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Morphologic and Metabolic Development of Human Fetal Epiphyseal Chondrocytes in Primary Culture

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ABSTRACT. Primary chondrocyte culture was carried out after enzymatic digestion of femoral and tibial epiphyseal cartilage of human fetuses, collected with informed parental consent within 12 h postmortem. Chondrocytes were cultured in HAM F-12 medium with penicillin and 15% serum. Three types of serum were used: human placental cord serum (HPS), fetal calf serum, and human male adult serum. Chondrocytes cultured with HPS grew as monolayers, formed abundant colony groups with a highly metachromatic pericellular matrix, and floating round cells were observed in the culture medium. By the 10th day of culture the great majority of proteoglycans present in the culture medium were found as aggregates. Chondrocytes cultured with fetal calf serum or human male adult serum grew as monolayers, were polygonal in shape, and the pericellular matrix was far less developed than in HPS cultures. By the confluent phase of growth, only approximately a third of the proteoglycans present in the culture medium were found as aggregates. Chondrocytes cultured with HPS proliferated significantly more rapidly than those cultured with fetal calf serum or human male adult serum. The results suggest that certain, as yet unidentified, factors are present in sufficient amount in HPS to allow chondrocytes in culture to retain phenotypic morphological and biochemical characteristics. HPS also facilitates growth of human fetal epiphyseal chondrocytes in culture. Primary human

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fetal epiphyseal chondrocyte culture could be a suitable experimental tool for the *in vitro* study of biochemical characteristics of cartilage and factors involved in fetal cartilage metabolism. (*Pediatr Res* 19: 720–727, 1985)

Abbreviations

HPS, human placental cord serum
FCS, fetal calf serum
HAS, human adult male serum
CPC, cetyl piridinium chloride

Human cartilage is a widespread tissue in the body with well-defined functions according to its location. The role of epiphyseal cartilage in promoting skeletal growth has been recognized since the endochondral calcification process was first described (1). Skeletal growth is particularly important during human fetal life. However, little is known of the biochemical characteristics of human fetal epiphyseal cartilage and the factors involved in cartilage metabolism.

Recently, chondrocyte cultures have been used for the *in vitro* study of biochemical characteristics of cartilage (2–10) and factors involved in cartilage metabolism (11–19).

The aim of this work was to study the morphological and metabolic development of human fetal epiphyseal chondrocytes in primary culture.

METHODS

Chondrocyte culture. Primary chondrocyte culture was carried out with subtle modification of the method of Corvol *et al.* (20). Tibial and femoral epiphyseal cartilage from human fetuses (12–32 wk old), products of miscarriage or premature delivery, were collected with informed parental consent within 12 h postmortem (Table 1). Fetuses with skeletal malformations were excluded. Under sterile conditions the epiphyseal cartilage was cleaned of surrounding tissue and the articular surface and calcifying zones were removed by dissection. The remaining cartilage was cut in thin slices and finely chopped with a surgical blade. This tissue mixture was digested at 37° C for 30 min with trypsin (Difco Laboratories, Detroit, MI) 0.2% in HAM F-12 medium (Grand Island Biological Co., Grand Island, NY) followed by two collagenase (Worthington Biochemical Corp, Freehold, NJ) digestions, 0.2%, in HAM F-12 medium, the first for 60 min and the second for 30 min. A pure chondrocyte suspension was obtained and chondrocytes were plated at different cell densities (0.5×10^5, 1×10^5, and 2×10^5) in 25 cm^2 plastic tissue culture flasks (Corning Glass Works, Corning, NY) with 4 ml of HAM F-12 medium supplemented with penicillin (25 IU/ml) and 15% serum (Table 1). Three types of serum were used: HPS, HAS, and FCS and chondrocytes were cultured in four different serum conditions (Table 1). The flasks were maintained at 37° C in an atmosphere of 5% CO_2 in air with humidity and the cultures were fed by changing the medium every 2 days.

HPS was collected from placental cord of normal deliveries at term, passed through 0.22 μm Millex-GS filters (Millipore, Molsheim, France) and kept frozen at −20° C in sterile conditions until used. Fourteen different batches were made, one for each culture (Table 1). HAS was obtained from five male adult volunteers, filtered through 0.22 μm Millex-GS filters and frozen at −20° C in sterile conditions until used; one pooled batch was made. Four different batches of FCS (Grand Island Biological

Table 1. *Human fetuses used for chondrocyte cultures*

				Morphological studies				
				Initial cell density plated				
No.	Age (wk)	Sex	Serum culture conditions	0.5×10^5	1×10^5	2×10^5	Cell growth studies (days of culture)	Proteoglycan characterization
1	12	M	15% HPS		+			+
2	14	M	10% HPS + 5% FCS		+			+
			15% FCS		+			+
3	17	M	15% HPS	+	+	+		
			15% FCS	+	+	+		
4	18	F	15% HPS	+	+	+		
			15% FCS	+	+	+		
5	18	M	10% HPS + 5% FCS		+		8, 10, 12, 14	+
			15% FCS		+		8, 12, 14	+
6	20	F	15% HPS		+		6, 8	+
			10% HPS + 5% FCS		+		6, 8, 10, 12, 15, 18	+
			15% FCS		+		6, 8, 10, 12, 15, 18	+
			15% HAS		+			+
7	23	M	10% HPS + 5% FCS		+		8, 12	
			15% HAS		+		8, 12	+
8	24	F	15% HPS		+		6, 8	
			10% HPS + 5% FCS		+		6, 8, 10	
			15% FCS		+		6, 8, 10, 12	
9	26	F	15% HPS		+			
			10% HPS + 5% FCS		+			
			15% FCS		+		8, 12	+
			15% HAS		+		8, 12	+
10	27	F	10% HPS + 5% FCS		+		8, 10, 14	
			15% FCS		+		8, 10, 14	
11	27	F	10% HPS + 5% FCS	+	+	+		
12	28	M	10% HPS + 5% FCS		+			
13	32	F	15% HPS		+			+
			10% HPS + 5% FCS		+			+
14	32	M	10% HPS + 5% FCS	+	+	+	8, 14	
			15% FCS	+	+	+	8, 12, 14	

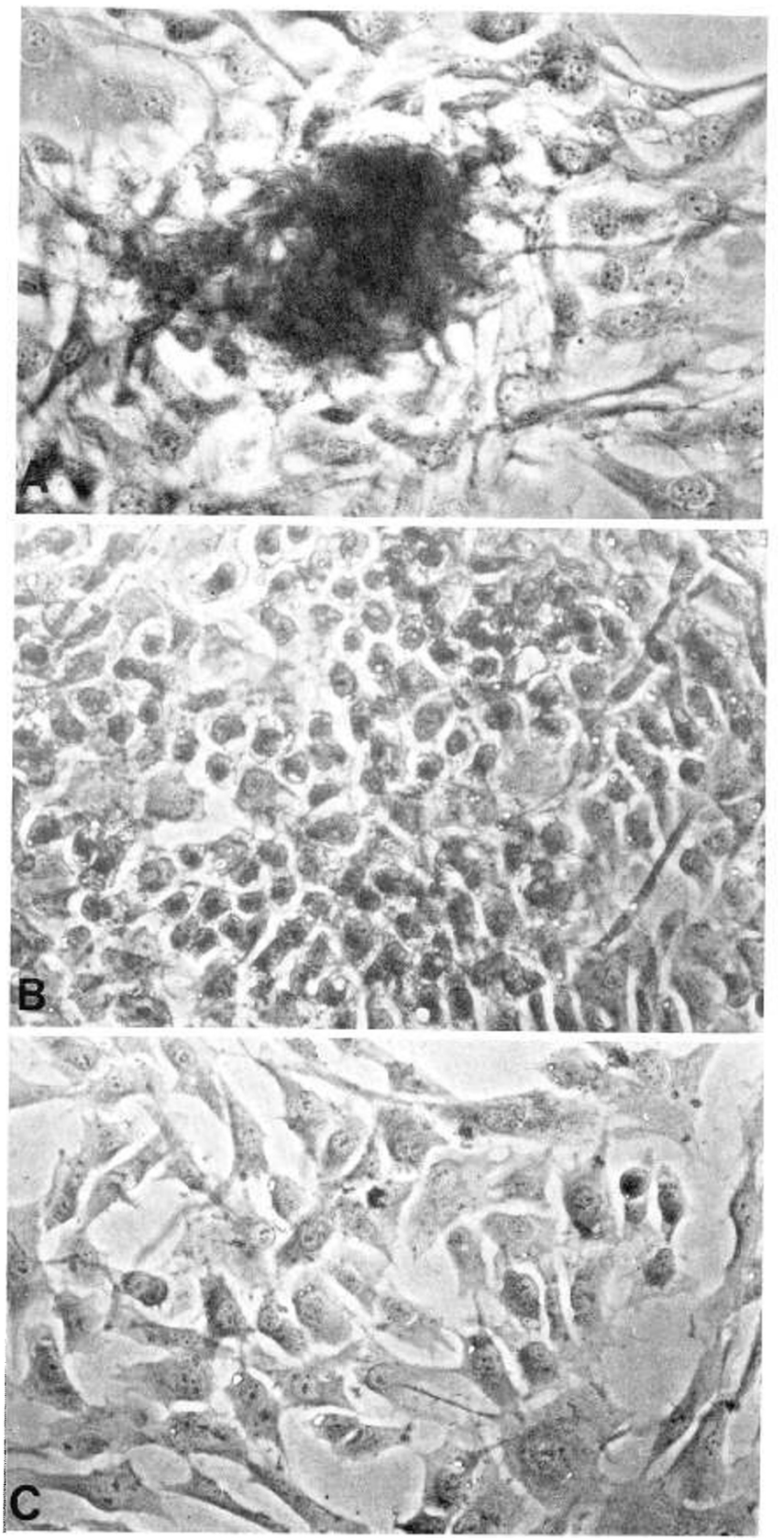

Fig. 1. The phase-contrast picture of chondrocytes in primary culture for a 20-wk-old fetus. *A*, chondrocytes cultured with 15% HPS, 10th day of culture. Note the intense metachromatic pericellular matrix of one colony group. *B*, chondrocytes cultured with 10% HPS plus 5% FCS, 12th day of culture. Chondrocytes are polygonal in shape. Note grouping in colonies with pericellular metachromatic matrix. *C*, chondrocytes cultured with 15% FCS by the confluent phase of growth. Chondrocytes were polygonal and the metachromatic pericellular matrix was far less developed than in 15% HPS cultures.

Co, Grand Island, NY) were used: batch 1 in cultures 1, 2, and 6; batch 2 in cultures 3, 4, 5, and 11, batch 3 in cultures 7, 9, 10, and 12, batch 4 in cultures 8, 13, and 14.

Morphological studies. The morphology of chondrocytes plated at different cell densities and cultured in different serum conditions (Table 1) were observed by phase contrast microscopy. On the 10th day of culture for chondrocytes cultured with 15% HPS and at the beginning of confluency for chondrocytes cultured in the other serum conditions, the culture medium was aspirated and the cells were stained directly with 0.25% aqueous toluidine blue in sodium acetate-acetic buffer, pH 4.5. The morphology and metachromacy of chondrocytes were then observed by phase contrast microscopy.

Cell growth studies. One hundred thousand chondrocytes released by enzymatic digestion from epiphyseal cartilage were plated in a 25 cm^2 culture flask with 4 ml of HAM F-12 medium, penicillin (25 IU/ml), and 15% serum. Chondrocytes were cultured in four different serum conditions: 15% HPS, 10% HPS plus 5% FCS, 15% FCS, and 15% HAS (Table 1). The cultures were fed by changing the medium every 2 days. As mentioned above the HPS batches used in these cultures differed from one culture to another. A common batch of HAS and four batches of FCS were used.

On different days of culture (Table 1) three flasks were trypsinized (0.25% trypsin in phosphate-buffered saline, 37° C, 10 min). From each separate flask cells were collected, rinsed in phosphate-buffered saline, resuspended in order to obtain a single cell suspension, counted by means of a hemocytometer, centrifuged, and the DNA measured following Burton's method (21).

Characterization of proteoglycans synthesized by epiphyseal cartilage. Fetal epiphyseal cartilage was collected under sterile conditions, cut in fine slices, and chopped by hand with a surgical blade. An average of 0.5 g of tissue was incubated in 4 ml Dubelcco's (Grand Island Biological Co.) with 6 μCi of $Na_2{}^{35}SO_4$ (SA 232.44 mCi/mmol, New England Nuclear, Boston, MA) for 20 h. The medium was recovered and labeled proteoglycans were precipitated and eluted in a Sepharose 2B (Pharmacia Fine Chemicals AB, Upssala, Sweden) column according to Wiebkin's method (22). CPC 5% (Sigma Chemical Co, St. Louis, MO) was added to the medium to a final concentration of 1% in the presence of 50 mM EDTA (E Merck AG, Darmstadt, FRG), 0.5 M 6-aminohexanoic acid (E Merck AG), and 25 mM benzamidine HCl (Sigma Chemical Co.) as proteinase inhibitors, after which the mixture was gently shaken overnight at room temperature. The CPC precipitates thus formed were centrifuged at 3640 × *g* at 15° C, washed once with 5 ml of 0.1 M Na_2SO_4, then dissolved in 2 ml of 1.25 M $MgCl_2$; the material was reprecipitated with 8 ml of ice-cold ethanol and left at 4° C for 16 h. The ethanol precipitates were centrifuged at 3640 × *g* at 4° C for 10 min, redissolved in 0.5 M sodium acetate (pH 6.8), and samples containing approximately 3000 cpm of labeled proteoglycans were applied to a column (30 × 0.8 cm) of Sepharose 2B. The column was eluted with 0.5 M sodium acetate (pH 6.8) at a flow rate of 10 ml/h. Fractions of 0.350 ml were collected and radioactivity counted in 10 ml of Unisolve (Koch Light Laboratories, Colnbrook Berks, England) in a β-counter (Isocap 300-Searle) for 10 min.

Characterization of proteoglycans synthesized by chondrocytes in primary culture. On the 10th day of culture for chondrocytes cultured with 15% HPS and at the beginning of the confluent phase of growth for chondrocytes cultured in the other different serum conditions, the culture medium was aspirated and replaced by 4 ml of Dubelcco's serum-free medium with penicillin (25 IU/ml). Twenty-four hours later the culture medium was again replaced by 4 ml of Dubelcco's antibiotic and serum-free medium with 6 μCi of $Na_2{}^{35}SO_4$. After 20 h of incubation the culture medium and the cells were collected separately. The medium was aspirated and the cells were washed twice with 0.9% NaCl and collected in 2 ml of distilled water. Labeled proteoglycans present in the culture medium and the cells were precipitated by CPC and ethanol as described above. Samples containing 3000–5000 cpm of labeled proteoglycans were applied and eluted in a Sepharose 2 B column as described above.

Statistics. Mean values and SDs were calculated. The differences between groups were analyzed by Student's *t* test.

RESULTS

Chondrocytes of human fetal epiphyseal cartilage are able to grow in primary culture, but culture characteristics differ with the type of serum used.

Morphological studies. Chondrocytes cultured with 15% HPS had become attached to the bottom of the flask by the second day after plating. They developed as monolayers, were polygonal in shape, and formed separate colony groups in which cells were surrounded by an intense metachromatic pericellular matrix (Fig. 1). Between the 6th and 8th days of culture single round cells and clusters of cells appeared floating in the culture medium.

Table 2. *Means ± SD of total cell number and DNA on different days of culture; chondrocytes were plated at initial cell density of 1 × 10^5 cells per flask and cultured under varied serum conditions (+)*

Day of culture	Serum culture conditions	No. of flasks studied	Cell no. (×10^3)	DNA (μg)
6	15% HPS	6	371.6 ± 39.7**	3.50 ± 0.60***
	10% HPS + 5% FCS	6	368.3 ± 44.4**	3.28 ± 0.29****
	15% FCS	6	225.5 ± 53.4	2.30 ± 0.34
8	15% HPS	6	766.1 ± 117.8***	7.03 ± 1.14****
	10% HPS + 5% FCS	18	758.3 ± 162.5****	7.07 ± 1.08****
	15% FCS	18	434.3 ± 105.6	3.82 ± 0.83
	15% HAS	6	423.3 ± 37.7^{NS}†	3.91 ± 0.31^{NS}
10	10% HPS + 5% FCS	12	1529.7 ± 240.8****	12.11 ± 1.51****
	15% FCS	9	674.2 ± 157.6	6.11 ± 1.60
12	10% HPS + 5% FCS	9	2513.3 ± 275.3****	16.34 ± 1.02****
	15% FCS	15	1113.0 ± 398.0	8.96 ± 2.24
	15% HAS	6	1094.5 ± 177.4^{NS}	8.51 ± 1.05^{NS}
14	10% HPS + 5% FCS	9	2602.2 ± 614.4*	17.56 ± 1.98****
	15% FCS	9	2022.2 ± 281.7	14.01 ± 1.17

* $p < 0.02$; ** $p < 0.01$; *** $p < 0.005$; **** $p < 0.001$ *versus* 15% FCS cultures.

† Not significant *versus* 15% FCS cultures.

The number of floating cells increased with time and colonies never became confluent even when the primary culture was maintained for 30 days or more. When floating chondrocytes were replated with 15% HPS in secondary culture they developed characteristics similar to those observed in primary culture.

Chondrocytes cultured with 15% FCS were adhering to the flask by the second day, grew as monolayers, and were polygonal in shape. The pericellular matrix was far less developed than in 15% HPS cultures (Fig. 1). When culture was prolonged beyond confluency the chondrocytes acquired a fibroblast-like shape. No cells or clusters of cells were observed floating in the medium during culture. When floating cells from primary HPS cultures were replated with 15% FCS, they developed in the same way as FCS primary cultures.

Chondrocytes cultured with 15% HAS developed similarly to chondrocytes cultured with 15% FCS.

Chondrocytes cultured with 10% HPS plus 5% FCS were attached to the flask by the second day, grew as monolayers, were polygonal in shape, and formed metachromatic colony groups (Fig. 1). If the culture was extended beyond confluency, fibroblast-like cells appeared among the colonies. At confluency, few cells were observed floating in the culture medium.

These morphological serum-dependent characteristics of human fetal epiphyseal chondrocytes in culture were observed in all three initial cell densities plated (0.5×10^5, 1×10^5, and 2×10^5 cells per flask) and at any gestational age studied (12–32 wk).

Cell growth studies. Table 2 shows the mean (± SD) number of cells and DNA present on different days of culture in 25 cm^2 flasks when chondrocytes were plated at initial cell density of 1×10^5 cells per flask, and cultured under different serum conditions and with different serum batches. Chondrocytes cultured with 15% HPS or 10% HPS plus 5% FCS proliferate significantly ($p < 0.01$) more rapidly than those cultured with 15% FCS or 15% HAS. No significant differences were observed between the proliferation rates of chondrocytes cultured with 15% HPS and those cultured with 10% HPS plus 5% FCS. Neither were there significant differences between the proliferation rate of chondrocytes cultured with 15% FCS and those cultured with 15% HAS.

Figure 2 shows the cumulative growth curve of 20-wk fetal epiphyseal chondrocytes in primary culture during the first 18 days of culture. Cells were plated at initial cell density of 1×10^5 cells per flask and cultured in parallel with 10% HPS plus 5% FCS or 15% FCS. Chondrocytes cultured with 10% HPS plus 5% FCS proliferate significantly ($p < 0.02$) more rapidly than those cultured with 15% FCS.

Characterization of proteoglycans synthesized by chondrocytes in cartilage incubations and in primary culture. Human fetal epiphyseal chondrocytes in primary culture synthesized proteoglycans. After precipitation by CPC and ethanol, approximately 80% of the total labeled proteoglycans were present in the culture medium and the remaining 20% in the cellular-pericellular pool. Toluidine blue staining of the cellular-pericellular pool is shown in Figure 1.

Figure 3 shows the elution profile in a Sepharose 2B column of labeled proteoglycans precipitated from the incubation medium of epiphyseal cartilage and from the culture medium of 20-wk fetal chondrocytes cultured under different serum condi-

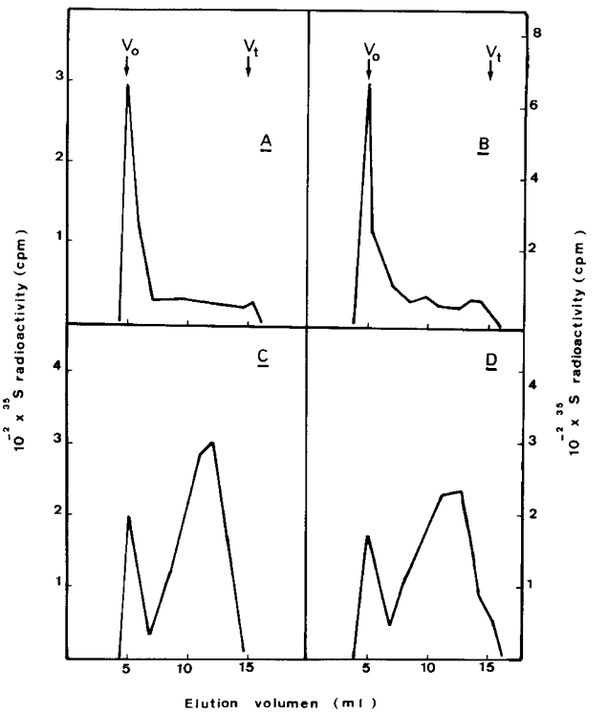

Fig. 3. Sepharose 2B elution profile of labeled proteoglycans from the incubation medium of epiphyseal cartilage and from the culture medium of epiphyseal chondrocytes in primary culture, for a 20-wk-old fetus. V_o is the void volume and V_t is the total volume of the column. *A*, labeled proteoglycans from the incubation medium of epiphyseal cartilage. *B*, labeled proteoglycans from the culture medium of chondrocytes cultured with 15% HPS. *C*, labeled proteoglycans from the culture medium of chondrocytes cultured with 15% FCS. *D*, labeled proteoglycans from the culture medium of chondrocytes cultured with 15% HAS.

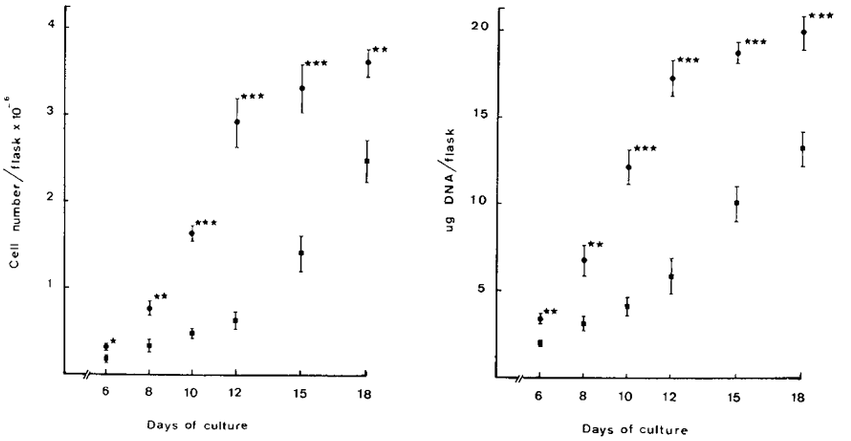

Fig. 2. The cumulative growth curve of fetal epiphyseal chondrocytes in primary culture during the first 18 days of culture, for a 20-wk-old fetus. Chondrocyes were plated at initial cell density of 1×10^5 cells per flask and cultured in parallel with 10% HPS plus 5% FCS (●) or 15% FCS (■). *Points* and *bars* are mean ± SD of three different flasks. $*p < 0.02$; $**p < 0.005$; $***p < 0.001$ *versus* 15% FCS culture.

tions. Labeled proteoglycans from 15% HPS culture showed the same elution profile as labeled proteoglycans from cartilage incubations, with the great majority eluting as aggregates in the void volume. However, only approximately a third of labeled proteoglycans from 15% FCS or 15% HAS cultures eluted as aggregates in the void volume and the remainder eluted in monomer form.

Figure 4 shows the elution profile of labeled proteoglycans precipitated from the cellular-pericellular pool of chondrocytes cultured under different serum conditions, for the same 20-wk-old fetus. Approximately a third of labeled proteoglycans from 15% HPS culture eluted as aggregates in the Vo and the remainder eluted in monomer form. However, labeled proteoglycans from 15% FCS culture eluted mainly in monomer form.

Table 3 shows the percentage of labeled proteoglycans eluting in the aggregate and monomer forms after chromatography in a Sepharose 2B column. Results from cartilage incubations and chondrocytes cultured under different serum conditions are quoted according to fetal age and are similar to those described above for a 20-wk-old fetus.

Recovery of radioactivity from the columns was higher than 70%.

DISCUSSION

Chondrocytes in culture need growth factors to develop, and FCS is widely used as a source of growth factors. As FCS is a heterologous serum for human fetal chondrocytes, HPS and HAS also were used in our cultures. Certain morphological and biochemical characteristics of human fetal epiphyseal chondrocytes cultured with these different types of serum were characterized.

Chondrocytes in culture grew as monolayers in all three sera, but in the presence of HPS, chondrocytes formed colony groups with an intense metachromatic pericellular matrix, and a considerable number of cells and clusters of cells were observed floating in the medium. These floating cells were living cells as shown by their capacity to develop with similar morphological characteristics in secondary culture as in primary culture. Corvol *et al.* (20) reported the ability to form colonies in primary culture as characteristic for chondrocytes from the proliferative zone of epiphyseal growth plate of immature rabbits. Yasumoto *et al.* (23) also reported that chick embryo sternal chondrocytes are able to develop in culture as floating cells retaining phenotypic characteristics. In our cultures, chondrocytes form colony groups and floating cells in the presence of HPS in the culture medium. These morphological characteristics were observed in all three initial cell densities plated and at any gestational age studied. This suggests the presence of unidentified factors in sufficient quantity in HPS (but not in FCS or HAS) to facilitate development of these morphological characteristics by human fetal epiphyseal chondrocytes in culture.

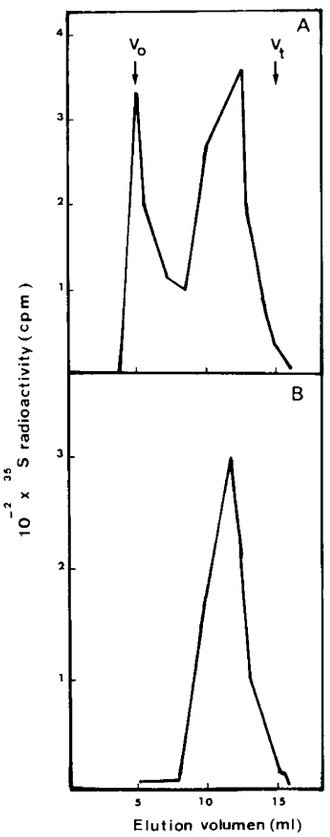

Fig. 4. Sepharose 2B elution profile of labeled proteoglycans from the cellular-pericellular pool, for a 20-wk-old-fetus. V_o is the void volume and V_t is the total volume of the column. *A*, labeled proteoglycans from 15% HPS culture. *B*, labeled proteoglycans from 15% FCS culture.

Human placental cord serum also contains factors (not apparently present in FCS or HAS) which enhance growth of human fetal epiphyseal chondrocytes in culture. Chondrocytes cultured with 15% HPS formed colony groups with a well-developed metachromatic pericellular matrix and it was often difficult to attain total cell release by trypsinization. Furthermore, in these cultures, from the 8th day onward, an increasing number of floating cells were lost when the medium was changed for culture feeding. For these reasons, only the cumulative growth curve was studied during the first 8 days of culture. Neither of these two problems was encountered when chondrocytes were cultured with 10% HPS plus 5% FCS, 15% FCS or 15% HAS, and it was therefore possible to study the cumulative growth curve during the first 18 days of culture.

Proteoglycans together with collagen are the major components of cartilage matrix. Proteoglycans in cartilage are present as large aggregates where many monomers are bound to a single large molecule of hyaluronic acid, and this union is stabilized by link proteins of small molecular weight (24). Human fetal chondrocytes in primary culture synthesized proteoglycans which are mainly found extracellularly in the culture medium. Sepharose 2B gel chromatography of labeled proteoglycans synthesized by chondrocytes in primary culture differed depending on the serum in which the chondrocytes were cultured. Labeled proteoglycans from the culture medium of chondrocytes cultured with 15% HPS were present principally as aggregates as was the case with the labeled proteoglycans from the incubation medium of epiphyseal cartilage. This indicates that human epiphyseal chondrocytes in primary culture retain this phenotypic characteristic even after 48 h of serum deprivation. However, only approximately a third of labeled proteoglycans from the culture medium of chondrocytes cultured with FCS or HAS were present as aggregates.

Labeled proteoglycans from the cellular-pericellular pool of chondrocytes cultured with FCS or HAS were found mainly as monomers. Labeled proteoglycans from the same pool of chondrocytes cultured with 15% HPS, while appearing as monomers, also were present (about 30%) as aggregates. Kimura *et al.* (5) and Björnsson and Heinegard (7) showed that in chondrocyte cultures, proteoglycan monomers, and hyaluronic acid were exported separately from the cell and combined extracellularly to form proteoglycan aggregates. In our cultures, chondrocytes cultured with 15% HPS formed a well-developed metachromatic pericellular matrix. We can assume that labeled proteoglycans came from two different sources: from inside the cells, where proteoglycans were present as monomers, and from the pericellular matrix, where proteoglycans were present as aggregates. However, when chondrocytes were cultured with HAS or FCS the pericellular matrix was far less developed and it can be assumed that labeled proteoglycans were mainly of intracellular origin.

Table 3. *Percentage of labeled proteoglycans present in aggregate and monomer forms for cartilage incubations and chondrocytes cultured under different serum conditions*

No. culture	Fetal age (wk)	Culture medium		Cellular-pericellular pool	
		Aggregates	Monomers	Aggregates	Monomers
Cartilage incubations					
	14	86.50	13.50		
	20	88.70	11.30		
	24	89.50	10.50		
	28	84.76	15.24		
Chondrocytes cultured with 15% HPS					
1	12	80.80	19.20	30.40	69.60
6	20	82.50	17.50	33.20	66.80
13	32	83.70	16.30	35.10	64.90
Chondrocytes cultured with 10% HPS + 5% FCS					
2	14	71.14	28.80	16.36	83.63
5	18	75.10	24.90	15.20	84.80
6	20	71.57	28.42	18.50	81.50
13	32	69.63	30.36	16.87	83.12
Chondrocytes cultured with 15% FCS					
2	14	22.80	77.20	8.40	91.60
5	18	22.60	77.40	8.00	92.00
6	20	23.20	76.80	10.50	89.50
9	26	26.24	73.75	9.60	90.40
Chondrocytes cultured with 15% HAS					
6	20	21.30	78.70	10.30	89.70
7	23	25.50	74.50	8.50	91.50
9	26	17.45	82.50	9.10	90.90

In conclusion, human fetal epiphyseal chondrocytes can be grown in primary culture and will retain morphological and biochemical phenotypic characteristics. However, to develop these characteristics, certain as yet unidentified factors must be present in the culture medium. These factors are found in sufficient amounts in HPS but not in HAS or FCS. Human placental cord serum, even at term, also provides a suitable pericellular environment for optimal growth of fetal chondrocytes.

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The Effect of Short-Term Starvation on Mucosal Barrier Function in the Newborn Rabbit*

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ABSTRACT. The compromised human newborn frequently presents with overwhelming feeding problems which lead to inadequate intake. These problems may affect the development of the small intestine, especially mucosal barrier function, leading to increased infections and susceptibility to allergens. To study this, an animal model was established using neonatal rabbits deprived of nutrients from birth until 72 h. Mucosal barrier function was compared in deprived and control (naturally fed 72-h-old animals) rabbits by measuring immunoreactive bovine serum albumin in serum 4 h after intragastric infusion of crystalline bovine serum albumin (200 mg/100 g body weight). Trypsin activity was measured in rinse fluid obtained from the small intestine. Representative sections of jejunum from control and experimental animals were formalin fixed and stained with hematoxylin and eosin for morphologic comparison. Following the bovine serum albumin feeding, a significantly increased serum immunoreactive bovine serum albumin and significantly decreased trypsin-like activity of the small intestinal rinse fluid was noted in starved animals compared to controls. In addition, the enterocytes of malnourished animals were more cuboidal and contained fewer and smaller supranuclear granules on microscopic examination than the enterocytes of controls. This study suggests that short-term starvation in newborns affects mucosal barrier function. Acute starvation may place newborns at increased risk for infections and allergic disease. (*Pediatr Res* 19: 727–731, 1985)

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Abbreviations

BSA, bovine serum albumin
i-BSA, immunoreactive BSA
PBS, phosphate-buffered saline

The compromised human newborn frequently presents with overwhelming feeding problems and grossly inadequate intakes are common. The extent to which this affects the differentiation and development of the small intestine, especially in terms of barrier function, has not been studied. The intestine plays a major role in preventing a variety of intraluminal substances, such as food antigens, microorganisms, and toxins, from penetrating the mucosal barrier and entering the systemic circulation.

There is evidence that under conditions of chronic malnutrition intestinal barrier function is altered in older animals. This observation is based on the finding of increased uptake of both intact and large fragments of protein from the intestinal lumen into the circulation, suggesting that the gut is more permeable under these conditions (1, 2).

There is also indirect evidence that a similar phenomenon may occur in malnourished children. Chandra (3) reported an increase in circulating food antibodies in children with severe malnutrition, suggesting that food antigens may cross the gut to induce a systemic antibody response. Similarly, the uptake of pathogens may be increased and this may contribute to the high incidence of gastrointestinal and systemic infections in malnourished children.

Mucosal barrier function in the animal and human studies cited above was compromised only after long periods of nutrient deprivation in the postweaning state. There is no information on how the newborn responds to a similar insult or what effect a short-term period of starvation, which occurs commonly in the clinical setting, would have in the period immediately after birth when the intestine undergoes many changes while adapting to extrauterine life.