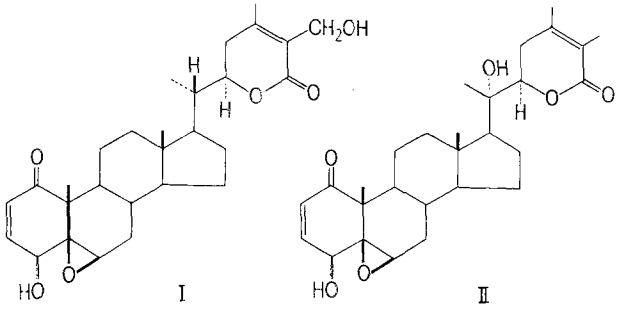
251–253°, $[\alpha]_D^{30°}$ + 80°($CHCl_3$). Yield, 0.03%. IR in cm^{-1}: 3500, 3390 (OH); 1709, 1680 (C = 0); *m/e* 470 (M^+). It formed an acetate, m.p. 245–248°. Preliminary studies showed that this compound was different from withaferin A, the antitumour lactone isolated previously. The compound has been identified as 4 *β*, 20*α*-dihydroxy-1-oxo-5*β*, 6*β*-epoxy-witha- 2,24-dienolide (II)[5] by comparison of physical constants i.e., mmp, specific rotation, thin layer chromatography (TLC) and finally by a superimposable IR-spectrum with that of an authentic sample. Compound (II) showed 95% tumour inhibition against Sarcoma 180 in mice[6] and was active against cells derived from human epidermoid carcinoma of the nasopharynx (KB), ED_{50} 1.0 μg/ml (NSC No. 156284). No other steroidal lactones could be isolated from this source.



The petroleum ether extract of the leaves on column chromatography (Silica gel) yielded a crystalline material in the petroleum ether-benzene (1:1) eluate. The product, on repeated crystallizations from chloroform-methanol, gave colourless crystals, $C_{29}H_{50}O$, m.p. 138°–140°. IR in cm^{-1}: 3440 (OH). It responded to Liebermann-Burchard test for a sterol and formed a crystalline acetate, $C_{31}H_{52}O_2$, m.p. 127–129°, $[\alpha]_D^{30°}$ -41°($CHCl_3$). IR in cm^{-1}: 1740 (C=O) and 1250 (C–O, acetate); *m/e* 456(M^+). The sterol was identical with *β*-sitosterol in all respects by mmp., TLC, and superimposable IR-spectra with that of an authentic sample.

The leaves of *W. somnifera*, collected from Tamil Nadu were examined. Following the same procedure as above, a colourless crystalline compound, $C_{28}H_{38}O_6$, m.p. 241–243°, $[\alpha]_D^{30°}$ + 114° ($CHCl_3$) has been isolated as the major product, yield, 0.04%. IR in cm^{-1}: 3350 (OH); 1709, 1685 (C=O); *m/e* 470 (M^+). It gave an acetate derivative, m.p. 201–202°. By comparison of the physical constants of the above product and its acetate with those of withaferin A and its acetyl derivative, the major product has been identified as withaferin A (I). All the above compounds gave satisfactory elemental analysis results. The TLC of the crude product after separation of withaferin A showed the presence of other minor constituents which could not be separated by column chromatography and crystallization. By preparative thin layer chromatography on silica Gel G in ethylacetate-benzene (7:3), the followign steroidal lactones could be isolated in trace amounts: 1. 27-Deoxy-14-hydroxy withaferin A; 2. dihydrowithaferin A; 3. 4*β*, 20*α*-dihydroxy-1-oxo-5*β*, 6*β*-epoxy- witha-2, 24-dienolide. The identity of these compounds was established by direct comparison of the authentic samples by mmp. and TLC. *β*-Sitosterol has also been found to occur in Tamil Nadu variety.

It may be mentioned that the plant occurring in Tamil Nadu contains predominently withaferin A (I) where as in the West Bengal variety compound (II) is the major antitumour constituent. The former specimen of *W. somnifera* conforms to chemotype I and the latter to chemotype II[3]. The plants studied were in the same developmental state.

Zusammenfassung. Nachweis, dass auch bei der indischen Varietät von *Withania somnifera* «chemische Rassen» mit unterschiedlicher Antitumoraktivität in verschiedenen Gegenden auftreten, die jeweils *β*-Sitosterol enthalten.

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Hyaluronidase in the Mucus Accessory Glands of the Drone (*Apis mellifera*)

In a previous work[1], we reported on the properties of the hyaluronidase (hyaluronate glycanohydrolase E.C. 3.2.1.35) in crude extracts of the testes of the drone. In common with the mammalian testicular hyaluronidase, the insect enzyme was found to have the capacity of depolymerizing both hyaluronic acid and chondroitin-6-sulfate and to cleave these substrates at the hexosaminidic bond. The effects of pH were somewhat, and those of temperature definetly, different for the enzymes from the 2 sources. In initial experiments it came to our attention that extracts of the mucus accessory glands of the reproductive system of the drone also possessed hyaluronidase activity. The possibility of contamination with adjoining testicular tissue was, naturally, envisaged, but histological examination of several preparations of these glands and of their liquid content failed to reveal any such tissue or spermatozoa.

The presence of hyaluronidase in the insect accessory glands was considered of interest since such an activity has not been reported so far in the male accessory organs, and it might be connected with the special features of the fertilization process in the bee, as will be discussed later. The present paper reports on the hyaluronidase in

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Table I. Colorimetric assay of hyaluronidase activity in extracts of mucus glands and testes of the drone

	Hyaluronidase activity						
	Hyaluronic acid (N-acetylglucosamine μmoles/mg protein)				Chondroitin-6- sulfate (N-acetylgalactosamine μmoles/mg protein)		
Time (min)	30	60	120	180	120	240	360
Mucus glands	0.16	0.32	0.56	0.78	0	1.50	5.48
Testes	0.03	0.04	0.08	0.12	0	0.18	0.96

The assay system consisted of 0.2 ml 0.1 *M* phosphate-citrate buffer pH 5.0, 0.05 ml high speed homogenate supernatant and 0.05 ml substrate solution containing 300 μg hyaluronic acid or chondroitin-6-sulfate. Incubation carried out at 37 °C. Colorimetric determination of the released N-acetyl-hexosamine-reacting products was carried out according to REISSIG et al.[4].

extracts of the mucus glands of the drone (*Apis mellifera*) and on some of its properties as compared to those of the testicular hyaluronidase from the same insect.

Materials and methods. Testes and mucus accessory glands were obtained from 7–10-day-old drones, either immobilized in the cold or which had been stored frozen, by incision through the abdomen and removal by the use of fine scissors and forceps under magnifying lens. All manipulations were carried out in the cold and the collected preparations kept on ice. Pools of homologous tissues were homogenized in 0.1 *M* phosphate-citrate buffer pH 5.0 in a glass-teflon homogenizer and the suspensions obtained centrifuged in a Spinco model L ultracentrifuge at 105,000 ×*g* for 1 h. Hyaluronidase was exclusively associated with the high speed supernatant, as the precipitates failed to provide any activity. The same procedure was used for the preparation of extracts from seminal vesicles and prostate from an adult albino rat weighing 180 g. Protein was determined by the method of LOWRY et al.[2], using crystalline bovine albumin (Nutritional Biochemicals Co.) as standard.

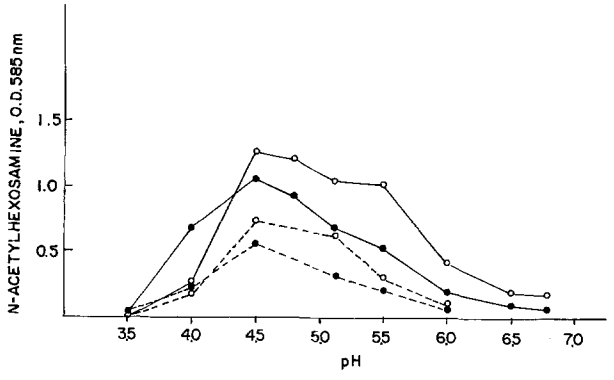

Fig. 1. Effect of pH on hyaluronidase activity of mucus glands and testes extracts from the drone. Incubation 18 h at 37 °C. Activity was assayed as described in Table I and expressed as optical density units at 585 nm using a microsample Gilford 300 spectrophotometer with a 10 mm light path. Protein contents in the samples: ○, mucus glands, 47 μg; ●, testes, 187 μg; ——, hyaluronic acid; – – –, chondroitin-6-sulfate.

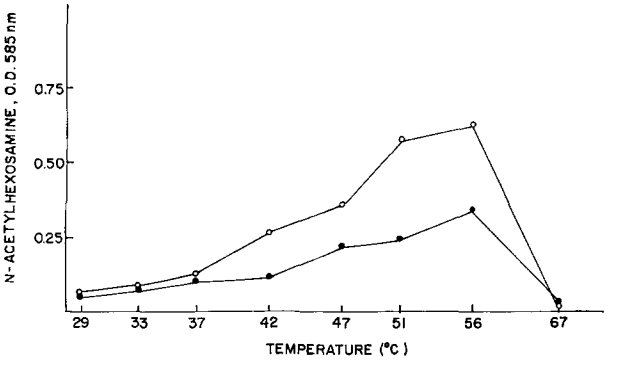

Fig. 2. Effect of incubation temperature on hyaluronidase activity of mucus glands and testes extracts from the drone. Incubation 4 h at 37 °C. Activity was assayed with hyaluronic acid as substrate and expressed as optical density units at 585 nm. ○, mucus glands; ●, testes.

Assays of hyaluronidase. 1. Release of N-acetylhexosamine-reacting products. Incubation was carried out in the presence of hyaluronic acid (Sigma) or chondroitin-6-sulfate, a gift of Dr. J. CIFONELLI, University of Chicago. The compositions of digestion mixtures and conditions of assay, carried out as described by MALES and TURKINGTON[3], are given in the Tables and Figures. N-acetylhexosamine was determined according to the method of REISSIG et al.[4], using as standards N-acetylglucosamine or N-acetylgalactosamine (Pfanstiehl).

2. Turbidimetric assay. The procedure of DI FERRANTE[5] consisting of the measurement of the turbidity developing upon addition of cetyltrimethylammonium bromide to the digestion mixture, was used. The fall in turbidity, which is a measure of the activity of the enzyme in depolymerizing hyaluronic acid or chondroitin-4-sulfate (Cifonelli), was expressed as percentage of the turbidity developping in a mixture containing the intact substrate.

Results and discussion. In common with the testicular extract, the accessory glands extract was found active in depolymerizing hyaluronic acid, chondroitin-4-sulfate and chondroitin-6-sulfate. Table I and II present the progressive activity with time of representative extracts assayed by the colorimetric and turbidimetric methods. It can be seen that the accessory glands extracts exhibited, on the basis of protein, a considerably higher activity than the testicular extracts. Parallel assays carried out on extracts from rat accessory organs failed to reveal any hyaluronidase activity in seminal vesicles and prostate.

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Table II. Turbidimetric assay of hyaluronidase activity in extracts of mucus glands and testes of the drone

	Protein (μg)	Hyaluronidase activity (% fall in turbidity)								
		Hyaluronic acid				Chondroitin-4-sulfate				
Time (h)		1	2	3	8	1	2	3	8	22
Mucus glands	47	0	10	31	80	2	5	7	20	45
Testes	187	0	0	14	37	6	6	6	13	28

The assay system consisted of 0.5 ml high speed homogenate supernatant and 0.5 ml substrate solution containing 200 μg hyaluronic acid or chondroitin-4-sulfate in 0.1 *M* acetate buffer – 0.15 *M* NaCl pH 5.0. After incubation at 37 °C 2 ml of a 2.5% solution of cetyltrimethylammonium bromide in 0.5 *M* NaOH were added and the turbidity developing was recorded at 400 nm. The fall in turbidity is expressed as a percentage of the turbidity developing in the intact control substrate.

Experiments consisting of the examination of the effects of pH and temperature showed similar characteristics of the enzymes of the 2 insect sources. Thus, pH profiles and optima were identical (4.5 with both hyaluronic acid and chondroitin-6-sulfate as substrates) as were also the profiles of enzymatic activities in relation to incubation temperatures (Figures 1 and 2). Of significance is the increase in activity from 37 °C to 56 °C, which is in contrast with the behavior of mammalian testicular hyaluronidase. The latter is gradually inactivated in this range[1]. This observation led us to examine the stability to heating of the insect hyaluronidase. Such experiments showed that it was almost unaffected by heating for at least 3 h at 50 °C, a temperature which promptly causes inactivation of mammalian testicular hyaluronidase[1,6]. Further work will be required in order to see whether this stability is a characteristic of the enzyme or is due to the presence of stabilizing factors in the crude extracts investigated.

The identical behavior of the hyaluronidases of accessory glands and testes of the drone, with respect to the parameters examined, raises the question whether they have a common origin. It would be difficult to assume, however, that the testicular tissue or the spermatozoa are the sources, since the accessory glands exhibited a higher specific activity than the testes.

Although it is generally supposed that in mammals the spermatozoal hyaluronidase is involved in the process of insemination, its precise role is still uncertain. The presence of hyaluronidase in the mucus accessory glands of the drone might be associated with the special features of the fertilization process in the bee. Thus, when drone and queen bee meet, the male, by explosive contraction of the abdomen, everts part of its reproductive system including spermatozoa and mucus into the genital tract of the bee. After each mating (multiple mating is common practice with the honey bee) the spermatozoa are forced out of the oviducts into the spermatheca where they are stored for very long periods of time (2 to 4 years). This latter process involves, in an interval of not more than 24 h after mating, the passage of spermatozoa through a very narrow channel, the spermathecal duct, whose particular structure permits control by the queen of the discharge of only small numbers of spermatozoa at the time of the discharge of eggs[7]. Possibly because of the resistance encountered during the flow through the narrow duct, only about 10% of the supply of spermatozoa received by the queen reach the spermatheca, the remainder being disposed off. The mechanism enabling the entry of a relatively large number of spermatozoa into the spermatheca is not clear. We suppose that the mucus of the male accessory glands might serve as the source of an enzyme capable of depolymerizing the mucopolysaccharides of the spermathecal duct, thus facilitating the flow of the spermatozoa to their site of storage. Further experiments are now in progress to test this hypothesis.

Zusammenfassung. Es wird in Hoden und akzessorischen Schleimdrüsen der Drohne das Vorkommen einer Hyaluronidaseaktivität nachgewiesen, die durch die Spaltung von Hyaluronsäure, Chondroitin-4- und -6-sulfat charakterisiert ist und eine bedeutend höhere Temperaturresistenz im Gewebeextrakt als die vergleichbaren Enzyme aus Säugergeweben zeigt.

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Arginine-rich Low Molecular Peptides in Human Neoplastic Serum

It has previously been found that, in the serum of patients with malignant neoplasms, the arginine content of the albumin fraction is increased[1]. These changes have not been observed in pathological conditions not due to neoplasma but with an increased tissue decomposition[2]. An increase in arginine content has also been observed in fibrin formed during the clotting of fibrinogen with thrombin from persons suffering from neoplasms[3]. It would therefore seem that in cases of neoplastic diseases, low molecular substances appear in the blood. These substances are probably peptides, either bound by serum

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