laboratory, Phosphocreatine disodium salt hydrate (Sigma), DL-Phosphoserine (Fluka), Na_2HPO_4 (Merck), Adenosine-5'-triphosphoric acid disodium salt (Merck). The compounds were used without further purification. All the drugs were dissolved in saline.

The animals received an i.v. infusion of 2 IU/kg of vasopressin at a rate of 1.6 IU/kg/min and phosvitin (750 mg/kg i.p. plus 250 mg/kg i.v. at a rate of 25 mg/kg/min respectively 25 and 10 min before starting the vasopressin infusion). The same schedule was followed with the drugs for comparison, which were administered in stoichiometric amounts relative to the phosphorous content. The ATP was only administered i.v. and in a non-stoichiometric dose (10 mg/kg) because of its side effects (depression of blood-pressure etc.). Control rats, under the same experimental conditions, only received saline in the same volume as used for the other drugs. For each compound at least 9 rats were tested.

Results and discussion. The results are given in the Figure. In all the control rats the ECG alterations as described in the literature were always present. Phosvitin appeared always to exert a protective action of ECG changes produced by vasopressin. In particular T wave was never inverted or flattened; similarly S-T segment alterations were always decreased or absent. On the contrary the comparison drugs phosphoserine, ATP and inorganic phosphates disclosed no activity, with the exception of phosphocreatine which only showed a slight and inconstant activity. The mechanism of protective effect of phosvitin is not known. The physiological role of this protein has been hypothesized as a supplier of energy-rich phosphate[23], an iron carrier[25], or in the oxidative generation of energy-rich phosphate with a reaction in which iron would be involved in some way. The product of this oxidation would be a serine enol-phosphate which can spontaneously liberate its phosphate to charge an ADP by means of an enzymatic system[26]. It has already been demonstrated[27] that phosvitin has no coronarodilator activity and that it does not decrease the blood pressure in the dose which have been employed. These results should be very interesting for any possible future research into the pharmocological aspects of phosvitin.

Riassunto. Viene dimostrata l'attività protettiva della fosvitina sulle alterazioni elettrocardiografiche prodotte dalla somministrazione endovenosa di vasopressina nel ratto. Risultati analoghi non si sono ottenuti impiegando fosfoserina, fosfati inorganici e ATP, fatta forse eccezione per la fosfocreatina per la quale è stata osservata una lieve ma trascurabile attività.

L. CAPRINO, F. BORRELLI and R. FALCHETTI[28]

Department of Pharmacology, Research Laboratories, Istituto Farmacologico Serono, Via Casilina 125, I-00176 Roma (Italy),
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The Acidic Glycosaminoglycans of the Synovial Fluid in Rheumatoid Arthritis

It is known that the principal acidic glycosaminoglycan (GAG) of synovial fluid in men and other species is hyaluronic acid. The presence of other GAG has been described by other authors[1,2]. Small amounts of chrondroitin-4-sulphate have also been described in some pathological human synovial fluids[3,4], although because of the fractionation methods used it was difficult to quantitate the isomeric chondroitin-sulphates.

This report presents data on the GAG content of the synovial fluid of the knee joint of patients with rheumatoid arthritis and healthy donors using techniques now available for the quantitation of GAG in order to get further insight into the changes in chemical composition of synovial fluid in rheumatoid arthritis.

Material and methods. The synovial fluids were obtained from 18 patients with classical rheumatoid arthritis[5] (males, age 18–39 years) and 12 male donors with apparently normal knews by puncturing the knee joints as described by BALASZ et al.[6]. All patients were not under corticosteroid treatment when samples were taken. As much fluid as possible was obtained from each joint (0.4 to 0.9 ml). Owing to the small amount of fluid obtained from the healthy donors, the respective fluid of 3 joints were pooled. Prior to analysis, samples were centrifuged at $75{,}000 \times g$ to remove the cells and stored at $-20\,^{\circ}C$. The GAG were precipitated by the addition of cetylpyridinium chloride to final concentration of 0.2% and incubated at 37 °C for 1 h.

The crude GAG were purified by dissolving them in $1.25\,M$ magnesium chloride. The resulting GAG were precipitated with 3 volumes of 2% sodium acetate in 95% ethanol during 24 h. Further purification was obtained by redissolving the GAG in 5% potassium acetate and reprecipitating them with 3 volumes of 95% ethanol for 12 h.

The purified GAG thus obtained were dissolved in $0.75\,M$ sodium chloride for further analysis. Total uronic acids were determined on an aliquot of the above by the method of BITTER and MUIR[7] and the original reaction described by DISCHE[8]. Total hexosamine was measured following the method of CESSI and PILLEGO[9] after hydrolysis of aliquot fractions in $4\,N$ HCl at 100 °C for 4 h using glucosamine HCl as standard. Sulphate was determined by an Antonopoulos modification of the benzidine method[10] and nitrogen by the Kjeldahl's method. Galactose was measured with the technique of DISCHE[11]. The purified GAG were then fractionated by chromatography on celulose microlumns by the technique

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Table I. Chemical analysis of the crude glycosaminoglycans form normal and rheumatoid synovial fluid

	Normal fluid	Rheumatoid fluid
Uronic acids		
Carbazol method	557.6[a]	230.8
Orcinol method	532.7	219.3
Hexosamine	529.4	222.1
Sulphate	8.2	4.6
Nitrogen	31.4	18.3

[a] Figures are expressed as mg of GAG/100 ml.

Table II. Concentration of acidic gag in synovial fluid from normal human and rheumatoid knee joints

	Normal fluid	Rheumatoid fluid	
Total GAG	249.6 ± 20.6	112.3 ± 9.8	−55.2%
Hyaluronic acid	219.2 ± 16.3	90.6 ± 8.3	−59.0%
Chondroitin-4-sulphate	4.2 ± 0.31	1.9 ± 0.007	−54.8%
Recovery (by addition)	223.4	92.5	

[a] 4 pooled samples. Concentration of GAG was based on 40% uronic acid content. Figures are expressed as mg of GAG/100 ml synovial fluid ± S.E.

of SVEJCAR and ROBERTSON[12]. In order to quantitate the different GAG, uronic acid concentration was also determined on each GAG fraction. GAG concentration was based on 40% uronic acid content. Identity of each GAG fraction was tested by the characteristic column chromatographic elution pattern and infrared spectra. Recoveries of 20–500 μg of hyaluronic acid alone or when added to 0.5 ml of synovial fluid and carried through the entire procedure, varied between 86–94%.

Results. Table I shows the chemical analysis of the crude GAG from normal and rheumatoid synovial fluid. Figures for uronic acids in both normal and rheumatoid fluids are very similar when measured with the BITTER and MUIR[7] reaction and the orcinol method, hence the uronic acid shoud be D-glucuronic acid.

Table II demonstrates the concentration of the GAG fractions of the normal and rheumatoid fluid expressed in mg of GAG/100 ml. It can be seen that the concentration of total GAG in the rheumatoid fluid was 44.8% below normal values. In the fractionation studies, hyaluronic acid and chondroitin-4-sulphate were 59% and 54.8% decreased in the rheumatoid fluid as compared with controls. Keratan sulphate (galactose) was not detected in any fraction.

Our studies and those of the SEPPALA et al.[4,13] confirm that hyaluronic acid is the main component of synovial fluid. Its concentration in both normal and arthritic fluid was more than 80% of the total GAG. Chondroitin-4-sulphate was also present in small amounts (less than 2%). We also detected in our fractionation studies, traces of a third component that elutes consistently with the magnesium chloride 0.75*M* acidified fraction. Infrared spectra of this component would be similar with that of chondroitin-6-sulphate. Further studies are in progress.

Resumen. Se estudiaron los glucosaminoglucanos del líquido sinovial de la rodilla de pacientes con artritis reumatoidea clásica. El nivel de glucosaminoglucanos totales desciende en la artritis un 55% comparado con los controles. Esto es debido principalmente a la disminución del ácido hialurónico (59%). Se confirmó la presencia de pequeñas cantidades de controidin-4-sulfato y se detectaron trazas de una sustancia similar al condroitin-6-sulfato.

J. A. KOFOED[14], A. A. TOCCI and A. C. BARCELO[15]

Cátedra de Fisiología, Facultad de Odontología, Universidad de Buenos Aires, M. T. de Alvear 2142, Buenos Aires (Argentine), 4 December 1972.

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Biochemical and Morphological Changes Induced by Triton-X 100 in Skeletal Muscle of Rats After Abdominal Aorta Ligation[1]

The possible vascular pathogenesis of some forms of muscular dystrophy[2,3] has been investigated recently with growing interest, culminating in the study of experimental myopathies caused by alteration of the circulatory equilibrium in muscular tissue[4–6].

In 1971 MENDELL et al.[6] obtained histological changes similar to those previously observed with microembolisation of the femoral artery[5], by i.p. administration of serotonin or noradrenalin to abdominal aorta ligated rats. In the author's opinion the myotoxic action might be due to 2 concomitant subthreshold vascular mechanisms: chronic hypoxia induced by aorta ligation, and acute hypoxia (functional and transient) induced by serotonin through vasoconstriction.

However, much evidence puts in question the vasoconstrictive effect of serotonin when given i.p. or s.c.[7–10]. It was therefore decided to investigate whether ligation of the abdominal aorta predisposes the muscular tissue to damaging action by substances whose mechanism is not vascular. The present study deals with the myotoxic effect of a non-ionic detergent, Triton-X 100 (*p*(1,1,3,3-tetramethylbutyl)phenylpolioxyethylene ethanol) whose damaging effect on cellular membranes is well known[11].

Methods. Male rats weighing 150–200 g were used. The following groups were investigated. 1. Controls: animals which were not treated; 2. Triton-X 100: animals treated with a single dose of Triton-X 100.(5 mg/kg, 1% solution in saline) i.p. The animals were sacrificed after 48 h. 3. Ligation of the abdominal aorta: animals operated 1 week before sacrifice. 4. Ligation of the aorta and Triton-X 100: Triton was administered at the same dose to animals with aorta ligation as in group 3.

Results. The Table gives the results of the biochemical studies. Whilst treatment with Triton alone had no