sis. Total uronic acids were determined on an aliquot of the above by the method of Bitter and Muir[9]. Concentration of total GAG was based on a 40% (carbazol) content. The GAG was then fractionated with the modificated technique of Antonopoulos et al.[10]. In order to quantitate the different GAG, uronic acid concentration was also determined on each GAG fraction. Identity of other GAG and hyaluronic acid was tested by the characteristic column chromatographic elution pattern, IR-spectra and the chondroitinase-sulfatase paper chromatographic method as described by Murata et al.[11]. Recoveries of 20–500 µg of hyaluronic acid alone, or when added to 1 ml of synovial fluid and carried through the entire procedure, varied from 86–95%.

Results and discussion. Table 1 shows the intrinsic viscosity of hyaluronic acid in normal and arthritic fluid. It can be seen that the viscosity of mild arthritic fluid, when compared with that of normal, is decreased by about 24%. In severe rheumatoid fluid, the decrease reached about 37%. This agrees with the data of Bollet[12] and Levine and Kling[13] but not with that of Balasz and Duff[14]. However, comparison of results obtained by different authors may differ because of variations in the methods used. On the other hand, hyaluronic acid concentration, as well as its total amount, varies continuously in the same joint depending on its functional state, since the amount of fluid has been found to increase during activity[16]. Table 2 shows the concentration of hyaluronic acid and other GAG in normal and arthritic fluids. Total GAG decreases significatively ($p < 0.001$ and $p < 0.001$) about 52% and 65% in mild and severe arthritis respectively as compared with normal fluids. This is mainly due to a decrease in hyaluronic acid, as can be seen in the table (57% and 70% for mild and severe arthritis, $p < 0.001$ and $p < 0.001$, respectively). It should be emphasized that the above findings are typical but not specific for rheumatoid arthritis. The presence of chondroitin-6-sulfate, first described by us[5], has been confirmed now in our laboratory by the methods already described. It should be noted, however, that GAG can be detected only in 58% of the normal fluids and in 30% of the pathological fluids studied. The reason for this particular behaviour cannot be induced from the above experiments. It may be concluded that intrinsic viscosity of hyaluronic acid is below normal in arthritic fluids, the above changes being more marked in the severe type. Hyaluronic acid concentration parallels the above findings.

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Age dependent changes in Na^+-K^+, activated ATPase activity of locust rectum

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Summary. Na^+-K^+, activated ATPase showed an increase in activity at the beginning of the 5th instar and adult life. This was followed by a relatively constant level of activity which, in larval preparations only, was not maintained but decreased with the onset of metamorphosis.

The movement of fluid across many secretory and absorptive epithelia is known to involve a sodium/potassium exchange pump, the biochemical manifestation of which is the Na^+-K^+, activated ATPase (E.C. 3.6.1.3).

This enzyme system has been implicated in fluid movements in the mammalian cornea[1,2], avian salt gland[3], insect salivary gland[4] and Malpighian tubules[5].

The rectum of terrestrial insects has long been known to be the site of selective reabsorption of solutes and water[6]. Recently, Na^+-K^+, activated ATPase has been demonstrated in rectal preparations from a variety of terrestrial insects[7–9]. Furthermore, Na^+-K^+, activated ATPase activity is many times higher in preparations from the rectum than from elsewhere in the hindgut. Consequently this enzyme system has been implicated in rectal reabsorption[7,9]. Although the properties of Na^+-K^+, activated ATPase and its distribution within the hindgut have been determined[9,10], other aspects of this enzyme's activity have not. Thus, the present study describes the effect of locust age on Na^+-K^+, activated ATPase activity of rectal preparations.

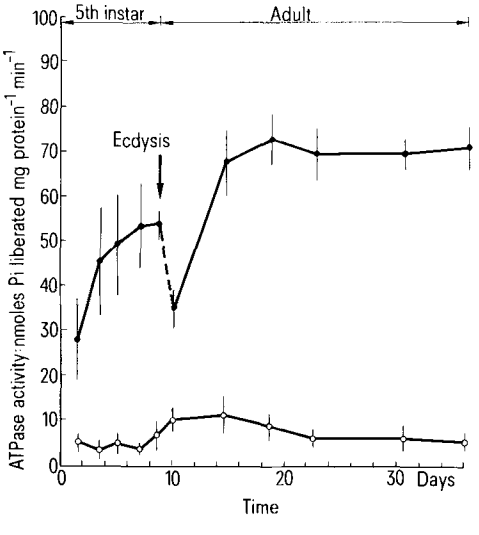
The age of individuals was determined as described by Clarke[11]. Male *Locusta migratoria* only were used throughout this study. 10–12 insects of known age were used per experiment. Following decapitation, the abdomen was removed and placed in ice cold (0–4 °C) homogenization medium consisting of 250 mM mannitol, 5 mM $MgCl_2$, 10 mM EDTA, 0.1% sodium deoxycholate in 30 mM histidine-HCl, pH 7.2. The rectum was then rapidly dissected out, cut open longitudinally and its contents removed. Homogenization was carried out in a Potter Elvehjem homogenizer with a teflon pestle (clearance 0.1–0.15 mm) with 15 passes of the plunger at 1000 rev/min. The homogenizer was surrounded by ice throughout this procedure. The homogenate was then extracted with sodium iodide following the method of Nakao et al.[12]. This extract was then spun at 50,000 × g for 30 min at 0 °C using a Beckman

L3-40 centrifuge, type 40 head. The pellet was discarded and the supernatant centrifuged at 100,000 × g for 60 min. The resulting pellet was resuspended in washing medium (5 mM NaCl and 1 mM EDTA) and recentrifuged at 100,000 × g for 30 min. This washing procedure was repeated twice more. The resulting pellet was resuspended in deionized water.

The reaction media used were as follows (mM/l): (1) 4 Mg^{2+}; (2) 4 Mg^{2+}, 20 K^+, 100 Na^+; (3) 4 Mg^{2+}, 20 K^+, 100 Na^+, 10^{-3} M ouabain all buffered in 50 histidine-HCl, pH 7.2. ATP (Tris salt) was added to the reaction media to give a final concentration of 2 mM. Reaction media were thermoequilibrated at 30 °C for 10 min and the reaction started by the addition of 0.5 ml of enzyme preparation. Incubations were carried out at 30 °C for 30 min and the reaction stopped by the addition of 4 ml of a mixture of 1% lubrol and 1% ammonium molybdate in 1.8 N H_2SO_4. Any protein which precipitated was removed by centrifugation. The tubes were then left at room temperature for 10 min to allow the yellow colour to develop, which was then read at 390 nm. The intensity of the yellow colour is proportional to the amount of inorganic phosphate[13].

Control tubes, assaying nonenzymatic hydrolysis of ATP, were run and contained either medium (1) or (2) above. These tubes were treated in an identical manner to the experimental tubes, except that the enzyme preparation was added after the addition of the lubrol/molybdic acid mixture. Protein determinations were made by the method of Lowry et al.[14] using bovine serum albumin-fraction V as standard. Na^+-K^+, activated ATPase activity was obtained as either the difference in the Pi liberated in the presence of Mg^{2+}, Na^+ and K^+ and in the presence of Mg^{2+} alone, or as the difference in activity in the presence of Mg^{2+}, Na^+ and K^+ and in the presence of all 3 cations plus 10^{-3} M ouabain. Mg^{2+} dependent ATPase activity was obtained as the difference in the Pi liberated in the presence of Mg^{2+} alone and the control tubes.

All solutions were made up in glass distilled water. All inorganic salts were present as chlorides and were AnalaR grade. Histidine, ATP, BSA, and ouabain were obtained from Sigma Chemical Co. Lubrol was a gift from ICI Dyestuffs Division.



Effect of age on ATPase activity of rectal preparations from 5th instar and adult *Locusta*. ATPase activity expressed as nmoles Pi liberated/mg protein/min. -●- Na^+-K^+, activated ATPase. -○- Mg^{2+} dependent ATPase. Vertical lines denote SE of the mean.

Considering first the ATPase activity of preparations from locusts of the 5th instar. From the figure it will be seen that the preparations were stimulated by magnesium ions (Mg^{2+} ATPase). This basic Mg^{2+} ATPase activity was low and showed neither increase or decrease during the stadium, varying between 3 and 7 nmoles Pi liberated/mg protein/min. The preparation was dramatically stimulated when, in addition to magnesium, sodium and potassium ions were present together (Na^+-K^+, activated ATPase). The stimulated activity due to sodium and potassium ions varied during the stadium, being about 28 nmoles Pi liberated/mg protein/min at 1.5 days, increasing to approximately 53 nmoles Pi liberated/mg protein/min at 7–8.5 days. At the time of metamorphosis, Na^+-K^+, ATPase activity decreased to about 35 nmoles Pi liberated/mg protein/min.

In adult preparations Mg^{2+} ATPase activity, although low, was slightly higher than that found in the 5th instar preparations varying between 5 and 11 nmoles Pi liberated/mg protein/min. Na^+-K^+, activated ATPase activity was low immediately after metamorphosis at about 35 nmoles Pi liberated/mg protein/min and increased to 68 nmoles Pi liberated/mg protein/min at 5.5 days and remained around this level for a further 21.5 days.

The pattern of Na^+-K^+, activated ATPase activity is very similar to the pattern of growth exhibited by 5th instar[11] and adult locusts[15]. It may well be that Na^+-K^+, activated ATPase activity merely reflects the normal growth of the locust. If this is the case, it would not be unreasonable to expect Mg^{2+} ATPase activity to follow a similar pattern, especially since both Mg^{2+} dependent and Na^+-K^+, activated ATPases are membrane bound and are biochemically closely related to one another[16]. This does not happen and suggests that some factor(s) is affecting one and not the other of the ATPases. In this respect, it is of interest that the method of obtaining microsomes with Na^+-K^+, ATPase activity, does so, by reducing the Mg^{2+} ATPase component of the membranes to almost zero[12]. Hence any pattern evident in the Mg^{2+} ATPase activity may well have been removed although it is as well to bear in mind that even using this method, alterations in Mg^{2+} ATPase activity are still detectable since this enzyme's activity is higher in adult than in larval preparations.

In conclusion, it appears that the pattern of Na^+-K^+, activated ATPase activity is closely related to the pattern of growth of the larval and adult locusts although it is not impossible that other factors are also involved.

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