

It should be remembered that fluorescence fading can occur during the initial selection and positioning of each region to be examined, so it is possible that, for a series of measurements each of which is taken during a short discrete period of irradiation, partial recovery of fluorescence fading would result in an initial measurement being lower than the final value of fluores-

cence intensity in the series. Although such a finding could, in theory, be at least partly due to irradiation-induced decomposition of the fluorophore having led to a diminution of concentration-dependent quenching¹, this could not explain the present finding of an increase in fluorescence intensity during a period when the specimen is not irradiated.

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The defensive secretion of the tiger beetle *Cicindela flexuosa* (F.) (Cicindelinae; Carabidae)

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Summary. The pygidial gland secretion of the tiger beetle *Cicindela flexuosa* consists of tetradecylacetate and hexadecylacetate. It functions as a defense mechanism.

Tiger beetles (Carabidae, Cicindelinae) are predatory beetles living in sandy areas and marshes along streams and seacoasts. Often brilliantly colored, they are favorites among collectors although they are swift runners and fast flyers and are difficult to capture. In common with many other members of the Carabidae they possess a pair of pygidial glands which secrete a substance used for defense. The chemistry of the secretion of a number of carabid beetles has been extensively investigated and has revealed a variety of compounds ranging from hydrocarbons to carboxylic acids, esters, aldehydes, ketones, phenols and quinones²⁻⁴. However tiger beetle secretions are still mostly unexplored; only a few species, all of the genus *Megacephala*, have been examined. *Megacephala australis*⁵ produces benzaldehyde in its pygidial glands while in *M. virginica* and *M. carolina*⁶ this aromatic aldehyde is accompanied by hydrogen cyanide. Small amounts of mandelonitrile were also detected in *M. virginica* indicating a cyanogenic pathway in the glands. In this paper we report on the pygidial gland secretion of a tiger beetle of the genus *Cicindela*, a secretion entirely different from that of the *Megacephala* beetle.

Cicindela flexuosa is an amphotilous tiger beetle which is found in most of the sand dunes along the Mediterranean shores of Israel. The structure of its pygidial glands is similar to that described for *C. campestris*⁷. The 2 glands are elongated and open on the outer side of the abdomen between the 8th and 9th tergites. When disturbed or handled roughly, the beetles often protrude their abdominal tip to expose the intersegmental membrane and in this position they discharge the contents of the glands.

Materials and methods. Adult beetles were collected in early spring near Ma'agan Michael, Israel, refrigerated and brought to the laboratory where the pygidial glands of live beetles were dissected and then extracted with methylene chloride. The ex-

tracts were analyzed by combined gas chromatography-mass spectrometry on a LKB-2091 instrument using a 1.8-m 3% OV-17 column programmed from 60 to 300°C and 1.8-m 10% SP-1000 column programmed from 60 to 240°C. Identification of the components was based on comparison with authentic standards. Tetradecylacetate and hexadecylacetate were synthesized from the corresponding alcohols and acetyl chloride. Two compounds were detected in the extract. The first component which accounted for more than 75% of the volatiles eluted at 210°C, the other at 230°C on the OV-17 column. Their mass spectra were similar and consistent for acetates, with a base peak at m/z 43 and a substantial diagnostic peak at m/z 61. The highest mass peaks at 196 and 224 respectively (M-60) spoke for mol. wts of 256 and 284, and the remainder of the spectrum in each case was typical for aliphatic chain structure. The compounds were identified as tetradecylacetate and hexadecylacetate by comparison with authentic samples (mass spectra and retention times).

Tetradecylacetate and hexadecylacetate are quite common in the defensive secretions of arthropods. They have been found in the pygidial glands exudates of several carabids; tetradecylacetate was detected in Psydriinae and also in Panagaeinae⁴ where it is accompanied by the hexadecyl homologue. Both acetates are present in large amounts in the Dufour's glands of the ants *Formica pergandei* and *F. subintegra*⁸ and serve as alarm pheromones and also as disarming agents during slave raids on other ant colonies. In *Lasius niger*⁹, *Camponotus ligniperda*¹⁰ and *C. herculeanus*¹¹, these acetates are used in alarm and in defense and it is very probable that the pygidial gland secretion of *C. flexuosa* has this same dual function. Other *Cicindela* have yet to be studied to determine if acetates are common to this genus just as benzaldehyde appears to be characteristic of the *Megacephala* genus of tiger beetles.

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Mechanical simulation of renal pelvic wall peristalsis in the rat¹

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Summary. A urinary concentrating defect was induced in Munich-Wistar rats by removing the renal pelvis from 1 kidney. This defect was partially corrected by crudely simulating the actions of pelvic wall peristalsis with a mechanical system that cyclically compressed the exposed renal papilla.

It has been shown, in rodents, that the upper portion of the ureter (i.e. the renal pelvic wall) physically 'milks' the renal papilla³⁻⁵. The peristaltic contractions of the pelvic wall cause discontinuous fluid flow in the papillary collecting ducts and vasa recta⁴ and appear to alter fluid distributions within the loops of Henle, medullary interstitium, vasa recta and collecting ducts⁶. These observations have led to a variety of observations concerning the role of the upper ureter in the renal concentrating process⁴⁻⁷.

Methods. A concentrating defect was created in Munich-Wistar female rats (Simonsen Laboratories, Gilroy, CA, USA) by removing the upper ureter from the right kidney and then, intermittency of fluid flow was simulated by a mechanical system that periodically compressed the exposed renal papilla. The changes in urine osmotic concentrations were compared during each of these periods.

On the evening before experimentation food and, in some cases, water were withheld from the animals. Surgical procedures, which have been described in detail elsewhere^{4,8}, were performed to expose the right kidney. Approximately 90 min after the administration of anesthesia (Inactin, i.p., 150 mg/kg) the right renal pelvic wall was removed with iridectomy scissors and the ureteral stump ligated; the exposed papillae ranged from 1250–1500 µm in length. Urine samples were then taken during the next 45 min from the openings of the ducts of Bellini with a micropipette. The osmotic concentrations of the uncontaminated samples (i.e. erythrocyte free) were determined on a Clifton nanoliter osmometer. During the next 45 min the action of the renal pelvic wall was crudely simulated with a 'mechanical pump' and urine samples continued to be collected for analysis. This pump consisted of a fiber optic probe (tip diameter = 1 mm) that was pushed against the side of the papilla 10 times per min by a motorized Zeiss micromanipulator. A stationary glass rod (diameter = 1 mm) was also positioned to prevent lateral movement of the papilla so that during each cycle there was sufficient compression to temporarily occlude blood flow in the vasa recta (about 20% of the 1000 µm papilla width).

Major drawbacks of this technique were bleeding of the vasa recta, loss of vasa recta blood flow and loss of urine flow during pumping. Of 25 attempts only 6 experiments produced

enough uncontaminated urine samples for analysis (these rats weighed between 79 and 94 g).

In order to correct for variations in urine concentration from animal to animal, the results were expressed as a ratio of the osmolalities of urines from the exposed papillae and the bladder (i.e. urine from the contralateral control kidney) collected at the same time. The rate of change of this ratio was compared before and during pumping with a paired Student t-test.

Results and discussion. Exposure of the renal papilla produced a concentrating defect that was more pronounced with increasing control urine osmolality (range of control urine osmotic concentrations = 0.4–2 Osm/kg; ratio of the exposed:control urines = 1.1–0.4 × control urine Osm/kg; N = 6; r² = 0.75). Extrapolation of these data suggests that the pelvic wall has no effect when urine is isotonic to blood (i.e. exposed:control = 1; osmotic concentration = 0.3 Osm/kg). Since the presence of the upper ureter is of lesser importance at lower urine osmolalities, only those animals having control kidney osmotic concentrations greater than about 1 Osm/kg were used for determining the effects of mechanical compression.

Mechanical compression of the renal papilla reversed the falling trend in urine osmolality caused by papillary exposure (table). The actual increases in urine osmolality during the 45 min of pumping were modest (about 0.2 Osm/kg) and indicate that our mechanical system is a poor substitute for the complex peristaltic actions of the upper urinary tract. In addition, the pumping rate used (10 cycles/min) was about 50% slower than the observed contraction rate of the Munich-Wistar rat ureter.

These findings imply that the physiological 'milking' of the renal papilla may result in increased urine concentration. One

Rate of change of exposed : control urine osmolalities (ΔE/C per h)

Control kidney urine osmolality (mOsm/kg H ₂ O)	Rate of change following exposure	Rate of change during compression
1386	– 0.151	0.106
1924	– 0.052	0.097
1546	– 0.041	0.068
978	– 0.164	– 0.076
Mean ± SEM	– 0.102 ± 0.032	0.049 ± 0.042

The means shown are significantly different (p = 0.028).