the absence of the protonating agent H_3PO_4. The partial oxidation of the protein takes place, on the contrary, only in the presence of phosphoric acid as indicated in the Table.

The large activity change concurrent with the partial oxidation of threonine and serine is very likely due to aggregation of RNase A induced by DCCI. Of course a partial denaturation of the protein responsible for the activity loss cannot be under-estimated. Figure 2 reports the chromatographic pattern of oxidized RNase A, which shows a residue 15% enzymatic activity, on the G-75 Sephadex column. Also in this case more proteic components were obtained by gel-filtration.

The presence of an altered conformation of RNase A caused by the above treatment was investigated by circular dichroism measurements in the far ultraviolet (Roussel-Jouan dichrograph II). The dichroic spectra of native RNase A and oxidized RNase A having 15% enzymatic activity were compared. The CD spectrum for native RNase reproduces the dichroic band with a minimum at 222 nm (characteristic of n-π^* peptide transition of α-helix) and the larger band at 208 nm associated with the π-π^* peptide transition of α-helix[6,7]. A slight shift toward lower wavelengths of the dichroic bands relative to oxidized RNase, i.e. to 220 and 207 nm respectively, and a dramatic decrease in their magnitude were observed. The large $[\theta]$ values diminution from 8600 to 1600 and from 10,500 to 2500 at 220 nm and 207 nm respectively is evidently due to a loss of ordered structure.

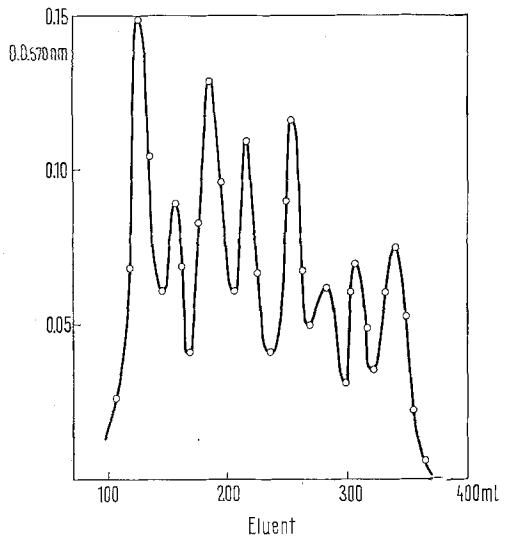

Fig. 2. Gel-filtration of RNase A reacted with the oxydizing system DCCI/DMSO/H_3PO_4. The experimental conditions are the same as in Figure 1.

The IR-spectrum of KBr pellets (Perkin-Elmer Mod. 337 Spectrophotometer) of RNase modified by the oxydizing system shows no appreciable difference from that of native RNase A. In both the cases maxima at 1650, 1530, 1450, 1405, 1240 cm^{-1} were present.

The results reported in this note allow us to conclude that a chemical modification procedure, successfully employed with models, cannot often be apted to protein because it influences dramatically the secondary or tertiary structure.

The present method, however, can be useful in sequence studies together with other chemical or enzymatic methods of cleavage.

Riassunto. È stata studiata la possibilità di modificazione dei residui di Treonina e Serina presenti nella RNase A da parte del sistema ossidante DCCI/DMSO/H_3PO_4 già utilmente impiegata in peptidi modello[1,2].

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[6] G. HOLZWARTH and P. DOTY, J. Am. chem. Soc. *87*, 218 (1965).
[7] A. M. TAMBURRO, A. SCATTURIN and L. MORODER, Biochim. biophys. Acta *154*, 583 (1968).
[8] A. MARZOTTO, F. MARCHIORI, L. MORODER, R. BONI and L. GALZIGNA, Biochim. biophys. Acta *147*, 26 (1967).
[9] P. DESNUELLE and A. CASAL, Biochim. biophys. Acta *2*, 64 (1968).
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The Binding of Some Sodium Substitutes to Chondroitin Sulfate

The need for sodium-free physiologic solutions calls for the use of substitutes to maintain isotonicity. The most commonly used ones are lactose or sucrose, choline chloride and lithium chloride. Such substitution, however, may alter the properties of the tissue incubated or perfused by affecting the state of the protein-polysaccharides which constitute the 'ground substance' and interstitium in general. Whether in the native form of protein-polysaccharides or in partly degraded forms such as chondroitin sulfate, the polysaccharides of the connective tissues are important polyelectrolytes, more precisely, polyanions. These macromolecules will immobilize cations as counter-ions and the kind and concentration of these will determine their domain and their electrical field and, in turn, determine hydration as well. These polyanions are broadly distributed and are present in relatively large amount in the arterial wall[1]. FRIEDMAN and FRIEDMAN[2] have stressed the role that this 'paracellular matrix' can play in altering vascular geometry and affecting peripheral vascular resistance. Equally important physiological effects may be anticipated in other tissues. It seemed thus pertinent to examine the affinities of the commonly used sodium substitutes for such polyanions, of which chondroitin sulfate was chosen as an appropriate representative. It was observed that while lithium and choline substituted efficiently for sodium on the binding sites of this macromolecule, lactose left these sites available to other ions of the medium, that is, in the case of physiological solutions, essentially to polyvalent ions which possess an intrinsically high binding affinity.

[1] J. E. KIRK, in *The Arterial Wall* (Ed. A. I. LANSING; Williams and Wilkins, Baltimore 1959), p. 161.
[2] S. M. FRIEDMAN and C. L. FRIEDMAN, Circulation Res. *21*, Suppl. 2, 147 (1967).

Methods. Sodium activity was determined by means of a sodium-sensitive glass electrode (G 502 Na, Radiometer) referring to a saturated calomel electrode. The electromotive force (EMF) of the cell was measured with a Vibron 33B electrometer and recorded with a Grass Polygraph. The electrodes were mounted in a thermostated (20 °C) microtitration assembly (Radiometer). Titrations were performed with continuous stirring by adding to 2 ml of chondroitin sulfate (sodium form) in the atmosphere of nitrogen portions of 0.01 ml of 2 *M* solutions of sucrose, choline chloride, lithium chloride or 1 *M* calcium chloride, at 2 min intervals. Blank titrations were run in the same way, using NaCl-*Tris* (same sodium activity) instead of chondrotin sulfate, in order to take into account the effect of dilution as well as the direct effect of the titrant on the EMF of the cell. The whole set of titrations was also performed automatically with a Radiometer titrator and autoburette; this yielded similar results. Total sodium content of the working solution of chondroitin sulfate was determined by flame photometry.

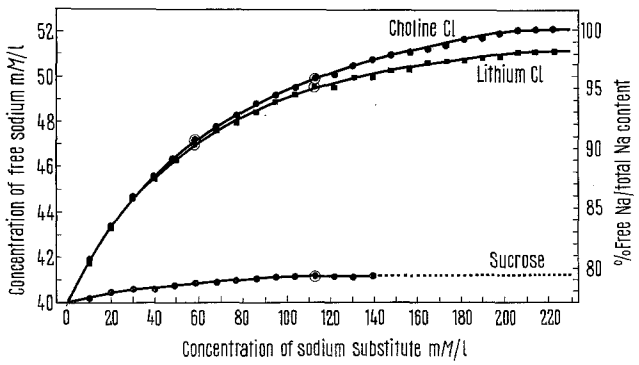

Fig. 1. Release of Na^+ from chondroitin sulfate by addition of sodium substitute. 2 ml of 0.020 *M* (monomer) chondroitin sulfate (sodium-form) titrated with 2 *M* solution of the substitute. Reading are corrected for change in the volume. Circles represent the variation corresponding to ± 0.1 mv, the precision of the assembly.

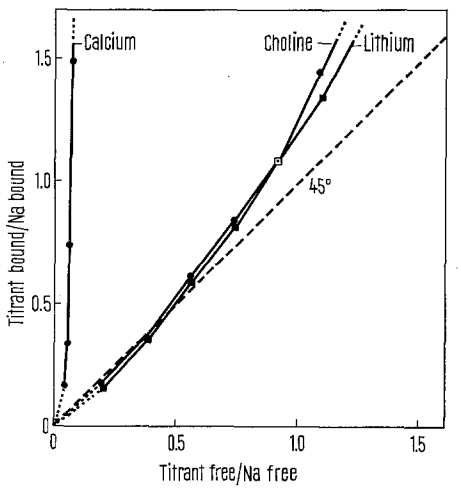

Fig. 2. The ratio of bound titrant/bound sodium as a function of the ratio of the free ions. Calculations are for concentrations. 'Titrant bound' = Na liberated. 'Titrant free' = titrant added – Na liberated. 'Na free' = Na concentration from Na^+ activity in the medium. 'Na bound' = total sodium content (Flame photmetry) – Na free. (For calcium the ratios are: γ/Ca Free/Na Free; γ/Ca Bound/Na Bound).

Chondroitin sulfate in the sodium form isolated from bovine nasal septa was obtained from Mann Research Laboratory. The free acid in preparations of lithium chloride and of choline chloride was neutralized with the respective hydroxide, that in calcium chloride by calcium carbonate. All reagents were analytical grade.

Results. The addition of lithium or choline ions to the titration medium displaced sodium ions from the binding sites of chondroitin sulfate. This was readily apparent in the increase of EMF as shown in Figure 1. This property is not shared by sucrose which produced a barely significant increase of EMF; the minor change in potential observed in this case is due to an unavoidable change of bridge potential and to the fact that the blank (NaCl-*Tris*) may not be an ideal replacement for chondroitin sulfate. It was confirmed in this study that the binding affinity of calcium ion, even on an equivalence basis, is much higher than that of either lithium or choline. This is illustrated in Figure 2 which represents the distribution of the 2 states, free or bound, of the titrant ion added to the titration medium. While essentially no calcium ion exists in the free state so long as binding sites are available, a certain amount of lithium and choline does remain unbound. At the beginning of the titration the slope representing the effect of lithium or choline is close to 45°, which means that their affinity for binding sites is at that moment equal to the affinity of sodium. As titration progresses their slope becomes, however, more acute indicating that the affinity of both choline and lithium ions is actually greater than that of sodium ion. The affinity of calcium for the binding sites of the chondroitin sulfate molecule is considerably greater than that of sodium, lithium or choline.

Discussion. These experiments have shown that lithium and choline ions are equally able to displace sodium ion from the binding sites of the chondroitin sulfate molecule. Their affinity for binding appears to be a little greater than that of sodium ion but considerably less than that of calcium ion. As expected, sucrose does not share this property and in these experiments no ion was available in the medium to displace sodium from its sites on the chondroitin sulfate molecule. Lithium and choline ions, used as substitutes in physiological salt solutions, are thus not likely to alter the conformation of a macromolecule such as chondroitin sulfate to any great extent; the total change of hydration, or of the volume occupied by such molecules will then be more dependent upon other properties of the specific counter-ion rather than on its binding affinity. Sucrose, or lactose, should not of itself change the conformation of the macromolecule, except insofar as it alters the colligative properties of the medium. When, however, sucrose is substituted for sodium in a physiological medium it will leave the binding sites readily available to divalent cations such as calcium and magnesium. Such ions, binding to 2 sites at a time may produce a 'shrinkage' of the polyanion and in so doing could reduce the capacity for holding water of protein-polysaccharide complexes in tissues. Such a direct correlation between the size of the free fluid of the extracellular space and the ratio of monovalent to divalent ions in physiologic solution bathing the isolated artery has recently been observed and stressed[3].

The present study reproduced in vitro what has previously been inferred from in vivo experiments. Thus

[3] V. Palatý, B. K. Gustafson and S. M. Friedman, Can. J. Physiol. Pharmac. *48*, 54 (1970).

FRIEDMAN et al.[4] showed that the rat tail artery perfused with partial (30 m*M*/l) substitution of sodium by lactose produced a loss of sodium from the 'cellular space' (this term includes paracellular matrix); such a withdrawal did not occur with choline chloride as substitute. In this case, it is likely that sodium was lost for 2 reasons: the taking over by divalent cations of the binding sites previously occupied by sodium ions, and shrinkage of the extracellular space.

It is concluded that the ion exchange properties of the polyanionic paracellular matrix can be involved in the responses of tissues which are observed during experiments involving ion substitution in the medium. This possibility must be especially carefully considered in the case of tissues such as arteries which contain substantial amounts of these materials[5,6].

Résumé. Il arrive souvent que l'on doive substituer l'ion de sodium dans les solutions physiologiques. Un effet particulier en est la compétition du substitut vis-à-vis les ions de sodium liés au sulfate de chondroitine. Nous démontrons que le sucrose ne déplace pas cette portion du sodium lié, alors que les ions de lithium et de choline possèdent une grande affinité pour ces sites de liaison, affinité moindre cependant que celle de l'ion de calcium. Les conséquences physiologiques qui en ressortent sont discutées.

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[4] S. M. FRIEDMAN, M. NAKASHIMA and C. L. FRIEDMAN, Proc. Soc exp. Biol. Med. *117*, 480 (1964).

[5] The authors wish to thank Dr. V. PALATÝ for his valuable suggestions and discussions.

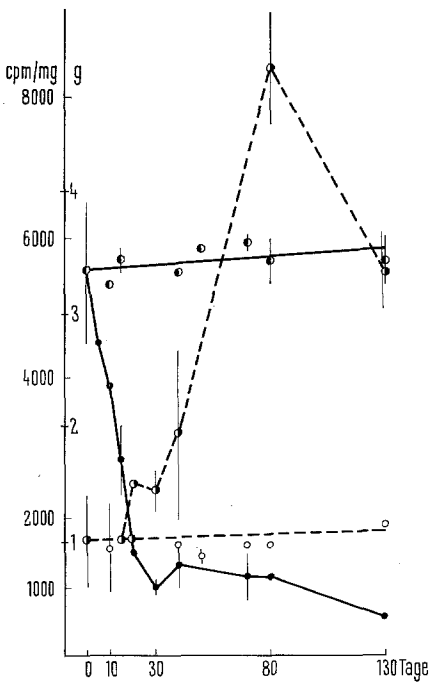
[6] This work was supported by grants from The Medical Research Council of Canada and The British Columbia Heart Foundation.

[7] Postdoctoral fellow of the Québec Medical Research Council.

Zur DNS-Synthese in Spermatogonien hypophysektomierter Ratten

65 männliche Ratten (Sprague-Dawley) mit einem Gewicht von 280–310 g (10. Woche) wurden nach der Methode von REISS, DRUCKREY und HOCHWALD[1] hypophysektomiert und zu verschiedenen Zeitpunkten (10, 15, 20, 30, 40, 80 und 130 Tage) nach der Operation gleichzeitig mit den Kontrolltieren getötet. Eine Stunde vorher erhielten die Tiere 0,8 μCi/g Körpergewicht H^3 Thymidin (spez. Aktivität 5 Ci/m*M*) i.p. injiziert.

Von jedem Tier wurde ein Teil der Hodenkanälchen (20–60 mg) in Soluene gelöst und im Liquidscintillationsspektrometer gemessen. Vom restlichen Gewebe wurden Autoradiogramme mit Ilford G5 Emulsion hergestellt und anschliessend H.E. gefärbt.



◐, Hodengewicht, Kontrollen (g); ●, Hodengewicht, hypophysectomierte Tiere (g); ○, cpm/mg Kontrollen; ◑, cpm/mg hypophysectomierte Tiere.

Der Hoden verliert nach Hypophysektomie deutlich an Gewicht, es atrophieren die Samenkanälchen, die Leydigschen Zwischenzellen, die Spermatiden und Spermien[1]. Ungeachtet dieser Reduktion lässt sich bei den hypophysektomierten Tieren ein markanter Anstieg der DNS-Synthese pro mg Hoden feststellen (Figur). Eine Erklärung dafür liefern die Ergebnisse der Autoradiographie, die zeigen, dass das markierte Thymidin ausschliesslich in die Spermatogonien – deren Anteil pro Gewichtseinheit infolge der Atrophie der anderen Zellklassen ansteigt – eingebaut wird. In den Spermatogonien konnten auch zahlreiche Mitosen beobachtet werden, die offenbar zu einer Vermehrung des Zelltyps, ohne Spermatohistogenese führten.

Bei der Autoradiographie besteht im Schnittpräparat vollständige Übereinstimmung mit den Ergebnissen früherer Untersuchungen[2]. Die dort auf die zusätzlich durchgeführte partielle Hepatektomie bezogene Stimulierung der DNA-Synthese im Hoden hypophysektomierter Ratten ist offensichtlich ausschliesslich das Ergebniss der Hodenatrophie nach Hypophysektomie, weil nur die weiterhin Nukleinsäure synthetisierenden Spermatogonien übrigbleiben.

Summary. Atrophy of the testes was induced by hyposectomy of rats. While the differentiation of spermatids and of spermatozoons was inhibited, DNA-synthesis and mitoses of the spermatogones continued.

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18. Februar 1970.

[1] M. REISS, H. DRUCKREY und A. HOCHWALD, Endokrinologie *12*, 243 (1933).

[2] H. BRÄNDLE, H. WRBA und H. RABES, Naturwissenschaften *53*, 85 (1966).