± 0·18	0·56 ± 0·15
		Isotonic saline control 2 ml. I.P.		14	0·65 ± 0·35	0·66 ± 0·27
6	Replete (intact)	Renin 10 u. I.P.	6	9	1·60 ± 0·50*	0·14 ± 0·10
		Isotonic saline control 2 ml. I.P.		9	0·20 ± 0·10	0·01 ± 0·01

through nephrectomy was probably not caused by the loss of renin, since intraperitoneal injection of renin did not restore sodium intake. This has been shown previously using formalin-induced sodium appetite (Fitzsimons & Stricker, 1971).

Bilateral ureteric ligation, which releases large amounts of renin from the kidneys, inhibited, rather than stimulated, sodium appetite. Sodium retention again was diminished ($0{\cdot}31 \pm 0{\cdot}09$ *vs.* $1{\cdot}15 \pm 0{\cdot}31$ m-mole Na/100 g body weight, $P < 0{\cdot}05$). Unilateral ureteric ligation, which is less debilitating, and which should also increase renin release, did not augment sodium intake.

Stimulation of renin release by isoprenaline did not cause sodium intake in the Na-deplete or Na-replete rat although it did increase water intake. Phentolamine, which releases renin reflexly, actually diminished sodium intake.

Increasing the level of circulating renin by the intraperitoneal injection of renin failed to induce sodium intake in Na-deplete or Na-replete adrenalectomized rats. Intact rats drank water in response to intraperitoneal renin but did not take sodium.

These results do not support the hypothesis that peripheral renin-angiotensin activation induces sodium appetite.

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The effect of biliary obstruction on the concentrating activity of the rabbit gall bladder

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It has been suggested that the gall bladder, by concentrating bile during biliary obstruction, exerts an influence on the composition of the bile contained in the obstructed biliary system (Rous & McMaster, 1921; Rains, 1964). In the absence of a normally concentrating gall bladder the bile becomes de-pigmented, i.e. 'white bile'. The purpose of this study was to test the assumption that the gall bladder concentrates bile during biliary obstruction.

Obstruction of the common bile duct was produced in 38 rabbits. Complete obstruction was effected in 24 and incomplete obstruction in 14. The gall bladders were excised for *in vitro* testing of concentrating activity, using the technique described by Diamond (1962), at intervals

of either 6 hr, 12 hr, 24 hr, 1 week, 2 weeks or 3 weeks, when the intra-biliary pressures were also measured. Twelve further rabbits were used as controls, the gall bladder being removed for testing after administration of the same anaesthetic (Hypnorm, dose 0·6 ml./kg body wt.) as in the obstructed group. Four of the control animals were allowed to recover without removal of the gall bladders which were then excised at intervals of either 24 hr or 1 week.

The mean fluid absorption of the 12 gall bladders in the control group was $88{\cdot}0 \pm 10{\cdot}7$ mg/100 mg of gall bladder per hr. Of the 24 gall bladders from rabbits with complete biliary obstruction the 4 tested 6 h after the onset of obstruction showed a mean fluid absorption of $67{\cdot}3 \pm 14{\cdot}4$ mg/100 mg gall bladder per hr, but in 3 tested after 12 hr and those tested after 24 hr, 1 week, 2 weeks, and 3 weeks no fluid absorption was recorded. Of the 14 gall bladders from rabbits with incomplete obstruction, 12 showed no fluid absorption and 1 at 2 weeks and 1 at 3 weeks showed minimal fluid absorption.

Intra-biliary pressures were significantly higher in complete obstruction, mean $228{\cdot}4 \pm 11{\cdot}2$ mmH_2O, than in incomplete obstruction, mean $156{\cdot}6 \pm 10{\cdot}5$ mmH_2O ($P < 0{\cdot}01$).

In complete or incomplete biliary obstruction the gall bladder ceases to concentrate bile and in complete obstruction this takes place between 6 and 12 hr after the onset of obstruction. These changes do not appear to be related to the difference in intra-biliary pressure between complete and incomplete obstruction. As the gall bladder ceases to absorb fluid during biliary obstruction it is unlikely to exert an influence on the composition of bile in the obstructed biliary system.

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The effect of dietary modifications on blood lactate during exercise

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Dietary modifications are known to affect carbohydrate (CHO) metabolism during exercise (Christensen & Hansen, 1939) and also appear to influence blood lactate concentration (Saltin & Hermansen, 1967; Rennie & Johnson, 1974). This effect has been further investigated.

Three subjects were studied during graded submaximal exercise on a bicycle ergometer after a normal diet, a low CHO diet and a high CHO diet; blood lactate was measured after each of four 5 min work periods. Oxygen uptake ($\dot{V}_{O_2}$) and respiratory exchange ratio were also determined. At all work loads, blood lactate was lower after the low CHO diet and higher after the high CHO diet than on the normal diet (Fig. 1).

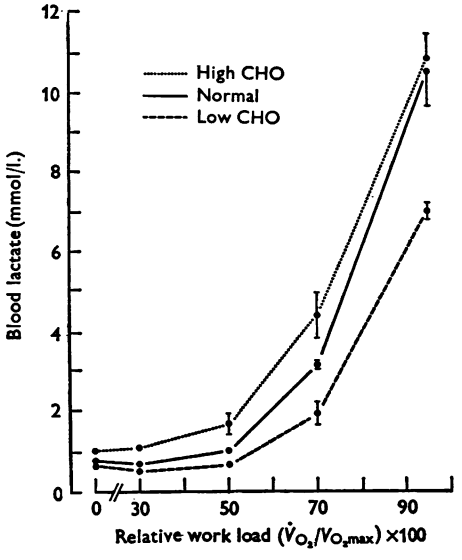

Fig. 1. Blood lactate concentrations (mean ± S.E.) after graded exercise on normal, low and high carbohydrate (CHO) diets.

In a second series of experiments, four subjects performed prolonged exercise at 75% of $\dot{V}_{O_2\,max}$. Blood lactate reached a steady level after 30 min of exercise and was again lower after a low CHO diet (3·0 ± 0·2 mmol/l.; mean ± S.E.) and higher after a high CHO diet (5·2 ± 0·5 mmol/l.) than on a normal diet (4·6 ± 0·5 mmol/l.).

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Responses of type A atrial vagal receptors to changes in atrial dynamics

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The mechanical stimulus which excites type A atrial vagal receptors during atrial systole is undetermined (Paintal, 1973). The electrical activity of twenty-two right atrial receptors was recorded simultaneously with instantaneous pressure and dimensional changes of the right atrium (Recordati, Lombardi, Malliani & Brown, 1974) in cats anesthetized with sodium pentobarbitone and immobilized with gallamine. The nervous activity was analysed in terms of peak and mean frequency of discharge in the burst and number of spikes per burst. Atrial dynamics during systole was characterized in terms of initial and peak systolic pressure, amplitude of the 'a' wave (ΔP), mean rate of change in pressure ($\Delta P/\Delta t$), initial and end-systolic diameters, amount and mean rate of diameter shortening. Receptor activity and atrial dynamics were studied during acute volume loading of the right atrium, electrical stimulation of the right stellate ganglion and isoproterenol infusion, electrical stimulation of the left thoracic vagus, acetylcholine administration and heart rate changes.

Atrial volume changes did not consistently alter the discharge of the receptors during atrial systole. Positive inotropic interventions significantly increased the systolic discharge. The amount of excitation was inversely related to spontaneous activity under control conditions. Negative inotropic interventions markedly decreased the activity of the receptors. The effects of inotropic interventions were independent of heart rate changes as they were still present in paced hearts. Pacing of the right auricle excited the receptors only when the atrium was contracting against the closed A–V valves (relative 'isovolumic 'contractions) at heart rates above 200 beats/min. During different haemodynamic conditions peak and mean frequency of discharge in the burst were found to be better related to the amplitude of the 'a' wave and to the mean rate of change in pressure, rather than to initial and peak systolic pressures, initial diameter, amount and rate of shortening.

These results suggest that the systolic discharge of type A receptors is a function of the active tension developed by atrial muscles during contraction.

To analyse the role of the tonic efferent sympathetic activity, three receptors were studied before and after removal of the stellate ganglia and three receptors before and after administration of propranolol. Both interventions markedly reduced impulse activity during systole, thus

indicating that the efferent sympathetic tone to the heart contributes to maintain the spontaneous discharge of receptors. This may explain the difficulty encountered in recording the activity of type A atrial receptors in the dog, which possesses a higher vagal and a lower sympathetic tone with respect to the cat.

On the basis of our results and following previous suggestions (Whitteridge, 1953) type A atrial vagal receptors may be defined as functionally 'in series' with atrial muscles.

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The form of the respiratory interaction between an alternate-breath oscillation of P_{A,CO_2} and hypoxia in man

BY D. J. C. CUNNINGHAM and SUSAN A. WARD.* *University Laboratory of Physiology, Oxford*

In man administration of alternate inspirates of high and low P_{CO_2} is usually associated with a reflex breath-by-breath alternation in respiration, especially when hypoxia is present (Marsh, Lyen, McPherson, Pearson & Cunningham, 1973). A similar response in the cat is mediated by the arterial chemoreceptors (Wolff, 1975). We have attempted to characterize the form of such hypoxia-dependence. Thirteen respiratory output variables (inspiratory and expiratory times, volumes, flows and accelerations) were measured in nine experiments on four subjects. Each experiment comprised 5–7 runs (between 40–150 breaths) at various steady P_{A,O_2} (between 200 and 47 torr) in each of four conditions: test (CO_2 oscillation present) and control (CO_2 oscillation absent) at rest and in mild exercise (HR $\sim$ 120 min^{-1}).

Incidence of alternating responses. Alternating responses were more frequent on passing from hyperoxia ($P_{A,O_2} \sim 200$ torr), through euoxia (P_{A,O_2} 129–75 torr), and in hypoxia ($P_{A,O_2} < 72$ torr). This effect was more marked in exercise.

The form of the hypoxia-dependence. A priori, flow and the time for which it occurs may be regarded as the primary variables of inspiration and expiration. Hypoxia-dependence was assessed by means of a regression analysis of the amplitude of alternation upon hypoxia (expressed as $1/(P_{A,O_2}-32)$) for the composite flow variables of inspiration and expiration ($\dot{v}'_I$, $\dot{v}'_E$) and

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T_E. T_I, being very stable in the face of the P_{A,CO_2} oscillation, was excluded. Thus for the rest and exercise 54 regressions were performed altogether (3 variables × 9 experiments × 2) Two criteria were examined: the mean amplitude of alternation ($\bar{y}$) and the regression coefficient (b).

In a little over half of the cases neither $\bar{y}$ nor b was significantly different from zero, reflecting possibly a low signal-to-noise ratio and the small number of points in each regression. In the remainder $\bar{y}$ was significant but in only half of these was b also significant (in accord with a multiplication between the CO_2 oscillation and hypoxia). Exercise increased the incidence of these latter two patterns.

These results are consistent with two views: (i) the two patterns of response described are the extremes of a continuous distribution of the hypoxia-dependency, the apparent dichotomy being due merely to experimental scatter, and (ii) there are two separate mechanisms involved in detecting the CO_2 oscillation, one which is dependent upon hypoxia, and the other a CO_2 receptor unaffected by hypoxia, perhaps in the airways (Boushey & Richardson, 1973; Bartoli, Cross, Guz, Jain, Noble & Trenchard, 1974). The potentiating effects of exercise might reflect a more faithful transmission of the blood-borne CO_2 oscillation. This possibility, together with the findings of Pearson & Wolff (1975), is more appropriate to the first hypothesis.

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The effects of abnormal oxygen tensions on breath by breath tidal volume changes in cats alternately breathing CO_2-rich and CO_2-free gas mixtures

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In these experiments anaesthetized cats were given 5% CO_2 and CO_2-free gas mixtures in alternate inspirates. An intra-arterial pH electrode was used to monitor the CO_2 induced swings in arterial pH (Band, Cameron & Semple, 1969).

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Nine experiments were performed in which 24 runs were recorded when the CO_2-rich and CO_2-free mixtures were both hypoxic (10 % O_2). These were compared with 146 runs from the same experiments using normal oxygen mixtures.

In thirteen experiments 33 runs were recorded with the two mixtures hyperoxic (95 % and 100 % O_2) and were compared with 167 runs using normal oxygen mixtures.

The incidence of runs individually showing a highly significant pattern of alternately large and small tidal volumes ($P < 0{\cdot}01$) was about 50 %. There were no significant differences in the incidence of such runs at the different oxygen levels.

Hypoxic runs (P_{a,O_2} 40–60 mmHg) showed average tidal volumes larger than those of the paired euoxic runs ($P < 0{\cdot}02$). Average breath by breath tidal volume differences in hypoxic runs were also significantly larger than in paired euoxic runs. This applied both to actual volumes ($P < 0{\cdot}01$) and also when these breath by breath changes were expressed as a percentage of the average tidal volume of the run ($P < 0{\cdot}05$). In all but one experiment the hypoxic run followed the normal oxygen control.

Hyperoxic runs (P_{a,O_2} 420–555 mmHg) showed average tidal volumes smaller than those of paired euoxic runs ($P < 0{\cdot}01$), but average breath by breath differences in paired hyperoxic and euoxic runs did not differ significantly.

In man it has been shown that the response to breaths of alternately high and low CO_2 is hypoxia dependent (Marsh, Lyen, McPherson, Pearson & Cunningham, 1973). Although the cat and man differ in that a significant response is present in the cat in hyperoxia as often as it is in hypoxia the enhanced magnitude of the response in hypoxia agrees with the findings in man.

The presence of an alternate breath response in hyperoxia would not be predicted from studies of discharge in the cut sinus nerve of the cat since there does not appear to be sufficient mean discharge for an oscillating signal to be carried. However, with the sinus nerve intact mean discharge under hyperoxic conditions may be different as shown for hypoxic conditions (Neil & O'Regan, 1971).

Other experiments support the view that the results reported here result from oscillations in carotid arterial P_{CO_2} (at a frequency half that of respiration) rather than from any local effect of CO_2 on the lungs (Wolff, 1975).

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The pattern of stimulated breathing in man during non-elastic expiratory loading

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In order to study factors influencing breathing pattern, six normal subjects underwent CO_2 rebreathe experiments within a constant volume whole-body plethysmograph; initially unloaded and later with an added expiratory non-elastic resistance.

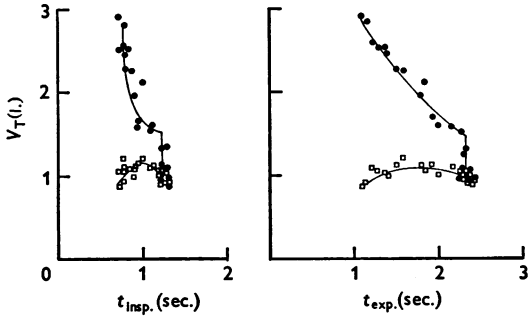

Fig. 1. A representative experiment. CO_2 rebreathe with expiratory resistance. Inspired tidal volume (V_T) plotted against inspiratory and expiratory duration. The open symbols indicate mean absolute values of V_T; the closed symbols the sum of the mean absolute V_T plus the change in V_{tg} for those breaths.

Intermittent measurement of end expiratory thoracic gas volume (V_{tg}) was made approximately every tenth breath during the rebreathe period.

The characteristic pattern of breathing as represented by the tidal volume/breath duration plot was established for each subject under non-loaded conditions. The maximum tidal volume attained ($V_{T\,max}$) in all six subjects was found to be equal to, or just less than the inspiratory capacity of each subject. No significant change in V_{tg} could be detected during rebreathing. Expiratory reserve volume was therefore not encroached upon even at the largest V_T.

Introduction of a non-elastic expiratory load equivalent to 4·5 cm H_2O at 1 l. sec^{-1} produced several changes in breathing pattern. The relationship inspiratory duration ($t_{insp.}$) to expiratory duration ($t_{exp.}$) was altered in the manner described by Zechman, Hall & Hull (1957). The most striking feature was a diminution in the V_T response associated with a loss of the break point (Clark & Euler, 1972). V_{tg} was unaltered at resting levels of ventilation but increased progressively as the rebreathe period continued. The maximum increase recorded being 2·1 l. Preliminary data indicate a similar phenomenon occurring in patients with airflow obstruction under unloaded conditions.

When V_T values were corrected for the change in V_{tg} and replotted against $t_{insp.}$ and $t_{exp.}$, a pattern not dissimilar from the unloaded studies reappeared. A break point was present and the total lung capacity appeared to limit $V_{T\,max}$ in five of the six subjects.

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Changes in intracranial pressure during brief exposure to hypoxia in anaesthetized cats

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Ponte & Purves (1974) have presented evidence that arterial chemoreceptors are involved in the cerebral vasodilatation response to hypoxia, which has generally been regarded as a locally mediated effect (Betz, 1972; Harper, 1972).

Information on the time course of changes in cerebral blood flow or of vasodilatation following abrupt alterations in blood gases might assist the distinction between these mechanisms.

The present experiments were designed to test the chemoreflex hypothesis indirectly in the relatively intact animal, without surgical procedures in the carotid region or prolonged cerebral hypoxia or ischaemia, which could alter the normal behaviour of the vascular bed. Responses were studied in terms of the latency of changes in intracranial pressure (ICP) following alterations in inspired gas concentrations. Changes in ICP were taken to reflect changes in intracranial blood volume and therefore in vasodilatation/vasoconstriction (in the absence of changes in central venous pressure).

Cats were anaesthetized with pentobarbitone. A cannula was inserted through a small parietal burr-hole so that the end 1 cm lay subdurally, and was connected to a transducer for ICP recording; also recorded continuously

were tidal volume (pneumotachograph), breath-by-breath CO_2 and O_2 concentration (infra-red and modified paramagnetic analysers), femoral arterial and central venous blood pressures and instantaneous heart rate. Arterial pH, P_{CO_2} and P_{O_2} were measured intermittently (Radiometer electrodes).

Chemoreceptor stimulation/inhibition was effected in these ways: (1) changing inspired gas to 10% oxygen for 2–3 min, followed by abrupt re-oxygenation with 100% oxygen, (2) rebreathing from 100 ml. 5% CO_2 with 14% oxygen, for $\frac{1}{2}$–1 min, (3) two to three breaths of nitrogen whilst breathing air, (4) two to three breaths of oxygen whilst breathing 10% oxygen. Responses occurred after a latency of 4–12 sec from the first change in end-expired gas concentration; alterations in ventilation, in arterial B.P. and in ICP were in the same direction and were simultaneous or within 3 sec of each other, during both 'on' and 'off' transients. When animals were paralysed and ventilation kept constant, ICP and B.P. responses still occurred with similar latencies. When by chance there was no hypertensive response, or when it was prevented by phenoxybenzamine, the ICP increase still occurred in close relation to the ventilatory response. When both ventilatory and B.P. responses were prevented, the ICP response occurred alone, with similar latency.

In circumstances other than hypoxic and/or hypercapnic stimulation, ICP changed in the opposite direction to ventilation and to arterial B.P., representing expected vasodilatation/vasoconstriction of the cerebral vessels.

These results show that, in conditions causing chemoreceptor stimulation, cerebral vasodilatation can occur independently of changes in ventilation or in arterial B.P.; the latency is consistent with a chemoreflex component in the response to hypoxia, but does not exclude other sites of action.

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Changes in aerobic power in patients undergoing elective surgery

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A deterioration in man's physical condition as a consequence of prolonged bed rest is well documented. One physiological change which occurs is a reduction in physical working capacity as reflected in the individual's maximum oxygen consumption (aerobic power). Most studies in this field

have involved the artificial immobilization of healthy subjects (Deitrick, Whedon and Storr, 1948).

This study involved male patients (24–45 years) undergoing abdominal surgery (vagotomy and pyloroplasty), and was undertaken to determine the degree of cardiovascular deterioration after surgery, and the subsequent recovery to normal. After surgery they remained in bed for 2 days and so were recumbent for a total of 3 days.

Aerobic power was calculated from the results of two 5 min submaximal tests on a bicycle ergometer employing a continuous series of increasing loads (Andersen, Shephard, Denolm, Varhauskas & Masironi, 1971). Values for oxygen consumption were extrapolated to a theoretical maximum heart rate of 220/min.age of subject (yr).

Patients were tested initially on admission (the day before operation), then at 4 and 6 days post-operatively. All were discharged after 7 days, but were retested after 28 and 42 days while still convalescing. Four days post-operatively aerobic power was reduced to 80% of the initial value. After 6 days there was no significant difference between pre- and post-operative values which were now 96% of the initial measurement. It is concluded that the initial fall in aerobic power is rapidly reversed on return to moderate activity. During the next period of outpatient convalescence a positive improvement was made and values increased to 113% of the initial measurements. By interpolation, the pre-operative 'normal' was reached about 31 days post-operatively.

All subjects showed a weight loss from initial mean weight of $67{\cdot}8 \pm 3{\cdot}5$ to $64{\cdot}2 \pm 3{\cdot}0$ kg. Fat content fell from 20 to 19%. These results indicate that such patients suffer no long-term cardiovascular deterioration as a result of surgical trauma and its associated immobilization. This agrees with results of another study involving the testing of patients 14 days after menisectomy (Bassey, Bennet, Birmingham, Fentem, Fitton & Goldsmith, 1972).

It would seem that in this present investigation the limiting factor to physical effort was abdominal discomfort during heavy breathing and trunk movement, which was reflected in their pattern of ventilation. In order to distinguish the effect of surgical trauma from simple immobilization, studies on medical patients, immobilized for clinical testing, will be carried out. Meanwhile the present regime of early ambulation after surgery would seem to have sound physiological support.

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Analysis of the gas exchange function of the gills in an elasmobranch fish, *Scyliorhinus stellaris*

By J. Piiper. *Department of Physiology, Max Planck Institute of Experimental Medicine, Göttingen, Federal Republic of Germany*

Based on physiological measurements of branchial gas transfer (Baumgarten-Schumann & Piiper, 1968) and morphometrical studies of the gill apparatus (G. M. Hughes, S. F. Perry & J. Piiper, unpublished), both performed in the same elasmobranch species, *Scyliorhinus stellaris*, and on theoretical studies on diffusion in interlamellar space water (Scheid & Piiper, 1971), a quantitative analysis of the branchial O_2 transfer in this species was attempted. The analysis was founded on an idealized gill model with counter-current flow of respired water and branchial lamellar blood.

In particular, the relative roles of diffusion limitation in interlamellar water and in the water–blood barrier of the secondary lamellae as derived from theory and morphometrical data were compared with experimental results of gas exchange measurements. It was estimated that in the resting conditions 42% of the total resistance to gill O_2 transfer was in the interlamellar water, 49% in the water–blood barrier. The relatively small remaining fraction, 9%, was attributable to unaccounted factors like diffusion and chemical reaction in blood, shunting of water or blood, cyclic variations of water and blood flow, and functional inhomogeneity of the system.

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Measurement of diffusivity of O_2 and CO_2 in respiring tissues: results in rat skeletal muscle

By T. Kawashiro, J. Piiper and P. Scheid. *Department of Physiology, Max Planck Institute of Experimental Medicine, Göttingen, Federal Republic of Germany*

In previous determinations of diffusivity of O_2 and CO_2 in tissues, the disturbing metabolic consumption of O_2 and production of CO_2 have been either suppressed by pharmacological or physical means, or taken into account by introducing corrections, or neglected. We have devised a new method allowing determination of Krogh's diffusion constant K for O_2 and CO_2 in tissues consuming O_2 and producing CO_2 at normal rates.

The tissue sample (which must be a uniformly thin layer) forms the diffusion barrier between a chamber flushed with gas of given composition and a closed chamber in which O_2 and CO_2 partial pressures are con-

tinuously measured by electrode techniques. The O_2 and CO_2 pressures in the closed chamber approach equilibrium values which are independent of their initial values. From the kinetics of this equilibration process, in connexion with geometrical dimensions, the K values can be calculated. In addition, the rates of O_2 consumption and CO_2 production can be obtained from the same experimental data.

The following mean values were found for rat abdominal muscle at 37° C:

$$K_{O_2} = 1{\cdot}31 \times 10^{-9} \text{ mmol cm}^{-1} \text{ min}^{-1} \text{ torr}^{-1},$$

$$K_{CO_2} = 28{\cdot}9 \times 10^{-9} \text{ mmol cm}^{-1} \text{ min}^{-1} \text{ torr}^{-1}.$$

The values are in reasonable agreement with comparable literature data.

Effect of age on oxygen binding to haemoglobin

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The relationship between the position of the oxygen binding curve as measured by the P_{O_2} at 50% saturation (P_{50}) and the red cell 2,3-diphosphoglycerate concentration (2,3-DPG) has often been studied in normal and diseased man but little attention has been given to the effect of age on this relationship. We have measured P_{50}, plasma pH (pH_e) and inorganic phosphate, haemoglobin concentration, erythrocyte pH (pH_i) and 2,3-DPG in the venous blood of forty-seven healthy, non-smoking adults of both sexes aged between 18 and 90 years. The variability of these values within individuals was determined by repeating measurements in three resting subjects on five consecutive mornings and in a further three non-resting subjects once weekly for 5 weeks at various times of the day. In each of the six subjects P_{50} expressed at pH 7·40, zero base excess and

Table 1. P_{50} and related factors in healthy, non-smoking adults

Age range (years)	P_{50} (mmHg)	DPG (μM/g Hb)	Inorganic phosphate (mg/100 ml. plasma)	Haemoglobin (g/100 ml. blood)	Plasma pH–erythrocyte pH
18–39	27·5	13·34	3·2	14·2	0·21
(n = 22)	S.D. 1·2	S.D. 1·62	S.D. 0·5	S.D. 1·2	S.D. 0·02
40–59	27·8	12·51	3·1	14·8	0·20
(n = 10)	S.D. 0·7	S.D. 1·20	S.D. 0·3	S.D. 0·8	S.D. 0·03
60–90	28·8	13·07	3·2	13·7	0·21
(n = 15)	S.D. 1·2	S.D. 1·64	S.D. 0·5	S.D. 1·1	S.D. 0·03

37° C, varied by 1·7–2·2 mmHg. 2,3-DPG, however, varied by 0·47–0·99 μM/g Hb on consecutive days and 1·32–1·67 μM/g Hb over the 5 weeks, but did not correlate with the P_{50}.

P_{50} tended to rise with age (Table 1), the mean value for the 15 subjects aged 60–90 years being significantly higher than that in the 22 subjects aged 18–39 years. No significant variation with age was noted for 2-3-DPG, inorganic phosphate, haemoglobin concentration or $(pH_e - pH_i)$. Correction of P_{50} to a constant pH_i 7·20 reduced the variance of P_{50} in each age range. This corrected P_{50} then showed a significant correlation with age ($r = 0{\cdot}60$, $P < 0{\cdot}01$) but did not correlate significantly with 2,3-DPG concentration.

Dynamic properties of stretch receptors in the trachea of the dog

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We have studied the activity of stretch receptors situated in an isolated, *in situ* segment of trachea in dogs artificially ventilated through the lower lobes of the right lung. The aim of this research was to evaluate the dynamic behaviour of these stretch receptors.

Two parameters were measured: tracheal pressure and the frequency of discharge of individual stretch receptors as evaluated from action potentials recorded from filaments separated from the peripheral cut end of the left vagus nerve. Thirty-two receptors have been studied in thirteen dogs.

In twenty-eight of our receptors the instantaneous frequency of discharge during the inflation phase of imposed sinusoidal pressure waves exceeded that measured in static conditions. At the lowest rate of oscillation used (17 c/min), the average discharge frequency at the mid-point of the pressure excursion was 126% of the corresponding static value. This figure increased with higher rates of oscillation, up to 161% at 220 c/min.

During the deflation phase of sinusoidal oscillations the frequency of discharge at the mid-point of the pressure excursion did not differ from the corresponding static value at the lowest rate of oscillation, but became lower with increasing rates of oscillation.

The remaining four receptors had discharge frequencies which did not exceed the static values during inflation, but in contrast these receptors showed higher frequencies of discharge during the deflation phase at all rates of oscillation.

In agreement with these findings, the adaptation indices, derived from responses to square wave pressure changes, were higher for the first mentioned group of receptors than for the second.

Consideration of the pressure–volume characteristics of normal airways indicates that the dynamic behaviour of most of the receptors studied is not consistent with their being simple transducers of circumferential tension in the airway wall. The static properties of these receptors would give an indication of airway transmural pressure, whereas their dynamic properties would provide information about its rate of change.

Grey squirrel dichromatic colour vision shown by flicker photometry

By Priscilla H. Silver. *M.R.C. Vision Unit, Centre for Research on Perception and Cognition, University of Sussex, Falmer, Brighton BN1 9QG*

By the use of a flicker photometer (Silver, 1969) it is shown that two grey squirrels (*Sciurus carolinensis leucotis*) can discriminate wave-lengths longer than 590 nm from those below 500 nm, the lights being matched in brightness simultaneously. One of the animals can also discriminate wave-lengths below 480 nm from most above 500 nm. This is the type of performance to be expected from a dichromatic visual system.

In the experiments, two monochromatic lights replace each other according to a $\sin^2$ law. For man, the sensation of flicker is least when the two are equally bright, and absent when they are also matched in hue. For squirrel, the animal's ability to detect flicker, indicated by its automatically recorded performance, is worst at same intensity ratio of the two lights, taken to indicate a brightness match. In a case where the alternated lights are of the same wave-length, performance drops to chance level. The same thing would happen if the two lights had identical effects on the visual system, a situation analogous to 'silent replacement'.

The residual ability to detect flicker when two alternating wave-lengths are equally bright, is taken as a measure of their hue difference, as seen by the animal.

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The visual pigments of the grey squirrel, *Sciurus carolinensis leucotis*

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There is ample evidence that the grey squirrel, *Sciurus carolinensis leucotis*, possesses at least two visual pigments (for review see Green & Dowling, 1975). However, only one of these has been certainly identified and that is a $P502_1$ extracted by Dartnall (1960). Microspectrophotometric

(MSP) examination of single visual cells from the grey squirrel retina has been undertaken to identify any other visual pigments present, and to localize specific pigments to morphologically distinct receptor cell types.

The retinas from three grey squirrels were isolated under dim red light and prepared for MSP examination using methods previously described (Bowmaker, Loew & Liebman, 1975). Only two classes of visual cells were

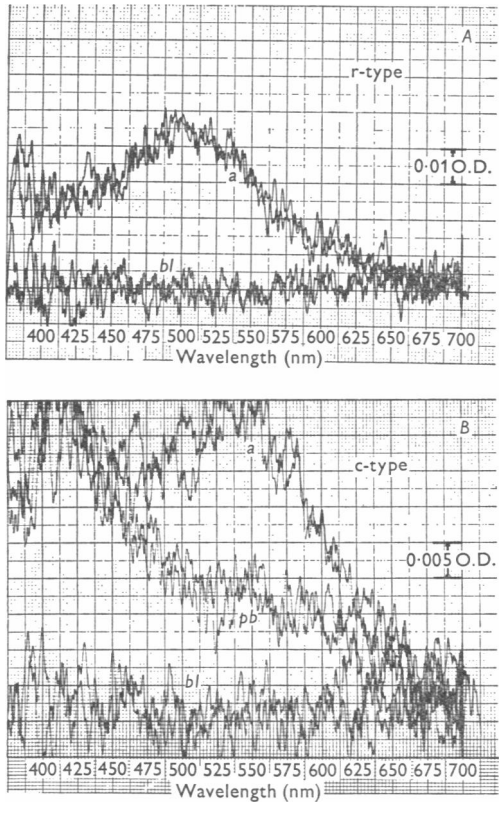

Fig. 1. Original absorbance spectra for single r-type (*A*) and c-type (*B*) outer segments. *a*, absorbance; *pb* post-bleach; *bl*, base line.

observed in fresh preparations – the c-type and r-type receptors described by Cohen (1964) and West & Dowling (1975). The r-type outer segments contain a visual pigment with a λ_{max} at about 500 nm, which is probably the same pigment as that found in extracts (Fig. 1*A*). Over 50 r-type cells were measured and all contained the 500 nm pigment. Over 70 c-type outer segments were measured and all contained a visual pigment with a λ_{max}, based on difference spectra, at 540–545 nm (Fig. 1*B*). In neither the c- nor

r-type receptors did bleaching of the visual pigment result in any measurable photoproducts absorbing above 400 nm.

Systematic examination of cells from different retinal areas showed no other visual pigments than those described. Assuming that those c- and r-type cells that were measurable are representative of the entire retinal receptor population, a blue receptor, if present in the grey squirrel, can amount to no more than 5 % of the total visual cell population, or, alternatively, such receptors must be restricted to a very small retinal area.

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Absence of spontaneous variability of orientational and directional tuning in cat visual cortical cells

BY D. P. ANDREWS, P. HAMMOND and C. R. JAMES. *Research Department of Communication, University of Keele, Keele, Staffordshire ST5 5BG*

Donaldson & Nash (1975) have recently reported that, over a period, most visual units in areas 17 and 18 of the adult cat show substantial fluctuations in their preference for direction and orientation. These results are at variance with our own extensive data and with those of most established groups (e.g. Hubel & Wiesel, 1962; Henry, Bishop, Tupper & Dreher, 1973; Ikeda & Wright, 1974; Rose & Blakemore, 1974).

We have collectively analysed several hundred cells in areas 17 and 18 of the adult cat's visual cortex (receptive field eccentricity up to 15°) and measured their directional and orientational tuning. Animals were prepared conventionally for visual experimentation and cell activity was recorded with 4 M-NaCl- or dye-filled micropipettes under light anaesthesia – pentobarbitone, chloralose, halothane/O_2, N_2O/O_2 + Halothane, or N_2O/O_2 + trace pentobarbitone – or from the unanaesthetized *cerveau isolée*. Stimulation ranged from hand-held wands and front- or rear-projection to pseudorandomized, computer-controlled X–Y displays.

All units were identified as 'simple', 'complex' or 'hypercomplex'. Receptive fields of simple and, where possible, complex cells were mapped with stationary flashed stimuli, or alternatively their minimum response fields (Barlow, Blakemore & Pettrigrew, 1967) were defined with moving bars of optimal orientation. Orientation tuning curves were obtained with light or dark bars or edges, moved back and forth across the receptive field at orientations 3–12° apart, and the directional bias was noted. Care was

taken to ensure that bar width and velocity were optimal, that the stimulus path was centred over the receptive field, and that stimulus traverse and length were adequate to cover the field.

In many cells, orientation tuning was measured at varying intervals over periods of up to 8 hr. *Responsiveness* varied spontaneously in all preparations, but in no instance have we observed shifts in orientation of more than a few degrees (i.e. within experimental error); nor have we seen dramatic shifts in directional bias, except in experiments involving interacting stimuli. We suspect that Donaldson & Nash's results may be due to several factors – excessively deep pentobarbitone anaesthesia, frequent use of binocular stimulation without correction of interocular alignment, and coarseness of orientation tuning measurements.

We conclude, in line with most other researchers in the field, that gross shifts of orientational or directional tuning are not features of cells in areas 17 and 18 of the cat.

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Pinna reflex activated γ-efferents in the conduction velocity spectrum to hind-limb muscles in the rat

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In an earlier communication (Andrew, Leslie & Part, 1975) we reported that γ-efferents activated by the pinna reflex in the anaesthetized rat produced a static fusimotor action in hind-limb muscles. We have now examined the conduction velocity spectrum of γ-efferents to soleus and peroneus brevis muscles in the rat. Conduction velocities and number of fibres were obtained by stimulation of the motor nerve at its point of entry to the muscle and recording both from the complete ventral roots (L4, L5 and L6) and later from filaments dissected from the peripheral end of cut ventral roots. Because of the small diameter of some of the efferents, signal averaging was necessary to pick out individual fibres in complete root recordings. Soleus had 18–25 γ-fibres ranging from 5 to 27 m sec^{-1} and