Ces résultats correspondent à ceux obtenus par VAN GELDER et al.[10] sur le cerveau de souris. Nous avons également mis en évidence une charge nettement plus anionique pour la fraction S en migration sur gel de polyacrylamide (Figure 1) par rapport à la fraction M. Les constantes de sédimentations évaluées par rapport aux

Fig. 4. Représentation de Lineweaver et Burk de l'affinité des fractions M (———) et S (- - - -) pour l'acide γ-aminobutyrique.

Fig. 5. Variation de l'activité enzymatique en fonction du pH du milieu réactionnel. M, ———; S, - - - -.

3 marqueurs suivants: l'alcool-déshydrogénase (S20 = 7,4), l'aspartate aminotransférase (S20 = 5,5) et le cytochrome c (S20 = 1,9) ont leur valeur comprise entre 5,5 et 5,7 pour les 2 enzymes (Figure 2).

Contrairement aux deux formes identifiées chez la souris[10], les pH_0 d'activité sont également très voisins (Figure 5). L'affinité par rapport au GABA est la même alors qu'elle était nettement distincte chez la souris (Figure 4).

En ce qui concerne le deuxième substrat impliqué dans la transamination, c'est-à-dire l'acide α-cétoglutarique, les affinités des deux formes (S) et (M) pour ce substrat sont nettement différentes. Cette propriété pourrait avoir une implication importante in vivo car la concentration en acide α-cétoglutarique est basse dans le compartiment synaptique, inférieure à la K_m (Figure 3). Les deux formes de la $GABA_T$ que nous avons séparées à partir de l'enzyme purifié[1] et qui pourraient correspondre l'une à l'enzyme mitochondriale ($GABA_T$ I), l'autre à l'enzyme cytoplasmique ($GABA_T$ II) se différencient elles aussi essentiellement par leur affinité pour l'activité α-cétoglutarique, métabolite essentiel puisqu'il est au centre de tous les processus de transamination.

L'affinité de la $GABA_T$ II pour ce substrat est identique à celle de l'enzyme synaptosomale et pourrait représenter une forme cytoplasmique de $GABA_T$. Cette enzyme pourrait occuper un site sur la membrane pré ou post synaptique. Elle interviendrait ainsi directement dans les phenomènes de régulation synaptique des pools du GABA alors que l'enzyme mitochondriale, extrasynaptique ($GABA_T$ I) ne paraît pas impliquée directement dans ce mécanisme.
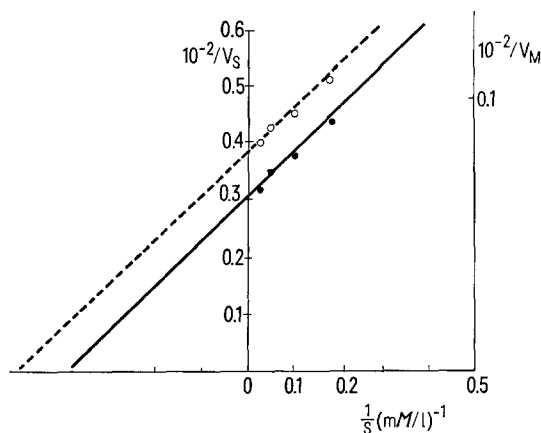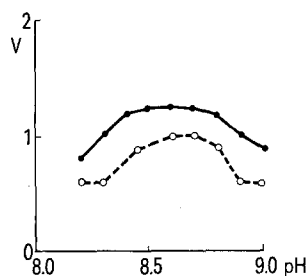
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Acrosomal Hydrolases in Buffalo Spermatozoa[1]

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Summary. Buffalo sperm acrosome resembles its counterpart in other species, being rich in hydrolytic enzymes. Of the enzyme activities estimated, acid phosphatase, β-N-acetylglucosaminidase and hyaluronidase were low compared to those of ram semen. However, the aryl sulphatase activity was high. GOT activity estimated in sperm preparation may not be of acrosomal origin.

Acrosome, a membraneous cap-like structure covering the nucleus of the spermatozoa, is considered to be a modified lysosome. It is a repository of a number of hydrolases and has been shown to play a vital role in the process of fertilization[3]. The levels of 6 hydrolases were estimated in buffalo sperm acrosome, which forms the subject matter of the present communication.

Materials and methods. Semen was collected from Murrah buffalo bulls with an artificial vagina. Samples showing high initial motility with a score of 3.5 to 4.5 (0, no motility; 5, the best motility) were used and sperm counts were made with haemocytometer. Semen was diluted with 3 vol. of 50 mM tris-HCl buffer, pH, 7.4

and centrifuged at 1100 g for 10 min. The sperm pellet was washed 3 times more with the same buffer. The acrosomal preparations (H_1 and H_2) were prepared by treating a saline suspension of washed sperm with an equal volume of 0.05% hyamine 2389 and 0.05% triton X-100 in 0.067 M tris-maleate buffer[3], pH 6.1. The seminal plasma free of spermatozoa was prepared by centrifuging fresh semen at 12,000 g for 30 min at 4 °C. For total sperm enzyme activities, washed sperm suspension was sonicated for 3 min in the cold with Branson Sonifier Model B-12. The sonicate was centrifuged at 25,000 g for 50 min and the enzyme activities were estimated in the supernatant.

The levels of hydrolases in buffalo sperm acrosome

Enzyme activity[a]	Seminal plasma	1 + 2 + 3 W	4th W	H_1	H_2	$H_1 + H_2$	Total sperm extract
Acid phosphatase	87.99 ± 8.96	75.48 ± 21.80	0.39 ± 0.05	1.46 ± 0.12	0.70 ± 0.12	2.16 ± 0.12	1.84 ± 0.16
Alkaline phosphatase	34.88 ± 6.13	56.60 ± 4.54	0.21 ± 0.03	0.45 ± 0.05	0.35 ± 0.06	0.80 ± 0.05	1.70 ± 0.31
Hyaluronidase	1.70 ± 0.20	2.20 ± 0.06	0.35 ± 0.01	8.54 ± 1.34	2.81 ± 0.08	11.35 ± 0.07	9.83 ± 3.20
β-N-Acetyl-glucosaminidase	30.88 ± 4.55	25.55 ± 4.81	0.145 ± 0.01	1.13 ± 0.13	0.52 ± 0.06	1.65 ± 0.07	1.64 ± 0.11
Aryl sulphatase A	465.50 ± 52.27	701.50 ± 30.50	58.0 ± 13.00	311.33 ± 34.00	29.10 ± 3.63	340.43 ± 18.81	446.67 ± 20.07
GOT	615.00 ± 59.88	1293.20 ± 86.84	101.00 ± 3.66	209.55 ± 26.31	116.15 ± 11.54	325.70 ± 18.92	450.45 ± 30.85

[a] Enzyme activities are expressed in units/ml. A unit is defined as the μmoles of substrate (μg for arylsulphatase) hydrolyzed in 30 min at 37 °C. For GOT, a unit is the amount of activity that results in the formation of 1 μg pyruvate/h at 37 °C.
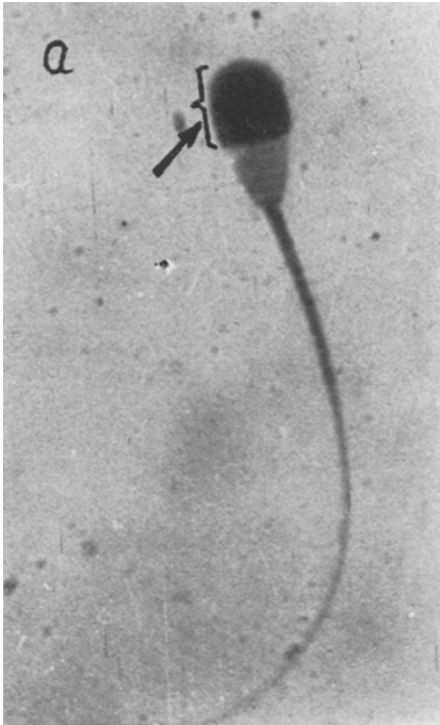
a) The arrow indicates violet Giemsa stained intact buffalo sperm acrosome. × 1,500.
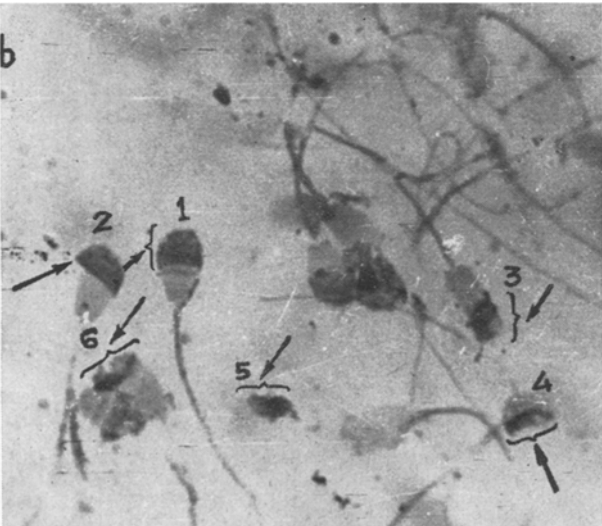
b) Buffalo sperm stained after 5 min incubation with hyamine. × 1,500. 1. Acrosome in the process of detachment, 2. Elevation and swelling of the acrosomal membrane, 3–6. Giemsa stainable acrosomal particles of varying dimensions.

Citric acid was determined according to the procedure of WHITE and DAVIS[4] and DNA by the method of BURTON[5]. Acid and alkaline phosphatases were assayed at pH 4.8 and 8.5, respectively with sodium p-nitrophenyl phosphate as the substrate[6]. Hyaluronidase and β-N-acetylglucosaminidase were estimated as described by Khar and ANAND[7] and aryl sulphatase as described by BAUM et al.[8] GOT activity was measured according to YATZIDIS[9].

Samples of washed and detergent-treated spermatozoa were fixed with 5% formaldehyde and then stained with Gimesa stain. The structural alterations were followed by examination under phase contrast microscope[10].

Results and discussion. To ensure that the enzyme activities being detected and estimated were of acrosomal origin, seminal plasma and cytoplasmic droplets contributing to these activities were first removed. The cytoplasmic droplets have been reported to be rich in hydrolytic enzymes[11]. Citric acid is known to be derived exclusively from seminal plasma[12] and its estimation in washings (1 + 2 + 3 + 4W) showed that the seminal plasma was completely removed by 3rd wash. The enzyme levels of combined washings over the seminal plasma were much higher than could be expected from about 4% cytoplasmic droplets estimated[11] in ejaculated buffalo spermatozoa. The higher enzyme activities may be accounted for by leakage from spermatozoa into the seminal plasma during suspension in hypotonic buffer, as JONES and HOLT[13] have reported that washing with Krebs-Ringer solution alone effects the sperm plasma membrane. The absence of DNA in H_1 and H_2 indicated that nucleus was not effected by the detergent action. Thus, the enzyme activities in H_1 and H_2 represent the contribution of the acrosomal contents. This was further supported by morphological changes occurring during the detergent treatment, which showed clearly the dislodging

[1] NDRI publication No. 75-129.

[2] We should like to thank Dr. D. SUNDARESAN, the Director for encouragement and the Indian Council of Agricultural Research, New Delhi for partially funding this work. One of the authors (PSC) was recipient of a UGC junior fellowship.

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of the acrosome (Figure 1) and the enzyme activities in H_1 and H_2 were higher than 4W.

At a hyamine concentration of 0.05% (0.025% final concentration), maximum enzyme release was obtained from buffalo acrosome with minimum damage to other
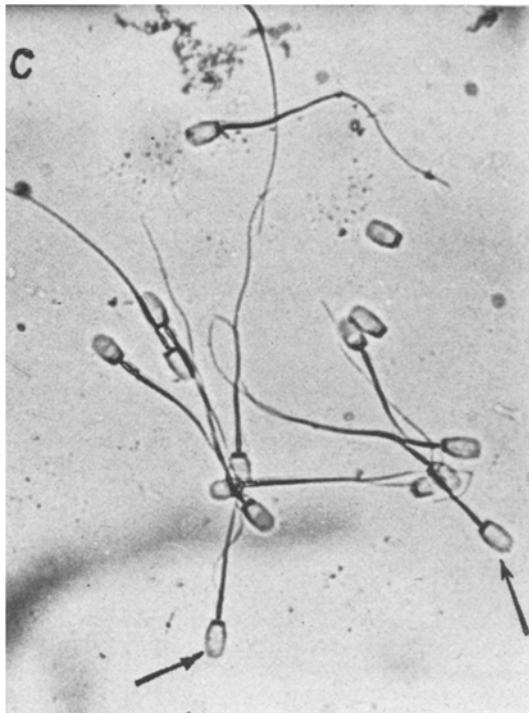
c) Buffalo sperm stained after 90 min incubation with hyamine. ×900. The cells have lost their acrosomes.

sperm organelles. The distribution of 6 enzymes in washings and acrosomal preparations shown in the Table are based on the calculations of 1 ml of semen. Compared to ram semen[3], buffalo acrosomal extracts had lower activities of acid phosphatase, β-N-acetylglucosaminidase and hyaluronidase. This was probably due to lower concentration of spermatozoa in buffalo semen[12]. The levels of hyaluronidase, however, were close to those found in bull acrosome[14]. CHAUHAN and SRIVASTAVA[15] have estimated the acid phosphatase and GPT in buffalo seminal plasma, but their values were lower than found by us. GOT has been shown to be associated only with spermatozoa and its leakage into extracellular fluid could be an index of sperm cell damage[16]. Higher centrifugation speed used by us to separate the seminal plasma from the spermatozoa could be a factor contributing to higher enzyme activities in buffalo seminal plasma. Aryl sulphatase was estimated in ram acrosome[3], but its levels in buffalo acrosome are very high. YANG et al.[17] has stated that this enzyme activity, besides others, may play an important role in the penetration of sperm through the zona pellucida, because the latter is rich in organic sulphate. The levels of alkaline phosphatase have not been reported in acrosomal extracts of any other species.

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Ecdysone 20-Hydroxylase from the Midgut of the Tobacco Hornworm (*Manduca sexta* L.)

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Summary. An ecdysone 20-hydroxylase enzyme system that converts α-ecdysone to 20-hydroxyecdysone was prepared from the midgut of the tobacco hornworm prepupa. This partially purified enzyme is NADPH dependent and is localized in the mitochondrial fraction of the midgut tissue.

The conversion of α-ecdysone (I) to 20-hydroxyecdysone (Ia) is known to be an essential step in molting hormone biosynthesis[2,3]. Consequently this is a possible metabolic control point for molting hormone synthesis, titer regulation, and inactivation, as well as a candidate target site for disrupting the ecdysone regulated processes. Previous studies of the metabolism of the insect molting ·hormones have utilized a variety of in vivo and in situ techniques. However, more refined biochemical studies are necessary to extend our knowledge of the mechanisms controlling the biosynthesis and metabolism of the ecdysones and the relationship of the juvenile hormones and hormonal chemicals to the molting processes of insects. We now report the first in vitro study of a partially purified enzyme system that converts α-ecdysone to 20-hydroxyecdysone.

The appearance of 20-hydroxyecdysone *as an enzymatic product* was first noted during the investigation of an ecdysone dehydrogenase-isomerase in the midgut of the tobacco hornworm, *Manduca sexta* (L.)[4]. Enzyme preparations from midgut tissue were assayed as previously described for the ecdysone dehydrogenase-isomerase

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