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.

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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

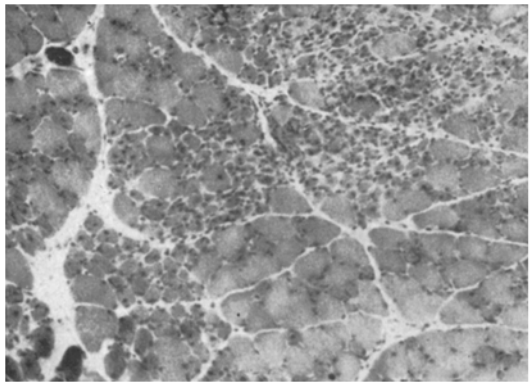

Fig. 1. Triton X-100 and aorta ligation. A damaged area with degenerative and regenerative aspects, surrounded by undamaged tissue. Hematoxylin-Eosin × 100.

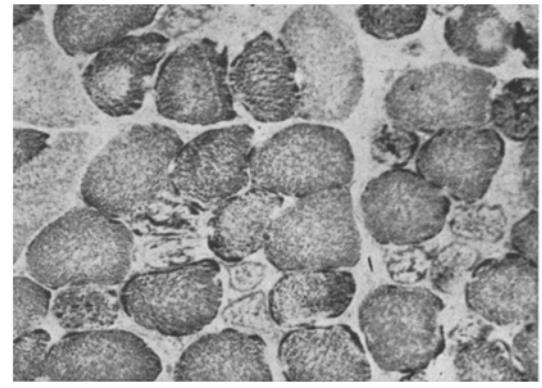

Fig. 2. Triton X-100 and aorta ligation. A marked alteration of succinate-dehydrogenase distribution is predominant in type I fibres. SDH × 250.

effect on any of the enzymes tested, ligation of the aorta induced a significant increase of the enzymatic activity. An even more remarkable increase of the hydrolitic enzymes was observed after injection of Triton into aorta-ligated animals.

Conventional histological methods (Figure 1) in all the animals of the group 4 showed that several groups of fibres were undergoing necrosis or atrophy, with an increase in sarcolemmal nuclei, generally localised in the centre of the fibres. Histoenzymological methods show that necrotic and atrophic fibres exhibit a marked decrease in succinate dehydrogenase activity. At the periphery of the necrotic fields, the type I fibres show a moth-eaten appearence (Figure 2). The myofibrillar ATPase and phosphorylase tend to disappear in the necrotic areas, while in the nearly areas there appears to be an alteration in the enzyme distribution in type I fibres. Acid phosphatase is increased remarkably in the necrotic fibres, but is almost absent in the surrounding normal fibres.

The muscles of aorta-ligated rats show only a few sarcolemmal nuclei in the centre of the fibres, with a slight increase in perinuclear acid phosphatase. No changes are observed in the controls, nor in Triton-treated animals.

Discussion. The results of our investigation lead to the following tentative conclusions.

Ligation of the abdominal aorta affects neither the morphology nor the histoenzymatic pattern of the muscular tissue, but induces an increase of its hydrolytic enzymes. This fact may be considered as an effect of a subthreshold lesion of the muscle of limbs caused by alteration in the blood supply. Administration of Triton-X 100 to aorta-ligated rats induces marked morphological lesions, and a sharp increase of muscle hydrolytic enzymes. Therefore, in this case, the action of a membrane disruptor (at a subthreshold dose, non-active under normal conditions) becomes clearly evident in tissues in which chronic hypoxia has been induced.

The lesions observed show some analogy to that observed by MENDELL et al.[6], although our experimental conditions were entirely different. Bearing in mind the facts known about the effect of serotonin on blood flow, we wonder whether a better interpretation of MENDELL's results would be that the observed muscular lesions could be accounted for by a direct effect of serotonin on cellular membranes. In our opinion even the muscle lesion by zones, usually regarded as an effect of a vascular mechanism, probably ought to be considered as a more general and non-specific form of muscular damage, and not necessarily as an effect of ischemia.

Riassunto. Il Triton-X 100 somministrato in vivo a ratti dopo legatura dell'aorta addominale provoca l'aumento di alcuni enzimi lisosomiali e la comparsa di focolai necrotici nei muscoli degli arti posteriori, simili a quelli osservabili nelle lesioni di tipo ischemico.

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	Acid phosphatase	β-glucuronidase	Ribonuclease	Acid maltase
Controls (9)	140.8 ± 3.6	4.3 ± 0.3	0.9 ± 0.3	141.6 ± 3.4
Triton X-100 (9)	142.3 ± 3.4	2.9 ± 0.7	1.1 ± 0.3	143.7 ± 3.1
Ligated (9)	185.6 ± 11.1	15.7 ± 1.4	2.9 ± 0.5	207.4 ± 6.6
Ligated + Triton X-100 (18)	242.4 ± 10.9	42.8 ± 2.2	8.2 ± 0.6	250.2 ± 6.3

Results are expressed as follows. Acid phosphatase: μg substrate hydrolized/100 mg tissue 15 min^{-1} β-glucuronidase: μg substrate hydrolised/100 mg tissue 30 min^{-1} Ribonuclease: units/1 g tissue 1 h^{-1} Acid maltase: μg glucose formed/100 mg tissue/1 h ± S.D. Numbers of animals in brackets.

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