extensively. As discussed above, the amino acid compositions as well as the electrophoretic behaviour of both forms are not different, indicating that the two forms of the enzyme are identical. However, only few data are known about the properties of the medullary DBH of other species. Ross et al.[3] showed that DBH of crude homogenates of tissues from ox, man and rat exhibited two electrophoretically distinguishable peaks of activity. The slow-moving peak, which has been eliminated prior to electrophoresis by high-speed centrifugation, could be converted into the faster migrating peak, suggesting that this peak is corresponding to the membrane-bound DBH. Although the mobility of the main peaks from human, rat and bovine adrenal medulla differed somewhat, the K_m-values for all species were similar. This is in agreement with our results. We found no statistically significant differences between the K_m-values of the soluble and membrane-bound as well as the total DBH of the species studied. The K_m-values for tyramine are lower than those for phenylethylamine, since tyramine has a higher affinity to the DBH than phenylethylamine has. The present results strongly support the view that soluble and membrane-bound DBH are identical enzymes. However, the physiological significance of the separation of DBH into soluble and membrane-bound form is not known so far.

Na, K-ATPase in the Salivary Gland of the Ixodid Tick *Amblyomma hebraeum* (Koch) and its Relation to the Process of Fluid Secretion[1]

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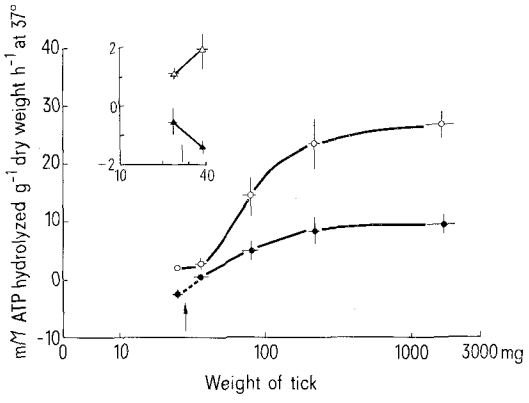
Summary. Total and ouabain-sensitive ATPase activities were determined in the salivary glands of ticks throughout the feeding cycle. Activities were very low in unfed specimens. In the glands of feeding females, the activities rose until a maximum was reached for both ATPase components at approximately 200 mg. The activities remain low in males throughout the feeding period. These findings are discussed in relation to the fluid secretory process of the salivary glands.

A common characteristic among haematophagous arthropods is the elimination of excess fluid taken in with the blood meal. Whereas in insects such as the bug, *Rhodnius*, the Malpighian tubules play the fundamental role in fluid excretion[2], in female ixodid ticks the salivary glands function in this capacity[3,4]. KAUFMAN and PHILLIPS[5] demonstrated that salivary secretion in females depended on active solute transport and that it could be inhibited by very low concentrations of ouabain. It was also shown that sodium and potassium were necessary in specific ratios for fluid secretion to proceed at a maximal rate, suggesting that an Na, K-ATPase was an important component of the secretory mechanism. KAUFMAN[6] demonstrated that salivary glands from unfed female ticks could secrete fluid only at a slow rate. Salivation augmented with increased tick weight, but the enhancement was not attributable merely to hypertrophy of the glands. The present paper confirms the existence of a Na, K-ATPase in the salivary gland of the female *Amblyomma hebraeum* (Koch); activity of this ATPase increased with time spent by the tick on its host. A similar Na, K-ATPase could not be detected in salivary glands of males.



ATPase activity in salivary glands of *A. hebraeum* plotted against logarithm of tick weight. Data for females are depicted in the main graph, those for males in the inset. Females: ○, total ATPase; ●, Na, K-ATPase. Males: △, total ATPase; ▲, Na, K-ATPase. Points appearing to the left of the arrow in each graph are for unfed specimens, those appearing to the right are for fed specimens. Horizontal and vertical bars denote SE of the mean weight and mean activity respectively, when the SE exceeds the dimension of the point. The curves have been fitted by eye. Ouabain slightly stimulated ATP hydrolysis by homogenates of unfed female glands and by unfed and fed male glands, hence the negative values for Na, K-ATPase activity in these cases. Whereas the female tick may attain a replete: unfed weight ratio approaching 100 after a normal 7 to 10 day feeding period, the male barely doubles its weight over the same time.

Material and methods. Unfed adult ticks were placed on the backs of rabbits[4] and specimens were removed at various stages of the feeding period. Salivary glands were dissected out under Hank's balanced saline (composition in g/l: 8.0 NaCl; 0.4 KCl; 0.14 $CaCl_2$; 0.06 KH_2PO_4; 0.98 $MgSO_4$; 0.048 Na_2HPO_4; 1.6 D-glucose; 0.01 phenol red) and non-salivary tissue including most of the tracheae were carefully dissected away. Preparation of the crude enzyme was essentially according to BONTING[7]. Each pair of glands was transferred to a small glass homogenizer containing 0.1 ml (equivalent to 4 or 5 volumes of tissue) of the homogenizing medium (1 m*M Tris* in distilled water adjusted to pH 7.5). The piston

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was operated manually following a standard procedure as closely as possible (20 passes of the piston including a twisting motion). The homogenate along with 2 washings of the homogenizer were transferred to tubes and centrifuged for 15 min at 1000 *g*. The supernatant along with 2 further washings of the 1000 *g* pellet were transferred to glass ampoules and lyophilized. During the total procedure from dissection to lyophilization, the tissue was kept on ice or held at 4 °C. For enzyme assay, the lyophilizate was reconstituted in distilled water, between 0.5 and 3.0 ml according to anticipated activity. Assay tubes containing 250 µl of the incubation medium (see below for composition) were brought to 37 °C, and 10 or 25 µl of tissue extract was added and incubated for 60 min. Under these conditions substrate utilization did not exceed 20% and the rate of inorganic phosphate liberation was constant for at least 90 min. Reaction was stopped by the addition of 2 ml 10% trichloroacetic acid and the tubes were transferred to ice. Non-incubated controls were run in parallel for each experiment. Each sample and non-incubated control consisted of 3 replicates. Inorganic phosphate was determined according to BONTING[7] using a Zeiss PM 4 Spectrophotometer. ATPase activity was determined at pH 7.4 in a medium containing 90 m*M* *Tris*, 0.1 m*M* EDTA, 2 m*M* Mg^{2+}, 60 m*M* Na^+, 5 m*M* K^+ and 2 m*M* Na_2ATP. Chloride was the predominant anion. A series of preliminary experiments had shown that either the elimination of sodium, potassium or both from the above medium, or the addition of 1 m*M* ouabain all resulted in equivalent degrees of inhibition. Thus for the following experiments the ouabain-sensitive component alone was used as the measure of Na, K-ATPase. Known volumes of enzyme solution were placed in pre-weighed glass tubes, thoroughly dried at 100 °C and weighed on a Mettler M5 microbalance to within 2 µg. After correction was made for the small amount of *Tris* known to be in solution, the dry weight of tissue per enzyme sample could be calculated. Enzyme activity is expressed as m*M* ATP hydrolyzed/h/g dry weight of tissue at 37 °C.

Results. In the present study, ouabain-sensitive ATPase of the salivary glands of feeding females was 34.5 ± 2.5% (SE of mean, $n = 15$) of the total ATPase (range = 15 to 50%). The average Na, K-ATPase activity for females weighing more than 170 mg was 8.3 m*M* ATP · g^{-1} dry weight · h^{-1}. The water content of this tissue is high – 82 ± 1.2% (SE of mean, $n = 14$), therefore the activity per g wet weight is about 1.5. Experimental results are presented in the Figure. We summarize as follows: Salivary glands from male ticks, whether unfed or fed for up to 12 days, possess a very low ATPase activity. Ouabain appeared to stimulate the activity slightly, and thus Na, K-ATPase could not be identified in this study. Salivary glands from unfed females behaved in much the same manner as those from males. In feeding females the ATPase activity increased steadily, and a ouabain-sensitive component appeared. By the time the females had attained 200 mg a plateau was reached for both ATPase components.

Discussion. In the present work we demonstrate a development of salivary gland Na, K-ATPase, the specific activity of which increases with feeding duration. Maximum activity of the enzyme is achieved when the female tick's fed: unfed weight ratio is approximately 8. As would be expected for an actively secreting tissue, the level of this plateau is comparatively high. For example, an extensive study of the quantitative distribution of Na, K-ATPase in tissues of the cat[8] showed highest activity in brain gray matter and in kidney medulla (respectively 1.52 and 0.44 m*M* ATP split/g wet weight/h at 37 °C.). Our high figure of 1.5 cannot be compared directly with the above since our extracts were partially purified to the extent of a single centrifugation, whereas their figures were for crude homogenates. We do, however, have a comparative figure for one crude homogenate of salivary glands – 0.62 m*M* ATP/g wet weight/h.

Concommittent with the increase of Na, K-ATPase, there is an enhanced ability to secrete fluid in vitro[6], also reaching a maximum at a fed: unfed ratio of 8. Moreover, current work reveals that the cell-type of the salivary gland in *A. hebraeum*, which is homologous to the 'water cell' of *Dermacentor andersoni*[9], undergoes radical ultrastructural changes during feeding, notably an enormous development in plasma membrane invagination and mitochondrial multiplication[10]. We feel that these correlations are related functionally. In harmony with this hypothesis, we note that salivary glands from well-fed males 1. possess an Na, K-ATPase activity which rests at a level below the sensitivity of the present procedure, 2. secrete fluid in vitro only feebly[6] and 3. undergo ultrastructural modifications in the secretory cell-type which are insignificant when compared to those occurring in salivary glands from females[10].

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Novel Metabolite of Nitrazepam in the Rabbit Urine

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Summary. Two novel metabolites appearing mainly as conjugated form in the urine of rabbits fed nitrazepam have been isolated as deconjugated form. From the data of elemental and spectral analysis, the structure was confirmed as 2-amino-3-hydroxy-5-nitrobenzophenone (M-I) and 2'-benzoyl-4'-nitro-2-hydroxyacetanilide (M-II).

Nitrazepam is widely used in clinical practice as a sleep-inducing agent. In the previous studies of nitrazepam metabolism, it has been reported that 7 metabolites were identified in man[1], 7-amino nitrazepam [I], 7-acetamido nitrazepam [II], 3-hydroxy-7-amino nitrazepam [III], 3-hydroxy-7-acetamido nitrazepam [IV], 2-amino-5-nitrobenzophenone [V] and 2-amino-3-hydroxy-5-nitrobenzophenone [VI]. And in rats[2], 4'-hydroxy nitrazepam [VII] and 2-amino-4'-hydroxy-5-nitrobenzophenone [VIII], in rabbits[3], 3-hydroxy nitrazepam [IX] were identified in addition to [I], [II], [III] and [V].

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