PRO EXPERIMENTIS

# Determination of uric acid and hematin in a single sample of excreta from blood-fed insects

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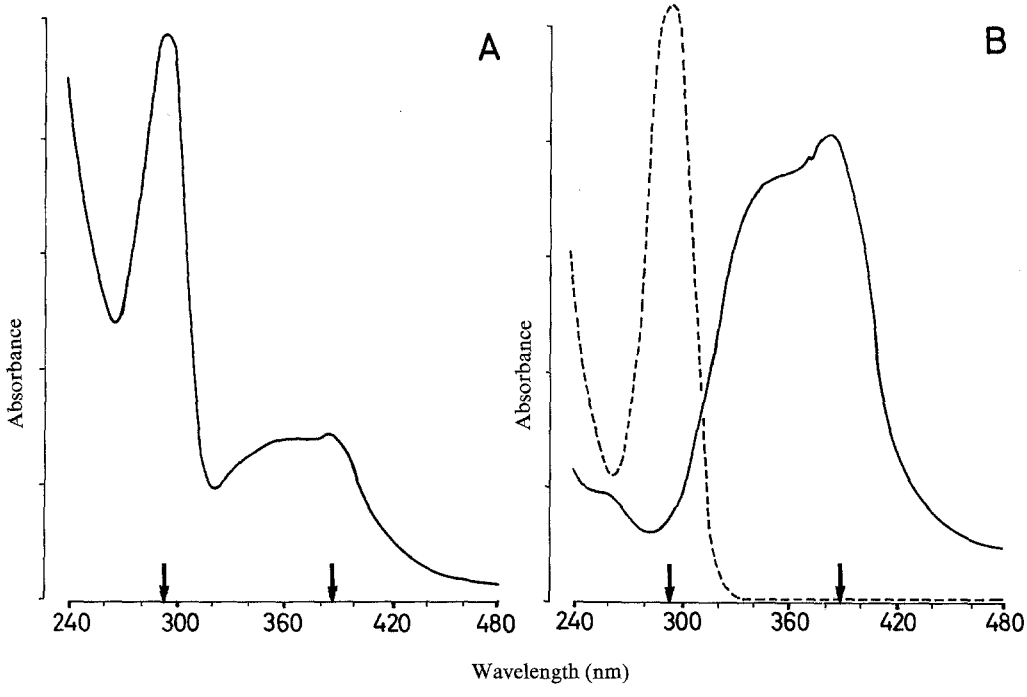
*Summary.* Uric acid and hematin, the principle excretory products in blood-fed mosquitoes, can be determined quantitatively in the presence of each other in fecal material from individual females.

Adult insects require a protein diet for their physiological needs in the process of reproduction. Various terrestrial insects have evolved successfully towards hematophagy; the vertebrate blood offers a source very rich in protein. For female mosquitoes it has been found that more nitrogen is ingested from a blood meal than is deposited in the yolk material[2]. Surplus nitrogen is excreted as uric acid while hemoglobin is quantitatively converted to hematin, which is defecated at the end of the digestion period[3].

For the determination of uric acid a simple colorimetric technique has been elaborated by Van Handel[4]. This method is insensitive to proteolytic enzymes, also present in mosquito feces[5]. In order to measure hematin, which prevails in the excreta of blood-fed mosquitoes, fecal material of such mosquitoes was analyzed qualitatively. 10 blood-fed females of *Aedes aegypti* were kept in a screen-covered beaker for 3 days after the blood meal. The yellowish and black pellets were then dissolved in 1 ml of a 1% lithium carbonate solution. An aliquot from this eluate was appropriately diluted with the same lithium solution and scanned in a quartz cell from 900 to 220 nm in a double-beam spectrophotometer (Beckman DB) in order to find the absorption maxima. A typical result is given in the figure. For comparison, solutions of uric acid (1%), hematin (0.1%), hemin (0.1%), hematinchloride (0.1%), and hemoglobin (1%), all in 1% lithium carbonate, were treated in the same way. It was found that the peaks in the figure, A exactly correspond to uric acid and hematin: absorption maxima are at 292 nm for uric acid and at 387 nm for hematin (figure, B). These absorption spectra illustrate that hematin can be determined directly at 387 nm without interference by uric acid or other excretory products. But conversely, the presence of hematin interferes with the absorbance of uric acid at 292 nm. Based on these observations it is advisable to determine uric acid by colorimetry[4] and hematin by spectrophotometry, i.e. by converting the extinction of the eluate at 387 nm to µg by means of a standard curve (e.g. 1-10 µg per sample). The same material can then be used for the neocuproin reaction with uric acid[4]. The contribution of hematin to this latter procedure can be subtracted easily, as pointed out by Van Handel[4]. Errors caused by the presence of hematin, which occurs in all excreta from blood-feeding insects, can lead to overestimations of uric acid concentrations from 20 to 150%, depending on the proportion of hematin to uric acid produced during blood digestion.

This procedure has been applied in our laboratory to quantitative work on the excretion of waste products by *A. aegypti* after blood meals of various sizes[3].




*A* Absorption spectrum of a sample of excreta from blood-fed *A. aegypti*. Fecal material was dissolved in 1 ml of 1% lithium carbonate and absorbances recorded from a double-beam spectrophotometer against water.
*B* Absorption spectrum of pure hematin (----) and urio acid (——). Solutions of 1% in 1% lithium carbonate were scanned in the same way. Arrows indicate the maxima at 292 nm for uric acid and at 387 nm for hematin. They coincide with the peaks in A.

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