Metabolism of cartilage proteins in cultured tissue sections

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The biosynthesis and turnover of cartilage proteins was studied in organ cultures of bovine tracheal-cartilage sections. In cultures labelled with [^3H]leucine, more than 99% of the labelled macromolecules were retained in the sections. About half of the [^3H]leucine-labelled protein was extracted with 4 M-guanidinium chloride. The incorporation of [^3H]leucine into protein extractable with guanidinium chloride was linear with time, after an initial delay of 20–25 min. The 148 kDa and 36 kDa cartilage proteins were major labelled components in this extract. The elimination of the proteins was studied by using a pulse–chase protocol. The 148 kDa protein was found to have a very slow turnover, similar to that of proteoglycans, whereas the 36 kDa protein was eliminated more rapidly.

The cartilage extracellular matrix contains collagen, proteoglycans and a limited number of non-collagenous proteins (Paulsson & Heinegård, 1979, 1982). Among the non-collagenous proteins, the link proteins have a well-defined function in stabilizing the interaction between proteoglycan monomers and hyaluronic acid, important for the formation of proteoglycan aggregates (Heinegård & Hascall, 1974; Hardingham, 1979; Franzén *et al.*, 1981).

In recent years two additional non-collagenous proteins have been identified in cartilage extracts. One of these, the 148 kDa protein [this protein has in two previous papers (Paulsson & Heinegård, 1979, 1981) been referred to as the ‘cartilage matrix protein’], is a trimer of similarly sized disulphide-bonded subunits (Paulsson & Heinegård, 1981). It is quantitatively prominent in guanidinium chloride extracts of bovine tracheal and nasal cartilages, but it is not detected in extracts of either articular cartilage or intervertebral disc (Paulsson & Heinegård, 1982). Available data indicate that the 148 kDa protein interacts with proteoglycans (Paulsson & Heinegård, 1979), although the specificity of the interaction has not been demonstrated. It is likely that the 148 kDa protein is a structural component of the extracellular matrix, particularly in tracheal cartilage, where it constitutes as much as 1% of the tissue wet weight.

Another non-collagenous cartilage protein, the 36 kDa protein, consists of a single polypeptide chain with a high content of leucine (about 140 residues/1000 residues; M. Paulsson, Y. Sommarin & D. Heinegård, unpublished work). It appears to be

present in all bovine cartilages (Paulsson & Heinegård, 1982).

Extracellular components of cartilage are synthesized by the chondrocyte or may enter the cartilage by diffusion from surrounding tissues or the blood, even though most cartilages contain few blood vessels. Examples of structural components synthesized by the chondrocyte are proteoglycans and collagen (for references see Eyre, 1980; Heinegård & Paulsson, 1983). These molecules have a very slow turnover once they are integrated in the cartilage matrix. In different studies of their turnover, half-lives ranging from 1 week to months and perhaps even years have been determined (Mankin & Lipiello, 1969; Maroudas, 1975, 1979; Lohmander, 1977). Other cartilage macromolecules, if structural components of the tissue matrix, would by analogy be expected to be synthesized by the chondrocyte and to show a slow turnover in the tissue. The present work was initiated to study the synthesis and turnover of non-collagenous cartilage proteins, particularly the 148 kDa and the 36 kDa proteins, and to relate the findings to simultaneously obtained data on proteoglycan core protein and link proteins.

Experimental

Materials

Ham's F12 H medium (GIBCO, Grand Island, NY, U.S.A.) was buffered with 10 mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] (Sigma Chemical Co., St. Louis, MO, U.S.A.). The unlabelled leucine normally included in the medium

was omitted, to allow a high specific radioactivity of [^3H]leucine.

Ham's F12 HA medium, used in chase experiments, was buffered with 10 mM-Hepes and supplemented with '50 $\times$ essential amino acids for Eagle's minimum essential medium' (20 ml/l) (Flow Laboratories, Rockville Pike, MD, U.S.A.) and 2 mM-leucine. The medium was further supplemented with penicillin (50 i.u./ml) and streptomycin (50 μg /ml) (both from Flow Laboratories).

[^3H]Leucine (65 or 160 Ci/mmol) and carrier-free [^{35}S]sulphate were from The Radiochemical Centre, Amersham, Bucks., U.K.

Cartilage sections were cultured in 50 ml Falcon conical polypropylene tubes (Becton Dickinson Labware, Oxnard, CA, U.S.A.) or in Linbro tissue-culture multi-well plates (Flow Laboratories) with 16 mm-diameter wells.

Organ cultures

Bovine tracheas from animals about 2 years old were obtained within 1 h after slaughter. Exposed surfaces were briefly washed with 70% (v/v) ethanol, and the tracheal rings were dissected free from mucosa and perichondrium under aseptic conditions. Transverse sections, 1 mm thick, were cut from the central portions of the rings, by using two fixed parallel blades. The sections thus obtained were of reproducible size and had an average wet weight of about 50 mg.

Four types of protocols were used for culture.

Pulse experiments. Sections were incubated in a 50 ml tube in 1 ml of Ham's F12 H medium (containing 20 μCi of [^3H]leucine and 10 μCi of [^{35}S]sulphate/ml)/section. To allow equilibration with radioisotope, the cultures were preincubated under gentle agitation at 3°C for 60–90 min before starting the pulse. The temperature was then rapidly raised by heating the tube for 15 min at 37°C in a water bath, whereafter the pulse was continued in a 37°C incubator. Sections were removed and immediately frozen at the times indicated.

In a subset of the pulse experiments, diffusion of radioisotopes into the cartilage as well as the time course of their early incorporation into macromolecules was studied. In this case the sections were preincubated in Ham's F12 H medium for 3 h at 37°C. At this time [^3H]leucine (20 μCi /ml) and [^{35}S]sulphate (10 μCi /ml) were added to the culture. Sections were then removed at the times indicated and immediately frozen.

Pulse-chase experiments. Sections were incubated in a 50 ml tube in medium containing radioisotopes as discussed above for pulse experiments. After 6 h the medium was replaced by Ham's F12 HA (2 ml/section) supplemented with leucine (2 mM) but not containing radioisotopes. Medium

was changed every 4 days. Sections were removed at the times indicated and immediately frozen.

In a separate experiment release of labelled macromolecules to the medium was studied. Both pulse and chase were done with one section in each well of a multi-well plate. Labelling was done with 1 ml of medium/section and chase with 2.5 ml/section, by the protocol described above for pulse-chase.

Isolation of cartilage proteins

Radiolabelled cartilage sections were cut into pieces with no side exceeding 2 mm. In one experiment the sections were instead cut into 0.2 mm-thick slices, with a Mickle gel slicer. Both procedures gave the same extraction yield. Each section was extracted under continuous stirring at 3°C for 24 h with 4 ml of 4 M-guanidinium chloride/50 mM-sodium acetate buffer, pH 5.8, containing the proteinase inhibitors 5 mM-benzamidinium chloride, 0.1 M-6-aminohexanoic acid, 10 mM-EDTA and 4 mM-*N*-ethylmaleimide. The extraction residue was pelleted by centrifugation for 15 min at 3000 g_{av} , and the extract was carefully removed. In some experiments the residue was extracted for two more 24 h periods, each time with 2 ml of the guanidinium chloride extraction buffer. Subsequently, no more labelled material could be released by guanidinium chloride extraction. The final residues were dissolved by incubation overnight at 60°C in 1 ml of 1 M-NaOH. The samples were neutralized by the addition of 0.3 ml of 5 M-acetic acid and adjusted to a final weight of 5 g by the addition of water. Duplicate samples were hydrolysed in 6 M-HCl at 100°C for 24 h, and the content of hydroxyproline was determined by the procedure of Stegeman & Stalder (1967). Other samples were taken for liquid-scintillation counting to determine the amount of radioactivity incorporated into unextractable protein. A ratio of scintillant (Instagel; Packard Instruments, Downers Grove, IL, U.S.A.) to sample of 10:1 was used for all determinations of radioactivity.

Extracts of cartilage sections were fractionated by density-gradient centrifugation in CsCl/4 M-guanidinium chloride. Solid CsCl and extra extraction solvent were added to give a density of 1.36 g/ml in a final volume of 13.5 ml. The samples were centrifuged in a Beckman 70.1 Ti angle rotor at 38 000 rev./min (100 000 g_{av} , r_{av} , 6.1 cm) for 60 h at 17°C. The tubes were divided in four fractions of equal volume with a Beckman tube-slicer.

The amount of radioactive macromolecules in extracts and CsCl-density-gradient fractions was determined by ethanol precipitation. Samples (50 μl) were diluted with 150 μl of water and precipitated with 1800 μl of 95% (v/v) ethanol. The precipitates were washed with additional ethanol and dissolved in

1 ml of 4 M-guanidinium chloride. Scintillant was added and the radioactivity determined. Free radioisotope in the sections was quantified by concomitant determination of the radioactivity remaining in the supernatant from the ethanol precipitation step. The radioactivity was always related to the amount of hydroxyproline present in the extraction residue.

Gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was performed as described by Neville (1971) on 3 mm-thick slab gels. The gels were 8.0% $\times$ 2.5%, where the first numeral (T) designates the total amount of monomer (% w/v) and the second numeral (C) designates the amount of *NN'*-methylenebisacrylamide, expressed as a fraction (% w/w) of total monomer in the gel casting solution. Samples of the pooled two top fractions from the CsCl density gradients, corresponding to 1 mg wet wt. of tissue, were precipitated with ethanol, as described above, redissolved in 0.5 M-sodium acetate buffer, pH 7.0, and again precipitated with ethanol to remove the last traces of CsCl and guanidinium chloride. The precipitates were then dissolved in 50 μ l of sample 'cocktail' (Neville, 1971) and incubated at 37°C for 2 h before electrophoresis. Samples to be reduced contained 5% 2-mercaptoethanol. The gels were stained for protein with Kenacid Blue R (BDH Chemicals, Poole, Dorset, U.K.). The positions of protein standards and proteins endogenous to samples were recorded with a Zeineh soft-laser densitometer. The lanes containing radioactive samples were then excised and sliced in 2 mm sections with a Mickle gel-slicer. Slices were dissolved by treatment with 500 μ l of 30% (w/v) H_2O_2 , either at 80°C for 2 h or at 37°C overnight. Scintillant was added and the radioactivity determined in a liquid-scintillation counter.

Results and discussion

Retention of the [3H]leucine-labelled macromolecules in the tissue

More than 99% of the incorporated [3H]leucine was retained in the cartilage when sections were labelled for up to 6 h. When identical sections were prelabelled for 6 h and then 'chased' for 190 h, only 3% of the total macromolecular radioactivity appeared in the medium. The [3H]leucine-labelled proteins were retained in the matrix of the sections in a manner similar to the situation *in vivo*, of importance for studies of the metabolism of these proteins. Because of the small proportion of the radioactive material released from the sections, media were not analysed in subsequent experiments.

Extraction of [3H]leucine-labelled macromolecules

About 50% of the [3H]leucine incorporated into the cartilage sections during labelling for up to 6 h was extracted with 4 M-guanidinium chloride. When sections prelabelled for 6 h were 'chased' for up to 188 h, the proportion of extractable radioactivity decreased to about 40%, even though the absolute amount of radioactivity decreased also in the residue. In separate experiments it was shown that the yield of 148 kDa protein was not higher when the guanidinium chloride concentration was increased to 5 or 6 M. The extraction yield of 3H -labelled proteins could be increased by about 25% of the total 3H label in the continuously labelled sections and by about 15% in the pulse-chase-labelled cartilage by repeated extraction with 4 M-guanidinium chloride (see the Experimental section). Most of the radioactive material recovered by a second extraction was high-molecular-weight polypeptides, distinct from the 148 kDa and 36 kDa proteins (results not shown), as shown by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The amount of [3H]leucine label in the 148 kDa and 36 kDa proteins was low compared with that in the first extract and appeared to be of a constant proportion, probably representing a small amount of first extract trapped in the extraction residue. Only the first guanidinium chloride extracts were analysed in subsequent experiments.

Kinetics of [3H]leucine and [^{35}S]sulphate incorporation

Cartilage sections were incubated with [3H]leucine and [^{35}S]sulphate and extracted with 4 M-guanidinium chloride. Macromolecules were separated from unincorporated radioisotope by ethanol precipitation, and the amounts of incorporated and of free radioisotope were determined. Labelled macromolecules appeared in the guanidinium chloride-extractable pool after less than 10 min, with both [3H]leucine (Fig. 1a) and [^{35}S]sulphate (Fig. 1b). The increase in both 3H - and ^{35}S -labelled macromolecules became linear with time after about 40 min and remained so for the duration of the experiment. When the linear portions of the curves were extrapolated to zero radioactivity, lag times of 20–25 min were obtained for both radioisotopes (Fig. 1). Determination of the radioactivity in the supernatants from the ethanol precipitation step indicated rapid equilibration of both radioisotopes between medium and cartilage sections, i.e. the amount of free radioisotope in the sections had already reached 70% of the plateau value after 5 min (Fig. 1).

The lag phase must therefore at least partly be due to factors other than isotope diffusion. In similar experiments, with slices of pig laryngeal cartilage, Hardingham & Muir (1972) demonstrated a delay of

40 min before ^{35}S -labelled proteoglycans became extractable. From this observation and radioautography results, they concluded that proteoglycans did not become extractable with guanidinium chloride until after secretion from the cells. The lag phase of 22 min that we observed for the extractable

Fig. 1. Kinetics of incorporation of ^3H leucine (a) and ^{35}S sulphate (b) into guanidinium chloride-extractable macromolecules

Radiolabelled cartilage sections were extracted with 4 M-guanidinium chloride/50 mM-sodium acetate buffer, pH 7.4, and the radioactivity in macromolecules (O) as well as free radioisotope (●) was determined after ethanol precipitation. The delay in appearance of labelled macromolecules in the extractable fraction was determined by extrapolation of the linear portion of the incorporation curves.

Fig. 2. Fractionation of ^3H leucine- and ^{35}S sulphate-labelled macromolecules by CsCl-density-gradient centrifugation in 4 M-guanidinium chloride

The example given is of sections labelled for 6 h, before extraction with 4 M-guanidinium chloride/50 mM-sodium acetate buffer, pH 5.8. The separations obtained at other labelling times and in chase experiments were closely similar. —, ^3H Leucine; ----, ^{35}S sulphate; O—O, density (g/ml).

Fig. 3. Accumulation of ^3H leucine-labelled macromolecules in the tissue

Synthesis of proteoglycans (●) and proteins (O) was determined as the ^3H radioactivity in the D1 fraction and the pooled D3 and D4 fractions respectively obtained by CsCl-density-gradient centrifugation of 4 M-guanidinium chloride extracts (Fig. 2).

[^3H]leucine-labelled protein is similar to the time from introduction of radioisotope to export of labelled protein when determined for typical secretory proteins. For example, the lag phase for [^3H]leucine-labelled serum albumin has been deter-

mined to be 19–21 min in isolated hepatocytes and 22–26 min in perfused rat liver (Feldhoff *et al.*, 1977). It therefore appears that the labelled molecules in the guanidinium chloride extracts are predominantly extracellular in origin.

Fig. 4. Fractionation of [^3H]leucine-labelled cartilage proteins by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis

Samples were obtained by ethanol precipitation of the pooled D3 and D4 fractions from CsCl-density-gradient centrifugation of 4 M-guanidinium chloride extracts. Cartilage sections had been labelled with radioisotope for the times indicated in the Figure. Electrophoresis was done on samples corresponding to 1 mg wet wt. of tissue, either reduced with 2-mercaptoethanol (a–d) or not reduced (e–h). Radioactivity profiles were obtained by liquid-scintillation counting of solubilized gel slices. The amounts of [^3H]leucine incorporated into individual proteins were calculated as indicated by the hatched areas. In the lower frame examples are given of the protein profiles obtained by Kenacid Blue staining of the samples reduced with 2-mercaptoethanol (left) or not reduced (right). Link 1 refers to the link protein of highest molecular weight.

Accumulation of [^3H]leucine-labelled macromolecules in the tissue

Cartilage sections were incubated with [^3H]leucine for up to 6 h and extracted with 4M-guanidinium chloride. The extracts were fractionated by direct dissociative CsCl-density-gradient centrifugation. By using a starting density of 1.36 g/ml, all proteins were recovered in the two top fractions (D3 + D4), whereas virtually all proteoglycans were present in the bottom D1 fraction (Heinegård *et al.*, 1981). An example of the fractionation obtained is shown in Fig. 2. The rates of [^3H]leucine incorporation into protein and proteoglycan were determined (Fig. 3). At all times more than 90% of the radioactivity was present in the protein fractions (D3 + D4), and about 5% was incorporated into proteoglycan core protein (D1). Labelling with a single radioactive amino acid does not necessarily give a correct measure of the relative rates of synthesis of different proteins, as their specific radioactivities may vary. Still, it appears certain that the proteoglycan core protein constitutes only a minor portion of the total protein synthesized.

Macromolecules in samples of the protein-rich D3 + D4 fractions were precipitated with ethanol and electrophoresed on sodium dodecyl sulphate/polyacrylamide gels (Fig. 4). The 148 kDa protein, the link proteins and the 36 kDa protein were identified by comparison of electrophoretic mobilities of radioactive components with those of corresponding Kenacid Blue-stained bands (Fig. 4, lower frame), as well as with the mobilities of purified preparations of these proteins. To substantiate further the identification of the proteins, electrophoresis was done under reducing (Fig. 4, traces *a-d*) as well as under non-reducing (Fig. 4, traces *e-h*) conditions. Additional criteria for identity were obtained for the 148 kDa protein, where radiolabelled molecules co-migrated with the intact trimeric protein as well as with the reduced subunits. Both the 148 kDa protein and the 36 kDa protein could already be distinguished as major components after 1.5 h of pulse (Fig. 4, traces *a* and *e*). These proteins were best separated from other components by using non-reducing conditions for the electrophoresis. The major link protein could under reducing conditions be seen as a shoulder on the peak of the 148 kDa-protein subunits (Fig. 4, traces *a-d*), and more clearly as a separate peak under non-reducing conditions (Fig. 4, traces *e-h*). Some unidentified radiolabelled material with a mobility somewhat lower than that of the subunits of the 148 kDa protein was observed. It appears as two major peaks, more clearly visible after long labelling times (Fig. 4, traces *c* and *d*). The mobility of this material is not significantly changed by reduction. It seems to be of rather high specific radioactivity, as

Fig. 5. Accumulation of individual [^3H]leucine-labelled proteins in the tissue

The rates of synthesis of the 148 kDa protein (●), the link proteins (□) and the 36 kDa protein (○) were calculated from sodium dodecyl sulphate/polyacrylamide-gel electrophoretograms, indicated by the hatched areas in Fig. 4.

Fig. 6. Elimination of [^3H]leucine-labelled macromolecules from the tissue

The rate of elimination of proteoglycans (D1 fraction, ●) and proteins (pooled D3 and D4 fractions, ○) was determined as the decrease in [^3H]leucine during a non-radioactive chase of cartilage sections that had been prelabelled for 6 h.

this region of the gel is only weakly stained by Kenacid Blue (Fig. 4, lower frame).

The radioactivity corresponding to the 148 kDa protein, the link proteins and the 36 kDa protein was calculated as indicated in Fig. 4 (traces e–h) and plotted versus labelling time (Fig. 5). Even though the rather low amounts of radioactivity obtained with the shorter labelling times were difficult to determine with accuracy, the kinetics of the synthesis of the individual proteins appeared to be linear and were similar to those of the whole protein fraction and of the proteoglycan core protein. This indicates that neither of the proteins is a degradation fragment of other molecules. Most label was accumulated in the 36 kDa protein. It should, however, be noticed that this protein has a higher

content of leucine (about 140 residues/1000 residues) than any of the other proteins quantified (M. Paulsson, Y. Sommarin & D. Heinegård, unpublished work).

Elimination of [^3H]leucine-labelled macromolecules from the tissue

Cartilage sections were pulse-labelled with [^3H]leucine for 6 h and then 'chased' with non-radioactive Ham's F12 HA medium containing 2 mM-leucine for up to 188 h. The sections were extracted with 4 M-guanidinium chloride and centrifuged in CsCl density gradients as described above. A plot of [^3H]leucine label in guanidinium chloride-extractable proteins (D3 + D4) and proteoglycans (D1) as a function of chase time is shown in Fig. 6. No

Fig. 7. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of the extractable [^3H]leucine-labelled cartilage proteins after different periods of chase

The sample preparation and electrophoresis was as described in the legend to Fig. 4, but only radioactivity profiles of the non-reduced samples are shown. The length of the non-radioactive chase is given for each sample in the Figure. The hatched areas in the electrophoretograms were used for calculation of radioactivity in individual proteins.

significant turnover of proteoglycans was observed during an experimental period of about 1 week. In contrast, the radioactivity in proteins decreased to about half in this period. The decrease was especially marked during the first 50 h of the chase. Only small amounts of labelled macromolecules could be recovered from the medium. Therefore most of the decrease must be due either to degradation of labelled proteins to fragments too small to be precipitated with ethanol or possibly to integration of labelled proteins into the guanidinium chloride-insoluble material. The latter alternative is unlikely, as the radioactivity in the extraction residue decreased during the chase at a rate only slightly lower than that of the guanidinium chloride extract.

Samples of the protein-rich top fractions were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. The radioactivity profiles from the non-reducing gels (Fig. 7) were used to quantify individual proteins. The elimination rates of the various proteins were markedly different (Fig. 8). Whereas the 36 kDa protein had a relatively rapid turnover, the 148 kDa protein was eliminated very slowly, with a turnover rate more similar to that of

the proteoglycans. The slow elimination of the 148 kDa protein explains why larger amounts of this protein accumulate in tracheal cartilage (Fig. 4, lower frame), even though its rate of synthesis is similar to that of the other proteins. Interestingly, the link proteins, although quantified with greater error (cf. Fig. 7), appear to have a higher turnover rate than the proteoglycans. It is possible that excess link proteins synthesized are eliminated at a higher rate (Kimura *et al.*, 1980).

General discussion

Our initial experiments were aimed at identification of the 148 kDa and 36 kDa proteins in cultures of isolated bovine tracheal-cartilage chondrocytes, grown either as primary cells in suspension or in monolayer. Except for the use of chondrocytes from 6-month-old animals rather than foetuses, the culture conditions were those described previously (Björnsson & Heinegård, 1981). The cells were derived from a tissue known to contain large amounts of these proteins (Paulsson & Heinegård, 1982), and they retained the chondrocyte phenotype as expressed by production of cartilage proteoglycans and link proteins. Nevertheless we could demonstrate neither the 148 kDa protein nor the 36 kDa protein in the media or in guanidinium chloride extracts of the cells, by using sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. In contrast, we could demonstrate their biosynthesis by using organ culture of cartilage sections, as described in the present paper. As the methodology used was identical except for the culture system, it appears that the synthesis of these proteins can be suppressed in isolated chondrocytes. Possibly production of the 148 kDa and 36 kDa proteins is induced by factors such as the interaction of the cells with a surrounding matrix.

Although having the advantage of allowing studies of cell function in an intact matrix, the use of cartilage sections presents some limitations. Thus in organ culture one problem is to minimize the variability between different cultures. Therefore several precautions were taken to achieve good repeatability in experiments. Sections were only taken from the central portion of tracheal rings from animals of a defined age. In most experiments measurements were based on pools of more than one section. Some variability in the mass of the cartilage sections can, however, not be eliminated. To correct for this variation, we found that hydroxyproline contents of the extraction residues provided reliable results, as could be expected since collagen is not likely to be lost to the medium, even during prolonged culture. By taking all these precautions, the relative rates of synthesis and turnover observed for the different macromolecules were highly reproducible between experiments.

Fig. 8. Elimination of individual [^3H]leucine-labelled proteins from the tissue

The rates of elimination of the 148 kDa protein (●), the link proteins (□) and the 36 kDa protein (○) were calculated from sodium dodecyl sulphate/polyacrylamide-gel electrophoretograms, as indicated by the hatched areas in Fig. 7.

One drawback remaining is that the extraction of labelled molecules is selective, i.e. some classes of proteins are extracted in good yield, whereas others are retained in the residue. It is therefore not possible to ascertain the nature of all the labelled material. Secondly, it is not possible to distinguish between matrix proteins and cellular proteins with the same certainty as when isolated cells are used. The kinetic data do, however, strongly indicate that most of the labelled proteins in the guanidinium chloride extracts are derived from the extracellular matrix. It should also be noted that the 148 kDa and 36 kDa proteins are major biosynthetic products of the tracheal cartilage chondrocyte, each incorporating considerably more [³H]leucine than does the cartilage proteoglycan core protein.

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