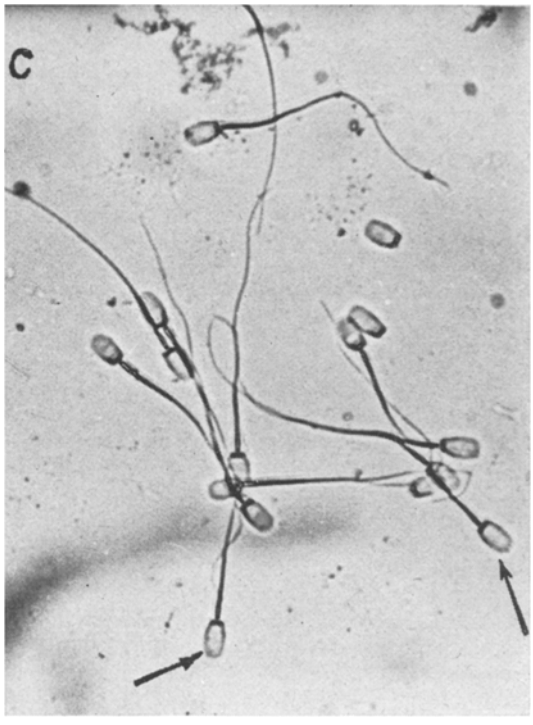
of the acrosome (Figure 1) and the enzyme activities in H_1 and H_2 were higher than 4W.

At a hyamine concentration of 0.05% (0.025% final concentration), maximum enzyme release was obtained from buffalo acrosome with minimum damage to other 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.



c) Buffalo sperm stained after 90 min incubation with hyamine. ×900. The cells have lost their acrosomes.

[14] L. J. D. ZANEVELD, K. L. POLAKOSKI and G. F. B. SCHUMACHER, J. biol. Chem. *248*, 564 (1973).
[15] R. A. S. CHAUHAN and R. K. SRIVASTAVA, J. Reprod. Fert. *34*, 165 (1973).
[16] M. M. PACE and E. F. GRAHAM, Biol. Reprod. *3*, 140 (1970).
[17] C. H. YANG, P. N. SRIVASTAVA and W. L. WILLIAMS, Fedn. Proc. *32*, 310 (1973).

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

H. N. NIGG[1], J. A. SVOBODA, M. J. THOMPSON, S. R. DUTKY, J. N. KAPLANIS and W. E. ROBBINS

Insect Physiology Laboratory, Plant Protection Institute, Agricultural Research Service, U.S. Department of Agriculture, Beltsville (Maryland 20705, USA), 30 September 1975.

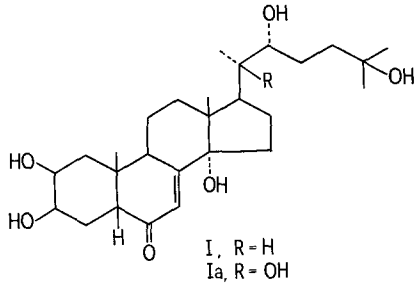
Summary. An ecdysone 20-hydroxylase enzyme system that converts α-ecdysone to 20-hydroxyecdysone was prepared from the midgut of the tobacco hornworm prepupa. This partially purified enzyme is NADPH dependent and is localized in the mitochondrial fraction of the midgut tissue.

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

[1] NRC-ARS Postdoctoral Associate. Present address: Agricultural Research and Education Center, IFAS, University of Florida, P.O. Box 1088, Lake Alfred, Florida 33850, USA.
[2] L. I. GILBERT and D. S. KING, in *The Physiology of Insecta* (Ed. M. ROCKSTEIN; Academic Press, New York 1973), p. 249.
[3] M. J. THOMPSON, J. N. KAPLANIS, W. E. ROBBINS and J. A. SVOBODA, in *Advances Lipid Research* (Academic Press, New York 1973), p. 219.
[4] H. N. NIGG, J. A. SVOBODA, M. J. THOMPSON, J. N. KAPLANIS, S. R. DUTKY and W. E. ROBBINS, Lipids *9*, 971 (1974).

except that a pH 7.8 phosphate buffer was used[4]. Ecdysones were extracted and fractionated according to previously described methods[5], and extracts were analyzed by high-pressure liquid solid chromatography (HPLSC)[6]. The product 20-hydroxyecdysone was identified by comparative thin-layer chromatography (TLC) and HPLSC (Zorbax-Sil)[7] and by NMR and mass spectroscopy.



I, R = H
Ia, R = OH

Hornworm midgut ecdysone 20-hydroxylase showed the following properties: 1. An absolute requirement for NADPH; 2. A possible mitochondrial location based on activity present only in the 10,000 *g* pellet; and 3. Activity, based on our current findings, present only during the late 5th-larval and early prepupal stages. A number of the other tissues of the early prepupa was examined for enzyme activity and, based on TLC and HPLSC analyses, only the fat body possessed slight 20-hydroxylase activity. No activity was detected in the foregut, hindgut, Malpighian tubules, brain, or blood or in the ventral nerve cord. However, these results may in part reflect the titer of enzyme present in each tissue and the limitations of the analytical methods used. It is also possible that the time of appearance of ecdysone 20-hydroxylase in various tissues may differ from that in the midgut and thus it may not have been detected. The titer of the midgut 20-hydroxylase during the ages in which it is present is appreciable and the production of 25 µg of 20-hydroxyecdysone from 250 µg substrate by preparations from 20 prepupal midguts during the 4-hour incubation period was not uncommon.

The disappearance of the midgut 20-hydroxylase activity during the period between the prepupal and pupal stages of the hornworm is particularly striking. This enzymic activity is detectable until the time of appearance of 2 sclerotized bars on the dorsal thorax of the prepupa, which under the rearing regime used in our laboratory, typically indicates that the insect will molt to a pupa within 24 h. Moreover, midgut ecdysone 20-hydroxylase activity was found to be absent on days 1, 2, 3, 4, 6, 8, 10, 12, 14 and 16 of pupal life.

Other stages and ages examined and found to lack the ecdysone 20-hydroxylase activity in the midgut were: 24-hour-old 1st-, 2nd, 3rd, 4th and 5th-instar larvae and 24-hour-old adult males and females. In addition, the total homogenates of 24-hour-old eggs and of 1-day-old and 6-day-old pupae minus the midgut lacked the capacity to convert α-ecdysone to 20-hydroxyecdysone.

The results of the present in vitro studies are supported by in vivo studies that indicate the presence of appreciable quantities of 20-hydroxyecdysone in the 5th-instar larva[8] and the prepupa of the hornworm[9] and by in vitro studies that demonstrate the conversion of α-ecdysone to 20-hydroxyecdysone by tissues of the hornworm prepupa[10]; they do not provide information concerning either the biosynthesis or the source of the high titer of 20-hydroxyecdysone found in the pupa of this insect[9,11]. Interestingly, this enzyme is similar to mammalian cholesterol 20-α-hydroxylase with its activity dependent upon NADPH and with a possible mitochondrial location[12,13]. This similarity warrants further study, particularly with other substrates.

No inference can be made as to whether this enzyme produces 20-hydroxyecdysone from α-ecdysone for secretion into the blood or for the metamorphosis of the midgut[14] itself or for excretion[8]. Due to the sudden appearance and disappearance of midgut ecdysone 20-hydroxylase activity, the midgut should be investigated further in relation to the biochemical control of ecdysone 20-hydroxylase activity and as an assay organ for candidate insecticides designed for a hormonal mode of action.

[5] J. N. Kaplanis, M. J. Thompson, S. R. Dutky, W. E. Robbins and E. L. Lindquist, Steroids *20*, 105 (1972).
[6] H. N. Nigg, M. J. Thompson, J. N. Kaplanis, J. A. Svoboda and W. E. Robbins, Steroids *23*, 507 (1974).
[7] Mention of a commercial or proprietary product in this paper does not constitute an endorsement of this product by the U.S. Department of Agriculture.
[8] J. N. Kaplanis, M. J. Thompson, W. E. Robbins and E. L. Lindquist, Steroids *20*, 621 (1972).
[9] J. N. Kaplanis, M. J. Thompson, R. T. Yamamoto, W. E. Robbins and S. J. Louloudes, Steroids *8*, 605 (1966).
[10] D. S. King, Am. Zool. *12*, 343 (1972).
[11] J. N. Kaplanis, W. E. Robbins, M. J. Thompson and A. H. Baumhover, Science *166*, 1540 (1969).
[12] S. Ichii, S. Omata and S. Kobayashi, Biochim. biophys. Acta *139*, 308 (1967).
[13] G. Constantopoulos, P. S. Satoh and T. T. Tchen, Biochem. biophys. Res. Commun. *8*, 50 (1962).
[14] K. J. Judy and L. I. Gilbert, J. Morph. *131*, 277 (1970).

Inhibition of Prodigiosin Formation in *Serratia marcescens* by Adenosine Triphosphate

A. O. Lawanson and F. O. Sholeye

Biological Sciences Department, University of Ife, Ile-Ife (Nigeria), 15 September 1975.

Summary. ATP, inorganic phosphate and ribose inhibited prodigiosin formation in *Serratia marcescens*, but adenine did not. ATP was not hydrolyzed by the organism during the experiment.

Materials and methods. The Commonwealth mycological institute type collection number 89668 of *Serratia marcescens* was utilized. Cultures were maintained on liquid peptone-glycerol medium (pH 7.2) according to the procedure of Goldschmidt and Williams[1]. ATP, inorganic phosphate, ribose or adenine (0 to 1 µmole/ml), sterilized by filteration through membrane filters, was introduced into the culture medium immediately after inoculation.

[1] M. E. Goldschmidt and R. P. Williams, J. Bact. *96*, 609 (1968).