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Inability of Varied-Carpet Beetle Larvæ (*Anthrenus verbasci* L.) to digest Chitin

A FEW animals are known to possess in their digestive juices a chitinase enabling them to digest chitin. The occurrence of a chitinase in the gut of the snail (*Helix pomatia* L.) was demonstrated long ago[1]. A similar enzyme is present in the intestinal tract of different earthworms[2], eelworms[3], snails and slugs[4], and in the terrestrial isopod *Porcellio scaber* Latr.[5]. Among the snails, and probably also other terrestrial pulmonate gastropods, the intestinal chitinase is not produced by the glands of the animal, but appears to be of microbial origin[4].

It is often thought by entomologists that pests of collection specimens digest chitin since they normally live on a diet containing a high percentage of chitin. However, it has never been demonstrated that this chitin is actually utilized. The following investigations were carried out using varied-carpet beetle larvæ (*Anthrenus verbasci* L.) (Coleoptera, Dermestidae), in order to check their view.

(1) *Absence of chitinase in glycerol extracts of digestive tracts.* Larvæ of *A. verbasci* were reared on dried insects. Fully grown larvæ (about 5 mm. long) were cleaned and carefully dissected under a binocular microscope. Sixty-four isolated digestive tracts were ground in a small mortar with 5 ml. of 5 per cent glycerol and clean sand, at 0° C. After centrifugation, the chitinolytic activity of the clear supernatant fluid was tested, using both nephelometric and chemical methods previously described[4]. After 22 and 72 hr. of incubation, at *p*H 6·5 and 36° C., under aseptic conditions, no chitinolytic activity could be detected.

(2) *Absence of chitinolytic bacteria in the digestive tract.* Four larvæ, grown on dried insects, were cleaned and aseptically dissected in a sterile chamber. The digestive tracts were ground in a sterile mortar and suspended in 2 ml. sterile distilled water. 1 mgm. of excreta of larvæ was similarly suspended in 2 ml. distilled water.

The suspensions were then added to a sterile agar–agar medium containing finely powdered chitin, and plated in Petri dishes[6]. After ten days, no chitinolytic bacteria could be detected: no typical clarification of the medium, such as that usually observed with all chitinolytic bacteria of different sources[4,6], was noticed. Digestive tracts and also

excreta are particularly poor in micro-organisms, and seem to be entirely devoid of chitinolytic bacteria.

(3) *Chitin balance in the diet of larvæ reared in controlled conditions.* When reared on a diet consisting of dried insects (adults of *Musca* sp., *Palomena prasina* and *Doryphora decemlineata*), larvæ of *A. verbasci* are able to utilize a maximum of 30 per cent (in dry weight) of the available diet. Two sets of experiments were carried out with larvæ reared for three months on a diet of a known content of chitin, at 28° C. and 50 per cent relative humidity. One control experiment consisted of exposing the same food in the same incubator without larvæ.

In one experiment, chitin was estimated by purification of the material, using the method of Black and Schwartz[7], the residue being weighed as pure chitin. The loss of chitin in the food seemed rather important (28 per cent with dried *Musca* and *Palomena* as diet, 8·5 per cent with *Doryphora*). But this method was found afterwards to be quite unsatisfactory in this particular case owing to the fact that purification is not completed (some proteins remain unhydrolysed) and the excreta, being of a very finely powdered material, are not easily retained during manipulations.

In a second experiment, chitin was estimated by purification of the material with 6 per cent potassium hydroxide at 100° C. for 2 hr.; *N* hydrochloric acid at 20° C. for 2 hr.; again 6 per cent potassium hydroxide at 100° C. for 2 hr.; 1 : 1,000 potassium permanganate at 60° C. for 20 min.; sodium metabisulphite; hot water, alcohol, ether. These different chemical treatments and the washings were performed in centrifuge tubes, the residue being centrifuged after each treatment for a long time, to prevent any loss of small particles. Estimation of chitin in the final residue was by nitrogen determination (Kjeldahl method).

In these conditions, the loss of powdered material was considerably reduced. The difference in chitin–nitrogen between controls and a diet consisting of dried *Palomena prasina* on which fifty larvæ were reared for three months reached 4 per cent only, this value being within the normal limits of error of the method. Thus chitin is not digested and can be nearly completely recovered in a controlled diet, although the cuticles of the dried insects are partially destroyed and reduced to powder by the larvæ.

Hence we conclude that, using an adequate method for the estimation of chitin balance in dried insects eaten by *Anthrenus verbasci* larvæ, no significant loss of chitin could be observed. The digestive tracts of these larvæ are devoid of chitinase and of chitinolytic

micro-organisms. Such larvæ are unable to digest chitin, although there is a high content of chitin in their usual diet.

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[1] Karrer, P., and Hofmann, A., *Helv. Chim. Acta*, **12**, 616 (1929).
[2] Tracey, M. V., *Nature*, **167**, 776 (1951).
[3] Tracey, M. V., 2nd Int. Congr. Biochem., Paris, Résumé des Comm., 242 (1952).
[4] Jeuniaux, Ch., *Mém. Acad. Roy. Belg., Classe Sci.*, **28**, fasc. 7 (1954).
[5] Jeuniaux, Ch., *Arch. internat. Physiol. Biochem.* (in the press).
[6] Jeuniaux, Ch., *Bull. Soc. Roy. Sci. Liège* (in the press).
[7] Black, M. M., and Schwartz, H. M., *Analyst*, **75**, 186 (1950).

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