¹⁴C-GLUCOSE AND ³⁵S-METABOLISM OF PIG EPIPHYSIAL CARTILAGE AND ITS VARIATIONS AFTER OSTEOTOMY

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On *in vitro* incubation of articular and epiphyseal cartilage of the ulna of the domestic pig 70 to 80 per cent of [U-¹⁴C] glucose was metabolised to ¹⁴C-lactate, but cartilage of the epiphyseal plate produced up to five times as much ¹⁴C-CO₂ as articular cartilage, and the specific radioactivity of ¹⁴C (or ³⁵S)-chondroitin 4 (6)-sulphate isolated from epiphyseal cartilage (following ³⁵S-sulphate incorporation) was about twice as high as that of articular cartilage. Six weeks after an osteotomy on both sides of the proximal epiphyseal plate of the left ulna, the glucose uptake, lactate production, and the specific radioactivity of the glycosaminoglycans displayed no significant differences when compared with those of the corresponding epiphyseal plate of the control right ulna, whereas a 50 per cent increase in the oxidation of ¹⁴C-glucose to ¹⁴C-CO₂ was observed.

The longitudinal growth of bone is primarily a function of the growth plate and a result of cell reproduction and elaboration of intercellular matrix. Experimental studies on the epiphyseal plate of rats and rabbits revealed that proliferation of epiphyseal cartilage cells—and hence longitudinal growth—depends on the age of the animal and on an adequate blood circulation (Haas 1917; Wu and Miltner 1937; Brashear 1963; Siffert 1966).

Many attempts have been made experimentally to transplant the epiphysis and the plate. Though freely transplanted ends of young bones usually fail to continue to grow, other results indicate that the plate is able to survive an avascular interval and to retain its potential for continued growth (Ring 1955a; Harris, Martin and Tile 1965).

Whereas many biochemical details are known about the hyaline cartilage of joints, trachea and nasal septa (Balazs 1970), no adequate results are available on the chemical composition and the metabolism of the cartilage of the growth plate. Handley and Phelps (1972) described the biosynthesis of glycosaminoglycans and their UDP-activated monosaccharide precursors in neonatal rat epiphyseal cartilage, but more detailed information about the activity and the metabolic features of different types of epiphyseal plates is required if the efficacy of autologous transplantation of this type of cartilage is to be improved.

In the present study some basic chemical metabolic features of the distal and proximal ulna epiphyses of domestic pigs and their variation after bilateral osteotomy will be reported.

MATERIAL AND METHODS

All chemicals used were of analytical grade and purchased from Boehringer (Mannheim), Merck (Darmstadt) and

Serva (Heidelberg). [U-¹⁴C] glucose (specific activity 3.0 mCi/mmol) and Na₂-³⁵SO₄ (carrier free) were products of the Biochemical Centre, Amersham, England. Pronase E (activity 70 000 PUK-E/mg) was obtained from Merck (Darmstadt).

Domestic pigs aged six and twelve weeks were used. For operation the animals were anaesthetised with inactin and on each side of the epiphyseal cartilage a transverse osteotomy parallel to and 5 millimetres from the proximal growth plate was made on the right ulna. After six weeks the animals operated upon and an equal number of age-matched control animals were killed. Cartilage of the proximal and distal epiphyseal plates of the right and the left ulna and articular cartilage of the elbow joint were obtained by careful dissection. The specimens were kept in ice-cold Ringer solution and used for the incubation experiments within two hours.

Incubation experiments *in vitro*—The epiphyseal or articular cartilage was sliced into 0.3 to 0.5 millimetre sections and batches of 30 to 100 milligrams were incubated according to the method of Handley and Phelps (1972) under a gas phase of 95 per cent oxygen and 5 per cent carbon dioxide at 37 degrees Celsius in 5.2 millilitres of a modified buffered Ringer solution containing 0.1 per cent glucose (Kresse and Buddecke 1968) for three to six hours: 100 µCi [U-¹⁴C] glucose or 50 µCi ³⁵S-sulphate was added to the incubation medium. The incubation was stopped by rinsing the cartilage three times with ice-cold Ringer solution.

Preparation of acidic glycosaminoglycans—Articular and epiphyseal plate cartilage were processed identically. The isolation of the total glycosaminoglycans was achieved by standard methods (Kresse and Buddecke 1968). The tissue was exhaustively digested by pronase E in 3 millilitres of 0.1 M Tris-HCl buffer, pH 7.6 containing 0.01 M

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CaCl₂ for 24 hours at 50 degrees Celsius. The digest was precipitated by ethanol in the presence of Na₂SO₄ and the precipitate redissolved in 5 millilitres distilled water. Aliquots were used for determination of DNA (Burton 1956), uronic acid and hexosamine (see below) and for further purification of the glycosaminoglycans by ion-exchange chromatography on Dowex 1 X 2 (Von Figura, Kiowski and Buddecke 1973). The purified glycosaminoglycans were eluted from the Dowex 1 X 2 column (Cl-form 0.8×5 centimetres) by 3 M sodium chloride, dialysed, precipitated with ethanol and redissolved in water.

Analyses—Determination of uronic acids, hexosamine, sulphate, and radioactivity was as previously described (Von Figura *et al.* 1973). Fractionation of the individual

glycosaminoglycans on an analytical scale was by thin-layer chromatography according to Humbel and Chomles (1972). Glucose and lactate were assayed from aliquots of the incubation medium by enzymatic methods (Bergmeyer 1965) after separation by anion exchange chromatography.

Measurements of radioactivity—Ten millilitres Instagel[®] (Packard Instruments, Frankfurt/Main) were added to 100 µl aqueous solutions and measured for radioactivity in a Tracerlab scintillation spectrometer (Model LSC 40).

RESULTS

The results listed in Tables I, II and III are based on a comparison of articular and epiphyseal cartilage, the

TABLE I

[U-¹⁴C] glucose metabolism of articular and epiphyseal cartilage of pig's ulna. 150–200 mg cartilage slices were incubated *in vitro* in 5.2 ml buffered Ringer solution in the presence of 20 µCi [U-¹⁴C] glucose (specific activity 10.6×10⁶ cpm/mg) over 3 h. Gas phase 95 per cent O₂ and 5 per cent CO₂. The specific radioactivity of the lactate ranged from 8.4–9.7×10⁶ cpm/mg. The values are means of four (articular cartilage), seven (distal) and twenty (proximal growth plate) experiments. Standard deviations in brackets. Statistically significant differences were examined by means of the t-test with reference to articular cartilage.
aGAG=acid glycosaminoglycans.

Type of cartilage	Glucose uptake µmol/mg DNA/h	Lactate ^b formation µmol/mg DNA/h	Percentage of incorporated total radioactivity		
			¹⁴ C-lactate	¹⁴ C-CO ₂	¹⁴ C-aGAG
I Articular cartilage	10.3 (±0.97)	17.9 (±3.8)	87.6 (±19.4)	0.27 (±0.05)	0.12 (±0.06)
II Cartilage of distal growth plate	21.9 (±6.3) ^a	32.7 (±8.6) ^a	75.4 (±21.1)	1.11 (±0.18) ^a	0.32 (±0.10) ^a
III Cartilage of proximal growth plate	21.4 (±7.4) ^a	31.0 (±7.9) ^a	72.6 (±22.6)	0.78 (±0.16) ^a	0.42 (±0.14) ^a

^a p I/II or I/III<0.01.

^b Specific radioactivity of the lactate varied from 8.4 to 9.7×10⁶ cpm/mg.

TABLE II

Glycosaminoglycan content and specific glycosaminoglycan radioactivities of articular and growth plate cartilage (pig's ulna) after labelling with ¹⁴C or ³⁵S. Values are means of 9 (I), 7 (II) and 11 (III) ¹⁴C-glucose experiments and ³⁵S experiments. Radioactivity was incorporated over three hours *in vitro* incubation of cartilage in the presence of [U-¹⁴C] glucose or ³⁵S-sulphate. The ratio µmol sulphate/µmol hexosamine of the isolated glycosaminoglycans were 0.98 (I), 1.09 (II) and 1.05 (III).

Type of cartilage	mg glycogen/ mg DNA ^c	mg aGAG/ mg DNA	Specific radioactivities after labelling by			
			³⁵ S-sulphate		¹⁴ C-glucose	
			cpm × 10 ⁻⁴ / µmol sulphate	per cent	cpm × 10 ⁻⁴ / µmol HexN	per cent
I Articular cartilage	3.5 (±0.68)	90 (±17.3)	80.8	100	37.0 (±6.7)	100
II Cartilage of distal growth plate	7.0 (±0.94) ^b	105 (±22.1)	193.9	240	85.1 (±19.5) ^a	250
III Cartilage of proximal growth plate	12.0 (±1.7) ^a	150 (±24.3) ^a	153.5	190	77.7 (±21.2) ^a	220

^a p I/II and I/III, respectively, <0.01; ^b p I/II and I/III, respectively, <0.05; ^c determined *before* incubation.

TABLE III

In vitro incubation of articular and growth plate cartilage of the ulna (twelve-week-old pigs) in the presence of ³⁵S-Sulphate. Glycosaminoglycan pattern and ³⁵S-radioactivities of glycosaminoglycans and individual glycosaminoglycan types. Mean values of three experiments. C 4-S, chondroitin 4-sulphate; C 6-S, chondroitin 6-sulphate; KS, keratan sulphate.

Type of cartilage	³⁵ S-radioactivity incorporated into total GAG cpm × 10 ⁶ /mg DNA	g/100 g total GAG			Ratio of specific radioactivities		
		C 4-S	C 6-S	KS	C 4-S	C 6-S	KS
I Articular cartilage	72.5	43	43	14	1.0	0.7	0.4
II Cartilage of distal growth plate	203.5	32	57	11	2.4	0.7	0.5
III Cartilage of proximal growth plate	230.5	53	38	9	1.9	0.8	0.2

tissue samples deriving from the same animal in each experiment and being processed identically. The percentage differences between articular and epiphyseal cartilage are highly reproducible, but a variation of absolute values was observed from experiment to experiment.

[U- ^{14}C] glucose metabolism—The kinetic of glucose uptake and glycolytic production of lactate was found to be linear with time up to six hours after a short initial delay (glucose uptake), thus indicating the adaptation of epiphyseal and articular cartilage to a steady state of *in vitro* metabolism (Fig. 1). Figures for the metabolic fate of

from ^{14}C -glucose oxidation, while other oxidable substrates releasing unlabelled CO_2 are not considered in this balance. It appears from the CO_2 values that the growth plates exhibit a more active oxidation metabolism than the articular cartilage. Although the percentage of radioactivity incorporated into the acid glycosaminoglycans is less than 1 per cent of the total radioactivity, there are regular and statistically significant differences between epiphyseal and articular cartilage.

Specific radioactivity and distribution of acid glycosaminoglycans—The glycosaminoglycans of cartilage make up

FIG. 1

Time course of glucose uptake (○, □) and lactate production (●, ■) of epiphyseal (○, ●) and articular (□, ■) cartilage during incubation *in vitro* over a period of 6 h. 80.4 milligrams (proximal epiphysis) and 94.8 milligrams (joint) slices of cartilage were incubated in 5 millilitres medium containing 0.1 per cent glucose over a gas phase of 95 per cent O_2 /5 per cent CO_2 .

FIG. 2

Incorporation of ^{35}S -sulphate into the total glycosaminoglycans of epiphyseal (△) and articular (□) cartilage. Incubation conditions as in Figure 1. Each of the cartilage samples (20 to 40 milligrams) contained 100 Ci ^{35}S -sulphate. The specific radioactivities refer to the sulphate content of the total glycosaminoglycans.

the ^{14}C -glucose after a three-hour incubation are given in Table I. Though a complete removal of mineralised cartilage zones was secured, all values refer to the DNA content of the tissue and hence reflect the cellular metabolic capacity.

The observations in Table I revealed that the glucose consumption of growth plate cartilage exceeded that of articular cartilage. The difference was statistically significant. In all types of cartilage, however, 70 to 80 per cent of the ^{14}C -glucose utilised was converted to ^{14}C -lactate, thus indicating a high rate of anaerobic glycolysis in these tissues. The specific radioactivity of the ^{14}C -lactate was lower compared with that of the added ^{14}C -glucose. It must therefore be concluded that the exogenous labelled ^{14}C -glucose is diluted by carbohydrates from endogenous sources.

The low percentage of labelled ^{14}C - CO_2 is to be expected from the low requirement of cartilage for molecular oxygen. The values listed in Table I, however, refer exclusively to the portion of ^{14}C - CO_2 that derives

20 to 30 per cent of the dry weight of tissue. The rate of incorporation of labelled precursors is considered to be an indicator of their metabolic activity. On incubation *in vitro* in the presence of ^{35}S -sulphate the incorporation of ^{35}S -radioactivity into the sulphated glycosaminoglycans of epiphyseal and articular cartilage proceeds at a constant rate for at least six hours (Fig. 2). The specific radioactivity depends on the rate of cellular synthesis and the quantity of glycosaminoglycans already present which dilute the newly synthesised labelled material.

When the total acid glycosaminoglycans were isolated from cartilage slices after *in vitro* incubation in the presence of ^{14}C - as well as ^{35}S -radioactivity, the specific radioactivity of epiphyseal glycosaminoglycans was about twice as high as that of the articular glycosaminoglycans (Table II). Since the glycosaminoglycan content of the distal and proximal growth plate was equal to, or even higher than, that of articular cartilage, it must be concluded that there is a higher rate of biosynthesis of glycosaminoglycans in the epiphyseal cartilage.

TABLE IV

Effect of double transverse osteotomy on the ¹⁴C-glucose metabolism and ³⁵S-sulphate incorporation of proximal growth plate. Osteotomy was carried out in six-week-old pigs and the epiphysial cartilage was obtained six weeks after osteotomy for *in vitro* incubation experiments. Values for ¹⁴C-glucose experiments are means of eleven control and five osteotomy experiments. Specific radioactivities of ³⁵S-labelled acid glycosaminoglycans are calculated from three independent experiments.

Status of proximal growth plate	Glucose uptake μmol/mg DNA/h	Per cent of incorporated total radioactivity		Specific radioactivity of aGAG cpm × 10 ⁴ /μmol	
		¹⁴ C-lactate	¹⁴ C-CO ₂	¹⁴ C	HexN and SO ₄ ³⁵ S
I Normal cartilage of proximal growth plate	21.4 (±4.3)	72.6 (±19.4)	0.78 (±0.21)	77.7 (±23.1)	153.3
II Cartilage of proximal growth plate after osteotomy	19.2 (±3.9)	80.7 (±22.4)	1.15 ^b (±0.18)	65.7 (±18.9)	146.9

^b p I/II < 0.05.

On fractionation of the total ³⁵S-glycosaminoglycans the distribution pattern of the individual glycosaminoglycans and their specific radioactivity could be determined (Table III). The major glycosaminoglycans of articular and epiphysial cartilage are chondroitin 4-sulphate and chondroitin 6-sulphate, but their ratio varied in the different cartilage types. In the distal growth plates the chondroitin 6-sulphate predominates, while in the proximal the chondroitin 4-sulphate is predominant. Though these differences were not statistically evaluated, they are of interest with regard to the fact that the specific radioactivity of chondroitin 4-sulphate exceeds that of chondroitin 6-sulphate two or three fold. The lower metabolic activity of articular cartilage is expressed by the relatively low specific activity of its individual glycosaminoglycans. The percentage of keratin sulphate as well as its specific radioactivity was regularly found to be smaller than that of the two chondroitin sulphates.

Effect of osteotomy—In a series of experiments the proximal epiphysis was isolated by osteotomy on both sides of the growth plate. The purpose of this surgical treatment was to simulate an autogenous transplantation of the epiphysis and to study the metabolic changes occurring under these conditions. The figures in Table IV were obtained six weeks after osteotomy. They reveal a slightly elevated lactate production but a remarkable increase of ¹⁴C-CO₂ formation, thus indicating increased oxidation processes. No change or a slight fall of the specific radioactivity of the total glycosaminoglycans was observed. In these experiments the animals served as their own controls, growth plate cartilage of the operated left ulna and the non-treated right ulna being compared.

The morphological changes following osteotomy and the stimulation of longitudinal growth will be reported elsewhere.

DISCUSSION

From the ¹⁴C-glucose balance studies it appears that the epiphysial cartilage follows predominantly an anaerobic glycolytic metabolism, though the rate of its glucose oxidation as judged by the ¹⁴CO₂-formation is remarkably higher than that of articular cartilage. The work of

Brighton and Heppenstall (1971), however, indicates that each zone of the epiphysial plate has a distinct oxygen tension that is significantly different from that of adjacent zones. Specified metabolic data for the different morphological zones of the epiphysial plate would be expected to yield valuable information as to whether the observed oxygen gradient is related to the rate of glycolysis, to peculiarities in the biosynthesis of proteins and proteoglycans, to the process of cartilage matrix calcification or to special enzymatic activities. Handley and Phelps (1972) concluded that a 95 per cent O₂/5 per cent CO₂ gas phase definitely preserved the physiological integrity of the isolated epiphyses *in vitro*.

The differences between articular and epiphysial cartilage in glucose uptake, glucose oxidation, glycogen content, and incorporation of ¹⁴C- and ³⁵S-radioactivity into the acidic glycosaminoglycans characterise epiphysial cartilage as a metabolically more active cartilage, when compared with articular cartilage. Analogous differences in the glucose utilisation and the oxygen consumption between rat epiphysial and costal cartilage have been described (Handley and Phelps 1972).

Though the differences in the distribution pattern of chondroitin 4-sulphate and 6-sulphate between the distal and proximal epiphysis were not confirmed by statistical methods, they may be related to their different functional properties: whereas the distal growth plate is subjected to compressive mechanical forces (as a result of weight-bearing), the proximal growth plate may be referred to as a traction epiphysis since there is a point of muscle attachment.

Successful autogenous transplantation of epiphysial cartilage depends on its ability to survive after interruption of the blood supply. From available information, species, age and the thickness of the transplant seem to be the important factors, variation of which may explain differing results from various investigators. Whereas excision and reimplantation of epiphysial cartilage in rabbits did not disturb the subsequent growth of the bone (Ring 1955b), affections of the epiphysis which interfered with its blood supply resulted in a failure of chondrogenesis and cessation of growth (Siffert 1966) and were described as definitive and irreversible (Brashear 1963).

From the present results, however, it appears that six weeks after an osteotomy that interrupts the blood supply through the epiphysial and diaphysial arteries, the metabolic activity of the epiphysial cartilage is not only restored but accelerated as far as the oxidation of ^{14}C -glucose is concerned. This is in accordance with reports of an increase in longitudinal growth after periosteal stripping (Wu and Miltner 1937) and the common know-

ledge that osteomyelitis and fractures of the long bones in children tend to accelerate longitudinal growth. Also in our experiments, biometrical investigations (Rodegerdts, unpublished) indicate that osteotomy induces a stimulation of growth resulting in a statistically significant enlargement of the epiphysial plate and an increase in the total length of the ulna by about 1 centimetre as compared with the control ulna.

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