Acid Glycosaminoglycans of Pig Aorta

A method for the separation of aortic glycosaminoglycans by a column procedure using quaternary ammonium ions has recently been described[1]. Microscale adaptations of this method have been used in studies of the composition of healing skin wounds[2] and human retrobulbar tissue[3]; in such fractionations, identification of the different polysaccharide fractions was based on the similar solubility of chemically identified fractions from larger scale experiments. In studies of the role of glycosaminoglycans in arterial disease, such microscale separations offer means whereby the composition of small sections of arterial tissue may be obtained[4]. In recent years, increasing use has been made of pigs as experimental animals in cardiovascular research[5,6], presumably because of their ability to develop lesions comparable to those of the pre-atheromatous phase in human atherosclerosis and to produce an atherosclerosis in response to dietary alterations. Little is known of the distribution of the glycosaminoglycans in pig aortic tissue; a quarternary ammonium ion fractionation of the acid glycosaminoglycan components of pig aortic intima using a method closely similar to that described[1] is now reported. The sample size has been reduced by a factor of about 1:400; this allowed preliminary identification of the various fractions without the need for a larger scale experiment.

Aortas from young pigs (4–6 months old) were collected from the abattoir, the intimal layers (intima + some media) were dissected from the tissue and frozen; portions (1–2 g) were homogenized in cold acetone, extracted with boiling acetone and air dried. The dry defatted tissue was incubated in 2.5 ml 0.1 *M* phosphate buffer containing EDTA (250 μmoles) at pH 8.0 and 37 °C for 1 h. The pH was adjusted to 6.0 with *M* HCl; papain (0.25 mg) and cysteine (60 μmoles) were then added, and the mixture incubated for 16 h at 65 °C. The pH was again adjusted to 6.0 and the papain-cysteine treatment repeated. Undissolved material was removed by centrifuging and 2.5 volumes of a 2% w/v cellulose suspension in 1% w/v cetylpyridinium chloride (CPC) was added to the supernatant solution. After standing for 3 h at 25 °–30 °C, the precipitate was recovered by centrifuging, and washed with 1% w/v CPC solution. Elution of the glycosaminoglycan-CPC complexes was carried out by washing the precipitate three times with 1.5 ml portions of each of the following eluting solutions: 0.3 *M* NaCl, 0.3 *M* $MgCl_2$, 0.5 *M* $MgCl_2$ + 0.1 *M* CH_3COOH, 0.6 *M* $MgCl_2$ + 0.1 *M* CH_3COOH, 0.7 *M* $MgCl_2$ + 0.1 *M* CH_3COOH, 0.9 *M* $MgCl_2$ + 0.1 *M* CH_3COOH, 0.75 *M* $MgCl_2$, 6 *M* HCl. Each eluting solvent contained 0.05% w/v CPC. 3.0 ml 1% w/v CPC and 13.5 ml water were added to each fraction (except the 6 *M* HCl fraction) and the mixtures allowed to stand at 25 °-30 °C for 30 min. The precipitates were recovered by centrifuging (1 h, 4000 rev/min) and dissolved in 6 *M* HCl. Uronic acid[7,8], total hexosamine[9] (as glucosamine) and galactosamine[10], were determined on portions of these solutions.

The results of the fractionation of 5 small samples, each from a separate collection of aortas, are given in the Table. The general distribution of the glycosaminoglycans resembles that reported by ANTONOPOULOS et al.[1] for pooled human aortas, but with a somewhat less satisfactory separation due to some overlap between the fractions. The major components of the various fractions were: fraction 1, hyaluronic acid; fraction 2, heparan sulphate; fractions 3–6, chondroitin sulphate; fraction 7, dermatan sulphate, in general agreement with the results of ANTONOPOULOS et al.[1] except that dermatan sulphate was not detected in fraction 6.

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Analysis of papain digests of pig aortic intima by precipitation with cetyl pyridinium chloride on cellulose

Fraction No.	Eluting solvent	Uronic acid[7] μg/g wet tissue	Uronic acid ratio DISCHE[7]/BITTER and MUIR[8]	Uronic acid[7]/total hexosamine[9]	Galactosamine[10]/total hexosamine[9]
1	0.3 *M* NaCl	68 ± 8	0.9	0.95	0.15
2	0.3 *M* $MgCl_2$	175 ± 14	1.1	1.9	0.40
3	0.5 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	113 ± 13	1.0	1.2	0.95
4	0.6 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	118 ± 24	1.0	1.0	0.95
5	0.7 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	180 ± 16	1.0	1.05	0.95
6	0.9 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	137 ± 15	1.0	1.0	1.0
7	0.75 *M* $MgCl_2$	30 ± 6	0.7	0.55	1.0
8	6 *M* HCl	36 ± 16	–	–	–
		Total 857 ± 77			
	Reference Compounds: hyaluronic acid		1.0	1.09	0.02
	heparan sulphate		1.1–1.2	1.67	0 (theory)
	chondroitin 6-sulphate		1.0	1.08	1.0 (theory)
	dermatan sulphate		0.6	0.64	1.0 (theory)

Results are mean values (± S.E.) of fractionations carried out on 5 separate collections of pig aortas. 1–2 g of wet tissue was used in each fractionation. The dry defatted weight was 29.7 ± 0.8% of the wet weight. 4.8 ± 1.0% of the dry defatted material remained undigested after papain treatment. 75–80% of the uronic acid (determined by the DISCHE method[7]) originally present in the digest was recovered from the fractionation procedure.

On the basis of uronic acid values, the total amount of glycosaminoglycan in the intimal layers of pig aorta was about 10–12 mg/g of the dry defatted tissue. The approximate proportions of the component glycosaminoglycans were: hyaluronic acid, 8%; heparan sulphate, 15%; chondroitin sulphate, 65%; dermatan sulphate, 4%. The total amount agreed with KAPLAN and MEYER's values[11] for human aorta, but was somewhat less than values reported by other authors who have used human, guinea-pig and rabbit material[12–15]. The low value for dermatan sulphate and the corresponding high chondroitin sulphate value appear to be characteristic of young aortic tissue[11], although the absolute value for the dermatan sulphate was lower than expected[16]. The hyaluronic acid value was also lower than those previously reported for tissues of similar age from other species[11–15,17].

Zusammenfassung. Mittels einer modifizierten Fällungsmethode mit Cetylpyridiniumchlorid wurde die Verteilung der Glykosaminoglykanen in den innern Schichten junger Schweineaorten festgestellt. Typisch für junges Aortengewebe waren niedrige Dermatansulfat- und entsprechend hohe Chondroitinsulfatwerte. Dermatansulfat- und Hyaluronsäurewerte blieben niedriger als bei bisher beschriebenem Aortengewebe anderer Tierarten in vergleichbarem Alter.

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The Fine Structure of *Corynebacterium minutissimum*

Erythrasma, a superficial bacterial infection of the skin, is caused by proliferation of a diphtheroid in the horny layer[1,2]. In view of the relatively little information available on the microanatomy of the corynebacteria[3] and as antecedent to an investigation of the ultrastructure of the horny layer in erythrasma, we have undertaken to study the fine structure of the causative agent, *Corynebacterium minutissimum*, the type strain of which was recently accepted by the National Collection of Type Cultures, London (England)[2].

10 strains originally isolated from patients with erythrasma were used. 8 were supplied by the National Collection of Type Cultures, London (England), another was provided by D. TAPLIN, University of Miami (Florida), and one was isolated from one of our patients. The medium described by SARKANY et al.[4] was used for isolation and maintenance of the organisms. For electron microscopy, they were subcultured in nutrient broth (Difco) and in blood agar, and fixed using the RYTER-KELLENBERGER[5] procedure. As described before, the same fixation technique was used for biopsied skin obtained from lesions of erythrasma[6]. Embedding was performed in a mixture of Epon and Araldite[7], sections were cut with a Porter-Blum microtome equipped with a diamond knife and observed in a RCA EMU3F electron microscope using an accelerating voltage of 50 kv.

C. minutissimum is seen in sectioned cultured cells (Figure 1) as slightly elongated rods surrounded by an electron-dense, surface-adhering material. Underneath this material lies the cell wall, a tripartite structure consisting of 2 bands (35 Å wide) separated by a light zone 50 Å wide. The plasma membrane was only occasionally well resolved in our preparations. Large, irregularly shaped, dense granules (volutin) were a striking feature

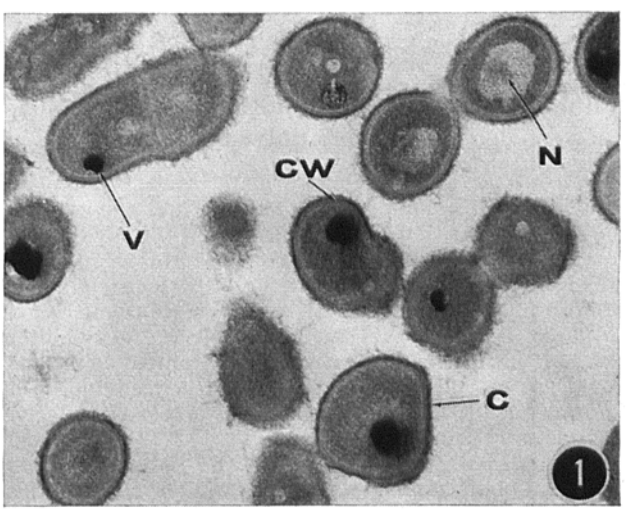

Fig. 1. Thin section through cells of *C. minutissimum*. The cell wall (CW) and the electron-dense surface adhering material (C) are shown. Several volutin granules (V) and nucleoplasms (N) are seen also. Broth culture incubated for 16 h. × 45,900.

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