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THE EFFECT OF SOME NEUROKININ A (NKA) ANALOGUES ON NKA-INDUCED CONTRACTION OF GUINEA- PIG TRACHEAL SMOOTH MUSCLE *IN VITRO*

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The peptide neurokinin A is found in the lung and is known to cause bronchoconstriction both *in vivo* and *in vitro* (Theodorsson-Nerheim *et al,* Acta. Physiol. Scand. 1985: 124, 129, Joos *et al,* Thorax. 1987: 243, 901), but an understanding of its role in control of airway function in health and disease has been difficult because of the lack of specific antagonists. It was the purpose of this study to synthetise analogues of NKA and to test them for pharmacological antagonism of NKA-induced contraction of guinea-pig tracheal smooth muscle. We have previously reported that NKA analogue in which glycine^{8} was replaced by aminoisobutyric acid (Aib) is an antagonist of NKA, but not Substance P, activity on tracheal smooth muscle from guinea-pig (Abu Shanab *et al,* Biochem. Soc. Trans. 1990: 18, 286). We now report the effect on biological activity of deletions at the N-terminus.

NKA was purchased from Biosyn Ltd. (Belfast, N. Ireland) and the peptides [Ala^{5}, Aib^{8}, Leu^{10}] NKA (4-10), [Ala^{5}, Aib^{8}, Leu^{10}] NKA (2-10) and [Ala^{5}, Aib^{8}, Leu^{10}] NkA, hereafter referred to as peptides 1, 2 and 3 respectively, were synthetised by solid phase methods. Isometric contraction of isolated guinea-pig tracheal rings in response to exogenous NKA (10^{-10} to 10^{-6}M) was recorded as described previously (Abu Shanab *et al,* Biochem. Soc. Trans. 1990: 18, 286) and the effect of the peptide analogues on the NKA dose-response relationship examined.

In the presence of peptide 3 (10^{-8} to 10^{-6}M) NKA evoked contractions were reduced at all doses producing a characteristic shift in the NKA dose-response curve suggestive of classical non-competitive antagonism, although contractions to substance P (SP; 10^{-8} to 10^{-6}M) were unaltered by analogue. In contrast, peptide 2 (10^{-8} to 10^{-6}M) caused a dose-dependent shift to the right of the NKA dose-response curve with no depression of the maximum NKA response. Peptide 2 showed no pharmaclogical antagonism to SP. A schild regression plot for peptide 2 antagonism of NKA evoked contractions yielded a straight line of slope -0.4 with an interscept (PA_2) of 7.7 on the abscissa. Unlike peptides 2 and 3, peptide 1 (10^{-6}M) itself caused contraction of the tracheal preparation.

In conclusion, peptide analogues that selectively antagonise the activity of NKA on tracheal smooth muscle have been produced by replacement of Gly^{8} in a NKA analogue that also had the substitutions Ala^{5} and Leu^{10}. Removal of the N-terminal residue did not abolish this selectivity although the nature of the antagonism was altered. Removal of a further two amino acid residues from the N-terminus resulted in the generation of agonist activity.

IRON STATUS AND BLOOD LIPIDS IN MALE AND FEMALE RATS

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Sex differences have been observed in the incidence of ischaemic heart disease (IHD), with males being more susceptible (Lerner *et al,* Am. Heart J. 1986: 111, 383). Higher iron levels in men have been implicated as a possible explanation (Sullivan, The Lancet, 1986: ii, 1162) and iron intake has been shown to influence lipid composition of the plasma in male rats (Cunnane & McAdoo, J. Nutr. 1987: 117, 1514).

In this study, two levels of dietary iron (Fe), 15 mg/kg (LFe) and 400 mg/kg (HFe), were fed to 12 male and 12 female weanling Sprague-Dawley rats for 42 days. Animals were anaesthetised with ether and sacrificed by exsanguination using cardiac puncture. Plasma lipids and iron indices were measured and the data analysed by two-way analysis of variance using iron and sex as main effects.

As expected, plasma Fe was significantly higher ($p<0.001$) in HFe rats (mean, 2.32; SE, 0.13 mg/l), compared with the LFe rats (mean, 1.52; SE, 0.28 mg/l). Plasma Fe was significantly higher ($p<0.001$) in female rats (mean, 2.48; SE, 0.20 mg/l) compared with the males (mean, 1.36; SE, 0.18 mg/l), and transferrin saturation (TS) was significantly higher ($p<0.01$) in females, with a mean of 48% (SE, 4.5), in comparison with the male group mean of 29% (SE, 4.1). (TS) was also significantly higher ($p<0.01$) in the HFe rats, with a mean of 47% (SE, 3.1) compared with 29% (SE, 5.4) in the LFe rats.

Plasma cholesterol (CHOL), and triglycerides (TRIG) were significantly higher ($p<0.001$) in the HFe rats, (CHOL; HFe, mean, 1.47; SE 0.07; LFe, mean, 1.20; SE, 0.08 mmol/l: TRIG; HFe, 1.32; SE, 0.07; LFe, mean, 0.93; SE, 0.08 mmol/l). CHOL was also significantly higher ($p<0.001$) in males (males, 1.53; SE, 0.05; females, 1.15; SE, 0.06 mmol/l).

These results indicate that there may be sex differences in the relationship between Fe status and plasma lipids which appear consistent with a role for dietary Fe in the aetiology of (IHD).

THE NATURE OF LOCALISED CONSTRICTOR RESPONSES TO ISOPRENALINE IN THE ISOLATED *IN SITU* LATERAL SAPHENOUS VEIN OF THE DOG

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When the canine lateral saphenous vein is relaxed, fibroelastic sphincters in its tributaries block the reflux of venodilator drugs from the lumen to the vasa vasorum of the vein. I have attempted to overcome the block in the case of isoprenaline (ISO) by injecting it through a catheter (Portex tubing 1.52 mm o.d.) into the mouths of the compressed venules which carry reflux to the vasa; these lie deep in the cusps of the tributaries' cardinal valves. The technique has been used to examine the response of a relaxed vein to a 1 ml bolus of 1 mM ISO injected into a cusp of the cardinal valve of both the lateral plantar and the calcaneal tributary. Tests with Methylene Blue dye indicated that 97.5% of the injected bolus flowed ineffectively to waste, leaving a maximum of 2.5% of the bolus to cause a constrictor effect. The vein was perfused at 38 ml/mn constant flow with oxygenated Tyrode solution at 34°C, in an amputated leg.

ISO caused a constrictor response which had a shorter latency than a response of equivalent strength to a 1 ml bolus of 1 mM noradrenaline; the effect was invariably localised to a single section (about 1 cm long) of the vein immediately upstream or downstream from a catheterised tributary. Commonly, the change in perfusion pressure was 35-60 mm Hg but individual responses varied greatly and

unpredictably. The constrictor effect was uneven, with some quadrants of the wall of an affected section being more constricted than others. Prazozin 1 μg/ml blocked the response; propranolol 2 μg/ml, and phentolamine 10 μg/ml had no effect. Both guanethidine (Ismelin), perfused at 4 μg/ml for 12 minutes, and reserpine (Serpasil), perfused at 5 μg/ml for 170 minutes, abolished the response to electrical stimulation but strengthened the response to ISO. ISO had no effect when injected into the vein lumen proper.

The findings confirmed that ISO did not cause a constrictor effect through its intraluminal action; they contradicted the belief that high concentrations of ISO cause an indirect constrictor effect by stimulating the release of neuronal noradrenaline. The evidence indicated that ISO directly stimulated the smooth muscle cells of the vein by activating their alpha$_1$ receptors. Four observations suggested that the drug acted after it had been released from the vasa vasorum of the vein: (1) the constrictor effect occurred only if the drug was injected into a venule which carried reflux to the vasa vasorum; (2) the vein wall constricted asymmetrically and, sometimes, upstream from the point of injection; (3) the sections of the vein which constricted when the plantar tributary had been catheterised had the same distribution as the sections of the vein whose vasa had filled with ink by reflux through the plantar tributary (Crotty, Microvasc. Res. 1989: 37, 119) and (4) the length of a section constricted by ISO was the same as that of a section dilated by noradrenaline released from the vasa vasorum (Crotty, Br. Hom. J. 1989: 78, 127).

PATCH CLAMP STUDY OF OPENINGS OF POTASSIUM CHANNELS IN RAT SKELETAL MUSCLES

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Sarcolemmal vesicles were prepared from isolated soleus, EDL and vastus lateralis muscles of rat by the method of Burton *et al.* (J. Physiol. 1987: 394, 12P). Muscles were removed from rats under sodium pentobarbitone anaesthetic (40 mg/kg). A bundle of fibres was pinned to a silastic block and immersed in 140mMKCl buffered by HEPES at pH 7.8 after removal of connective tissue and fat. It was then transferred to a similar solution containing Sigma collagenase Type 1A (100 units/ml) and incubated for about 40 min at 30°. The vesicles were detached into a solution containing 140mM KCl 5mM K_2ATP, 1mM EGTA 0.5 mM $MgSO_4$ at pH 7.4. Where Cl-free fluid was required the anion was replaced by glucuronate. Micropipettes were prepared from washed haematocrit tubes, their tips were coated with Sylgard to within 50 microns of the openings which were then fire polished. The micropipettes were filled with fluid similar to that bathing the vesicles but with 1-3mM Ca gluconate replacing EGTA. They had a tip impedance of 10MΩ increasing to about 20GΩ on sealing with vesicular membrane. Attached cells or whole cell clamps were used. Soleus muscle yielded few channel openings except in presence of 10^{-6}M salbutamol in the micro pipette or bath, whereupon inward current was found on slight hyperpolarisation of the patch. Their open time average 20ms and they seemed to have weak voltage dependence. In vesicles from EDL hyperpolarisation of about 30mV opened channels with mean open time of 10ms. This inwardly rectifying channel had a conductance of about 20 pS.

In Cl-free conditions with 3mM Ca in the pipette hyperpolarisation opened channels with conductance of 164 pS and open time of 1-3ms. Channel openings in response to suction applied to the patch was also noted at times.

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THE REFLEX CHANGES IN RENAL VASCULAR RESISTANCE IN RESPONSE TO CAROTID OCCLUSION AS A TEST OF RENAL DENERVATION

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Unloading the carotid sinus baroreceptors induces a reflex increase in vascular resistance in the kidney and other vascular beds (Acta Physiol. Scand. 1972: 85, 464). Electrical stimulation of the efferent nerves to the kidney is a common way of establishing denervation of this organ. However, it is difficult by this means to be certain of total denervation. In this study we have measured the reflex changes in renal vascular resistance in response to carotid occlusion as a possible test of renal denervation.

Dogs were surgically prepared under sodium pentobarbitone anaesthesia (30 mg/kg I.V.). Supplemental doses were administered as required through a 20-gauge IV placement unit in the cephalic vein. Both kidneys were exposed through flank incisions and surgical blood loss minimized by electro-surgical bipolar coagulation. The right renal pedicle was thoroughly cleaned with saline soaked swabs and stripped of all visible nerves. The region was then coated with a solution of 10% phenol in absolute alcohol. Noncannulating electromagnetic flow transducers were positioned about the right and left arteries. Both common carotid arteries were exposed for clamping through a mid line neck incision. A homeothermic blanket system was used to stabilize the animal's temperature. Blood pressure was recorded with a Statham P23AA pressure transducer attached to an arterial line advanced into the abdominal aorta. All signals were displayed on a Grass model 7 polygraph. Statistical analysis was by Students t-test.

One minute after clamping the common carotid arteries a significant increase in renal vascular resistance (R.V.R.) was observed on the innervated side but no significant change occurred on the 'denervated' (Dx.) side. It was concluded that the sympathetic efferent nerves to the 'denervated' kidneys were totally disrupted.

		Control	CCA clamp	p
B.P.	Innervated	138.3 (12)	182.7	<0.001
(mm Hg)	Dx.	126.6 (9)	170.3	<0.001
R.B.F.	Innervated	128.8 (12)	130.4	n.s.
($ml.min^{-1}$)	Dx.	132.2 (9)	170.6	<0.001
R.V.R.	Innervated	12.48 (12)	16.57	<0.01
($kPa.\ ml^{-1}.S$)	Dx.	11.92 (9)	10.51	n.s.

THE RENAL RESPONSE IN THE ANAESTHETISED WATER-LOADED PROSTAGLANDIN E_1

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Intrarenal prostaglandin E_1 has both direct saluretic and indirect antidiuretic actions on the kidney (J. Physiol. 1978: 281, 1). The potential release of arginine vasopressin (AVP) by overspill of prostaglandin E_1 from the infused kidney, might mediate the latter effect ('The Posterior Pituitary' by Baylis & Padfield, 1985, p. 72). In this study we have investigated whether intracarotid prostaglandin E_1 produces an antidiuresis of greater magnitude than intrarenal prostaglandin E_1 and whether a measurable increase in plasma AVP occurs.

In ten dogs surgical anaesthesia was induced with sodium pentobarbitone, 30 mg/kg IV. Both ureters were cannulated through flank incisions and a hooked 21-gauge needle inserted into the common

carotid artery for infusion of a prostaglandin E_2, 2 $\mu g.min^{-1}$. The animals were hydrated by IV infusion of a 1 : 1 mixture of glucose (0.3M) and isotonic saline at a rate of 10 $ml.min^{-1}$. Experiments consisted of control, test and recovery periods, each of 30 min. and divided into 10 min. collection intervals. Arterial blood for RIA of pAVP was collected at the mid-point of the third control interval and at the mid-point of the third test interval.

Plasma AVP at the mid-point of the third 10 min. infusion interval did not differ from control values. A decrease in urine output occurred from both kidneys. On the right side urine output fell from a median value of 2.2 to 1.4 $ml.min^{-1}$ ($p<0.01$, Wilcoxon signed rank test for paired data). This decrease did not differ in time-course or magnitude from the decrease which occurs on the right side on infusion of the same dose of prostaglandin E_1 into the left renal artery (Ir. J. Med. Sci. 1988: 157, 25, the Mann-Whitney test for determining differences between two groups, $p=0.69$). There was a significant increase in urine osmolality from a median value of 182 to 223 mosmoles/kg H_2O ($p<0.05$), with downward trends which did not achieve statistical significance in sodium ($U_{Na}.V$) and solute (Uosm.V) excretion. The decrease in free water clearance (C_{H2O}) was significant at $p<0.05$ level. The delivery of sodium from the proximal to the distal nephron [$(C_{Na} + C_{H2O})$. pNa] was decreased at $p<0.05$ level. The findings suggest that the decrease in volume and the increase in Uosm may follow a decrease in filtrate delivery from the proximal tubule rather than an increase in the water permeability of the distal and collecting tubules.

ANATOMICAL LANDMARKS AND THE ACCURACY OF EXPERIMENTAL TOTAL HIP ARTHROPLASTY

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Acetabular cup malposition is a major cause of dislocation following total hip arthroplasty. Nevertheless, accurate cup placement still depends on the subjective judgement of the surgeon rather than objective criteria, and may be rendered difficult by obesity or the position of the body at operation. The anterior superior iliac spines and the pubic tubercles are readily identifiable landmarks which lie in the vertical plane of the body and can be used as reference points to establish the recommended angles of acetabular anteversion (0°) and abduction(45°) which is independent of body position.

A metal frame, on which was mounted a light source, was placed on these bony points and used to assist the insertion of Charnley cups in 14 dissection room cadavers. Optimum positioning was achieved when a collimated beam of light was reflected back to the source from a metal mirror fixed to the cup introducer. Cups inserted conventionally on the contralateral side served as controls and the accuracy of the two procedures was compared both radiologically and by direct measurement. The use of the device produced statistically significant decreases in the mean deviations from the desired angles of both abduction ($p<0.001$) and anteversion ($P<0.01$). When the total range of cup positions was considered use of the experimental technique resulted in a threefold increase in acccuracy.

These results provide an example of the practical application of anatomical principles and of the potential usefulness of the dissection room in clinical research.

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SELENIUM SUPPLEMENTATION AND THYROID FUNCTION IN HEALTHY VOLUNTEERS

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Recent work involving rats and cattle has shown that selenium affects thyroid function (Arthur *et al*, Res. Vet. Sci. 1988: 45, 112; Beckett *et al*, Biochem. J. 1987: 248, 443). In Se deficient animals plasma concentrations of thyroxine (T_4) are increased whilst triiodothyronine (T_3) concentrations are decreased. Se deficiency inhibits both type I and type II 5' deiodinase activity in the brain, liver and kidney and may also inhibit 5 deiodinase activity. The increased levels of T_4 are due to impaired conversion to T_3 and the absence of inhibition of thyroid stimulating hormone (TSH) production normally controlled via type II 5'-deiodinase activity (Beckett *et al*, Biochem J. 1989: 259, 887).

The current study assessed the affect of Se supplementation on thyroid function in 14 healthy volunteers. The subjects, mean age (SD) was 22.2 (1.6) years, received a Se supplement (200 ug) daily for six weeks. Three measurements were taken at (i) baseline, (ii) the end of the supplementation period and (iii) four weeks post supplementation. Mean (SD) serum Se significantly ($p<0.001$) increased on supplementation from (i) 1.09 (0.04) to (ii) 1.41 (0.06) uM/l and returned to the initial level four weeks after the end of the Se supplementation. However red cell glutathione perioxidase activity was unaffected by supplementation [(i) 159 (21), (ii) 153 (25), (iii) 164 (25) U/gm haemoglobin]. Similarly there were no significant differences on supplementation levles [mean (SD)] of free T_3 [(i) 5.4 (0.2), (ii) 5.9 (0.28), (iii) 5.5 (0.27 pmol/l] total T_3 [(i) 2.1 (0.08),)ii) 2.1 (0.10), (iii) 2.0 (0.07) nmol/l] free T_4 [(i) 18.3 (0.77), (ii) 18.6 (1.2), (iii) 19.8 (1.1) pmol/l] total T_4 [(i) 96.7 (3.9), (ii) 99.2 (5.1), (iii) 96.9 (5.3) nmol/l].

Results would suggest that Se status of these subjects was sufficient not only for optimum GSH-Px activity but also for other possible physiological requirements for Se such as thyroid function.

Ethical approval for the study was obtained from the University of Ulster Ethical Committee and subjects gave informed consent.

THERMOREGULATORY DISTURBANCE OF THE STEADY-STATE RESPONSE TO BICYCLE EXERCISE AT CONSTANT-LOAD

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During a previous study on the exercise at a constant work load in the heat, a steady-state heart rate was only briefly achieved before commencing to rise steadily. A rise in skin temperature was associated with the onset of this secondary increase in heart rate. The aim of this study was to determine the pattern of physiological responses to different levels of steady-state exercise and to see whether there was any relationship between the oxygen consumption, minute ventilation and heart rate responses and deep body and skin temperatures. The individual degree of fitness of four subjects was first determined using a progressive bicycle exercise test. 30 min bouts of constant-load bicycle exercise were then studied at 3 load levels (LOW)L), MEDIUM (M) and HIGH (H)) related to individual fitness. Ambient temperature was 20°C, relative humidity 50%. The mean oxygen consumption at the three levels of exercise was equal to 44.4 (L), 56.1 (M) and 75.2 (H) of maximum oxygen consumption.

Deep body temperature (DBT) - recorded from an insulated thermistor probe inserted in the external auditory meatus), with a delay of about 5 to 10 min after beginning exercise, showed a continuous rise, reaching significantly higher levels during the high intensity exercise (dT_{core}: 1.33±0.28 (H), 0.7±0.13 (M) and 0.022 (L) ($P<0.05$). Temperature of the back of the right hand (recorded by a calibrated thermocouple) showed a different pattern of change with an initial decrease (vasoconstriction) followed by an increase (vasodilation) concurrent with the onset of rise in DBT. On start of exercise the minute oxygen consumption, minute ventilation and heart rate quickly reached initial steady values. However, at the time of commencement of elevation in DBT and of vasodilatation all of these variables began to rise. The incremental increase in heart rate was significantly higher at H load ($P<0.05$ than at M or L (22.67±1.97, 8.67±4.26, 2.83±2.42 bpm, respectively). A positive correlation was found between core temperature and heart rate elevation at all work rates, but a much higher correlation coefficient was found in H and M than in L (r^2: 0.932±0.032, 0.636±0.177 and 0.291±0.201 respectively).

In summary we can say that during constant load exercise a brief cardiorespiratory steady-state condition is reached even at high work loads, but it is transient in nature. Commencement of disturbance of this steady-state coincides well with the beginning of concurrent elevation in core temperature and skin temperature.

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IN VITRO ASPIRIN INHIBITION OF PLATELET AGGREGATION IN ORAL CONTRACEPTIVE USERS AND CONTROLS

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Epidemiological studies have shown that in women taking combined oestrogen/progestogen oral contraceptives, the incidence of both venous and arterial thromboembolic disease is increased. Increased platelet aggregation has been reported in women taking combined oral contraceptives and also changes in the levels of prostaglandins. These changes may be related to the enhanced platelet aggregation found.

Whole blood platelet aggregation was measured in 36 women aged between 18 and 34 years; 18 of these women were taking triphasic oestrogen/progesterone oral contraceptives (oral contraceptive group), the remaining 18 women, who were not taking any hormone therapy, acted as healthy controls (control group).

Whole blood platelet aggregation stimulated by ADP (0.5 uM), platelet-activating-factor (PAF) (0.05 uM), collagen (0.5 ug/ml), adrenaline (1.0 uM) and arachidonic acid (AA) (0.2 mM) was measured both in the presence and absence of the cycloxgenase inhibitor aspirin (100 uM).

Significantly higher levels of aggregation were found in response to collagen, adrenalin and AA ($P<0.01$, $P<0.05$, $P<0.001$) in the oral contraceptive group compared with the control group, however when whole blood was incubated with aspirin there were no significant differences between the groups. In the oral contraceptive treated group collagen, adrenalin, PAF and AA induced aggregation were significantly reduced by incubation of whole blood with aspirin ($P<0.001$, $P<0.05$, $P<0.03$, $P<0.01$). In the control group, collagen and AA induced aggregation was significantly reduced by aspirin ($P<0.001$).

The results of this study indicate that there is an increase in platelet aggregation in women taking oral contraceptives and that this increase could be prevented by inhibiting the cyclo-oxygenase enzyme and thus preventing synthesis of thromboxane A2. It would appear therefore that the increases in platelet aggregation observed may be mediated by the enhancement of the cyclo-oxgenase pathway leading to an increase in the formation of pro-aggregatory prostaglandins.

RELATIONSHIPS OF ENDOMETRIAL $PGF_2\alpha$ WITH NORMAL MENSTRUATION AND DYSFUNCTIONAL UTERINE BLEEDING

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We have recently shown that patients with dysfunctional uterine bleeding (DUB) have generally lower than normal levels of 13, 14-dihydro-15 Keto $PGF_2\alpha$ PGFM) in their endometria (Sharma *et al*, Proc. Roy. Acad. Med. (Biol. section), January, 1990). PGFM is a metabolite of $PGF_2\alpha$ and is formed by the action of prostaglandin 15-dehydrogenase 14 reductase on $PGF_2\alpha$. In the present study we have investigated if levels of $PGF_2\alpha$ in the endometrium of patients with DUB are also lower than normal and if they bear any relationship to menstrual blood loss in the two populations.

Subjects were recruited following menstrual blood loss studies over a minimum of two consecutive cycles. Those with a mean blood loss of more than 80 ml per cycle were regarded as DUB; others were classed as normal subjects. Women with any uterine pathology were excluded from the study. Endometrial samples were collected immediately after hysterectomy and stored at -80°C in indomethacin containing ethanol (5 µg/ml w/v). These were later extracted and estimated for $PGF_2\alpha$ using radioimmunoassay techniques described earlier (Sharma *et al*, Prostaglandins II(3), 555, 1976). In each case the uterine tissue was also examined histologically to confirm the phase of the menstrual cycle.

TABLE I

Endometrial tissue levels of $PGF_2\alpha$ in women with DUB and normal menstrual bleeding (ng/g; mean ± SEM)

Subjects	Phase of the menstrual cycle				Full menstrual cycle
	Proliferative	Early-mid secretory	Late secretory	Menstrual	
Normal	1.71±0.29 (n=5)	13.07±2.73 (n=7)	9.92±3.41 (n=6)	5.87±1.05 (n=6)	8.16±1.42 (n=24)
'r_{test}' value*	-0.37	-0.35	-0.59	-0.66	-0.39
DUB	11.40±4.40 (n=12)	10.73±6.26 (n=5)	29.18±15.03 (n=4)	12.85±6.63 (n=5)	4.29±3.52 (n=26)
'r_{test}' value*	-0.19	-0.88	+0.73	+0.52	>1

*between menstrual blood loss and endometrial $PGF_2\alpha$

The results in Table I indicate that, with the exception of (early to mid) secretory phase, the endometrial levels of $PGF_2\alpha$ in DUB are higher than normal. $PGF_2\alpha$ is a vasoconstrictor of most blood vessels and its rise in the endometrium of DUB patients is therefore somewhat surprising. Although this may reflect the body's own defence mechanism to control bleeding it may indicate a distrubed metabolism of the eicosanoid in DUB patients. From these results it however seems unlikely that excessive menstrual bleeding is the result of a reduced endometrial level of $PGF_2\alpha$. Table I also indicates that there is a uniform (although small) negative correlation between endometrial $PGF_2\alpha$ and menstrual blood loss in the normal subjects. This uniformity is not seen in patients suffering from DUB. In them there is a (small) negative correlation in the first two phases which is changed to a positive correlation during the last two phases of the cycle. Furthermore there is a negative correlation between endometrial $PGF_2\alpha$ and blood loss in normal subjects when values from the full menstrual cycles (irrespective of phase) are taken into consideration. This is absent in the DUB group. From these results it appears that $PGF_2\alpha$ plays a role in normal menstruation but has an abnormal biosynthesis in DUB.

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BLOOD BIOCHEMISTRY OF ALCOHOL DRINKERS IN THE GENERAL POPULATION

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Various laboratory tests have been proposed as indicators of alcohol abuse. However, many of these lack sufficient sensitivity and specificity as biochemical markers of excessive drinking and there are few data on how alcohol consumption affects such markers in unselected populations. In this study a random sample of the Northern Ireland population, aged 18-64 years, gave a blood sample and alcohol consumption was elicited by questionnaire and by a seven day weighed dietary record. Subjects were classified as either drinkers (drink at least once/week) or non-drinkers (including occasional drinkers) using questionnaire data. Blood was analysed for: haematological profile, including haemoglobin (Hb); iron, folate and vitamin B_{12} status including serum Fe and serum ferritin (SF); electrolytes, including calcium; a range of liver and kidney function tests including gamma-glutamyl-transferase (GGT); lipids, including high density lipoprotein cholesterol (HDL-C); and antioxidant enzyme activities, including erythrocytre superoxide dismutase (SOD).

Only those measurements, which were significantly affected by alcohol consumption in either sex by two-way analysis of variance testing for the main effects of alcohol consumption are expressed as means with standard deviations in parentheses.

When smoking status was also included with the other independent variables of sex, age and socioeconomic status in multiple regression analysis, alcohol consumption remained a significant independent variable in explaining the variance of each of the measurements (and vitamin B_{12}) given in the Table. Spearman correlates with the quantity of alcohol consumed (7-day weighed records) were significant for HDL-C, SF and vitamin B_{12} in male drinkers and for urate, GGT, Fe, Hb and Ca in female drinkers.

	GGT (U/l)	urate (mM/l)	HDL-C (mM/l)	Hb (g/l)	Fe (μM/l)	SF (μg/l)	Ca (mM/l)	SOD (U/mgHb)
Male								
Drinkers (n, 134)	28.4**	0.34	1.48	150	17.2*	121***	2.44	17.1
	(19.1)	(0.09)	(0.39)	(11)	(7.1)	(100)	(0.66)	(3.2)
Non-Drinkers (n, 84)	23.0	0.32	1.35	147	14.0	83	2.35	17.4
	(11.1)	(0.07)	(0.25)	(11)	(3.9)	(60)	(0.10)	(2.9)
Female								
Drinkers (n, 105)	18.8**	0.26**	1.80*	134*	14.9	50**	2.37*	17.3*
	(10.6)	(0.05)	(0.41)	(11)	(4.8)	(59)	(0.08)	(4.1)
Non-Drinkers (n, 176)	16.3	0.24	1.66	130	13.4	39	2.33	18.8
	(10.4)	(0.06)	(0.34)	(11)	(4.4)	(30)	(0.10)	(4.8)

* P<0.05; ** P<0.01; *** P<0.001 significantly different from non-drinkers.

Ethical approval was obtained from the Queen's University Ethical Committee and subjects gave informed consent.

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PENTOBARBITONE AND VALPROATE ENHANCE PUNISHED DRINKING IN RATS BY ACTIONS ON NEURONAL ION CHANNELS

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It is generally agreed that benzodiazepines and related drugs bring about their effects by acting at a receptor complex containing a benzodiazepine receptor, a GABAa receptor and a chloride ion channel which functions as the "effector" for the complex. This model predicts that GABAa agonists, as well as benzodiazepines and "channel agonists" such as barbiturates, should induce anxiolytic effects in humans and anticonflict effects in appropriate animal paradigms. However a large number of studies using both directly- and indirectly-acting GABA agonists have failed to confirm this prediction. The single exception appears to be valporate, which consistently produces anticonflict effects in animals

We report data from two experiments using punished drinking in rats, a widely-used conflict bahaviour. Both pentobarbitone (10.0 mg/kg) and valporate (200.0 mg/kg) significantly increased punished drinking, these being maximally-effective doses. In both cases the increases were significantly attenuated by the "channel antagonist" picrotoxin (1.5 or 2.0 mg/kg) and Ro 5-3663 (5.0 mg/kg) and also by the benzodiazepine antagonist Ro 15-1788 (10.0 mg/kg). The similarity between these interactions, together with other findings, argue for the chloride ion channel as the site of valporate action. It is less clear why, in these and several other experiments, the actions of "channel agonists" should be attenuated by benzodiazepine antagonists. An hypothesis based on the behavioural processes involved in conflict paradigms is offered.

BIDIRECTIONAL TEMPERATURE CONTROL IN A SINGLE HUMAN SUBJECT USING A PROGRAMME OF BIOFEEDBACK AND INTERMITTENT REINFORCEMENT

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Using a programme of biofeedback, a single subject was trained to increase the peripheral digital temperature of the index finger of his dominant hand. The temperature was monitored with LM35D sensors and feedback was provided by a BBC Master computer. Initially the feedback consisted of a continuous bidirectional tone and stars which were awarded for the correct response on a Fixed Ratio-1 schedule (FR-1). Once responding was initiated the subject was transferred to a Fixed Interval-2 minute (FI-2 min.) schedule of star availability, while the tone was given according to an ABA design across sessions. An exclamation mark on the VDU screen acted as a discriminative stimulus for star availability.

The subject responded during both schedules of feedback delivery, however the response pattern was different in each. During the FR-1 schedule there was a single increase of approximately 2 degrees centigrade (2°C), which was held until the end of the feedback session. However, during the FI-2 min. schedule, a bidirectional response pattern of approximately 2°C emerged. Initially the temperature would rise by approximately 2°C and be held to the end of the first reinforcement interval. Once a star was awarded it would drop rapidly to the starting temperature and remain there for approximately one minute, when it would increase once more and be held until the next star was obtained. This pattern was repeated every two minutes until the end of the feedback session.

The pattern appeared only in the digit receiving contingent feedback (on which the subject was concentrating) and was not affected by the presence or absence of auditory feedback. The temperature in the contralateral hand tended to fall during each feedback period.

The results show that fine differential control of digital temperature can be achieved in human subjects using a programme of biofeedback. It is also concluded that the schedule of feedback delivery will affect the response pattern achieved.

LASER INDUCED MACROPHAGE RESPIRATORY BURST ACTIVITY

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Low level laser therapy has been promoted as a safe, effective and non-invasive therapeutic modality in recent years (Basford, J. R., Mayo Clin. Proc. 1986: 61, 671). Recent evidence (Young, S. *et al,* Lasers Surg. Med. 1989: 9, 497) suggests that laser irradiation of the human macrophage cell line, U937, causes release of a factor(s) which stimulate fibroblast proliferation *in vitro*. Oxygen radical (O_2-) production by macrophages is known to be a useful biochemical correlate of macrophage activation (Pick, E., Meth. Enzymol. 1986: 132, 407). The purpose of the current study was to detemine if laser irradiation caused U937 activation as assessed by O_2- production.

U937-GTB cells were maintained in culture in RPMI 1640, supplemented with 10% foetal calf serum and antibiotics at an initial concentration of 5×10^4 cells/ml. Cells were removed to 96- well flat bottomed microtitre plates, 100 µl per well (4×10^6 cells). These cells were exposed to 10, 30 or 60 s irradiation, (15 mW, 660 nm, pulsed at 5,000 Hz) giving energy densities of 1.2, 3.6 and 7.2 J/cm^2 respectively. Production of O_2- was assessed by cytochrome C reduction over a 90 minute period against appropriate controls. Positive controls involved treatment of cells with phorbol myristate acetate (PMA, 10 µg/ml), a potent inducer of respiratory burst activity.

Results indicate that laser irradiation induced O_2- production in a dose dependent fashion. Following exposure to 3% dimethyl sulphoxide (DMSO) for 24 hours to induce cell differentiation, laser induced O_2- production was enhanced. Table I shows results from a typical experiment.

TABLE I

Treatment	Maximum O_2- production (nmol per 10^6 cells)	
		+DMSO
PMA (10µM)	0.514	13.035
Laser 10s	0.000	0.395
Laser 30s	0.908	1.303
Laser 60s	0.908	0.908

Further, laser stimulated O_2- production was immediate while there was a time delay following chemical stimulation with PMA. This may suggest that laser stimulation bypasses some intracellular pathway to O_2- production. Laser's effect was seen to be most consistent with doses greater than 1.2 J/cm^2 while, overall, a dose of 3.6 J/cm^2 gave the most reproducible results. These results suggest that therapeutic laser irradiation causes activation of U937 cells *in vitro* and may indicate a possible role for laser in macrophage activation *in vivo*.

LASER MEDIATED INCREASE IN MEDIAN NERVE CONDUCTION LATENCIES

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Nerve conduction studies are a realtively common test of peripheral nerve function in clinical neurophysiology. These have been used recently by a number of groups to assess the effect of low level laser irradiation (<30 J/cm^2) upon human peripheral nerves *in vivo*. The results of these studies are contentious with some finding no effect (Greathouse *et al,* Phys. Ther. 1985: 65, 1184), while others report increase in conduction latencies after relatively short periods of irradiation (Snyder-Mackler & Bork, Phys. Ther. 1988: 68, 223).

For the current study, healthy human volunteers (n=24) were recruited and screened for peripheral neuropathy. The procedure was explained, a simple consent form signed, and subjects allowed to rest for 10 minutes. After this, the skin over the forearm was prepared with an abrasive (Omniprep) and alcohol. A bipolar muscle stimulator was then used to identify the median nerve at the elbow for antidromic stimulation. The site was marked and hydrogel electrodes affixed. A velcro supported earth was then fastened approximately three inches above the wrist. Finally, digital ring recording electrodes prepared with electrode gel were positioned on the second digit.

Electrodes were attached to a Mystro$^+$ recording and stimulation system (Medelec, Woking). Stimulation consisted of 100 µs pulses, using stimulation voltages of 35-55V (nominal). Stimulus voltage was increased until response amplitude was maximum. At this time, 16 stimuli were delivered at a frequency of 1 Hz and the responses averaged and stored.

For the experimental condition, subjects were irradiated at ten points along the course of the nerve for 30 s each point, using a continuous wave mW laser diode operating at 830 nm (1.2 J/cm^2). For placebo condition, the procedure was repeated without switching

on the laser diode. For controls, the subjects rested for five minutes. At the end of this period, all subjects had nerve conduction tests repeated as described above, and again after 1, 2 and 5 minutes.

Subsequent analysis for stored recordings showed a small but reproducible increase in conduction latency after irradiation. Before irradiation, conduction latency was 7.135 ± 0.185 ms (s.e. mean), 5 minutes post irradiation, 7,418 ± 0.191 ms. No such increase was observed for control or placebo conditions.

The results suggest a direct neurophysiological response to laser irradiation at therapeutic doses *in vivo*.

BIOCHEMICAL CHANGES WITH SUXAMETHONIUM IN CHILDREN WITH STRABISMUS

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It has been suggested that children with strabismus may have latent muscle disease and more susceptible to development of masseter spasm and malignant hyperthermia following suxamethonium administration (Tammisto, T. *et al*, Ann. Clin. Res. 1970: 2, 126). As a group they might be expected to show exaggerated biochemical changes with its use, which may be a pointer to more serious sequelae of suxamethonium administration in such children. This has been investigated prospectively in the present study

One hundred and twenty children between the ages of 3 and 12 years were included in this study. Half were scheduled to undergo surgery for correction of strabismus and the remainder, undergoing tonsillectomy, were the controls. Within each group, children were randomly allocated to be anaesthetised with thiopentone 4-5 mg kg^{-1} (T), halothane (H) or isoflurane (I) with oxide and oxygen. The T and H groups received suxamethonium 1 mg kg^{-1}.

Venous blood samples (2 ml) were withdrawn prior to administration of suxamethonium and at 1, 3 and 5 min later for estimation of serum potassium (K) and total calcium (Ca) levels. Baseline samples and a sample taken 24 hrs later were analysed for serum creatinine phosphokinase levels (CPK).

Twenty patients had to be excluded from the analysis because of the unsatisfactory condition of the blood samples. The results shown in Table I are thus based on 100 children. Administration of suxamethonium resulted in a significant increase in Serum K and CPK levels in the H groups. Rise in CPK was more modest in the T groups and minimal in the I groups. Serum K decreased in the T groups receiving suxamethonium. Our results suggest that biochemical changes following suxamethonium are dependent on the anaesthetic agents employed rather than on the presence or absence of strabismus. These results support recent work suggesting that children with strabismus do not respond differently to suxamethonium (Leary, N. P, Ellis, F. R., Br. J. Anaesth. 1990: 64, 488).

TABLE I
Mean (SD) maximum biochemical changes

	Strabismus			Control		
	T	H	I	T	H	I
n	16	19	17	16	13	19
Ca ($mmol\ l^{-1}$)	−0.044 (0.06)	−0.051 (0.07)	−0.004 (0.05)	−0.043 (0.05)	−0.028 (0.04)	−0.019 (0.06)
K ($mmol\ l^{-1}$)	−0.35 (0.38)	+0.26 (0.40)	+0.03 (0.49)	−0.13 (0.33)	+0.53 (0.33)	+0.08 (0.42)
CPK ($\mu\ \mu l^{-1}$)	+56 (119)	+596 (956)	+11 (117)	+279 (474)	+406 (661)	+29 (79)

STUDY OF PREOXYGENATION IN THE ELDERLY

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Although there are many studies of preoxygenation (Gold, M. I. Br. J. Anaesth. 1989: 62, 241) the effect of this in the elderly has not been thoroughly investigated. We therefore assessed oxygen saturation during a modified sequence induction in ASA I or II elderly patients, after several preoxygenation techniques.

With Ethical Committee approval 60 patients over 65 years of age, were randomly allocated to 5 groups of 12 each; "A", "B", "C", "D" or "E". Patients with proven ischaemic heart disease, chronic obstructive airays disease, weight greater than 120% of ideal, anaemia, and smoking within 3 months of surgery were excluded. Premedication consisted of 10 mg of diazepam, 90 minutes preoperartively. All patients had ECG, non-invasive blood pressure and oxygen saturation monitored routinely from their arrival in the anaesthetic room until the end of surgery. The pulse oximeter used was a Nellcor N-100 averaging over 3 seconds, with an accuracy of ±2%. The standard anaesthetic technique consisted of vecuronium 0.1 mg/kg, followed 30 seconds later by thiopentone 3-5 mg/kg and fentanyl 1 μg/kg, injected over 10 seconds. Intubation was attempted 90 seconds after administration of vecuronium.

The preoxygenation technique for group "A" consisted of 4 deep breaths of 100% oxygen, over 30 seconds from a non-rebreathing system, at a fresh gas flow of 10 l/min. The standard technique for the other groups involved preoxygenation periods of 1, 2, 3 and 4 minutes for "B", "C", "D" and "E" respectively. After induction, no ventilatory assistance was given to any group either before or after intubation, until the patient's oxygen saturation reached 93%. During this period, the patient's endotracheal tube was left open to the atmosphere. The period of acceptable apnoea was defined as the time from the injection of thiopentone to the study end-point. Results were analysed using the one way analysis of variance, the Kruskal-Wallis test, and the Chi square test.

For technical reasons, two patients were excluded from group B leaving fifty-eight patients in the study. There was no statistical difference between the groups in terms of age, sex and weight, or oxygen in saturation at rest, after preoxygenation and at intubation. The duration of apnoea varied between 4.2 and 5.2 minutes in the five groups.

TABLE I
Demographic/Saturation Data

Data (SD)	A	B	C	D	E
Age/Years	78 (6.2)	77 (7.6)	77 (6.8)	77 (6.8)	77 (5.2)
Weight/kg	59 (10.4)	64.4 (10.0)	63 (8.2)	60 (9.3)	65 (17.0)
Resting Saturation *Range*	94-100	94-98	94-99	95-99	94-99
Saturation in Oxygen *Range*	98-100	99-100	99-100	99-100	98-100
Intubation Saturation *Range*	98-100	99-100	99-100	99-100	99-100
Mins to Saturation of 93%	4.3 (1.8)	4.2 (1.0)	5.0 (1.4)	5.2 (1.6)	5.0 (1.8)

Our results indicate that prolonging preoxygenation beyond four deep breaths does not produce a statistically significant improvement in the duration of apnoea. Nevertheless, clinically a period of at least 2 minutes is optimum as it gives a mean extra 42 seconds period of apnoea prior to intubation.

INHALATION INDUCTION OF ANAESTHESIA WITH ISOFLURANE IN ADULTS

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Inhalation induction of anaesthesia offers several possible advantages. These include cardiovascular stability (Wilton, Thomas, Anaesthesia 1986: 41, 472), maintenance of airway safety and, especially when isofluorane is used, negligible residual effects of anaesthesia (Editorial, Br. J. Anaesth. 1988: 61, 373), making it particularly suitable for day-case procedures.

An old technique using a low concentration of carbon-dioxide to promote regular respiration for inhalational induction in adults (Coleman in Wylie and Churchill Davidson's A Practice of Anaesthesia, 5th edition, 1984: 188) has been revised and tested.

Sixty healthy patients (ASA 1-2) scheduled for minor surgery were studied after approval by the University Ethical Committee and informed consent. Most were not pre-medicated according to our day-case practice.

Monitoring of blood pressure and ECG were commenced prior to induction. The patients were asked to hold the anaesthetic mask themselves and were not encouraged to breathe deeply. A standard anaesthetic gas mixture of 37% oxygen in nitrous-oxide was delivered at a fixed minute volume of 81. Thirty patients were randomised to receive added carbon-dioxide at $0.41.min^{-1}$ for the first 90 seconds and in each of these two groups, patients received either halothane or isoflurane by random allocation. This was increased by 0.5% every 10 seconds to a maximum of 5% but if the patient started coughing this was adjusted clinically. Time to loss of eyelash reflex was recorded. Coughing and excitation were separately graded and scored as 0 (None), 1 (Mild) and 2 (Severe).

Results were interpreted using analysis of variance, Kruskall Wallis and the Mann Whitney-U test where appropriate. The acceptance of the technique was very high, only 2 patients stating that they would not use a mask induction again. Fourteen others stated that they would prefer the mask induction to a previous intravenous anaesthetic. Recall in all patients was limited to the first few breaths from the mask.

Loss of eyelash reflex was achieved some 16 seconds earlier in the isoflurane patients ($p<0.05$), while carbon-dioxide made no significant alteration to this time.

When comparing the two anaesthetic agents, there was a significantly higher incidence of cough in the group receiving isofluorane ($p<0.01$), but no difference in excitatory phenomena. However carbon-dioxide caused a highly significant reduction in excitatory phenomena compared with the non CO_2 group ($p<0.01$) but made no difference to the incidence of cough.

Isoflurane with carbon-dioxide produced significantly less excitation than halothane alone.

Addition of 5% carbon-dioxide to the anaesthetic gas mixture facilitates inhalation induction in adults by reducing the excitatory phenomena even when isoflurone is used.

PROLONGATION OF ANTIEMETIC ACTION OF P6 STIMULATION BY ACUPRESSURE

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The antiemetic action of stimulation of P6 (Neiguan) point has been proven beyond reasonable doubt. However, the action of invasive stimulation does not last longer than 8 hours, while that of non-invasive techniques (transcutaneous electrical stimulation (TCES) and acupressure) lasts for only 2-3 hours. Since acupressure is easy to apply, using the commecially available Sea Bands, its use in extending the duration of action of acupuncture (ACP) has been studied (Dundee and Yang, J. Roy. Soc. Med. 1990, in press). This paper reports its efficacy in prolonging the action of both invasive and non-invasive stimulation of P6.

Patients who were sick after a previous course of chemotherapy, despite the use of conventional antiemetics had either 5 minutes of ACP or TCES carried out before the next course, with continuation of the antiemetics. This was followed by pressure on the stud of the Sea Band for 5 minutes every 2 hours during waking hours. The benefit was assessed at 8 hours and 24 hours using a simple 4 point scale: A (complete relief); B (good relief); C (slight relief) and D (no benefit).

Acupressure following Acupuncture			Transcutaneous stimulation		
n	8 hr	24 hr	n	8 hr	24 hr
54	A	A	56	A	A
21	B	B	23	B	B
2	A	B	3	A	B
1	A	C	1	A	C
1	A	D	2	B	C

Prolongation of antiemetic action of invasive (ACP) or non-invasive (transcutaneous electrical) stimulation of P6 by acupressure, as shown by grade of benefit recorded at 8 and 24 hours after initial treatment.

By the use of a Sea Band, pressed every 2 hours a good antiemetic effect was sustained for 24 hours in 74/79 (94%) of patients after acupuncture and in 79/85 (93%) after transcutaneous electrical stimulation at P6 point.

Two hourly use of stimulation of P6 by acupressure is a simple method of prolonging the antiemetic action of both ACP and TCES requiring only patient motivation.

FEASIBILITY OF SELF ADMINISTRATION OF P6 STIMULATION AS AN ANTIEMETIC IN CANCER CHEMOTHERAPY

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Any method of self-administration of a therapy motivates a patient to play an active role in their treatment. Some, but not all patients respond positively to this challenge.

The use of large diffuse surface electrodes for transcutaneous electric stimulation (TCES) has removed the necessity of exact placement and made feasible the self-administration of a stimulus to the P6 (Neiguan) antiemetic point. This has been studied in 24 inpatients having 5 day courses of highly emetic cytotoxic drugs. TCES was used in addition to conventional antiemetics, when these by themselves proved inadequate. The commercially available battery driven portable TENS machine (15 Hz) was used and stimulation was started 2 hours before chemotherapy and continued for 5 minutes every 2 hours when awake for the duration of the chemotherapy. It was easy for patients to adjust the current control on the machine and elicit Qi.

The benefit was assessed on a 4 point scale as described by Dundee and Jang (J. Roy. Soc. Med. 1990, in press). The results given below are based on inpatient studies and give the overall assessment of benefit over 4-5 days. Data from ACP and doctor administered

TCES have been published by Dundee and colleagues (J. Roy. Soc. Med. 1989: 82, 268; ibid 1990, in press).

	Doctor administered		Self-administered
Benefit	ACP	TCNS	TCNS
n	34	27	29
Very good	18	9	9
Good	15	13	17
Slight or nil	1	5	3
Mean ± Sd			
Current mA		7.6±1.6	11.5±1.9

On individual day to day results the incidence of good and very good benefit with doctor administrated TCES was 87% (n=104) and 90% (n=137) with self-administration. The mean current used by the patients was significantly greater than that required by the doctors ($P<0.05$).

There were no major problems with self-administration. Some difficulty arose from benzodiazepine amnesia and drowsiness, the use of the dominant hand for application of the electrode with an inability to use the machine with the other hand and inability of a minority of patients to operate the controls. These are not unsuperable and the development of a custom built stimulator may overcome these. The concept is worthy of further study.

LUNG INJURY IN EXPERIMENTAL PANCREATITIS

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Acute lung injury is a major factor contributing to the mortality in acute pancreatitis. The lung injury varies from atelectasis in the mildest form to acute respiratory failure in the most severe form. In this latter form the injury is indistinguishable both clinically and pathologically from adult respiratory distress syndrome.

We studied the microcirculation of the rat lung in experimental pancreatitis by a resin casting technique. Haemorrhagic pancreatitis was induced in rats by retrograde intraductal installation of bile salts (0.2 ml of 5% sodium taurocholate) and oedematous pancreatitis was induced by intra arterial infusion of caerulein (5 ug/Kg/hr). At timed intervals retrograde infusion through the right atrium was commenced with saline until all blood cells were removed, followed by 2.5% buffered gluteraldehyde. The fixative was washed out with saline and then the capillaries were filled with Mercox resin and the cast allowed to solidify. Inflammation of the pancreas was evident in the animals at three and twelve hours post induction of haemorrhagic pancreatitis. On scanning electron microscopy some of the lung looked normal but large areas of the capillaries were disrupted and incompletely filled. At twelve hours there was gross capillary disruption. In the animals with oedamatous pancreatitis the capillary baskets of the lungs failed to fill properly and capillaries ended blindly. At four hours the abnormalities were accentuated, abruptly terminating vessels were a notable feature.

These findings support the suggestion that experimental pancreatitis is a disease of the microcirculation in several organs.

THE EFFECT OF PHORBOL 12,13-DIBUTYRATE AND EXTERNAL CALCIUM ON LONG-TERM POTENTIATION AND AN NMDA-INDUCED SHORT TERM POTENTIATION IN THE CA1 REGION OF THE RAT HIPPOCAMPUS *IN VITRO*

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Phorbol esters and external calcium are known to effect long-term potentiation (LTP) in the hippocampus. In this study the actions of phorbol 12,13-dibutyrate (PDBu) and external Ca^{2+} were examined on both LTP and an NMDA-induced short-term potentiation (STP) which has only previously been produced using iontophoresis (Kaur, J. A. *et al,* Nature 1988: 334, 250).

Hippocampal slices were routinely prepared and superfused at 33°C with a physiological solution containing (mM): NaCl 120, KCl 2.5, $CaCl_2$ 2.0 or 4.0, $NaHPO_4$ 2.0, $MGSO_4$ 1.0, $NAHCO_3$ 26, glucose 10. Extracellular recordings of excitatory post-synaptic potentials (epsp) were obtained from the stratum radiatum of CA1 in response to field stimulation of the radiatum. LTP was initiated by a 250Hz tetanus consisting of 10 trains, each train of 40ms duration, at 0.5Hz. STP was induced by bath applying 130mM NMDA for 10 seconds at a flow rate of 12 ml/min.

The NMDA-induced STP consisted of a rapid abolition of the epsp following NMDA application with a subsequent recovery to control levels at 7 minutes, a potentiation peaking at 11 minutes and returning to control levels by 20-30 minutes. Under control conditions a peak potentiation of 46.3 ±6.1% (n=15) was obtained. The application of 100nM PBBu caused an increase in epsp amplitude of 65.9±7.5% (n=3) at 1 hour. After 2 hours in PDBu the epsp amplitude was readjusted to the control amplitude and NMDA applied as before which produced a similar abolition and recovery of the epsp but with no potentiation, the percentage change being −7.3±4.6% (n=6). Similarly, under control conditions LTP was found to be 27.8±3.6% (n=4), while in 100nM PDBu for 2 hours LTP was found to be 3.7±1.4% (n=6). Changing the external calcium from 2 - 4mM caused an increase in the epsp amplitude of 46.2±7.0% (n=9). In the presence of 4mM calcium the NMDA-induced STP was abolished, with a percentage change of 0.0±1.5% (n=5). However, LTP in 4mM calcium was not different from control levels, being 27.6±6.0% (n=4).

The similar actions of PDBu and high calcium on the NMDA-induced STP suggests the involvement of protein kinase C and/or the inositol phosphate system and a possible synergism between the two systems. However, the differing effect of calcium on LTP and the NMDA-induced STP seems to indicate that the STP may not be an early decremental phase of LTP as previously thought.

CREATINE KINASE AND NEURON SPECIFIC ENOLASE IN SERUM-MARKERS OF CELL DAMAGE IN THE HUMAN CENTRAL NERVOUS SYSTEM

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Creatine kinase isoenzymes CK-BB and CK-MB have been used as serum markers for brain and cardiac injury. CK-BB is found in high concentrations in the brain where it is produced by astrocytes. Previous radioimmunoassay procedures for CK-BB and CK-MB involved polyclonal antisera that recognise the B subunit with a resultant degree of cross reaction between the two species. In this study CK-BB and MB isoenzymes were detected using an enzyme linked immunabsorbent assay employing monoclonal antibodies. No detectable cross reactivity occurred between the species in the measuring range 0-1000 ng/ml and detection limit of 0.01 ng/ml. Neuron specific enolase is a brain specific isoenzyme of the glycolytic enzyme enolase and is characteised by its consistent occurrence in the cytoplasm of mature neurons. It was detected using a double antibody radioimmunoassay with measuring range and detection limits of 2.0 - 200 µg/ml and 2.0 µg/ml respectively.

Blood samples were obtained with informed consent from adult amateur oarsmen (n=17) before and after exercise. The oarsmen undertook a rowing ergometer test simulating a 2000 metre flat water course, achieving maximal exercise as defined by final heart and respiratory rates and serum lactate levels. No significant changes were found between the pre and post exercise CK-BB serum levels in the oarsmen, indicating the absence of astrocytic or neuronal injury. This is in contrast to findings of significantly raised CK-BB in athletes on completion of a marathon (Philips *et al*, Lancet, 1982: i, 1310).

THE URETER *IN VITRO*: NORMAL MOTILITY AND RESPONSE TO COMMON URINARY PATHOGENS

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Previous studies (King, W. W., Cox, C. E., J. Urol. 1972: 108, 700), based on the contractility of isometric ureteric muscle strips have shown that several species of pathogenic bacteria may have an inhibitory effect on ureteric smooth muscle contractility. The aim of the present study was to assess the contractility of the isolated denervated canine ureter exposed to pathogenic bacteria using a new model for assessment of ureteric activity *in vitro*.

Forty segments of normal canine ureter were evaluated. A cannula attached to a pressure measuring transducer and chart recorder was ligated into the proximal end of a 2 cm ureteric segment. The distal ureter was ligated to form a closed pressure monitoring system. The ureter was suspended in an organ bath containing Krebs Henseleit buffer at physiological pH and temperature. Following onset of spontaneous ureteric activity, broths of E. coli, Proteus Pseudomonas, and Staphylococcus aureus were added to the bathing buffer solution in doses of 100,000 - 100,000,000 organisms/ml. These experiments were repeated using heat killed organisms and filtrates of the same bacteria. The effects of E. coli endotoxin in doses of 0.25 - 25 ug./ml were assessed.

Each of the organisms studied produced a dramatic excitatory effect on ureteric motility with an apparent resetting of the intrinsic pacemaker activity. This effect was absent with the heat killed bacteria, endotoxin and live bacterial filtrates. The effect was reversed by addition of Gentamicin in doses of 0.2 - 0.4 mg/ml which resulted in death of all organisms present in the organ bath. The isolated denervated canine ureter exhibits a marked stimulatory response to the presence of common pathogenic urinary bacteria. Active metabolising bacteria are required to produce this response which appears to be due to a heat labile bacterial cell wall component. This response may represent a ureteric mechanism for expelling pathogenic infecting organisms *in vivo*.

A STUDYOF THE MICROAPPENDAGE POPULATION OF THE ATRIOVENTRICULAR VALVES OF THE GUINEA PIG

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Recent work (Gilloeaux and Linz, Am. J. Anat. 1989: 186. 161) has shown that the microappendages on the endothelium of the atrium have an active biological role in growth and in metabolic exchange between the atrial myocytes and the blood. Such microappendages have been documented on the atrioventricular valves. However, there is no agreement as to which surface of a valve has more microappendages. We statistically analysed the number of microappendages on the atrial and ventricular surfaces of the mitral tricuspid valves.

Ten guinea pigs were anaesthetised with sodium pentobarbitone (60 mg/kg/ip) and perfused with Karynovsky's fixative through the left ventricle. The anterior cusp of the mitral and tricuspid valves were dissected, post-fixed in osmium tetroxide, critically point dried and sputter coated with gold. With scanning electron microscopy, counts were made of the number of microappendages in three 75 square micron areas from the base, intermediate and distal zones of the valve surface.

Analysis with a paired "t" test showed more microappendages on atrial surface (32±19.2 S.D.) than the ventricular surface (26±14.1 S.D.) of the tricuspid valve ($P<0.05$). In contrast on the mitral valve the atrial surface had 18 (±16.7 S.D.) microappenndages and the ventricular surface had 13 (±11.2 S.D.) which was not significantly different at the 5% level. A comparison of counts from the right side of the heart with the left side revealed a significant difference ($P<0.05$). When analysing each valve surface separately, the distal edge of the valves had a greater number of microappendages than the base and intermediate regions of the valve surface ($P<0.05$).

These results suggest that the microappendage population is a reflection of the haemodynamic forces acting on the surface of the mitral and tricuspid valves, the greater the force the fewer the number of microappendages. As these microappendages are also proposed to be involved in metabolic exchange, the presence of more microappendages at the valve edge may help to explain why the lesion of bacterial endocarditis develops at the valve edge.

DNA REPAIR OF DAMAGE INDUCED BY REACTIVE OXYGEN SPECIES (ROS) IN THE FRIEND ERYTHROLEUKAEMIC CELL-LINE 707

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Oxidants, (or Reactive Oxygen Species – ROS) are highly reactive derivatives of molecular oxygen which have been implicated in carcinogenesis. These oxidants include O^2 (the superoxide anion), H_2O_2 (hydrogen peroxidase), and OH^* (the hydroxyl radical). There are a variety of sources of these oxidants including radiation, sunlight and cigarette smoke. Oxidant damage to the genetic material of a cell is known to have a variety of deleterious effects such as single and double strand breaks and cross-linkages. These may lead to lethal or sub-lethal mutations which may or may not be repaired. Obviously, the ability of a cell to repair potentially carcinogenic damage is crucial. The aim of this study was to assess the capacity of a murine cell-line to repair oxidative damage.

The cells used were of the 707 Friend erythroleukaemic lineage. The properties of these cells regarding repair of UV-induced DNA damage have already been assessed and they are generally assumed to be repair proficient. The methods used to assess excision repair of oxidative DNA damage included (i) an incorporation assay (an adaptation of the method by Larcom and Smith, J. Nat. Can. Inst. 1988: 80 (14)) and (ii) autoradiography. Exponentially growing cells were exposed to a 1mM/mU concentration of oxidants generated by the enzymatic system xanthine/xanthine oxidase (x/xod) for $1^1/_2$ hrs. The cell population was divided into two – one part for incorporation studies and the other for autoradiography. (Autoradiography results are shown in Table I below). A 'rest' period of variable duration was included to allow for the initiation of repair enzymes prior to exposure to ^{3}HdT.

Results to date indicate that excision repair of oxidant damage occurs in 707 cells – the extent of which depending upon the length of the cell 'rest' period and the time exposed to ^{3}HdT.

TABLE I
Autoradiography Results.

	1 hr rest + 1 hr ^{3}HdT		1½ hrs. xanthine/xanthine oxidase (x/xod) 1½ hrs rest + 1 hr ^{3}HdT		1½ hrs rest + 1½ hrs ^{3}HdT	
	Control	Test	Control	Test	Control	Test
%S	67.8 ± 3.6	52.9 ± 2.1	69.25 ± 3.5	58.2 ± 4.6	71.6 ± 2.8	51.9 ± 3.1
%R	20.95 ± 4.0	36.7 ± 3.3	21.6 ± 1.8	39.6 ± 2.0	25.8 ± 2.6	41.17 ± 1.8
%B	11.25 ± 2.1	10.4 ± 0.65	9.15 ± 1.2	2.2 ± 0.8	2.6 ± 2.1	6.86 ± 0.86
Mean NNGC	36.2 ± 0.9	49.15 ± 6.2	31.8 ± 1.3	45.4 ± 0.95	35.4 ± 4.2	51.0 ± 3.6
Increase in NNGC	12.95		13.6		15.62	

Results for autoradiography expressed as the % of cells in S (replicating) phase, R-repair phase, or B-background phase, with standard deviation values. NNGC refers to the Net Nuclear Grain count in all non-S-phase cells addressed.

AN ADAPTIVE RESPONSE TO OXIDANT STRESS IN HUMAN LYMPHOID CELLS

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During immune or inflammatory responses cells act not in isolation but with interaction at many levels. At inflammatory sites phagocytes such as polymorphonuclear leucocytes (PMNL) discharge their lysosomal contents and exhibit respiratory burst activity. This respiratory burst gives rise to an array of reactive oxygen species (ROS) including the superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•). It may be hypothesised that when released into the cellular enviroment ROS may prove cytotoxic not only to pathogens but also to host cells such as lymphocytes. This may affect cell function leading to altered immunity and possibly transformation to malignancy.

We have already shown that ROS generated enzymatically by xanthine + xanthine oxidase (X + XOD), by H_2O_2 or by stimulated PMNL (stimulation by phorbol myristate acetate, PMA), causes DNA damage in the human lymphoid cell line Molt-3 (T-cell) (Cromie and Hannigan, unpublished observations).. DNA damage was manifested as an increase in the frequency of chromosomal aberrations and sister chromatid exchanges (SCES). In addition the cell's proliferative capacity was significantly diminished in a dose dependent manner following exposure to X + XOD or H_2O_2. On exposure to stimulated PMNLs the reduction in lymphoid cell proliferation was seen to increase with increasing concentrations of stimulus (PMA) and with increasing target: effector cell ratio (Table I). Phagocyte generated O_2- was measured by the SOD-inhibitable reduction of cytochrome C and H_2O_2 was measured using the HRPO assay (Table II).

TABLE I
The effect of activated PMNLs on MOLT-3 proliferation

PMA ng/ml	PMN:Molt-3 (1 : 1)	PMN:Molt-3 (2 : 1)
0	14107 ± 1106	12778 ± 1401
10	10961 ± 1081	10124 ± 1061
50	8964 ± 981	2961 ± 208
100	3261 ± 102	

TABLE II
Phagocyte-generated oxidants

PMA ng/ml	O_2 nM	H_2O_2uM
5	11.3	2.0
10	21.8	10.0
50	30.0	50.1
100	37.6	>65.0
500	44.9	>65.0

When target cells had been pretreated with low non-lethal doses of H_2O_2 they became less susceptible to ROS-mediated DNA damage and cell proliferation approached normal control levels. This was recorded as a reduction in the number of aberrations and SCEs unpublished observations and an increase in proliferation capacity relative to non pretreated cells (Table III), indicative of an adaptive response to oxidative damage existing in human lymphoid cells. Such a response has previously been noted only in bacteria and in phagocytes.

Morgan *et al.* Proc. Natl. Acad. Sci. 1986: 83, pp. 8059-8063.
Polla, B. S. Immumol. Today. 1988: 9, pp. 134-137.

TABLE III
3H-dT incorporation in MOLT-3 cells

H_2O_2uM	control	pretreated
0	22205 ± 1468	22230 ± 1440
5	20125 ± 1201	21518 ± 1109
50	6186 ± 540	11349 ± 981
100	1841 ± 61	5600 ± 462

A COMPARISON OF MACROPHAGE TYPES IN THE INDUCTION OF DNA SINGLE STRAND BREAKS IN HUMAN LYMPHOID TARGET CELLS

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Macrophages (MO) are important cells of the immune system. They occur in most tissues including solid malignant tumors. MO have a variety of functions which vary according to the state of differentiation and activation of the MO. Functions include phagocytosis, antigen presentation, cytokine production and release of reactive oxygen species (oxidants) such as the superoxide anion (O_2^-) and its reduction products hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH). Oxidants have cytotoxic and genotoxic functions and have been implicated as possible aetiological agents in a number of disease states including cancer. We have shown previously that human lymphoid cells are susceptible to oxidant-induced DNA damage. MO and lymphoid cells occur in close proximity at inflammatory sites and within tumour tissues. The present study investigated oxidant production by resident, activated and tumour-associated murine MO and the ability of such cells to induce DNA single strand breaks (DNA SSB) in T-lymphoblastoid target cells, MOLT-3. Effective oxidant species were characterized by using

specific oxidant scavengers (antioxidants) superoxide dismutase (SOD, scavenges O_2^-) and catalase (Cat, scavenges H_2O_2). Resident or activated MO were obtained by peritoneal lavage of normal or *Cornebacteruim Parvum* inoculated Balb/c mice respectively. Tumour associated macrophages (TAM) were isolated from excised 21-28 day transplanted adenocarcinomas of Balb/c mice by enzyme disaggregation and selective adherance. O_2^- and H_2O_2 production from phorbol myristate acetate (PMA)-stimulated MO were estimated by cytochrome C and horseradish peroxidase reduction respectively. DNA SSB were quantitated in target lymphoid cells following exposure to various numbers of PMA-stimulated MO ± SOD or Cat. Of the three MP types examined only activated MO secreted detectable amounts of O_2^- (Table I), H_2O_2 and SSB were further increased in the presence of SOD whle both events were decreased by Cat (Table I & II) (data is presented as mean ± standard deviation).

Our results indicate (i) that H_2O_2, rather than O_2^- is an effective MO-derived oxidant and (ii) that TAM resemble resident MO in their inability to produce oxidants or to induced DNA damage. Thus any intratumoural interaction between MO and lymphoid cells probably does not involve oxidant production.

TABLE I

O_2^- and H_2O_2 production by 1 x 10^6 C. parvum activated mouse peritoneal MOs ± antioxidants.

Time/min	nmol O_2^-	nmol H_2O_2			
		PMA.MO	SOD.PMA.MO	Cat.PMA.MO	MO
30	1.570±0.064	7.047±1.453	11.757±0.701	5.962±0.489	0.687±0.158
60	2.990±0.410	13.792±1.479	>33.400	10.053±0.284	0.463±0.434

TABLE II

%DNA SSB induced in MOLT-3 cells by mouse Peritoneal MOs & TAM for indicated MO or TAM : MOLT-3

	0 : 1	2.5 : 1	5 : 1	10 : 1
MO+PMA		37.508±1.390	42.078±1.370	44.148±1.380
MO+PMA+SOD		40.158±3.700	47.428±3.030	51.128±3.360
MO+PMA+Cat		36.958±2.400	38.158±2.470	40.288±2.520
MO		34.092±1.690	35.148±1.260	37.378±1.980
TAM+PMA		33.930±1.867	33.562±1.992	34.340±3.575
TAM+PMA+SOD		33.930±1.867	33.562±1.992	34.340±3.575
TAM+PMA+Cat		33.471±1.291	33.110±1.101	34.046±1.810
TAM		34.619±2.143	34.985±0.465	33.834±2.573
MOLT-3+PMA	34.768±3.545			
MOLT-3	33.272±2.638			

THE EFFECT OF NON IRON STATUS AND TUMOUR INDUCTION ON THYMIDINE KINASE ACTIVITY IN RAT COLONIC TISSUE

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The effect of iron status and 1,2 dimethyhyldrazine (DMH) treatment on tumourigenesis in rat colon was investigated. Two levels of dietatry iron, 15 mg/kg (LFe) and 400 mg/kg (HFe), were fed to two groups each comprising 18 male and 18 female Sprague-Dawley rats. After 42 days, 6 male and 6 female rats from each of the LFe and HFe groups were sacrificed and acted as controls for the DMH treated rats. The remaining rats in each dietary group were given a weekly injection (30 mg/kg body weight) of DMH for 14 weeks and sacrificed after a further six weeks. Levels of the nucleotide salvage pathway enzyme, thymidine kinase (TK) were measured in colonic tissue and serum of all rats and also in the colonic tumours of the DMH-treated rats. Increased TK are frequently observed in tumours and in the serum of tumour-bearing rats and humans.

Although diet had little effect on TK activity in DMH-treated colonic tissue, the number of tumours found were significantly ($P<0.001$, t-test) higher in the male LFe rats (mean 9.1, SE 1.5) compared with the male HFe rats (mean 4.5, SE 0.7) and also higher ($P<0.01$) in male rats (mean 6.8, SE 1.0) compared with female rats (mean 2.7, SE 0.3). Total TK activities in counts/min/mg protein in colonic tissue of the DMH-treated rats (mean 1,933, SE 127) were higher ($P<0.001$) than controls (mean 926, SE 171). However, no correlations were found between serum and tumour nor between serum and colonic TK activities.

CIGARETTE SMOKING AND SERUM HEAT STABLE ALKALINE PHOSPHATASE ACTIVITY

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Cigarette smoking is the main risk factor for lung cancer and environmental tobacco smoke exposure in childhood has been linked to subsequent respiratory morbidity (Britten *et al*, Br. Med. J. 1987: 294, 1317). Further evidence suggests that "passive smoking" increases the risk of lung cancer in non-smokers by 10-30% (U.K. Independent Scientific committee on Smoking and Health, Fourth Report 1988, London HMSO). Clearly a simple yet reliable marker of chronic tobacco smoke inhalation would be an invaluable aid in preventative medicine.

We have recently developed a sensitive, uncomplicated and inexpensive assay for the measurement of heat stable alkaline phosphatase (HSAP) activity in non-pregnancy serum and here report our findings for current cigarette smokers, ex-smokers and non-smokers.

Non-specific alkaline phosphatase activity is eliminated by preincubation of serum at 60°C and residual HSAP activity is measured at 37°C with the fluorogenic substrate 4-methyl umbelliferyl phosphate. Uncompetitive stereospecific inhibition of serum HSAP by L-amino acids homoarginine phenylalanine and leucine indicates that the activity of smokers and non-smokers is due to the placental-like Nagao isoenzyme of pulmonary origin.

Current cigarette smokers demonstrate significaantly higher serum HSAP levels than non-smokers ($p<0.001$) whilst ex-smokers have similar values to non-smokers ($p<0.1$). Males and females show no HSAP differences in all categories ($p<0.1$) (Table I).

TABLE I
Serum HSAP activity (mean ± standard deviation) of cigarette smokers, ex-smokers and non-smokers.

		Serum HSAP activity (U/ml)
Cigarette smokers	Male (n=27)	88±79
	Female (n=27)	93±84
Ex-smokers	Male (n=45)	17±7
	Female (n=7)	19±4
Non-smokers	Male (n=56)	16±8
	Female (n=38)	15±7

Although most young smokers aged 18-24 years have low activity, serum HSAP increases with duration (r=0.45) and frequency (r=0.38) of cigarette smoking. A large majority (86%) of smokers aged 25-65 have elevated values (≥35 U/ml) that are correlated to the urinary excretion of the main nicotine metabolite cotinine (r=0.51 for HSAP < 140 U/ml and r=0.79 fr HSAP > 140 U/ml). Conversely, most long term heavy smokers revert to "normal" HSAP values within one year of abstinence from smoking but a minority (11%) of ex-smokers and non-smokers have raised serum activity

EVALUATION OF SERUM MYOGLOBIN AS AN AID TO THE RAPID DIAGNOSIS OF MYOCARDIAL INFARCTION

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Clinicians usually make a diagnosis of myocardial infarction (MI) if two of the following criteria are "positive": 1. an abnormal ECG. 2. a clinical history suggestive of heart disease. 3. elevated serum cardiac enzyme activities. In 10-20% of patients suffering a myocardial enzyme infarction the ECG is equivocal and in such cases cardiac enzyme measurements are essential. However even the serum activity of the creatine kinase MB isoenzyme (CK-MB) may not be raised until 8-12 hours after the infarct (Lott and Stang, Clin. Chem. 1980: 26, 1241). As it has been reported that serum myoglobin is elevated after a myocardial infarction but before the cardiac enzymes appear (Roxin *et al,* Acta Med. Scand. 1984: 215, 417) it was decided to investigate whether a rapid method for measuring serum myoglobin would provide a superior diagnostic indicator for myocardial infarction.

Samples were obtained (with consent) over the course of one year from 112 patients with chest pains who were admitted to the coronary care ward. Blood was obtained on admission and again 4, 12, 24 and 36 hours later. Myoglobin concentration and cardiac enzyme activities were measured on all samples. ECGs, biochemistry reports and clinical histories were examined by a consultant physician before a definitive diagnosis was made in the case of any particular patient.

Retrospective diagnoses have shown that 33 of the 112 (29%) patients did in fact suffer a myocardial infarction. 32 of these MI patients had raised serum myoglobulin 4 hours post admission, the other MI patient having a raised serum myoglobin 12 hours after admission. The negative predictive value for myoglobin was 0.98 at 4 hours post admission and the false negative fraction was 0.03. By comparison the negative predictive value for CK-MB was only 0.82. The positive predictive values for myoglobin and CK-MB were found to be 0.74 and 0.84 respectively. Nine of the MI patients did not have raised cardiac enzyme activities until twelve hours after admission whilst in a minority of cases the AST or CK-MB activities did not rise at any stage.

These results indicate that, subsequent to an MI, myoglobin appears in serum much sooner than CK-MB. Myoglobin is not a specific marker for cardiac muscle and appears in the serum of "non-MI" patients who receive intramuscular injections or suffer other muscle trauma. It would appear that the main role of serum myoglobin measurements is in the early exclusion of a diagnosis of myocardial infarction as the cause of chest pain rather than its confirmation.

DEFIBRILLATION SUCCESS AND FIBRILLATION FREQUENCY

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Is transthoracic defibrillation easier with a high dominant frequency of ventricular fibrillation (VF), after a short duration of VF, than with a lower dominant frequency, after 90 seconds?

Ventricular fibrillation was induced by RV endocardial electrical stimulation in 10 pentobarbitone-anaesthetised greyhounds (35 mg/kg iv). The Defibrillation threshold (DFT) was determined by decreasing the delivered energy in steps from 30 J until failure first occurred (duration of VF <10 seconds). Curves for percentage success versus stored energy and transthoracic current were then determined for this duration of VF using 5 stored energy levels (15-50 J) each administered between five and eight times in a Latin Square design. A second, final DFT was then determined for the same duration of VF. The initial and final DFTs did not differ significantly for stored energy (30.5±4.2 J; 34.3±8.8 J) but did for current (15.8±1.3 Amps; 18.1±2.1 A) due to a fall in transthoracic impedance (42.5±1.1 Ohms; 31.7±1.0 Ohms). On individual % success: energy curves, these DFTs corresponded with 43.7±10.5% and 40.3±9.0% success rates.

In 6 dogs fibrillated for 90 seconds, the dominant frequency in the first 8 seconds of VF (10.2±0.2 Hz) fell to 6.0±0.9 Hz after 82-90 sec. At a stored energy of 50 J, the percentage success was 81.2±10.2% (21.9±0.2 A) at 7 sec of VF, and 45.5±15.0% (22.5±0.2 A) after 90 sec. At a higher stored energy (75 J), the delivered current (27.6±0.3 A) achieved only 53.8±13.8% success for 90 sec duration of VF.

This study shows a higher energy requirement for defibrillation after 90 sec compared with that for less than 10 sec duration of ventricular fibrillation. This higher energy requirement is associated with a drop in the dominant frequency of fibrillation at this time. It underlines the ease in assessing ability to defibrillate by DFT step determinations, and the difficulty of determining the percentage success versus energy curves. These curves have the major disadvantage of requiring many more fibrillation - defibrillation sequences.

UPPER AIRWAY AND DIAPHRAGM MUSCLE REFLEX RESPONSES TO CHANGES IN INTRALARYNGEAL CO_2 CONCENTRATION IN THE ANAESTHETISED CAT

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Bannon *et al.* (Ir. J. Med. Sci. 1990: 1559, 32) reported that increases in intralaryngeal CO_2 ($F_{IL}CO_2$) in an artificially ventilated larynx of the anaesthetised cat induced marked changes in the discharges of pressure sensitive sensory fibres in the superior laryngeal nerve (SLN). Application of pressures to the isolated upper airway has been shown to reflexly alter the pattern of respiration and upper airway dilator muscle activity (Mathew *et al*, J. Appl. Physiol. 1982: 52, 438). We have investigated that possibility that changes in $F_{IL}CO_2$ might exert similar effects.

Nine adult cats were anaesthetised with pentobarbitone sodium (induction, 30 mg/kg i.p.; maintenance 6 mg i.v. hourly) and breathed spontaneously via a low cervical tracheostomy. The isolated larynx was artificially ventilated in synchrony with the animal's spontaneous respiratory cycle using a specially designed ventilator. This ventilator permitted gas at room temperature and humidity to be drawn caudally through the larynx during the animal's spontaneous inspiration whilst heated (37°C), humifidied gas was pumped cranially through the larynx as the animal expired through the tracheostomy. Gases ventilating the larynx were either room air or mixtures of 5 or 9% CO_2, 21% O_2 in N_2. We continuously recorded systemic arterial blood pressure, tracheostomy airflows, subglottic laryngeal values of $F_{IL}CO_2$, airflows and pressures and, also, electromyographic activity recorded from the diaphragm (DIAEMG) and genioglossus (GGEMG) muscles.

In the conditions of our experiments phasic GGEMG was not present while the larynx was unventilated. On artificial ventilation of the larynx phasic GG EMG was recorded together with a reduction in respiratory frequency. Raising $F_{IL}CO_2$ from 0.05 to 0.09 throughout the cycle of laryngeal artificial ventilation induced marked increases in phasic GGEMG together with a further decrease in respiratory rate. These reflex responses were abolished by bilateral SLN section.

The reflex responses to increases in $F_{IL}CO_2$ could serve to preserve airway patency during the negative airway pressures normally present in inspiration. Thus, increased GG EMG would tend to dilate and stabilise the pharynx while a reduction in respiratory rate would tend to reduce the magnitude of the negative pressures developed in the upper airway with each inspiration. We propose that reflex responses to upper airway pressures and CO_2 concentrations may be important in the prevention or limitation of obstructive apnoea, and as such may be of major significance in the pathophysiology of the obstructive sleep apnoea syndrome.

PN was in receipt of a HRB Summer Student Grant during the time this work was carried out. The Wellcome Trust provided some of the equipment which was used.

A PRELIMINARY HISTOLOGICAL STUDY OF THE EFFECTS OF ULTRASOUND ON THE FREE GRAFTED RAT EXTENSOR DIGITORUM LONGUS MUSCLE

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Although therapeutic ultrasound is commony used in the management of musculoskeletal injuries, surprisingly little is known about its biological effects on skeletal muscle. In the present investigation, bilateral free grafting of the whole extensor digitorum longus muscle was performed in 42 mature male Sprague Dawley rats using the technique described by Carlson (Fed. Proc. 1986: 55, 1456) and the effects of treatment with ultrasound on the characteristic pattern of centripedal degeneration and regeneration studied. The animals were divided into two groups and received, under ether anaesthesia, a daily dose of either 0.5 W/cm^2 or 2.5 W/cm^2 pulsed 1:5 for 5 minutes to the right limb for periods up to 10 days. The untreated left legs served as controls. At assay, rats were killed by cervical dislocation under ether anaesthesia and the muscles were excised, processed and examined by light microscopy.

In the immediate postoperative period the histological picture was dominated by the appearance of an inflammatory exudate, fibre degeneration and phagocytosis. Ultrasound, at both dosages, produced small but discernible differences in these processes. Between five and ten days there was capillary invasion, the formation of scar tissue and the commencement of fibre regeneration. While small differences were observed between untreated muscles and muscles receiving 0.5 W/cm^2, these phenomena were markedly stimulated by the higher dosage level, one commonly employed in clinical practice.

A dosage of 0.5 W/cm^2 was found to produce optimal stimulation of tissue repair in the rabbit ear pinna (Dyson *et al*, IEEE trans. son. ultrason. 1970: SU-17, 133). The results of the present study suggest that other tissues respond optimally to different intensities. It is clear, however, that therapeutic ultrasound stimulates the regeneration of skeletal muscle. Further studies are planned to identify and quantify the cellular process involved.

THE EFFECTS OF HIGH POTASSIUM AND STIMULATION PARAMETERS ON FATIGUE OF THE RAT SOLEUS MUSCLE

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Tension-time curves were investigated in the soleus muscle following either continuous high frequency stimulation (at 10, 30 and 100 Hz) or a series of high frequency trains, both in control and in a high potassium medium. Increasing the frequency of stimulation caused the rate of fatigue to become faster during continuous high frequency stimulation ($1.06 \cdot 10^{-2}$% tension decrease per second at 10 Hz and $2.99 \cdot 10^{-2}$% tension decrease per second at 100 Hz). The rate of fatigue during high frequency trains of stimulation was much slower than with continuous stimulation, with a faster rate ($6.95 \cdot 10^{-3}$% tension decrease per second) followed by a slower rate ($9.96 \cdot 10^{-4}$% tension decrease per second) of fatigue.

The rate of fatigue caused by both continuous high frequency stimulation and by trains of high frequency stimulation became faster in the presence of a high potassium medium ($1.134 \cdot 10^{-1}$% tension decrease per second at 10 Hz continuous stimulation compared to $4.4 \cdot 10^{-3}$% tension decrease per second with trains of high frequency stimulation).

PLASMINOGEN ACTIVATOR INHIBITORS 1 AND 2 IN PRE-ECLAMPSIA AND INTRAUTERINE FOETAL GROWTH RETARDATION

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An adequate blood supply through uterine spiral arteries to the

intervillous space of the placenta is an important prerequisite for the maintenance of a healthy foetus in normal pregnancy. Excess fibrin deposition in the uteroplacental spiral arteries leading to placental ischaemia and infarction is found in pre-eclampsia (PET) and intra-uterine foetal growth retardation (IUGR). It is suggested that disordered fibrinolysis plays an important role in the pathogenesis of these pregnancy complications.

In this study plasminogen activator inhibitors PAI-1 (endothelial type) and PAI-2 (placental type) were measured in peripheral blood in late pregnancy, delivery, and the puerperium in 21 healthy primigravidae and were compared with the inhibitor levels in a group of 26 women whose pregnancies were complicated by PET and/or IUGR (pathology group). Biopsies were obtained from placentae in both groups: the biopsies from the pathology group were taken from areas in the placenta which appeared macroscopically normal (avoiding infarcted areas). Placental tissue extracts were prepared and antigenic levels of PAI-1 and PAI-2 were measured.

Plasma PAI-1 levels were consistently higher in the pathology group than in normal pregnancy at 36 weeks gestation, delivery, 1hr and 24hr post partum. Compared to normal pregnancy plasma PAI-2 levels were lower in the pathology group at 36 weeks gestation, delivery, 1hr and 24 hr post partum ($P<0.001$, $P<0.001$, $P<0.0001$, $P<0.05$). Levels of PAI-1 in placental tissue extracts were significantly higher in the pathology group ($P<0.001$) compared to normal pregnancy. Placental PAI-2 levels were similar in both the control and pathology group.

The results in this study suggest that the decreased levels of PAI-2 in the peripheral circulation in PET and IUGR is a pathological reaction resulting from placental infarction. Raised plasma and placental levels of PAI-1 however, appear to play a more primary role in the pathophysiology of PET and IUGR, by increasing fibrin deposition and reducing uteroplacental blood flow.

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DOPEXAMINE HYDROCHLORIDE AUGMENTS TISSUE PERFUSION FOLLOWING CARDIAC SURGERY

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Impaired tissue perfusion relative to demand occurs in patients undergoing major general surgical operations, and results in the development of an acute lactic acidosis. This correlates with increased morbidity and mortality, while augmentation in oxygen delivery and consumption results in significant increases in patient survival (Shoemaker *et al.* Chest. 1988: 94, 6). Dopexamine Hydrochloride is a recently developed inotrope that has been advocated for the management of low cardiac output states following cardiac surgery (Van Der Starre and Rosseel, Am. J. Cardiol. 1988: 62, 78c).

The aim of this study was to compare haemodynamic and tissue perfusion indices in patients undergoing elective coronary artery bypass grafting.

Following ethics committee approval and informed patient consent, 16 patients with left ventricular ejection fraction (LVEF) > 0.5 were randomly allocated to one of two treatment groups. After induction of anaesthesia baseline measurements of heart rate, arterial pressure, CVP, pulmonary capillary wedge pressure, and cardiac output were measured and cardiac index (CI), systemic and pulmonary vascular resistance were calculated. Arterial and mixed venous oxygen content were measured using a co-oximeter and oxygen delivery (DO2) and oxygen consumption (VO2) were calculated. Serum lactate was measured by fluorescent polarization immunoassay. Eight patients received an infusion of dopamine hydrochloride at 2 ug/kg/min, and 8 received an infusion of 0.9% saline for 24 hours. Measurements were recorded at 12 intervals and statistical analysis was by analysis of variance.

Significant elevations in serum lactate occurred in both groups following surgery. In the dopexamine group, CI and DO2 were significantly higher, $P<0.05$, and were associated with significant increases in VO2, $p<0.05$, and a more rapid reduction in serum lactate to control values. Significant increases in heart rate occurred in both groups. Haemoglobin concentration was similar in both groups.

This study confirms the occurrence of inadequate tissue perfusion in major surgical operations, in particular for cardiac surgery. Even in relatively low risk patients (LVEF > 0.5), benefit in tissue perfusion is achieved by improved cardiac index and DO2.

Peri-operative infusion of dopexamine is clinically efficacious by improving haemodynamic and tissue oxygenation in patients undergoing cardiac surgery.

INTRA-THECAL MORPHINE IN CARDIAC SURGERY – A PILOT STUDY ON THE CONTRIBUTION OF MORPHINE METABOLITES TO ANALGESIA

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In 1971 opioid receptors were discovered by Goldstein and others and in 1976 opioid receptors were located in the spinal cord. Both intra-thecal and extradural routes have been advocated for the administration of opioids to provide post-operative analgesia. Fitzpatrick has shown that 2 mg of intra-thecal morphine provides effective and sustained analgesia for patients following cardiac surgery (Fitzpatrick and Moriarty, Br. J. Anaesth. 1988: 60, 639). Hand confirmed the analgesic contribution of the morphine metabolite, morphine 6 glucuronide, which was detected in the cerebro-spinal fluid (CSF) following both oral and intra-muscular administration of morphine sulphate (Hand *et al.* Lancet, 1987: 2, 1207).

The aim of this study was to examine the cerebro-spinal fluid and determine the analgesic contribution of morphine metabolites following intra-thecal administration of morphine sulphate.

Following Ethics Committee approval and informed patient consent, 15 male patients undergoing elective cardiac surgery were entered into the trial. Each patient received a standardised pre med and anaesthetic. Following induction each patient received 2 mg preservative free morphine intrathecally after a sample of CSF was withdrawn via a 26 gauge spinal needle. Plasma levels were sampled at 4 hourly intervals. Post operatively patients were transferred to the intensive care for 72 hours. At 24 hours following induction each patient was assessed and, if indicated, a repeat lumbar puncture was performed, a second sample of CSF was taken, and 1 mg preservative free morphine was injected to provide additional analgesia. All plasma and CSF samples were analysed by gas liquid chromatography for the following: 1. Morphine, 2. Morphine-3-Glucuronide, 3. Morphine-6-Glucuronide, 4. Normorphine, 5. Codeine.

In addition pain scores (0-10) were assessed using a visual linear analogue pain score; respiratory status, PaCO2 and respiratory rate; sedation scores (0-4); and requirements for additional analgesia (I.V. pethidine) were recorded.

Ten patients satisfied the requirements of the protocol and repeat lumbar puncture was performed after 24 hours. Only 2 patients had morphine and morphine-3-glucuronide in the plasma samples after 4 hours and only 1 of these had morphine-3-glucuronide detectable

at 8 hours. Morphine sulphate was detected in the CSF of 9 patients after 24 hours and no morphine metabolites were detected in any of the 10 patients. Analgesia was deemed excellent as documented by low pain scores.

Morphine metabolites do not contribute to analgesia following intrathecal administration.

STATIC PRESSURE-RESPONSE RELATIONSHIPS OF SINUS NERVE BARORECEPTOR ACTIVITY DURING APPLICATION OF FELODIPINE TO THE CAROTID SINUS IN THE ANAESTHETIZED CAT

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The antihypertensive effects of CA^{2+} antagonist, felodipine, could arise from changes induced on baroreflexes in addition to its direct action on precapillary resistance vessels (Hallbëck-Nordlander and Thalen, J. Hypertension, 1983: 1, S2, 217). In this study we investigate the possibility that felodipine can alter baroreceptor sensitivity by direct effects on the receptor complex.

Sixteen cats were anaesthetized with pentobarbitone sodium (induction 48 mg/kg i.p.; maintenance 6-12 mg i/v/ hourly), paralysed with pancuronium bromide (0.8 i.v. hourly) and artificially ventilated. The carotid bifurcation was vascularly isolated (Neil and O'Regan, J. Physiol, 1971: 215, 15) and cannulae were inserted into branches of the common and external carotid arteries to permit pathways for artificial perfusion of the isolated segment with Ringer-Locke or Krebs-Henseleit solutions and the monitoring of carotid sinus pressures. Activity was recorded from single or a few active barosensory fibres prepared from the cut sinus nerve. Static pressure-barosensory activity relationships (0-240 mm Hg) were constructed with the isolated segment perfused with saline solutions containing either no drug or felodipine (10^{-8M}) or noradrenaline (10^{-5M}). Blood was intermittently readmitted to the carotid sinus. Statistical analysis was carried out using a student's t-test with $P \leq 0.05$ considered as statistically significant.

Twelve satisfactory barosensory preparations were studied. In 7 preparations, a progressive and statistically significant shift downwards and to the right was observed in static pressure-responses of barosensory activity during saline perfusion of the isolated bifurcation. This behaviour was present during perfusion with drug-free solutions and was partially reversed by readmission of blood to the sinus area. In the remaining 5 preparations, felodipine did not statistically affect either the threshold, slope (gain) or maximal activity of the static pressure-response curves of these preparations. Noradrenaline reversible decreased the threshold and increased the maximal activity of baroreceptor discharges, confirming previous findings (Munch *et al*, Circ. Res. 1987: 61, 409).

The cause of the reduced barosensory responsiveness noted in more than half the preparations studied is obscure. It may be due to increased stiffness of the carotid sinus wall consequent on an increase in its water content during saline perfusion (Bradford and McDermott, unpublished observations). Alternatively, a normal CO_2 tension in the perfusing medium may be important since Krebs-Henseleit solution was less liable to induce reduced barosensory responsiveness than the Ringer-Locke counterpart. In preparations exhibiting no change in barosensory responsiveness during saline perfusion of the isolated sinus, felodipine applications in therapeutic concentrations had no effect on static-pressure baroreceptor responses. It is unlikely, therefore, that the antihypertensive effect of this agent involves any changes in baroreflexes due to direct effects on the baroreceptor endings.

Some of the equipment used in this study was provided by the Wellcome Trust.

SIMPLIFICATION OF P6 ACUPUNCTURE ANTIEMESIS IN CANCER CHEMOTHERAPY

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While the addition of P6 acupuncture (ACP) to standard antiemetics produces benefit in more than 90% patients receiving potent cytotoxic drugs, the technique is invasive and requires special skill. Furthermore, in conjunction with standard antiemetic the benefit only lasts for 6-8 hours. To increase acceptability by both patients and medical and nursing staff the following modifications have been introduced.

1. Replacement of large multichannel ACP machines by a small single channel Transcutaneous Electrical Nerve Stimulator (TENS). This must have a slow (10-15 Hz) frequency band. It can be attached to both the needle and a skin electrode.
2. Use of transcutaneous electrical stimulation (TCES). Although the results are not quite as good as with ACP, more than 70% patients benefitted. In contrast with ACP, the benefit only lasts 2-3 hours.
3. Prolongation of effect of ACP and TCES by acupressure, with pressure for 5 minutes every 2 hours when awake.
4. Use of large diffuse surface electrodes (such as for ECG) rather than the conventional small magnestised copper studs. This removes the need for accuracy of placement over P6. Such electrodes are robust and can be worn for 4-5 days. They also reduce the current necessary for skin penetration and thus save batteries.
5. Self administration using a conventional TENS machine (15 Hz). Patient uses the stimulator for 5 minutes every 2 hours when awake.

Benefit is similar for 2, 3 and 5 and not quite as good as for ACP. The ultimate aim is a miniaturised purpose-built time-controlled stimulator to be worn on the forearm similar to Sea Bands.

DIFFERENTIAL ASPECTS OF TOLERANCE TO MUSCLE RELAXATION AND ANTI-CONFLICT ACTIONS OF THE BENZODIAZEPINES IN ANIMAL MODELS

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Benzodiazepine effects in man may be classified broadly as sedative hypnotic and they possess anxiolytic, anticonvulsant and muscle relaxant properties to varying degrees. Conventional wisdom holds that tolerance develops to the sedative and muscle relaxant effects but not to the anxiolytic action.

The Pull-up test has been proposed to measure sedation/muscle relaxation effects of drugs in rats while conflict procedures are commonly used to determine possible "anxiolytic" or behavioural

disinhibitory effects. In the first part of this study the actions of a number of psychotropic compounds were determined in the Pull-up test and Shock Probe Conflict procedure after acute oral administration in rats. The results of each test are presented in terms of ED50 values. Some differences in profile of action emerged. Flurazepam, clonazepam, oxazepam and meprobamate (and to a lesser degree, phenobarbital) provided markedly lower ED50's for anticonflict action than for sedative/muscle relaxant effect. The reverse was true for clobazam and pentobarbital. ED50 values for the other drugs tested were similar in both tests (nitrazepam, lorazepam, midazolam, diazepam) or lower in the Pull-up test (flunitrazepam, bromazepam, chlordiazepoxide). No ED50 could be calculated for carbamazepine within the tested dose range.

The second part of the study aimed to determine whether tolerance develops to the sedative/muscle relaxant effects of the prototypical benzodiazepine chlordiazepoxide (CDP). CDP (40 mg/kg s.c.) was injected daily in rats and performance in the Pull-up test evaluated. Latency to pull-up was increased on day 1 but not on day 5. A robust anticonflict effect in the Shock Probe Conflict procedure was however still obtained on day 5. In another group of rats tested daily in the latter procedure after CDP treatment response rate decreased in an orderly manner over time to near zero on day 5. However, as the response rate of saline controls also declined over time in a similar fashion, the decrease may be ascribed to habituation to the test environment. Tolerance therefore developed to the sedative/muscle relaxant effects of CDP but not its anticonflict action.

DEVELOPMENT AND GROWTH OF INTRACELLULAR SURFACTANT INCLUSIONS

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Pulmonary surfactant, the material which helps stabilise the distal air spaces of the lung, is produced by the type II cells of the alveolar epithelium. Before its release from the alveolar cell, surfactant is stored within osmiophilic inclusion bodies. The method of growth and maturation of these inclusions was investigated in this study.

Small blocks of lung tissue from adult rats were fixed in buffered glutaraldehyde and then postfixed in a solution of osmium tetroxide, osmium-ferrocyanide or osmium-imidazole. The tissue sections were examined in a transmission electron microscope.

The earliest evidence of surfactant was small masses of osmiophilic material associated with the smooth endoplasmic reticulum of the type II alveolar cells. As these masses increased in size, they appeared to coalesce with multivesicular bodies. Composite bodies, possessing both lamellated and vesicular material, were frequently encountered. The larger inclusion bodies were located in the superficial zones of the cells and their lamellated contents had a complex arrangement. Rod-like structures, consisting of small elongated masses of granular material, were occasionally seen in contact with the inclusions.

These observations indicate that the phospholipid component of lung surfactant is synthetised in the endoplasmic reticulum and is sequestered within inclusion bodies prior to its expulsion from the cell. The multivesicular bodies are probably the vehicles by which hydrolytic enzymes are transferred to the developing inclusions. The rod-like structures, although few in number, appear to be normal components of the alveolar cells. The microscopic appearances suggest that they contribute material to the larger inclusion bodies.

PLASMA ENDOTOXIN LEVELS IN TRAUMA PATIENTS ESTIMATED BY THE CHROMOGENIC LIMULUS AMEOBOCYTE LYSATE ASSAY

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Endotoxins are lipopopolysaccharide-protein complexes derived from the cell wall of gram negative bacteria and are responsible for many of the pathological alterations seen during gram negative infections. The observation that lysates of blood ameobocytes from Limulus polyphemus (horse shoe crab) gelate on exposure to minute amounts of endotoxin (Levin and Bang, Thromb. Diath. Haemorrh. 1968: 19, 186) generated considerable interest in the development of an endotoxin assay for clinical use. More recently, the gelation assay has been greatly improved by the introduction of synthetic peptide chromogenic substrates (Iwanaga *et al*, Haemostasis 1978: 7, 183). Reagents for the colourimetric limulus ameoboycte lysate (LAL) assay are now available commercially and these have been used to develop an enzymatic procedure for the quantitative estimation of plasma endotoxin.

The analytical performance of this biochemical method has been optimised and evaluated using an endotoxin standard prepared from Escherichia coli. The absolute sensitivity of the assay is 10 pg/ml and it exhibits a linear calibration up to 100 pg/ml. Within-day and between-day imprecision at 10 pg/ml is ± 10% and ± 12% respectively whilst at 100 pg/ml it is ± 2% and ± 13% respectively. Best results are obtained with freshly reconstituted LAL reagent as a gradual loss of sensitivity is observed when the reagent solution is stored at 4°C for more than 24 hours. Reconstituted LAL reagent stored at –70°C for three days shows marked loss of peptidase activation ability. The performance of different preparations of LAL from the same manufacturer varies unpredictably which can increase the cost per test significantly.

No detectable endotoxin was found in the plasma of healthy adult volunteers (n=10) nor in a group of ten ward patients with no history of gastro-intestinal disease. Elevated levels of plasma endotoxin were found in ten ICU patients with multiple trauma (range 18-52 pg/ml). These increased levels may be due to alterations in the gastro-intestinal tract of trauma patients which cause the release of endotoxin into the peripheral circulation. However, the clinical sepsis score of the trauma group (range 4-21) correlated poorly (r=0.34) with plasma endotoxin level.

SERUM THYMIDINE KINASE LEVELS IN ACUTE LEUKAEMIA

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Thymidine kinase (TK) catalyses the phosphorylation of deoxythymidine to thymidine monophosphate, an essential precursor for DNA synthesis. Among the different isoenzymes of TK present in human cells, TK1, the cytosolar form of TK occurs in large amounts in dividing cells and is more or less absent from resting differentiated cells. TK2, the mitochondrial form of the enzyme, is also present in the cytosol at relatively constant levels throughout the cell cycle.

In this preliminary study serial blood serum samples were obtained from 9 patients with leukaemia; 5 with acute myeloid leukaemia (AML) and 4 with acute lymphoblastic leukaemia (ALL). Serum samples were obtained before and during the course of treatment and assayed for the relative contribution of the TK isoenzymes TK1 and TK2 to total TK activities. In the patients with ALL the results indicate that as treatment progresses the total serum TK falls and remains low relative to the original value. All of the ALL patients appear well at over 360 days from commencement of treatment. This trend is also reflected in the AML patients except in two cases where a transition to progressive disease was paralleled by an increase in total serum TK. From these preliminary results it can be suggested that serum TK levels may be used as a marker for the effects of treatment in AML and ALL patients. Furthermore serum TK levels may be useful for longitudinal follow-up studies of disease status both in indolent disease and in progressive disease during treatment.

APRT DEFICIENCY, NUCLEOTIDE POOL LEVELS AND MUTAGEN SENSITIVITY IN FRIEND ERYTHROLEUKAEMIA CELLS

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APRT deficient Friend erythroleukaemia cells (subclones 707DAP8 and 707DAP10) have significantly increased sensitivity, relative to wild-type cells (clone 707) to the induction of mutations to 5-bromo-2-deoxyuridine and 6-thioguanine resistance following treatment with ethyl methanesulphonate and methyl methanesulphonate. Although the APRT deficient subclones have similar growth rates to wild-type cells, nucleotide pool measurements reveal that they have significantly decreased levels of ATP, CTP, GTP and UTP plus decreased levels of dATP, dGTP and dTTP relative to wild-type cells. It is suggested that the increased mutagen sensitivity in the APRT deficient cells may be the result of deoxyribonucleotide pool imbalance. Additionally the deficiency of ATP may result in an inhibition of ATP-dependent DNA repair processes. These hypotheses are currently under investigation. The possibility that the APRT deficient cells may be simultaneously deficient in O^6-alkylguanine methyltransferase and that this may be a key factor in their increased sensitivity to alkylating agents can be discounted since wild-type Friend cells appear to be deficient in this enzyme.

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RELATIONSHIPS BETWEEN DIET AND ANTIOXIDANT STATUS

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Antioxidant defence systems, which prevent tissue damage evoked by free radical species, are considered to be dependent on the body's nutritional state. The current study investigated possible correlations (Pearson) between dietary intakes of copper, zinc, vitamin E, selenium and monounsaturated (MFA) polyunsaturated (PUFA) and saturated (SFA) fatty acids measured by laboratory analyses on daily weighed duplicate samples of selected nutrients and blood measures of antioxidant status *viz:* serum caeruloplasmin, B-carotene, total carotenes, vitamin E, erythrocyte superoxide dismutase (SOD), catalase, glutathione peroxidase (GSH-Px) and glutathione reductase (GR). The study group comprised 32 healthy adults (16 women, mean age 35.3, SEM 2.8 years; 16 men, mean age 31.5, SEM 1.8 years) who were non-smokers and not on any medication.

No correlations were found between intakes of Cu or Zn and measures of antioxidant status nor between any of the dietary measures and serum carotenoids or caeruloplasmin activity. Dietary intake of vitamin E was positivley correlated with both serum levels of vitamin E (r, 0.50; $P<0.05$) and erthyrocyte GR activities (r, 0.58; $P<0.01$) in the men. Similarly Se intake was positively correlated with both serum vitamin E (r, 0.58, $P<0.01$) and erythrocyte GR activity (r, 0.46; $P<0.05$) in men and also with erythrocyte GSH-PX activity (r, 0.47; $P<0.05$) in women. The only other significant correlation in women was between SFA intake and erythrocyte SOD activity (r, 0.61; $P<0.01$). In men intake of MFA was negatively correlated with erythrocyte SOD (r, –0.54; $P<0.05$) and catalase (r, –0.44; $P<0.05$) activities whilst dietary PUFA were negatively correlated with erythrocytes SOD activity (r, –0.45; $P<0.05$).

Although one day duplicate food samples may not be representative of habitual food intake, results from this pilot study, nevertheless, suggest that dietary intakes of both vitamin E and selenium, may influence serum levels of vitamin E and erythrocyte glutathione-dependent enzymes. Further fatty acid intake may affect erythrocyte antioxidant enzyme activities, though correlation trends were not always consistent in the men and women investigated.

Ethical approval was obtained from the University of Ulster Ethical Committee and subjects gave informed consent.

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EFFECTS OF SURGERY ON SERUM TK LEVELS IN PATIENTS WITH BREAST CANCER

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The nucleotide salvage pathway enzyme, thymidine kinase (TK) occurs in two isozyme forms in the cytosols of mammalian cells. TK1 levels are closely associated with rates of DNA synthesis while TK2 levels remain relatively constant throughout the cell cycle. Pre-surgery levels of these isozymes were assayed in the blood serum of operable breast cancer patients (N=17) and compared to the levels of TK in the serum of fibroadenoma patients (N=6) and control patients (N=15) who were scheduled to undergo surgery for a variety of non-malignant conditions. All patients were female and within a similar age range (29-78). Samples were also obtained serially post-surgery in the breast cancer patients and the effects of surgery on the TK levels were then observed. It was noted that there was no significant difference pre-surgery between the total TK levels in control patients and fibroadenoma patients. There was also no significant difference in the TK2 levels or the % contribution of TK1 to the total TK. There was however a significant difference ($P=0.001$) between the breast cancer patients pre-surgery and fibroadenomas and control patients with the breast cancer patients exhibiting higher total TK levels due largely to an increase in TK1. Measuring the levels of TK in pre and post surgery samples from breast cancer patients and control patients it was shown that surgery had no significant effect on the TK levels. These preliminary findings indicate that surgery does not significantly reduce serum TK levels in patients with operable breast cancer. It is therefore possible that the elevated serum TK levels

observed in breast cancer patients may not be a direct result of the primary tumour mass.

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PATHOPHYSIOLOGY OF THE PARTIALLY OBSTRUCTED/ REIMPLANTED CANINE URETER

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Changes in ureteric motility following complete ureteric obstruction and recovery of function after relief of obstruction are well described. However, the pattern of ureteric motility following relief of partial ureteric obstruction is unclear. The aim of this study was to compare the function of the canine ureter prior to and following a period of experimental partial ureteric obstruction.

Partial ureteric obstruction was created in 12 female mongrel dogs weighing between 15 and 20 kg. The animals were anaesthetised with pentobarbitone given intravenously in a dose of 0.5 mg/kg. Two weeks prior to obstruction, a left sided nephrostomy was inserted using a 14 gauge Nutricath cannula which was placed in the renal pelvis via the renal cortex. The proximal end of the cannula was tunnelled subcutaneously and connected to a heplock, allowing chronic access to the collecting system. Partial ureteric obstruction was created for 4 weeks by insertion of a stent of 0.3 mm internal diameter in the lower end of the left ureter via a small ureterotomy. At the end of this period, the stent was removed, a segment of proximal ureter harvested for histology and in vitro analysis and the ureter reimplanted into the bladder. Ureteric motility was recorded before and during the period of obstruction and after reimplantation, by passage of a ureteral catheter percutaneously via the nephrostomy tube into the ureter. This cannula was then attached to a pressure transducer. These studies were performed during normal urine output and provoked diuresis. At the end of the recovery period, a segment of ureter was again harvested and studied in vitro.

Before obstruction, the resting intraureteric pressure ranged from 0 - 5 mm Hg and the ureter demonstrated regular contractions with an average rate of 5.7 contractions per minute and a mean peristaltic amplitude of 27.2 mm Hg. At the end of the obstruction period, abnormal ureteric rhythmicity was observed with multiphasic irregular contractions at both normal and increased urine flow rates, with a significant increase in mean amplitude of contraction. Resting intraureteric pressure was not significantly changed. *In vitro* experiments confirmed the disordered pattern of ureteric motility seen *in vivo*. Histology of the obstructed ureter demonstrated hypertrophy of the muscularis and lamina propria layers with chronic inflammatory infiltrate. Following reimplantation the ureterer demonstrated a return towards pre-obstruction type rhythm and rate but with persisting evidence of increased contractility at 2 months post surgery. Long term recovery of the partially obstructed canine ureter requires further assessment.

EXPRESSION OF VIMENTIN AND CYTOKERATIN INTERMEDIATE FILAMENT PROTEINS IN BENIGN AND MALIGNANT BREAST EPITHELIUM – AN IMMUNOHISTOCHEMICAL STUDY

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Intermediate filament protein expression was studied in malignant and benign lesions of the breast using the Avidin Biotin Complex technique Vimentin was detected using a monoclonal antibody originating from the V9 clone (Dakopatts). Cytokeratins were detected using the monoclonal antibodies AE1 and AE3 (ICN).

Vimentin expression was detected in 5 of 12 grade I carcinomas (43%), 13 of 25 grade II carcinomas (52%), 2 of 7 grade III carcinomas (29%), 8 of the 12 fibroadenomas (67%) and 3 of the 6 cases of fibroadenosis (50%). In the carcinomas Vimentin was distributed diffusely in the cytoplasm of the epithelial cells in most cases (17 cases) with, in some instances accentuation in the perinuclear (2 cases) and subplasmalemmal location (3 cases). In 3 other cases Vimentin expression was solely perinuclear. In the benign lesions the staining was diffusely cytoplasmic in 5 and solely perinuclear in 3 of the fibroadenomas. In 2 cases of fibroadenosis, Vimentin staining was perinuclear and in 1 other case it was subplasmalemmal.

Cytokeratin expression was detected with both antibodies in the epithelium in all the carcinomas, fibroadenomas and cases of fibroadenosis. In both benign and malignant lesions it was distributed diffusely in the cytoplasm, with perinuclear or subplasmalemmal accentuation also being identified.

These results confirm the work of other authors who have identified Vimentin and cytokeratin coexpression in benign and malignant lesions of the breast.

A COMPARISON OF THE DETECTION OF INTERMEDIATE FILAMENT EXPRESSION ON CYTOLOGICAL AND HISTOLOGICAL PREPARATIONS

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Intermediate filament expression as detected on fine needle aspiration cytology was compared with their detection on histological sections. 28 carcinomas of the breast (Grade I – 9 cases, Grade II – 14 cases, Grade III – 5 cases), 9 fibroadenomata and 3 cases of fibroadenosis were examined. Cytokeratin expression was detected using the monoclonal antibodies AE1 and AE3 (ICN) and Vimentin was detected using a monoclonal antibody originating from the V9 clone (Dakopatts).

Cytokeratin expression was detected on histological examination with both antibodies, in every case and involved most of the epithelial cells. Cytokeratin expression, which was detected in most epithelial cells, was present on cytological examination with AE1 in all but 1 case, a carcinoma which occurred in an early female; and with AE3 in all but 1 case, a fibroadenoma.

In the carcinomas, Vimentin was detected both histologically and cytologically in 9 cases, only histlogically in 2 cases, only cytologically in 7 cases and by neither technique in 10 cases. In the benign lesions Vimentin was detected both histologically and cytologically in 3 cases, only histologically in 4 cases, only cytologically in 2 cases and by neither technique in 3 cases. Vimentin expression within a lesion can be quite focal, providing a possible explanation for this finding.

Immunoblotting confirmed the presence of Vimentin of presumed epithelial origin in 14 aspirates (10 carcinomas, 4 fibroadenomas) and identified it in a further 5 aspirates where Vimentin had not been detected on immunohistochemical staining.

STATISTICAL PROPERTIES OF PHARMACOKINETIC PARAMETER ESTIMATES

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The compartmental approach to pharmacokinetic parameter estimation requires that a complete mammillary model be specified. This requirement may lead to model misspecification errors in the parameter estimates. The noncompartmental approach is an attempt to overcome this problem. The quality of the parameter estimates depends on the mean and variance of their sampling distributions and may be characterised by the mean squared error (MSE). The compartmental and noncompartmental (using the linear trapezoidal numerical integration algorithm) methods for estimation of the area under the plasma drug concentration/time curve (AUC) were studied by computer simulation using the Monte Carlo method. The table shows the MSE for compartmental and noncompartmental methods when the data were generated using monoexponential (an example of bolus intravenous drug administration) and biexponential (an example of extravascular drug administration) models. For the monoexponential case the two methods produce estimators with similar MSE's whereas in the case of the biexponential model the MSE for the compartmental estimator is extremely large despite the fact that the model is correctly specified. This large MSE can be attributed to the fact that the AUC is expressed in terms of ratios of the basic model parameters and these parameters are negatively correlated in the case of the biexponential model. This observation provides another reason besides model misspecification error for choosing a noncompartmental method for pharmacokinetic data analysis.

TABLE

Method	Model	
	Monoexponential	Biexponential
Compartmental	38947	730715
Noncompartmental	34718	33928

The mean squared errors for compartmental and noncompartmental (linear trapezoidal algorithm) estimators of the area under the plasma drug concentration/time curve for mono and biexponential models.