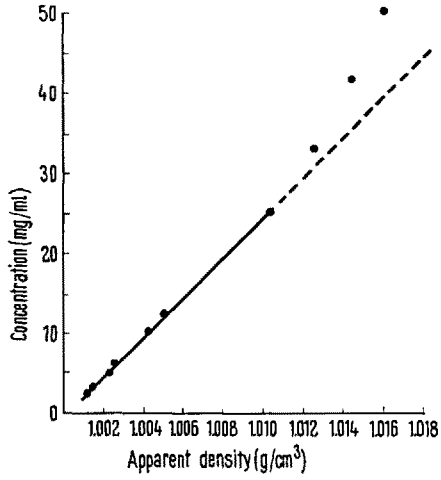
of freshly prepared stock solutions of each preparation were determined by the falling drop method[6] using calibrated xylene:bromobenzene (79:21, v/v) columns for the densiometer liquid phase (to provide a constant rate of shear) and a specially designed, micrometer-driven microliter syringe assembly. The time of descent of droplets of uniform size was determined to ± 0.1 sec by a stopwatch for both the solvent and solute phases. Apparent density was then calculated by the algebraic sum of the true density of water and the difference between the apparent densities of the water and chondroitin sulfate solutions, corrections being made for temperature effects. All studies were performed at 25 ± 1°C.

The mean values of 4–10 separate determinations of the apparent density of each of the three preparations (reproducibility, ± 0.00005) were then plotted for concentrations up to 50 mg/ml, which proved to be the upper limit of usefulness of the experimental method.

As demonstrated in the Figure, a remarkably linear relationship was found to exist between apparent density and concentration over the range of 2.5–25 mg/ml. Non-Newtonian behavior was clearly demonstrated beyond this range. At low concentrations, where randomly distributed chains presumably do not overlap and no entanglement couplings are formed, density increases monotonically with concentration. However, at higher concentrations, above 25 mg/ml, free rotation of chain segments appears to be constrained by the formation of entanglement couplings (and perhaps other electroviscous and charge interactions as well) so that, despite the increase in the total number of chains present in solution, each occupies a smaller relative volume than do freely orienting chains. The overall effect is thus a significant departure of the concentration-density relationship from linearity and a corresponding increase in the complexity of the kinematic viscosity formulation.



The resulting elastic as well as viscous properties[7,8] of such gel-like materials (which, in physiological articular cartilage, may exist in concentrations approximating 200 mg polysaccharide/ml 'tissue water'[9]) almost certainly accounts for the characteristic physical properties of specific native connective tissues. Selective loss of the carbohydrate moiety from connective tissues generally during the course of natural aging or disease processes, reported by a large number of investigations, would then also be predicted to result in profound alterations in the physical properties and hydration of these tissues, which accords with an expanding body of experimental evidence.

Résumé. La densité du sulfate de chondroitine-protéine en solution aqueuse a été déterminée par la méthode du «falling drop» utilisant des colonnes de xylène:bromobenzène. Un comportement Newtonien fut observé à des concentrations de 25 mg/ml. A des concentrations plus élevées (au niveau physiologique), les polysaccharides se comportent comme des liquides visco-élastiques, probablement à cause de l'enchevêtrement des différentes chaînes polymères.

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[6] H. G. BARBOUR and W. F. HAMILTON, J. biol. Chem. *69*, 625 (1926).
[7] J. H. FESSLER, Biochem. J. *76*, 124 (1960).
[8] J. D. FERRY, *Viscoelastic Properties of Polymers* (J. Wiley and Sons Inc., New York 1961), p. 82.
[9] L. EICHELBERGER, T. D. BROWER, and M. ROMA, Am. J. Physiol. *166*, 328 (1951). – L. EICHELBERGER, Clin. Orthopedics *17*, 77 (1960).

Effect of pH on Active Sodium Excretion

There are some data concerning the effects of changes in H^+-concentration on sodium transport in the frog[1–3]. This fact could be of functional importance, considering that the metabolic end-products of mammals are mostly of an acid nature. We tried to study this aspect following principally a well-known method[4].

We isolated the M. soleus and M. extensor digit. long., red and white muscles respectively, of rats. They were loaded in a modified K-free Krebs-Henseleit solution with a concentration of 160 m*M* Na at 3°C and pH 7.48 for $2^1/_4$ h whilst bubbling with a gas mixture of 93% O_2-7% CO_2. The changes in the Na and K content of the muscles (mEq/kg wet weight ± S.E. of mean) are shown in Table I. In these and the following experiments, we always compared companion muscles.

In the next series we recovered such Na-rich muscles in a fluid at 22°C containing 137 m*M* Na and 10 m*M* K at different pH values with adequate gas-bubbling for $2^3/_4$ h.

[1] E. SCHOFFNIELS, Arch. int. Physiol. *63*, 513 (1956).
[2] L. B. KIRSCHNER, J. cell. comp. Physiol. *53*, 85 (1959).
[3] A. LEAF, A. KELLER, and E. F. DEMPSEY, Am. J. Physiol. *207*, 547 (1964).
[4] E. J. CONWAY, R. P. KERNAN, and J. A. ZADUNAISKY, J. Physiol. *155*, 263 (1961).

Table I

	M. soleus			M. extensor digit. long.		
	n	Na	K	n	Na	K
Before loading	14	39.7 ± 0.7	81.8 ± 1.3	10	31.9 ± 3.1	102.0 ± 2.6
After loading	14	75.3 ± 3.7	68.3 ± 1.6	10	56.3 ± 4.5	88.9 ± 1.5

Table II

	Recovery at pH	M. soleus						M. extensor digit. long.					
		Na			K			Na			K		
		n	mEq/kg Δ		n	mEq/kg Δ		n	mEq/kg Δ		n	mEq/kg Δ	
a	7.38	11	58.3	15.2 ± 3.6	11	70.4	16.3 ± 2.6	5	37.8	10.9 ± 3.5	5	90.0	13.4 ± 1.2
	5.88	11	73.5	$p < 0.01$	11	54.1	$p < 0.01$	5	48.7	$p < 0.05$	5	76.6	$p < 0.01$
b	7.38	24	58.0	8.4 ± 1.7	24	68.7	4.9 ± 1.2	19	44.1	3.0 ± 1.3	19	89.1	4.5 ± 1.3
	6.94	24	66.4	$p < 0.01$	24	63.8	$p < 0.01$	19	47.1	$p < 0.05$	19	84.6	$p < 0.01$
c	7.38							14	46.8	4.3 ± 1.4	14	88.5	7.6 ± 1.3
	7.38 + insulin + lactate							14	42.5	$p < 0.01$	14	96.1	$p < 0.01$
d	6.94							18	50.7	1.3 ± 1.6	18	82.6	4.5 ± 1.2
	6.94 + insulin + lactate							18	52.0	$p < 0.50$	18	87.1	$p < 0.01$

The pH of fluids was altered by changing the $NaHCO_3$-NaH_2PO_4 ratio. In Table IIa the cation contents of muscles recovered at pH 7.38 and 5.88 are presented. The values are related to the weights after loading. The differences of the results obtained at different H^+-concentration were treated statistically. Though the fluids contained 30 mM/l glucose, the weight of muscles continually increased during both loading and recovery. This did not exceed 15–20% during the whole 5 h and there was no correlation with pH values.

Since performing the experiments at 22°C did not prevent a strong contraction (contracture?) of the muscles in acid medium, we also accomplished these investigations at a lower pH difference (Table IIb).

It will be seen in Table I that the M. soleus gained about 35.6 mEq/kg Na during loading. During recovery (Table II) in a medium of pH 7.38, the sodium content decreased by about 17 mEq/kg in both series. On the other hand, when recovering at pH 5.88 the muscle lost only 1.8, and at pH 6.94 it lost 8.9 mEq/kg Na (11 and 52%). The differences in the extensor muscles are similar. Thus an inhibition of the Na-pump occurred also in the absence of contraction. As can be seen, K-uptake was also inhibited on account of lower pH values.

These results demonstrate the inhibitory effect of increasing H^+-concentration on the sodium pump in mammalian muscle. Considering the slow diffusion of H^+, one is led to think that processes on boundary surfaces are mainly causing the inhibition. This idea is corroborated by the absence of any connection between the initial Na content of the individual muscles and the degree of inhibition produced in their companions by pH shift.

Insulin seems to promote K-uptake in rat muscle[5–7], and insulin and lactate stimulate the sodium pump in frog muscle. KERNAN concluded that lactate oxidation might be connected with the sodium pump[8].

In the following experiments we demonstrated the increase of Na-excretion and K-uptake at pH 7.4 on the rat extensor muscle when recovery fluid was supplemented by 100 IU insulin and 5 mM sodium lactate per litre (Table IIc). In the soleus muscle, only the K-content increased, while there was no difference in the sodium pump. To see whether a more intensive carbohydrate metabolism might increase Na-excretion in the presence of higher H^+-concentration, we put one of the sodium-rich companion muscles in a medium of pH 6.94 and the other in a medium of the same pH but containing also insulin and lactate in the above concentration.

In Table IId it can be seen that, though K-accumulation ensued in the extensor muscle, Na-excretion did not. This suggests that some step necessary to stimulate sodium excretion is being separately inhibited by the lower pH values[9].

Zusammenfassung. Die aktive Na-Ausscheidung Na-reicher quergestreifter Muskeln konnte durch eine mässige Steigerung der Wasserstoffionenkonzentration gehemmt werden. Obgleich der intensivere Kohlehydratstoffwechsel an und für sich die Na-Abgabe steigert, wurde dieser Effekt durch die Herabsetzung des pH unterdrückt.

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[5] J. C. SOMOGYI and F. VERZÁR, Helv. med. Acta 7, Suppl. no. 5, 30 (1940).
[6] F. VERZÁR, Schw. med. Wschr. *1941 II*, 878.
[7] K. L. ZIERLER, Am. J. Physiol. *197*, 515 (1959).
[8] R. P. KERNAN, J. Physiol. *162*, 129 (1962).
[9] My thanks are due to Professor E. J. CONWAY for a personal grant and to Dr. R. P. KERNAN for helpful discussions and laboratory facilities.
[10] On leave from Department of Urology, University of Medicine, Budapest (Hungary).