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MOULT CYCLE OF A CENTIPEDE *ETHMOSTIGMUS SPINOSUS* (CHILOPODA : MYRIAPODA)

STUDIES on the moult cycle of millipedes[1,2] reveal that these myriapods resemble remarkably the Onychophora[3], in that there is no secretion of moulting fluid and no part of the exuvial cuticle is digested, resulting in the shedding of the old cuticle intact. This similarity would substantiate the theory proposed by Manton[4] that Diplopoda and Onychophora are phylogenetically closely related. It will be of interest to know in this context, how far the moult cycle in centipedes resembles that in millipedes and onychophorans. In view of the paucity of information on the same[5] the present study has been undertaken.

The moult cycle was followed in a population of 48 centipedes of the species *Ethmostigmus spinosus*, reared in the laboratory as described elsewhere in detail[6]. The following is the account of the moult cycle of the centipede, the stages of which are designated according to the scheme proposed by Drach[7].

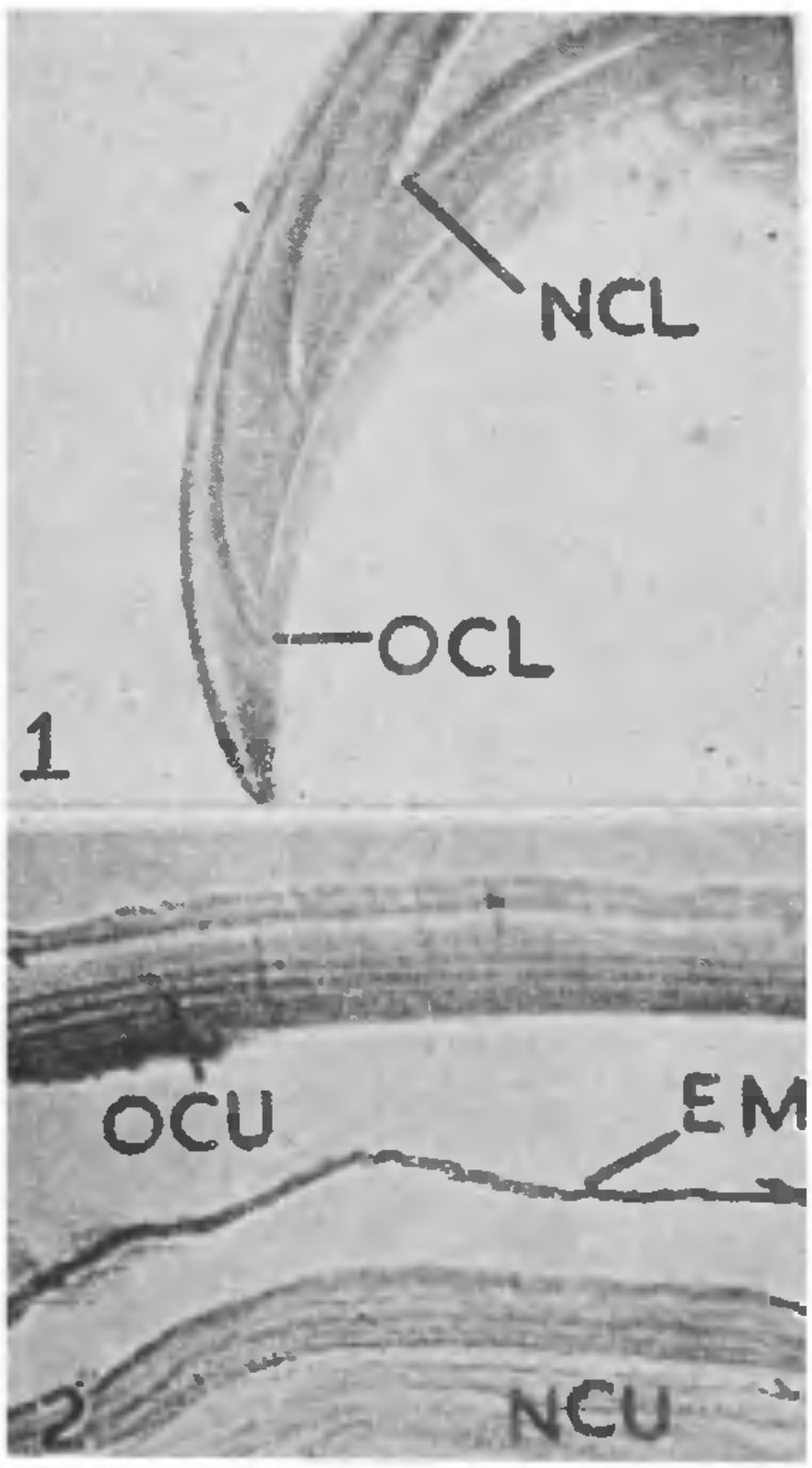
Stage A : This stage represents the condition immediately after the shedding of the old cuticle. The new cuticle is wet, soft and supple. The animals do not feed nor are they able to move even when disturbed, as their legs are not functional. The general colour of the animals is pale orange. This stage lasts from 2 to 5 hrs.

Stage B : The cuticle in this stage begins to harden progressively. Hardening starts in the legs, mandibles and cephalic capsule and then spreads along the tergites and sternites. The legs are functional and the animals crawl slowly when disturbed. No feeding. The colour of the animals becomes bright orange. Duration of this stage is 9 to 12 hrs.

Stage C : This is the intermoult stage when the animals lead normal life. The cuticle in all the regions of the body becomes hard and achieves its definitive colouration, *i.e.*, bright orange alternated with black stripes. The animals feed normally on the food provided (disabled spiders and flies) but not on the exuvium. This is the period of longest duration, 17 to 35 days.

Stage D : This is the stage preparatory to moult. The animals stop feeding and seek refuge in dark crevices where they remain stationary. The epidermis becomes swollen, indicating the intense activity and it starts digesting the inner layers of the old cuticle apart from laying the new cuticle. Therefore, this stage can be subdivided as follows.

D_1 *Stage* : The cuticle colour becomes dull orange. New claws start forming in legs (Fig. 1). The epidermis in the other regions of the body retracts a little from the cuticle so that there is a small gap above it. It takes 2 to 3 days to complete this stage.



FIGS. 1–2. Fig. 1. A claw of *Ethmostigmus spinosus* in D_1 stage. Fig. 2. Transverse section through the cuticle of *Ethmostigmus spinosus* in D_2 stage, stained in Heidenhain's haematoxylin.
EM, ecdysial membrane ; NCL, new claw ; NCU, new cuticle ; OCL, old claw ; OCU, old cuticle.

D_2 *Stage* : The epidermis starts secreting the new cuticle to the extent of 2 to 3 μ in thickness over the entire body, and it simultaneously secretes the moulting fluid in the space between the old and new cuticles. In the legs, the new claws are fully formed and they start retraction. This period lasts from 3 to 4 days.

D_3 *Stage* : Retraction of the new claws continues. The moulting fluid digests about half of the region of the old endocuticle and now an ecdysial

membrane is present in between the old and new cuticles (Fig. 2). The new cuticle increases in thickness to about 8 to 10 μ. Duration of this stage is from 2 to 3 days.

D_4 Stage: The new claws are retracted completely. The fresh cuticle increases in width to about 12 to 16 μ and becomes pale orange pigmented. The entire old endocuticle is digested by the moulting fluid and the ecdysial membrane is now attached to the inner surface of the old cuticle. This stage lasts from 6 to 9 hrs.

Stage E: This is the stage in which the animals actually shed the old cuticle starting from the cephalic region to the anal legs. This process takes place in about 70 to 100 minutes during nights.

It may be seen from the foregoing results that in centipedes there is secretion of moulting fluid, unlike in Diplopoda and Onychophora, and the old cuticle is partly digested. Therefore the feature noted in millipedes is not of general applicability to the whole of Myriapoda.

An interesting feature is that the duration of stage D is about 10 to 11 days in centipedes, compared to millipedes where this stage lasts only 3 to 4 days[1]. Even in insects where there is secretion of moulting fluid the stage D is never more than 6 days[8]. Therefore, centipedes will be ideal materials for the study of physiology of moulting in arthropods in greater detail.

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EFFECTS OF AN ACUTE DOSE OF BIOTIN ON THE REPRODUCTIVE ORGANS OF THE FEMALE RAT

THE adverse effects of biotin deficiency on the reproductive capacity and general metabolism of mammals are well documented[1,2]. On the other hand, there are no reports regarding the effects of an excess amount of biotin either in acute or chronic form on the reproductive ability and metabolism of the mammal. In certain insects, however, an excess intake of biotin causes severe degenerative effects on the ovary which leads to complete sterility[3,4]. It was of interest, therefore, to study the effects of an acute dose of biotin on the reproductive cycle, morphology of the ovary and the hepatic and uterine glycogen of the adult female rat.

Colony bred adult female rats of the Holtzman strain (approximately 3 months old) were selected after observing their normal oestrus cycle for three cycles. Eighteen rats thus selected were given biotin at a dose of 5 mg/100 g body weight dissolved in 0·2 ml of 0·1 N NaOH subcutaneously in two injections, morning and evening, at the dioestrus stage of the cycle. Groups of 6 rats each were sacrificed 7, 14 and 21 days after biotin injections. Another group of 6 rats was given an equal volume of 0·1 N NaOH at the dioestrus stage and was sacrificed 7 days after the injections. The entire experiment was performed under standardized light and dark, temperature and feeding conditions which are designed for best reproductive performances. The oestrus cycle of all the animals was studied daily on the basis of the microscopic examination of the vaginal smears. The leucocyte concentration of the vaginal smears was determined by the use of a haemocytometer after appropriate dilutions with normal saline. The histology of the ovary was studied following routine techniques and staining with haematoxylin and eosin. The liver and uterine glycogen was estimated according to the method of Montgomary[5].

The results of the present investigation showed that the NaOH-treated controls maintained normal oestrus cycles and the vaginal leucocyte numbers varied normally in relation to the stages of the oestrus cycle (Fig. 1). Since NaOH did not affect the normal oestrus cycle upto 7 days after treatment, running the controls upto 21 days was not considered necessary. The biotin-treated rats showed complete irregularity of the cycle and the vaginal leucocyte numbers progressively increased up to 14th day and sharply declined thereafter (Fig. 1). An increase in leucocyte infiltration in the vaginal lumen normally occurs during dioestrus stage in relation to the appearance of the luteal tissues in the ovary. This luteal phase of the cycle can be considered as an infertile stage of the mammalian reproductive cycle.

Therefore, biotin-induced irregularities of the oestrus cycle and massive infiltration of leucocytes in the vaginal lumen may be considered as an indication of its sterilizing effect. Biotin treatment