of analog **Ii**) which, to a rough approximation, can be correlated with their inhibitory power. The strongest inhibitor of all the products synthesized is analog **Ih** which completely eliminates the sensitivity of the uterus to oxytocin (figure). This is the result of a specific effect of analog **Ih** since the action of prostaglandin $F_{2\alpha}$ was not affected. The examination of oxytocin effect as a function of the dose after the application of the inhibitor and its washing off the bath clearly indicate the noncompetitive nature of the inhibition[8] characterized by a decrease of the maximal response to oxytocin. The properties of an irreversible inhibitor require the proper location of the reactive groups in the peptide molecule[2,3], the chemical character of the groups is of lesser importance. The fact that all the analogs tested in this study are irreversible inhibitors leads us to believe that analogs **Ic–Ii** are covalently bonding to various groups of the receptor in the target tissue (or its immediate neighbourhood) rather than to react with 1 functional group only. We cannot, however, disregard the possibility that the testing of these analogs is paralleled by nonspecific bonding which manifests itself by differences in quantitative parameters (such as activity itself and inhibitory power). An answer to these questions could provide the isolation of the receptor macromolecules with covalently bonded inhibitors, which in this particular case can be regarded as affinity label compounds.

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The synovial fluid hyaluronic acid in rheumatoid arthritis[1]

J.A. Kofoed[2] and Ana C. Barceló

Cátedra de Fisiología, Facultad de Odontología, Universidad de Buenos Aires, M.T. de Alvear 2142, Buenos Aires (Argentina), 2 May 1978

Summary. The intrinsic viscosity of hyaluronic acid in synovial fluid decreases significatively in mild and severe arthritis (24% and 37% respectively). Variation in hyaluronic acid concentration parallels the above results. Chondroitin-6-sulfate can be detected in about 30% of the arthritic fluids.

Chemically, the principal acidic glycosaminoglycen (GAG) in human synovial fluid is hyaluronic acid, although the presence of other GAG has been described[3–5]. It is presumably formed in the periarticular connective tissue by the same cells which produce hyaluronic acid in all other connective tissues. Most of the studies have been carried out in larger animals, because of the minute amounts of fluid that can be obtained from the largest human joint, the knee joint, in vivo. It has been shown to contain up to 2 ml of fluid.

This report presents further data on hyaluronic acid in normal human synovial fluid obtained in vivo and from human joints with mild and severe rheumatoid arthritis[6], in order to get further insight into the hyaluronic acid alteration in this disease.

Material and methods. The fluids were obtained from 27 donors (male, age 18–40 years) with clinically normal joints; from 6 patients with severe rheumatoid arthritis (male, age 24–38 years) and from 18 cases of mild rheumatoid arthritis (male, age 21–32 years) by puncturing the knee joint as described by Balasz et al.[7]. All patients were not under corticoid treatment when samples were taken. As much fluid as possible was obtained from each normal jount (0.4–1.0 ml). Owing to the small amount of fluid obtained from the controls, the fluids of 3 joints were pooled. The fluid volume obtained from the pathological joints varied from 1.5 to 5.2 ml and was studied individually. Prior to analysis, all samples were centrifuged at 75,000 × g to remove the cells, and stored at −20 °C. Intrinsic viscosity of hyaluronic acid was determined as described by Sundblad[8]. The GAG were precipitated by the addition of cetylpiridinium chloride to final concentration of 0.2% and incubated at 37 °C for 1 h. The centrifuged crude GAG were purified by dissolving in 1.25 M magnesium chloride. The resulting GAG was then precipitated with 3 vol. of 2% sodium acetate in ethanol 95% for 24 h. Further purification was obtained by redissolving the GAG in 5% potassium acetate and precipitating with 3 vol. of ethanol 95% for 12 h. Purified GAG thus obtained was dissolved in 0.75 M magnesium chloride for further analy-

Table 1. Intrinsic viscosity of hyaluronic acid in normal and arthritic synovial fluids

	Cases	Intrinsic viscosity*	
Normal fluid	27	3850	
Arthritic fluid, mild	18	2920	−24%
Arthritic fluid, severe	6	2340	−37%

*(cc/g) mean. The measurements were made at velocity gradients of 500 sec^{-1}.

Table 2. Concentrations of acid glycosaminoglycans in normal and pathological synovial fluid

	Normal fluid*	Pathological fluid	
		mild	*severe*
Total GAG	251.4 ± 18.3	121.3 ± 8.3	88.1 ± 5.6
Hyaluronic acid	228.3 ± 14.3	98.2 ± 5.8	68.7 ± 4.8
Chondroitin-4-sulfate	5.3 ± 0.3	2.8 ± 0.4	2.7 ± 0.2
Chondroitin-6-sulfate	2.6 ± 0.1	2.6 ± 0.1	2.1 ± 0.1
Recovery (by addition)	236.2	103.6	73.5

*Average of 9 pooled samples. Concentration of GAG was based on 40% uronic acid (carbazol) content. Figures are expressed as mg of GAG/100 ml of fluid ± SE. For statistic analysis see the text.

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

A.J. Peacock

Department of Zoology, University of Rhodesia, P.O. Box MP 167, Salisbury (Rhodesia), 13 April 1978

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