Acid Glycosaminoglycan Composition of Aortic Tissue from Chickens Fed on Commercial and on Cholesterol-Supplemented Diets

Studies in our laboratory[1,2] have been concerned with the properties of glycosaminoglycan-protein complexes of pig aorta and their possible significance in cardiovascular disease. We have extended these studies to the glycosaminoglycans of chicken aorta. The present communication deals with the determination of the distributions of the various glycosaminoglycans in different sections of the chicken aorta; because of the ready response of this species to dietary change, an examination of the effect on these distributions of cholesterol + oil feeding has also been made.

One-day-old chickens were reared on commercial chick starter mash for 8 weeks. The birds were divided randomly into 2 groups of equal number. One group continued to feed on the starter mash while the other group fed on a diet consisting of cholesterol (1%), cottonseed oil (5%) and starter mash (94%)[3]. Diets and water were supplied ad libitum. At times 2, 4, 7, 10 and 13 weeks after the diet alteration, birds (6) were randomly selected from each group and killed by breaking their necks. The aortas were removed and divided into ascending (including aortic arch), thoracic and abdominal sections; by pooling the corresponding sections from the 6 aortas, samples of wet weight 1.5–2.5 g were obtained. The distributions of the glycosaminoglycans in these samples were determined in the manner described previously for pig aorta[1], where papain digestion was followed by precipitation with cetylpyridinium chloride (CPC) on cellulose and elution with salt solutions. The fractions were identified from analytical values for uronic acid[4,5], total hexosamine (as glucosamine)[6] and galactosamine[7].

Table I gives a chemical analysis of the fractions obtained from a papain digest of chicken aorta. Fraction 1 is clearly hyaluronic acid. Fraction 2, which contains heparan sulphate[1], also contains a galactosaminoglycan, probably a chondroitin sulphate. Fraction 3 contains a chondroitin sulphate, probably the 6-sulphate. Fractions 4 and 5 appear to be composed primarily of dermatan sulphate but the presence of other galactosaminoglycans is suggested by the higher values of the uronic acid ratio and the uronic acid/total hexosamine ratio.

Table I. Analysis of fractions obtained from a papain digest of chicken aorta by precipitation with cetyl pyridinium chloride on cellulose

Fraction No.	Eluting solvent	Uronic acid ratio DISCHE[4]/ BITTER and MUIR[5]	Uronic acid[4]/ total hexosamine[6]	Galactosamine[7]/ total hexosamine[6]
1	0.3 *M* NaCl	0.8	1.0	0.05
2	0.3 *M* $MgCl_2$	0.9	1.1	0.5
3	0.7 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	0.8	0.9	1.0
4	0.9 *M* $MgCl_2$ + 0.1 *M* CH_3COOH	0.6	0.7	1.0
5	0.75 *M* $MgCl_2$	0.7	0.8	1.0
6	6 *M* HCl	0.6	1.2	0.5

Values for the ratios for the reference compounds hyaluronic acid, heparan sulphate, chondroitin sulphate and dermatan sulphate are given elsewhere[1].

The results of the experiments are reported in Table II.

In the ascending aortas of the test group the total glycosaminoglycan content of the tissue showed an initial rise from about 1 mg uronic acid/g dry tissue to 3.7 mg uronic acid/g dry tissue in the first 2 weeks on the supplemented diet. This was followed by a steady decrease to 1.8 mg uronic acid/g dry tissue over the subsequent 11 weeks. The ascending aortas of the control group showed a steady increase from the same initial level to about 4 mg uronic acid/g dry tissue at the end of the experiment. The other measured quantities varied little in tissues of animals killed at various times, therefore, mean values ± S.E.M. are reported.

The dry defatted weights (% of wet weight) were lower than those found earlier for pig aorta (30%)[1]. The amount of dry material remaining after papain treatment was greater in the aortas of cholesterol-fed animals than in those of the control animals. The proportion of material

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Table II. Dry weights and glycosaminoglycan distributions in sections of chicken aorta

	Aorta section	Basal diet	Basal diet + supplement
Dry defatted weight (% of wet weight)	Ascending	17.2 ± 1.5	19.5 ± 0.3
	Thoracic	15.1 ± 0.9	15.1 ± 0.8
	Abdominal	12.8 ± 1.0	15.7 ± 0.9
Material not digested by papain (% of dry defatted material)	Ascending	6.5 ± 0.4	8.6 ± 0.1
	Thoracic	8.8 ± 0.8	9.5 ± 0.3
	Abdominal	13.5 ± 1.4	13.9 ± 0.5
Total glycosaminoglycan (mg uronic acid[4]/g dry defatted material)	Ascending	see text	see text
	Thoracic	3.9 ± 0.25	3.8 ± 0.24
	Abdominal	2.3 ± 0.03	1.6 ± 0.13
Fraction 1[a]	Ascending	0.08 ± 0.01	0.05 ± 0.004
	Thoracic	0.14 ± 0.01	0.12 ± 0.01
	Abdominal	0.08 ± 0.01	0.07 ± 0.01
Fraction 2[a]	Ascending	0.09 ± 0.003	0.08 ± 0.005
	Thoracic	0.08 ± 0.004	0.08 ± 0.003
	Abdominal	0.24 ± 0.01	0.24 ± 0.01
Fraction 3[a]	Ascending	0.30 ± 0.01	0.31 ± 0.02
	Thoracic	0.28 ± 0.01	0.34 ± 0.02
	Abdominal	0.28 ± 0.01	0.33 ± 0.02
Fractions 4 + 5[a]	Ascending	0.40 ± 0.04	0.42 ± 0.03
	Thoracic	0.42 ± 0.03	0.36 ± 0.03
	Abdominal	0.23 ± 0.03	0.17 ± 0.01

[a] Uronic acid values expressed as fractions of the total uronic acid content of the section of tissue. Mean values ± S.E.M. are given (see text).

undigested by papain increased as the arterial tree was descended.

The results show the following important characteristics of the glycosaminoglycan pattern.

(a) Only slight alterations in the distributions are produced as a result of feeding a diet known to produce atherosclerosis in this species[3]. (b) The total glycosaminoglycan content is greater in the thoracic aorta than in the abdominal aorta. (c) The hyaluronic acid fraction (1) is present in greatest proportion in the thoracic section of the aorta. (d) The heparan sulphate fraction (2) comprises a much greater proportion of the glycosaminoglycans in the abdominal aorta than it does in the ascending and thoracic aorta segments. (e) The proportion of the chondroitin sulphate fraction (3) is relatively constant in all sections of the aorta. (f) The dermatan sulphate fraction (4 + 5) is present in lowest proportion in the abdominal aorta.

Thus, the distribution of glycosaminoglycans in chicken aorta varies in the different sections of the tissue; the variation does not parallel that observed by other workers with human aortic tissue[8]; only slight alterations in the distributions are produced as a result of feeding a diet known to produce atherosclerosis in this species[3,9].

Zusammenfassung. Es wird gezeigt, dass die Verteilung von Glukosaminoglykane in der Hühneraorta offenbar im Unterschied zu menschlichem Aortengewebe in Abhängigkeit der topographischen Lage steht. Höchste Gesamtwerte für Glykosaminoglykan wurden in der Brustaorta, niedrigste in der abdominalen Aorta gefunden.

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Detection of Fusaric Acid in the Mycelium and Conidia of *Fusarium oxysporum* f. *vasinfectum*

Fusaric acid (FA) (5-*n*-butyl picolinic acid) is produced by a large number of *Fusarium* spp. and the toxin was so far not detected in the mycelium[1]. Recently we found[2] that *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyder and Hansen produced the toxin in the cotton plants within 12 h of inoculation which suggested that the toxin was readily released by the pathogen. The detection of fusaric acid in the mycelium and conidia is reported in this note.

The fungus was grown in Czapek's medium for 10 days at room temperature (28 ± 2°C). The mycelium was removed, washed 3 times with distilled water and the excess moisture was removed by blotting. 50 g of the mycelium was continuously extracted in a Soxhlet apparatus for 14 h with ethyl acetate (10 ml/g). The ethyl acetate extract was divided into 2 aliquots: 1 aliquot (I) was evaporated to dryness and the residue was dissolved in 2 ml methanol. The other aliquot (II) was also evaporated to dryness but the residue was dissolved in 25 ml distilled water, acidified to pH 3.0 with 2*N*-HCl and extracted with equal volumes of ether 3 times. The ether extract was evaporated and the residue was dissolved in 2 ml methanol.

About 100 µl of the fractions were spotted on Whatman No. 1 filter paper and developed descendingly in *n*-butanol-acetic acid-water (4:1:1). The papers were air dried and separately sprayed with bromo phenol blue (BPB); 1% Cu So_4-BPB and modified DRAGENDORFF's reagent[3].

A spot corresponding to authentic FA (courtesy of Prof. Dr. H. KERN, Eidg. Technische Hochschule, Zurich, Switzerland) which matched in Rf value (0.87) and colour reactions was detected. Whereas aliquot II contained only FA, aliquot I contained 4 more copper chelating compounds (Rf 0.72, 0.65, 0.57, 0.46) in addition to FA.

The toxicity of FA was bioassayed by aseptically applying the extract to filter paper discs of 1.1 cm diameter, air dried, placed on *Bacillus subtilis* seeded nutrient agar plates and incubated at room temperature for 12 h. The area of inhibition measured by the method of SMALE and KEIL[4] revealed that 100 µl of the extract caused an inhibition of 114 mm² while at 300 µl, it was 267 mm². The spot corresponding to known FA cut from paper chromatograms inhibited the bacterial growth by 147 mm².

The presence of FA in the conidia was also investigated. The fungus was grown in potato dextrose agar medium in petri plates for 10 days and the conidia were suspended in sterile distilled water. 150 ml of the suspension (ca. 3,000 spores/ml) was incubated at room temperature for 24 h, filtered off, the cell free filtrate was acidified and extracted thrice with equal volumes of ether. The solvent was flash evaporated and the residue was dissolved in 2 ml methanol. When the extract was chromatographically analysed, a spot corresponding to the authentic FA was detected. The methanol extract possessed strong antibiotic activity; at 100 µl the area of inhibition was 220 mm² while at 300 µl it was 575 mm².

In conclusion, the wilt toxin fusaric acid was detected in the mycelium and conidia of *Fusarium oxysporum* f. *vasinfectum*.

Résumé. Le champignon *Fusarium oxysporum* f. *vasinfectum* contient de l'acide fusarique (l'acide 5-*n*-butyle picolinique) dans les filaments et spores.

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