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Topical Review

Surviving in a Matrix: Membrane Transport in Articular Chondrocytes

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Introduction

The separation of the extracellular milieu from the cytosol is especially important for articular chondrocytes, given the atypical and arguably challenging environment that these cells inhabit [77, 110, 117]. Chondrocytes, like all other cells, must possess effective membrane transport systems to minimize changes of cellular composition in the face of fluctuating surroundings [53, 82, 108]. They must also scavenge adequate precursor molecules for matrix macromolecule synthesis, a task hindered by their remoteness from the vasculature [62, 110].

The realization in the last decade that characterization of the cellular physiology of chondrocytes will further our understanding of cartilage pathologies such as osteoarthritis has led to increasing interest in the field of chondrocyte membrane transport [2, 51, 53, 55, 82, 132]. In this short, and necessarily selective, review we will consider the challenges faced by chondrocytes in articular cartilage, the ways in which these cells control their intracellular composition, and the role of membrane transporters in maintaining matrix integrity.

The Extracellular Matrix Presents Challenges to the Chondrocyte

Although chondrocytes occupy less than 1% of articular cartilage, they are responsible for maintaining the integrity of the extracellular matrix by balancing macromolecular synthesis with breakdown [110]. The matrix that surrounds them confers a mechanically resilient surface to the articulating bones within joints, and comprises collagens (principally collagen II), other noncollagenous proteins, and proteoglycans (Fig. 1) [88]. There have been many surveys of the biochemistry of articular cartilage [for example, 14, 26, 58, 88] and a detailed consideration of these individual components is beyond the scope of this review. Here, it is sufficient to note that the hydrated proteoglycans confer the matrix with resistance to compression, and are constrained by the collagen fibrillar meshwork (the so-called 'string and balloon' model) [8, 9, 68, 113].

The proteoglycan structure, with highly sulfated glycosaminoglycan (GAG) side chains, underlies the compressive stiffness of the cartilage matrix. The high numbers of fixed negative charges on the GAGs attract free cations and exclude free anions from the matrix. With cation accumulation, water is osmotically imbibed, resulting in a hydrated matrix which has a raised osmolarity and lowered pH in comparison with other extracellular environments (Table 1) [8, 9, 14, 68, 76, 113, 117].

Two further, related issues compound the challenges facing the chondrocyte. First, the avascularity of articular cartilage means that movement of hormones, cytokines, nutrients and metabolites between the blood and the cells is across large distances and along steep gradients [110]. As a consequence, chondrocytes experience low partial pressures of oxygen, undergo predominantly anaerobic glycolysis and must endure high concentrations of the lactic acid they produce (and hence an acidic extracellular pH) [53, 74, 110].

Second, mechanical loading during routine activi-

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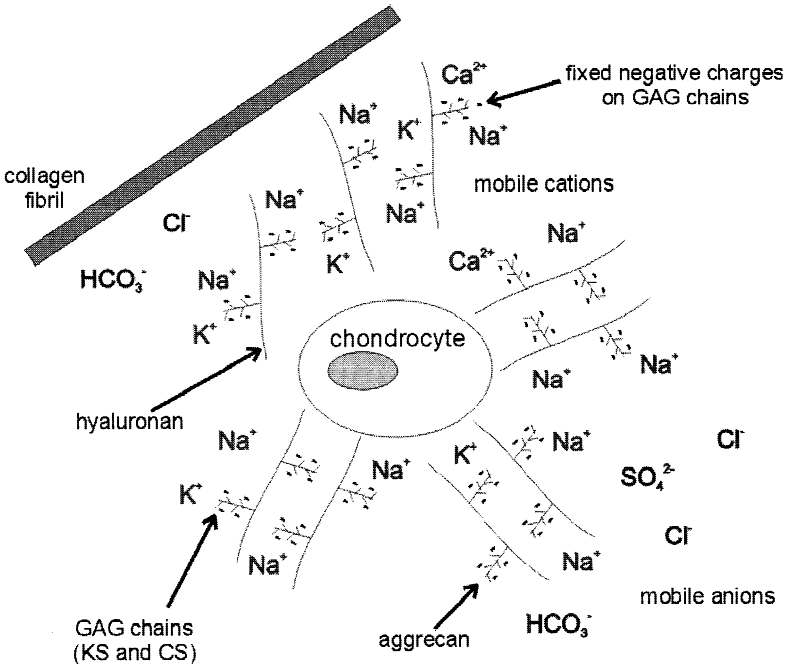

Fig. 1. The extracellular environment of articular chondrocytes. The high numbers of fixed negative charges on the keratan sulfate (KS) and chondroitin sulfate (CS) of glycosaminoglycan (GAG) chains attracts mobile cations and excludes mobile anions from the extracellular matrix. Adapted from [117].

ties, such as walking, exposes chondrocytes to profound fluctuations in their physicochemical environment [87, 117]. The processes underlying these changes are to some extent interrelated, making their identification and characterization complex. Nonetheless, a number of dynamic and static consequences of loading can be listed. On joint loading hydrostatic pressure within the matrix is raised, tissue and cell deformation ensues and fluid flows arise as a consequence of the hydraulic pressure gradients that are established. On removal of the load, pressure falls and the matrix regains its steady-state conformation. If loading is maintained, however, these dynamic components are followed by slower osmotic consequences. Hence, static loading results in expression from the matrix of interstitial fluid along the pressure gradients, raising the GAG fixed negative charge density, the concentrations of extracellular cations and osmolarity. This fluid expression will continue until hydrostatic and osmotic pressures balance within the matrix, or until the load is removed and fluid can be imbibed [47, 49, 76, 117]. While the proportion of total cartilage fluid expressed during a walking cycle is low [34], the incomplete recovery of fluid with repeated cycles means that the amount of fluid lost may become significant. Studies suggest that articular cartilage may lose up to 5% of the interstitial fluid during normal walking cycles [51, 117].

Such a difficult environment presents significant challenges to the chondrocyte. Effective homeostasis to counter the chronic hyperosmotic and acidic conditions must occur, while transport processes to supply scarce substrates for the anabolic processes in which the cell is engaged and remove the products of this metabolism will be required. Furthermore, the changes in the extracellular environment of the matrix associated with mechanical load must be effectively countered. Intracellular cation (Na^+, K^+, Ca^{2+}, H^+) concentrations fluctuate with load, in response to altered electrochemical gradients for each ion across the plasma membrane and altered activity of membrane transport pathways for these ions. Aside from mechanically-induced changes in membrane permeability associated with deformation, other variables such as pressure, osmolarity and pH are all well-recognized regulators of membrane transport systems in other cell types [22, 36, 54, 72, 100].

Despite the challenges presented by their extracellular environment, chondrocytes can prosper within the matrix. Their survival is fundamental to the maintenance of cartilage integrity, and the sections which follow will consider the homeostatic mechanisms by which they do so.

Studies of Chondrocyte Physiology are Complicated by the Surrounding Matrix

The extracellular matrix not only provides challenges for chondrocytes, but also for scientists seeking to study the cellular physiology of these cells. The use of well-established techniques of radiotracer fluxes, fluorescence imaging and electrophysiological measurements is at best seriously complicated and at worst impossible for cells *in situ* in matrix. Recent advances using confocal microscopy [37] have allowed some limited fluoroprobe

Table 1. The composition of articular chondrocytes and of their extracellular matrix

	Cytoplasm	Matrix	Serum/synovium
Na^+ (mM)	40	240–350	140
K^+ (mM)	120–140	7–12	5
Ca^{2+} (mM)	8×10^{-5}	6–15	1.5
Cl^- (mM)	60–90	60–100	140
HCO_3^- (mM)	20	15	23
SO_4^{2-} (mM)	0.17	0.30	0.81
pH	7.1	6.6–6.9	7.4
Osmolarity (mOsm)		350–450	300

For comparison, the composition of serum and synovial fluid is also tabulated. Table adapted from values cited by [12, 117]. Other values R.J. Wilkins (*unpublished observations*).

studies of chondrocyte composition and volume for cells in intact cartilage slices, but most of the information concerning chondrocyte membrane transport has derived from experiments using isolated cells. There are drawbacks to such experiments, notably the harsh enzymatic digestion [40] to which the cells are subjected (reported to destroy cell membrane proteins [41]), and the arguably unphysiological environment [53, 120] of the culture media into which cells are liberated during digestion. As Table 1 shows, isolation media are relatively lacking in cations whereas there is an excess of anions, and osmolarity is lowered. To some extent, these disparities can be minimized by supplementation of the extracellular medium so that it more accurately reflects the composition of the matrix [for example, 55]. Such an approach cannot, however, entirely reproduce the environment of chondrocytes *in situ*, and in any case there is evidence that some chondrocyte parameters, such as regulatory volume responses, reset to match the environment of the isolation medium [51]. The instability of the chondrocyte phenotype under culture conditions is an additional problem [4], and to overcome the laborious digestion and subsequent dedifferentiation of the cells, some workers have turned instead to immortalized cell lines to study chondrocyte cellular physiology [11]. Undoubtedly these cells offer an easily-obtained, stable preparation, although comparison with primary cells suggests that their properties reflect only an approximation of those of true chondrocytes. Furthermore, chondrocytes cultured to monolayer confluency may not accurately reflect *in situ* conditions; the appearance of gap junctions in these cells for example [27, 44] suggests that their usefulness is questionable.

Nevertheless, a combination of studies using intact cartilage slices, isolated primary chondrocytes and cultured cell lines has provided some basic information about chondrocyte intracellular composition (Table 1). It is worth noting the additional descriptions of the properties of growth plate cells and intervertebral disc cells. Although there is value in comparing the properties of these cells with articular chondrocytes, they are essentially different cells and a single 'cartilage cell' does not exist. We shall therefore focus almost exclusively on articular chondrocytes in this review.

In keeping with a cell inhabiting a cation-rich, anion-poor environment, intracellular $[Na^+]$ and $[K^+]$ are raised [120] in comparison with other cell types such as erythrocytes [107] (Table 1), although values reported for intracellular pH and $[Ca^{2+}]$ are little different from those commonly observed [11, 12, 132]. Chondrocyte membrane potential has been determined in a variety of studies, with values between −44 mV and −15 mV measured [39, 139, 140]. The magnitude of the challenges presented to chondrocytes has encouraged recent characterization of their membrane transport processes, and although the available information is far from complete, a picture is emerging of a cell equipped to contend with its extracellular environment (Table 2).

We shall now turn our attention to the systems by which chondrocytes control cellular volume, pH and $[Ca^{2+}]$—three parameters which are highly relevant in the context of the extracellular environment experienced by these cells—and then consider the membrane transport processes for metabolite movement between the cell and the matrix which have been characterized.

Chondrocytes Possess Volume Regulatory Transporters

Chondrocyte cell volume, as for other cell types, will be determined by a pump-leak model, a double Donnan equilibrium existing between the intracellular compartment and the matrix [110]. The effective exclusion of Na^+ ions from the cell is achieved by the activity of the Na^+-K^+ ATPase and volume is maintained by altered balance of leaks and pumps to hold cell water constant. Unsurprisingly, given the elevated $[Na^+]$ of their surroundings, chondrocytes have been reported to possess high Na^+-K^+ ATPase activity, with expression and functional activity upregulated in response to raised extracellular $[Na^+]$ [81]. Furthermore, application of physiological levels of hydrostatic pressure is reported to inhibit Na^+-K^+ ATPase activity in isolated bovine chondrocytes [52].

There have been reports that bovine and human chondrocytes express multiple isoforms of the subunit proteins of the ATPase (α1, 2, 3; β1, 2, 3) [80, 115]. In other cells there have been elegant expositions considering the consequences of multiple isoform expression [109]; in chondrocytes, however, the functional relevance of potentially nine ATPase variants has yet to be elucidated. Nevertheless, it has been suggested that maintenance of a low $[Na^+]_i$:$[K^+]_i$ ratio is vital to maintain chondrocyte viability [115], and it is certainly the

Table 2. Membrane transport pathways identified in articular chondrocytes

System	Method employed Cell type tested					Proposed function	References
	Flux	Fluorescence	Immunoblot	PCR	Electrophysiology		
Na^+-K^+ ATPase	Bovine		Bovine; Human	Bovine		Maintenance of Na^+ : K^+ ratio	55, 79, 80, 81
						Maintenance of gradients	115
Na^+-K^+-$2Cl^-$ symport (NKCC)	Bovine		Human			Regulatory volume increase	55, 116
$Na^+ \times H^+$ antiport (NHE)		Bovine; Avian; Human	Bovine; Human	Bovine; Human		pH_i regulation	11, 28, 53, 116, 131, 132, 133, 136, 137
H^+ ATPase		Human; Avian				H^+ extrusion	11, 28
	Na^+-$2HCO_3^-$ × Cl^- carrier	Avian				pH_i regulation	28
Ca^{2+}-ATPase					Avian	$[Ca^{2+}]_i$ regulation	144
$Na^+ \times Ca^{2+}$ antiport		Porcine				$[Ca^{2+}]_i$ regulation	96
Anion exchanger (AE)	Bovine	Avian	Bovine; Human	Bovine; Human		pH_i regulation $SO_4^{2-} \times OH^-$ antiport? Zn^{2+} uptake?	25, 28, 43, 127, 128
SAT1	Bovine; Rodent			Bovine; Rodent; Human		SO_4^{2-} uptake?	24, 84, 85
DTDST				Bovine; Human; Rodent		SO_4^{2-} uptake?	24, 102
System A	Bovine					Na^+-dependent proline, glycine, and glutamine uptake	2, 19
System ASC (ASCT)	Bovine					Na^+-dependent proline, glycine, and glutamine uptake	2, 19
System GLY (GLYT)	Bovine			Bovine		Na^+-dependent, Cl^--dependent glycine uptake	2
System N	Bovine					Na^+-dependent glutamine uptake	2
System L (LAT) ?b^{0+} (NBAT)	Bovine					Na^+-independent leucine and tryptophan uptake	2, 19
Monocarboxylate carrier (MCT)		Bovine	Bovine; Human			Lactic acid extrusion	135
Stretch-activated *g*cation		Bovine; Rodent; Porcine			Ovine; Human	Mechanotransduction?	44, 63, 139, 140, 142, 143
Calcium-activated *g*K^+					Equine; Ovine; Porcine; Human	Regulatory volume decrease?	45, 46, 123, 139, 140
Ca^{2+} channels		Porcine			Porcine; Human	Signaling?	45, 64, 139
Na^+ channels			Human		Ovine	Mechanotransduction? Maintenance of cell volume?	116, 140
Volume-sensitive organic osmolyte anion channel	Bovine					Regulatory volume decrease	51

A variety of systems mediating exchange of solutes between the extracellular matrix and the cytoplasm have been characterized. These processes confer regulatory capacity for cellular volume, pH and Ca^{2+} concentration, and permit the uptake and extrusion of metabolites. *See* text for discussion of specific transport pathways.

case that high cytoplasmic [K^+] facilitates the activity of many enzymes in other cell types [70].

Disturbances to chondrocyte volume occur — unlike those experienced by most other cells in the body — from anisosmotic challenges. Fluid expression will raise extracellular osmolarity by up to 20mOsm [117] although the onset of this osmotic shock will be gradual (seconds to minutes), unlike the step changes imposed to study volume regulation in many cell types. There is now evidence that the rate of change of osmolarity can dictate the responses activated by the cell [42] and this should be borne in mind given the nature of the osmotic shocks experienced by chondrocytes *in situ.* Notwithstanding this question, *in vitro* flux studies show that the bumetanide-inhibitable Na^+-K^+-$2Cl^-$ cotransporter can perform pseudoregulatory volume increases (pseudo-RVI) in bovine chondrocytes, while there are reports of expression of the NKCC1 isoform of the cotransporter in human chondrocytes [116]. The activity of this system is reduced by hydrostatic pressures equivalent to those associated with joint loading (up to 150ATA) [52], although to date there is no clear molecular explanation for the effects of pressure on either this cotransporter or on the Na^+-K^+ ATPase.

Whether activity of the cotransporter can effectively minimize changes of chondrocyte volume *in situ* is unclear; confocal microscope measurements of chondrocyte volume in cartilage slices show that the cells perform RVI only following prior swelling and regulatory volume decrease (RVD) suggesting that under physiological conditions they behave as osmometers [37]. Similarly, chondrocytes in intact matrix slices that have been subjected to static loads are observed to have reduced cellular volume in comparison with those from unloaded tissue [17]. The absence of RVI *in situ* is not uncommon, and for other cell types has been ascribed to unfavorable Cl^- gradients [61, 129]. It is possible, therefore, that the low levels of Cl^- ions in the extracellular matrix consign chondrocytes to remain shrunken for the duration of static loading.

Raised extracellular osmolarity has also been shown to activate $Na^+ \times H^+$ exchange in chondrocytes [141]. In other cell types the amiloride-sensitive $Na^+ \times H^+$ exchange operates in concert with $Cl^- \times HCO_3^-$ exchange to mediate Na^+Cl^- uptake [60]. There is no evidence that such a system plays a part in RVI processes in chondrocytes [53], and there are reasons to discount such a possibility (*see below*).

The response to reduced extracellular osmolarity has also been characterized in chondrocytes. In common with all animal cells, pathways have been identified by which chondrocytes can offload osmolytes if swollen to effect RVD [51, 55]. Since *in situ* chondrocytes appear to behave as osmometers, these pathways are probably not implicated in restoration of cell volume in the aftermath of static loading. Nevertheless, they may be activated in chondrocytes which have been isolated into media in which the extracellular osmolarity is lower than that of the matrix, exemplifying the complexities of working with isolated cells. It is worth noting that RVD systems may have a significant role during the onset of osteoarthritis, where loss of proteoglycan decreases extracellular osmolarity and subsequent cell swelling occurs [51].

Flux studies reveal that the K^+-Cl^- cotransporter does not seem to be important functionally in the chondrocyte RVD response [55]. Instead, RVD can be effected by channel-mediated efflux of K^+ and Cl^- ions. The efflux of both ions is tamoxifen-sensitive, and this route also appears to mediate the efflux of organic osmolytes, similar to the volume-sensitive organic osmolyte anion channel (VSOAC) described in other cell types [51, 111]. Although taurine is considered the classical organic substrate for VSOAC, there is persuasive evidence that chondrocytes accumulate betaine, and lose this compound on swelling [29, 51, 121]. The threshold for activation of osmolyte efflux from bovine chondrocytes through volume-sensitive channels has been shown to adapt to prevailing osmotic conditions. Hence, *in vitro* the set-point for activation is found to be close to the osmolarity experienced during cell preparation [51].

$Na^+ \times H^+$ Exchange is an Important Determinant of Chondrocyte pH

The regulation of pH_i has been characterized *in vitro* for several types of cartilage cells, including bovine and human articular chondrocytes, avian growth plate chondrocytes and bovine intervertebral disc cells [11, 28, 53, 98]. Experiments show that the acidic extracellular environment (approximately pH 6.8) of chondrocytes promotes the inward leak of H^+ ions, so chondrocytes will be subjected to chronic acid loading of their cytoplasm [134]. Acid extrusion to counteract this inward leak will therefore be vital if pH_i is to be held steady. In addition to acid extrusion by a bafilomycin-sensitive H^+-ATPase, pH_i has been reported to be tightly regulated by the amiloride-sensitive $Na^+ \times H^+$ exchanger [11, 28, 53, 98].

That this system should play a dominant role in the regulation of pH_i in chondrocytes is not surprising, given its ubiquity in mammalian cells and the steep inward gradient of Na^+ ions which can energize H^+ extrusion. The exclusion of anions from the extracellular matrix might similarly suggest that HCO_3^--dependent systems have little functional role in chondrocyte pH_i regulation, and to some extent this appears to be true. There is some variability in the degree to which chondrocyte pH_i regulation is HCO_3^--dependent, most notably for bovine chondrocytes which show minimal HCO_3^--dependent pH_i regulation [133]. In contrast, a Na^+ and HCO_3^--

dependent component similar to that reported in many other cells including avian growth plate chondrocytes [28] has been detected in human chondrocytes [11]. Regardless of these differences, given the K_m values for HCO_3^- ions of these systems in other cell types [6], it seems that the low pH, HCO_3^--poor environment of the extracellular matrix would in any case minimize their activity in chondrocytes, or suggest a novel, adapted isoform.

The failure to detect any functionally significant HCO_3^--dependent component of pH_i regulation in bovine articular chondrocytes is puzzling, especially given that expression of the anion exchange isoform AE2 can be demonstrated in these cells [43]. HCO_3^- uptake on simple anion exchange is often restricted by the Cl^- gradient [122], and it may be that HCO_3^--dependent systems in chondrocytes are constrained by the Cl^- transmembrane distribution in a similar fashion to that already outlined for the Na^+-K^+-$2Cl^-$ cotransporter. An alternative explanation for these findings might lie in minimal carbonic anhydrase activity since the uncatalyzed hydration of CO_2 has been reported to occur at a rate some 600 times slower than the enzyme-catalyzed reaction [20]. In accord with this, it has been reported that expression of carbonic anhydrase in chondrocytes is very weak [35].

The central role implicated for $Na^+ \times H^+$ exchange in the regulation of cartilage cell pH_i has understandably meant that most attention has focused on the properties of this transporter in chondrocytes. Hence, functional studies show that acid extrusion mediated by $Na^+ \times H^+$ exchange is sensitive to mechanical load. We have already noted the activation of $Na^+ \times H^+$ exchange by hyperosmotic shock [141]. Characterization of this response indicates, in common with other cell types in which osmotic sensitivity of the antiporter has been described, the involvement of phosphorylation reactions, and more particularly the activation of myosin light chain kinase [141]. Although the value of $Na^+ \times H^+$ exchange activation is not apparent with regard to volume regulation, it may provide a mechanism by which the chondrocyte can protect cytoplasmic pH against the extracellular acidification predicted to arise on joint loading.

In chondrocytes, levels of hydrostatic pressure comparable to those experienced upon joint loading have also been shown to modify acid extrusion. Exposure to hydrostatic pressures of 100–300 ATA are found to stimulate acid extrusion by $Na^+ \times H^+$ exchange; phosphorylation processes are again involved in this response, although in this case there is no indication of a role for myosin light chain kinase [10]. The observation that cAMP production is elevated in chondrocytes [7] upon exposure to hydrostatic pressure suggests instead that classical adenylate cyclase/protein kinase A (PKA) pathways may be involved.

In addition, studies characterizing the molecular identity of the $Na^+ \times H^+$ exchanger in chondrocytes suggest that while the 'housekeeper' NHE1 is the dominant isoform expressed by these cells, other isoforms may also be present. Hence NHE3, more typically an epithelial isoform, has been detected in bovine cells [136, 137], and both NHE2 and NHE3 are declared to be present in human cells [116]. No correlation of expression with function has been performed in human cells, and the reasons for multiple NHE isoforms in these cells remain unexplained. Nevertheless, in bovine cells expression of NHE3 is enhanced by exposure to serum and to proinflammatory cytokines [137], and this expression correlates with the detection of functional NHE3 activity [131]. In contrast to NHE1, NHE3 is inhibited by hypertonicity and by PKA pathways, but activated by hypotonicity [5, 126], so it is possible to envisage altered pH_i responses to mechanical load under conditions where exposure to cytokines is increased.

Intracellular Calcium Concentrations are Sensitive to Mechanical Load

Chondrocyte cytosolic Ca^{2+} levels have been studied less than cellular volume and pH. Nevertheless, there have been recent advances in our understanding of the processes that determine intracellular $[Ca^{2+}]$ in these cells. In common with all mammalian cells, $[Ca^{2+}]_i$ is maintained at low levels, approximately 80 nM [12]. There is now good evidence for the operation of a vanadate-sensitive Ca^{2+}-ATPase, similar to that involved in Ca^{2+} homeostasis in many other cell types [50, 108, 144]. In addition, there are reports of a Na^+-dependent component of Ca^{2+} extrusion, which is sensitive to analogues of amiloride such as benzamil [13, 96]. It is perhaps not surprising that — given the high concentrations of Na^+ ions in the extracellular matrix — such a system has a role to play in chondrocyte Ca^{2+} homeostasis. Despite the observation that cytosolic Ca^{2+} ion levels are sensitive to extracellular $[Na^+]$, this has yet to be supported by the demonstration of $Na^+ \times Ca^{2+}$ expression in chondrocytes [82]. There are now indications that both hyperosmolarity and hydrostatic pressure can modify $[Ca^{2+}]_i$ [R.J. Wilkins, *unpublished observations*], suggesting that Ca^{2+} is also sensitive to these variables.

Chondrocyte $[Ca^{2+}]_i$ will be set by the balance between Ca^{2+} extrusion by these systems, influx via membrane channels, and Ca^{2+} release from intracellular stores. Although there is no evidence for voltage-activated Ca^{2+} channels, several channel pathways for Ca^{2+} influx have been identified. Intracellular Ca^{2+} concentration has been found to be modified by extracellular Ca^{2+} levels, in a La^{3+}-sensitive fashion [45], observations interpreted as evidence for the presence of 'tonically active' Ca^{2+} channels. Aside from a histamine-

activated verapamil-inhibited Ca^{2+} influx in porcine chondrocytes [64], there are also reports that components of mechanical load can modulate the Ca^{2+} permeability of the plasma membrane. High levels of cyclical pressurization induce a Gd^{3+}-inhibited $[Ca^{2+}]_i$ increase in ovine chondrocytes, which can also be achieved by cell swelling [140]. The demonstration that chondrocytes possess a quinidine-sensitive Ca^{2+}-activated K^+ channel explains the hyperpolarization of membrane potential which is associated with such pressurization [46]. In addition, there are reports that fluid flow is able to induce rises of $[Ca^{2+}]_i$ in bovine chondrocytes [142, 143]. These rises reflect both Gd^{3+}-inhibited Ca^{2+} influx from the extracellular medium, and a release of Ca^{2+} from intracellular stores. The latter is initiated by inositol triphosphate mobilization and can be inhibited by prior depletion of stores using thapsigargin. Finally, there have been descriptions of mechanically- and ATP-induced oscillations of $[Ca^{2+}]_i$ in monolayer cultures of porcine cells. Gap junction-mediated fluxes of Ca^{2+} have been suggested to play a role in this response [27, 44] although, as outlined earlier, the relevance of confluent cultures of chondrocytes to *in vivo* circumstances is not clear.

Recent studies suggest that intracellular Ca^{2+} levels can also be modulated by changes in pH. Intracellular alkalosis has been shown to elevate $[Ca^{2+}]_i$, a process that is not dependent on the presence of Ca^{2+} ions in the extracellular solution, and which can be inhibited by the depletion of intracellular Ca^{2+} stores by thapsigargin [12]. In contrast extracellular alkalinization activates a Ni^{2+}-inhibited $[Ca^{2+}]_i$ rise, dependent on extracellular Ca^{2+} levels [13].

Transport of Metabolites Remains Largely Uncharacterized for Articular Chondrocytes

Although we have up until this point concentrated on the regulation of the key cellular parameters associated with cation composition, the transport of metabolites across the plasma membrane will also have an important role to play in maintaining chondrocyte biosynthetic output and hence matrix integrity [110]. For such essential processes, surprisingly little is known about the systems present in the chondrocyte membrane that bring about metabolite transport. Here, we will concentrate on the transporters that have been identified in chondrocytes for the essential nutrients sulfate and amino acids — both precursors for matrix macromolecule synthesis — and one metabolic waste product, lactic acid.

The divalent anion **sulfate** is required by chondrocytes for proteoglycan synthesis, and as we have already pointed out, the fixed negative charges on chondroitin and keratan sulfate chains are key to the establishment of the compression-resistant hydrated extracellular matrix [14]. Whether the SO_4^{2-} availability within the cytoplasm ever limits the rate of sulfation of these GAGs in healthy cells is a matter of discussion, since there seems to be ample SO_4^{2-} for this process within intracellular pools including the sulfate donor PAPS (3′-phosphoadenosine 5′-phosphosulfate) [127]. Notwithstanding this question, an effective route for SO_4^{2-} entry will be required. The recent rapid expansion of information about SO_4^{2-} membrane transporters in other cell types had led to the identification of a 'superfamily' of SO_4^{2-}-carrying systems [69]. Integrating this information with what is known about chondrocyte SO_4^{2-} transport is not straightforward, but nevertheless certain conclusions can be drawn.

Functional studies of SO_4^{2-} uptake in bovine articular chondrocytes reveal large fluxes via a Na^+-independent system, which is inhibited by stilbenes such as DIDS and SITS [38, 84, 85]. Hence, a role for the sodium-dependent NaSi system identified in other cell types [75] is precluded, and this is consistent with molecular studies which fail to demonstrate the expression of this transporter [R.J. Wilkins, *unpublished observations*].

Instead, SO_4^{2-} uptake is mediated by a low affinity ($K_m \approx 16$ mM) system, reminiscent of the anion exchange family of transporters [38]. Flux studies show that although SO_4^{2-} can be exchanged for HCO_3^- or Cl^- ions, it is not dependent on these ions and still occurs following their replacement. Furthermore, the process appears to be sensitive to the transmembrane H^+ gradient, being stimulated by acidic extracellular pH [38]. These observations are taken as evidence for the operation of $SO_4^{2-} \times OH^-$ exchange, similar to that previously reported for hepatocyte and enterocyte brush border membrane SO_4^{2-} uptake [65, 104]. The ion preferences and kinetic properties of the SO_4^{2-} uptake observed in chondrocytes do not correlate with those of a single SO_4^{2-} transporter identified at the molecular level [84, 85, 127], suggesting that several parallel pathways exist in these cells to mediate SO_4^{2-} permeation. In agreement with this proposition, the SO_4^{2-} transport-systems SAT1 and DTDST have been shown to be expressed in human and bovine chondrocytes in addition to the simple anion exchanger AE2 identified as a sometime SO_4^{2-} transporter [43, 102, 106], although their relative contributions to SO_4^{2-} uptake remain uncharacterized.

The matrix undersulfation by growth plate chondrocytes associated with chondrodysplasias may, however, be relevant in this context. The SO_4^{2-} uptake properties of undersulfating growth plate chondrocytes show some differences from those of properly sulfating cells, with the apparent K_m for SO_4^{2-} being increased [84, 85, R.J. Wilkins, *unpublished observations*]. In parallel, there is a differential expression of the candidate SO_4^{2-} transporters; while SAT-1 and DTDST expression remains

Table 3. The effects of mechanical load on chondrocyte matrix macromolecule synthesis

	Parameter	Effects on matrix synthesis	Comments	Reference
Dynamic effects	↑ Hydrostatic pressure	↑ ↓ if t > min		56, 57, 93, 101, 114, 121
	↑ Flow	↑		16, 18
	↑ Cell/matrix deformation	↑		16
	↑ Streaming potentials	↑		16
Static effects	↑ Osmolarity	↓ at t > min	Optimal synthesis occurs at tissue osmolarity	66, 118, 119, 121
	↑ $[Na^+]_o$ (Constant osmolarity)	↓		121
	↑ H^+	↓	Optimal synthesis occurs at tissue pH	91, 121, 134
	↑ $[Ca^{2+}]_o$	No direct effect	Extracellular Ca^{2+} required to maintain chondrocyte phenotype	3, 30, 31, 32, 33, 73, 89, 90
			Intracellular Ca^{2+} promotes synthesis	23

The various components of mechanical load can be broadly grouped as dynamic (immediate onset following load) or static (delayed onset, seconds-hours) phenomena.

unchanged, AE2 expression is markedly reduced [25]. These undersulfating cells may therefore help discriminate between the various systems for SO_4^{2-} uptake in articular chondrocytes.

Transport of **amino acids** in mammalian cells is complicated by the number and variety of transporters which have been described, but a consensus — with four or five superfamilies of transporters — is emerging, with molecular biology contributing to our knowledge in this context [1, 86]. For once, there are good detailed studies of transport of these nutrients into chondrocytes [2, 19]. Their significance lies in the provision of sufficient substrate to satisfy collagen and proteoglycan turnover, and there is some evidence that inadequate uptake may compromise the maintenance of cartilage integrity [112]. In bovine chondrocytes, functional *in vitro* studies indicate that amino acid uptake is mediated by both Na^+-dependent and independent transporters [2, 19]. Thus, transport of proline, glycine and glutamine has been shown to be mediated by the Na^+-dependent systems A (inhibited by α-methylaminoisobutyric acid) and ASC (alanine-sensitive). Although little is known regarding the sensitivity of amino acid transport to mechanical load, experiments in which porcine chondrocytes were exposed to long-term hypertonicity revealed a transient upregulation of the activity of this transporter [29]. Although the relevance of such a response is unclear, the stimulation of Na^+- and Cl^--dependent betaine uptake which follows this event suggests that load (and presumably the osmotic effects associated with it) can modify the amino acid transporters under consideration here. In addition, a highly specific Na^+-dependent uptake of glycine, also dependent on extracellular Cl^- ions has been observed and is ascribed to system Gly (sarcosine-inhibited) [2]. In accord with this functional finding, expression of the glycine transporter GLYT1 has been demonstrated in bovine articular chondrocytes [2]. In addition, Na^+-independent uptake of leucine, inhibited by BCH (2-amino-2-norbornane carboxylic acid) was ascribed to system L (although system b^{0+} could equally be responsible for this uptake [21]). These systems, as might be expected for a cell type situated at some distance from the vasculature, have low K_m values for their substrates and hence confer high affinity uptake properties on chondrocytes [2, 19]. Finally, the transport of arginine which, as the synthetic precursor of nitric oxide must be taken up at significant rates, has yet to be characterized.

It would be remiss to conclude consideration of nutrient uptake systems without addressing the membrane pathways present for **glucose** uptake. We have already indicated that the vascular environment of chondrocytes means that exchange between the plasma and the cytoplasm must occur across large distances. Such an arrangement might be expected to indicate high affinity transport systems by which to scavenge glucose from the extracellular matrix [95]. Aside from one report indicating that insulin-like actions of cytokines such as IGF-1 can modulate glucose metabolism by a pathway involving the glucose carrier GLUT4 [124], we are not aware of any published studies that have addressed the question. Nevertheless, glucose must permeate the chondrocyte membrane, and — in the relatively hypoxic environment of the extracellular matrix — enter glycolytic pathways, with consequent production of lactic acid.

There have been a variety of studies demonstrating high rates of **lactic acid** production by chondrocytes [74, 110], which are modified by levels of load and by Po_2. In contrast to reports in which lactic acid production by intervertebral disc cells has been shown to rise as Po_2

Table 4. The effects of components of mechanical load upon chondrocyte cellular physiology

↑ Mechanical deformation/flow	↑ Hydrostatic pressure	↑ Osmolarity	↑ $[Na^+]_o$	↑ $[H^+]_o$	↑ $[Ca^{2+}]_o$
	↓ Na^+/K^+-ATPase[52]	↑ Na^+/K^+-ATPase[79] ↓ g*Ca*$^{2+}$[60, 78]	↑ Na^+/K^+-ATPase[55, 81]		
↑ gCa^{2+}[142, 143] ↑ gK^+[139] ↓ *VSOAC*[111]	↓ gK^+[52]	↓ gK^+[55] ↓ VSOAC[51]	↓ gK^+[55]		
	↓ NKCC[52]	↑ NKCC [55]			
	↑ $Na^+ \times H^+$ exchange[10]	↑ $Na^+ \times H^+$ exchange[141]	↑ $Na^+ \times H^+$ exchange[132]	↓ *$Na^+ \times H^+$ exchange*[48]	
	↑ Ca^{2+}-ATPase	↑ Ca^{2+}-ATPase		↑ *Ca^{2+}-ATPase*[105]	
					↓ $Na^+ \times Ca^{2+}$ exchange[96]
			↑$[Na^+]_i$	↑ $[Na^+]_i$[48]	
			↓ $[H^+]_i$[132]	↑ $[H^+]_i$[134]	
			↓ $[Ca^{2+}]_i$[96]	↓ $[Ca^{2+}]_i$[13]	↑ $[Ca^{2+}]_i$[12]
	↑ cAMP[7]	↑ *cAMP*[94]			

Each of the components identified will increase following the application of load. The reported effects of such increases on the activity of a variety of transport systems and signaling pathways are listed, although discrimination between the actions of interrelated cues (osmolarity and cation concentration, for example) are not necessarily clear cut. Refer to text for details. Numbers are references which exemplify the effect described; all unnumbered effects R.J. Wilkins (*unpublished observations*). Italicized effects indicate reports of effects on cells other than articular chondrocytes.

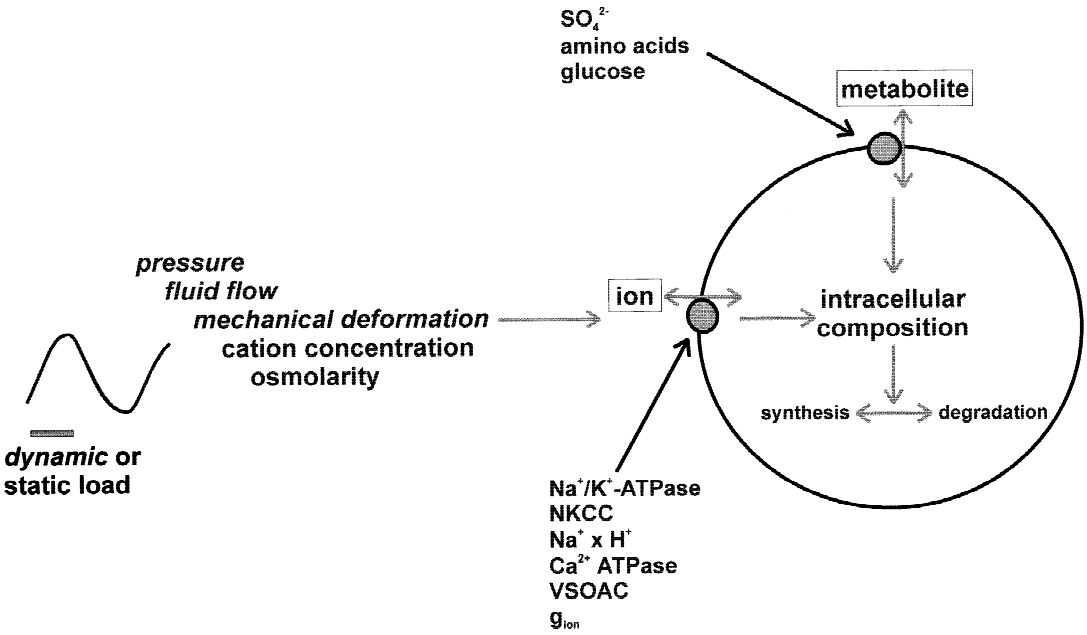

Fig. 2. The involvement of plasma membrane transport processes in the maintenance of cartilage integrity. The balance between synthetic and degradative processes is modulated by intracellular composition. The application of load elicits a variety of changes to the physical and chemical environment experienced by chondrocytes. Plasma membrane transport processes determining the composition of the intracellular milieu are sensitive to such changes, and their altered activity could modify cytoplasmic composition and thereby cartilage turnover. In addition, the activity of transport pathways for nutrients such as SO_4^{2-}, amino acids and glucose will dictate the availability of substrates for matrix macromolecule synthesis. For clarity, other determinants of cytoplasmic composition, such as intracellular stores, and other potential transduction pathways of mechanical load have been excluded (adapted from [130]).

decreases (a 'positive Pasteur' effect [62]), low Po_2 inhibits lactic acid production in articular chondrocytes (a 'negative Pasteur' effect [74]). Regardless of the response of glycolytic metabolism to Po_2 levels, the cell must possess transporters for lactic acid efflux. We have already seen that membrane transporters that can extrude H^+ ions operate in chondrocytes. Other dedicated systems will exist, however, by which lactate$^-$-H^+ can be

transported out of chondrocytes. Studies in which lactic acid transport has been measured in chondrocytes show that these cells possess a saturable transport system, inhibitable by α-CHC, an inhibitor of H^+-monocarboxylate transport [125, 135]. A family of monocarboxylate transporters, termed MCT, has been identified [97], and in chondrocytes it appears that two of these carriers are expressed. In addition to the 'housekeeper' isoform MCT1, expression of MCT4 has been demonstrated in both human and bovine articular chondrocytes [135]. MCT4 has previously been identified in glycolytic tissues such as 'red' skeletal muscle fibers, and this finding is in keeping with the kinetic properties determined for these cells [97].

Mechanical Load Modifies Chondrocyte Composition and Influences Cartilage Synthesis

There is little doubt that mechanical load is required to maintain cartilage integrity. Matrix proteoglycan is lost from cartilage in immobilized joints, while within an individual joint, the matrix shows distinct differences between loaded and unloaded areas with increased proteoglycan content correlated to loaded regions. Mechanical cues may therefore enable chondrocytes to maintain the matrix to withstand the customary loads and forces associated with a particular joint [59, 71, 87, 99, 101].

Many *in vitro* studies characterizing the effects of the various aspects of mechanical loading on the maintenance of matrix integrity by chondrocytes have been made (Table 3), and although diverse in nature, trends have become apparent. Thus, dynamic loading is observed to stimulate proteoglycan and protein synthesis (although excessively high hydrostatic pressures or strains reduce synthesis), whereas static components are associated with decreased synthesis [47, 56, 67, 83, 92, 103, 138].

In contrast to the many studies summarized in Table 3, until recently very few studies addressed directly the manner in which load is translated into altered macromolecule turnover. Several pathways have been implicated in mechanotransduction [110, 130], although here we are primarily concerned with the consequences of load for chondrocyte intracellular composition.

As the earlier sections have shown, the homeostatic mechanisms that control chondrocyte intracellular composition are, to varying degrees, sensitive to components of load. The sensitivities summarized in Table 4 may be one route in which load could be translated into a cellular response [121, 130] (Fig. 2). When chondrocytes are exposed to simulated load (hyperosmolarity, acidity, pressure), changes in $[Na^+]_i$, $[K^+]_i$, pH_i and $[Ca^{2+}]_i$ occur as a result of altered transporter activity, and such disturbances modify macromolecular synthesis in a similar fashion to the component of load being simulated. For example, by actions on transporters which we have outlined, hyperosmolarity or prolonged hydrostatic pressure can raise $[Na^+]_i$ and in turn depress matrix synthesis in isolated bovine chondrocytes [10, 141]. Similarly, *in vitro* studies in which bovine chondrocyte pH_i is acidified by incubation in acidic media show reduced matrix synthesis under these conditions [119, 121, 134]. A complicated interdependence between intracellular variables must exist: given the effects of pH on $[Ca^{2+}]_i$ described earlier, the effects of joint loading on extracellular and intracellular pH could drive matrix synthesis indirectly via changes in $[Ca^{2+}]_i$.

Concluding Remarks

In this review, we have outlined the challenges faced by articular chondrocytes, and the homeostatic mechanisms that they employ to meet them. Our knowledge of the processes involved has grown in recent years and continues to improve. Understanding how chondrocytes endure the insults of their extracellular environment, while at the same time sustaining cartilage matrix integrity, is fundamental. Defective membrane transporter function is now known to underlie many disease processes; establishing whether the same is true for cartilage pathologies such as osteoarthritis remains the goal for physiologists and pharmacologists who work on the transport systems we have described in this review.

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References

1. Barker, G.A., Ellory, J.C. 1990. The identification of neutral amino acid transport systems. *Exp. Physiol.* **75:**3–26
2. Barker, G.A., Wilkins, R.J., Golding, S., Ellory, J.C. 1999. Neutral amino acid transport in bovine articular chondrocytes. *J. Physiol.* **514:**795–808
3. Bashey, R.I., Iannotti, J.P., Rao, V.H., Reginato, A.M., Jimenez, S.A. 1991. Comparison of morphological and biochemical characteristics of cultured chondrocytes isolated from proliferative and hypertrophic zones of bovine growth plate cartilage. *Differentiation* **46:**199–207
4. Benya, P.D., Shaffer, J.D. 1982. Dedifferentiated chondrocytes reexpress the differentiated collagen phenotype when cultured in agarose gels. *Cell* **30:**215–224
5. Bookstein, C., Musch, M.W., Xie, Y., Rao, M.C., Chang, E.B. 1999. Regulation of intestinal epithelial brush border Na^+/H^+ exchanger isoforms, NHE2 and NHE3, in C2bbe cells. *J. Membrane Biol.* **171:**87–95
6. Boron, W.F., Knakal, R.C. 1992. Na^+-dependent Cl-HCO_3 exchange in the squid axon. Dependence on extracellular pH. *J. Gen. Physiol.* **99:**817–837
7. Bourret, L.A., Rodan, G.A. 1976. The role of calcium in the

inhibition of cAMP accumulation in epiphyseal cartilage cells exposed to physiological pressure. *J. Cell. Physiol.* **88:**353–361

8. Broom, N.D., Marra, D.L. 1985. New structural concepts of articular cartilage demonstrated with a physical model. *Connect. Tissue Res.* **14:**1–8
9. Broom, N.D., Poole, C.A. 1983. Articular cartilage collagen and proteoglycans. Their functional interdependency. *Arthritis Rheum.* **26:**1111–1119
10. Browning, J.A., Walker, R.E., Hall, A.C., Wilkins, R.J. 1999. Modulation of $Na^+ \times H^+$ exchange by hydrostatic pressure in isolated bovine articular chondrocytes. *Acta Physiol. Scand.* **166:**39–45
11. Browning, J.A., Wilkins, R.J. 1998. The characterization of mechanisms regulating intracellular pH in a transformed human articular chondrocyte cell line. *J. Physiol.* **513P:**54
12. Browning, J.A., Wilkins, R.J. 1999. Effects of intracellular alkalinization on Ca^{2+} homeostasis in a human articular chondrocyte cell line, C-20/A4. *J. Physiol.* **520P:**54
13. Browning, J.A., Wilkins, R.J. Effects of extracellular alkalinisation on Ca^{2+} homeostasis in articular chondrocytes. *J. Physiol.* **S26P:**105
14. Buckwalter, J.A., Mankin, H.J. 1998. Articular cartilage: tissue design and chondrocyte-matrix interactions. *Instr. Course Lect.* **47:**477–486
15. Burg, M.B. 1997. Renal osmoregulatory transport of compatible organic osmolytes. *Curr. Opin. Nephrol. Hypertens.* **6:**430–433
16. Buschmann, M.D., Gluzband, Y.A., Grodzinsky, A.J., Hunziker, E.B. 1995. Mechanical compression modulated matrix biosynthesis in chondrocyte/agarose culture. *J. Cell. Sci.* **108:**1497–1508
17. Buschmann, M.D., Hunziker, E.B., Kim, Y.J., Grodzinsky, A.J. 1996. Altered aggrecan synthesis correlates with cell and nucleus structure in statically compressed cartilage. *J. Cell. Sci.* **109:**499–508
18. Buschmann, M.D., Kim, Y.J., Wong, M., Frank, E., Hunziker, E.B., Grodzinsky, A.J. 1999. Stimulation of aggrecan synthesis in cartilage explants by cyclic loading is localized to regions of high interstitial fluid flow. *Arch. Biochem. Biophys.* **366:**1–7
19. Byers, S., Handley, C.J., Lowther, D.A. 1983. Transport of neutral amino acids by adult articular cartilage. *Connect. Tissue Res.* **11:**321–330
20. Cardenas, V., Heming, T.A., Bidani, A. 1998. Kinetics of CO_2 excretion and intravascular pH disequilibria during carbonic anhydrase inhibition. *J. Appl. Physiol.* **84:**683–694
21. Castagna, M., Shayakul, C., Trotti, D., Sacchi, V.F., Harvey, W.R., Hediger, M.A. 1997. Molecular characteristics of mammalian and insect amino acid transporters: implications for amino acid homeostasis. *J. Exp. Biol.* **200:**269–286
22. Chalfant, M.L., Denton, J.S. Berdiev, B.K., Ismailov, I.I., Benos, D.J., Stanton, B.A. 1999. Intracellular H^+ regulates the alpha-subunit of ENaC, the epithelial Na^+ channel. *Am. J. Physiol.* **276:**C477–C486
23. Clark, C.C., Iannotti, J.P., Misra, S., Richards, C.F. 1994. Effects of thapsigargin, an intracellular calcium-mobilizing agent, on synthesis and secretion of cartilage collagen and proteoglycan. *J. Orthop. Res.* **12:**601–611
24. Corkey, K., Wilkins, R.J. 2000. Undersulfation of cartilage matrix proteins in Ng cells is not due to impaired sulfate transport. *FASEB J.* **14:**A352
25. Corkey, K.D., Wilkins, R.J. 1998. Increases in extracellular sulfate concentration decrease AE2 protein levels in the transformed human chondrocyte cell line C-20/A4. *J. Physiol.* **513P:**54
26. Cremer, J.A., Rosloniec, E.F., Kang, A.H. 1998. The cartilage collagens: a review of their structure, organization, and role in the pathogenesis of experimental arthritis in animals and in human rheumatic disease. *J. Mol. Med.* **76:**275–288
27. D'Andrea, P., Calabrese, A., Grandolfo, M. 1998. Intercellular calcium signaling between chondrocytes and synovial cells in co-culture. *Biochem J.* **329:**681–687
28. Dascalu, A., Nevo, Z., Korenstein, R. 1993. The control of intracellular pH in cultured avian chondrocytes. *J. Physiol.* **461:**583–599
29. de Angelis, E., Petronini, P.G., Borghetti, P., Borghetti, A.F., Wheeler, K.P. 1999. Induction of betaine-gamma-aminobutyric acid transport activity in porcine chondrocytes exposed to hypertonicity. *J. Physiol.* **518:**187–194
30. Deshmukh, K., Kline, W.G., Sawyer, B.D. 1977. Effects of calcitonin and parathyroid hormone on the metabolism of chondrocytes in culture. *Biochim. Biophys. Acta* **499:**28–35
31. Deshmukh, K., Kline, W.H. 1976. Characterization of collagen and its precursors synthesized by rabbit-articular-cartilage cells in various culture systems. *Eur. J. Biochem.* **69:**117–123
32. Deshmukh, K., Sawyer, B.D. 1977. Synthesis of collagen by chondrocytes in suspension culture: modulation by calcium, 3′: 5′-cyclic AMP, and prostaglandins. *Proc. Natl. Acad. Sci. USA.* **74:**3864–3868
33. Deshmukh, K., Sawyer, B.D. 1978. Influence of extracellular pyrophosphate on the synthesis of collagen by chondrocytes. *FEBS. Lett.* **89:**230–232
34. Eckstein, F., Tieschky, M., Faber, S., Englmeier, K.H., Reiser, M. 1999. Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. *Anat. Embryol.* **200:**419–424
35. Ekman, S., Ridderstrale, Y. 1992. Carbonic anhydrase localization in normal and osteochondrotic joint cartilage of growing pigs. *Vet. Pathol.* **29:**308–315
36. Ellory, J.D., Gibson, J.S., Stewart, G.W. 1998. Pathophysiology of abnormal cell volume in human red cells. *Contrib. Nephrol.* **123:**220–239
37. Errington, R.J., Fricker, M.D., Wood, J.L., Hall, A.C., White, N.S. 1997. Four-dimensional imaging of living chondrocytes in cartilage using confocal microscopy: a pragmatic approach. *Am. J. Physiol.* **272:**C1040–C1051
38. Gehl, K.A., Ellory, J.C. 1992. Sulfate:hydroxyl exchange in isolated bovine chondrocytes. *J. Physiol.* **446P:**532
39. Gehl, K.A., Harvey, B.J., Ellory, J.C. 1991. Sulfate uptake by isolated bovine chondrocytes. *J. Physiol.* **435P:**90
40. Glade, M.J., Kanwar, Y.S., Hefley, T.J. 1991. Enzymatic isolation of chondrocytes from immature rabbit articular cartilage and maintenance of phenotypic expression in culture. *J. Bone Miner. Res.* **6:**217–226
41. Glant, T., Mikecz, K. 1986. Antigenic profiles of human, bovine and canine articular chondrocytes. *Cell Tissue Res.* **244:**359–369
42. Godart, H., Ellory, J.C., Motais, R. 1999. Regulatory volume response of erythrocytes exposed to a gradual and slow decrease in medium osmolality. *Pfluegers Arch.* **437:**776–779
43. Golding, S., Mobasheri, A., Wilkins, R.J., Ellory, J.C. 1997. Molecular identification of anion exchanger isoform AE in articular chondrocytes. *Trans. Orthop. Res. Soc.* **22:**10
44. Grandolfo, M., Calabrese, A., D'Andrea, P. 1998. Mechanism of mechanically induced intercellular calcium waves in rabbit articular chondrocytes and in HIG-82 synovial cells. *J. Bone Miner. Res.* **3:**443–453
45. Grandolfo, M., D'Andrea, P., Martina, M., Ruzzier, F., Vittur, F. 1992. Calcium-activated potassium channels in chondrocytes. *Biochem. Biophys. Res. Commun.* **182:**1429–1434
46. Grandolfo, M., Martina, M., Ruzzier, F., Vittur, F. 1990. A po-

tassium channel in cultured chondrocytes. *Calcif. Tissue Int.* **47:**302–307
47. Gray, M.L., Pizzanelli, A.M., Grodzinsky, A.J., Lee, R.C. 1988. Mechanical and physiochemical determinants of the chondrocyte biosynthetic response. *J. Orthop. Res.* **6:**777–792
48. Green, J., Yamaguchi, D.T., Kleeman, C.R., Muallem, S. 1988. Cytosolic pH regulation in osteoblasts. Interaction of Na^+ and H^+ with the extracellular and intracellular faces of the Na^+/H^+ exchanger. *J. Gen. Physiol.* **92:**239–261
49. Grodzinsky, A.J. 1998. Electromechanical and physicochemical properties of connective tissue. *Crit. Rev. Biomed. Eng.* **9:**133–199
50. Guerini, D., Garcia-Martin, E., Zecca, A., Guidi, F., Carafoli, E. 1998. The calcium pump of the plasma membrane: membrane targeting, calcium binding sites, tissue-specific isoform expression. *Acta Physiol. Scand.* **643:**265–273
51. Hall, A.C. 1995. Volume-sensitive taurine transport in bovine articular chondrocytes. *J. Physiol.* **484:**755–766
52. Hall, A.C. 1999. Differential effects of hydrostatic pressure on cation transport pathways of isolated articular chondrocytes. *J. Cell. Physiol.* **178:**197–204
53. Hall, A.C., Horwitz, E.R., Wilkins, R.J. 1996. The cellular physiology of articular cartilage. *Exp. Physiol.* **81:**535–545
54. Hall, A.C., Pickles, D.M., MacDonald, A.G. 1993. Aspects of eukaryotic cells. *Adv. Comp. Environ. Physiol.* **17:**29–85
55. Hall, A.C., Starks, I., Shoults, C.L., Rashidbigi, S. 1996. Pathways for K^+ transport across the bovine articular chondrocyte membrane and their sensitivity to cell volume. *Am. J. Physiol.* **270:**C1300–C1310
56. Hall, A.C., Urban, J.P., Gehl, K.A. 1991. The effects of hydrostatic pressure on matrix synthesis in articular cartilage. *J. Orthop. Res.* **9:**1–10
57. Handa, T., Ishihara, H., Ohshima, H., Osada, R., Tsuji, H., Obata, K. 1997. Effects of hydrostatic pressure on matrix synthesis and matrix metalloproteinase production in the human lumbar intervertebral disc. *Spine* **22:**1085–1091
58. Heinegard, D., Sommarin, Y. 1987. Proteoglycans: an overview. *Methods Enzymol.* **144:**305–319
59. Helminen, H., Jurvelin, J., Kiviranta, I., Paukkonen, K., Saamanen, A-M., Tammi, M. 1987. Joint loading effects on articular cartilage: a historical review. H.J. Helminen, I. Kirivanta, M. Tammi, A.-M. Saamanen, K., Paukkonen, editors. pp. 1–46. Wright, Bristol
60. Hoffmann, E.K., Simonsen, L.-O. 1989. Membrane mechanisms in volume and pH regulation in vertebrate cells. *Physiol. Rev.* **69:**315–382
61. Hoffmann, E.K., Sjoholm, C., Simonsen, L.O. 1983. Na^+, Cl^- cotransport in Ehrlich ascites tumor cells activated during volume regulation (regulatory volume increase). *J. Membrane Biol.* **76:**269–280
62. Holm, S., Maroudas, A., Urban, J.P.G., Selstam, G., Nachemson, A. 1981. Nutrition of the intervertebral disc: solute transport and metabolism. *Connect. Tissue. Res.* **8:**101–119
63. Horwitz, E.R., Harvey, B.J. 1995. Mechanosensitive calcium increase in porcine chondrocytes. *J. Physiol.* **489P:**112
64. Horwitz, E.R., Higgins, T.M., Harvey, B.J. 1996. Histamine-induced cytosolic calcium increase in porcine articular chondrocytes. *Biochim. Biophys. Acta* **1313:**95–100
65. Hugentobler, G., Meier, P.J. 1986. Multispecific anion exchange in basolateral (sinusoidal) rat liver plasma membrane vesicles. *Am. J. Physiol.* **251:**G656–G664
66. Ishihara, H., Warensjo, K., Roberts, S., Urban, J.P. 1997. Proteoglycan synthesis in the intervertebral disk nucleus: the role of extracellular osmolality. *Am. J. Physiol.* **272:**C1499–C1506
67. Jones, I.L., Klamfeldt, A., Sandstrom, T. 1982. The effect of continuous mechanical pressure upon the turnover of articular cartilage proteoglycans in vitro. *Clin. Orthop.* **165:**283–289
68. Ker, R.F. 1999. The design of soft collagenous load-bearing tissues. *J. Exp. Biol.* **202:**3315–3324
69. Kere, J., Lohi, H., Hoglund, P. 1999. Genetic Disorders of Membrane Transport III. Congenital chloride diarrhea. *Am. J. Physiol.* **276:**G7–G13
70. Kernan, R.P. 1980. Cell Potassium. John Wiley & Sons, New York
71. Kiviranta, I., Tammi, I., Jurvelin, J., Arokoski, J., Saamanen, A.-M., Helminen, H. 1988. Moderate running exercise augments glycosaminoglycans and thickness of articular cartilage in the knee joint of young beagle dogs. *J. Orthop. Res.* **6:**188–195
72. Lang, F., Busch, G.L., Ritter, M., Volkl, H., Waldegger, S., Gulbins, E., Haussinger, D. 1998. Functional significance of cell volume regulatory mechanisms. *Physiol. Rev.* **78:**247–306
73. Layman, D.L., Sokoloff, L., Miller, E.J. 1972. Collagen synthesis by articular chondrocytes in monolayer culture. *Exp. Cell. Res.* **73:**107–112
74. Lee, R.B., Urban, J.P. 1997. Evidence for a negative Pasteur effect in articular cartilage. *Biochem. J.* **321:**95–102
75. Markovich, D., Forgo, J., Stange, G., Biber, J., Murer, H. 1993. Expression cloning of rat renal $Na^+/SO4^{2-}$ cotransport. *Proc. Natl. Acad. Sci. USA* **90:**8073–8077
76. Maroudas, A. 1979. Physico chemical properties of articular cartilage. M.A.R. Freeman, editor. pp. 215–290. Adult Articular Cartilage. Academic Press, New York
77. Maroudas, A. 1980. Physical chemistry of articular cartilage and the intervertebral disc. L. Sokoloff, editor. pp. 239–291. Joints and Synovial Fluid, vol. 2. Academic Press, New York
78. McCarty, N.A., O'Neil, R.G. 1992. Calcium signaling in cell volume regulation. *Physiol. Rev.* **72:**1037–1061
79. Mobasheri, A. 1998. Correlation between [Na^+], [glycosaminoglycan] and Na^+/K^+ pump density in the extracellular matrix of bovine articular cartilage. *Physiol. Res.* **47:**47–52
80. Mobasheri, A., Errington, R.J., Golding, S., Hall, A.C., Urban, J.P. 1997. Characterization of the Na^+, K^+-ATPase in isolated bovine articular chondrocytes; molecular evidence for multiple alpha and beta isoforms. *Cell Biol. Int.* **21:**201–212
81. Mobasheri, A., Hall, A.C., Urban, J.P., France, S.J., Smith, A.L. 1997. Immunologic and autoradiographic localization of the Na^+, K^+-ATPase in articular cartilage: upregulation in response to changes in extracellular Na^+ concentration. *Int. J. Biochem. Cell Biol.* **29:**649–657
82. Mobasheri, A., Mobasheri, R., Francis, M.J., Trujillo, E., Alvarez de la Rosa, D., Martin-Vasallo, P. 1998. Ion transport in chondrocytes: membrane transporters involved in intracellular ion homeostasis and the regulation of cell volume, free [Ca^{2+}] and pH. *Histol. Histopathol.* **13:**893–910
83. Mohtai, M., Gupta, M.K., Donlon, B., Ellison, B., Cooke, J., Gibbons, G., Schurman, D.J., Smith, R.L. 1996. Expression of interleukin-6 in osteoarthritic chondrocytes and effects of fluid-induced shear on this expression in normal human chondrocytes in vitro. *J. Orthop. Res.* **14:**67–73
84. Morcuende, J.A., Kimura, J.H., Plaas, A.H.K. 1996. Identification of the sulfate transport channel SAT-1 in chondrocytes. *Trans. Comb. Orthop. Res. Soc.* **2:**50
85. Morcuende, J.A., Plaas, A.H.K., Kimura, J.H. 1996. Altered functional activity of the sulfate transport channel SAT-1 in a mutant rat chondrosarcoma clone. *Trans. Orthop. Res. Soc.* **21:**764
86. Moseley, R.H. 1996. Hepatic amino acid transport. *Semin. Liver Dis.* **16:**137–145
87. Mow, V.V., Wang, C.C., Hung, C.T. 1999. The extracellular

matrix, interstitial fluid and ions as a mechanical signal transducer in articular cartilage. *Osteoarthritis Cartilage* **7:**41–58
88. Muir, H. 1995. The chondrocyte, architect of cartilage. Biomechanics, structure, function and molecular biology of cartilage matrix macromolecules. *Bioessays* **17:**1039–1048
89. Muller, P.K., Lemmen, C., Gay, S., Gauss, V., Kuhn, K. 1977. Immunochemical and biochemical study of collagen synthesis by chondrocytes in culture. *Exp. Cell. Res.* **108:**47–55
90. Norby, D.P., Malemud, C.J., Sokoloff, L. 1977. Differences in the collagen types synthesized by lapine articular chondrocytes in spinner and monolayer culture. *Arthritis. Rheum.* **20:**709–716
91. Ohshima, H., Urban, J.P. 1992. The effect of lactate and pH on proteoglycan and protein synthesis rates in the intervertebral disc. *Spine* **17:**1079–1082
92. Palmoski, M.J., Brandt, K.D. 1984. Effects of static and cyclic compressive loading on articular cartilage plugs in vitro. *Arthritis Rheum.* **27:**675–681
93. Parkkinen, J.J., Ikonen, J., Lammi, M.J., Laakkonen, J., Tammi, M., Helminen, H.J. 1993. Effects of cyclic hydrostatic pressure on proteoglycan synthesis in cultured chondrocytes and articular cartilage explants. *Arch. Biochem. Biophys.* **300:**458–465
94. Pena-Rasgado, C., Kimler, V.A., McGruder, K.D., Tie, J., Rasgado-Flores, H. 1994. Opposite roles of cAMP and cGMP on volume loss in muscle cells. *Am. J. Physiol.* **267:**C1319–C1328
95. Pessin, J.E., Bell, G.I. 1992. Mammalian facilitative glucose transporter family: structure and molecular regulation. *Annu. Rev. Physiol.* **54:**911–930
96. Ponte, M.R., Hall, A.C. 1994. Effect of extracellular Ca^{2+} and Na^+ on $[Ca^{2+}]_i$ of porcine articular chondrocytes. *J. Physiol.* **475P:**105
97. Price, N.T., Jackson, V.N., Halestrap, A.P. 1998. Cloning and sequencing of four new mammalian monocarboxylate transporter (MCT) homologues confirms the existence of a transporter family with an ancient past. *Biochem. J.* **329:**321–328
98. Razaq, M.S., Urban, J.P.G., Wilkins, R.J. 2000. Regulation of intracellular pH by bovine intervertebral disc cells. *Cell Physiol. Biochem.* **10:**109–115
99. Roberts, S., Weightman, B., Urban, J.P.G., and Chapell, D. 1986. Mechanical and biochemical properties of human articular cartilage in osteoarthritic femoral heads and in autopsy specimens. *J. Bone Joint. Surg. Br.* **68:**278–288
100. Sabirov, R.Z., Okada, Y., Oiki, S. 1997. Two-sided action of protons on an inward rectifier K^+ channel (IRK1). *Pfluegers Arch.* **433:**428–434
101. Sah, R.L.-Y., Grodzinsky, A.J., Plaas, A.H.K., Sandy, J.D. 1992. Effects of static and dynamic compression on matrix metabolism in cartilage explants. K.E. Kuettner, R. Schleyerbach, J.G. Peyron, and V.C. Hascall, editors. pp. 373–392. Raven Press, New York
102. Satoh, H., Susaki, M., Shukunami, C., Iyama, K., Negoro, T., Hiraki, Y. 1998. Functional analysis of diastrophic dysplasia sulfate transporter. Its involvement in growth regulation of chondrocytes mediated by sulfated proteoglycans. *J. Biol. Chem.* **273:**12307–12315
103. Schneiderman, R., Keret, E., Maroudas, A. 1986. Effects of mechanical and osmotic pressure on the rate of glycosaminoglycan synthesis in the human adult femoral head cartilage: an in vitro study. *J. Orthop. Res.* **4:**393–408
104. Schron, C.M., Knickelbein, R.G., Aronson, P.S., Della Puca, J., Dobbins, J.W. 1985. pH gradient-stimulated sulfate transport by rabbit ileal brush-border membrane vesicles: evidence for SO4-OH exchange. *Am. J. Physiol.* **249:**G607–G613
105. Schwiening, C.J., Kennedy, H.J., Thomas, R.C. 1993. Calcium-hydrogen exchange by the plasma membrane Ca-ATPase of voltage-clamped snail neurons. *Proc. R. Soc. Lond. B.* **253:**285–289
106. Sekler, I., Lo, R.S., Mastrocola, T., Kopito, R.R. 1995. Sulfate transport mediated by the mammalian anion exchangers in reconstituted proteoliposomes. *J. Biol. Chem.* **270:**11251–11256
107. Sperelakis, N. 1994. Cell Physiology Source Book. Academic Press, San Diego
108. Stein, W.D. 1990. Channels, Carriers and Pumps. Academic Press, San Diego
109. Stengelin, M.K., Hoffman, J.F. 1997. Na,K-ATPase subunit isoforms in human reticulocytes: evidence from reverse transcription-PCR for the presence of alpha1, alpha3, beta2, beta3, and gamma. *Proc. Natl. Acad. Sci. USA.* **94:**5943–5948
110. Stockwell, R.A. 1991. Cartilage failure in osteoarthritis: relevance of normal structure and function. A review. *Clin. Anat.* **4:**161–191
111. Strange, K. 1999. Regulation of a swelling-activated anion channel by intracellular ionic strength and ATP concentration. R. Kozlowski, editor. pp. 65–78. Chloride Channels. Isis Medical Publishing, Oxford
112. Svedstrom, E., Parto, K., Marttinen, M., Virtama, P., Simell, O. 1991. Skeletal manifestations of lysinuric protein intolerance. A follow-up study of 29 patients. *Skeletal. Radiol.* **22:**11–16
113. Szirmai, J.A. 1970. Structure of the intervertebral disc. *In:* Chemistry and Molecular Biology of the Intercellular Matrix. E.A. Balazs, editor. pp. 1279–1308. Academic Press, New York
114. Takahashi, K., Kubo, T., Arai, Y., Kitajima, I., Takigawa, M., Imanishi, J., Hirasawa, Y. 1998. Hydrostatic pressure induces expression of interleukin 6 and tumor necrosis factor alpha mRNAs in a chondrocyte-like cell line. *Ann. Rheum. Dis.* **57:**231–236
115. Trujillo, E., Alvarez de la Rosa, D., Mobasheri, A., Avila, J., Gonzalez, T., Martin-Vasallo, P. 1999. Sodium transport systems in human chondrocytes. I. Morphological and functional expression of the Na^+, K^+-ATPase alpha and beta subunit isoforms in healthy and arthritic chondrocytes. *Histol. Histopathol.* **14:**1011–1022
116. Trujillo, E., Alvarez de la Rosa, D., Mobasheri, A., Gonzalez, T., Canessa, C.M., Martin-Vasallo, P. Sodium transport systems in human chondrocytes. II. Expression of ENaC, $Na^+/K^+/2Cl^-$ cotransporter and Na^+/H^+ exchangers in healthy and arthritic chondrocytes. *Histol. Histopathol.* **14:**1023–1031
117. Urban, J.P. 1994. The chondrocyte: a cell under pressure. *Br. J. Rheum.* **33:**901–908
118. Urban, J.P., Bayliss, M.T. 1989. Regulation of proteoglycan synthesis rate in cartilage in vitro: influence of extracellular ionic composition. *Biochim. Biophys. Acta* **992:**59–65
119. Urban, J.P., Hall, A.C., Gehl, K.A. 1993. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. *J. Cell. Physiol.* **154:**262–270
120. Urban, J.P.G., Hall, A.C. 1992. Physical modifiers of cartilage metabolism. *In:* Articular Cartilage and Osteoarthritis. K.E. Kuettner, R. Schleyerbach, J.G. Peyron, and V.C. Hascall, editors. pp. 393–406. Raven Press, New York
121. Urban, J.P.G., Wilkins, R.J. 1999. Extracellular ions and hydrostatic pressure: their influence on chondrocyte intracellular ionic composition. S. Tanaka and C. Hamanishi, editors. pp. 3–20. Advances in Osteoarthritis. Springer, Tokyo
122. Vaughan-Jones, R.D. 1979. Regulation of chloride in quiescent sheep-heart Purkinje fibers studied using intracellular chloride and pH-sensitive micro-electrodes. *J. Physiol.* **295:**111–137
123. Vittur, F., Grandolfo, M., Fragonas, E., Godeas, C., Paoletti, S., Pollesello, P., Kvam, B.J., Ruzzier, F., Starc, T., Mozrzymas, J.W., Martina, M., De Bernard, B. 1994. Energy metabolism,

replicative ability, intracellular calcium concentration, and ionic channels of horse articular chondrocytes. *Exp. Cell Res.* **210:**130–136

124. Wang, J., Zhou, J., Bondy, C.A. 1999. Igf1 promotes longitudinal bone growth by insulin-like actions augmenting chondrocyte hypertrophy. *FASEB J.* **13:**1985–1990
125. Wang, X., Levi, A.J., Halestrap, A.P. 1996. Substrate and inhibitor specificities of the monocarboxylate transporters of single rat heart cells. *Am. J. Physiol.* **270:**H476–H484
126. Watts, B.A., Good, D.W. 1994. Apical membrane Na^+/H^+ exchange in rat medullary thick ascending limb. pH-dependence and inhibition by hyperosmolality. *J. Biol. Chem.* **269:**20250–20255
127. Whittaker, K.A. 1992. Ion transport by isolated bovine chondrocytes. D. Phil. thesis, University of Oxford
128. Wilkins, R.J., Browning, J.A., Crossfield, A.L., Ross, E., Liou, C. Ellory, J.C. 2000. Investigation of the pathways for zinc transport across the chondrocyte plasma membrane. *Trans. Orthop. Res. Soc.* **25:**916
129. Wilkins, R.J., Browning, J.A., Ellory, J.C. 1999. Cell volume regulation reduced to size. *In:* Chloride Channels. R. Kozlowski, editor. pp. 19–33. Isis Medical Publishing, Oxford
130. Wilkins, R.J., Browning, J.A., Urban, J.P.G. 2000. Chondrocyte regulation by mechanical load. *Biorheology* **37:**67–74
131. Wilkins, R.J., Crossfield, A.L. 2000. Functional identification of NHE isoform 3 in bovine articular chondrocytes. *J. Physiol.* **526P:**26
132. Wilkins, R.J., Hall, A.C. 1992. Measurement of intracellular pH in isolated bovine articular chondrocytes. *Exp. Physiol.* **77:**521–524
133. Wilkins, R.J., Hall, A.C. 1993. Bovine articular chondrocytes demonstrate only minimal bicarbonate-dependent recovery from changes to intracellular pH. *J. Physiol.* **459P:**289
134. Wilkins, R.J., Hall, A.C. 1995. Control of matrix synthesis in isolated bovine chondrocytes by extracellular and intracellular pH. *J. Cell. Physiol.* **164:**474–481
135. Wilkins, R.J., McClure B., Browning, J.A. 2000. Characterization of lactic acid transport in bovine articular chondrocytes. *Trans. Orthop. Res. Soc.* **25:**925
136. Wilkins, R.J., Mobasheri, A., Hall, A.C. 1996. Bovine articular chondrocytes possess two isoforms of $Na^+ \times H^+$ exchange. *Trans. Orthop. Res. Soc.* **21:**35
137. Wilkins, R.J., Mobasheri, A., Hall, A.C. 1996. Characterization of $Na^+ \times H^+$ exchange in isolated bovine articular chondrocytes. *FASEB J.* **10:**666
138. Wong, M., Wuethrich, P., Eggli, P., Hunziker, E. 1996. Zone-specific cell biosynthetic activity in mature bovine articular cartilage: a new method using confocal microscopic stereology and quantitative autoradiography. *J. Orthop. Res.* **14:**424–432
139. Wright, M., Jobanputra, P., Bavington, C., Salter, D.M., Nuki, G. 1996. Effects of intermittent pressure-induced strain on the electrophysiology of cultured human chondrocytes: evidence for the presence of stretch-activated membrane ion channels. *Clin. Sci. Colch.* **90:**61–71
140. Wright, M.O., Stockwell, R.A., Nuki, G. 1992. Response of plasma membrane to applied hydrostatic pressure in chondrocytes and fibroblasts. *Connect. Tissue. Res.* **28:**49–70
141. Yamazaki, N., Browning, J.A., Wilkins, R.J. 2000. Modulation of $Na^+ \times H^+$ exchange by osmotic shock in isolated bovine articular chondrocytes. *Acta Physiol. Scand.* **169:**221–228
142. Yellowley, C.E., Jacobs, C.R., Donahue, H.J. 1999. Mechanisms contributing to fluid-flow-induced Ca^{2+} mobilization in articular chondrocytes. *J. Cell. Physiol.* **180:**402–408
143. Yellowley, C.E., Jacobs, C.R., Li, Z., Zhou, Z., Donahue, H.J. 1997. Effects of fluid flow on intracellular calcium in bovine articular chondrocytes. *Am. J. Physiol.* **273:**C30–C36
144. Zanetti, M., Camerotto, R., Romeo, D., De Bernard, B. 1982. Active extrusion of Ca^{2+} from epiphysial chondrocytes of normal and rachitic chickens. *Biochem. J.* **202:**303–307