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## Calcium citrate in an insect

The oothecae of all Praying Mantids so far examined contain small rectangular crystals adhering to or embedded in the protein membranes of which the ootheca is formed. The crystals originate in a large gland located in the body wall of the seventh ventral sternite or sub-genital plate, and are added to the secretions from the colleterial glands at the time of laying. The purpose of this note is to describe the chemical nature of the crystals, each of which is contained in a protein envelope of complicated structure.

Under the microscope the crystals within the ventral body-wall gland appear identical with those found in the oothecae. They vary in size but average dimensions are  $5 \times 3 \times 1 \mu$ , and when allowed to settle on a slide they appear rectangular and strongly birefringent with the optic axis at about 45° to the edge of the crystals. Sometimes the crystals have cleaved parallel to the long edge and these thin plates lie on their sides. In this view they are trapeziform and are birefringent with the optic axis in a plane parallel to the long edges of the crystal. Just before laying, the gland is packed full, i.e. there is a volume of several cubic mm of crystals. Within the gland a few crystals of another substance were observed, having no birefringence and a more clongated form.

By X-rays and infra-red the crystals have been identified as calcium citrate. By reaction of calcium chloride and sodium citrate insoluble citrates were obtained that had a variety of structures according to X-ray and infra-red tests. The calcium citrate identical with the crystals in the insect was formed when 150 ml o.t M calcium chloride was mixed with 100 ml o.t M sodium citrate and allowed to stand in a tall cylinder, whereupon the calcium citrate slowly precipitated. This precipitate was washed dried in air, and the rate of dehydration followed over P<sub>2</sub>O<sub>5</sub>. Changes in the vapour pressure occurred at weights corresponding to the hexahydrate and the tetrahydrate, while heating at 100° C gave an approximately anhydrous product. Three types of X-ray powder diagram were obtained corresponding to the hexahydrate, tetrahydrate and the anhydrous form as just defined. On the basis of their X-ray diagram and infra-red absorption the crystals produced by the Mantis were identified as the hexahydrate.

Several things are noteworthy concerning these crystals. They are formed within protoplasmic membranes. They show that a biological mechanism exists for a very great concentration of citrate as calcium citrate. These special devices suggest that the citrate plays an important part in the transformation of the colleterial gland protein into the highly characteristic ribbon-like elements of the ootheca. In most respects the Mantis ootheca differs markedly from the better known cockroach ootheca; but we may recall that the left colleterial gland in the cockroach contains the structural protein of the ootheca and large quantities of calcium oxalate. The function of this calcium oxalate is unknown—suppression of calcium ions is one possibility. In contrast, the Mantis shows no oxalate (or citrate) crystals within the colleterial glands, but calcium citrate is added from a separate gland during production of the ootheca.

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