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ATP Sensitive K^+ Channels and Regulation of Microvascular Tone

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ATP-sensitive K^+ channels (K_{ATP}) play an important role in the regulation of arteriolar tone and reactivity in the microcirculation. We have shown that glibenclamide (GLIB), a selecive K_{ATP}-antagonist, causes sustained arteriolar constriction. Coupled with the observation that K_{ATP}-agonists, such as pinacidil and cromakalim, cause arteriolar vasodilatation, these data indicate that there are both open and recruitable K_{ATP} in the microcirculation that play an important role in determining resting tone. GLIB also inhibits arteriolar vasodilatation induced by adenosine, isoproterenol and prostacyclin. Thus, several important classes of vasodilators act, at least in part, by opening K_{ATP} in the microcirculation. Patch clamp studies of single cremasteric arteriolar muscle cells revealed active, GLIB-sensitive K^+ currents around the resting membrane potential. Inhibition of these currents decreased membrane conductance and led to depolarization consistent with our functional studies. We have also found that hypoxia (P_{O_2} 20 mm Hg) increases membrane conductance and inhibits contractile reactivity of single cremasteric arteriolar muscle cells which could be reversed by GLIB. Therefore, K_{ATP} channels play an important role in the regulation of membrane potential and tone of arterioles and likely participate in the neural, hormonal and local controls of blood flow in the microcirculation.

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Mechanisms of Myogenic Activity

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The ability of blood vessels to constrict in response to a rise in intravascular pressure is well-established. Here, the mechanism has been investigated in isolated, slightly preconstricted rat mesenteric small arteries (RMSA), with particular attention to the possibility that arachidonic acid metabolites mediated by cytochrome P450 (CyP450) could be involved. After cannulation, RMSA were held at 60 mm Hg and slightly preconstricted (noradrenaline and neuropeptide Y). Pressure was then raised to 100 mm Hg for 10 min, and then reduced again to 60 mm Hg, and the activating cocktail was washed out. The process was then repeated in the presence of 17-octadecanoic acid (17-O, 20 μM) or ketoconazole (ket, 100 μM), inhibitors of CyP450; or miconazole (mic, 1 μM), epoxygenase inhibitor; or charybdotoxin (CTX, 0.1 μM) or iberiotoxin (IBX, 0.1 μM; or methoxyverapamil (D600, 0.2 μM), calcium channel blocker. The table shows diameters measured 2 and 10 min after raising the pressure, n = 5–8 vessels per drug. * indicates difference to control (cont) value ($p < 0.01$).

Time	Diameter change, µm ± SEM, from diameter at 60 mm Hg						
	cont	17–O	ket	mic	CTX	IBX	D600
2 min	−8±1	−6±1	−8±2	−5±2	−17±9	−10±2	+26±3*
10 min	−11±1	+13±3*	+10±3*	−6±2	+21±3*	+21±5*	+28±5*

Other K-channel inhibitors (apamin, 0.1 μM; barium, 100 μM; glibenclamide, 1 μM; 4-aminopyridine, 500 μM) had no effect at either time point. The results suggest that the sustained, but not the initial, phase of the myogenic response is mediated through K_{Ca} channels inhibited by non-epoxygenase metabolites of CyP450, e.g. 20-HETEs.

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Cellular Mechanisms of Angiogenesis

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The pattern of capillary growth (angiogenesis) is generally believed to involve disruption of the endothelial cell (EC) basement membrane (BM), followed by migration and mitosis of ECs, leading to the formation of sprouts that make new connections. The role of perivascular pericytes (PCs) in this process is diverse and is different under pathological circumstances or during development, where EC mitosis may not be involved and PCs may guide EC migration. We studied the cellular mechanisms involved in physiological angiogenesis and the interaction of ECD and PC, using quantitative electron microscopy and bromodeoxyuridine (BrdU) labelling index in three different animal models that emphasise either luminal (sustained vasodilatation) or abluminal (muscle stretch) mechanical stimuli, or a combination of both (chronic electrical stimulation). Capillary supply increased to a similar extent in all three cases, but the mechanism was different. There was no evidence for the breakage of the BM or EC proliferation with chronic vasodilatation, but vacuolisation of EC and intraluminal capillary division by cytoplasmic processes was seen, this longitudinal splitting being confirmed by confocal imaging. The interdigitation of EC and PC increased but there was no change in the relative capillary perimeter coverage or PC morphology. Prolonged muscle stretch induced EC activation prior to migration to form abluminal sprouts while PC coverage was increased, indicating a possible supportive role for PCs. Finally, chronic electrical stimulation evoked widespread EC activation and proliferation, occasional disturbance of BM, and formation of both luminal and abluminal sprouts, while PC coverage was decreased concomitant with capillary growth. Thus, angiogenesis in adult skeletal muscle may occur with or without breakage of BM and/or EC proliferation, and PCs may either assist it by withdrawing or supporting the migration of sprouts.

Adenosine as a Regulator of Muscle Microcirculation in Systemic Hypoxia

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During acute systemic hypoxia vasodilatation mediated by the local effects of tissue hypoxia tends to overcome reflex vasoconstriction mediated by the sympathetic nervous system. Our experiments have shown that in skelatal muscle, locally released adenosine is largely responsible for the vasodilatation of systemic hypoxia and that it acts preferentially on the terminal arterioles rather than the proximal arterioles. This adenosine-dependent dilatation is partly attributable to the opening of ATP-sensitive K^+ (K_{ATP}) channels, which may be on the skeletal muscle fibres, vascular smooth muscle and/or endothelium, but it is largely dependent on the synthesis of NO by the endothelium. Our recent experiments with specific pharmacological agonists and antagonists of the adenosine receptor subtypes indicate that although exogeneous adenosine can induce vasodilatation in skeletal muscle by acting on adenosine A_2 receptors, it is the A_1 receptors that play the major part in the muscle vasodilatation of acute hypoxia. We are currently investigating the role of K_{ATP} channels and NO in A_1-dependent dilatation.

During the adaptation to *chronic* systemic hypoxia it seems the arterioles of skeletal muscle become more readily affected by the dilator influences of adenosine that is released by an acute hypoxic challenge, but less sensitive to the constrictor influences of noradrenaline. Our evidence suggests that this may be explained by up-regulation of NO synthesis by the vascular endothelium which facilitates the influences of adenosine, but antagonises the action of noradrenaline.

Endothelium-Derived Relaxing Factors and Their Interaction

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Endothelial cells control vascular tone by synthesising and releasing the vasoactive autacoids nitric oxide (NO), prostacyclin (PGI_2) and the endothelium-derived hyperpolarising factor (EDHF). Fluid shear stress, exerted by the streaming blood at the endothelial surface, is the physiologically most important stimulus for the continuous production of NO. Both shear stress and isometric stretch activate endothelial NO production via a mechanism which differs from the classical activation pathway in that it is independent of an increase in the concentration of intracellular free Ca^{2+} and insensitive to calmodulin antagonists. This Ca^{2+}-independent NO production is abolished by tyrosine kinase inhibitors and is associated with the tyrosine phosphorylation of cytoskeletal and caveolar proteins. The endothelium of certain microvascular beds is also able to release an EDHF which hyperpolarises vascular smooth muscle cells by activating Ca^{2+}-dependent K^+ channels. This factor, which may be an arachidonic acid metabolite, is released not only by receptor-dependent and -independent agonists but also in response to pulsatile changes in transmural pressure. The production of EDHF is not detectable in the absence of NO synthase- and cyclo-oxygenase-inhibitors. Moreover, NO donors attenuate EDHF-induced dilation, suggesting that EDHF synthesis is suppressed by the continuous production of NO. Thus this factor, which is synthesised in response to both mechanical and humoral stimuli, may contribute mainly to the regulation of vascular tone in situations associated with a dysfunction of the endothelium.

Regulation of Endothelial Function in Diabetes

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Endothelium-dependent relaxation is impaired in established diabetes mellitus, yet in the early stages of the disease blood flow is increased in various organs. This vasodilatation in diabetic patients appears to be influenced by the degree of hyperglycaemia and is attenuated upon lowering of blood glucose levels. As recently reviewed (Jaap and Tooke, Clin Sci 1995;89:3–12), endothelial cells may be the site of a primary defect in diabetes contributing to the onset of diabetes-related vascular diseases.

We have previously reported that basal activity of the *L*-arginine-nitric oxide synthase signalling pathway was elevated in human umbilical vein endothelial cells isolated from gestational diabetic pregnancies (Sobrevia et al, J Physiol 1995;489:183–192) and in non-diabetic cells cultured in high *D*-glucose (Sobrevia et al, J Physiol 1996;490:775–781), whereas basal release of prostacyclin was impaired. *L*-Arginine transport was increased in diabetic cells or non-diabetic cells exposed to hyperglycaemia for 24 h. Unlike gestational diabetic cells, hyperglycaemia-induced stimulation of transport was time- (3–12 h) and protein synthesis-dependent and reversible. Elevated rates of basal NO synthesis in diabetic and hyperglycaemic cells were abolished by 100 μM *L*-NAME. Although diabetic cells exhibited a sustained membrane hyperpolarization, membrane potential was unaffected by elevated glucose. Hyperglycaemia and diabetes were associated with an increase in basal $[Ca^{2+}]_i$. Insulin (1 nM, 8 h) and adenosine (10 μM, 2 min, A_2-purinoceptor) stimulated *L*-arginine transport and NO synthesis in nondiabetic cells (Sobrevia et al, J Physiol 1997;490:775–781), but in diabetic and hyperglycaemic cells insulin downregulated the activity of the *L*-arginine-NO pathway. Thus, the normal stimulatory action of insulin on vasodilator pathways in human fetal endothelial cells may be impaired under conditions of sustained hyperglycaemia.

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Cellular Mechanisms Regulating the Passage of Leucocytes across Venular Walls in Inflammation

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The migration of leucocytes from the vascular lumen to the extravascular tissue involves a number of sequential cellular responses mediated by the interaction of adhesion molecules expressed on the leucocytes and endothelial cells. These distinct but overlapping events include rolling of leucocytes along the vessel wall, flattening and firm adhesion of leucocytes to venular endothelial cells and migration of leucocytes through the vessel wall. Although much progress has been made in our understanding of the cellular and molecular events that mediate leucocyte rolling and firm adhesion, very little is known about the events that mediate the passage of leucocytes through the vessel wall in vivo. A direct approach to investigating this response is by intravital microscopy which allows direct viewing of events within a microvascular bed. Using this technique, to date we have shown distinct roles for endogenously generated platelet activating factor and the adhesion molecule PECAM-1 (CD31) in the process of leucocyte extravasation from rat mesenteric venules (Nourshargh et al, Blood 1995;85:2553–2558; Wakelin et al, J Exp Med 1996;184:229–239). A better understanding of the in vivo events that mediate leucocyte extravasation will shed much light on our understanding of the overall process of leucocyte migration, and considerably facilitate the identification of potential targets for the development of novel antiinflammatory strategies.

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Effects of Acute and Chronic Cold Exposure on Tissue Blood Flow

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On acute cold exposure most mammals show an impaired oxygen delivery to skeletal muscle, due to a reduced muscle blood flow (MBF) and altered vascular reactivity (Brown et al, J Physiol 1993; 467:36P). Although chronic exposure may increase capillary density (Bratcher and Egginton, J Physiol 1995;483:132P), it is unclear to what extent this increased peripheral resistance may be overcome by activity and/or acclimation. Male Wistar rats were subjected to a progressive reduction in environmental temperature and photoperiod for a period of 6–8 weeks, from an ambient of 20 °C to a final of 4 °C, and compared to age matched controls. There was little difference in cardiac output or regional blood flow (BF), estimated by the radiolabelled microsphere technique, between groups of animals at normal (36 °C) body temperature. For example, BF in tibialis anterior (TA) at rest was 4 ± 1 and 5 ± 1 ml/min/100 g for control and cold-acclimated animals under euthermic conditions, and 161 ± 27 and 112 ± 23 ml/min/100 g during 4 Hz isometric contractions induced by indirect electrical stimulation (n.s). Acute cooling to levels used in some forms of surgery (25 °C) decreased visceral and cutaneous BF in both groups but increased it in brown adipose tissue due to thermogenesis, a response that was enhanced after cold acclimation (when BF doubled). Hindlimb fast muscle (TA) BF was 5 ± 1 and 12 ± 4 ml/min/100 g at rest ($p < 0.05$) and 128 ± 40 and 188 ± 35 ml/min/100 g during stimulation in non-acclimated and acclimated animals, respectively ($p < 0.05$), while slow muscle (soleus) BF decreased in both groups. This higher muscle BF likely reflects enhanced muscle thermogenesis, cold-induced angiogenesis (C:F was 1.57 ± 0.04 and 1.90 ± 0.07, respectively, $p < 0.01$) and recovery of vascular reactivity in cold-acclimated animals.

Cardiovascular Responses to Mild Cooling in Human Subjects with Primary Raynaud's Disease

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In 9 controls and 9 primary Raynaud's patients (PR) we studied the response of the digital and forearm vasculature to 5 repetitions of mild cooling (16 °C for 2 min) of the contralateral hand on each of days 1, 3 and 5. Cooling in session 1 evoked a rise in arterial pressure (ABP) (6.1 ± 1.1 ($p < 0.001$) and 5.1 ± 1.5 mm Hg ($p < 0.01$, one sample t test)) in controls and PR respectively. Furthermore, there was a decrease in digital cutaneous vascular conductance (DCVC), measured by laser Doppler, in the contralateral hand in controls (0.62 ± 0.18 pu/mm Hg, $p < 0.01$) and PR (1 ± 0.4 pu/mm Hg, $p < 0.05$). Five control subjects showed a decrease in forearm vascular conductance (FVC), determined by plethysmography, in response to cool immersion in the 1st session, indicating muscle vasoconstriction, whilst the remainder showed no change or an increase in FVC, indicating muscle vasodilation. In contrast only 1 PR patient responded with a vasoconstriction in the forearm. On this basis, the control and PR patients were divided into 2 groups: constrictor and dilator. On days 3 and 5 the changes evoked in controls and PR patients in ABP and DCVC were maintained. The decrease in FVC seen in the 'constrictor' control group was still evident on day 5 (a fall of 0.006 ± 0.002 vs. 0.003 ± 0.001 conductance units), but the increase in FVC in the control dilator group was reversed to a decrease in FVC (0.003 ± 0.001 vs. a fall of 0.007 ± 0.002 conductance units, $P < 0.05$ regression analysis). By contrast, in PR patients the increase in FVC persisted (0.004 ± 0.001 vs. 0.005 ± 0.002 conductance units). These results suggest that mild cooling initially evokes the forearm muscle vasodilation of the alerting response in ~ 50% of controls; on repetition habituation occurs and eventually the primary reflex vasoconstrictor response to cooling predominates. However, the majority of PR patients show the muscle vasodilation of the alerting response and do not habituate. These findings are consistent with our previous results on responses to sound in PR patients and with the idea that the alerting response is more readily evoked and is more persistent in PR patients (Edwards and Marshall, Int J Microcirc Clin Exp 1996;16:204).

The Effect of Small Cumulative Increases in Venous Congestion Pressure on Blood Flow in the Human Calf

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We have shown that the relationship between fluid filtration and small (8 mm Hg) cumulative venous congestion pressure steps (cVCP) is linear (Gamble et al, J Physiol 1993;464:407–422). However, Darcy's Law made us reason that this could not occur unless local vascular resistance decreased as venous pressure rose. This study investigates the relationship between cVDP and limb blood flow (Qa).

We used strain gauge plethysmography on 13 healthy females (age 20–23 years) and estimated Qa from the initial slope of the limb circumference trace when a 10 s duration 80 mm Hg congestion step was superimposed towards the end of each 6–7 min duration cVCP. The maximum cVCP was always < mean arterial pressure (MABP) measured in each study with a Dinamap® monitor.

Since Qa varied widely (median 2.34, range 1.5–11.5 $ml \cdot min^{-1} \cdot 100\ ml^{-1}$) the results below were normalised as a percentage of the initial value. We also predicted the decrease in Qa (DAQA%) from the initial Qa using Darcy's Law equation, assuming that MABP and limb vascular resistance remained unchanged during the protocol.

	cVCP, mmHg				
	5	20	30	40	50
Qa% ± SEM	100	99.3±6.6	99.1±7.5	82.1±6.8	102.0±9.5
DAQa%	100	78.2±0.6	67.1±0.9	44.7±1.4	35.0±2.2

We believe that the significant differences between measured and Darcy-predicted Qa values at each cVCP ($p < 0.0001$, Mann-Whitney) reflect the activation of local vasodilator mechanisms during the small cumulative congestion pressure step protocol.

Distinct Morphological Responses of Endothelial Cells to Extracellular Matrix Gels in vitro

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When endothelial cells are exposed to extracellular matrix proteins such as collagen, laminin or fibrin in vitro, they are stimulated to develop morphological responses resembling angiogenesis or vasculogenesis in vivo. The process has been described as a reciprocal interaction involving cell-driven reorganisation of the matrix which then creates guidance pathways around which cells organise. The contribution of specific cell adhesion events to these processes is not clear.

We compared the effects of two types of extracellular matrix, interstitial (type I collagen) and basement membrane extract (Matrigel) on the behaviour of human placental microvascular endothelial cells (HPMEC) and human umbilical vein endothelial cells (HUVEC) in two different configurations: 1. Gelling solutions of either collagen I or Matrigel in culture medium, at varying concentrations, were laid onto confluent cell monolayers. 2. Gels of collagen I or Matrigel were first prepared, and then cells were overlaid at a density of $1 \times 10^5/cm^2$. Cultures were observed at intervals thereafter. Both matrices caused concentration-dependent changes in cell morphology. Both cell types responded to Matrigel by retraction and alignment into cords. Vacuoles appeared and sometimes fused to develop tube-like structures. Collagen I caused monolayer retraction and cell elongation. Vacuolation occurred later and lumina developed in cell clusters. HUVEC showed similar elongation of cells but contrasted with HPMEC in forming cords. When cells were overlaid, the differences

in morphology between the matrices were more dramatic. Lumina were found in cryosections through Matrigel plugs infiltrated by HPMEC.

We conclude that different matrices provoke different morphological responses. Interstitial collagen gels seem to produce a migratory angiogenic response whereas basement membrane gels cause the development of tubes. Moreover, there are cell-specific differences in the responses to extracellular matrix components. Our data suggests that although the matrix influences morphology by providing a three-dimensional structure, specific adhesion mechanisms shape the angiogenetic process.

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Active Secretion of Hyaluronan into Synovial Cavity, Turnover Time and Comparison with Plasma Albumin Kinetics

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The objective was to measure the rate of active secretion of hyaluronan (lubricant, 2.95×10^6 D) into the joint cavity, leading to an estimate of its turnover time. The latter is of interest because other evidence leads us to believe that hyaluronan escapes through the interstitial pathways in the lining less freely than water or plasma albumin (0.07×10^6 D). Here, multiple washouts of the knee joint cavity were used to determine the endogenous intraarticular hyaluronan mass, followed by its secretion rate in vivo over 4 h in anaesthetised rabbits. Plasma albumin permeation into the joint cavity was determined similarly. Experiments post mortem acted as controls.

Endogenous hyaluronan mass, measured by high performance liquid chromatography, was 182 ± 9.9 μg (mean ± SEM; n = 21). Since the hyaluronan concentration averages 3.62 ± 0.19 μg μl^{-1}, mean endogenous synovial fluid volume was 50.3 μl (mass/concentration). This is twice the aspiratable volume.

Hyaluronan secretion rate over 4 h averaged 4.80 ± 0.77 μg h^{-1} (n = 5). The rate was significantly increased in contralateral joints expanded by 2 ml Ringer solution (5.80 ± 0.84 μg h^{-1}, n = 5, p = 0.01, paired t test). The size of the newly secreted chains ($2.05–2.48 \times 10^6$ D) was not significantly different from endogenous hyaluronan (2.95×10^6 D).

Hyaluronan turnover time, calculated as mass/secretion rate, was ≥31.4 h. This is more than an order of magnitude longer than turnover times for plasma albumin, 2.1 ± 0.4 h (n = 6), calculated from intra-articular albumin mass and entry rate. The results thus support the view that, relative to plasma albumin, hyaluronan molecules are partially sieved out and retained in the joint cavity by the synovial lining. Since synovial cells are separated by wide, interstitium-filled gaps of 1–2 μm, a further corollary is that interstitial matrix can selectively impede the movement of hyaluronan relative to albumin.

Altered Capillary Fine Structure in Chronically Stimulated Muscles after Dorsal Root Section

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Muscles electrically stimulated for 7 days, as well as contralateral muscles, showed swelling of capillary endothelium (Egginton and Hudlická, J Physiol 1991;435:16P). This type of stimulation activates both somatic efferent and afferent fibres. To determine the extent to which changes in contralateral muscles reflect activation of sensory fibres, dorsal roots (L3–L6) were sectioned two weeks prior to indirect electrical stimulation (10 Hz, 8h/day, for 7 days) of lower limb flexors in rats. Under sodium pentobarbitone anaesthesia, extensor hallucis proprius muscles were superfused in situ with 2.5% glutaraldehyde in phosphate buffer, and blocks ca. 1 mm^3 processed for electron microscopy. Micrographs of 40 capillaries per block were taken from stimulated and contralateral muscles to evaluate the proportion of modest (<1/3 of circumference) or substantially (>1/3) damaged endothelium.

Percentage of damaged capillaries in muscles with intact (DR+) or sectioned (DR–) dorsal roots

	Control	Stimulated		Contralateral	
	DR+	DR+	DR–	DR+	DR–
<1/3	21±2	41±5	12±3*	40±5	8±2*
>1/3	5±1	42±3	34±2	23±7	5±3

Although deafferentation did not interfere with angiogenesis in chronically stimulated muscles (capillary/fibre ratio increased by 30% in both groups), it significantly reduced the proportion of capillaries with damaged endothelium in both limbs (* $p < 0.01$ v. DR+). It is possible that stimulation of afferent fibres results in release of humoral factors which modify the capillary endothelium. Contralateral muscles are clearly an inadequate control for the effects of stimulation.

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Angiogenesis during Muscle Development – Effects of Activity

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Capillary growth in skeletal muscle is very rapid during early postnatal development, depending on both the genetic program and activity pattern. To differentiate between these influences we estimated the capillary supply of the major flight muscle (pectoralis) in two populations of barnacle geese, *Branta leucopsis*. One group was caught on the breeding grounds in Svalbard prior to their 2500 km migration and the other a captive, flight-restricted colony at Birmingham. Muscle was sampled for histochemistry and electron microscopy at weekly intervals post-hatch, and showed a sigmoidal growth curve with a marked increase in mass between age 5 and 7 weeks (Bishop et al, J Zool 1996;239:1–15). Fibre cross sectional area was 1,080 ± 350, 1,568 ± 192, 2,218 ± 397 and 3,176 ± 232 μm^2 at 3, 5, 7 and 11 weeks, respectively. Capillary supply was initially very sparse, only one vessel per fascicle, with a capillary to fibre ratio (C:F) of 0.11 ± 0.02 at 3 weeks. By 7 weeks (when the birds begin to fly) this had risen to 1.8 ± 0.1, similar to that found in adult birds. This dramatic capillary growth represents an 8-fold greater change than seen in fibre size. Moreover, there was a dissociation between mitochondrial volume density which showed an initial predominance of oxygen demand over supply that was reversed by 11 weeks. Since there were no significant differences between the two populations of birds before then, capillary growth in this muscle is primarily under genetic control, with activity level having a relatively minor influence.

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The Changes in Interleukin-6 and Tumour Necrosis Factor-α in Response to Venous Hypertension in Patients with Chronic Venous Disease

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The mechanism by which ambulatory venous hypertension leads to leucocyte trapping and activation in the microcirculation of the leg remains unexplained. It has been suggested that cytokines released locally in response to venous hypertension are responsible for initiating leucocyte and endothelial activation. We measured the cytokines interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) in the microcirculation of the leg in 30 patients with chronic venous disease before and after short-term experimental venous hypertension. The patients were divided into 2 groups: group 1, varicose veins with skin changes (LDS, n = 15); and group 2, varicose veins without skin changes (VVs, n = 15). Blood samples were taken from a foot vein after lying supine for 20 min, and standing supported for 30 min so as to raise the venous pressure. Plasma was separated by centrifugation and the cytokines were measured by commercially available ELISA.

Median (IQR)	IL-6 (pg/dl)		TNF-α (pg/dl)	
	LDS	VVs	LDS	VVS
Basal	270 (105–450)	145 (133–155)	205 (135–248)	120 (83–300)
Standing	325 (153–473)	185 (115–255)	270 (183–300)	165 (113–303)
p (Wilcoxon)	0.009	0.01	0.009	n.s.

There was a significant rise in the plasma concentration of IL-6 in both patient groups in response to experimental venous hypertension, while with TNF-α a significant rise was seen only in the LDS group. No significant difference was noted between the two patient groups.

We conclude that IL-6 and TNF-α are released in the microcirculation of the leg in patients with chronic venous disease in response to experimental venous hypertension and may be the initiator of leucocyte and endothelial activation.

The Role of the Epidermis in the Microangiopathy of Chronic Venous Disease

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Neo-vascularisation of the dermis occurs in lipodermatosclerosis (LDS) in patients with chronic venous disease (CVD). The molecular angiogenic stimulus causing this is not known. We studied the expression of angiogenic growth factors in the skin of patients with chronic venous disease who had never suffered ulceration. Skin biopsies were obtained from patients with LDS (n = 10) and patients with varicose veins and unaffected leg skin (n = 10). Using an indirect immunohistochemical technique we discovered that transforming growth factor beta and fibroblast growth factor expression in CVD skin paralleled that seen in normal skin. The platelet derived growth factor BB isoform was upregulated in the epidermis of skin exposed to venous insufficiency while PDGF-AA demonstrated increased expression at the junction of the epidermis and dermis, which was particularly noticeable in lipodermatosclerotic skin. Vascular endothelial growth factor was upregulated in the cytoplasm of keratinocytes in LDS (n = 10). It was also strongly upregulated in 5/10 specimens of patients with clinically unaffected skin. VEGF is one of the most powerful angiogenic growth factors. The presence of the neovascularisation of chronic venous disease occurring adjacent to the epidermis suggests that epidermally derived VEGF may be the angiogenic stimulus causing the cutaneous microangiopathy of chronic venous disease.

Vitamin C Inhibits Oxidized LDL Mediated Activation of the *L*-Cystine-Glutathione Pathway in Human Smooth Muscle Cells

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Oxidatively modified low density lipoproteins (LDL) modulate vascular cell function and are involved in the pathogenesis of atherosclerosis, which may be ameliorated by antioxidants (Steinberg, New Engl J Med 1993;328:1487–1489). We have previously shown that oxidized LDL enhances the expression of stress proteins heme oxygenase-1 and MSP23 (Siow et al, FEBS Letts 1995;368:239–242), elevates intracellular glutathione (GSH) levels and transport of its precursor *L*-cystine (system x_c^-) (Siow et al, J Physiol 1996;491P:31P–32P) in cultured vascular smooth muscle cells. In the present study we have investigated whether vitamin C affects GSH levels and cystine transport in human umbilical artery smooth muscle cells (SMC) exposed to oxidized LDL.

SMC were cultured from explants in medium MCDB 131 supplemented with 10% fetal calf serum. *L*-[^{14}C]Cystine transport (2 μCi/ml, 50 μM, 2 min) and GSH levels were determined in cell monolayers exposed for 24 h to 100 μg/ml active (nLDL), minimally modified (mmLDL) or highly oxidized (oxLDL) LDL. GSH and cystine uptake was also measured in cells pretreated for 24 h with 100 μ*M* vitamin C before exposure to the LDL. GSH levels (nmol/mg protein, mean ± SE) were elevated in mmLDL (20.9 ± 0.7) and oxLDL (25.6 ± 1.5) treated cells compared to nLDL-treated (18.4 ± 0.7) and untreated (18.9 ± 1.0) cells. Basal GSH levels in SMC were reduced by 24 h pretreatment with 100 μ*M* vitamin C and abolished the GSH elevation mediated by mmLDL and oxLDL. *L*-Cystine transport (nmol/mg protein/min, mean ± SE) was increased by mmLDL (0.534 ± 0.055) and oxLDL (0.731 ± 0.047) compared to nLDL-treated (0.449 ± 0.024) and untreated (0.453 ± 0.023) cells. Vitamin C pretreatment reduced both basal and LDL mediated increases in *L*-cystine transport. These findings suggest ascorbic acid may reduce oxidative stress in cultured human SMC and may protect SMC from oxidant insult by oxidatively modified LDL.

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Vascular Endothelial Growth Factor (VEGF) Increases Permeability by Inducing Openings Through Endothelial Cells

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When perfused through frog mesenteric microvessels, VEGF transiently increases hydraulic permeability (L_p). Maximum L_p is seen within 30 s and returns to baseline at 2 min (Bates and Curry, Am J Physiol 1996;271:H2520–H2528). We have investigated the ultrastructural changes in microvascular endothelium which accompany this transient increase in L_p. In three pithed frogs, mesenteric microvessels were perfused in situ via glass micropipettes with a Ringer solution containing bovine serum albumin (BSA, 10 mg·ml^{-1}). Vessels were transiently occluded to measure the L_p of the vessel walls (Michel, J Physiol 1980;309:341–335) to ensure it was normal. The Ringer/BSA solution was exchanged with a similar perfusate containing 1 n*M* VEGF by a fine tube in the micropipette. During perfusion with the VEGF solution, rapid transient occlusions were made; when it was clear that L_p had risen (20–40 s), the vessels were immediately fixed with buffered 2.5% glutaraldehyde (4°C) and processed for electron microscopy. Reconstructions from serial sections covering a total of 42 μm length from the vessels revealed 3 transcellular gaps (holes), diaphragmed fenestrations and some enlarged cytoplasmic vesicles. No intercellular openings have been found so far.

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Microvascular Hydraulic Conductivity (L_p) Is Chronically Increased by Acute, Transient Perfusion with Adenosine Triphosphate (ATP)

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We have shown previously that 10 min exposure to vascular endothelial growth factor results in an acute, transient increase in L_p followed by a chronically increased baseline L_p 24 h later. To determine whether this chronic increase can be brought about by other agonists which transiently increase permeability, we measured L_p of 7 microvessels 24 h after exposure to ATP, which is known to result in a similarly transient increase in L_p. L_p was measured in mesenteric microvessels of *Rana pipiens* (anaesthetised by 0.15 mg/g body weight MS222) as previously described. Microvessels were perfused with 1% BSA in frog Ringer's solution and L_p measured. The vessel was recannulated and perfused with 1% BSA in Ringer's with or without 100 μM ATP, and L_p measured. The vessel was perfused for 10 min, before the pipette was removed. The mesentery was then replaced in the animal which was allowed to recover. 24 h later the animal was reanaesthetised, the vessel relocated, and L_p measured again. In 7 microvessels perfusion with ATP resulted an initial transient increase in L_p from 3.4 ± 1.4 (median + IQR) to a peak of 25 ± 27 × 10^{-7} cm·s^{-1}·cm H_2O^{-1} which resolved to baseline within 5 min. The baseline L_p was increased 24 h later in all 7 vessels (7.5 ± 3.2 fold) compared to the previous day (to 21 ± 4.1 × 10^{-7} cm·s^{-1}·cm H_2O^{-1}, $p < 0.02$). In contrast, there was no increase in baseline L_p after 24 h in 5 vessels which were treated as above, but did not receive ATP. Chronically increased L_p can therefore be brought about by inflammatory mediators such as ATP as well as vascular endothelial growth factors.

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N^G-Monomethyl-*L*-Arginine (*L*-NMMA) Abolishes the Increase in Permeability Induced in Rat Mesenteric Venules by Histamine

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Using the red cell micro-occlusion technique (Kendall and Michel, Exp Physiol 1995;80:359–372), we have determined the hydraulic permeability (Lp) and effective oncotic pressure ($\sigma\Delta\Pi$) exerted across the walls of single mesenteric venules perfused in situ in anaesthetised rats. When histamine was added to either the perfusate or superfusate at concentrations of 1.0–1.5 mmol l^{-1}, L_p increased and $\sigma\Delta\Pi$ was reduced. Changes in the permeability reached their maxima between 6–12 min and attenuated at later times, with L_p returning to its baseline value at 18 min. Following the addition of 1.0 mmol l^{-1} histamine to the superfusate, L_p rose from a mean ± standard error of 2.27×10^{-7} (± 0.54) cm s^{-1} cm H_2O^{-1} to a maximum value of 7.55×10^{-7} (± 1.65) cm s^{-1} cm H_2O^{-1} at 6 min, while $\sigma\Delta\Pi$ fell from a mean of 20.52 (± 0.65) cm H_2O to 11.27 (± 2.02) cm H_2O (n = 6). By contrast in six experiments where the arginine analogue, *L*-NMMA, was added to the superfusate, histamine (1.0 mmol l^{-1}) failed to change L_p or $\sigma\Delta\Pi$ from the control values of 2.87×10^{-7} (± 0.43) cm s^{-1} cm H_2O^{-1} and 18.35 (± 1.48) cm H_2O respectively. This is consistent with the report of Yuan et al. (Am J Physiol 1993;264:H1734–H1739) and strengthens the hypothesis that nitric oxide formation is a crucial step in the cascade of reactions between the binding of histamine to its receptor and the increase in microvascular permeability which ensues.

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Bradykinin-Induced Permeability Changes Observed in Single Pial Venular Capillaries

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Bradykinin (Bk) is an inflammatory mediator with a variety of physiological and pathological actions, and we have evidence that it is involved in the considerably raised microvascular permeability following cerebral ischaemia (Kurokawa and Fraser, J Physiol 1995; 483:140P). The acute (ca. 1 min exposure) responses to Bk are small, of similar magnitude as histamine and of the order of 2×10^{-6} cm·s^{-1}. We are investigating the mechanisms by which the larger responses are produced, and here we report a method by which they may be investigated. The permeability to sulforhodamine of single venular capillaries of rats (anaesthetized with pentobarbitone: 60 mg kg^{-1} i.p.) was measured from the rate of decrease of fluorescence in an occlusion experiment, and the effects of Bk on single pial venular capillaries were investigated by applying it to their abluminal surface (see Easton and Fraser, 1994;475:147–157). Permeability responses were followed before, during and after 10 µ*M* bradykinin was applied for 10 min. Permeability increased by 2×10^{-6} cm s^{-1} during the application, but did not fall when Bk was removed, as it does after an acute application. Permeability remained at this level for a further 20 min and then rose steadily to about 7×10^{-6} cm·s^{-1} over the course of 60 min. The subsequent administration of 1 µ*M* HOE 140, the bradykinin B_2 receptor antagonist, reduced the permeability back to the 2×10^{-6} cm·s^{-1} level. Thus exposure of microvessels to Bk produces permeability results in responses which depend on the duration of exposure, and longer exposure times appear to set off more complicated mechanisms.

Selective Depletion of Interstitial Glycosaminoglycans Greatly Elevates Hydraulic Permeability of Joint Lining

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The resistance of synovium to leakage of lubricating fluid out of a joint cavity is thought to be created partly by the glycosaminoglycans (chondroitin sulphate, heparan sulphate and hyaluronan) found in the broad interstitial spaces between the synovial lining cells. This was tested here by selective enyzmatic removal of hyaluronan and chondroitin sulphate. Ringer solution was infused into the synovial cavity of knee joints in anaesthetised rabbits and the rate of fluid escape across the synovium (Q_s) was measured at controlled joint pressures (P_j). One joint cavity was injected with 500 µl enzyme solution (500 units) for 30 min prior to measurements and the other with 500 µl Ringer solution (control). The enzymes were testicular hyaluronidase (THase, 5 rabbits), which hydrolyses both hyaluronan and chondroitin sulphates, or Streptomyces hyaluronidase (SHase, 5 rabbits), which hydrolyses only hyaluronan.

Both enzymes rapidly (in minutes) and dramatically increased the fluid escape rate e.g. by 5.32 ± 0.58 times for THase (n = 34, mean ± S.E.M.). The steepness dQ_s/dP_j of the pressure-flow plot, which represents synovial hydraulic conductance, was raised 5.36 ± 1.86 times by THase at low pressures and 6.65 ± 2.83 times by SHase over the same pressure range. THase did not have any significantly bigger effect on conductance than SHase despite its wider action.

The results show that hyaluronan is a major contributor to synovial hydraulic resistance. Yet hyaluronan comprises only 20% of synovial interstitial glycosaminoglycans (Price et al. J Physiol 1996; 395:821). The dramatic effect of its removal is therefore difficult to explain, quantitatively, merely by removal of uniformly distributed resistive molecules (model of Levick, Microvasc Res 1994;47:90). Their removal could, in principle, have a greater effect on resistance if hyaluronan plays a structural role in interstitial organization, or if hyaluronan distribution is markedly non-uniform.

Flow Dependence of Potassium Permeability in Single Perfused Microvessels of Pithed Frogs

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By using a development of the single bolus microperfusion technique (Crone et al., J Gen Physiol 1978;71:195–220), we have shown that microvascular permeability to potassium ions (P_{K^+}) increases linearly with the flow velocity (U) in single perfused frog mesenteric capillaries (Int J Microcirc 1996;16(suppl 1):216). In these experiments the change in U was achieved by changing perfusion pressure. We have now examined whether the increase in P_{K^+} is determined by the changes in pressure or the changes in flow. To do this, P_{K^+} was first estimated at several U under conditions of free flow through a single vessel (control). The vessel was then partially occluded downstream from the second K^+ sensitive microelectrode and the vessel was reperfused over a range of U comparable to the control but at microvascular pressures 10–20 cm H_2O higher than under control conditions. Mean values for P_{K^+} ($\mu m \cdot s^{-1}$) were related to U by $P_{K^+} = 5.1 \pm 0.0038$ U during partial occlusion and by $P_{K^+} = 5.0 \pm 0.0050$ U under conditions of free flow. The positive correlation between P_{K^+} and U was mantained after the vessel was partially occluded and there was no suggestion that a higher perfusion pressure resulted in a higher P_{K^+} (n = 4). Thus the observed change in P_{K^+} appears to be attributable to 'flow' but not to 'pressure'.

In our standard protocol, P_{K^+} is measured from the fall in K^+ concentration, $[K^+]$, of a K^+ rich bolus (20 mmol·l^{-1}) of perfusate as it flows along a microvessel between two K^+ sensitive microelectrodes. As the flow rate increases, the mean $[K^+]$ between the electrodes is increased and there is a possibility that the increase in P_{K^+} with increased U reflects a relationship between P_{K^+} and $[K^+]$. To test this, we reversed the high and low $[K^+]$ in our protocol. The mesentery was perfused and superfused with 20 mmol $K^+ \cdot l^{-1}$ and P_{K^+} was measured from the rise in $[K^+]$ in a bolus of perfusate of low $[K^+]$ (2 mmol·l^{-1}). With this protocol the positive correlation between P_{K^+} and U was maintained and values of P_{K^+} were comparable to those obtained before (n = 8). Thus, the flow dependence of P_{K^+} in single vessels appears to be independent of both pressure and $[K^+]$ within the ranges investigated.

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Surface Presented CD31 and P-Selectin Transduce Signals That Regulate Neutrophil Migration

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The adhesive mechanisms underlying capture and immobilisation of circulating neutrophils in inflammed blood vessels have been well described. Factors such as chemotactic agents and adhesion molecules controlling subsequent migration over and through the endothelium are, however, poorly understood and little is known about their relative importance or mode of integration.

We examined the kinetics of neutrophil migration after formyl tripeptide or platelet-activating factor was perfused over neutrophils which were already rolling on P-selectin, presented either on immobilised platelets or in purified form, coated on glass capillaries. Upon activation neutrophils stopped rolling, spread and began to migrate, all of which were dependent on β2-integrin (CD11b/18). The rate of migration increased over a period of about 8 min and was directly modulated by both P-selectin and CD31. Antibody blockade of either CD31 or P-selectin on platelets resulted in a reduction in the velocity of migration, while simultaneous blockade of both reduced velocity further. Purified CD31 and P-selectin (but not a control adhesion molecule ICAM-1) increased migration velocity in a concentration-dependent and additive manner that reconstituted migratory behaviour observed on platelets.

These studies show that ligation of CD31 and/or P-selectin modifies the rate of integrin-supported neutrophil migration. This novel example of 'cross-talk' suggests that cell adhesion molecules might quite generally transduce accessory signals that act in a juxtacrine manner to modify migratory responses of cell responding to chemotactic signals. It seems, therefore, that in the microcirculation 'adhesion molecules' on endothelium may have dual and unexpected roles in modulating responses to activation as well as in mechanical support.

The Role of Endothelial Cell-Derived Reactive Oxygen Species in Hypoxia/Reperfusion-Induced Leukocyte Adhesion

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Increased leukocyte adhesion to small blood vessels is a prominent feature of reperfusion injury following ischemia. We have used cultured human umbilical vein endothelial cells to model the possible mechanisms involved. Cells were exposed to 6–18 h hypoxia (95% N_2:5% CO_2) followed by requilibration with 95% air:5% CO_2 for up to 24 h. Hypoxia alone, or hypoxia followed by 30 min reoxygenation, did not increase the surface expression of the adhesion molecule VCAM-1, but over longer periods of reoxygenation VCAM-1 expression was consistently enhanced, reaching levels 8 ± 2 (means ± SEM, 8 experiments) fold higher than basal levels after 24 h. Levels of ICAM-1, but not of E-selectin, were also increased. In separate experiments the addition of exogenous xanthine oxidase (XO: 10 mU/ml, with or without 100 μ*M* xanthine) significantly increased endothelial VCAM-1 expression at 24 h, indicating that reactive oxygen species (ROS) generated during reoxygenation may contribute to upregulation of cell adhesion molecules. The XO inhibitor SL301 (0–100 μ*M*) significantly reduced hypoxia/reoxygenation (HR)-induced VCAM-1 expression, implicating endogenous XO as a source of ROS, while inhibitors of NADPH oxidase (apoxyanin or acetosyringone, 100 μ*M*) had no effect. The antioxidants pyrrolidine- or diethyl-dithiocarbamate (10 μ*M*) also reduced HR-induced VCAM-1 expression. However, neither H_2O_2 (up to 100 μ*M*) nor menadione (0.01–1 μ*M*; which induced detectable superoxide release from endothelial cells) increased VACM-1 expression. We conclude that ROS

generated by HR are necessary but not sufficient to upregulate VCAM-1 expression, and that other as yet unknown transduction signals must be activated by HR.

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The Role of Nitric Oxide in Ischaemia-Reperfusion Injury to Tumour Microcirculation

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Using two murine tumour types of differing responsiveness to ischaemia-reperfusion we have investigated the role of nitric oxide (NO) in tumour microcirculatory control and response to I-R injury. Following I-R injury, relative tissue perfusion (measured 24 h after clamp removal) remained significantly less than controls and was correlated with reduced tumour cell survival. This evidence indicates vascular damage as a major determinant of I-R toxicity.

Since NO is a major agent controlling vascular tone, e.g. vessel dilatation and leukocyte adhesion, we investigated whether NO was involved in tumour response to I-R injury. The intrinsic capacity of tumours to produce NO was measured from in vitro production of nitrite and nitrate by tumour pieces. Elevated NO production was found in those tumours which were resistant to I-R, supporting the hypothesis that NO maintains vessel dilatation and assists in microcirculating flow. Ex vivo NO production was also found to be stimulated by increased oxygen tension; an important observation considering local variations in tissue oxygenation are known to occur within solid tumours. Reduction of plasma NO levels was achieved by intravenous injection of nitric oxide synthase (NOS) inhibitor, N^{ω}-nitro-*L*-arginine (20 mg/kg) during the initial phase of reperfusion. NOS inhibition resulted in greater cytotoxicity in both tumour types however injection of a NO donor (diethylamine-N-oxide) reduced the cytotoxicity of the I-R insult.

In conclusion, NO acts to reduce the vascular component of I-R injury in tumours. Intrinsic production of NO may be both tumour type and oxygen dependent and this may predict the therapeutic response to other anti-cancer therapies targeted against tumour microcirculation.

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L-Arginine Both Protects and Exacerbates Ethanol-Induced Rat Gastric Mucosal Injury

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Introduction: We have previously demonstrated that 60% ethanol (ETOH) increases macromolecular leakage and induces focal lesion formation in areas of permanent flow stasis within gastric mucosal vessels (Kalia et al, Int J Microcirc 1995;15:204). Nitric Oxide (NO may prevent lesion formation by inhibiting leakage. This study used fluorescent in vivo microscopy to investigate (i) whether *L*-arginine (NO precursor) affect ETOH induced injury (ii) the mechanism of any protection. *Methods:* Experiments were carried out on anaesthetised rats (hypnorm/diazepam) receiving intra-arterial (i.a) fluorescein isothiocyanate-bovine serum albumin (FITC-BSA; 0.2 ml/100 g), a marker for quantitating leakage. Animals then received 100, 300 or 500 mg/kg *L*-arginine (i.a.) alone or followed by 60% ETOH topically applied to the gastric mucosa (n = 6 for each group). Vessel diameter and leakage from post capillary venules (PCV) and capillaries were quantitated using image analysis. *Results:* 100 mg/kg *L*-arginine did not increase vessel diameter or prevent ETOH-induced lesion formation and leakage. Both 300 mg/kg and 500 mg/kg alone induced significant and sustained increases in PCV diameter after 15 ($p < 0.01$) and 5 min ($p < 0.001$) respectively. Lesion formation was prevented by 300 mg/kg *L*-arginine but 500 mg/kg resulted in haemorrhagic lesion formation throughout the exposed mucosa. Neither 300 mg/kg or 500 mg/kg prevented leakage following ETOH. *Conclusion:* 300 mg/kg *L*-arginine prevented lesion formation, without inhibiting leakage but possibly by increasing blood flow in dilated PCVs. In these studies macromolecular leakage and lesion formation appear independent.

Remodelling of the Submucosal Microvasculature in the Asthmatic Airways

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The aim of this study was to quantitate the changes that occur in the ultrastructure of the microvasculature of the submucosal plexus in the asthmatic airway. Bronchial biopsies were taken from the subcarina between the right middle and lower lobe during fibreoptic bronchoscopy. The tissues were fixed, embedded in epon and ultrathin sections of the bronchial mucosa cut perpendicular to the luminal epithelial cell surface for examination using transmission electron microscopy. The asthmatic bronchus showed a marked deposition of collagen below the epithelial basement membrane to give a total thickness of 9.8 ± 0.8 μm (mean ± SEM, n = 8) compared with 3.2 ± 0.4 μm (n = 3) in the healthy airways. There was a small but not significant increase in the number of vessels per unit area of tissue to a depth of 100 μm from the basal surface of the epithelium (199 ± 20 and 135 ± 29 vessels/mm^2 for asthmatic and control, respectively). The mean diameter of the asthmatic vessels (6.6 ± 0.5 μm) was not significantly different from that of vessels from healthy bronchus (5.8 ± 0.6 μm). The endothelium of asthmatic vessels was highly attenuated with up to 24 fenestrae per vessel profile concentrated within the part of the vessel wall facing the epithelium of the bronchial lumen. Fenestral density was significantly higher ($p < 0.04$) in the asthmatic vessels (15.1 ± 1.6 per μm^2 luminal endothelial cell surface) from those in control vessels (5.42 ± 2.5 per μm^2) exceeding values reported in the fenestrated vessels of the synovium and salivary gland. A number of interendothelial cell gaps of between 0.05 and 2 μm in diameter were also seen in vessels from all the asthmatic biopsies and in one control biopsy. These data suggest that there are long term changes in the submucosal microvascular plexus which may contribute to the pathogenesis of airways disease through changes in the bronchial mucosa, vascular proliferation and, most importantly, changes in microvascular permeability.

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Microcirculation, Capillary Supply and Fibre Type Changes in Skeletal Muscle of Rats Following Myocardial Infarction

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The cause of impaired performance in skeletal muscles of patients with heart failure following myocardial infarction (MI) is somewhat controversial. We showed that in rats with small infarcts (19% necrosis of left ventricular mass), diameters of small arterioles in extensor digitorum longus (EDL) muscles were less and vasodilation to adenosine and acetylcholine impaired, indicating deficiency in nitric oxide release (Thomas et al., Int J Microcirc 1996;16:207). Similar changes were seen in rats with larger infarcts (37%) but no heart failure. We investigated whether these microcirculatory changes are compensated for by improved capillary supply and oxidative capacity in EDL which would maintain muscle function before the onset of heart failure. Muscle isometric twitch peak tension and fatigue index, FI (under pentobarbitone anaesthesia), capillary supply (alkaline phosphatase staining) and proportions of oxidative and glycolytic fibres (SDH staining) were measured in EDL ten weeks after ligating the left descending coronary artery. Muscles in control/sham-operated animals and those with MI had similar peak tensions and fatigued to a similar extent (FI = 52 ± 2% and 51 ± 9% respectively, means ± SEM, both n = 5). Capillary/fibre ratio was 1.40 ± 0.06 in sham vs. 1.47 ± 0.03 and 1.49 ± 0.02 (n.s.) in rats with small and medium infarcts respectively but the latter had a higher proportion of oxidative fibres, 59.8 ± 3.7% vs 49.1 ± 2.4 in sham ($p < 0.05$). This modest adaptation in muscles, similar to that described in peripheral vascular diseases, might explain the preservation of normal muscle function despite impaired microcirculation.

Use of Fluorescence Microlymphography and Stereology to Quantify the Dermal Lymphatic Network

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Lymphatic capillaries of human skin can be studied by injecting fluorescein isothiocyanate (FITC)-dextran, Bollinger's fluorescence microlymphography method (Circulation 1981;64:1195–1200). We have adapted this method to study the forearm, using stereology to quantify the lymphatic network. Ethics Committee approval was given.

The normal arms of 17 subjects (8 male, 9 female, 20–73 years) were studied. 6% FITC-dextran was infused, using a 36G needle, into the upper dermis of the ventral forearm (skin temperature 33 ± 1 °C, mean ± SD). The site was monitored with a fluorescence microscope. Lymphangiograms printed from videotapes were analysed by line intersection (concentric circles every 1 cm from edge of dye depot on magnified image) to give radial lymphatic density (LD_r, $cm \cdot cm^{-2}$) and total vessel length (cm). Maximum lymphatic spread from the depot was measured (mm). LD_r peaked close to the edge of the depot, diminishing radially. The results (table) indicate greater density, total length and spread in men than in women. Preliminary findings (in men) suggest that the lower leg, a dependent region, has a higher dermal lymphatic density (peak LD_r 25.0 ± 6.1 $cm \cdot cm^{-2}$) than the forearm (19.6 ± 6.9 $cm \cdot cm^{-2}$, n = 5). Stereology has proved a valuable method for quantifying the dermal lymphatic network.

	Peak LD_r	Total lymphatic length	Spread
Men	14.8 ± 7.1	15.5 ± 8.8	9.2 ± 3.0
Women	10.1 ± 7.6	7.0 ± 8.8	5.8 ± 2.7
p	0.10	0.016	0.003

Unorthodox Angiogenesis under Physiological Circumstances

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Angiogenesis under pathological circumstances begins by disturbance of the endothelial basement membrane followed by abluminal endothelial migration and sprouting. Proliferation at the base of the sprouts follows later. During development, angiogenesis can occur by longitudinal splitting or by intussusceptive growth. We present data demonstrating capillary growth in adult skeletal muscle which does not confirm with the above sequence of events, where the structural response depends on where the mechanical stimulus acts. The alpha-1 blocker prazosin (a vasodilator which increases shear stress) increased C/F ratio without any disturbance of the basement membrane, endothelial cell proliferation or abluminal sprouting. Electron microscopic studies demonstrated intraluminal cytoplasmic processes, which sometimes divided capillaries into two. Long-term stretch, induced by extirpation of an agonist, increased sarcomere length by 20% without affecting blood flow, thus distorting capillaries by acting on the abluminal side. There was extensive abluminal sprouting, whith basement membrane breakage at the tip of the sprouts, while mitotic nuclei were seen. Muscles exposed to chronic electrical stimulation (which increases both blood flow and shear stress, and distorts the abluminal side of capillaries by activity of muscle fibres) displayed both luminal and abluminal sprouting, with occasional disturbance of the basement membrane. Proliferation of ECs was ween prior to capillary growth (Pearce et al, J Physiol 1995; 483:146P). Thus, similar degrees of angiogenesis can be achieved with or without initial breakage of the basement membrane, by luminal sprouting without prominent proliferation when the initial stimulus is acting from the luminal side, abluminal sprouting when it acts mainly from the abluminal side, or a combination of both with EC proliferation preceding sprouting.

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In vitro Microcarrier Bead Based Angiogenesis Assay

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An in vitro angiogenesis assay has been developed based on microcarrier bead using human microvascular endothelial cells (HMVEC) which allows the effects of growth factors and cytokines on to be quantified. The assay has been adapted from a previously published method using bovine endothelial cells (Nehls and Drenckhahn, Microvasc Res 1995;50:311–322) and has been optimised for human endothelial cells (EC's) to permit the study of anti-angiogenesis regimes.

HMVEC were isolated and cultured via the method of Hewett et al. (In vitro Cell Dev Biol 1993;29a). One 75 cm^2 tissue culture flask of passage 3–6 HMVEC were seeded onto 300 µl of packed microcarrier beads, prepared according to the manufacturers recommendations (Cytodex 3, Pharmacia), and agitated every 30 min for 6 h. 200 beads coated with HMVEC were washed 3 times in human endothelial serum free medium (SFM) and mixed with 2 ml of a 2 mg/ml fibrinogen solution (pH 7.6) containing 200 U/ml aprotonin, and placed in a 35 mm petridish. A fibrin clot was formed by the addition of 1 U of thrombin, at 37 °C for 30 min. After 24 h sprouts of EC's could be seen growing from the beads. By day 3 capillary-like tubes were observed and by day 6 these tubes had grown in length and cell division occurred. Tubes had a lumen, determined by sectioning, stained positive for von Willebrand factor and were multicellular. Tube lengths varied from 100 µm to 1,000 µm by day 6, with some branching. The assay was quantified by counting the number of capillary-like tubes greater than 150 µm in length radiating from 50 randomly selected microcarrier beads, and expressed as a tube:bead ratio. This microcarrier bead based angiogenesis assay is therefore relatively simple to perform and quantify.

Can Lower Peripheral Vascular Resistance in Working Muscle Be Maintained at Very Low Temperatures?

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The increase in slow muscle blood flow (MBF) during sustainable exercise of rainbow trout was maximal at 11 °C, and hence was lower following acclimatisation to both high and low seasonal temperatures (Taylor, Egginton and Taylor, J Exp Biol 1996;199:835–845). It is unclear to what extent species adapted to a continuous cold environment retain control of regional blood flow during activity. The Antarctic teleost *Notothenia coriiceps* (living at <0 °C) was therefore compared with trout (at 11 °C). MBF was measured in conscious fish using the radiolabeled microsphere method, with injection via dorsal aorta and withdrawal via caudal artery (Wilson and Egginton, J Exp Biol 1994;192:299–305). *Notothenia* had a lower cardiac output (11 vs. 30 ml/min/kg) although resting blood flow was similar (around 0.08 ml/min/g). Following maximal exercise there was a significant increase in specific blood flow to slow, but not fast muscle in both species. Interestingly, the functional hyperaemia was far greater in trout (111 vs. 3-fold increase in MBF), despite a greater relative increase in cardiac output in *Notothenia* (9- vs. 5-fold). This lower MBF may reflect less oxygen demand and/or increased capillary supply. Peripheral resistance was 2,429 and 6,482 dyn·sec/cm^5 for trout and *Notothenia* at rest, respectively. The increase in blood flow to slow muscle, and decease in peripheral resistance, during maximal exercise therefore appears to be better regulated in trout than in *Notothenia*. However, regional hyper- and hypoperfusion can occur within a single locomotory muscle during exercise in *Notothenia* suggesting that, unlike trout, adequate control over vascular tone can be maintained at very low temperatures.

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Skin Blood Flow Following Longitudinal Groin Incision

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Longitudinal groin incision for the exposure of the femoral artery in vascular bypass surgery may disrupt skin blood supply. The aim of this study was to investigate the hypothesis that such an incision results in a drop in blood flow and therefore haemoglobin saturation ($SatO_2$) across the wound.

Microvascular $SatO_2$ was measured with lightguide spectrophotometry (EMPHO II, BGT, Überlingen, Germany) in 21 patients undergoing femoro-popliteal or femoro-distal bypass procedures. A series of measurements were made in the groin, medial and lateral to the surface marking of the femoral artery. The mean $SatO_2$ on each side was calculated, and the contra-lateral groin was used as a control. The measurements were repeated at 2 and 7 days post-op.

The results are shown in the table below. $SatO_2$ in the skin of the operated groins was increased significantly from baseline at 2 days post-op ($p < 0.001$) and had begun to return to normal by day 7. The rise was more marked on the lateral side of the wound ($p < 0.001$) and consequently, post-op $SatO_2$ was significantly higher on the lateral side than on the medial ($p < 0.01$). By contrast, $SatO_2$ in the control groins fell steadily from baseline over the 7 day period ($p < 0.001$) and there was no difference between the medial and lateral sides. All wounds healed at 10 days.

These results suggest that skin blood flow is in a lateral to medial direction across the groin, and is disrupted by longitudinal incision. This may have some significance to the relatively high incidence of infection in such a wound.

	Operated		Control	
	Medial	Lateral	Medial	Lateral
Pre-op	24.1±11.6	23.1±12.2	26.6±12.2	23.8±11.1
2 days	33.3±11.1	45.4±11.5	17.1±7.9	15.4±7.7
7 days	23.6±14.1	30.0±15.8	13.3±9.3	10.7±8.6

Characterisation of Two Human Endothelial Cell Lines for Use in in-vitro Paracellular Permeability Studies

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Copious research on endothelial paracellular permeability has used primary endothelial cell culture. Given the limited supply of human primary-derived endothelial cells, these investigations could be furthered by the use of immortalised cell lines. This study demonstrates the usefulness of two human endothelial cell lines; ECV304 cells derived from primary human umbilical vein endothelial cells (HUVEC), and HMEC-1 cells derived from primary human dermal microvascular endothelial cells (HDMEC), and compares them with primary cultures of HUVEC.

The morphology of the cultures was assessed using light and electron microscopy. Both cell lines grew to confluence and formed multilayers, with cell clumping in some areas whilst the HUVECs grew as a monolayer. This may affect the paracellular permeability of these cell lines as the passage rate of macromolecules could feasibly be decreased.

Molecules implicated in the formation of paracellular junctions were visualised using immunofluorescence. The HMEC-1 cell line expressed Platelet Endothelial Cell Adhesion Molecule-1 and Vascular Endothelial Cadherin. Both localised at cell-cell borders with no staining seen at any free cell edges. This distribution is similar to that seen in HUVEC cultures. Positive staining for these important molecules was not observed in the ECV304 cell line. Additionally staining for von-Willebrand Factor, a marker of endothelial phenotype, in the HMEC-1 cells was punctate and located in the cytoplasm. This was comparable to that seen in the HUVECs. The ECV304 cells showed very weak staining.

These results imply that the ECV304 cell line may have limitations in permeability studies. However, the HMEC-1 cell line, by expressing the relevant adhesion molecules important for endothelial barrier function, may prove a more useful resource in studies on paracellular permeability.

Evidence that Serum Albumin but Not Plasma Albumin Increases Cerebral Microvascular Permeability in the Anaesthetized Rat

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Serum albumin increases intracellular calcium in human umbilical vein endothelial cells whereas plasma albumin has no effect (Fuentes et al, J Physiol 1996;491:10P) and it seems that lysophosphatidic acid (LPA) released from platelets and binding to albumin during blood clotting is the responsible factor. We have carried out experiments to test the idea that serum albumin and LPA can increase cerebral microvascular permeability. The permeability to Lucifer Yellow (P_{LY}) of single venular capillaries of rats (anaesthetized with pentobarbitone: 60 mg kg^{-1} i.p.) was measured from the rate of decrease of fluorescence in an occlusion experiment. When serum albumin was applied to either luminal or abluminal surfaces of a venular capillary, P_{LY} increased rapidly and reversibly. With abluminal application the half maximal effect was at 0.25 mg/ml, and of similar maximum magnitude to that of histamine. The effect was not observed when plasma albumin was applied. Plasma albumin to which LPA had been bound did, however, increase P_{LY}. Free LPA was also found to increase P_{LY} with half maximal effect at about 0.5 n*M*. When calcium was removed from the superfusing medium, addition of serum albumin resulted in only a transient increase in P_{LY}. These results suggest that events during blood clotting are likely to have important inflammatory consequences.

Crosslinking of Basement Membrane (Matrigel) Results in a Decease in Its Permeability to Both Glycated and Nonglycated Albumin

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The observed increase in capillary pressure and/or the development of advanced glycosylation end product (AGE) associated crosslinks in capillary basement membrane could explain the increased transcapillary flux of albumin seen in diabetes. Since it is not possible to isolate the effects of these two factors in vivo we have developed an in vitro model for the study of basement membrane permeability.

Basement membrane films formed from Matrigel (mouse sarcoma basement membrane) at the base of stirred pressure chambers were extensively crosslinked with dimethyl adipimidate, which reacts selectively with lysine ε-NH_2 groups. The permeability of native and crosslinked films to extensively glycated albumin (ALBg) and non glycated albumin (ALB) was measured at 6 different pressures within a physiological range (13–78 cm H_2O).

In comparison to native membranes, the permeability of crosslinked membranes to ALB was reduced by a similar amount at all pressures. For example at 63 cm H_2O protein flux (Js) was 3.02 ± 0.178 mg/cm^2/s across control membranes and only 2.32 ± 0.19 across crosslinked membranes. Such changes reached significance at only 3 pressures ($p < 0.05$). In comparison to ALB, the flux of ALBg across native membranes was significantly reduced at all pressures ($p < 0.05$); this effect became greater as pressure increased such that Js of ALBg was approximately half that of ALB at 63 cm H_2O. Finally, in comparison to the flux of ALB across native membranes, a combination of membrane crosslinking and albumin glycation resulted in approximately a four-fold decrease in Js at all pressures ($p < 0.05$). In all studies Js increased with increasing pressure.

In conclusion, these results do not support the concept that crosslinking (at least to the extent utilised in these experiments) can explain the increased transcapillary albumin flux observed in diabetes.

Non-Uniformity in the Permeability of the Endothelium around the Renal Bifurcation

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Early atherosclerotic lesions are distributed non-uniformly around the aorto-renal bifurcation, being most prevalent on the cranial side of the ostium and very rare within the renal artery itself. High resolution blood flow measurements suggest that blood flow patterns are quite different in these regions and so the endothelial cells are likely to experience quite different temporally and spatially varying shear stresses. To investigate whether shear influences endothelial permeability, we have measured the short-term uptake of lissamine-rhodamine-labelled albumin in young (2–2.5 kg) rabbits.

The protein circulated for 10–13 min in vivo before being washed out with saline and 20% formaldehyde. This procedure removed the protein from the vessel surface but precipitated it within the tissue. 10 μm thick longitudinal and transverse sections were cut and viewed in a fluorescence microscope with a scanning colour video camera. After digitization, the images were examined with an image processing package (Khoros) which enabled intensity measurements to be made in strips of tissue, 10 μm wide at various sites around the bifurcation. Within the time period of the experiment, negligible quantities of the protein had reached the middle of the wall and it was possible to determine how much had entered across the luminal surface.

The highest luminal uptake was seen in the aorta, downstream of the bifurcation (8.6 ± 3.7 arbitrary units) but was only slightly lower at the flow divider (5.6 ± 1.5). Lower values were seen opposite the flow divider (1.9 ± 0.7) and in upstream regions of the aorta (2.8 ± 1.4). Values in the renal artery itself were all less than 1.5 units. There is, therefore, marked non-uniformity of permeability in this region, although in these animals it only correlates in part with atherosclerosis susceptibility.

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A Study of the Inflammatory Response in Human Skin using Scanning Laser Doppler Imaging (SLDI)

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The aim of this study was to investigate agonist induced changes in blood flow in human skin using SLDI. Basal levels of cutaneous perfusion and changes in blood flux during the development of weal and flare responses in the volar surface of the forearm were measured during both the morning and the afternoon. Repeat images were collected every 30 s before and during the response to 10 μl of intradermal histamine (3 μ*M*) or bradykinin (1 μM). Measurements were made of flare and weal areas, mean and peak perfusion rates within the flare, and the time course of the development of the inflammatory response. The response to histamine and bradykinin showed similar time courses, reaching a steady state within 5 min. Perfusion rates within the flare were heterogeneous, with sites of increased perfusion (0.2–1.0 cm^2) lasting between 1 and 3 min. In the morning, maximum perfusion rates within the flares were not different although the fraction of the flare perfused at these rates was grater with histamine (11.5 ± 2.2%) than with bradykinin (5.1 ± 1.2%). At 10 min, the flare areas were: histamine 26.03 ± 0.98 cm^2 (mean ± SEM, n = 17), bradykinin 11.7 ± 0.17 cm^2 (n = 17) and saline 0.20 ± 0.03 cm^2 (n = 11). The weal area with histamine was 2.29 ± 0.66 cm^2 (n = 11) compared with 1.17 ± 0.13 cm^2 (n = 12) with bradykinin. In the afternoon basal blood flux increased four-fold and the response to histamine (3 μ*M*) by 39 ± 11%. Both flare area and perfusion rates within the flare decreased in a dose related fashion following intradermal injection of histamine (30–100 n*M*). Together these data reflect the differences in the cutaneous vascular response to histamine and bradykinin.

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Focal Changes of Permeability Found in Single Cerebral Microvessels (with Demonstration)

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We have previously reported on a method for localizing permeability changes in a single microvessel which uses a modification of the occlusion technique (Easton and Fraser, Int J Microcirc 1992;11: 452), and now we describe an improvement which is being used to investigate the relationship between leucocyte diapedesis and permeability changes in cerebral pial microvessels in the rat. The cerebral surface of the anaesthetized rat was exposed and a bolus of fluorescent dye (either Lucifer Yellow, 474 D, or rhodamine-labelled albumin, ca. 68 kD) was either injected directly into the selected venular capillary (diameter 10–27 mm) and trapped by lowering a microprobe, or introduced into the cerebral circulation via the perfusion pipette. Images were captured in a Pulsar frame-grabber board (Matrox, Canada) in a fast microcomputer. This arrangement allows floating point software (Image Hopper, Samsara, Dorking, UK) to be used for image integration, rationing and arithmetic. This improved accuracy over the previous technique which was restricted to 8-bit processing. This system has been tested in a series of experiments where arachidonic acid (1 m*M*) was focally applied to the abluminal surface of the capillary. Sequences of the processed images which indicate rapid changes in local dye loss will be shown.

The Restricted Areas of Small Transendothelial Cell Gaps

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Recently, using reconstruction made from serial sections we discovered that gaps induced by mild hyperthermia, high luminal pressure, histamine, calcium ionophore (A23187) or VEGF, in the endothelium of singly perfused frog and rat microvessels, passed through rather than between the cells (e.g. Neal and Michel, J Physiol 1996; 492.1:39–52). Reconstructed transcellular gaps were found associat-

ed with diaphragmed fenestrations and vacuoles. If a transcellular hole develops from an expanding fenestration or vacuole then cytoskeletal filaments may interfere with expansion restricting the size of holes through the cells. A Digit image analysis program was used to measure the areas of 102 reconstructed frog transcellular gaps to see if there were any size restrictions. Gap boundaries were traced to obtain the area exposed by endothelial cell retraction. Thirty five of the smallest gap areas showed a punctuated quantal frequency distribution. The quantal nature was explored by constructing a curve in a similar way to Boyd and Martin (J Physiol 1956;132:74–91) to see where 35 area values would occur in a quantal distribution. The smallest transcellular gaps or endothelial holes appear to be size restricted to multiples of a unit area. This size restriction occurs irrespective of the stimulus used to induce gap formation.

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The Cadherin-Catenin Complex in Normal and Histamine-Perfused Microvessels of the Human Term Placenta – A Confocal Study

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It has been shown that ex vivo perfusion of human placental microvessels with histamine results in increased endothelial permeability and redistribution of juctional VE-cadherin and non-junctional PECAM-1 (Leach et al, Microvasc Res 1995;50:323–337). In vitro studies have shown that the adherens junctional molecule VE-cadherin is linked to the actin cytoskeleton by peripheral cytoplasmic molecules including α- and β-catenin. Using confocal microscopical techniques we have investigated the distribution and association of VE-cadherin, catenins and PECAM-1 in normal and histamine-perfused microvessels. Term human placentae were perfused for 30 min with medium either with (n = 3) or without histamine (n = 3; 100 μ*M*) followed by perfusion fixation. An indirect double-labelling immunofluorescence technique (monoclonal + rabbit primary antibodies; FITC + TRITC conjugated secondary antibodies) was used to localise the adhesion molecules present in the endothelium prior to confocal analyses.

In control microvessels, VE-cadherin was visualised as (red) punctate staining along the paracellular clefts of endothelial cells. The linking molecules, α- and β-catenin (green), were co-localised with VE-cadherin as yellow spots. Optical rotation of the clefts showed that this co-localisation was present all along the cleft. β-Catenin was also seen as discrete staining peripheral to the abluminal surface. PECAM-1 was seen both in the clefts and on the luminal surface, appearing to be more diffuse along the clefts but in regions distinct from the catenins. In microvessels perfused with histamine, no VE-cadherin staining was observed. There was also no α- and β-catenin staining in the paracellular cleft. PECAM-1 staining was present on the luminal surface but absent from the paracellular clefts.

This work is a first demonstration of the presence of catenins in the human placenta. Histamine perfusion appears to affect endothelial cadherin-catenin co-localisation and PECAM-1 staining, implicating a regulatory role for these molecules in junctional permeability.

Effect of a Hyperosmotic Concentration of Glucose and Mannitol on Vascular Permeability in the Isolated Rat Heart

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The early effects of a high glucose concentration on vascular permeability and ultrastructure were compared with those obtained using a high mannitol concentration. The multiple indicator dilution method was employed to determine the PS products for ^{125}I-albumin and ^{57}Co-cyanocobalamin in hearts perfused at constant flow with well-oxygenated Krebs solution containing either 5 or 25 m*M* glucose or 5 m*M* glucose with 20 m*M* mannitol. The PS products, measured at 15 min intervals, for albumin and cyanocobalamin in hearts perfused with normal Krebs solution (5 m*M* glucose) were 0.2 ± 0.04 and 3.4 ± 0.8 ml min^{-1} g^{-1} (n = 8; mean ± SE) which remained stable throughout the 75 min perfusion period and 0.75 ± 0.14 ($p < 0.05$; n = 6) 15 min after perfusion with 25 m*M* glucose. These values were constant throughouth the perfusion period. PS values were 0.26 ± 0.04 ($p > 0.05$) and 11.1 ± 0.9 ($p < 0.05$; n = 6) 15 min. after perfusion with high mannitol concentrations which remained stable throughout. The effect of mannitol was associated with a 31 ± 2% reduction in aortic perfusion pressure which could be reversed in a concentration-related manner with L-NMMA. Electron microscopy of these hearts showed endothelial gaps (~ 1 μm) and an absence of lanthanum binding sites on the glycocalyx in hearts perfused with a high glucose concentration but not in those perfused with normal glucose or a high mannitol-containing solution. We conclude that perfusion with a high glucose concentration produced a fast and sustained increase in coronary microvascular albumin permeation and that the likely route of this effect is the endothelial gaps. The effects of glucose cannot be explained by hyperosmolarity. Furthermore, mannitol per se may cause vasodilatation.

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Effects of Propofol on the Microcirculation during Controlled Haemorrhage

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The effects of haemorrhage on the microcirculation during propofol anaesthesia is unknown, but propofol is thought to cause vasodilation of microcirculatory vessels (Holzman et al, Br J Anaesth 1995;75:452–456). This study aimed to investigate the possible beneficial effects of propofol on the compensatory mechanisms induced during haemorrhage and reinfusion. Rats (120–220 g) were anaesthetised with a sleep dose of propofol (20–30 mg/kg) and fentanyl (6–8 μg/kg) via the tail vein (iv) and maintained with an iv infusion of propofol (20–60 mg/kg/h) and fentanyl (20–30 μg/kg/h). Animals were ventilated with 30% oxygen, cardiovascular variables were monitored and the cremaster muscle prepared for in vivo microscopy (Brown et al, Br J Cancer 1994;69:706–710). Rats were allowed 30 min equilibration prior to study. In all groups (n = 15) vessel diameters, flow and macromolecular leak were recorded every

10 min for 120 min. During haemorrhage (n = 5) and haemorrhage-reinfusion (HR, n = 5), blood was removed (10% body volume) over 10 min. In the HR group (n = 5), 30 min after blood removal, saline and blood (2:1) was reinfused over 10 min.

Haemorrhage induced hypotension and tachycardia and resulted in either vasoconstriction of A1 vessels (n = 6) with vasodilation of smaller arterioles ($p < 0.05$), or no response in A1 (n = 4) vessels with vasoconstriction of small arterioles ($p < 0.05$). Deceased bloodflow was observed qualitatively in all vessels, but no leak was observed. Following reinfusion, vessel diameters and flow returned to pre-haemorrhage levels, but this was transient. Controls demonstrated vasomotion, with vessel diameters and leak remaining stable throughout the study, but control animals were not haemodynamically stable. Therefore propofol anaesthesia is not beneficial during haemorrhage and does not appear to be a suitable long-term anaesthetic in this model.

Immuno-Expression of Intracellular Adhesion Molecule ICAM-1 and White Blood Cell Adherence in Chronically Ischaemic Rat Muscles

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After ischaemia/reperfusion in skeletal muscle, antibodies to ICAM-1 reduce adherence of white blood cells (WBCs) to post-capillary venules (Ferrante et al, J Vasc Surg 1996;24:187–193) and restore the impaired perfusion of capillaries (Jerome et al, Am J Physiol 1994;266:H1316–H1321). We have studied whether there is a relationship between WBC adhesion an immuno-expression of ICAM-1 in chronically ischaemic muscles. Unilateral ischaemia of hind limb muscles was induced by ligation of one common iliac artery in adult male rats, and rolling and adhesion of WBCs observed directly by intravital microscopy (under pentobarbitone anaesthesia) in post-capillary venules (~ 20 μm diameter) in ischaemic (ISC) and contralateral (CL) extensor digitorum longus muscles 3 and 7 days later. Immuno-expression of ICAM-1 was evaluated as the number of vessels showing positive ICAM-1 antibody staining in two areas (total 0.5 mm^2) in frozen muscle sections. There was no ICAM-1 staining in control muscles or in those ischaemic for 3 days. After 7 days, ISC muscles (n = 4) had 2.5 ± 0.7 ICAM-1 positive vessels. WBC rolling and adhesion were also elevated in ISC muscles at 7 but not 3 days after ligation, suggesting a temporal link. However, in CL muscles, ICAM-1 stain was seen after both 3 (2.2 ± 1.1 vessels, n = 5) and 7 (2.0 ± 0.4) days, yet WBC adherence was not different from control in either group, although more rolling was observed in both. Although there is no evidence that ICAM-1 is involved in rolling of WBCs in vitro, its enhancement in CL muscle may reflect systemic effects of ischaemic trauma.

Elevated *D*-Glucose Alters Actions of Insulin on the *L*-Arginine-NO Pathway in Human Umbilical Vein Endothelial Cells from Gestational Diabetes

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Elevated *D*-glucose and human insulin induce the activity of system y^+ and nitric oxide (NO) synthase in human fetal endothelial cells (Sobrevia et al, J Physiol 1996;490:775–781). We now have examined the effects of elevated *D*-glucose on the action of insulin on the *L*-arginine-NO pathway in human umbilical vein endothelial cells from gestational diabetic pregnancies. Endothelial cells were exposed to either 5 or 25 m*M* *D*-glucose (0–48 h) in the absence or presence of human insulin (0–10 n*M*, 8 h). *L*-Arginine transport was increased in diabetic cells cultured in 5 m*M* *D*-glucose (V_{max} 9.0 ± 1.1 pmol (μg protein)$^{-1}$ min^{-1}, mean ± SEM., unpaired Student t test) or 25 m*M* *D*-glucose (V_{max} 9.5 ± 0.5) compared with non-diabetic cells in 5 m*M* *D*-glucose (V_{max} 4.9 ± 0.7, n = 4, $p < 0.03$). Unlike in non-diabetic endothelium, diabetes-stimulated *L*-arginine transport was not reversed by re-exposing endothelial cells to 5 m*M* *D*-glucose. Basal NO synthesis and *L*-[^{3}H]citrulline production were elevated ~ 2.5-fold in diabetic cells in 5 and 25 m*M* *D*-glucose, compared with non-diabetic cells in 5 m*M* *D*-glucose. Diabetic cells were hyperpolarized (E_m −79 ± 0.9 vs −67 ± 0.4 mV, n = 3, $p < 0.05$) and exhibited an increase in basal $[Ca^{2+}]_i$ (165 ± 25 vs 46 ± 12 n*M*, n = 43–49 cells, $p < 0.01$), but E_m and $[Ca^{2+}]_i$ were not altered by elevated *D*-glucose. Histamine (10 μ*M*, 5 min) stimulation of NO synthase was lower in diabetic endothelium in 25 m*M* *D*-glucose. Although insulin (1 n*M*) downregulated the elevated rates of *L*-arginine transport (via a protein synthesis dependent mechanism) and NO synthesis in diabetic cells cultured in 5 n*M* *D*-glucose, insulin has no such effect in cells cultured in 25 m*M* *D*-glucose. Elevated *D*-glucose seems to alter the sensitivity to human insulin in vitro in fetal endothelial cells obtained from gestational diabetic pregnancies.

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Vascular Effects of Nitric Oxide Synthase versus Haemoxygenase Inhibition in the Rat P22 Carcinosarcoma

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Ex vivo perfusions of 'tissue-isolated' tumours were used to measure changes in tumour vascular resistance induced by the nitric oxide snythase (NOS) inhibitor, N^{ω}-nitro-*L*-arginine (*L*-NNA) and the haemoxygenase (HO) inhibitor zinc protoporphyrin IX (Zn-PP). *L*-NNA caused a dose-dependent increase in tumour vascular resistance which was partially reversed by *L*-arginine. At 50 μ*M*, *L*-NNA increased tumour vascular resistance to >400% in 80% of prepara-

tions. HO activity in the P22 tumours was found to be high (1,500 pmol bilirubin/mg cell protein/h) and could be stimulated further by administration of hemin or bacterial lipopolysaccharide. HO inhibition by ZnPP produced tumour vasoconstriction but this effect was non-specific since copper protoporphyrin IX (CuPP), which does not directly inhibit HO, had a very similar effect to ZnPP.

Effects of *L*-NNA and ZnPP on blood flow to tumour vs. normal tissue were investigated in the whole animal using a radiotracer technique. *L*-NNA caused a dose-dependent decrease in tumour blood flow, reaching 45% of control, which was reversed by 200 mg/kg *L*-arginine. Of the normal tissues, only the effect in the skeletal muscle approached that in the tumour. ZnPP (30 mg/kg) decreased tumour blood flow to 60% of control. However, this effect was nonspecific since CuPP had a very similar effect. Interestingly, both ZnPP and CuPP caused a significant increase in blood flow to skeletal muscle which is opposite to the effect observed for NOS inhibition.

In conclusion, direct vascular effects of *L*-NNA and ZnPP in ex vivo perfused tumours were similar to those observed with systemic administration in the whole animal. NO production by tumours appears to be an important factor in maintenance of tumour blood flow. CO production via HO is less important. NOS inhibition has potential for enhancing therapeutic regimes which would benefit from a selective reduction in tumour blood flow.

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Transport of *L*-Arginine in Red Blood Cells from Septic Patients

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Sepsis is a pathological condition in which, in common with uraemia and heart failure, there is a disturbance of the *L*-arginine-nitric oxide signalling pathway (Knowles and Moncada, Biochem J 1994; 298:249–258). This lead us to investigate the plasma profile and transport of *L*-arginine in red blood cells from septic patients. We have shown previously that *L*-arginine transport is increased in red blood cells from uraemic and heart failure patients, whilst plasma concentrations of *L*-arginine are reduced and levels of monomethyl-*L*-arginine increased (Hanssen et al, J Physiol 1996;494:112).

In the present study, we examined *L*-arginine transport in red blood cells from 3 septic patients and 5 controls and compared the plasma amino acid profiles in 12 controls and 20 septic patients. Transport of *L*-arginine via system y^+ was increased in septic patients ($438 \pm 58\ \mu M \cdot l \cdot h^{-1}$) compared to controls ($281 \pm 69\ \mu M \cdot l \cdot h^{-1}$, $p = 0.036$, Student's t test). The amino acid plasma concentrations in patients with septic shock were generally significantly lower compared to controls. *L*-arginine was significantly reduced in septic patients ($36 \pm 6\ \mu M$) compared to controls ($125 \pm 5.5\ \mu M$). *L*-NMMA was increased in septic patients ($3 \pm 0.7\ \mu M$) compared to nondetectable levels in controls. Our results confirm that *L*-arginine-nitric oxide signal transduction pathway is impaired in sepsis, and suggest that red cells may be a useful tool to study the transport disturbances of *L*-arginine associated with this syndrome.

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Angiogenesis in Atherosclerosis: Association between Circulating Endothelial Cell Stimulating Angiogenesis Factor (ESAF) Vascular Endothelial Cell Factor (VEGF), von Willebrand Factor (VWF) and E-Selectin

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Endothelial cell injury is an important early step in the pathogenesis of atherosclerosis and there is evidence of endothelial cell dysfunction in the risk factors for this disease. Such dysfunction or injury inevitably must lead to proportional increase in repair if the intima is to avoid being de-endothelialised. The current study was designed to determine the levels of circulating factors such as ESAF, VEGF, soluble E-selectin and vWF which might reflect endothelial cell growth, regeneration, or damage in patients with peripheral vascular disease (PVD) and patients with ischaemic heart disease (IHD). Any meaningful relationship between the levels of these factors might contribute to the understanding of angiogenesis in these conditions.

	Healthy Controls	IHD Patients	PVD Patients
vWF(IU/dl)	102 ± 17	133 ± 30*	140 ± 35*
E-selectin (ng/ml)	51 ± 17	57 ± 20	53 ± 22
VEGF (ng/ml)	1.5	8.0*	5.0*
ESAF (U/ml)	9.4	19.6*	17.1*

* raised ($p < 0.01$) relative to healthy controls, but not between patient groups.

Twenty four subjects (seven women) with PVD and in whom atherosclerosis was confirmed by Doppler or angiography (stenosis >70% or occlusion and who were asymptomatic for IHD) were recruited. Twenty five patients (five women) had blood samples withdrawn at least six weeks after a confirmed myocardial infarction.

Levels of ESAF, vWF and VEGF were all significantly raised ($p < 0.01$) in both groups of patients with no difference between the two groups. Soluble E-selectin however was weakly raised but only in patients with IHD. Increased levels of VEGF and ESAF in patients with long standing IHD and PVD indicate ongoing angiogenesis. Whether this occurs in the endothelium of large arteries or in the microvessels of the vasa vasorum or in the capillary beds is uncertain. However these preliminary studies appear to warrant further studies of the the roles of VEGF and ESAF in atherosclerosis.

Lesional Levels of Endothelial Cell Stimulating Angiogenesis Factor (ESAF) and VEGF Are Elevated in Psoriasis

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Local and controlled dissolution of the capillary basement membrane is a prerequisite for the initiation of the angiogenic process. Endothelial cell stimulating angiogenesis factor (ESAF), a low molecular weight, non enzymic, non protein, specific for microvascular endothelial cells, is able to activate three major neutral promatrix metalloproteinases including progelatinase-A. Levels of ESAF are elevated in patients with severe proliferative diabetic retinopathy where extensive neovascularisation is occurring. VEGF is a protein angiogenic factor like ESAF specific for microvascular endothelial cells. Since expansion of the dermal microvasculature, due partly to angiogenesis, is an important feature in psoriasis, we measured ESAF and VEGF levels in lesional and nonlesional skin from patients with psoriasis. Fifteen patients (9 males, 6 females; age range 18–72), with untreated chronic plaque psoriasis were enrolled into the study. The severity of psoriasis was assessed using the psoriasis area and severity index (PASI) (range 10–34.8,). Six mm punch biopsies were taken from lesional and non-lesional skin, snap frozen and stored at −70 °C prior to use. Tissue levels of ESAF were assayed utilising its ability to activate latent collagenase. VEGF levels were measured using a quantikine kit. Levels of ESAF and VEGF were significantly elevated in lesional skin: 13.72 ± 8.7 and 28.12 ± 17.6 U/mg respectively as compared to uninvolved nonlesional skin of psoriatics: 5.27 ± 2.9 U/mg; $p = 0.001$, and 8.8 ± 5.3 U/mg; $p < 0.001$, respectively. Both ESAF and VEGF tissue levels were significantly related to PASI scores: $r = 0.60$ and 0.89 respectively. Thus it appears that ESAF and VEGF levels are elevated in plaques of patients with chronic plaque psoriasis, where microvascular changes are thought to play a crucial role in pathogenesis. Modulation of these biological factors may be a future therapeutic strategy in the treatment of this disease.