*J. Reprod. Fert.* (1986) **76**, 239–250

*Printed in Great Britain*


# β-*N*-Acetylglucosaminidase in the reproductive organs and seminal plasma of the bull

A. Jauhiainen and T. Vanha-Perttula

*Department of Anatomy, University of Kuopio, P.O. Box 6, 70211 Kuopio, Finland*

**Summary.** The highest specific activity of β-*N*-acetylglucosaminidase (β-NAG) was found in the different parts of the epididymis, where the activity seemed to be partly in secretory and partly in non-secretory, tissue-bound form. Epididymal spermatozoa also contained moderate β-NAG activity.

The β-NAG was separated by chromatofocussing and anion exchange chromatography with HPLC into multiple forms with distinct pI values (8·0–4·0). The cauda epididymidis, ampulla and the seminal vesicles formed the major secretory sources of the high β-NAG activity in bull seminal plasma. The major secretory forms of β-NAG in caput and cauda epididymidis showed distinct elution profiles. In the fractionation with gel filtration on Sepharose 6B, the β-NAG activities derived from bull testis and caput epididymidis had smaller molecular weights than did the secretory enzymes in seminal plasma, seminal vesicle secretion and cauda epididymidis. Maximum activity of all β-NAG isoenzymes was observed at pH 5·0. They were almost totally inactivated at 60°C and about 75–80% of the activity was lost at 55°C. All the isoenzymes were strongly inhibited by thiol reagents but not with other metal ions and chelating agents.

Histochemical studies showed a strong granular (lysosomal) reaction for β-NAG in basal cells and basal parts of the principal cells in all but the initial segment of the epididymis. An apical (secretory) reaction was prominent in the distal caput and corpus as well as in distal cauda. After the distal caput the luminal sperm mass became increasingly mixed with a β-NAG-positive material. The epithelial cells of the ampulla and seminal vesicle displayed a moderate apical (secretory) reaction.

## Introduction

The presence of high amounts of β-*N*-acetylglucosaminidase (β-NAG, 2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30) in the mammalian male genital tract is well known (Conchie & Mann, 1957; Conchie & Findlay, 1959; Boström & Öckerman, 1971). β-NAG is a hydrolytic enzyme, which is involved in the degradation of β-*N*-acetylglucosamine and β-*N*-acetylgalactosamine residues from glycolipids (e.g. gangliosides), glycoproteins and mucosubstances. A specific function for this enzyme in the male reproductive tract is not yet apparent, although a role in processes leading to fertilization has been suggested (Farooqui & Srivastava, 1980).

Multiple forms of β-NAG in the male reproductive system have been reported, e.g. in ram testis and/or epididymis (Caygill, Roston & Jevons, 1966; Bullock & Winchester, 1973), in bull semen (Khar & Anand, 1974; 1977a, b), in rat and mouse testis (Majumder & Turkington, 1974; Majumder, Lessin & Turkington, 1975; Gupta & Kapur, 1981), in rabbit (Farooqui & Srivastava, 1980), chimpanzee (Srivastava, Farooqui & Gould, 1981) and human semen (Gupta & Agarwal, 1982), as well as in boar epididymis (Parkes, Stirling & Calvo, 1984). Some studies have also shown that the testicular, epididymal and semen activities can be separated into numerous isoenzymes

which differ in their pI values (Bullock & Winchester, 1973; Conary, Thompson & Rodén, 1982). With neuraminidase treatment the multiple electrophoretic β-NAG activities of the bull epididymis have been transformed into a few slower moving bands (Hayase, Reisher & Kritchevsky, 1973).

Quantitative studies have indicated that the enzyme activity varies greatly along the length of the epididymis with clear differences between species (Bamberg, Hanna & Stöckl, 1975; Sinowatz, Bamberg, Lipp & Stöckl, 1975; Bamberg, Sinowatz & Schafelner, 1978; Milne, Williams & Rudolph, 1978; Bamberg, Sinowatz & Kanout, 1979; Skolek-Winnisch, Lipp, Stöckl & Bamberg, 1981). Similarly, variations have been reported in the intensity and distribution of the histochemical β-NAG reaction in different epididymal segments of the bull (Sinowatz *et al.*, 1975; Sinowatz, Lipp & Perfler, 1976), boar (Bamberg *et al.*, 1978) and dog (Bamberg *et al.*, 1979; Sinowatz, Fischer, Skolek-Winnisch & Chandler, 1979). In many studies the hormone-dependence of testicular (Majumder *et al.*, 1975) and epididymal (Bamberg *et al.*, 1975; Milne *et al.*, 1978) activities was emphasized.

In the present paper we describe the separation of β-NAG activities from seminal plasma, spermatozoa and different reproductive organs of the bull by using chromatofocussing and anion exchange chromatography with high-performance liquid chromatography (HPLC) on Mono Q. We also report some properties of the isoenzymes, their histochemical distribution and the possible origin of bull seminal plasma β-NAG activities.

## Materials and Methods

*Semen and reproductive organs.* All the bull semen samples and reproductive organs were obtained as described earlier (Jauhiainen & Vanha-Perttula, 1985). The epididymides were divided into six segments: $E_1$, $E_2$ and $E_3$ formed the caput epididymidis, $E_4$ the corpus epididymidis and $E_5$ and $E_6$ the cauda epididymidis (Jauhiainen & Vanha-Perttula, 1985).

*Chemicals.* The chemicals were obtained as indicated by Jauhiainen & Vanha-Perttula (1985). The substrates 4-methylumbelliferyl-*N*-acetyl-β-D-glucosaminide (4-MU-GlcNAc), *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (*p*-NP-GlcNAc) and naphthol AS-BI *N*-acetyl-β-D-glucosaminide (*N*-ASBI-GlcNAc) were purchased from Sigma Chemical Company (St Louis, MO, USA). 4-Methylumbelliferyl-*N*-acetyl-β-D-galactosaminide (4-MU-GalNAc) was from Boehringer Mannheim GmbH (Mannheim, West Germany).

*Tissue homogenization.* The tissue homogenates, epididymal secretions and sperm samples were prepared as described earlier (Jauhiainen & Vanha-Perttula, 1985) except that 0·02 M-sodium citrate, pH 7·0, containing 0·1% (v/v) Triton X-100 was used as the homogenization buffer instead of sodium acetate, which inhibits β-NAG activity.

*Fractionation.* Gel filtration on Sepharose 6B, chromatofocussing and anion exchange chromatography with HPLC were carried out as indicated by Jauhiainen & Vanha-Perttula (1985). However, in chromatofocussing, after the usual Polybuffer 74–HCl elution, an NaCl gradient (0–0·5 M) was formed in 0·02 M-sodium citrate, pH 5·0, instead of sodium acetate. For HPLC, all the samples were first diluted in 0·025 M-imidazole–HCl buffer, pH 7·4, and filtered through a Millipore-filter (pore size 0·45 μm). The β-NAG isoenzymes were then eluted with the same buffer and an NaCl gradient (0–0·3 M) from the anion exchange column as reported earlier (Jauhiainen & Vanha-Perttula, 1985).

*Measurement of enzyme activity.* If not otherwise mentioned, β-NAG activity in the bull seminal plasma, spermatozoa, different reproductive organs and in the fractions of gel filtration, chromatofocussing and anion exchange chromatography was measured with *p*-NP-GlcNAc as the substrate. The standard incubation medium contained 100 μl 0·1 M-sodium citrate buffer, pH 5·0, 100 μl enzyme solution and 100 μl 1·5 mM-substrate in water. After incubation at 37°C the reaction was

stopped with 500 µl 0·1 M-NaOH and the absorbances were measured with a Gilford Stasar III spectrophotometer at 410 nm. With *N*-ASBI-GlcNAc as substrate the reaction was stopped with 100 µl Fast Garnet GBC (1 mg/ml with 10% Tween 20 added) and 300 µl 1·0 M-sodium acetate buffer, pH 4·2. The red colour was measured at 540 nm with spectrophotometer. The hydrolysis of 4-MU-GlcNAc or 4-MU-GalNAc was studied in the same incubation medium or with 0·1 M-citric acid/0·2 M-sodium phosphate, pH 6·5. After incubation the reaction was terminated with 500 µl 0·1 M-NaOH and the liberated 4-methylumbelliferone was measured in a fluorescence spectrophotometer (Hitachi 650–60) at 365 nm for excitation and 450 nm for emission.

The protein concentration of the enzyme samples was estimated according to the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as the standard. The enzyme activities in the chromatographic fractions are given as absorbances and in the seminal plasma, organ secretions, spermatozoa and reproductive organs as nmol/min·mg protein$^{-1}$.

*Studies on enzyme characteristics.* The pH optimum for the pooled enzymes after chromatofocussing was determined in a series of 0·2 M-sodium citrate buffers having a pH range between 3·0 and 7·0. The effect of temperature was tested by preincubating the pooled enzyme preparations from chromatofocussing in 0·1 M-sodium citrate, pH 5·0, for 15 min at the temperature indicated. After cooling the mixtures on ice, the substrate (*p*-NP-GlcNAc) was added. The remaining enzyme activity was then assayed and compared with the control preincubated at 37°C.

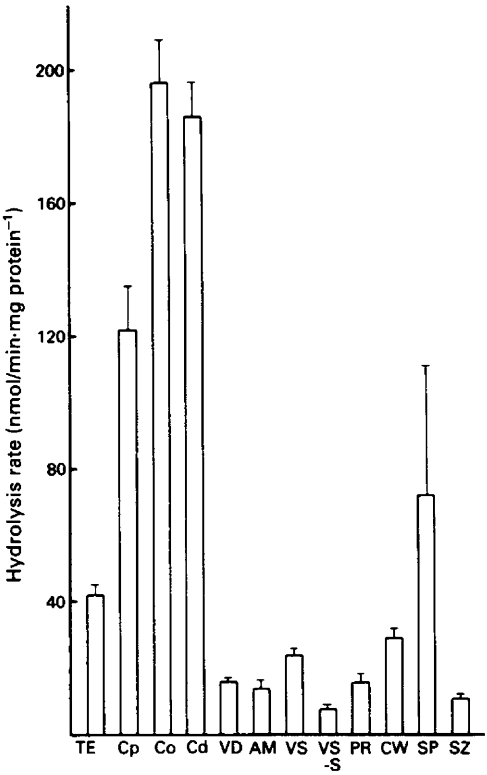


**Fig. 1.** Hydrolysis rate of β-NAG (substrate *p*-NP-GlcNAc) in bull testis (TE), caput (Cp), corpus (Co) and cauda (Cd) epididymidis, vas deferens (VD), ampulla (AM), seminal vesicle (VS), seminal vesicle secretion (VS-S), prostate (PR), Cowper's gland (CW), seminal plasma (SP) and ejaculated spermatozoa (SZ) of adult (about 1 year of age) bulls.

Various modifier substances were dissolved in 0·1 M-sodium citrate buffer, pH 5·0, and the incubation of enzyme pools from chromatofocussing was carried out as described earlier (Jauhiainen & Vanha-Perttula, 1985). The absorbance readings were converted to percentages using the pooled enzyme without any addition as the control (100% value).

The tentative molecular weights of β-NAG isoenzymes were estimated as described earlier (Jauhiainen & Vanha-Perttula, 1985).

*Histochemistry.* β-NAG was demonstrated in 10 μm unfixed tissue sections prepared with a Cryo-Cut microtome (American Optical Corp., Buffalo, NY, USA) with *N*-ASBI-GlcNAc as substrate and hexazotized-*p*-rosanilin as a coupling agent at pH 5·0 according to Hayashi (1965). The sections were studied and photographed with a Leitz Orthoplan microscope.

## Results

### *β-NAG activity in semen and in reproductive organs*

The hydrolysis rates of β-NAG in different reproductive organs, seminal vesicle secretion, seminal plasma and ejaculated spermatozoa are given in Fig. 1 with *p*-NP-GlcNAc as substrate at 0·5 mM final concentration. Table 1 shows the hydrolysis rate of β-NAG in different segments of the epididymis (segments $E_1$–$E_6$) in immature (about 2 months of age), young (5–7 months of age) and adult (about 1 year of age) bulls as well as in the epididymal cells, secretions and spermatozoa. In the adult bulls the highest level of β-NAG was found in different parts of the epididymis (particularly in segments $E_3$, $E_4$ and $E_6$). Part of this very high epididymal enzyme activity seemed to be secretory ($E_6$ secretion) and part of it seemed to be in non-secretory, tissue-bound form (segments

**Table 1.** The hydrolysis rate of β-*N*-acetylglucosaminidase in different segments of the epididymis ($E_1$–$E_6$) in immature (about 2 months of age), young (5–7 months of age) and adult (about 1 year of age) bulls and in epididymal cells, secretions and spermatozoa of different segments ($E_1$, $E_2+E_3$, $E_6$) in adult bulls

| | β-*N*-acetylglucosaminidase activity (nmol/min·mg protein$^{-1}$) | | |
|---|---|---|---|
| Sample | Immature bulls | Young bulls | Adult bulls |
| Epididymis | | | |
| $E_1$ | 21·74 ± 0·05 (3) | 24·05 ± 5·38 (3) | 36·56 ± 5·66 (5) |
| $E_2$ | 21·59 ± 2·85 (3) | 32·15 ± 2·52 (3) | 99·15 ± 25·62 (5) |
| $E_3$ | 21·94 ± 3·34 (3) | 136·98 ± 5·30 (3) | 115·99 ± 36·25 (5) |
| $E_4$ | 55·70 ± 1·93 (3) | 156·59 ± 59·31 (3) | 144·07 ± 34·34 (5) |
| $E_5$ | 60·95 ± 10·45 (3) | 85·02 ± 18·75 (3) | 98·46 ± 25·01 (5) |
| $E_6$ | 55·62 ± 0·32 (3) | 77·70 ± 8·61 (3) | 179·59 ± 40·61 (5) |
| Epididymal cells | | | |
| $E_1$ | | | 43·78 ± 5·40 (3) |
| $E_2+E_3$ | | | 153·85 ± 37·78 (3) |
| $E_6$ | | | 157·81 ± 27·42 (3) |
| Epididymal secretions | | | |
| $E_1$ | | | 15·02 ± 4·30 (3) |
| $E_2+E_3$ | | | 24·69 ± 3·16 (3) |
| $E_6$ | | | 210·54 ± 46·06 (3) |
| Epididymal spermatozoa | | | |
| $E_1$ | | | 44·80 ± 15·46 (3) |
| $E_2+E_3$ | | | 46·01 ± 14·17 (3) |
| $E_6$ | | | 26·50 ± 9·79 (3) |

Values are given as mean ± s.d. of duplicate measurements for the number of animals in parentheses.

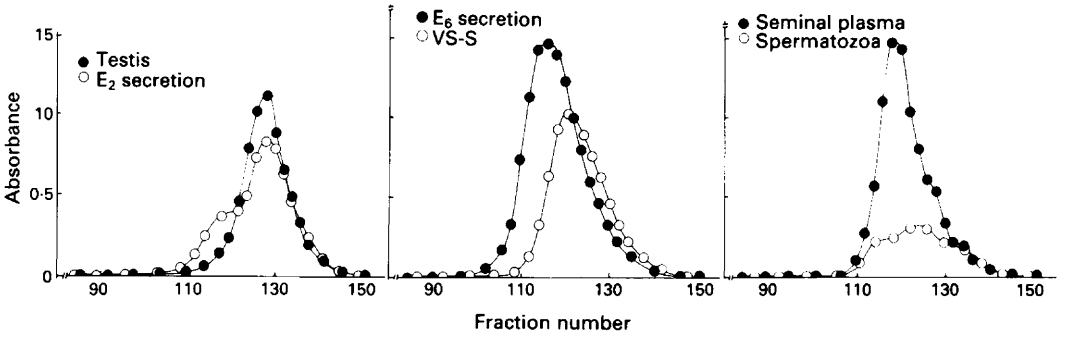


**Fig. 2.** β-NAG activity of bull testis, $E_2$ secretion, $E_6$ secretion, seminal vesicle secretion (VS-S), seminal plasma and ejaculated spermatozoa after gel filtration on Sepharose 6B. The hydrolysis was measured in the fractions with *p*-NP-GlcNAc as substrate as described in 'Materials and Methods'. The activities are given as absorbances at 410 nm.

$E_1$–$E_6$). The epididymal spermatozoa also contained moderate β-NAG activity. In immature animals the activity of β-NAG in the epididymis was significantly lower than in adult mature animals, thus demonstrating that the activity in the epididymis is probably hormone-dependent. The young bulls had epididymal enzyme levels nearly comparable to those of the adult animals. The most significant difference was found in the activity of the distal cauda ($E_6$).

*Sepharose 6B gel filtration*

A single peak for β-NAG was observed after gel filtration with all tissue homogenates and secretions (Fig. 2). However, the β-NAG activity occurring in bull testis and $E_2$ secretion (eluting in fractions 120–138) had a slightly smaller molcular weight ($M_r \sim 280 \times 10^3$) than did the secretory activity occurring in seminal plasma ($M_r \sim 380 \times 10^3$), seminal vesicle secretion ($M_r \sim 360 \times 10^3$) and $E_6$ secretion (eluting in fractions 110–130; $M_r \sim 410 \times 10^3$). The β-NAG activity in ejaculated spermatozoa eluted in gel filtration as a quite broad peak in fractions 110–138.

*Chromatofocussing*

Chromatofocussing of bull seminal plasma (Fig. 3) resulted in the appearance of four activity areas for β-NAG (CF-1 to CF-4). The first two peaks (CF-1, pI 7·0, and CF-2, pI 7·5–6·9) seemed to coelute with the major activities of the epididymis. However, the prostate, Cowper's gland, ampulla and seminal vesicles also had activities eluting at the same sites. The peaks CF-3 (pI 6·3–5·5) and CF-4 (pI 5·3–4·7) in seminal plasma had closely similar locations with activities in prostate, Cowper's gland, ampulla and seminal vesicles. These peaks were particularly prominent in the fractionation of seminal vesicle secretion. The largest testicular activities eluted also coincided with CF-3 and CF-4, while two smaller ones, CF-5 (pI 4·3–4·0) and CF-6 (eluting always with the NaCl gradient), were present in testis homogenates and in ejaculated spermatozoa. The largest sperm peak, however, coincided with CF-1. The comparison of the β-NAG pattern in different parts of the epididymis revealed an enzyme form with low pI in the caput, which was weak or absent in the more caudal parts. As with *p*-NP-GlcNAc, use of 4-MU-GlcNAc, 4-MU-GalNAc or *N*-ASBI-GlcNAc as substrates at pH 5·0 and 6·5 gave identical isoenzyme peaks.

*Anion exchange chromatography*

The results after anion exchange chromatography with HPLC (Fig. 4) were quite comparable to

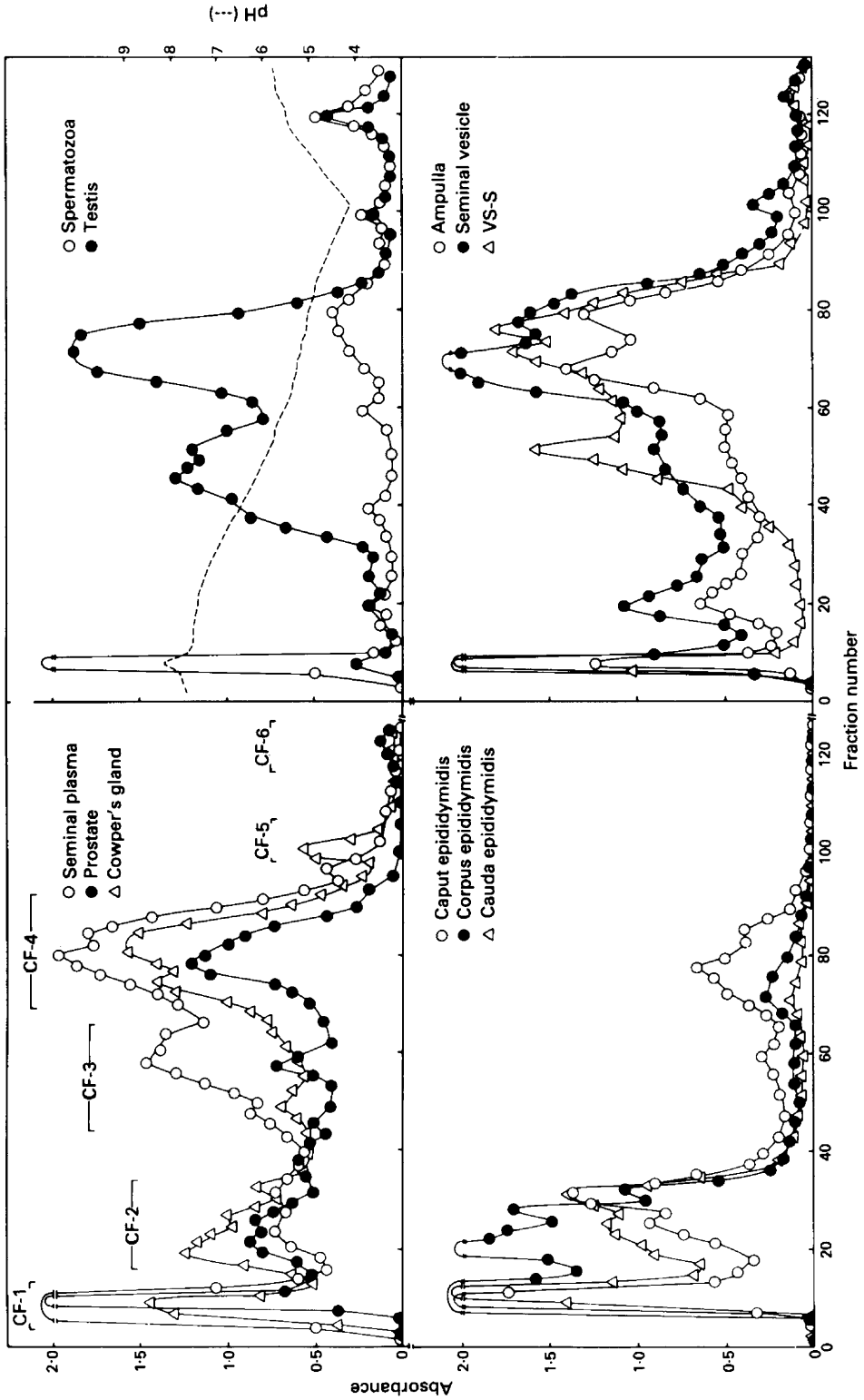


**Fig. 3.** β-NAG activity in chromatofocussing of bull seminal plasma, prostate, Cowper's gland, ejaculated spermatozoa, testis, caput, corpus and cauda epididymidis, ampulla, seminal vesicle and seminal vesicle secretion (VS-S). The hydrolysis of β-NAG was measured as indicated in Fig. 2. The pH gradient shown was identical for the other fractionations.

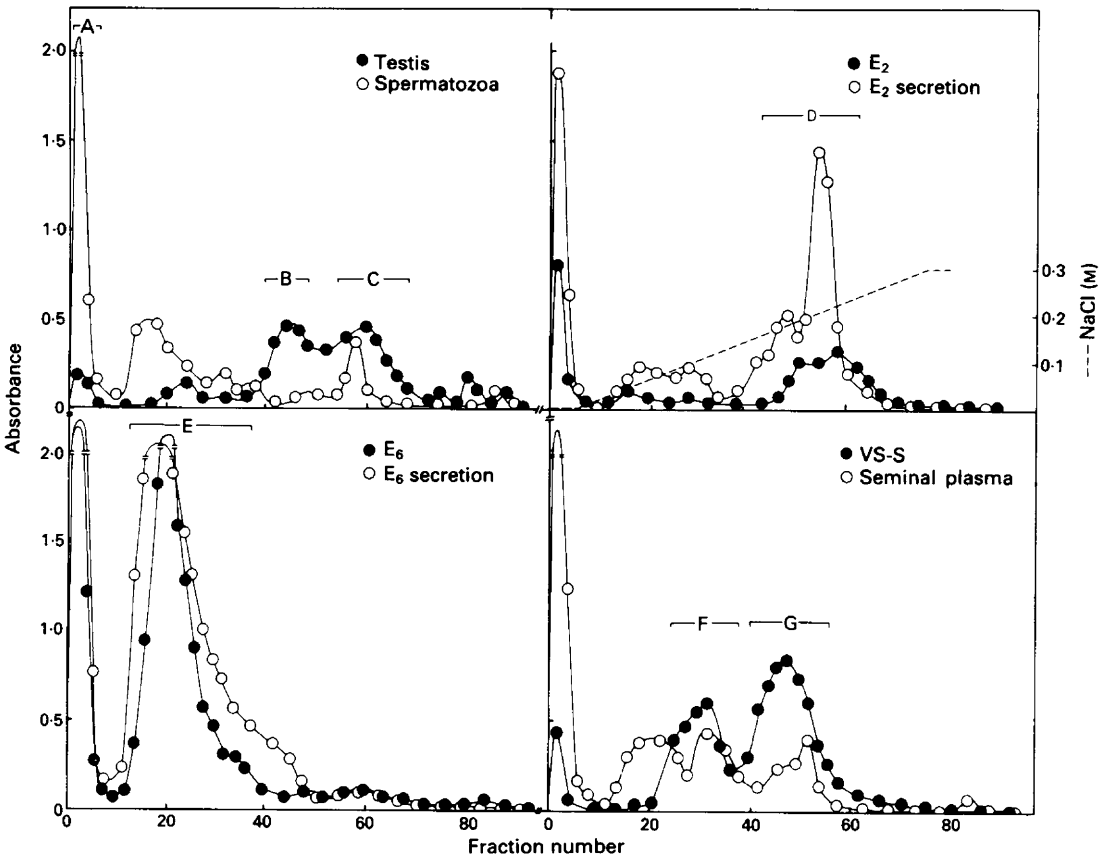


**Fig. 4.** β-NAG activity after anion exchange chromatography in HPLC (Mono Q) of bull testis, ejaculated spermatozoa, epididymal segment $E_2$, $E_2$ secretion, epididymal segment $E_6$, $E_6$ secretion, seminal vesicle secretion (VS-S) and seminal plasma. The hydrolysis of β-NAG was measured as indicated in Fig. 2. The NaCl gradient shown was identical for all fractionations.

those of chromatofocussing, thus indicating several distinct isoenzymes for β-NAG in bull seminal plasma and different reproductive organs. Of the 4 peaks found in the seminal plasma fractionation, two (eluted at the beginning and at 0·06 M-NaCl) coeluted with the major peaks of the epididymal cauda ($E_6$) secretion, while two others (peaks F and G eluted at 0·12 M- and 0·18 M-NaCl) coincided with the activities of seminal vesicle secretion. The major peak (D) of the epididymal caput ($E_2$) and its secretion was different from that (E) of the cauda ($E_6$) secretion. The major sperm activities coeluted with those of the epididymal cauda ($E_6$) and its secretion, while two testicular activities required higher sodium chloride concentrations (peak B, 0·15 M; peak C, 0·21 M) for elution.

*Optimal pH*

The β-NAG peaks pooled after chromatofocussing of bull seminal plasma (CF-1 to CF-4) and testis (CF-5 and CF-6) had their optimum at pH 4·5–5·5. Studies with MU-GlcNAc as substrate gave identical pH optima for these enzyme preparations.

*Thermal inhibition*

All the isoenzymes were quite stable up to 50°C, after which they were inactivated rapidly, so that about 75–80% of the activity was lost at 55°C and at 60°C hardly any activity remained after 15 min of exposure.

*The effect of modifiers*

$Hg^{2+}$ (1 mM) caused an inhibition of about 70–80% and *p*-chloromercuribenzoate (1 mM) one of about 40–50% of all the isoenzyme activities (CF-1 to CF-6). None of the various other divalent cations tested ($Ca^{2+}$, $Cd^{2+}$, $Cu^{2+}$, $Pb^{2+}$ and $Zn^{2+}$ at 1 mM and $Co^{2+}$, $Fe^{2+}$ at 0·1 mM) had such an obvious effect on β-NAG preparations. The activities were not affected by cysteine, dithioerythreitol, ethylenediamine tetra-acetic acid, iodoacetamide and *o*-phenanthroline (1 mM).

*Histochemistry*

The relative intensity of the histochemical reaction in basal cells and basal and apical parts of principal cells in the 6 epididymal segments as well as in the epithelial cells of the ampulla and seminal vesicle is semi-quantitatively shown in Table 2. The basal cells and the basal parts of the principal cells showed a strong reaction in segments $E_2$–$E_4$ (Fig. 5a) and $E_5$ (Fig. 5b). This reaction was granular and possibly corresponds to the distribution of the lysosomes. A conspicuous reticular distribution of the reaction product was seen in the apical parts of principal cells in segments $E_3$–$E_4$ and simultaneously the tubular sperm mass was mixed with reactive material. This was particularly abundant in $E_5$, which otherwise was rather inactive in the epithelial enzyme reaction (Fig. 5b). The apical reaction in $E_6$ (Fig. 5c) was very strong, partly granular and reticular in appearance. In this segment the intratubular reaction was also strong. The apical and intratubular histochemical β-NAG reaction was regarded as an indication of secretory enzyme activity. In the immature epididymis this reaction was conspicuously absent. The secretory epithelium of ampulla showed a weak and that in the seminal vesicle a moderate fine-granular apical reaction (Fig. 5d). The histochemical staining of the epithelium in bovine prostate and Cowper's gland was very weak or absent.

**Table 2.** Histochemical reaction for β-NAG in basal cells and different parts of principal cells as well as in the luminal content of the six ($E_1$–$E_6$) segments of the bull epididymis, in the ampullary (AM) and seminal vesicle (VS) epithelium

| Segment/tissue | $E_1$ | $E_2$ | $E_3$ | $E_4$ | $E_5$ | $E_6$ | AM | VS |
|---|---|---|---|---|---|---|---|---|
| Basal cells | 0 | ++ | +++ | ++++ | + | +++ | 0 | 0 |
| Principal cells | | | | | | | | |
| Basal part | + | ++ | +++ | +++ | + | +++ | − | − |
| Apical part | − | − | ++ | +++ | + | ++++ | + | ++ |
| Lumen | − | − | + | ++ | +++ | +++ | − | − |

−, Negative; +, weak; ++, moderate; +++, strong; ++++, very strong.
0 = cells absent.

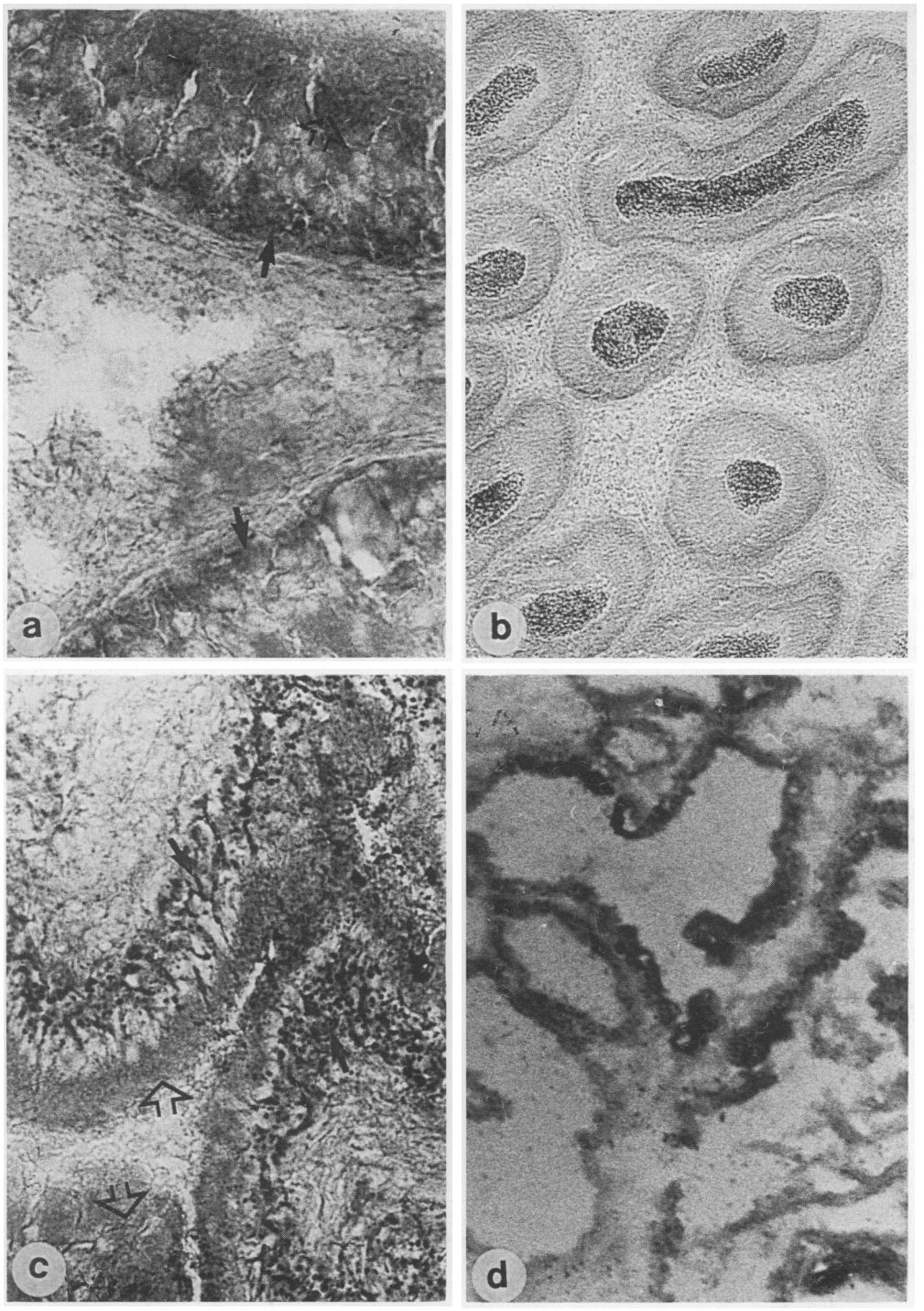


**Fig. 5.** Histochemical reactions for β-NAG with *N*-ASBI-GlcNAc as substrate and hexazotized *p*-rosanilin as the coupling agent. Incubation time was 60 min at 37°C. (**a**) Caput epididymidis ($E_2$) with granular reaction in the basal part of the epithelium (black arrows) and reticular reaction in the apex (open arrow). × 400. (**b**) Cauda epididymidis ($E_5$) with granular reaction in the basal part of the epithelium and a strong reaction in the tubular sperm mass. × 125. (**c**) Cauda epididymidis ($E_6$) with granular reaction in the basal part of the epithelium (black arrows) and granular/reticular reaction in the apex (open arrows). × 400. (**d**) Seminal vesicle with homogenous apical staining in the epithelium. × 125.

## Discussion

In many species the epididymis contains the highest specific activity of β-*N*-acetylglucosaminidase (β-NAG) in mammalian tissues. Our results with the bull reproductive organs are consistent with these results. Moreover, a detailed analysis showed that the activity gradually increased from the initial segment ($E_1$) to the corpus ($E_4$) with a decrease in the proximal cauda ($E_5$). Before the onset of maturity enzyme activity in the distal cauda ($E_6$) remained at about the same level as the previous region, but in the adult animals an increased specific activity was found in this part. Generally, the activities were greater in adult than immature bulls, but the increase was most apparent in the distal cauda. The biochemical results were also consistent with the histochemical observations, which showed a gradually increasing reaction in the epididymal cells from caput to corpus, a temporary decrease in the proximal cauda and again an increase. The cellular location of β-NAG was found in all parts close to the basal membrane both in the principal cells and in the distinct basal cells. Another location for β-NAG was found in the apical part of principal cells particularly in distal caput and distal cauda. After distal caput the intratubular sperm mass became increasingly mixed with β-NAG-positive material. Our histochemical results were quite consistent with those reported by Sinowatz *et al.* (1975, 1976). The basal and apical cell reactions as well as the intratubular activity contribute to the total biochemical activity but enzyme activity may be derived from the acrosomes, although the histochemical reaction remained negative in these structures.

The enzyme reaction in the basal part of principal cells and in the basal cells corresponded to the distribution of lectin-stained glycoproteins in the bull epididymis (Arya & Vanha-Perttula, 1985). It was assumed in that work that glycoproteins are absorbed from the tubular lumen and possibly degraded in the basal part of the epithelium. The high enzyme reaction is consistent with the possibility that in this location β-NAG participates in the splitting of β-*N*-acetylglucosamine residues from glycoproteins. The intracellular site of this reaction could be due to lysosomes which have been found in abundance in basal cells and basal extensions of the principal cells (Sinowatz, 1981). The strong reaction in the distal caput indicates that this is the area for secretion. The intratubular reaction found in more distal regions consequently may represent the secretory form of the enzyme, which then becomes intimately associated with spermatozoa. The secretory activity seemed to continue down to the corpus, but in the proximal cauda ($E_5$) the epithelium had only the basal activity. In the distal cauda, however, the apical staining was found again which supports an active participation of this region in β-NAG secretion.

The specific activity of β-NAG was much lower in the other bull reproductive organs and similarly also the histochemical reaction was much weaker. Consistently, however, a moderate staining was found in the epithelial cells of ampulla and seminal vesicles but very weak in prostate and Cowper's gland. Our biochemical study was aimed at disclosing the possible origin(s) of β-NAG in seminal plasma by comparing the elution pattern of the enzyme in different tissues and their secretions.

Gel filtration on Sepharose 6B of secretions and tissue homogenates disclosed mostly a symmetrical peak, which often was rather wide in profile. The maximum of the peak varied between the tissues, indicating a heterogeneity in the size of the enzyme. Further support to this assumption was obtained from results with chromatofocussing and anion exchange chromatography. In fact, these fractionation methods disclosed that β-NAG in different tissues and secretions is a complex containing enzymes differing from each other in their pI values and number of anionic determinants. The seminal plasma in both fractionations gave at least four separate peaks with distinct pI values and elution after the NaCl gradient. This pattern was not, however, identical with that obtained with secretions from the cauda epididymidis ($E_6$) or seminal vesicles, but apparently both were contributing to the formation of the enzyme complex. The secretion of the cauda epididymidis was rich in enzyme forms which had high pI values and were eluted at low NaCl concentrations while the enzymes derived from the seminal vesicles had low pI values and required higher salt concentrations for elution in anion exchange chromatography. Closely similar values

were obtained for the 4 peaks in seminal plasma. The ampulla, which had a β-NAG pattern identical with that of the seminal vesicles in both fractionations, and the prostate and Cowper's glands may contribute to the formation of the seminal plasma isoenzymes. On the basis of the relative size, however, the latter two organs obviously have a minimal part in supplying the secretory β-NAG. The molecular weights of the multiple forms of β-NAG were not separately analysed by gel filtration. Thus, a detailed comparison with the results of Khar & Anand (1977b) is not possible. In addition, their fractionation method differed from that used in the present work.

Except for the elution profiles in the three fractionation methods, β-NAG isoenzymes were very similar in their biochemical properties, i.e. substrate specificity, pH optima, thermal stability and modifier characteristics. These observations suggest that the active centres of these enzymes are possibly identical. The differences in the molecular weight, pI values and the amount of anionic determinants, however, indicate that other structural differences do exist. The wide heterogeneity in pI values can be ascribed to the variation in the sialic acid residues. This has been found as the major reason for the electrophoretic heterogeneity of bull epididymal β-NAG isoenzymes (Hayase *et al.*, 1973). Our results indicate that the enzyme forms considered as lysosomal (CF-1, partly CF-2) may have fewer sialic acid residues and/or anionic determinants than do other forms of the enzyme. The enzymes regarded as secretory in the caput (CF-4, peak D) and cauda (CF-2, peak E) epididymidis markedly differed in their elution by chromatofocussing and anion exchange chromatography. Besides the amount of sialic acid residues, these two enzyme forms may have larger structural differences, since the molecular weight of the activity in the cauda epididymidis ($E_6$) was clearly larger than that of major secretory enzyme in the caput ($E_2$). The analysis of these differences would, however, require an extensive purification of each isoenzyme form and subsequent structural analysis.

The role of the secretory forms of β-NAG in different parts of the epididymis, ampulla and seminal vesicles can obviously be regarded distinct from the participation of the lysosomal enzymes in phagocytic and degrading activity in the basal extensions of principal cells and basal cells of the epididymal epithelium. During the passage through the epididymis, spermatozoa become exposed to the secretory β-NAG and this exposure will continue when mixed with the secretions coming from ampulla and seminal vesicles. The bull sperm surface exhibits strong lectin staining by Concanavalin A with an affinity for *N*-acetylglucosamine in the epididymal caput, but this staining gradually fades during the transit through the epididymal duct (Arya & Vanha-Perttula, 1985). This phenomenon may be associated with the maturation processes taking place in spermatozoa within the epididymis. These enzymes are probably attached to the sperm surface and their effect may still continue after ejaculation. Some of the β-NAG peaks separated from sperm homogenates by chromatofocussing and anion exchange chromatography could be due to such enzymes. The major sperm activity peak was eluted at the start of anion exchange chromatography (peak A) and had a high pI value in chromatofocussing. This enzyme form may be acrosomal in origin and structurally correspond to the lysosomal enzyme in the tissues. In ejaculated bull spermatozoa the acrosomal β-NAG is highly soluble (Zahler & Doak, 1975) and can also be due to an activity adsorbed on the sperm surface. The acrosomal as well as the attached secretory enzymes could have some role in sperm capacitation and acrosomal reactions as well as in sperm–egg interaction, in which glycoproteins of the zona pellucida and plasma membrane of the ovum play a crucial role.

We thank Mrs Aino Niittynen and Dr Eero Remes (D.V.M.) from the AI Station of East and Middle Finland; Dr Veikko Rosendahl (D.V.M.) from Lihakunta Abattoir for organizing the supply of bull semen and reproductive organ samples; and Miss Eija Kettunen, Miss Arja Venäläinen and Mrs Rauni Peltonen for excellent technical and secretarial help. This work was supported by the Ministry of Agriculture and Forestry of Finland and the North-Savo Fund of the Finnish Cultural Foundation.

## References


**Arya, M. & Vanha-Perttula, T.** (1985) Lectin-binding pattern of bull testis and epididymis. *J. Androl.* **6**, 230–242.

**Bamberg, E., Hanna, A.A. & Stöckl, W.** (1975) Verteilung der N-Acetyl-hexosaminidase im Nebenhoden vom inflantilen, kryptorchen und adulten Eber. *Berl. Münch. Tierärztl. Wschr.* **88**, 242–244.

**Bamberg, E., Sinowatz, F. & Schafelner, J.** (1978) Aktivität der β-N-Acetyl-hexosaminidase im Nebenhoden von Ferkeln und Läufern. *Berl. Münch. Tierärztl. Wschr.* **91**, 379–382.

**Bamberg, E., Sinowatz, F. & Kanout, A.G.** (1979) Biochemische und histochemische Untersuchungen über die Verteilung der β-N-Acetylhexosaminidase im Nebenhoden des Hundes. *Zentbl. VetMed. A* **26**, 380–385.

**Boström, K. & Öckerman, P.-A.** (1971) Glycosidases in human semen and male genital organs. *Scand. J. Urol. Nephrol.* **5**, 117–122.

**Bullock, S. & Winchester, B.** (1973) The N-acetyl-hexosaminidase components of the ram testis and epididymis. *Biochem. J.* **133**, 593–599.

**Caygill, J.C., Roston, C.P.J. & Jevons, F.R.** (1966) Purification of β-acetyl-glucosaminidase and β-galactosidase from ram testis. *Biochem. J.* **98**, 405–409.

**Conary, J.T., Thompson, J.N. & Rodén, L.** (1982) Chromatofocusing of lysosomal hydrolases from bovine testis. *Carbohydrate Res.* **100**, C51–C55.

**Conchie, J. & Findlay, J.** (1959) Influence of gonadectomy, sex hormones and other factors on the activity of certain glycosidases in the rat and mouse. *J. Endocr.* **18**, 132–146.

**Conchie, J. & Mann, T.** (1957) Glycosidases in mammalian sperm and seminal plasma. *Nature, Lond.* **179**, 1190–1191.

**Farooqui, A.A. & Srivastava, P.N.** (1980) Isolation of β-N-acetyl-hexosaminidase from rabbit semen and its role in fertilization. *Biochem. J.* **191**, 827–834.

**Gupta, G.S. & Agarwal, P.** (1982) Immunological characteristics of β-N-acetylglucosaminidase from human seminal plasma. *Ind. J. Biochem. Biophys.* **19**, 37–40.

**Gupta, G.S. & Kapur, D.K.** (1981) Purification and characterization of mouse testicular β-N-acetylglucosaminidase. *Ind. J. Biochem. Biophys.* **18**, 187–191.

**Hayase, K., Reisher, S.R. & Kritchevsky, D.** (1973) Microheterogeneity of N-acetyl-β-D-hexosaminidase of bull epididymis. *Proc. Soc. exp. Biol. Med.* **142**, 466–470.

**Hayashi, M.** (1965) Histochemical demonstration of N-acetyl-β-glucosaminidase employing naphtol-AS-BI-N-acetyl-β-glucosaminide as substrate. *J. Histochem. Cytochem.* **13**, 355–360.

**Jauhiainen, A. & Vanha-Perttula, T.** (1985) Acid and neutral α-glucosidase in the reproductive organs and seminal plasma of the bull. *J. Reprod. Fert.* **74**, 669–680.

**Khar, A. & Anand, S.R.** (1974) Studies on the glycosidases of semen: purification and properties of two hexosaminidases of bull seminal plasma. *Ind. J. Biochem. Biophys.* **11**, 292–298.

**Khar, A. & Anand, S.R.** (1977a) Studies on the glycosidases of semen: purification and properties of β-N-acetylglucosaminidase from bull sperm. *Biochim. Biophys. Acta* **483**, 141–151.

**Khar, A. & Anand, S.R.** (1977b) Studies on the glycosidases of semen: further purification and characterization of two hexosaminidases from bull seminal plasma. *Biochim. Biophys. Acta* **483**, 152–159.

**Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J.** (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.

**Majumder, G.C. & Turkington, R.W.** (1974) Acrosomal and lysosomal isoenzymes of β-galactosidase and N-acetyl-β-glucosaminidase in rat testis. *Biochemistry, N.Y.* **13**, 2857–2864.

**Majumder, G.C., Lessin, S. & Turkington, R.W.** (1975) Hormonal regulation of isoenzymes of N-acetyl-β-glucosaminidase and β-galactosidase during spermatogenesis in the rat. *Endocrinology* **96**, 890–897.

**Milne, A.F., Williams, J.N. & Rudolph, F.B.** (1978) Changes in the specific activity of N-acetyl-β-D-hexosaminidase in the rat epididymis with age. *Comp. Biochem. Physiol.* **59B**, 199–200.

**Parkes, H.C., Stirling, J.L. & Calvo, P.** (1984) Affinity purification and subunit structures of β-N-acetylhexosaminidases A and B from boar epididymis. *Biochem. J.* **219**, 1009–1015.

**Sinowatz, F.** (1981) Ultrastrukturelle und enzymhistochemische Untersuchungen am Ductus epididymidis des Rindes. *Adv. Vet. Med.* **32**, 1–99.

**Sinowatz, F., Bamberg, E., Lipp, W. & Stöckl, W.** (1975) Verteilung der N-Acetylhexosaminidase im Nebenhoden vom Stier. *Wien. tierärztl. Wschr.* **62**, 287–290.

**Sinowatz, F., Lipp, W. & Perfler, B.** (1976) Histotopik von Glykosidasen in den akzessorischen Geschlechtsorganen des Bullen. *Acta histochem.* **55**, 325–333.

**Sinowatz, F., Fischer, M., Skolek-Winnisch, R. & Chandler, J.A.** (1979) Histochemical localization of glycosidases in dog epididymis. *Histochem. J.* **11**, 103–109.

**Skolek-Winnisch, R., Lipp, W., Stöckl, W. & Bamberg, E.** (1981) Untersuchungen über die Verteilung der β-N-Acetylhexosaminidase im Nebenhoden des Hengstes. *Acta histochem.* **69**, 77–84.

**Srivastava, P.N., Farooqui, A.A. & Gould, K.G.** (1981) Studies on hydrolytic enzymes of chimpanzee semen. *Biol. Reprod.* **25**, 363–369.

**Zahler, W.L. & Doak, G.A.** (1975) Isolation of the outer acrosomal membrane from bull sperm. *Biochim. Biophys. Acta* **406**, 479–488.


**Received 15 April 1985**