Isolation, Characterization and the Role of Rabbit Testicular Arylsulphatase A in Fertilization

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Arylsulphatase A was purified from rabbit testis. The purification was accomplished by a four-step procedure involving $(\text{NH}_4)_2\text{SO}_4$ fractionation, chromatography on DEAE-cellulose, SP(sulphopropyl)-Sephadex and affinity chromatography on concanavalin A-Sepharose. The specific activity of purified preparation was $135 \mu\text{mol/min}$ per mg of protein, which represented an increase of 900-fold above that of the crude homogenate. The purified enzyme ($20\text{--}50 \mu\text{g}$) was found to move electrophoretically as a single band on polyacrylamide gel at pH 7.2 and 8.4. The homogeneous enzyme was shown to be a glycoprotein with 0.8% (w/w) of *N*-acetylneuraminic acid and 20% neutral sugar. The treatment of purified enzyme with bacterial neuraminidase had no effect on enzyme activity or kinetic properties, but it changed the elution profile of rabbit testis arylsulphatase A through DEAE-Sephadex. The purified enzyme was strongly inhibited by Cu^{2+} , Fe^{3+} and Ag^+ . It hydrolysed several sulphate esters including cerebroside 3-sulphate, ascorbic acid 2-sulphate and steroid sulphates. Pure arylsulphatase was effective in dispersing the cumulus cells of rabbit ova.

Arylsulphatases (EC 3.1.6.1), the group of enzymes catalysing the hydrolysis of aryl sulphates, are universally distributed from bacteria to mammals. Three types of arylsulphatases, namely arylsulphatases A, B and C, distinguishable by their relative substrate specificities, response to inhibitors and intracellular localization, have been known to exist in mammalian tissues (Dodgson & Rose, 1975; Roy, 1976; Farooqui & Mandel, 1977). Arylsulphatases A and B have been extensively purified from various animal sources (Nichol & Roy, 1964; Breslow & Sloan, 1972; Bleszynski & Roy, 1973; Stevens *et al.*, 1975; Lee & Van Etten, 1975; Balasubramanian & Bachhawat, 1975; Shapira & Nadler, 1975; Draper *et al.*, 1976; Farooqui & Roy, 1976). Arylsulphatase A, an acidic glycoprotein (Graham & Roy, 1973), differs from the corresponding arylsulphatase B, which is a slightly basic glycoprotein (Farooqui & Roy, 1976), in many physicochemical and kinetic properties (Dodgson & Rose, 1975; Roy, 1976; Farooqui & Mandel, 1977). The physiological role of arylsulphatases A and B has been recently established. Arylsulphatase A is a cerebroside 3-sulphatase or, more precisely, a galactose 3-sulphatase, and its deficiency causes the disease metachromatic leucodystrophy, which is characterized by an abnormal accumulation of cerebroside 3-sulphate in various organs and by excessive excretion of this lipid in urine (Austin *et al.*, 1963; Mehl & Jatzkewitz, 1968; Farooqui & Bachhawat, 1973; Jerfy & Roy, 1973; Dulaney & Moser, 1978). Arylsulphatase B is a *N*-acetylgalactosamine 4-sulphatase (Fluharty *et al.*,

1975; Farooqui & Roy, 1976; Farooqui, 1976a), and its deficiency results in Maroteaux-Lamy syndrome (Stumpf *et al.*, 1973), a mucopolysaccharidosis characterized by a marked accumulation and excretion of dermatan sulphate (Maroteaux *et al.*, 1963).

The occurrence of arylsulphatases in mammalian testes is well known (Dodgson *et al.*, 1953; Allison & Hartree, 1970; Hook *et al.*, 1973). Arylsulphatase A has been purified from boar and rabbit testis (Yamato *et al.*, 1974; Yang & Srivastava, 1974, 1976). The purified preparation was not homogeneous and had a specific activity of $38 \mu\text{mol/min}$ per mg of protein. Bishayee *et al.* (1973) have reported that arylsulphatases bind concanavalin A. Further, the concanavalin A-arylsulphatase complex can be dissociated by concanavalin A-specific sugars such as α -methyl D-glucoside or α -methyl D-mannoside. The concanavalin A-Sepharose chromatography has been successfully used for the purification of arylsulphatases from various sources (Balasubramanian & Bachhawat, 1975, 1976). In the present paper we describe a rapid method for the purification of rabbit testis arylsulphatase A by concanavalin A-Sepharose chromatography. Some properties of a homogeneous preparation of the enzyme are also presented.

Materials and Methods

The following materials were obtained from indicated sources: *p*-nitrocatechol sulphate, *p*-nitrophenyl sulphate, D-glucose 6-sulphate, dehydro-

isoandrosterone sulphate, esterone 3-sulphate, *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide, neuraminidase (from *Clostridium perfringens*; type V, lot 96C-8125) and Coomassie Brilliant Blue G from Sigma Chemical Co., St. Louis, MO, U.S.A.; DEAE-cellulose DE52 (microgranular) from Whatman Chemicals, Maidstone, Kent, U.K.; DEAE-Sephadex A-50, SP(sulphopropyl)-Sephadex and concanavalin A-Sephadex from Pharmacia, Uppsala, Sweden; acrylamide, *NN'*-methylenebis-acrylamide and rhodizone dipotassium salt from Eastman Chemical Co., Rochester 3, N.Y. U.S.A.; cholesterol sulphate was a gift from Dr. K. D. Roberts (Hôpital Maisonneuve-Rosemont, Montreal, Que., Canada); cerebroside 3-sulphate and ascorbic acid 2-sulphate were kindly provided by Dr. L. Sarlieve and Dr. G. Rebel (Faculté de Médecine, Institut de Chimie Biologie, Strasbourg, France); all other chemicals used in the present study were of analytical grade.

Assay of rabbit testis arylsulphatase A

The enzyme was assayed by measuring the release of 4-nitrocatechol from 4-nitrocatechol sulphate (Yang & Srivastava, 1976). The incubation mixture (0.4 ml) consisted of 0.25 M-sodium acetate buffer, pH 5.2, 10 mM-4-nitrocatechol sulphate and 5–10 μ l of enzyme. The tubes were incubated for 10 min at 37°C and the reaction was terminated by the addition of 2.5 ml of 1 M-NaOH. The amount of 4-nitrocatechol liberated (ϵ_{510} 12600 litre $\cdot$ mol⁻¹ $\cdot$ cm⁻¹) was determined spectrophotometrically at 510 nm. A unit of enzyme is defined as the amount liberating 1 μ mol of 4-nitrocatechol/min under the above conditions.

Arylsulphatase A activity towards cerebroside 3-sulphate, ascorbic acid 2-sulphate and steroid sulphates was determined by the method of Waheed & Van Etten (1978).

Determination of protein

This was carried out as described by Bradford (1976), with bovine serum albumin as standard. The chromatographic profiles were based on A_{280} . The specific activity of the enzyme at each purification step was based on the 4-nitrocatechol sulphate assay and protein determination on pooled fractions by the method of Bradford (1976). The specific activity was expressed as units/mg of protein.

Neuraminidase treatment

Portions of purified rabbit testis arylsulphatase A (224 μ g of protein, containing 30 units) were incubated with neuraminidase from *Clostridium perfringens* (3.1 units) for 10 h at 37°C in 0.05 M-sodium acetate, pH 5.0. The neuraminidase was omitted from the control tubes. At the end of the incubation period the solutions were dialysed against 0.05 M-Tris/HCl

buffer, pH 7.4. The dialysed preparation was applied to a column (1 cm $\times$ 22 cm) of DEAE-Sephadex equilibrated in 0.05 M-Tris/HCl buffer, pH 7.4. The column was eluted with 125 ml of the same buffer and then with a linear gradient formed from 100 ml of 0.05 M-Tris/HCl buffer, pH 7.4, and 100 ml of 0.5 M-NaCl in the same buffer. Fractions (2.5 ml) were collected at a flow rate of 0.2 ml/min.

Carbohydrate analysis

The neutral-sugar content of the enzyme was determined by the anthrone method (Ashwell, 1957), with galactose as the standard. The *N*-acetylneuraminic acid content of the enzyme was determined by the method of Warren (1959).

Amino acid analysis

Samples of the purified enzyme (112 μ g) were placed in microcarius tubes and sufficient constant-boiling conc. HCl was added to give a final HCl concentration of 6 M. The ampoules were evacuated, sealed and heated at 110°C for 22 h. The hydrolysate was evaporated to dryness, dissolved in 0.2 ml of 0.2 M-sodium citrate buffer, pH 2.2, and analysed under standard conditions with a Beckman 120C analyser (Beckman 120C Manual, 1975).

Gel electrophoresis

Polyacrylamide-gel disc electrophoresis was performed at room temperature as described by Brewer & Ashworth (1969) with 7.5 and 10% gel in 0.03 M-Tris/HCl and Tris/glycine buffers, pH 7.2 and 8.4, for 3 h at 2 mA/tube. Bromophenol Blue was used as a tracking dye. The gels were stained by 1% Amido Black in 7% (v/v) acetic acid and destained with 10% acetic acid at room temperature. For activity determination an unstained gel was cut into 0.5 cm segments and incubated in the assay system for arylsulphatase for 2 h.

Dispersal of cumulus oophorus by the homogeneous preparation of arylsulphatase A

Freshly ovulated ova were obtained from rabbit tracts by flushing with 0.15 M-NaCl. The cumulus clots containing ova were transferred to small dishes in 2.0 ml of 0.05 M-Tris/HCl buffer, pH 7.0, containing 0.15 M-NaCl. Various amounts of purified arylsulphatase A were added to the dishes and the ova were observed for the dispersal of the cumulus matrix at room temperature under an Olympus inverted microscope. The control cumulus clots placed in 2.0 ml of 0.05 M-Tris/HCl buffer, pH 7.0, containing 0.15 M-NaCl, showed no evidence of dispersion after 24 h at room temperature.

Purification of rabbit testis arylsulphatase A

All operations were carried out at 0–4°C unless otherwise mentioned. Rabbit testes (200g) were cut into small pieces and homogenized in a Waring blender for 2min with 800ml of 0.05M-Tris/HCl buffer, pH7.4, containing 0.1M-NaCl. The homogenate was centrifuged for 30min at 27000g and the supernatant was kept. The pellet was suspended in 400ml of the same buffer and homogenized once again in a Waring blender for 1min. The homogenate was centrifuged as above. The pellet was discarded, and the two supernatants were combined and subjected to $(\text{NH}_4)_2\text{SO}_4$ fractionation.

 $(\text{NH}_4)_2\text{SO}_4$ fractionation

The supernatant was made 60% saturated by the addition of solid $(\text{NH}_4)_2\text{SO}_4$ with constant mechanical stirring. After equilibration for 2h the solution was left overnight at 4°C. It was then centrifuged at 27000g for 30min in a Sorvall centrifuge with a SS-34 rotor. The supernatant was discarded and the pellet was dissolved in 200ml of cold water and dialysed against water until sulphate-free. Finally the solution was dialysed against 0.02M-Tris/HCl buffer, pH7.4. The dialysed solution was centrifuged at 27000g for 30min and the clear supernatant was subjected to DEAE-cellulose chromatography.

DEAE-cellulose chromatography

The above solution was applied to a column (6.0cm×40cm) of DE-52 cellulose equilibrated with 0.02M-Tris/HCl buffer, pH7.4. The column was washed with 2 litres of the above buffer and the elution was continued with a stepwise gradient of NaCl in the same buffer at a flow rate of 0.5ml/min (Yang & Srivastava, 1976). Fractions (15ml) were collected. The 0.25M-NaCl fractions having arylsulphatase, *N*-acetylhexosaminidase and acid phosphatase activities were pooled, concentrated by ultrafiltration in an Amicon cell (membrane UM-10) and dialysed against 0.025M-sodium citrate buffer, pH4.5, for 20h with three changes. The precipitate that appeared during dialysis was removed by

centrifugation at 27000g for 30min and the clear supernatant was subjected to SP-Sephadex chromatography.

SP-Sephadex chromatography

The above supernatant (54ml) was applied to a column (1.5cm×30cm) of SP-Sephadex equilibrated in 0.025M-sodium citrate buffer, pH4.5. The column was washed with the same buffer at a flow rate of 0.2ml/min. The fractions containing arylsulphatase activity were pooled and concentrated by ultrafiltration as described above. This solution was exhaustively dialysed against 0.02M-Tris/HCl buffer, pH7.4, containing 1M-NaCl, 1mM-MnCl₂ and 1mM-CaCl₂. These salts were necessary for optimal binding of glycoproteins with concanavalin A-Sepharose (Karlstam, 1973).

Concanavalin A-Sepharose chromatography

The dialysed solution (14.5ml) was then applied to a column (1.5cm×13cm) of concanavalin A-Sepharose equilibrated in 0.02M-Tris/HCl buffer, pH7.4, containing 1M-NaCl, 1mM-MnCl₂ and 1mM-CaCl₂, at room temperature. The column was washed with 4.5ml portions of the same buffer at a flow rate of 0.15ml/min. After 70 fractions had been collected, the column was eluted with a gradient formed from 125ml of the starting buffer+125ml of 0.5M-methyl α -D-mannoside in the same buffer. The active fractions were pooled and concentrated to 5ml by ultrafiltration.

Results and Discussion

Rabbit testis arylsulphatase A was purified 900-fold from the homogenate, with a recovery of 18%, by using multiple column chromatography (Table 1). The yield of the enzyme was 14mg/1kg of rabbit testes. This is a much higher yield of the pure material than was reported for ox liver, human liver and sheep brain arylsulphatase A (Nichol & Roy, 1964; Balasubramanian & Bachhawat, 1975; Shapira & Nadler, 1975). DEAE-cellulose chromatography

Table 1. Summary of preparative scheme for rabbit testis arylsulphatase A

A unit of enzyme activity was defined as the amount liberating 1 μ mol of nitrocatechol/min under conditions described in the text.

Stages	Total protein (mg)	Activity (total units)	Specific activity	Yield (%)
Homogenate	14472	2124	0.146	100
27000g supernatant	8400	1506	0.179	71
0–60%-satd. $(\text{NH}_4)_2\text{SO}_4$ fraction	3365	1279	0.380	60
DEAE-cellulose fraction	713	1092	1.53	51
SP-Sephadex fraction	36	580	16.0	27
Concanavalin A-Sepharose fraction	2.8	380	135	18

Fig. 1. Elution profile of rabbit testis arylsulphatase A through a concanavalin A-Sepharose column

A linear gradient of methyl α -D-mannoside was applied as indicated by the arrow. Full experimental details are given in the Materials and Methods section. ●, Arylsulphatase A activity; ----, A_{280} .

indicated the presence of only one form of arylsulphatase, arylsulphatase A, in rabbit testes (Yang & Srivastava, 1976). The enzyme was completely retained on the column and could be eluted with 0.25M-NaCl. Similar results were obtained with arylsulphatase A from other sources (Bleszynski *et al.*, 1969; Gniot-Szulczycka, 1972). SP-Sephadex chromatography gave 10-fold purification of arylsulphatase A, with 53% recovery of enzyme activity over that in the previous step. The elution profile of rabbit testis arylsulphatase A on gel filtration through concanavalin A-Sepharose is shown in Fig. 1. About 85–90% of protein was washed out from the column and the enzyme was completely retained. The enzyme appeared as a sharp peak when the column was eluted with a linear gradient of methyl α -D-mannoside. The retention of enzyme on concanavalin A-Sepharose and its elution by methyl α -D-mannoside indicated that the binding of the enzyme with concanavalin A-Sepharose was probably through the carbohydrate moiety of this enzyme. A similar elution profile was also obtained for sheep brain and human placental enzymes (Balasubramanian & Bachhawat, 1975; Farooqui, 1976b). The purity of the final preparation was judged by polyacrylamide-gel disc electrophoresis at pH 7.2 and 8.4. One protein band was obtained with both systems. The enzyme activity on unstained gel corresponded to the protein band of the stained gel.

Carbohydrate analysis, neuraminidase treatment and amino acid composition

Carbohydrate analysis of the purified enzyme gave a value of 20% neutral sugar and 0.8% sialic acid. The ox liver enzyme (Graham & Roy, 1973) contained 3.85% neutral sugar and 2.18% sialic acid and sheep brain enzyme (Balasubramanian & Bachhawat, 1975) had 25% neutral sugar and 0.5% sialic acid. The treatment of the enzyme with neuraminidase removed

the sialic acid from the enzyme and thus altered the elution profile of arylsulphatase A through DEAE-Sephadex. The elution volumes of the native and the desialylated enzymes were 212 and 187 ml respectively. Further, the removal of sialic residues caused no obvious change in kinetic properties such as pH optimum (5.0 for both native and desialylated enzymes), K_m value (4.44 mM for native and 4.25 mM for desialylated enzymes) and V_{max} (135 μ mol/min per mg of protein for native and 129 μ mol/min per mg of protein for desialylated enzymes) of the native and the neuraminidase-treated testicular arylsulphatase A. Graham & Roy (1973) also reported that the electrophoretic mobility and the affinity of ox liver arylsulphatase A for DEAE-Sephadex was altered by the neuraminidase treatment. However, kinetic properties of the desialylated enzyme did not differ from those of the native enzyme.

The amino acid composition of rabbit testis arylsulphatase A is presented in Table 2. In general the amino acid composition of rabbit testis enzyme is not very similar to that of the human liver and urine enzymes (Breslow & Sloan, 1972; Draper *et al.*, 1976). The most obvious difference between rabbit testis and human liver enzyme is the presence of large amounts of threonine and serine. The occurrence of higher amounts of glutamic and aspartic acid is presumably reflected in the low isoelectric point, 4.6, of arylsulphatase A (Yang & Srivastava, 1976). Ox liver arylsulphatase A is reported to have an isoelectric point of 3.4 (Nichol & Roy, 1965). The u.v. spectra of purified rabbit testis arylsulphatase A were similar to that of the enzyme from ox brain (Bleszynski

Table 2. Partial amino acid composition of rabbit testis arylsulphatase A

The values are based on a mol.wt. of 100000 (Yang & Srivastava, 1976). No corrections were made for the possible destruction during hydrolysis.

Amino acid	Composition (residues/molecule)
Lysine	22
Histidine	25
Arginine	34
Aspartic acid	45
Threonine	91
Serine	130
Glutamic acid	121
Proline	89
Glycine	105
Alanine	85
Valine	91
Methionine	20
Isoleucine	35
Leucine	82
Tyrosine	35
Phenylalanine	38

et al., 1969) and human liver (Shapira & Nadler, 1975). The specific absorption coefficient at 280 nm was $6.8 \text{ litre} \cdot \text{g}^{-1} \cdot \text{cm}^{-1}$, which is similar to that of the ox liver (Farooqui & Roy, 1976b) and human liver enzymes (Shapira & Nadler, 1975).

Properties of rabbit testis arylsulphatase A

The kinetic properties of the rabbit testis enzyme were similar to those reported by Yang & Srivastava (1976). The enzyme showed the same abnormal time-activity relationship as shown by arylsulphatase A from other sources (Roy, 1953; Baum & Dodgson, 1958; Roy & Trudinger, 1970; Farooqui & Bachhawat, 1971). Metal ions such as Na^+ , Mn^{2+} , Ca^{2+} , Co^{2+} and Zn^{2+} had no effect on the enzyme activity, but Cu^{2+} , Fe^{3+} and Ag^+ at $25 \mu\text{M}$ caused 86, 33 and 25% inhibition respectively of the enzyme activity (Table 3). EDTA alone had no effect on the enzyme activity, but it protected the enzyme against the inhibition by Cu^{2+} . Carbonyl reagents and ascorbate were also strong inhibitors of rabbit testis arylsulphatase A, as well as of ox liver and chicken brain arylsulphatase A (Roy, 1970; Farooqui & Bachhawat, 1972).

The substrate specificity of rabbit testis arylsulphatase A was tested on various sulphate esters (Table 4). In common with pig kidney (Mehl & Jatzkewitz, 1968), ox liver (Jerfy & Roy, 1973), boar testis (Yamamoto *et al.*, 1974), human urine (Stevens *et al.*, 1975) and human placenta (Farooqui, 1976b) arylsulphatase A, rabbit testis enzyme hydrolysed cerebroside 3-sulphate at a rate of $5.39 \mu\text{mol/min}$ per mg of protein. The rate of hydrolysis of ascorbic acid 2-sulphate was over ten times that of cerebroside 3-sulphate. Similar rates of ascorbic acid 2-sulphate hydrolysis were reported for ox liver (Roy, 1975; Carlson *et al.*, 1977) human urine (Fluharty *et al.*, 1976) and enzymes. The rate of hydrolysis of steroid sulphates by arylsulphatase A was low compared with that of 4-nitrocatechol sulphate, ascorbic acid 2-sulphate and cerebroside 3-sulphate, but might, nevertheless, be significant. Waheed & Van

Table 3. *Effect of metal ions, carbonyl reagents and ascorbate on rabbit testis arylsulphatase A*

The purified enzyme was kept at 37°C for 15 min in 0.25 M-sodium acetate buffer, pH 5.2, at indicated concentrations of various compounds with occasional shaking. The reaction was started by the addition of 4-nitrocatechol sulphate in a final volume of 0.4 ml. The tubes were incubated for 5 min at 37°C . A control reaction mixture containing all the components except the compound used was treated in the same way. Carbonyl reagents and ascorbic acid were adjusted to pH 7.0 before use. Results are means $\pm$ s.d. for three determinations.

Added compound	Concn. (mM)	Activity (% of control)
None (control)	—	100
CuCl_2	0.025	14 ± 4
FeCl_3	0.025	67 ± 9
AgCl	0.025	75 ± 8
Hydroxylamine	1.25	48 ± 5
	2.50	26 ± 9
Hydrazine	12.5	47 ± 2
	25.0	34 ± 7
Semicarbazide	6.25	73 ± 7
	12.50	56 ± 9
EDTA	1.25	100
	12.5	94 ± 6
Ascorbate	0.01	90 ± 8
	0.025	64 ± 7
	0.05	26 ± 5
Ascorbate + EDTA	0.05	95 ± 3
	2.5	

Etten (1978) and Jerfy & Roy (1973) also reported the low rates of steroid sulphate hydrolysis by rabbit and ox liver arylsulphatase A. The rate of hydrolysis of various sulphate esters by native and desialylated rabbit testis arylsulphatase A were not very different from each other, indicating that sialyl residues did not play any role during hydrolysis of sulphate esters. Rabbit testis arylsulphatase A did not hydrolyse glucose 6-sulphate.

Table 4. *Substrate specificity of rabbit testis arylsulphatase A*

Asialoenzyme was prepared as described in the Materials and Methods section. The activity towards ascorbic acid 2-sulphate, cerebroside 3-sulphate and steroid sulphates was assayed by the method of Waheed & Van Etten (1978). Results are means $\pm$ s.d. for three determinations.

Substrate	Activity (nmol/min per mg of protein)	
	Native enzyme	Asialoenzyme
p-Nitrocatechol sulphate	135000 ± 1800	129000 ± 1600
Ascorbic acid 2-sulphate	52000 ± 920	46000 ± 800
Cerebroside 3-sulphate	5390 ± 70	5540 ± 90
Oestrone 3-sulphate	40 ± 2	42 ± 5
Dehydroisoandrosterone sulphate	50 ± 4	56 ± 8
Cholesterol sulphate	19 ± 3	20 ± 3
Glucose 6-sulphate	0	0

Table 5. *Dispersal of the cumulus oophorus by rabbit testis arylsulphatase A*

At least four cumulus clots were taken in 2ml of 0.05M-Tris/HCl buffer, pH7.0, containing 0.15M-NaCl, and incubated at room temperature (20°C) with purified arylsulphatase A. These values are means for four experiments.

Protein (μ g)	Enzyme (units)	Dispersal time (min)
3	0.4	150
14	1.9	90
28	3.7	60
56	7.5	15

The role of hyaluronidase in the dispersal of the cumulus oophorus is well established (Talbot & Franklin, 1974; Morton, 1977). That arylsulphatases readily leak out, like hyaluronidase, from non-detached acrosomes caused Allison & Hartree (1970) to suggest that sulphatase '... might conceivably contribute to the dispersal of the cumulus, although in the absence of knowledge of the natural sulphatase substrate, there is no evidence for this at present.' Our results in Table 5 present the first experimental evidence that the pure testicular arylsulphatase A indeed dispersed the cumulus of rabbit ova. There was no visible effect on the corona radiata and the zona pellucida of the ovum. There is an approximate linearity between the amount of the enzyme and the time taken for the dispersal of the cumulus. On the basis of protein, 28 μ g of the homogeneous arylsulphatase A, which is equivalent to 3.7 units, disperses the cumulus in 1 h at room temperature. The control cumulus in the buffer remained intact up to 24 h. Our unpublished data on the dispersal of the cumulus by pure hyaluronidase showed that 0.28 μ g of the homogeneous enzyme (1.25 munits) dispersed the cumulus in 1 h at room temperature. Although we cannot compare the two enzymes on the basis of their activities, as judged by the amount of pure protein required to disperse the cumulus under the identical conditions, there is no doubt that hyaluronidase is far more effective. These experiments were repeated four times over a period of several weeks and similar results were obtained. Our preliminary results show that the two enzymes act synergistically. In two experiments using cumulus clots from one super-ovulated rabbit, 1.5 units of arylsulphatase A took 105 min to disperse the cumulus, 0.5 munits of hyaluronidase required 130 min for the dispersal of the cumulus, and a combination of 1.5 units of arylsulphatase A and 0.5 munit of hyaluronidase removed the cumulus in 75 min. Thus there appears to be a decrease in the cumulus dispersal time when both the enzymes are present. These two enzymes may facilitate the passage of the spermatozoon through the cumulus of the ovum during fertilization process.

The crucial question as to whether cumulus oophorus contains a natural substrate for arylsulphatase A cannot be answered, since our present knowledge on the chemical composition of the cumulus matrix is limited, but hyaluronic acid is considered to be the major component (Piko, 1969). The dispersal of cumulus matrix by arylsulphatase A suggests that the cumulus contains sulphate esters (sulphated glycolipids or sulphated mucopolysaccharide), which are the substrates for arylsulphatase A.

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