10^{-5} M atropine (244 ± 12.8 beats/min) is not significantly different ($p > 0.5$) than the average basal rate (no atropine) of atria from sedentary animals (235 ± 8.8 beats/min).

Discussion. Exercise bradycardia cannot be attributed to an increase in vagal tone nor to a decrease in sympathetic tone since the decreased rate is present in atria isolated from exercised rats. The increased concentrations of acetylcholine in the heart reported by other workers[5,6], however, conceivably hold the rate of discharge of the pacemaker in abeyance, thus maintaining the bradycardia.

Our finding that atropine has no chronotropic effect on atria from sedentary animals is in agreement with the report of Grodner et al[10]. However, our finding of a significant increase in rate of atria from exercised animals in response to atropine has not been previously reported. The increases in atrial rate in response to atropine shift the atrial rate of exercised animals toward the basal atrial rates of sedentary animals, with 10^{-5} M atropine causing a rate in exercised atria which is not significantly different from the basal rate of atria from sedentary rats. These results may indicate that the increased amount of acetylcholine in atrial tissue reported by other workers plays an important role in producing exercise bradycardia.

1 This study was supported by a grant from the Genesee Valley Heart Association.
2 C.M. Tipton and B. Taylor, Am. J. Physiol. *208*, 480 (1965).
3 Y.C. Lin and S.M. Horvath, J. appl. Physiol. *33*, 796 (1972).
4 C.P. Bolter, R.L. Hughson and J.B. Critz, Proc. Soc. exp. Biol. Med. *144*, 364 (1973).
5 H.C. Herrlich, W. Raab and W. Gigee, Archs int. Pharmacodyn. *129*, 201 (1960).
6 C. de Schryver and J. Mertens-Strythagen, Experientia *31*, 316 (1975).
7 J. Ekstrom, Q. J. exp. Physiol. *59*, 73 (1974).
8 C.M. Tipton, R.J. Barnard and G.D. Tharp, Z. angew. Physiol. *23*, 34 (1966).
9 W.C. Schefler, Statistics for the Biological Sciences. Addison-Wesley Pub. Co., Reading 1969.
10 A.S. Grodner, H.-G. Lahrtz, P.E. Pool and E. Braunwald, Circulation Res. *27*, 867 (1970).

Concentration by the renal sac of *Molgula manhattensis* of homarine, a nitrogenous compound

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Summary. An important compound in the renal sac fluid of *Molgula manhattensis* is homarine. This substance may have a role in the osmotic adjustment of the molgulid renal sac.

The renal sac of ascidians contains large amounts of uric acid and purine compounds[1-4]. It was considered for a long time as the storage-excretion structure of these animals[4-6]. The importance of this excretion was reconsidered after the work of Goodbody[7,8] who demonstrated that ascidians liberate ammonia in water. Goodbody showed that ammonia is the main product of nitrogenous excretion in ascidians as in other marine invertebrates. This excretion coexists with storage excretion, as Sabbadin et al.[9] confirmed. Other roles than simple excretion were predicted by Nolfi[10]. Nolfi showed the purine origin of uric acid in *Molgula manhattensis*, and suggested that it had a reserve function. A more recent work[11] concerns a possible osmoregulatory participation of the renal sac and shows that the ionic composition of renal sac fluid differs from that of blood. Organic compounds could be basically involved in osmotic adjustment. Determination of very large concentration of homarine in the renal sac fluid confirms the basic importance of this compartment. Homarine is a methyled base (figure 1), which contains little nitrogen and its role in nitrogenous excretion appears to be very weak in comparison with that of uric acid (figure 1). Homarine should, however, play a part in intracellular osmotic equilibrium as do other nitrogenous compounds[13-15].

Molgula manhattensis specimens were collected in the Bay of Arcachon. Adult animals were collected at different seasons (February, May, July). The renal sac is a closed system which contains fluid and concretions. Renal sac fluid and blood withdrawals can be made on the living animals with the organs in situ. Supernatant fluid and precipitate of both renal sac fluid and blood were analyzed after centrifugation. Analyses were also made on isolated renal concretions, renal envelope, intact renal sac and on the whole animal. The renal concretions were ground in cold 0.1 N perchloric acid. Homarine was identified by UV spectrophotometry (max = 272–273 nm) independent of the pH) and TLC on cellulose plates (Schleicher and Schull, 114 LS 254). Comigration with a reference substance (Homarine hydrochloride, Aldrich-Europe, No. 16037-7) was checked in several solvent systems (e.g. Propanol-ammonium hydroxide 1%, 2:1 v/v). Uric acid was identified by UV spectrophotometry (λ max = 286 nm at pH 1, 297 nm at pH 13), and determinated as previously described by one of us[16].

The major compound in the renal sac fluid is homarine (R_f 0.53). We detect 3 other UV absorbing more polar substances, all differing from uric acid (R_f 0.05). Homarine is present in very large amounts and it was necessary to dilute 500 times renal sac fluid for recording its UV-spectrum (figure 2). This substance is found at a level of 5.7 mg/ml of renal sac fluid. This amount corresponds to 3 mg/g of renal sac. Renal concretions show some traces of homarine and its concentration in the blood is about 0.2 mg/ml. Uric acid cannot be directly detected in the renal sac fluid by UV spectrophotometry. Its presence is noted, in very low amounts, after purification by TLC. We found on the average 2 mg uric acid for each renal sac, which represents 40% of concretions wet weight. Its presence in the renal

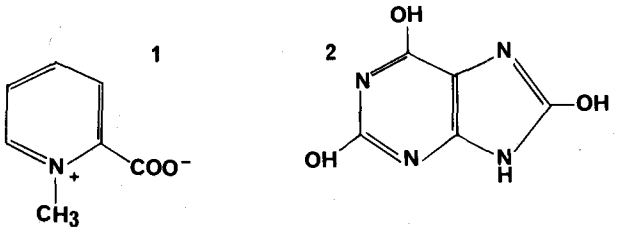

Fig. 1. Structural formula of homarine **1**, and uric acid **2**.

epithelium is evident. Very small quantities were found in the blood.

Our results confirm those of Goodbody[8] and Nolfi[10] on the amount of uric acid contained in renal concretions. Uric acid is the most important compound of the concretions and homarine is the essential UV absorbing substance of the renal sac fluid. Our results agree with those of Gasteiger et al.[12] when related to the wet weight of the whole animals. The homarine quantities in the isolated renal sac and in the whole animal are about equal: this substance is thus specifically located to the renal sac in *Molgula manhattensis*. Its absence from ascidians families which have no renal vesicles[13] supports this idea. The renal sac fluid of molgulids is not excreted outside and homarine concentrations may be the result of an accumulation. Its presence at a level of 40 mosmole/l should be related to a role in the osmotic adjustment of the renal sac medium to osmotic pressures of blood and sea water[11].

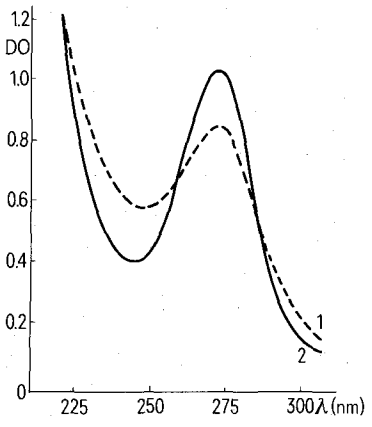

Fig. 2. UV absorption spectrum (λ in nm) of renal sac fluid: *1* in primary extracts of the renal fluid (dil. 500); *2* after TLC and elution of the R_f 0.53 band.

1 C. Kupffer, Arch. mikrost. Anat. EntwMech. *8*, 358 (1872).
2 H. Lacaze-Duthiers, Archs Zool. exp. gén. *6*, 457 (1877).
3 L. Roule, Annls Mus. Hist. nat. Marseille *2*, 457 (1885).
4 M. Azéma, Annls Inst. océanogr., Monaco *17*, 1 (1937).
5 R. Burian, Handb. vergl. Physiol. *2*, 607 (1948).
6 S. Das, Biol. Bull. *95*, 307 (1948).
7 I. Goodbody, J. exp. Biol. *34*, 297 (1957).
8 I. Goodbody, J. exp. Biol. *42*, 299 (1965).
9 A. Sabbadin and A. Tontodonati, Monitore zool. ital. *1*, 185 (1967).
10 J.R. Nolfi, Comp. Biochem. Physiol. *35*, 827 (1970).
11 F. Gaill and P. Lasserre, C. r. Acad. Sci. *285*, 1343 (1977).
12 E.L. Gasteiger, P.C. Haake and J.A. Gergen, Ann. N.Y. Acad. Sci. *90*, 622 (1960).
13 J.R. Beers, Comp. Biochem. Physiol. *21*, 11 (1967).
14 G. Bricteux-Grégoire, G. Duchâteau-Bosson, C. Jeuniaux and M. Florkin, Archs int. Physiol. Biochim. *70*, 273 (1962).
15 R. Duchâteau and M. Florkin, Archs int. Physiol. Biochim. *60*, 124 (1955).
16 R. Lafont and J.L. Pennetier, J. Insect Physiol. *21*, 1323 (1975).

Olfactory sensitivity in *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomellidae) in relation to its host plant

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Summary. Food-perception ability of *Aulacophora foveicollis* is adversely affected by bilateral antennectomy, but remains unaffected by unilateral antennectomy.

Interesting informations on the chemosensory relationship between *Epilachna vigintioctopunctata* Fabr. (Coleoptera: Coccinellidae) and its gost plant *Luffa aegyptiaca* Mill (Cucurbitaceae)[1] prompted us to undertake the study on the same aspect with respect to another beetle pest, *Aulacophora foveicollis*, and one of its cucurbitaceous hosts, *Lagenaria vulgaris*.

Methods and materials. This investigation was carried on adult *A. foveicollis* of mixed age and sex. The technique and the general lay out of the experiments, reported by Krishna and Sinha[1], were followed in the present study with alteration in the duration of the experimental period. Instead of 1 h observation period[1], it was restricted to 15 min, because the maiden biting response of normal as well as the antennectomized insects, occurring only during the first 15 min, was taken into account by Krishna and Sinha[1] to explain the chemosensory relationship between *E. vigintioctopunctata* and *L. aegyptiaca*.

The parts of *Lagenaria vulgaris* preferred in this study, were flowers and leaves - the best choices among all the parts of the plant[2]. Experiments, with respect to each category of beetles viz., normal, with left antenna amputed, with right antenna amputed and with both antennae amputed, involved 50 individuals, utilising one in each replicate.

Results and discussion. It is very obvious from the table that extirpation of either of the antenna of *Aulacophora foveicollis* hardly makes any difference in the biting response of this beetle, while removal of both drastically minimises it. These observations tally closely with the findings of Krishna and Sinha[1] on *Epilachna vigintioctopunctata* and thus fully conform to the interpretations given by them, which can be summarized in relation to *A. foveicollis*, as stated below. The number of olfactory receptors located on single

Relative numerical strength of the different groups of *Aulacophora foveicollis* showing the only biting response on either flower or leaf of *Lagenaria vulgaris*

Group of the insects	Number of individuals comprising each group	Number of individuals showing the biting response during 15-min observation period
A	50	23
B	50	24
C	50	21
D	50	4

A, Insects with both antennae intact; B, insects with right antenna removed; C, insects with left antenna removed; D, insects with both antennae removed.