the intrauterine locations or sexes of the fetuses and the incidences of ocular anomalies. Equal numbers of unilateral ocular anomalies were present on the right and left sides of the heads of these fetuses.
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Fertilization: A Uterine Glycosaminoglycan Stimulates the Conversion of Sperm Proacrosin to Acrosin

Abstract. *Acrosin is a proteinase required for mammalian fertilization, and in freshly ejaculated spermatozoa exists as an inactive zymogen, proacrosin. A factor present in uterine flushings of gilts stimulates the conversion of highly purified boar proacrosin to acrosin. Characterization of this factor indicates that its active component is a glycosaminoglycan.*

Acrosin (E.C. 3.4.21.10), a serine proteinase found in the acrosomes of mammalian spermatozoa, is used by spermatozoa for penetration of the zona pellucida of the ovum (*1*). The mechanism whereby proacrosin, the enzymatically inactive zymogen present at ejaculation (*2*), is converted to acrosin during the spermatozoa's residence in the female reproductive tract (*3*) is unclear. The work reported here shows that a glycosaminoglycan from porcine uterine flushings accelerates the conversion of highly purified proacrosin to acrosin.

Proacrosin was purified from ejaculated boar spermatozoa (*4*). Uterine fluid was obtained by flushing uteri with normal saline at laparotomy in gilts in which estrus was induced with pregnant mare serum gonadotropin and human chorionic gonadotropin (*5*). The generation of active acrosin from proacrosin was measured spectrophotometrically with *N*-α-benzoyl-L-arginine ethyl ester (BzArgOEt) at 253 nm (*6*).

Proacrosin is stable at *p*H 3.0, but when it is incubated at *p*H 7.0 it shows a sigmoidal conversion profile in which the time required for one-half maximum activity is 15 minutes ($t_{½}$). The $t_{½}$ is reduced to approximately 30 seconds when uterine flushings are included in the incubation medium (Fig. 1A). This acceleration of proacrosin conversion is dependent on the concentration of the uterine flushings (Fig. 1B). These results demonstrate the uterine flushings contain a factor that stimulates the conversion of proacrosin to acrosin.

Although proacrosin is converted to acrosin by trypsinlike proteinases (*4*), the uterine factor does not catalyze the hydrolysis of the synthetic trypsin substrate BzArgOEt, nor does the factor demonstrate proteolytic activity when measured with the general proteinase substrates Azocoll or Azoalbumin (lower limit of detection is 100 ng of trypsin equivalents per milliliter). Also, the activation of chymotrypsinogen and trypsinogen is not stimulated by the uterine factor. Furthermore, there is no demonstratable loss of the uterine factor's influence when it is incubated (24 hours at 37°C) with either trypsin (85 μg/ml) or pronase (83 μg/ml). Thus it is established that the uterine factor responsible for the stimulation of the conversion of proacrosin to acrosin is not a proteinase, and possibly not even a protein.

Anionic phospholipid vesicles also stimulate proacrosin conversion to acrosin (*7*). However, the uterine factor is not associated with subcellular organelles, nor is it a phospholipid, for it remains in the supernatant fluid after ultracentrifugation (2 hours at 150,000*g*), is not ether-extractable, and is resistant to digestion with phospholipase C (E.C. 3.1.4.3).

The ability to stimulate proacrosin conversion is rapidly lost when the uterine factor is incubated with testicular hyaluronidase (Fig. 2), an enzyme that specifically hydrolyzes the endo-*N*-acetylhexosamine bonds of hyaluronic acid

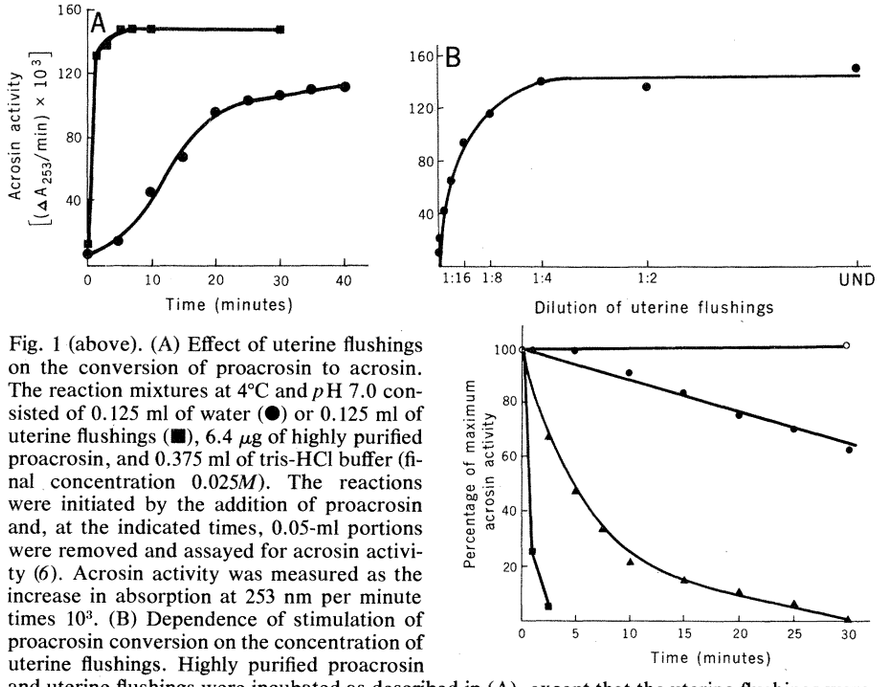

Fig. 1 (above). (A) Effect of uterine flushings on the conversion of proacrosin to acrosin. The reaction mixtures at 4°C and *p*H 7.0 consisted of 0.125 ml of water (●) or 0.125 ml of uterine flushings (■), 6.4 μg of highly purified proacrosin, and 0.375 ml of tris-HCl buffer (final concentration 0.025*M*). The reactions were initiated by the addition of proacrosin and, at the indicated times, 0.05-ml portions were removed and assayed for acrosin activity (*6*). Acrosin activity was measured as the increase in absorption at 253 nm per minute times 10^3. (B) Dependence of stimulation of proacrosin conversion on the concentration of uterine flushings. Highly purified proacrosin and uterine flushings were incubated as described in (A), except that the uterine flushings were diluted as indicated; *UND*, undiluted. After 3 minutes of incubation, 0.05-ml portions were removed and assayed for acrosin activity. Fig. 2 (right). Testicular hyaluronidase digestion of uterine factor. The reaction mixtures at *p*H 5.3 and 37°C consisted of 0.008*M* sodium acetate, 0.1 ml of undiluted uterine flushings, and testicular hyaluronidase, 5 μg (■), 0.5 μg (▲), 0.25 μg (●), and no hyaluronidase (○), in a total volume of 0.60 ml. Reactions were initiated by the addition of hyaluronidase and at the indicated times, 0.025-ml portions were removed and added to 0.11 ml of a *p*H 7.0 solution consisting of 0.08*M* tris-HCl and 2.89 μg of highly purified proacrosin, and were maintained at 4°C. After a 30-second incubation period, a 0.05-ml portion was removed and assayed for acrosin activity (*6*).

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and chondroitin sulfates A and C, but not chondroitin sulfate B, heparin, or keratosulfates (*8*). The uterine factor is also sensitive to digestion with chondroitin ABC lyase (E.C. 4.2.2.4) from *Proteus vulgaris*. These results indicate that the critical conversion of proacrosin to acrosin is stimulated by a uterine glycosaminoglycan, possibly through the interactions between the specialized proteinase zymogen (proacrosin) and the anionic surfaces of the glycosaminoglycan.

The presence of glycosaminoglycans in porcine uterine fluid is not surprising. Partial characterization of murine uterine secretions reveals a high carbohydrate content and the presence of a heparinlike substance (*9*), which is most likely a glycosaminoglycan. Furthermore, metachromic staining with basic dyes such as toluidine blue demonstrates the presence of glycosaminoglycans in rabbit endometrial stroma (*10*).

Female reproductive tract secretions are obligatory for fertilization since mammalian spermatozoa must reside in the female reproductive tract before they acquire the capacity to penetrate the zona pellucida of the ovum (*11*). During the incubation of boar spermatozoa in vivo for the time period established for boar spermatozoa to acquire zona pellucida-penetrating ability (*12*), 70 to 80 percent of the proacrosin is converted to acrosin. No conversion is observed in control spermatozoa incubated for the same time period in buffer (*13*), thus indicating that a female reproductive tract factor effects the physiological conversion of proacrosin to acrosin.

The present study documents the interaction of sperm proacrosin with a factor present in uterine flushings and indicates that this factor is a glycosaminoglycan. This interaction between proacrosin and the glycosaminoglycan may be one reason why spermatozoa must reside in the female reproductive tract prior to acquiring fertilization potential.

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A Regulatory Locus for Mouse β-Glucuronidase Induction, *Gur*, Controls Messenger RNA Activity

Abstract. *A regulatory locus in a higher organism has been shown to control a specific messenger RNA activity. The* Gur *locus in mice regulates the production of kidney β-glucuronidase messenger RNA activity after induction of the β-glucuronidase structural gene,* Gus, *by testosterone. β-Glucuronidase messenger RNA was assayed by its ability to direct the synthesis of catalytically active murine β-glucuronidase in* Xenopus *oocytes.*

In higher organisms, changes in messenger RNA (mRNA) activity underlie the alterations in protein synthesis that accompany hormonal induction (*1*) and the appearance of specialized proteins during differentiation. It has thus been an article of faith that a set of regulatory genes must exist in eukaryotes whose function is to modulate mRNA concentrations, but it has been frustrating to demonstrate this experimentally. This difficulty reflects on the one hand the lack of appropriate regulatory mutants for genes whose mRNA products can be readily assayed, and, on the other, the inability to assay the mRNA of genes that have characterized regulatory mutations. The recent development of a sensitive catalytic assay for mammalian β-glucuronidase mRNA (*2*) has made it possible to test for the presence of mRNA regulatory genes in a system with several characterized regulatory mutations (*3, 4*). In so doing we have found that the *Gur* regulatory locus (*5*) that controls rates of β-glucuronidase synthesis in kidneys in response to hormonal induction does, in fact, determine the response of mRNA activity.

Mouse kidney β-glucuronidase (E.C. 3.2.1.31) is induced by androgens such as testosterone (*4, 6*) when normal pituitary function is available (*7*). The response occurs in the epithelial cells of the proximal part of the convoluted tubule (*8*), and results from a dramatic increase in the rate of enzyme synthesis (*4*). The extent of induction is under the control of a regulatory locus, *Gur*, that is located in close proximity to or within the structural gene *Gus* (*4*). No recombination between *Gur* and *Gus* has been observed in conventional genetic crosses. The increase in rate of enzyme synthesis in mice of strain A/J carrying the Gur^a allele is about three times greater than in B/6 [see (*9*)] strain mice carrying the Gur^b allele. *Gur* acts in a cis manner (that is, it affects only the *Gus* gene on the same chromosome), and heterozygotes carrying Gur^a Gus^a on one chromosome and Gur^b Gus^b on the other synthesize a preponderance of GUS-A subunits (*4*).

β-Glucuronidase mRNA activity was assayed in mice of different genotypes to test whether the relative rates of enzyme synthesis seen in Gur^a and Gur^b mice actually reflect differences in mRNA activity. Assays were conducted on three strains of mice. Two of these were the strains originally used to define the *Gur* locus: A/J, which is homozygous for the Gur^a regulatory allele and Gus^a structural allele, and B/6, which is homozygous for the Gur^b and Gus^b alleles. The third strain, B/6 · Gur^a, carries a chromosome segment from strain A/J containing the associated Gur^a and Gus^a alleles transferred onto a B/6 genetic background by repeated backcrossing. This congenic line was developed by V. Chapman, advantage being taken of the close linkage between *Gur* and *Gus* and the ease of scoring *Gus* alleles in crosses. It is the result of six generations of backcrossing A/J onto a B/6 background with continued selection of $Gus^{a/b}$, $Gur^{a/b}$ heterozygotes, followed by an intercross between heterozygotes to produce $Gus^{a/a}$,

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