

TABLE XIII

Analytical Values of the Unsaturated Disaccharides Obtained From Chondroitin Sulfate A and B and Hyaluronate After Digestion with Flavobacterium Enzymes

Substrate	Carbazole	Hexosamine	Reducing Value	E 1 per cent 230 m_{μ}	SO ₄ (per cent)
Chondroitin sulfate B	46.9	30.1	61.3	120	
Chondroitin sulfate B	44.2	25.6	44.2	130	15.2
Chondroitin sulfate A	40.5	33.6	52.4	138	
HA	40.0	41.7	61.0	148	

of the flavobacterium. The sulfatase of these extracts also does not act on the polymers, but it does act on the sulfate groups after glycosidic hydrolysis. Kerato sulfate, for example, is not hydrolyzed by these extracts nor are the sulfate ester and the glycosidic bonds. The same applies to heparin. The extracts of the nonadapted organisms or organisms adapted to chondroitin sulfate A or B do not split heparin or heparitin sulfate and apparently do not hydrolyze the sulfate groups either. Extracts of organisms adapted to heparin will split glycosidic bonds of heparin or heparitin sulfate and will also hydrolyze sulfate of the sulfate esters. This we have interpreted as a confirmation of the findings of Dodgson and Lloyd (51).

Heidelberger: Does the sulfatase split the *N*-sulfate or the *O*-sulfate, or is that not known.

Meyer: In the heparin-adapted enzyme, it splits both *N*-sulfate and *O*-sulfate. Of course, the enzymes which are not adapted to heparin do not attack either the *N*-sulfate or the *O*-sulfate.

Bernardi: I studied the infrared spectra of some sulfated polysaccharides in the region 960 to 730 cm.^{-1} (Table XIV). The bands present in this region are assignable according to Barker *et al.* (52). The ring vibration and ring breathing bands appear at the expected

frequencies. The C₁-H deformation bands, which are correlated with the type of glycosidic bond present, are in agreement with the structures suggested by chemical and optical rotational data. A remarkable exception, however, is heparin (as well as mactin A and mactin B). Here an α -glycosidic linkage is indicated by the strong positive optical rotation [+47, +71, +41, respectively (53)], according to Wolfrom *et al.* (54), whereas the presence of a band at 890 cm.⁻¹ suggests a β -linkage. In this connection, I should like to point out that in the case of κ -carrageenan, which shows an optical rotation of +63° (55), both x-ray diffraction data (56) and infrared spectrum indicate the presence of β -linkages.

The most important feature, however, concerns the sulfate. The sulfate band appears near 820 cm.⁻¹ in some sulfated polysaccharides and near 850 cm.⁻¹ in others. This difference was noted for the first time by Orr (15). On the basis of the work of Jones *et al.* (57,58) on the infrared spectra of steroids, Orr suggested that this difference could arise from a different steric position of the sulfate group with respect to the sugar ring. This interpretation, although criticized by Egami (59), receives further support by the following considerations:

We can reasonably assume (60,61) that the hexoses forming the polysaccharides shown in Table XIV are in the Sachse C 1 chair form (62). In this case we know from Reeves' work (62) the steric position of the hydroxyls or of their substituents. Actually, we just need to know the positions of the hydroxyls on C₂, C₃, and C₄, because the C₁ hydroxyl is involved in the glycosidic linkage and the C₆ hydroxyl is substituted by the C₆ group which is always in an equatorial position (therefore, also the C₆ hydroxyl in some way occupies an equatorial position).

Glucose has equatorial hydroxyls on C₂, C₃, C₄ (and on C₆, of course). Therefore, the first seven polysaccharides of the table, whose hexose units are formed either by glucose or by its derivatives, glucosamine and glucuronic acid, have equatorial positions only for the substituting sulfate groups. They all show a sulfate band near 820 cm.⁻¹, which is therefore supposed to be correlated with the presence of equatorial sulfate groups. It is important to stress that it does not matter if there is more than one sulfate per hexose unit. Polysulfation will only increase the intensity of the band at 820 cm.⁻¹ as well of the S=O stretching vibrations near 1240 cm.⁻¹. Nor does it matter if the sulfate is bound to an amino group, as in heparin, because the only important thing is the steric position of the sulfate with respect to the sugar ring.

Galactose has equatorial hydroxyls on C₂, C₃ (and C₄) and an axial

TABLE XIV
Infrared Absorption Bands of Some Sulfated Polysaccharides in the 960 to 730 cm^{-1} Region

Sulfated Polysaccharides	Axial Hydroxyl on $\text{C}_2\text{-C}_4$	Ring Vibration	$\text{C}_1\text{-H}$ Deformation	Ring Breathing	Sulfate	Ref.
Hyaluronate polysulfate	None	935		775	820	15
Starch polysulfate	None	937		(740)	823	59
Cellulose polysulfate	None	937	(895)	767	815, (800)?	59
Dextran polysulfate	None	939	845	740	826, 809?	59
Heparin	None	935	890		825	53
Mactin A	None	935	890		825	53
Mactin B	None	935	890		825	53
Chondroitin sulfate C	C_4 galactosamine			775	820	63
Kerato sulfate	C_4 galactose	945	(895)	770	815	*
Chondroitin sulfate A	C_4 galactosamine	928			855	15
Chondroitin sulfate B	C_4 galactosamine	928			855, 840?	63
λ -Carrageenan	C_4 galactose	935		770	840	56
k -Carrageenan	C_4 galactose	930	890	770	850	56
Alginate polysulfate	C_2 manuronic	950	890	730	834, 802	15

*Bernardi G., unpublished result.

Note: Some of the above values were read on published tracings. Values in brackets indicate very weak absorptions. Values followed by a question mark indicate absorptions of doubtful assignment.

hydroxyl on C₄. Consequently, there are two possible positions for the sulfate group in the polysaccharides whose sulfate-carrying hexose is galactose or galactosamine; namely, those having a sulfate on C₄, like the carrageenans (64), and the chondroitinsulfates A and B (23,63) have the sulfate in an axial position and show a band near 850 cm.⁻¹. Chondroitin sulfate C, which has the sulfate in an equatorial position, on C₆ (63), shows, as expected, the sulfate band near 820 cm.⁻¹. About kerato sulfate, which also has the sulfate in an equatorial position—I can only say that its sulfate should not be on the C₄ of galactose.

Mannose has equatorial hydroxyls on C₃, C₄ (and C₆) and an axial hydroxyl on C₂. Polysulfated alginate, whose repeating hexose unit is mannuronic acid, may therefore have both axial and equatorial sulfate groups. This is probably the explanation of its double sulfate band (15).

A final remark I should like to make concerns the assignment of the sulfate band. The sulfate bands shown in Table XIV do not shift to different frequencies by passing from the salt forms to the free acids (15). The only known exception is polysulfated alginic acid, where a possible explanation is given by the contraction, shown by x-rays (65), that alginic acid undergoes while passing to the salt form. This failure to shift raised some doubts about the assignment of the sulfate band (59). However, as acid forms of the polysaccharides are obtained by casting them down from a dilute HCl solution, it is likely that only the carboxyl group if present becomes undissociated, whereas the sulfate may remain in the ionized form. The belonging of the sulfate band is well shown by its appearing after sulfation in nonsulfated polysaccharides (15) and its disappearing after desulfation in sulfated polysaccharides.*

Wolfrom: You say there are two bands for sulfate? Where is the second band for sulfate?

Bernardi: No, there is just one band except in the case of alginate polysulfate. Actually, a weak band also appears at 800 cm.⁻¹ in cellulose polysulfate, and at 809 cm.⁻¹ in dextran polysulfate, but I do not know whether or not they belong to the sulfate. I did not prepare any hyaluronate polysulfate. Its spectrum is given by Orr (15). The only original spectrum shown in the table is that of kerato sulfate, which I did using a sample you sent me, Dr. Wolfrom.

Hoffman: Following the publication of Orr's paper (15), we also began a study of the infrared spectra of the mucopolysaccharides. As Dr. Bernardi stated, Orr had shown that the absorption of chondroitin sulfate C was related to that of polysulfated hyaluronate—

Bernardi: Orr did not speak of chondroitin sulfate C.

*Meyer, K.: Personal communication.

Hoffman: The B isomer mentioned by Orr is what we call chondroitin sulfate C. It is important to clarify that point.

Meyer: I would like to stress that what he calls B is from nucleus pulposus and is chondroitin sulfate C, as confirmed in Sweden by Gardell (66) and isolated by us from a chordoma, a tumor originating like nucleus pulposus from notochord cells.

Bernardi: I should like to remind you that kerato sulfate also is present in nucleus pulposus, besides chondroitin sulfate C and A and perhaps chondroitin.

EDITOR'S NOTE: Dr. Bernardi would like to add the following "afterthought" to his remarks at the Conference:

Orr's B isomer (15), in my opinion, was a mixture of chondroitin sulfate C and kerato sulfate. The first evidence for this was given by Orr himself, who found in his preparation a ratio of the intensities of the band at 1736 ($-\text{COOH}$) to that at 1560 ($=\text{NH}$) 21 to 29 per cent lower than in hyaluronic acid. This means a rough ratio of 3 to 1 of chondroitin sulfate C to kerato sulfate in Orr's B isomer. Another finding reported by Orr (15) is very interesting in connection with what Dr. Meyer said about the presence of kerato sulfate in some types of hyaline cartilage, as well as about the electrophoretic mobilities of chondroitin sulfate and kerato sulfate. Orr (15) found that the faster moving component obtained by counter-current electrophoresis from a tracheal cartilage preparation of chondroitin sulfate showed a 1736/1560 ratio equal to hyaluronic acid, whereas the whole preparation had a ratio of 0.89. This suggests that the slower moving component probably was kerato sulfate.

Meyer: They isolated from nucleus pulposus two compounds, one of which appears to be identical with what we have called chondroitin sulfate C, the other identical or at least similar to kerato sulfate.

Hoffman: Orr's basis for the equatorial sulfate in C was the similarity of its absorption to that appearing after the polysulfation of hyaluronate, and, because A had a different absorption, he assumed that it was due to an axial sulfate group. Of course, at that stage, he did not know that the glycosidic linkages were the same for hyaluronate and the chondroitin sulfates, and he would not commit himself as to whether the differences were a result of the sulfate's being on different carbon atoms or of different glycosidic linkages. However, he favored the correct interpretation, that the difference was due to the sulfate linkage.

Mathews (16) carried the work further by obtaining the spectrum of chondroitin sulfate B, and he noted that it was similar to that of A. As a preliminary report by Jeanloz had shown that the sulfate on B could not be on C-6 of the galactosamine and was probably on C-4,

exists in salt solution as some kind of random coil, and that it will extend as we lower the salt concentration, because there are negative charges. Extension of this results because of repulsion of charges.

Mathews* found the minimum molecular weight of the complex to be about 4,000,000. About 3,200,000 of this is chondroitin sulfate A, and 1,800,000 is protein. Each of the chains of chondroitin has a molecular weight of 50,000.

We thought that this was going to be very easy, that if it were possible to remove the side chains with hyaluronidase the protein would remain and could be studied with all its physicochemical properties. Unfortunately, side chains are removed to a certain point and then there are still chondroitin residues, so this interferes with the physicochemical characterization of the nature of the protein.

In addition, these individual macromolecules seem to have a very high degree of association with other molecules, so that actually in nature they may not all be 4,000,000. This is the lowest estimate he has obtained. Many of the preparations show much higher molecular weights.

Pollard: There really are not any physicochemical methods that will deal with a molecule of that type. There is confusion between not knowing the axial ratio and the degree of hydration.

Dorfman: That is correct. All one can say is that on the basis of viscosity and light-scattering data this is a highly asymmetrical molecule. Dr. Bernardi has a little different idea about the shape of this molecule.

Bernardi: Mathews and Lozaityte (12) quite recently published a good paper on the mucoprotein from bovine nasal septa cartilage. The interpretation of their data and their conclusions on the molecular shape and structure of the chondroitin sulfate-protein complex were different from mine (16). I should like to recall that the model I proposed on the basis of results obtained with different techniques for cartilage mucoprotein was that of a coiled filament formed by chondroitin sulfate linear chains bridged by polypeptide chains in an end-to-end arrangement. A similar model was previously put forward by Webber and Bayley (17) who observed that mucoprotein molecules showed streaming birefringence in distilled water but not in salt solution. This criterion, however, was shown to be equivocal (18,19).

It is important to point out that Mathews and Lozaityte studied a product extracted by a method different from that which I used. This explains the apparent discrepancies in the results we got, particularly those concerning the molecular weight. Therefore, I will discuss only

*Unpublished data.

our main discrepancy, namely, our different interpretation and proposed model for the mucoprotein molecule.

Fremont-Smith: What did you start with?

Bernardi: Bovine nasal septum cartilage. The mucoprotein was extracted with 30 per cent potassium chloride. After dialysis against running tap water and precipitation with two volumes of ethanol in the presence of potassium acetate, the preparation was dried with ethanol and ether and dissolved in M/15 phosphate buffer of pH 7.0. At this point the solution was centrifuged for 2 hours at 40,000 rpm, in order to get rid of a component with higher sedimentation constant. The yield was very low, 0.36 per cent of the original wet cartilage.

Mathews and Lozaityte (12) proposed for their mucoprotein a rod-like basic molecular unit in which 62 units of chondroitin sulfate are distributed along a protein core 3700 m μ . long. This was proposed mainly on the basis of their findings after hyaluronidase treatment. But after this enzymatic treatment, they still got some 15 per cent polysaccharide in their preparation.

I am afraid there is no sound basis for the rod model proposed by Mathews and Lozaityte. Indeed, light-scattering does not give an unequivocal answer for the model in the case of polydisperse systems (20). A good indication for a polydisperse system of rods can be obtained from streaming birefringence. Unfortunately, the streaming birefringence of mucoprotein solutions is very low (16,17) and probably only due to deformation with shear. The very low shear dependence found by Mathews in the viscosity measurements is in agreement with this. Dr. Follis, will you tell us about the diameter of the particles in the mucoprotein fraction of your preparation?

Follis: It is possible to get different sizes, depending on how mucoprotein solution is put on the grid, so that I think this is of not much use, either. If it is sprayed on in very fine droplets, one size will be obtained, but if a big drop is put on and allowed to dry, an entirely different picture will result. There will be all sorts of peculiar strands which, I am sure, are artifacts. Are there any methods? What about x-ray diffraction, as was mentioned earlier?

Meyer: In cartilage, I would say that there is more than 20 per cent of mucoprotein or chondroitin sulfate. What figure would you give, Dr. Schubert?

Schubert: It is close to 40 per cent in the nasal cartilage. Let us decide what we are talking about. You are talking about the chondroitin sulfate of nasal cartilage?

Meyer: Yes.

Schubert: That would be about 40 per cent. The mucoprotein content will be about 50 or 55 per cent.

Meyer: Anyhow, if the electron microscope pictures which we have seen correspond to reality, then, at least in epiphyseal cartilage, the protein complexes of the sulfated polysaccharides are somehow oriented on the collagen fibers or bundles.

Studies on x-ray diffraction of cartilage have been done in J. T. Randall's laboratory (Dept. of Physics, King's College, London, England) and they have only shown the collagen pattern. One would expect a regular structure such as proposed by Mathews to show some evidence of crystallinity.

Another uncertainty in the data of Mathews (12) appears to me to be the molecular weight of the protein complex. The minimum molecular weight is based on the methionine value as the lowest amino acid. This value by paper chromatography has been estimated as 0.18 gm./100 gm. of complex. There must be considerable margin of error in this estimation. In fact, an amino acid in such low concentration may belong to a protein impurity. In a recent article (13) on the protein complex of chondroitin sulfate, methionine was not found among the amino acids. I would like to suggest that the protein complex is something like a glass of a globular protein which bridges chondroitin sulfate chains in irregular random fashion.

Dorfman: I think we are getting off on to a lot of theory about a fairly simple thing. It is possible to obtain a complex with a minimum molecular weight of 4,000,000 that has side chains of chondroitin sulfate, that has a high protein, and that is highly asymmetric. You may then try to draw a physical picture on the basis of the known facts. Beyond that, you may want to draw your own pictures whichever way looks better to you—a little globule stuck together in a line or a long chain. I do not think anybody realistically would think of this as being a perfectly rigid rod. That would be inconsistent with what we know about protein structure. It might be an extended protein. This is possible rather than thinking of it as a curtain rod sticking through the middle that is absolutely rigid. Nobody really means that.

Bernardi: I do not think you can conclude anything like that on that basis. In this connection I should like to recall what I found (19) by treating my preparation with trypsin and chymotrypsin. The weight average molecular weight determined by light-scattering fell from 2,000,000 to 800,000. Of course, this is not the molecular weight of chondroitin sulfate, and I am quoting this result to show how dangerous it is to rely only on a light-scattering measurement after enzymatic treatment.

Dorfman: Did you also do osmotic pressure molecular weights on that material after treatment with trypsin?

Bernardi: No.

Dorfman: Because, you see, if you have a very small contamination with some undigested material, the light-scattering molecular weight is raised tremendously. If you do osmotic pressure measurement, then you can estimate what that would be. You may actually have had mostly 50,000 material.

Catchpole: I would like to go back to Dr. Follis's presentation and, perhaps, try to link it up with some work that Dr. Schubert has done. Dr. Follis, you presented, in Figures 7 and 8, a PAS stain with which you intended to show glycogen, of course, and the matrix was quite heavily stained in that section. Without getting into what I know is an old argument about PAS, I was just wondering whether Dr. Schubert's chondroitin sulfate or his chondroitin sulfate-protein complex does give a positive PAS or does not, because, of course, you were trying to identify your material with Dr. Schubert's, and did it only on the basis of metachromasia and alcian blue.

Follis: This material does give a positive reaction with PAS.

Schubert: We have worked with the periodic acid, not PAS, and years ago we found what many others found, including Dr. Jeanloz, that chondroitin sulfate reacts very slowly with periodic acid. In the work we did in the course of 4 days, one mol of periodate is taken up per period of chondroitin sulfate. It is a very slow reaction. The usual PAS stain is run in a few minutes.

We have been working recently with the material that is left after extraction of mucoprotein; this contains a considerable amount of hexosamine and material that we will refer to now as simply carbohydrate; I am not even sure that it is polysaccharide. This stains much more rapidly than chondroitin sulfate, and it is not glycogen. It reduces periodate much more rapidly. I think, therefore, there is another carbohydrate material present in the cow nasal cartilage that is more likely to give the PAS stain than chondroitin sulfate or mucoprotein. What this is, I am not prepared to say, but it contains hexosamine.

Jeanloz: Many years ago, we were interested in the PAS staining of mucopolysaccharides (21). Dr. Schubert had mentioned in a private conversation the possibilities of glycoproteins' being a contaminant of preparations of chondroitin sulfate and we felt that impure preparations of hyaluronic acid would contain the same contaminant. Our impure hyaluronic acid gave a very positive reaction with the PAS stain, whereas after purification it did not react. We tested a series of carbohydrate-containing proteins and found that, based on the carbo-

hydrate content. α_1 -acid glycoprotein (orosomucoid) was 10 to 100 times more reactive than glycogen. If we could measure quantitatively the PAS stain, we would like to correlate its intensity with the amount of neuraminic acid (sialic acid) in glycoprotein, since it is known that this acid is oxidized extremely fast by the periodate ion. For quite some time glycoproteins have been recognized as the reactive substances with PAS in tissues whereas hyaluronic acid and chondroitin sulfate are negative, as shown in Dr. Leblond's laboratory (22).

Meyer: Does anybody here know whether aqueous extracts of cartilage or the residue give reactions for sialic acid?

Schubert: We have never found any sialic acid. It is interesting that the material that rapidly reduces the periodate remaining in the cartilage residue is not readily extractable with water. It is very hard to get out.

Meyer: We have not done staining reactions in tissue sections, but we have used periodic acid to spot the polysaccharides on paper. Hyaluronic acid and chondroitin sulfate A, B, and C do not react in this test with periodic acid. However, kerato sulfate does show up with periodic acid on paper.

György: Does it contain sialic acid?

Meyer: No. It is a polymer of unknown structure, consisting of N-acetylglucosamine, galactose, and sulfate in equimolar amounts which is present in rib cartilage at least in quite large concentrations, in fact, in shockingly high concentrations in normal costal cartilage of adults.

György: Why do you say "shocking"?

Meyer: We had missed it and so probably have others. It was, to us, rather unexpected.

EDITOR'S NOTE: Dr. Meyer would like to add the following "afterthought" to his remarks at the conference:

We have now found that the fractions isolated from rib cartilage contained over 2 per cent methylpentose calculated as fucose. Whether or not the methylpentose is present as a constituent of kerato sulfate will have to be determined.

Bernardi: Figure 22 is an electron micrograph of a mixture of mucoprotein and chondroitin sulfate from horse nasal septum cartilage.* The preparation was made following Dr. Schubert's method (23) but without using any thorough kaolin treatment. What we found were irregular filaments without any evident structure or periodicity, many thousands of Å units long, and probably formed by lateral and/or end-to-end aggregation of mucoprotein and polysaccharide molecules.

*Bernardi, G., Cessi, C., and Gotte, I.: Unpublished results.

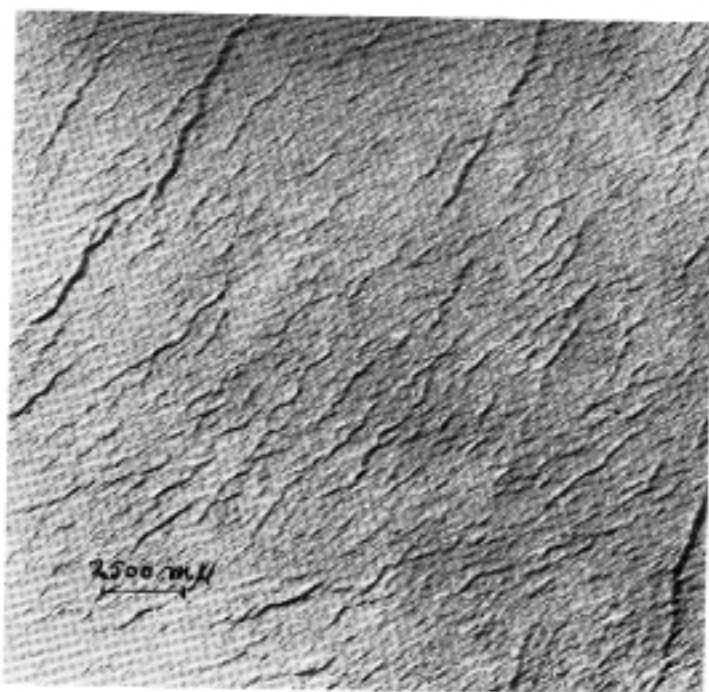


FIGURE 22. Electron micrograph of a mucoprotein-chondroitin-sulfate mixture obtained from hyaline cartilage. 0.75×10^{-5} gm./ml. in distilled water. Magnification 40,000x. Shadowing with gold manganese at 20 degrees.

Follis: About 10 years ago Gross (24) published an electron micrograph of hyaluronic acid, in which he pictured it as long filaments, but I do not think he meant to suggest that this was a representation of it in life. He told us recently that this could be interpreted entirely as some sort of artifact.

In studying our material, as I have indicated, if we put it on as a spray, we get filaments around the periphery of the drop, and then we get great blobs in the middle. I have no way of judging whether either one of these represents the way it looks in nature.

Bernardi: The same picture was obtained with nucleic acid, so very probably it is an artifact; blobs and filaments are coming out.

Dorfman: I might point out, Dr. Bernardi, in your model, if you had that kind of chain arrangement when you treated with hyaluronidase, you would break the chain, and the molecule would have to fall apart. It does not do that.

Bernardi: Yes, but what I said before is that this is a system where there is neither complete enzymatic breakdown nor absence of interactions between the residual macromolecules.

trouble on the paper strips and could not duplicate our original results.

We checked for glucosamine and galactosamine with Gardell's procedure and found out that we definitely had no galactosamine and that glucosamine was our amino sugar. The accuracy of the determination was ± 5 per cent.

The uronic acid was determined by Tracey's (57) method, liberation of CO_2 followed by manometric determination.

The *N*-acetyl and *O*-acetyl are things which we cannot explain. This involved hydrolysis with toluene sulfonic acid. We did not expect to find any *N*-acetyl. The samples, having been prepared as they were, should not contain any free acetate. If there had been acetate present, this should have been removed on passage through the strong base, strong acid monobed. But there it is. In the case of phosphorous, free amino-nitrogen, *N*- and *C*-methyl, we had nothing at all.

The rotations are even more strongly positive than heparin, which gave us a rotation of $+47^\circ$. This is the sodium salt. I think that your barium salts are $+47.5^\circ$, Dr. Wolfrom, but mactin A and B were $+71^\circ$ and $+61^\circ$ respectively.

The glucuronic acid was determined by paper chromatography, by the method of Fischer and Dörfel (58). Speaking of weak links, this is the only evidence we have for glucuronic acid, but, as it is, it checks very well.

Dorfman: How did you hydrolyze it?

Burson: For the paper strips, we used two procedures: 1 normal hydrochloric acid for 3 hours, and 3 normal hydrochloric acid for 1 hour. With both of these procedures, we got the spot which coincided with the glucuronic acid spot. I must say that it is much easier to see glucosamine on these paper strips than it is glucuronic acid, in our experience. Our glucosamine, in addition to being spotted, was isolated. We got no analytical elementary analysis, but the rotation checked with glucosamine.

Figure 40 is the infrared curve to which Dr. Bernardi referred earlier. There are really no gross differences between heparin or either of the two mactins. Do you have a comment about this, Dr. Bernardi? This, the peak at 890 cm.^{-1} , is what has bothered Dr. Bernardi, I think, because this indicates the β -configuration.

Bernardi: Yes, according to the results obtained by Barker *et al.* (59) in Birmingham.

Hoffman: They obtained their results on crystalline compound, and I do not think that is comparable.

Bernardi: They obtained the same results working with polysaccharides.

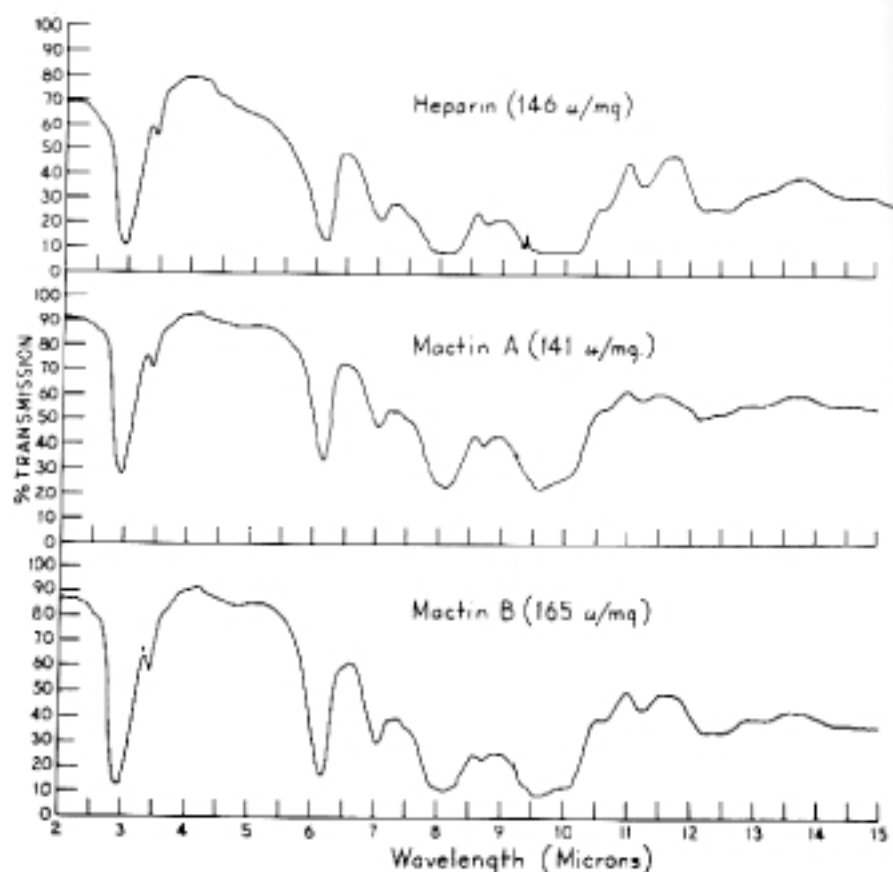


FIGURE 40. Infrared absorption. Reprinted, by permission, from Bursan, S. L., Jr., Fabrenbach, M. J., Frommhagen, L. H., Riccardi, B. A., Brown, R. A., Brockman, J. A., Lewry, H. V., and Stokstad, E. L. R.: Isolation and purification of mactins, heparin-like anticoagulants from mollusca. *J. Am. Chem. Soc.* **78**, 5874 (1956).

Hoffman: I do not believe they worked with amorphous material.

Bernardi: I doubt that the polysaccharides they used were crystalline.

Kabat: Not crystalline.

Heidelberger: Yes. We tried numerous polysaccharides in Birmingham, and got α -peaks when α was indicated, and β -peaks when β was indicated.

Hoffman: On amorphous material?

Heidelberger: Yes.

Kabat: They found the α -peaks in dextran, too.

Heidelberger: And β -peaks in the Type XIV pneumococcus polysaccharide.

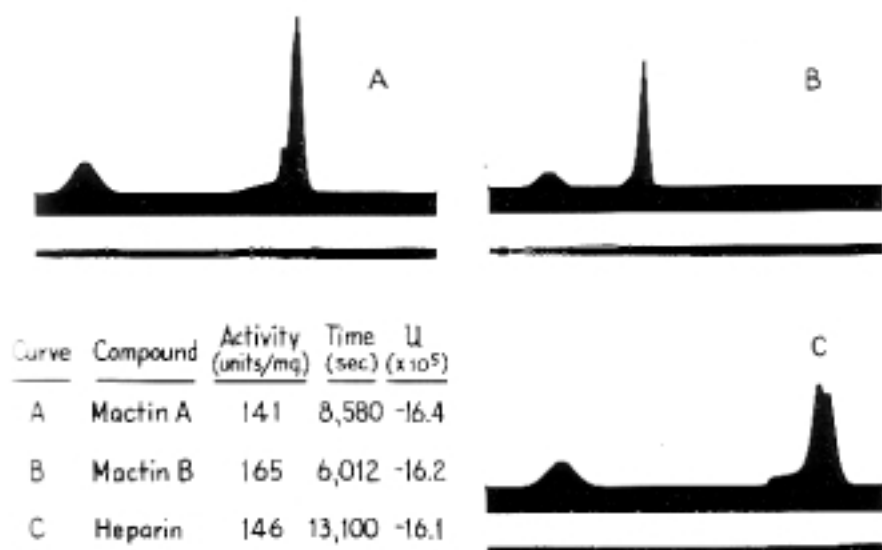


FIGURE 42. Electrophoretic patterns (ascending). Reprinted, by permission, from Burson, S. L., Jr., Fahrenbach, M. J., Frommhagen, L. H., Riccardi, B. A., Brown, R. A., Brockman, J. A., Lewry, H. V., and Stokstad, E. L. R.: Isolation and purification of mactins, heparin-like anticoagulants from mollusca. *J. Am. Chem. Soc.* 78, 5874 (1956).

crystalline barium salt is something of the order of 160 or so, usually.

Wolfrom: That is high. Our preparations ran 110 to 120 (barium acid salt basis) with an extrapolated value of 190 (sodium salt basis), as I have noted earlier in the discussion.

Burson: This heparin, as far as I know, was the product just as it came off the assembly line.

Table XXXI brings us still further into Dr. Brown's realm. I am not qualified at all to discuss this. The sedimentation constants and diffusion constants in the specific volume were used to determine average molecular weights of the two mactins and our commercial heparin. This particular commercial heparin does not compare; there is a discrepancy between this 14,000 and the minimum 20,000 that Dr. Wolfrom referred to. Using the same procedure, both mactins were considerably larger than the heparin sample.

Bernardi: I am afraid that the molecular weight obtained by sedimentation and diffusion data is not a weight average molecular weight as indicated in your table.

Burson: Dr. Brown, in his discussions with us, indicated that he preferred a weight average molecular weight to a number average. Another thing which he pointed to, to indicate the heterogeneity or homogeneity of these compounds, was the standard deviation of the

contained in the kappa fraction. The other fraction, called the lambda fraction, which is a catchall fraction, is composed entirely of D-galactose units, which are linked alpha 1,3.

EDITOR'S NOTE: Dr. Bernardi would like to add the following "afterthought" to his remarks at the Conference:

The Kappa fraction of carrageenan was so named by Smith and Cook (12) because of its sensitivity to potassium salts, a phenomenon later explained by Bayley (13). The lambda was chosen just because it was the following letter in the Greek alphabet.

Stoloff: The gelling property of the carrageenans appears to reside in the kappa component, which, except for the complexing effect with milk and methylene blue, is very similar to the major component of the gellans. The lambda component of carrageenan has no gel-forming properties by itself, but does possess some gelling properties in the presence of the kappa fraction. You do not get a complete addition of gel properties, but there is an increase in gel properties beyond that expected from just a diluting effect.

The proportion of kappa to lambda components of carrageenan is reported as approximately 3 to 2.

Springer: How do you homogenize kappa and lambda? Is it possible to subfractionate each subfraction again? Can you do this with your lambda and kappa fractions?

Stoloff: We have done very little work on the separation. Most of the work on separation has been done by the National Research Council in Halifax, Nova Scotia (14,15,16). The fractions are certainly not homogeneous with regard to size. They are homogeneous with regard to composition.

Bernardi: O'Neill in Halifax made a chemical study on kappa carrageenan (17,18) and arrived at a formula which is different from that given by you. Yours is a simplified formula, is it not?

Stoloff: This is a simplified version of what O'Neill has. O'Neill suggested a single unit side chain, and suggested another sulfate, but I do not entirely agree with him. I suspect that they are on their primarily as a bookkeeping procedure—to balance the books. The physical properties do not indicate a side chain, and I do not think it likely that there will be two sulfates on one hexose unit.

Springer: You must postulate that in the fucoidin molecule there are two sulfates on every fifth pyranose unit. Otherwise, not all of the sulfates are accounted for.

Stoloff: I think that the analytical procedures for sulfate are suspect, and until the controversy with regard to sulfate determination has been