MUCOPOLYSACCHARIDES OF THE ESTROGEN-STIMULATED CHICK OVIDUCT

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SUMMARY

Hyaluronic acid, heparin monosulfuric acid and a mixture of chondroitinsulfuric acid-A and -B have been isolated from the oviducts of stilbestrol-injected chicks.

INTRODUCTION

As part of a program concerned with the influence of hormones on connective tissues, an investigation was undertaken to identify the acid mucopolysaccharides in certain target organs. One such organ, the cock's comb, contains both hyaluronic acid (HA)[1,2] and a mixture of chondroitinsulfuric acid-A and -B (CSA-A and CSA-B)[2]. In the chick comb, the content of mucopolysaccharides is increased by the administration of androgens[3–5].

The substance which accumulates in the sex skin of monkeys during the estrous phase of the menstrual cycle is believed to be HA or a closely related compound[6]. In view of the capacity of the chick oviduct to hypertrophy in response to the administration of estrogens, it was considered worthwhile to examine this target organ for its content of acid mucopolysaccharides.

The present paper describes the isolation and characterization of three mucopolysaccharide fractions from oviducts of stilbestrol-treated chicks. One fraction is identical with HA, the second fraction contains a mixture of CSA-A and CSA-B, and the third fraction is a glucosamine-containing sulfated mucopolysaccharide which appears to be identical with the heparin monosulfuric acid isolated by JORPES AND GARDELL[7] from ox liver and lung.

METHODS AND MATERIALS

190 sex-linked Cross breed* chicks, raised on chick starter mash from birth, received daily subcutaneous injections of 1.0 mg of stilbestrol in 0.1 ml of mazola oil for 1 week starting at the age of 13 days and were killed 24 h after the last injection. The oviducts were removed and trimmed of adhering tissue. A total wet weight of 338 g was obtained. After defatting with acetone the dry tissue weighed 57 g.

* A cross between Barred Rocks and Rhode Island Reds.

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The acetone-defatted oviducts were extracted with 860 ml of 0.5 *N* NaOH by constant shaking for 24 h. A crude mucopolysaccharide mixture was prepared from the alkaline extract by a method described previously[8]. The nitrogen and hexosamine concentrations of this mixture (285 mg) were 4.65 and 16 %, respectively. Zone electrophoresis on Celite filter-aid was then used to separate the mucopolysaccharides[8]. 1 cm portions of the Celite slab were eluted with 15 ml of water in coarse sintered glass funnels. The eluates were analyzed for uronic acid by the carbazole method of DISCHE[9].

Three peaks of uronic acid-containing material were found (Fig. 1). Eluates corresponding to each peak were combined, dialyzed against distilled water, and concentrated to a volume of 25 ml. Hexosamine, as determined by a modification of the ELSON-MORGAN method[10], was found in all three fractions.

In order to obtain sufficient material for characterizing the mucopolysaccharides, an attempt was made to isolate these substances from oviducts of mature hens. Isolation of mucopolysaccharides from mature hens' oviducts was more difficult than from stimulated immature oviducts since crude extracts of the former would collect on the walls of the glass when precipitated with alcohol. Additional oviducts were obtained, therefore, from 300 White Leghorn chicks that had been injected with stilbestrol at the same age and in the same manner as described above for the original batch of Cross breed chicks. Oviducts from the White Leghorn chicks did not attain the size of those of the sex-linked Cross breed chick treated similarly. The 49.6 g of acetone-defatted dry oviducts obtained were processed as above and the 3 fractions isolated by zone electrophoresis were pooled with the appropriate samples from the first preparation.

The molar ratio of N to hexosamine was greater than the expected 1:1 in both the slowest migrating fraction and the middle peak. Purification of the former (Table I, HA) was effected by passage through a column of Darco G-60 and cellulose powder[11], concentration of the effluent and precipitation with glacial acetic acid. The precipitate was washed thoroughly with ethanol and dissolved in water. After dialysis against distilled water it was diluted to a volume of 50 ml.

TABLE I

ANALYSES OF ACID MUCOPOLYSACCHARIDES ISOLATED FROM OVIDUCTS OF STILBESTROL-TREATED CHICKS

Fraction	*N**	*Hexosamine***	*Uronic acid****	*SO_4§*	*Acetyl§§*	*Specific rotation*	*Turbidity reduction by testicular hyaluronidase§§§*	*Amino sugar†*
	μequivalents per ml					$[\alpha]_D$	*per cent*	
HA (acid form)	1.48	1.46	1.48	0	1.36	−81.5°	100	Glucosamine
Heparin monosulfuric acid (Na form)	1.03	1.00	0.93	1.22	0.47	+78.1°	10	Glucosamine
CSA (Na form)	1.27	1.44	1.04	1.49	1.45	−58.3°	71	Galactosamine

* By the micro-Kjeldahl method.

** By a modification of the ELSON-MORGAN method[10] following hydrolysis in 4 *N* HCl for 14 h at 100°.

*** By the colorimetric carbazole reaction[9].

§ By a colorimetric reaction with barium chloranilate[13] following hydrolysis in *N* HCl for 2 h at 100°.

§§ By a colorimetric reaction with hydroxamic acid[14].

§§§ By the method of DORFMAN AND OTT[15].

† By the method of STOFFYN AND JEANLOZ[16].

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The middle peak (Table I, heparin monosulfuric acid) was precipitated at 37° with a 1% aqueous solution of cetyl pyridinium chloride[12]. The cetyl pyridinium complex was dissolved in 3 *M* NaCl and the mucopolysaccharide precipitated by the addition of 4 volumes of ethanol. After 5 washings with ethanol, the precipitate was dissolved in water and dialyzed to eliminate the last traces of salt. The solution was then diluted to a volume of 50 ml.

RESULTS

Hyaluronic acid

The analytical values of the slowest migrating fraction are shown in Table I. Nitrogen, hexosamine, uronic acid, and acetyl are present in equimolar concentrations; ester sulfate is absent. The electrophoretic mobility on Celite (Fig. 1), the high negative rotation and the susceptibility to digestion by testicular hyaluronidase are consistent with the conclusion that this material is identical with HA from other sources.

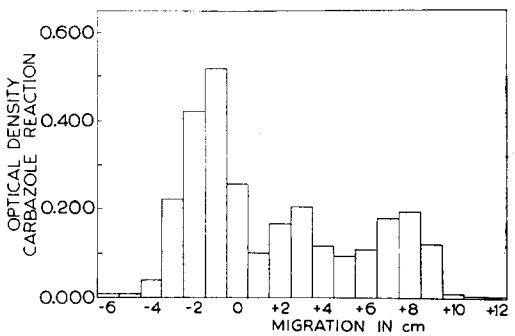

Fig. 1. Distribution of uronic acid after zone electrophoresis of a mucopolysaccharide preparation from chick oviducts.

Chondroitinsulfuric acid

The most rapidly migrating fraction is a mixture of CSA-A and CSA-B, as indicated by the analyses (Table I). The molar ratio of hexosamine:uronic acid (as determined by the carbazole reaction) is 1.0:0.72. This ratio is 1.0:1.0 for a pure sample of CSA-A and 1.0:0.38 for CSA-B. Unlike skin[8], CSA-A constitutes the major component of the mixture since some 70% of the material is susceptible to digestion by testicular hyaluronidase. An infra red spectrum indicated the presence of either CSA-A or -B or both when compared with the spectra published by MATHEWS[17].

Heparin monosulfuric acid

The analytical data of the material isolated from the middle peak are presented in Table I. They indicate an identity of this substance with the heparin monosulfuric acid isolated from ox lung and liver by JORPES AND GARDELL[7]. The disaccharide unit is thus composed of glucosamine, uronic acid and ester sulfate moieties in equimolar concentrations and an acetyl group in 0.5 *M* concentration. Like the heparin monosulfuric acid of JORPES AND GARDELL[7], the monosulfated compound is hydrolyzed more rapidly than is heparin but more slowly than are either CSA-A or -B. Rates of

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hydrolysis were determined by measuring the amount of hexosamine liberated at 1 and 14 hours from a solution of the substance in 1 *N* HCl maintained at 100°.

The infra red spectrum of the substance is illustrated in Fig. 2 and is compared with a sample of heparin monosulfuric acid obtained from the Upjohn Company. The commercial preparation was purified from contaminating heparin by fractionation with cetyl pyridinium chloride[18] followed by zone electrophoresis on Celite.

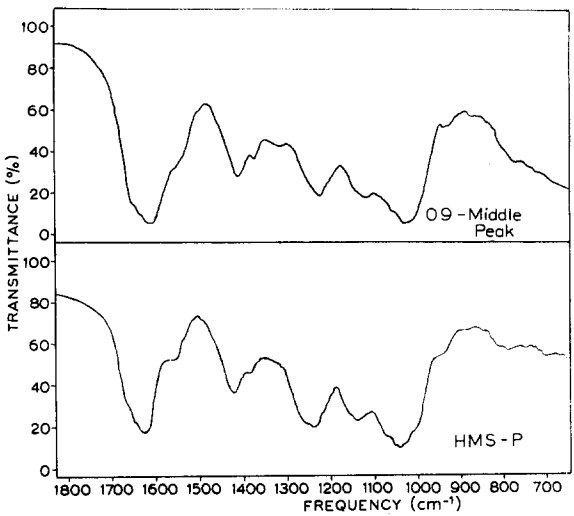

Fig. 2. Infrared spectra of heparin monosulfuric acid from chick oviducts (above) and from a purified commercial preparation (below).

DISCUSSION

It is now increasingly clear that connective tissues contain a greater variety of sulfated mucopolysaccharides than was hitherto suspected. Differences between them may be sufficiently subtle to escape detection by techniques commonly employed for their characterization.

The fraction that has an electrophoretic mobility on a Celite slab intermediate between HA and CSA, appears to be identical with the heparin monosulfuric acid of ox lung and liver[7]. This conclusion is based on the similarity of chemical analyses, the high positive specific rotation, the identity of the amino sugar as glucosamine and the rate of acid hydrolysis. Of particular interest is the low molar ratio of acetyl to hexosamine (0.5:1.0). It is possible that the incomplete acetylation is related to the rate of migration found by zone electrophoresis (Fig. 1).

A similar compound has been isolated from urine[19,20] and liver[20–22] of patients with the Hurler syndrome and from liver amyloid and abdominal aorta[23]. The low molar ratio of acetyl to hexosamine found in the present communication has also been reported by others[21,23]. On the other hand, variable ratios of S to hexosamine were obtained by BROWN[21] and by LINKER *et al.*[23], a finding at variance with the analysis reported here and with that of JORPES AND GARDELL[7] for heparin monosulfuric acid. It seems likely that elucidation of this discrepancy will provide evidence for the existence of additional sulfated glucosamine-containing acid mucopolysaccharides possessing a high positive rotation.

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Whether or not the level of acid mucopolysaccharides in oviducts is under the influence of estrogens cannot be ascertained from the present study. The chicks were injected with stilbestrol on the assumption that the stimulated organ would yield acid mucopolysaccharides in quantities sufficient for isolation and characterization. The assumption is supported by the studies of ANASTASSIADIS *et al.*[24] who found increased concentrations of hexosamine in oviducts of chicks following the administration of estradiol benzoate.

It may be of interest to note that the oviduct appears to be one of the few animal tissues from which both uridine nucleotides[25] and mucopolysaccharides have been isolated.

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REFERENCES

1 N. F. BOAS, *J. Biol. Chem.*, 181 (1949) 573.
2 S. SCHILLER AND A. DORFMAN, *Proc. Soc. Exptl. Biol. Med.*, 92 (1956) 100.
3 A. W. LUDWIG AND N. F. BOAS, *Endocrinology*, 46 (1950) 291.
4 S. SCHILLER, E. P. BENDITT AND A. DORFMAN, *Endocrinology*, 50 (1952) 504.
5 J. A. SZIRMAI, *Proc. Soc. Exptl. Biol. Med.*, 93 (1956) 92.
6 E. CHAIN AND E. S. DUTHIE, *Brit. J. Exptl. Pathol.*, 21 (1940) 324.
7 J. E. JORPES AND S. GARDELL, *J. Biol. Chem.*, 176 (1948) 267.
8 S. SCHILLER, M. B. MATHEWS, H. JEFFERSON, J. LUDOWIEG AND A. DORFMAN, *J. Biol. Chem.*, 211 (1954) 717.
9 Z. DISCHE, *J. Biol. Chem.*, 167 (1947) 189.
10 L. A. ELSON AND W. T. J. MORGAN, *Biochem. J.*, 27 (1933) 1824.
11 J. A. CIFONELLI AND M. MAYEDA, *Biochim. Biophys. Acta*, 24 (1957) 397.
12 J. E. SCOTT, *Biochim. Biophys. Acta*, 18 (1955) 428.
13 R. J. BERTOLACINI AND J. E. BARNEY, *Anal. Chem.*, 29 (1957) 281.
14 J. LUDOWIEG AND A. DORFMAN, unpublished results.
15 A. DORFMAN AND M. L. OTT, *J. Biol. Chem.*, 172 (1948) 367.
16 P. J. STOFFYN AND R. W. JEANLOZ, *Arch. Biochem. Biophys.*, 52 (1954) 373.
17 M. B. MATHEWS, *Nature*, 181 (1958) 421.
18 J. E. SCOTT, S. GARDELL AND I. M. NILSSON, *Biochim. J.*, 67 (1958) 7p.
19 A. DORFMAN AND A. E. LORINCZ, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 443.
20 K. MEYER, M. M. GRUMBACH, A. LINKER AND P. HOFFMAN., *Proc. Soc. Exptl. Biol. Med.*, 97 (1958) 275.
21 D. H. BROWN, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 783.
22 A. E. LORINCZ, *Federation Proc.*, 17 (1958) 266.
23 A. LINKER, P. HOFFMAN AND K. MEYER, *Federation Proc.*, 17 (1958) 264.
24 P. A. ANASTASSIADIS, W. A. MAW AND R. H. COMMON, *Can. J. Biochem. Physiol.*, 33 (1955) 627.
25 J. STROMINGER, *Biochim. Biophys. Acta*, 17 (1955) 283.