ing chloro-K was excellent. When six male and six female normal rats (weight, 160 to 180 g) were placed in a cage containing both the EPA 0.005 percent chloro-K diet and the EPA diet with only 5 percent corn oil added, the ratio of the chloro-K diet to that of the control diet consumed over a 4-day period was 0.93. In a similar test with four normal males (180 to 190 g) that were given a choice of the 0.02 percent chloro-K diet or the control diet the ratio was 0.89.

To be effective as a general rodenticide, an anticoagulant would have to kill all the rats in the population, not just the warfarin-resistant strain. As a rather high dietary concentration of chloro-K might be required to control the normal rats, a mixture of chloro-K and warfarin would appear to be an effective treatment. The results of the exposure of warfarin-resistant and normal rats to such mixtures are also shown in Fig. 1. The mixture of 0.025 percent warfarin and 0.02 percent chloro-K was effective in killing both strains of rats in about 1 week. The amount of this diet consumed by these 150- to 180-g rats before they died was 39 ± 3 g (mean $\pm$ standard error) for the normal rats and 42 ± 4 g for the warfarin-resistant rats. In these rats, the total intake of the anticoagulant chloro-K was 45 to 50 mg per kilogram of body weight and that of warfarin was 55 to 60 mg/kg.

Chloro-K therefore appears to hold great promise as a rodenticide. Relatively low concentrations are lethal, and its mechanism of action is known. As is the case with the use of warfarin as a rodenticide, the available information (*12*) would suggest that accidental exposure of other animals to the compound could be counteracted by administration of vitamin K. These studies also indicate that the acceptability of the compound in the diet is good. If used alone, chloro-K could control local pockets of warfarin-resistant rats, and if used in combination with warfarin, it could prevent new areas of resistance from developing.

J. W. SUTTIE

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin, Madison 53706

References and Notes

1. C. M. Boyle, *Nature* **188**, 517 (1960).
2. M. Lund, *ibid.* **203**, 778 (1964); D. Drummond, *New Sci.* **30**, 771 (1966).
3. W. B. Jackson and D. Kaukeinen, *Science* **176**, 1343 (1972).
4. J. H. Greaves and P. Ayres, *Nature* **215**, 877 (1967); *ibid.* **224**, 284 (1969).
5. J. G. Pool, R. A. O'Reilly, L. J. Schneiderman, M. Alexander, *Amer. J. Physiol.* **215**, 627 (1968).
6. M. A. Hermodson, J. W. Suttie, K. P. Link, *ibid.* **217**, 1316 (1969).
7. M. J. Thierry, M. A. Hermodson, J. W. Suttie, *ibid.* **219**, 854 (1970).
8. D. J. Lorusso and J. W. Suttie, *Mol. Pharmacol.* **8**, 197 (1972).
9. R. J. Davis and B. H. Davies, *Biochem. J.* **118**, 44P (1970); A. Taylor and M. G. Townsend, *ibid.*, p. 56P; L. Ernster, C. Lind, B. Rase, *Eur. J. Biochem.* **25**, 198 (1972).
10. J. H. Greaves and P. Ayres, *J. Hyg.* **67**, 311 (1969); D. C. Drummond and E. W. Bentle, in *Report International Conference on Rodents and Rodenticides* (European and Mediterranean Plant Protection Organization, Paris, 1965), p. 57.
11. J. Lowenthal and M. N. Roy Chowdhury, *Can. J. Chem.* **48**, 3957 (1970).
12. J. Lowenthal, in *The Fat-Soluble Vitamins*, H. F. DeLuca and J. W. Suttie, Eds. (Univ. of Wisconsin Press, Madison, 1970), p. 431.
13. The 2-chloro-3-phytyl-1,4-naphthoquinone used was synthesized (*11*) by Dr. C. Schroeder from 2-chloro-1,4-naphthoquinone, which was a gift from Dr. J. Lowenthal.
14. The aqueous chloro-K preparation used for injection contained 2 mg of chloro-K, 14 mg of Tween-80, and 10 mg of ethanol per milliliter.
15. The warfarin-resistant rats used were descendants of a colony homozygous for this trait which has previously been described (*6*). These rats are fed Purina laboratory chow and, except when on experiments, are given water containing 0.5 mg of menadione sodium bisulfate (trihydrate) per liter. Without this supplement the male rats do not maintain normal prothrombin concentrations. Prothrombin concentrations in these rats are not affected by an intraperitoneal injection of warfarin (5 mg/kg) or by feeding them a diet containing 0.25 percent warfarin.
16. The diet contained: 65 percent coarse ground corn, 25 percent ground oats, 5 percent powdered sugar, and 5 percent corn oil. The chloro-K or chloro-K and warfarin mixtures were dissolved in an additional 5 percent of corn oil and added to the diet.
17. S. S. Shapiro and D. F. Waugh, *Thromb. Diath. Haemorrhag.* **16**, 469 (1966).
18. Supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and in part by NIH grant AM-14881.

20 February 1973

Mucopolysaccharides: Comparison of Chondroitin Sulfate Conformations with Those of Related Polyanions

Abstract. *X-ray diffraction shows that chondroitin 6-sulfate, and some further sulfated derivatives, can occur in two ordered structures in stretched films. Both structures contain single helices with similar projected disaccharide lengths (9.6 and 9.8 angstroms) but with very different turn angles between successive disaccharides (120 and 45 degrees). In contrast, coaxial double helices of hyaluronates and ι-carrageenates have shorter projected disaccharide lengths (8.5 and 8.9 angstroms).*

We have obtained x-ray diffraction patterns from stretched films (*1*) of sodium salts of chondroitin 6-sulfate (*2*) and some closely related materials (*3*). The patterns (Fig. 1, A and B) indicate that, in these films, threefold or eightfold helical molecules (*4*) pack parallel to one another in regular arrays (*5*). Molecular conformations and interactions can therefore both be defined. Chondroitin sulfates occur in connective tissue as side chains on proteoglycans (*6*). We believe that our results may be useful in defining possible conformations and interactions of short lengths of these side chains.

Axial periodicities per disaccharide residue (h) are very similar (9.6 and 9.8 Å) for the two molecules and correspond to almost fully extended chain conformations. In contrast the turn angles per disaccharide (θ) are very different (120° for a threefold helix and 45° for an eightfold helix). We used computer methods (*7*), which combine stereochemical information (*8*) with the values of h and θ, to build preliminary molecular models (Fig. 2) (*9*). The resulting threefold helix (Fig. 2a) has no unacceptably short nonbonded distances, and the eightfold helix (Fig. 2b) has only one, which should be removed during detailed refinement (*10*). Packing considerations, supported by density measurements, indicate that the molecules are single helices (Fig. 2, a and b) packed so that their axes are 14.3 Å apart for threefold helices and 13.8 Å apart for eightfold helices. Intermolecular distances of about 14 Å are probably characteristic of sulfated mucopolysaccharides since they also occur for the seaweed polysaccharide ι-carrageenate, which has the same linkage pattern (*11*).

The two molecules have similar central cores of pyranose residues that are fringed with charged sulfate groups. Ionic bridges, involving counterions, are the only interactions possible between these molecules. The importance of counterions for the interaction of chondroitin sulfate chains is consistent with their envisaged biological functions in electrolyte physiology and in structural organization of the intercellular matrix (*12*). Although the threefold and eightfold helices have similar h values the azimuthal distribution of

charged side groups is very different in the two conformations. This difference is a result of the glycosidic bonds lying near, and having a similar direction to, the helix axis. Changes in glycosidic conformation angles, therefore, have little effect on h but have a great effect on θ and, consequently, the direction of the side groups. We would not be surprised to discover further chain conformations with h near 9.5 Å but with the charged side groups in some intermediate distribution. Whatever importance variability of charge distribution may have for their biological functions, chondroitin sulfates can achieve this is two ways: by differences in sites and degree of sulfation and by conformational differences of the kind we have observed.

Hyaluronate films (*13*) prepared similarly to chondroitin 6-sulfate films contain a molecular conformation which has been identified as a double helix (*14, 15*). The double helix persists with little change even when the specimen is dried over silica gel for 12 hours. Fig. 1C shows the diffraction pattern of this lower-humidity form, which has regular double helical molecules with h and θ equal to 8.5 Å and 90°, respectively (*16*). Coaxial double helices ($h = 8.9$ Å) are present also in ι-carrageenate (*11*). Although the hyaluronate molecule has antiparallel and ι-carrageenate parallel chains, both

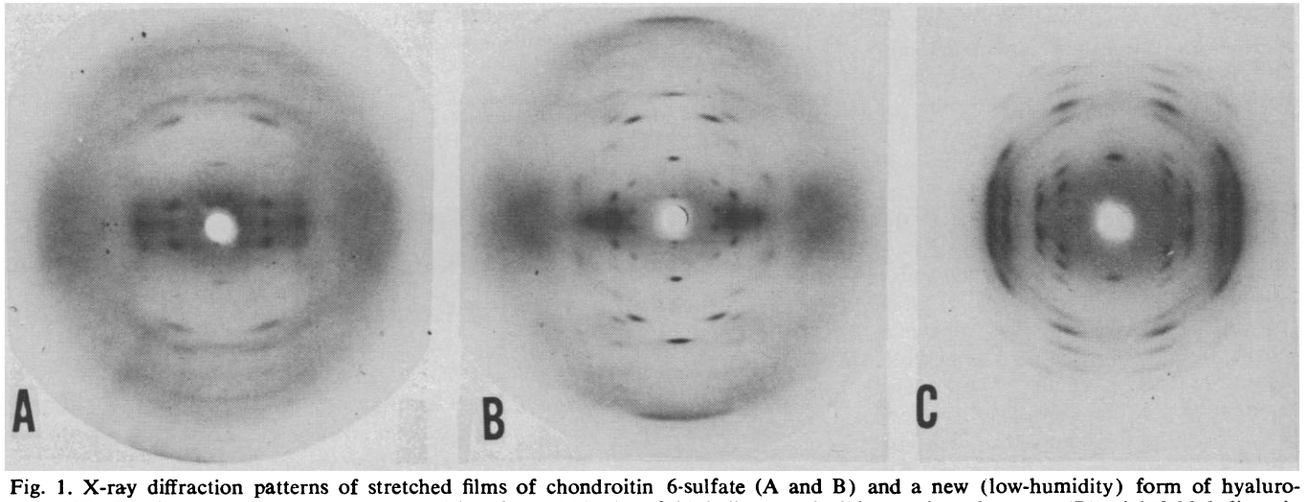

Fig. 1. X-ray diffraction patterns of stretched films of chondroitin 6-sulfate (A and B) and a new (low-humidity) form of hyaluronate (C). We interpret these patterns as arising from (A) threefold helices packed in a trigonal array, (B) eightfold helices in a tetragonal array, and (C) fourfold helices also in a tetragonal array. The direction corresponding to the parallel axes of the helices is vertical in all cases.

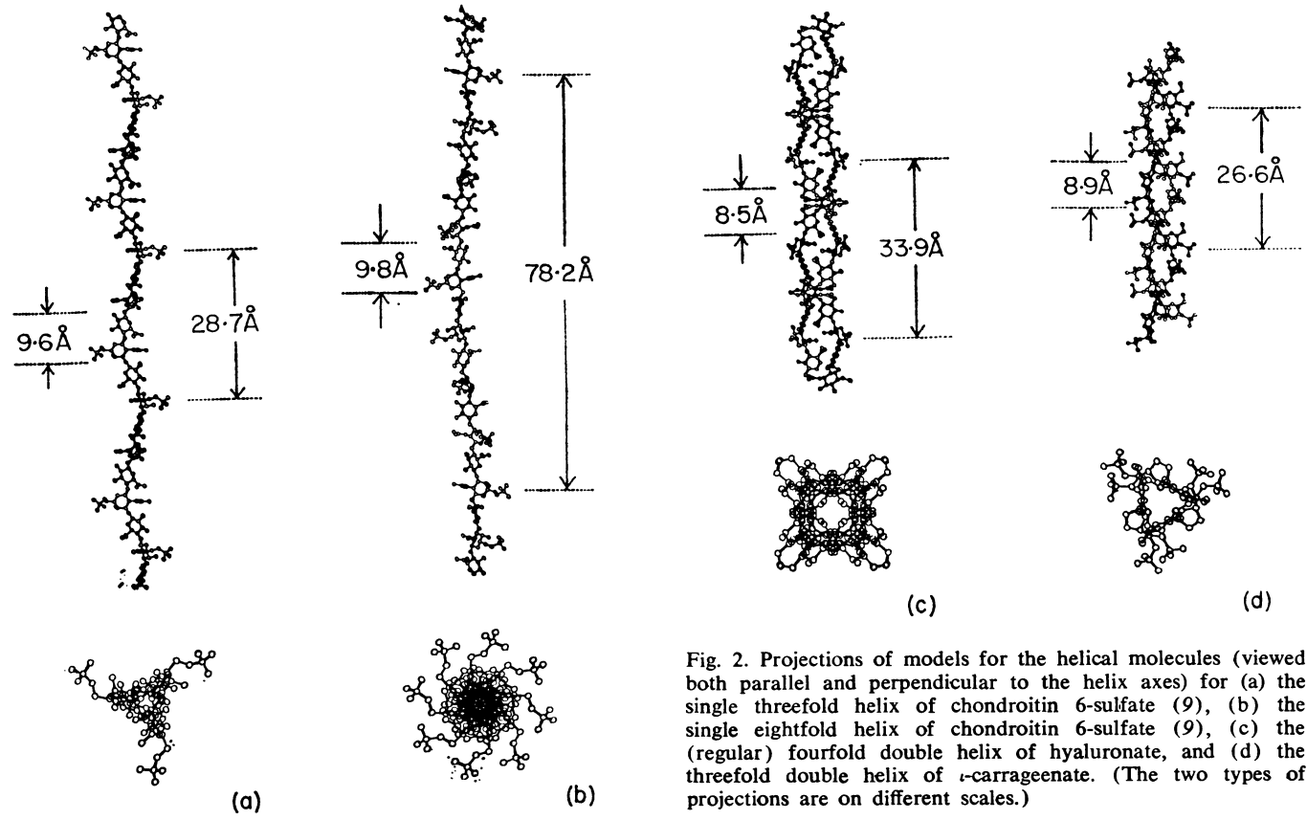

Fig. 2. Projections of models for the helical molecules (viewed both parallel and perpendicular to the helix axes) for (a) the single threefold helix of chondroitin 6-sulfate (*9*), (b) the single eightfold helix of chondroitin 6-sulfate (*9*), (c) the (regular) fourfold double helix of hyaluronate, and (d) the threefold double helix of ι-carrageenate. (The two types of projections are on different scales.)

double helices have h values about 1 Å shorter than the single helices of chondroitin 6-sulfate (*17*). This shortening leads to more obviously grooved, helical structures (Fig. 2, c and d) that are apparently necessary to accommodate the second chain of a double helix. In the lower-humidity form regular double helices of hyaluronate pack 9.9 Å apart, which is essentially the same as their distance of closest approach in the high-humidity form. This distance is much closer than has been observed for chondroitin 6-sulfate or ι-carrageenate, where the lattice has to accommodate protruding sulfate groups.

The conformational flexibility of the extended single chains of chondroitin 6-sulfate is in marked contrast to the relatively rigid molecular conformations imposed by the double helical structures of hyaluronate and ι-carrageenate. The existence of double helices for both of these shows that sulfation or its absence is not a prerequisite for coaxial helices. Chemical differences between chondroitin sulfate and hyaluronate are amplified by their secondary structures, which presumably provide additional specificity for their biological functions.

STRUTHER ARNOTT
J. M. GUSS
D. W. L. HUKINS
Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907
M. B. MATHEWS
Departments of Pediatrics and Biochemistry, La Rabida–University of Chicago Institute, and Joseph P. Kennedy, Jr., Mental Retardation Research Center, University of Chicago, Chicago, Illinois 60637

References and Notes

1. Films a few hundred micrometers thick are cast on Teflon blocks. Strips 0.5 by 0.2 cm are stretched under constant tension (about 3 g) at 80 to 90 percent relative humidity. The procedure is similar to that described in (*15*).
2. Chondroitin is a polydisaccharide in which a β-D-glucopyranuronic acid residue is linked 1 → 3 to a 2-acetamido-2-deoxy-β-D-galactopyranose residue that is in turn linked 1 → 4 to the next glucopyranuronic acid residue. In the 6-sulfate the site of esterification is the galactopyranose. Structural formulas are given in a review by H. Muir, *Amer. J. Med.* **47**, 673 (1969).
3. We have used chondroitin 6-sulfates extracted from ray cartilage (which are occasionally further sulfated at C2 or C3 of the glucopyranuronic acid) or from squid cartilage (which has some additional sulfation at C4 of the galactopyranose). We have also used chondroitin 6-sulfate from shark cartilage and skin, additionally sulfated at C2 or C3 of the glucopyranuronic acid (Sigma Chemical Co., St. Louis, and Seikagaku Kogyo Co., Tokyo).
4. Figure 1A corresponds to threefold helical molecules because there are meridional reflections only on layer lines 3, 6, and 9; Fig. 1B corresponds to eightfold helical molecules since there are meridional reflections only on layer lines 8, 16, and 24.
5. The threefold helices pack in a trigonal lattice ($a = 14.3$ Å and $c = 28.7$ Å), and the eightfold helices in a tetragonal lattice ($a = 13.8$ Å and $c = 78.2$ Å). Definitions of these terms are given by K. C. Holmes and D. M. Blow, in *Methods of Biochemical Analysis*, D. Glick, Ed. (Interscience, New York, 1965), vol. 13, p. 147. E. D. T. Atkins, R. Ganssen, D. H. Isaac, V. Nandanwar, J. K. Sheehan [*J. Polym. Sci. Part B* **10**, 863 (1972)] have just reported a threefold chondroitin 6-sulfate helix with $c = 28.5$ Å. Although they have tentatively interpreted their diffraction pattern as showing orthorhombic symmetry, we suspect that their structure is essentially the same as our trigonal form.
6. M. B. Mathews, *Biochem. J.* **125**, 37 (1971).
7. Conformation angles corresponding to the centers of "fully allowed" regions were determined by the method of D. A. Rees [*J. Chem. Soc. B* (1969), p. 217], but by using as fixed stereochemical parameters the averaged bond lengths, bond angles, and (C1 chair) pyranose geometry in (*8*). Model conformation angles were allowed to deviate minimally from these idealized values to attain the observed values of h and θ as described in (*8*).
8. S. Arnott and W. E. Scott, *J. Chem. Soc. Perkin Trans. II* (1972), p. 324.
9. Left-handed chains are shown. We have no good stereochemical reasons for preferring left-handed rather than right-handed helices. A preference must await detailed structure refinement involving the intensities of the x-ray diffraction data. The general features that we wish to discuss here are effectively the same for helices of either hand.
10. The unacceptably short distance (2.5 Å) is between the ring oxygen (O5) of the galactopyranose residue and C4 of the glucopyranuronic acid residue. This distance cannot be increased merely by adjusting conformation angles at the glycosidic bridges but will require some small change in the shape assumed for the pyranose rings.
11. N. S. Anderson, J. W. Campbell, M. M. Harding, D. A. Rees, J. W. B. Samuel, *J. Mol. Biol.* **45**, 85 (1969); S. Arnott, W. E. Scott, D. A. Rees, C. G. A. McNab, in preparation
12. A. G. Ogston, in *Chemistry and Molecular Biology of the Intercellular Matrix*, E. A. Balazs, Ed. (Academic Press, New York, 1970), pp. 1231–1240; M. B. Mathews, in *The Connective Tissue*, B. M. Wagner and D. E. Smith, Eds. (Williams & Wilkins, Baltimore, 1967), pp. 304–329.
13. In hyaluronate, 2-acetamido-2-deoxy-β-D-glucopyranose replaces the galactopyranose of chondroitin. It contains no sulfate groups. We have used sodium hyaluronate of high molecular weight (weight average, 1.3×10^6) extracted from human umbilical cord.
14. I. C. M. Dea, R. Moorhouse, D. A. Rees, S. Arnott, J. M. Guss, E. A. Balazs, *Science* **179**, 560 (1973).
15. E. D. T. Atkins and J. K. Sheehan, *ibid.*, p. 562.
16. This lower-humidity form gives a diffraction pattern very similar to that of the higher-humidity form (*14*). However, the tetragonal molecular packing ($a = 9.9$ Å and $c = 33.9$ Å) and the absence of meridional reflections on layer lines 2 and 6 indicate a regular fourfold helix. The higher-humidity form contains a perturbed version of this fourfold helix packed in an orthorhombic lattice ($a = 9.8$ Å, $b = 11.4$ Å, and $c = 33.7$ Å).
17. The double helices of hyaluronate can be destroyed by more extensive treatments; the result is molecular helices with h values in the higher range (9.3 to 9.5 Å), which have been interpreted as being single helices (*15*).
18. Supported by NSF grants GB-18835 and GB-35965 to S.A.; by grants from the Arthritis Foundation (to J.M.G.) and the Jane Coffin Childs Memorial Fund for Medical Research (to D.W.L.H.); and by PHS grants AM-05996 and HD-04583 to M.B.M.

8 January 1973

Ferromagnetic Contamination in the Lungs and Other Organs of the Human Body

Abstract. *Contaminating particles which are ferromagnetic have been found in the human body. Their distribution was measured by applying an external magnetic field to the torso for a short time, and then, in a shielded room, mapping the steady magnetic field around the torso due to the magnetized particles. Maps of subjects show various distributions, including particles in the stomach from food cans and in the lungs from arc welding. The fields from these two sources are strong enough to be detected with a flux-gate magnetometer, without the need for a shielded room. This simplicity of detection of larger amounts of ferromagnetic contamination suggests that this method may be used in two applications: in detecting the presence of large amounts of asbestos (ferromagnetic and harmful) in the lungs of asbestos workers, and in tests of the condition of the lung where FE_3O_4 dust (ferromagnetic and harmless) would be used as an inhaled tracer material.*

In recent years my colleagues and I have been investigating the steady (*1*) magnetic fields around the torsos of both humans and dogs produced by natural, internal direct current (d-c). Fields from d-c, although very weak, have been of interest because it has been believed that such fields can be a measure of heart injury (*2*). In mapping these fields around the torso I noticed that often there was another, steady field that was not due to internal d-c; instead, investigation showed that this field was produced by ferromagnetic particles in the lungs, stomach, and other organs. These particles entered the body through food and air. This other steady field, therefore, was the remanent field of these particles, magnetized by the earth's field and other sources. Since these particles are foreign to the body and also interfere with the magnetic measurement of internal d-c, they have been named ferromagnetic contamination (FC). It was then suggested (*3*) that magnetic measure-