Glycosaminoglycans of Bovine Lung Parenchyma and Pleura

Lung tissue has often been used as a source of glycosaminoglycans (heparin[1], heparan sulphate[2], dermatan sulphate[1,3]) and these polysaccharides are likely to be of importance in lung pathology[4] yet little has been done to describe all of the glycosaminoglycan components or to quantitate them. Due to the great anatomical complexity of pulmonary tissue most previous workers have been restricted to histochemical studies[5] which do not permit full chemical characterization of glycosaminoglycan components implicated in such structures as the alveolar membrane. In an attempt to pinpoint any glycosaminoglycan fraction which may be uniquely associated with the process of respiration, a comparison has been made in this communication between these components of the parenchyma of bovine lung (and their susceptibility to various extraction procedures) and those of the visceral pleura. Since this membrane includes connective tissue components similar to, and continuous with, pulmonary interstitial tissue[6], differences between the two closely juxtaposed tissues should reveal any glycosaminoglycan particularly concentrated at sites such as the tissue space of the blood-air barrier described by Low[7].

Materials and methods. Lungs from 2-year-old steers were perfused with water within hours of death to remove all traces of blood. The visceral pleura was stripped from all surfaces from which it could be obtained in coherent, uniform sheets. Bronchi and associated blood vessels were removed from the parenchyma and both tissues were airdried after immersion in ethanol, acetone, and diethyl ether (in that order). Digestions and extractions were performed after high-speed homogenization of tissue suspensions and, in the case of parenchyma, depolymerization of the nucleic acid present using crystalline pancreatic ribonuclease and deoxyribonuclease (British Drug Houses Ltd.).

Glycosaminoglycans were released from tissues by exhaustive papain digestion and assayed as hexosamine or uronic acid after fractionation on Dowex 1×2 (Cl^-)[8]. The identity of glycosaminoglycan fractions was confirmed by cellulose acetate electrophoresis[9] before and after treatment with testicular hyaluronidase or with nitrous acid. These treatments depolymerized hyaluronic acid and chondroitins 4- and 6-sulphate, or heparan sulphate and heparin[2], respectively. After digestion of the appropriate fraction with testicular hyaluronidase, chondroitins 4- and 6-sulphate were determined by the method of MATHEWS and INOUYE[10] and undegraded dermatan sulphate was recovered by chromatography on Dowex 1×2. Collagen and elastin were estimated from hydroxyproline content[11] before and after alkaline extraction of tissue samples[12].

Results. With the exception of keratan sulphate all major glycosaminoglycans were found in both tissues (Table 1). The glycosaminoglycan/fibrous protein ratio is higher in parenchyma by a factor of almost three with the extra glycosaminoglycan chiefly in the heparin and heparan sulphate fractions. The former polysaccharide is located in mast cells[1] and is not a component of connective tissue intercellular material. This is included for purposes of comparison in Table II where the other 2 major glycosaminoglycan components of parenchyma are compared with their counterparts in pleura using exhaustive salt extraction and digestion with protease specific for each of the fibrous proteins. As has been found in other

[1] M. STACEY and S. A. BARKER, *Carbohydrates of Living Tissues* (Van Nostrand, London 1962).
[2] J. A. CIFONELLI, Carbohyd. Res. *8*, 233 (1968).
[3] L.-Å. FRANSSON, *Chemistry and Molecular Biology of the Intercellular Matrix* (Ed. E. A. BALAZS; Academic Press, New York 1970), vol. 2, p. 823.
[4] E. BUDDECKE, Beitr. Silikosforsch., Supplement *4*, 291 (1960).
[5] H.-J. CLEMENS, Acta histochem. *2*, 170 (1956).
[6] W. S. MILLER, *The Lung* (Charles C. Thomas, Springfield Ill. 1937).
[7] F. N. LOW, Anat. Rec. *139*, 105 (1961).
[8] F. S. WUSTEMAN, D. B. JOHNSON, K. S. DODGSON and D. P. BELL, Life Sci. *7*, 1281 (1968).
[9] R. M. MASON and F. S. WUSTEMAN, Biochem. J. *120*, 777 (1970).
[10] M. B. MATHEWS and M. INOUYE, Biochim. biophys. Acta *53*, 509 (1961).
[11] H. STEGEMANN, Hoppe-Seyler's Z. physiol. Chem. *311*, 41 (1958).
[12] A. I. LANSING, T. B. ROSENTHAL, M. ALEX and E. W. DEMPSEY, Anat. Rec. *114*, 555 (1952).

Table I. Glycosaminoglycan and fibrous protein content of parenchyma and visceral pleura of bovine lung

Fraction	Amount per g dry weight of Pleura	Parenchyma
Collagen[a]	393±35	251±27
Elastin[a]	310±32	100±15
Total glycosaminoglycan[b]	6.67±0.45	10.8±0.9
Hyaluronic acid[b]	0.77	0.83
Heparan sulphate[c]	0.89	3.76
Chondroitin 4-sulphate[d]	0.53	1.53
Chondroitin 6-sulphate[d]	0.41	0.59
Dermatan sulphate[d]	2.52	2.35
Heparin[c]	0.88	2.26

Results are mean values (±S.E.M.) of 5–7 separate estimations using material derived from 4 animals. Further fractionations were performed at least in replicate on pooled glycosaminoglycan preparations. Results expressed in terms of mg fibrous protein[a], μmole glucuronic acid[b], μmole glucosamine[c], or μmole galactosamine[d].

Table II. Extractability of major glycosaminoglycan fractions of parenchyma and visceral pleura of bovine lung

Glycosaminoglycan	Tissue	Percent remaining insoluble after extraction with: Collagenase	Elastase	*M* NaCl
Heparan sulphate	Parenchyma	3	0	29
	Pleura	5	12	25
Dermatan sulphate	Parenchyma	15	39	39
	Pleura	12	65	79
Heparin	Parenchyma	85	27	11
	Pleura	39	27	0

Digestion with collagenase (Sigma Type III, Fraction A, 1000 U/g tissue) at 37 °C for 24 h in 0.15 *M* phosphate buffer pH 7.1 yielded products which, when dry, contained 1.6% (pleura) and 6.2% (parenchyma) collagen. Digestion with elastase (Sigma or Koch-Light crystalline, 10 mg/g tissue), of tissue before treatment with organic solvents, at 37 °C for 24 h in 0.1 *M* $NaHCO_3$ pH 8.8 yielded products which were virtually free of elastin. Repeated extraction with *M* NaCl (a total of 40 ml/g parenchyma and 200 ml/g pleura) yielded residues and soluble extracts which were both analyzed for glycosaminoglycans.

tissues[3], dermatan sulphate is associated closely with collagen fibres since collagenase digestion removes more of this glycosaminoglycan than either salt extraction or digestion with elastase, a less specific protease. By this criterion heparan sulphate is closely associated with neither collagen nor elastin and its distribution is clearly different to that of heparin, being least effectively solubilized by salt solution. As has been noted with the product from human aorta[13], an appreciable fraction of the heparan sulphate of bovine lung is eluted from Dowex 1×2 by relatively low concentrations of NaCl (38% from parenchyma and 57% from pleura eluted with 0.8 *M* NaCl). Also, a significant fraction is not precipitated by cetylpyridinium chloride in the presence of 0.5 *M* NaCl. This could indicate a variable degree of sulphation (CIFONELLI and DORFMAN[14]) or the presence of heparan sulphate of low molecular weight.

Discussion. The low levels of heparan sulphate found in the pleura can probably be attributed to the vascular layer since this glycosaminoglycan is found in arteries[15]. When found in bovine aorta[16] heparan sulphate is accompanied by much higher levels of glycosaminoglycans containing galactosamine. These are present in parenchyma but in insufficient quantities for the heparan sulphate there to be attributed solely to residual blood vessels which are difficult to remove from the tissue.

Thus bovine lung parenchyma contains large quantities of a readily-extractable, heterogenous, heparan sulphate fraction which could have a specific function in the connective tissue components involved in gas exchange.

Résumé. On décrit et compare les glycosaminoglycanes du parenchyme et de la plèvre du poumon bovin. Tous les glycosaminoglycanes principaux, sauf le kératan-sulfate, y ont été trouvés, mais il y a beaucoup plus d'héparan-sulfate dans le parenchyme.

F. S. WUSTEMAN[17]

Department of Biochemistry, University College, Cathays Park, Cardiff (U.K.), 18 October 1971.

[13] V. KUMAR, G. S. BERENSON, H. RUIZ, E. R. DALFERES and J. P. STRONG, J. Atheroscler. Res. *7*, 573 (1967).
[14] J. A. CIFONELLI and A. DORFMAN, J. biol. Chem. *235*, 3283 (1960).
[15] S. THUNELL, C. A. ANTONOPOULOS and S. GARDELL, J. Atheroscler. Res. *7*, 283 (1967).
[16] U. R. ENGEL, Atherosclerosis *13*, 45 (1971).
[17] Acknowledgement. The help of Dr. M. CRAIGMYLE of the Department of Anatomy, University College, Cardiff, is appreciated.

Genetic Evidence for the Tetramer Structure of Glyceraldehyde-3-Phosphate Dehydrogenase

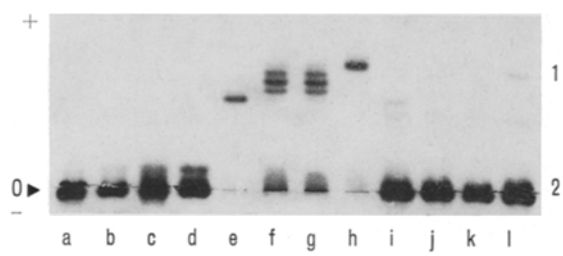
Crystallographic[1], molecular weight[2] and amino acid[3] studies have suggested that the active enzyme form of glyceraldehyde-3-PO_4 dehydrogenase (G3PD) is a tetramer, composed of 4 polypeptide subunits. Here we present genetic evidence using starch-gel electrophoresis to support that hypothesis.

If 2 electrophoretically different polypeptide chains (A and A′) are produced in cells and if these subunits combine randomly to form tetramer molecules, molecules AAAA, AAAA′, AAA′A′, AA′A′A′, and A′A′A′A′ should be produced in a 1:4:6:4:1 ratio[4].

Starch gel electrophoresis of tissue extracts[5] of *Xiphophorus maculatus* strain 163A, platyfish[6], and *X. helleri strigatus*, swordtail, followed by histochemical staining for G3PD[7] reveals 2 isozymes of G3PD in each species, a slow migrating form G3PD-2 present in liver and muscle and a faster migrating form G3PD-1 present in eye and brain (Figure). While G3PD-2 is the same in both species there is an electrophoretic difference in the G3PD-1 isozyme of the two species. These facts plus the observed tissue specificity indicate the 2 G3PD isozymes are specified by separate genetic loci.

In the F_1 hybrids[8] which contain 1 G3PD-1 allele specifying the platyfish subunit and 1 for the swordtail subunit, the expected 5 banded pattern consistent with the tetramer structure of G3PD was obtained (Figure). F_1 hybrids when backcrossed to swordtails produced 15 fish displaying the 5 banded hybrid pattern and 16 fish the 1 fast band pattern of the swordtail. This agrees with the expected 1:1 ratio ($\chi^2 = 0.03$, $P > 0.8$).

Five membered sets of G3PD bands have been reported as being usually seen following zone electrophoresis on



Glyceraldehyde-3-PO_4 dehydrogenase isozymes in platyfish, swordtails and their hybrids. a–d) Liver extracts of a) platy, b) and c) F_1 hybrids and d) swordtail. e–h) eye extract of e) platy, f) and g) F_1 hybrids and h) swordtail. i–l) muscle extracts of i) platy, j) and k) F_1 hybrids, l) swordtail. The hybrid eye patterns (channels f and g) were cut from the starch gel and the activity in each band was estimated using a Densicord recording densitometer. The ratio of the bands in the hybrid pattern was approximately 1:4:6:4:1.

[1] W. C. WATSON and L. J. BANASZAK, Nature, Lond. *204*, 918 (1964).
[2] W. F. HARRINGTON and G. M. KARR, J. molec. Biol. *13*, 855 (1965).
[3] J. I. HARRIS and R. N. PERHAM, J. molec. Biol. *13*, 876 (1965).
[4] C. L. MARKERT, Science *140*, 1329 (1963) and C. R. SHAW, Brookhaven Symp. Biol. *17*, 117 (1964).
[5] Tissue from eye, muscle, liver and brain was frozen on dry ice, homogenized in glass homogenizers in cold 1/20 dilution of Amphibian Ringer's solution that was buffered with 0.001 *M* *Tris*-HCl at pH 7.5. The ratio of tissue to homogenizing medium was 1:3 weight/volume.
[6] Both strains are the descendents of fish obtained from Klaus Kallman of the New York Zoological Society.
[7] The method of vertical starch gel electrophoresis and G3PD staining was essentially that of C. R. SHAW and R. PRASAD, Biochem. Genet. *4*, 297 (1970). Their buffer system I (*Tris*-citrate, pH 7.0) was used with the modification of adding 0.06 ml β-mercaptoethanol and 40 mg nicotinamide adenine dinucleotide for 600 ml of starch gel. These 2 ingredients proved essential for demonstration of G3PD activity in starch gels.
[8] Hybrids were obtained according to the artificial insemination method of E. CLARK, Science *112*, 722 (1950).
[9] H. G. LEBHERZ and W. J. RUTTER, Science *157*, 1198 (1967).