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HEXOSAMINES, THEIR DERIVATIVES, AND MUCINS AND MUCOIDS

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P. A. LEVENE

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PREFACE.

The purpose of this monograph is not to offer a complete review of all the literature on the subject, but to bring together that part of it which has appeared from this laboratory from time to time in the course of many years. Many of the earlier observations received a clear explanation only in the light of subsequent work. To correlate all the details might be a taxing enterprise for one who has not been personally engaged in the work.

The work collected in this monograph was done with the cooperation of Drs. F. B. La Forge, J. López-Suárez, G. M. Meyer, I. Matsuo, and Mr. E. P. Clark. The cooperation of Dr. La Forge was particularly valuable in the earlier part of the work; Dr. López-Suárez has devotedly and skillfully cooperated in the part of mucins and mucoids; Dr. Meyer took part in the work on the optical rotation of hexonic acids; Dr. Matsuo assisted in the synthesis of 3-aminoheptonic acids, and to Mr. Clark we are indebted for the preparation of the pentoses which were required for the synthetic part of the work.

P. A. L.

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HEXOSAMINES, THEIR DERIVATIVES, AND MUCINS AND MUCOIDS.

BY P. A. LEVENE.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, July 11, 1921.)

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PART I.

2-AMINOHEXOSES AND THEIR DERIVATIVES.

THEORETICAL.

1. CONFIGURATION OF CARBON ATOM 2.

The present work was undertaken with the object of procuring data for the purpose of identification of 2-aminohexoses. When this work was undertaken only one representative of this group was known, namely chitosamine (glucosamine). All the details of the configuration of chitosamine have been explained save one, namely that of the configuration of carbon atom 2. The difficulties which were in the way of solving the problem have as yet not been overcome. They are brought about principally by the phenomenon of Walden inversion. From the work of Fischer it was known that when glucosamine was deaminized into chitose and this further oxidized to the corresponding acid, chitonic acid formed. On the other hand, when chitosamine was first oxidized to chitosaminic acid and this subsequently deaminized, chitaric acid formed. Fischer (1911) surmised that the acids were epimeric, but the configuration of either one of them was not disclosed by him. Irvine and his coworkers (1911, 1912, 1914) have shown that from chitosamine may be derived either glucose or mannose, depending on the condition of the experiment. The difficulties which lay in the way of the discovery of the configuration of carbon atom 2 in chitosamine could be foreseen to recur whenever the question arose as to the configuration of carbon atom 2 of any other 2-aminohexoses.

Thus the problem of identification of any one of the still unknown 2-aminohexoses fell into two parts, one dealing with the configuration of carbon atoms 3, 4, and 5, and the other dealing with the configuration of carbon atom 2. *A priori*, the way towards the solution of the first part of the problem seemed clear, whereas great difficulties were foreseen in connection with the problem of the configuration of carbon atom 2.

Since the configuration of carbon atom 2 is identical in a sugar and in the corresponding hexonic acid, the solution of the problem of the configuration of one is in itself a solution of the configuration of the other. The configuration of carbon atoms 3, 4, and 5 of any natural hexosamine can be easily disclosed as soon as the synthesis of the entire series of the 2-aminohexonic acids and the corresponding anhydrotetroxyadipic acids is accomplished.

The question as to the configuration of carbon atom 2 cannot as yet be answered by direct chemical evidence. Hence, one is justified in the use of indirect evidence.

The entire series of *d*-2-aminohexonic acids has now been prepared synthetically. Certain analogies in the reactions leading to their synthesis and in the properties of the corresponding hexonic and hexosaminic acids were looked into. These analogies were then

TABLE I.

From.	Predominating form.	From.	Predominating form.
Arabinose.	Mannonic acid.	Arabinoside.	Chitosaminic acid.
Lyxose.	Galactonic acid.	Lyxoside.	Epichondrosaminic acid.
Xylose.	Gulonic acid.	Xyloside.	Dextro-xylohexosaminic acid.
Ribose.	Altronic acid.	Riboside.	Levo-ribohexosaminic acid.

utilized for a provisional explanation of the configuration of carbon atom 2 in the 2-aminohexonic acids and hence in the corresponding sugars.

The first analogy to which attention was drawn is the parallelism in the equilibria of the two epimers which form on the condensation of prussic acid with pentoses on the one hand and with amino pentosides on the other. This is shown in Table I.

This parallelism, of course, is in itself no proof that the predominating forms in both series have an analogous configuration of their carbon atom 2. It is, however, permissible to make this assumption, and then to inquire to what extent other properties of hexosaminic acids are consistent with the assumption.

Attention was then directed to the optical rotation of the hexonic and hexosaminic acids. First it was discovered that in a pair of

hexonic acids the rotation of carbon atom 2 was to the right when its hydroxyl was in the same position as in gluconic, and to the left when its position was as in mannonic. Analyzing the optical rotations of the hexosaminic acids, it was observed that also in each pair of epimers of this series carbon atom 2 rotated to the right in one epimer and to the left in the other. A comparison of the predominating forms from the viewpoint of the rotation of their carbon atom 2 gives the results shown in Table II.

The rotation of carbon atom 2 determines the direction of the rotation of the hexonic acids. If the same rule were applicable also to aminohexonic acid, one would observe that the conclusions reached on the basis of the equilibrium of the two epimers are identical with those based on the direction of the rotation of carbon atom 2. The fact that the two assumptions are mutually supporting adds weight to the original assumption.

TABLE II.

From.	Rotation of carbon atom 2 in predominating form.	From.	Rotation of carbon atom 2 in predominating form.
Arabinose.	Levo.	Arabinoside.	Levo.
Lyxose.	"	Lyxoside.	"
Xylose.	Dextro.	Xyloside.	Dextro.
Ribose.	Levo.	Riboside.	Levo.

E. Fischer and his coworkers in their work on the Walden inversion have observed that when an amino-acid and its ester are acted upon by nitrous acid, the inversion as a rule occurs in the acid but not in the ester. Admitting that this rule holds also for hexosaminic acids, one would expect that on deamination they will form acids rotating in the direction opposite to that of the original amino-acid. This expectation was realized for every one of the eight hexosaminic acids. On the other hand, when not the acid but the sugar or the acid lactone was deaminized, as a rule no change in the direction of the rotation was observed. Thus also the observations on the Walden inversion are consistent with the original assumption based on the equilibrium of the epimeric forms. It follows that on the basis of every one of the three assumptions, the configuration of the 2-aminohexonic acids is as shown in Table III. If eventually by direct

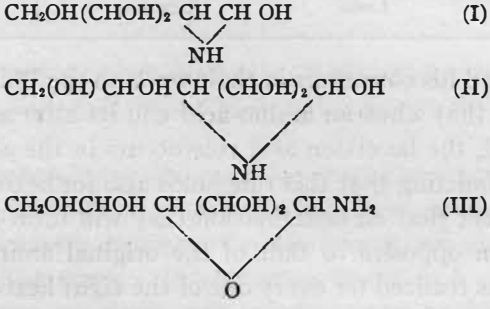
evidence the configuration of carbon atom 2 in any one of these acids should be found the reverse of that postulated above, then our conception of the configuration of the same carbon atom in every other hexosaminic acid will have to be reversed. In other words, the knowledge of the configuration of any one of the 2-amino-hexonic acids is sufficient to explain the configuration of every member of the entire series.

TABLE III.

Chitosaminic acid	2-aminomannonic acid.
Epichitosaminic acid	2-aminogluconic acid.
Chondrosaminic acid	2-aminotalonic acid.
Epichondrosaminic acid	2-aminogalactonic acid.
Dextro-*d*-xylohexosaminic acid	2-aminogulonic acid.
Levo-*d*-xylohexosaminic acid	2-aminoidonic acid.
Dextro-*d*-ribohexosaminic acid	2-aminoallonic acid.
Levo-*d*-ribohexosaminic acid	2-aminoaltronic acid.

2. STRUCTURE OF THE AMMONIA DERIVATIVES OF SUGARS.

Three alternative structures have been suggested for these derivatives.



Structure (I) is advanced by Lobry de Bruyn, (II) by Wohl, and (III) by Irvine, Thomson, and Garret. Hitherto none of these theories was supported by experimental evidence. This was furnished in the course of the present work (Levene, 1916, *b*); namely, it was demonstrated that the nitrogen in these compounds is present in the form of a primary amino group (Table IV). To

the butylene ring, preference is given over the ethylene structure because of the analogy with the structure of other glucosides.

TABLE IV.

Relation of Total Nitrogen to Amino Nitrogen in Aminopentosides. Theory = 9.40 per cent.

	Total N	NH_2N
	per cent	*per cent*
Aminoriboside	9.73	9.39
	9.06	8.78
Aminolyxoside	9.64	9.16

3. PREPARATION AND EQUILIBRIUM OF EPIMERS OF HEXOSAMINIC ACIDS.

When the natural hexosamine is readily accessible, the oxidation of it with bromine or with mercuric oxide often leads to the hexosaminic acid (Pringsheim and Ruschmann, 1915). Conditions of oxidation have to be adapted to each individual sugar. The method sometimes fails. Of the three crystalline hexosamines prepared up to the present date, two were oxidized readily; the third, epichitosamine, could not be oxidized to the corresponding acid.

When one 2-aminohexonic acid is obtained from a natural sugar, its epimer is readily prepared by heating with pyridine in the same manner as in the case of the formation of talonic from galactonic acid. Epichitosaminic acid was prepared in this way (Levene, 1917, *b*).

The synthesis of 2-aminohexonic acids from aminopentosides is accomplished by its condensation with prussic acid. Fischer and Leuchs have applied the process for the synthesis of chitosaminic acid from the aminoarabinoside. The yield, in their experience, was 10 per cent of the riboside. It was observed in the course of the present work that the conditions of the reaction must be adapted to each pentoside. The temperature and duration of the reaction are the conditions which determine the success of the operation. When the optimal conditions are found, the yield of the acid is about 55 per cent of the employed pentoside.

The separation of the two epimers formed by the synthesis can be accomplished by fractional crystallization. For final purification, whenever possible the acids are converted into their crystalline derivatives. The behavior of the acids towards benzaldehyde was exploited for this purpose. Individual acids react differently with this reagent. Often one epimer forms a benzilidene derivative, whereas the other epimer under identical conditions does not combine with the aldehyde and in its place a non-substituted lactone is formed. Thus chitosaminic acid forms a benzilidene-chitosaminic-ethyl-ester; its epimer forms a lactone. Dextro-*d*-xylohexosaminic gave a dibenzilidene-xylohexosaminic-ethyl-ester; its epimer, a monobenzilidene lactone. Dextro-*d*-ribohexosaminic gave the dibenzilidene lactone; its epimer, the lactone.

Lyxo-*d*-hexosaminic acids do not form crystalline lactones, esters, or benzilidene derivatives, but the levo-*d*-xylohexosaminic on reduction forms a crystalline hydrochloride of the sugar, whereas the epimer does not do so.

Thus it was possible to obtain sufficient proof of the purity of each individual epimer of four pairs of *d*-hexosaminic acids. The equilibria of the two epimers derived on the one hand from each pentose, and on the other from the corresponding aminopentoside, are compared in Table I.

4. RELATION OF OPTICAL ROTATION OF EPIMERIC MONOCARBOXYLIC SUGAR ACIDS AND OF HEXOSAMINIC ACIDS.

According to van't Hoff's theory, substances containing several asymmetric carbon atoms possess a rotatory power equal to the algebraic sum of the rotations of each group. Hudson (1909) has successfully applied van't Hoff's theory to the study of the configuration of the terminal carbon atom in glucosides. In application to the monocarboxylic acids, van't Hoff's rule could be made use of in the following way (Levene, 1915; Levene and Meyer, 1916, 1917). Given two epimeric acids differing in the configuration of their carbon atom 2, such as

1	COOH	COOH
2	OHCH	HCOH
3	OHCH	OHCH
4	HCOH	HCOH
5	HCOH	HCOH
6	CH_2OH	CH_2OH

the part of the molecule containing carbon atoms 1 and 2 may be designated by the letter B, and the part containing carbon atoms 3, 4, 5, and 6 by the letter A. The algebraic sum of one epimer is A + B, and of the other A − B. From this it follows that if

$$A + B = m, \text{ and } A - B = n, \text{ then } A = \frac{m + n}{2}$$

$$B = m - A = \frac{2m - m - n}{2} = \frac{m - n}{2}$$

$$-B = n - A = \frac{2n - m - n}{2} = -\frac{m - n}{2}$$

In this manner the optical rotation of carbon atom 2 was calculated in derivatives of several pairs of sugar acids. The results are presented in Tables V and VI.

From these data it is evident that there exists a constant correlation between the configuration and the direction of the optical rotation of carbon atom 2. In the acids with an allocation of the carbon atom as in gluconic acid, carbon atom 2 rotates to the right, and in those with the carbon atom as in mannonic, to the left.

Later, Hudson and his coworkers (1917, 1918, 1919), and also Weerman, calculated the optical rotation of carbon atom 2 in a series of sugar acids, and found that the direction of the rotation of carbon atom 2 determines the direction of the rotation of the acid. The rule of Hudson holds for most sugar acids, but is not as general as the first rule of the present writer. For the 2-5-anhydro acids the rule of Hudson does not hold, whereas the original rule holds. Whether the rule of Hudson or of the present writer is applied, the identical conclusion is reached regarding the correlation of the configuration of carbon atom 2 and the optical rotation of the acid. In the dextrorotatory acids, the configuration of this carbon is as in gluconic, and *vice versa* in the levorotatory as in mannonic.

TABLE V.

Inorganic salts.	C	$[\alpha]_D^{20}$	Rotation of carbon atom 2. $[\alpha]_D^{20}$	$[M]_D^{20}$
d-Na gluconate	20	+11.78	+10.29	+22.42 (10^2)
" mannonate	10	− 8.82	−10.29	−22.42 (10^2)
" gulonate	10	+12.68	+ 7.60	+16.55 (10^2)
" idonate	10	− 2.52	− 7.60	−16.55 (10^2)
" galactonate	10	+ 0.40	—	
" talonate	10	Not determined.	—	
" allonate	10	+ 4.30	+ 4.20	+ 9.15 (10^2)
" altronate	10	+ 4.05	− 4.20	− 9.15 (10^2)
d-Ca chitarate	10	+70.29	+18.32	+39.72 (10^2)
" chitonate	10	+33.65	−18.32	−39.72 (10^2)

TABLE VI.

Brucine salts.	M. P.	C	$[\alpha]_D^{20}$	Rotation of carbon atom 2. $[\alpha]_D^{20}$	$[M]_D^{20}$
	°C.				
d-Gluconate	155	2.5	−15.95	+4.85	+28.50 (10^2)
d-Mannonate	212	2.5	−25.70	−4.85	−28.50 (10^2)
d-Gulonate	162–4	2.5	−19.59	+3.1	+18.20 (10^2)
d-Idonate	188	2.5	−25.79	−3.1	−18.20 (10^2)
d-Galactonate	170	2.5	−21.01	+2.57	+15.10 (10^2)
d-Talonate	132	2.5	−26.15	−2.57	−15.10 (10^2)
d-Allonate	160	2.5	−21.28	+1.28	+ 7.51 (10^2)
d-Altronate	158	2.5	−23.82	−1.28	− 7.51 (10^2)
Chitarate	195	5.0	− 2.96	+2.72	+14.15 (10^2)
Chitonate	222	5.0	− 8.47	−2.72	−14.15 (10^2)

TABLE VII.

Acid.	$[\alpha]_D$ of carbon atom 2.	$[M]_D^{20}$	Phenyl-hydrazide.	$[\alpha]_D$ of carbon atom 2.	$[M]_D^{20}$
Epichitosaminic	+12.5	+24.37 (10^2)	Gluconic.	+14.25	+42.18 (10^2)
Chitosaminic	−12.5	−24.37 (10^2)	Mannonic.	−14.25	−42.18 (10^2)
Dextro-xylohexosaminic	+12.5	+24.37 (10^2)	Gulonic.	+14.25	+42.18 (10^2)
Levo-xylohexosaminic	−12.5	−24.37 (10^2)	Idonic.	−14.25	−42.18 (10^2)
Epichondrosaminic	+12.5	+24.37 (10^2)	Galactonic.	+ 8.25	+24.42 (10^2)
Chondrosaminic	−12.5	−24.37 (10^2)	Talonic.	− 8.25	−24.42 (10^2)
Dextro-ribohexosaminic	+19.12	+37.28 (10^2)	Allonic.	+20.8	+61.56 (10^2)
Levo-ribohexosaminic	−19.12	−37.28 (10^2)	Altronic.	−20.8	−61.56 (10^2)

Configuration and Rotation of Epimeric Hexosaminic Acids.—All of the eight possible *d*-hexosaminic acids have been prepared. Applying the above method for calculating the value and the direction of the rotation of carbon atom 2, it is observed that in the 2-aminohexonic acid also the rotation of carbon atom 2 is to the right in one of the epimers and to the left in the other. Comparing the numerical value of the rotation of this carbon atom in hexosaminic acids and the value of the rotation of the same carbon atom in the phenylhydrazides of the corresponding hexonic acids, one is struck by the identity of the values in the two series. The exception in the pair of galactonic-talonic epimers is undoubtedly due to the fact that talonic acid has not yet been prepared in pure state. The values are identical for the specific, but not for the molecular rotations.

Table VII contains the values of the rotations of carbon atom 2 in the hexosaminic acids and in the corresponding phenylhydrazides of hexonic acids.

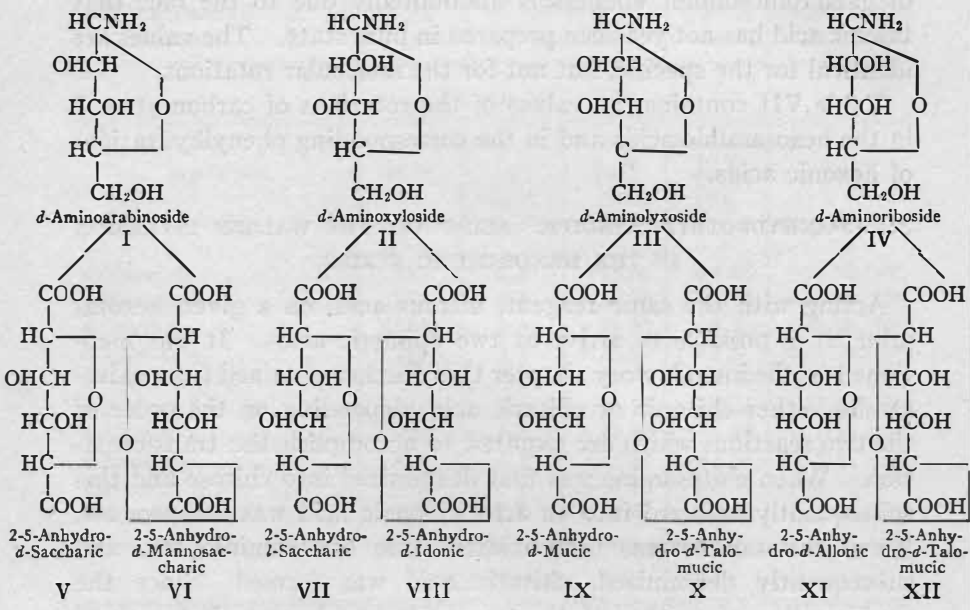
5. 2-5-ANHYDROTETROXYADIPIC[1] ACIDS AND THE WALDEN INVERSION IN THE HEXOSAMINIC SERIES.

Acting with the same reagent, nitrous acid, on a given hexosamine, it is possible to arrive at two epimeric acids. It was mentioned in the introductory chapter that Fischer obtained from chitosamine either chitonic or chitaric acid, depending on the order of the two reactions which are required to accomplish the transformation. When chitosamine was first deaminized into chitose and this subsequently oxidized into an acid, chitonic acid was the product. When chitosamine was first oxidized into chitosaminic acid and subsequently deaminized, chitaric acid was formed. Since the configuration of neither of the two acids was known, it remained impossible to determine in which of the two the direction of the rotation of carbon atom 2 was reversed from that of the original hexosamine. Chitonic and chitaric acids are 2-5-anhydrohexonic acids. It became necessary to establish the configuration of the series of these acids in order to establish in which of the two hydroxy acids formed from a hexosaminic acid, the rotation of carbon atom 2 was in a direction opposite to that of carbon atom 2 in the parent hexosaminic acid.

[1] The carbon atoms are numbered as in the corresponding hexoses.

The configuration of carbon atoms 2, 3, 4, and 5, remains the same in hexoses and in the corresponding hexonic and tetroxyadipic acids. Similarly, it remains the same in anhydrohexonic and anhydrotetroxyadipic acids. The configuration of the series of hexoses was made clear by Fischer on the basis of the mutual relationship of tetroxyadipic acids. In a similar way the configuration of 2-5-anhydrohexonic acids was established by the mutual relationship of the 2-5-anhydrotetroxyadipic acids.

The trend of thought which led to the solution of the problem is seen from the following structures.



It is seen that Structures (V) and (VI) are antipodal anhydrosaccharic acids, hence (VI) is mannosaccharic, and (VIII) idosaccharic. Of the pair of anhydro acids derived from the lyxoside, the inactive form is dihydromucic (IX) and the other is identical with (XII) derived from the riboside and has the structure of dihydro-*d*-talomucic. The second epimeric form from the riboside has the configuration of anhydro-*d*-allomucic. Thus the configuration of the anhydrotetroxy acids was established and simultaneously the config-

uration of the corresponding 2-5-anhydrohexonic acids and of the 2-5-anhydrohexoses was made clear.

Since it is permissible to refer to the anhydrotetroxyadipic instead of to the anhydrohexonic acid, it is possible to compare the rotation of carbon atom 2 in these and in the hexosaminic acids from which they are derived.

From Table VIII it is seen that the direction of the rotation of carbon atom 2 is reversed when the 2-aminohexonic acid is converted

TABLE VIII.

Acid.	Rotation of carbon atom 2.	Obtainable from.	Rotation of carbon atom 2.
Anhydro-*d*-gluconic* (chitaric).	Right.	Chitosaminic acid.	Left.
" -*d*-mannonic (chitonic).	Left.	Chitosamine.	"
" "	"	Epichitosaminic acid.	Right.
" "	"	Epichitosaminic lactone.	"
" -*d*-gulonic.	Right.	Levo-xylohexosaminic acid.	Left.
" "	"	Dextro-xylohexosaminic lactone.	Right.
" -*d*-idonic.	Left.	Dextro-xylohexosaminic acid.	"
" -*d*-galactonic.	Right.	Chondrosaminic acid.	Left.
" -*d*-talonic.	Left.	Chondrosamine.	"
" "	"	Epichondrosaminic acid.	Right.
" -*d*-altronic.	"	Dextro-ribohexosaminic acid.	"
" -*d*-allonic.	Right.	Levo-*d*-ribohexosaminic acid.	Left.

* Epichitosamine ought to form the same anhydro acid; however, it is the only substance of this group that does not form the anhydro substance. On oxidation it gives rise to saccharic acid. From the viewpoint of configuration the result is as expected.

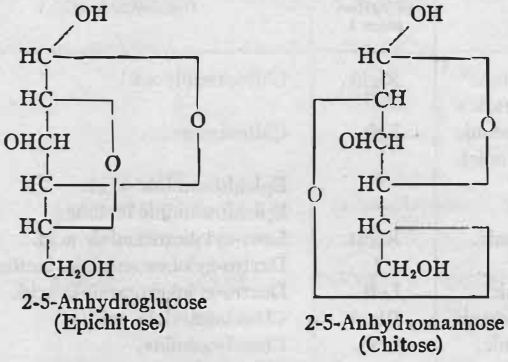
into the 2-5-anhydrohexonic acid. In the three instances when the amino sugar was deaminized prior to oxidation, the direction of the rotation of carbon atom 2 remained unaltered. Of the two occasions when lactones were deaminized, in one the direction of the rotation of carbon atom 2 was reversed, in the other not.

Thus, starting from the hexosamine, it is possible to arrive at two epimeric 2-5-anhydrohexonic acids. Consequently a Walden inversion has taken place on one occasion, probably when the hexosaminic acid was deaminized.

6. CONFIGURATION OF CHITOSE AND EPICHITOSE.

It has been known for a long time that on treatment with nitrous acid a nitrogen-free 2-5-anhydrohexose was formed. The configuration of the sugar remained unknown. On the basis of the present work (Levene and La Forge, 1915, *b*; Levene, 1919), namely in view of the fact that chitose is oxidized to 2-5-anhydromannonic acid, it is evident that chitose has the configuration of 2-5-anhydromannose.

Similarly the new anhydrohexose prepared in the course of this work from epichitosamine has the structure of 2-5-anhydroglucose.



7. 3-AMINOHEPTONIC ACIDS.

In conclusion, mention may be made of the synthesis of the two pairs of epimeric 3-aminoheptonic acids, namely 3-aminochitoheptonic and 3-aminochondroheptonic acids. These acids were prepared with the object of determining the configuration of their deaminized derivatives, as these in their turn may be of service in connection with further work on the configuration of the corresponding 2-aminohexonic acids.

EXPERIMENTAL.

1. CONVERSION OF HEXOSAMINIC ACIDS INTO THEIR EPIMERS.

This change can be accomplished by heating with an aqueous solution of pyridine at 100–105°C. for 4 hours (Levene, 1918, *a*; 1916, *e*).

In a preliminary way the formation of the epimer was shown by the fact that from both chitosaminic and chondrosaminic acids on heating with pyridine, fractions were obtained with a distinctly different optical rotation, thus:

Acid.	$[\alpha]_D$ Original.	After treatment with pyridine.
Chitosaminic	−15.02	+2.11°, +6.75°, +7.72°, +8.57°
Chondrosaminic	−16.15	−4.83°

Later by this process from pure chitosaminic, epichitosaminic acid was obtained. The details are as follows:

100 gm. of chitosaminic acid are taken up in 1,000 cc. of water to which 100 gm. of pyridine are added and heated in an autoclave at 105°C. for 4 hours. The solution is then evaporated under diminished pressure to a small volume, so that the greater part of chitosaminic acid crystallizes in the distillation flask. The residue is again brought into solution with a minimum amount of boiling water. To the solution while still hot an equal volume of 95 per cent alcohol is added. The solution then becomes filled with crystals of chitosaminic acid. To complete the crystallization the material is allowed to stand from 8 to 12 hours.

The filtrate is then concentrated under diminished pressure (the temperature of the water bath should not exceed 50°C.) to a small volume. To this, hot 95 per cent alcohol is added until the solution begins to show opalescence, and enough hot water is then added to clarify the solution. On standing, more readily after some scratching, a crystalline substance begins to deposit. The deposit reaches its maximum in about 24 hours. This substance is dissolved in a minimum amount of hot water, boiled with charcoal, and the filtrate treated with 95 per cent alcohol in the manner just described. The crystalline substance obtained is at times nearly colorless, and at times of very light tan. It consists of a mixture of varying proportions of chitosaminic and epichitosaminic acids. On one occasion it consisted of pure chitosaminic acid. The optical rotation of the substance varied from $[\alpha]_D^{20} = +3.0°$ to $+8.0°$, and on one occasion it had $[\alpha]_D^{20} = +10.0°$.

For further purification the epichitosaminic acid was converted into its lactone. This was accomplished in the following way: to 3 gm. of the acid 2 cc. of 99.5 per cent alcohol and 1 cc. of benzaldehyde were added, and dry hydrochloric acid gas was passed. The acid first goes into solution and subsequently a crystalline deposit begins to reappear. The treatment with the gas is continued for approximately 7 minutes. After standing 8 to 12 hours, 3 cc. of dry ether (dried over sodium) were added, and the material was allowed to stand an additional 12 or 24 hours in order to obtain the maximum yield of the lactone hydrochloride. The addition of benzaldehyde has for its purpose the conversion of the chitosaminic acid into the benzal of its ethyl ester. Under these conditions it remains in solution and permits the crystallization of the epichitosaminic acid lactone. In the absence of benzaldehyde an amorphous precipitate is formed which probably consists of a mixture of the two lactones.

The lactone is crystallized out of methyl alcohol. For this purpose it is advisable to dissolve the crude substance in a large excess of hot methyl alcohol, to decolorize the solution with charcoal, and then to concentrate it to a small volume. The lactone crystallizes in the form of colorless prismatic needles. The substance had a melting point of 203°C. (with gas evolution). Its composition was as follows:

0.1040 gm. substance: 0.1286 gm. CO_2 and 0.1040 gm. H_2O.
0.1000 " " required (Kjeldahl) 4.65 cc. 0.1 N acid.
0.1000 " " " (Volhard) 4.50 " 0.1 N $AgNO_3$.

$C_6H_{12}NO_5Cl$. Calculated. C 33.71, H 5.67, N 6.55, Cl 16.25.
Found. " 33.72, " 5.47, " 6.51, " 16.00.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.90 \times 100}{1 \times 2} = +45.0°$$

a. Epichitosaminic Acid.

2 gm. of the lactone were dissolved in 20 cc. of distilled water and the solution was rendered alkaline by means of a solution of barium hydroxide. The solution was allowed to stand over night, then the

barium was removed quantitatively by means of sulfuric acid, the hydrochloric acid by silver carbonate, and the excess of the latter reagent by hydrogen sulfide. The mother liquor of the silver sulfide was concentrated to a small volume; on addition of a little alcohol the epichondrosaminic acid crystallized immediately in prismatic needles. The substance had a melting point of 198°C. (uncorrected) with gas evolution. The composition of the substance was the following:

0.0992 gm. substance: 0.1336 gm. CO_2 and 0.0606 gm. H_2O.

$C_6H_{13}NO_6$.	Calculated.	C 36.92,	H 6.66.
	Found.	" 36.80,	" 6.72.

The optical rotation of the substance was

Initial. Equilibrium.

$$[\alpha]_D^{20} = \frac{+0.20 \times 100}{1 \times 2} = +10° \qquad [\alpha]_D^{20} = \frac{+0.78 \times 100}{1 \times 2} = +39°$$

2. PREPARATION OF HEXOSAMINIC ACIDS FROM *l*-AMINOPENTOSIDES.

The optimal concentrations of pentosides and prussic acid were found identical for all; namely, 30 gm. of the pentoside dried first over soda lime under diminished pressure at room temperature and finally at 50°C. also at diminished pressure, are taken up in 50 cc. of water to which 40 cc. of 80 per cent prussic acid and 5 cc. of ammonia water are added (Levene, 1918, *a*). The solution is warmed and kept at constant temperature until the reaction is ended. The colorless solution turns at first very light brown and gradually darkens. The originally thin solution gradually turns viscous. After some experience the end of the reaction can be determined by the appearance of the solution. The temperature and duration of the experiment varies with the configuration of the pentoside. Lyxoside is warmed to 35°C. and maintained at that temperature for 8 to 10 minutes. Xyloside is warmed to about 30°C. and maintained at that temperature for 15 to 20 minutes. Subsequent treatment is the same in every instance and is as follows:

The flask is immersed in a cooling mixture and the temperature of the solution allowed to fall to 0°C., and is poured into 300 cc. of

concentrated hydrochloric acid previously cooled to the same temperature. The solution is then saturated with hydrochloric acid gas and allowed to stand not less than 1 hour; it may be allowed to stand over night. The solution is concentrated under diminished pressure, the temperature of the water bath not exceeding 75°C. In the course of the concentration considerable ammonium chloride crystallizes out in the flask. When the solution becomes quite viscous the concentration is interrupted and the contents of the flask are poured into 2 liters of 95 per cent alcohol. Generally a small quantity of tar separates out which is removed by filtration through cotton wool.[2] The alcoholic filtrate is concentrated under diminished pressure, the residue is dissolved in about 1 liter of water, 200 gm. of moist barium hydroxide are added, and the mixture is repeatedly evaporated nearly to dryness, the water being renewed at this phase. When all ammonia is removed the evaporation is interrupted and the barium is removed quantitatively by means of sulfuric acid, the hydrochloric acid by means of lead and silver carbonate. The final solution is concentrated under diminished pressure to a volume of about 50 cc. To this solution hot methyl alcohol is added gradually in small portions and the solution is kept on a hot water bath until a crystalline deposit begins to form.

a. Chondrosaminic and Epichondrosaminic Acids.

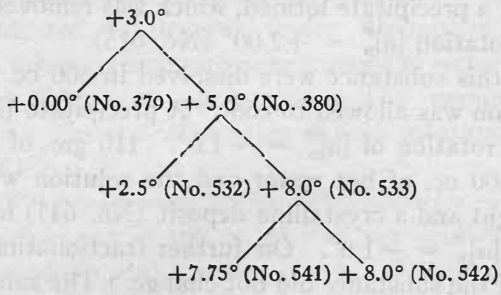
The specific rotation of the crude material was + 3.0°. Since the specific rotation of chondrosaminic acid is − 17.0°, it is evident that the material is a mixture of the two epimers (Levene, 1918, *a*; Levene and La Forge, 1915, *c*).

Separation of the Epimers.—250 gm. of mixed acid having a rotation of $[\alpha]_D^{20} = +3.0°$ were dissolved in 750 cc. of hot water and allowed to stand over night at room temperature. By far the greater part of the material crystallized out. The rotation of this substance (No. 379) was $[\alpha]_D^{20} = 0.00°$. The mother liquor was concentrated under diminished pressure to dryness and the residue again crystallized out of a minimum amount of water. A crystalline deposit formed on standing over night (No. 532) with a rotation of $[\alpha]_D^{20} = +2.5°$. The mother liquor was concentrated to dryness, taken up in a minimum amount of water, and hot methyl alcohol was added

[2] Treatment with alcohol may be omitted.

to opalescence. Soon a crystalline deposit formed (No. 533) which had a rotation of $[\alpha]_D^{20} = +8.0°$. This material was again dissolved in a minimum amount of hot water and allowed to stand at room temperature over night. A crystalline deposit formed (No. 541) with a rotation of $[\alpha]_D^{20} = +7.75°$. The mother liquor was concentrated under diminished pressure to dryness. The residue was taken up in a minimum amount of water, and hot methyl alcohol was added until the appearance of a slight opalescence; the solution soon turned into a crystalline mass. The substance (No. 542) had a rotation of $[\alpha]_D^{20} = +8°$, thus showing that the final treatment did not accomplish any further fractionation.

The progress of fractionation is represented in the following diagram.



The substance with the rotation $[\alpha]_D^{20} = 0.00°$ was continually recrystallized out of water until a fraction was obtained with a rotation $[\alpha]_D^{20} = -17.5°$. The dextro epimer had the following composition:

0.1004 gm. substance: 0.1358 gm. CO_2 and 0.0624 gm. H_2O.

$C_6H_{13}NO_6$.	Calculated.	C	36.92,	H	6.66.
	Found.	"	36.89,	"	6.96.

The melting point was 206°C. (uncorrected) with gas evolution. The optical rotation was

$$[\alpha]_D^{20} = \frac{+0.16 \times 100}{1 \times 2} = +8°$$

The synthetic chondrosaminic acid is identical with that prepared on oxidation of the natural chondrosamine.

b. Xylohexosaminic Acids.

The optical rotation of the mixed acids was (Levene, 1918, *a*; Levene and La Forge, 1915, *b*)

$$[\alpha]_D^{20} = \frac{+0.07 \times 100}{1 \times 2} = +3.5°$$

Separation of the Epimers.—280 gm. of the mixed acids were dissolved in a minimum amount of water, and methyl alcohol was added until a crystalline precipitate began to form. This was filtered off. It had a rotation of $[\alpha]_D^{20} = +5.0°$. The mother liquor was concentrated to dryness under diminished pressure. The residue was then taken up in a minimum amount of water and on addition of alcohol a precipitate formed, which was removed by filtration and had the rotation $[\alpha]_D^{20} = +2.00°$ (No. 615).

180 gm. of this substance were dissolved in 300 cc. of hot water, and the solution was allowed to cool. A precipitate (No. 630) thus formed had a rotation of $[\alpha]_D^{20} = -1.0°$. 110 gm. of No. 630 were dissolved in 400 cc. of hot water and the solution was allowed to stand over night and a crystalline deposit (No. 641) formed having a rotation of $[\alpha]_D^{20} = -1.0°$. On further fractionation of No. 641 the rotation of the substance did not change. The substance melted at 200°C. (uncorrected) with gas evolution. The composition was the following:

0.1000 gm. substance: 0.1354 gm. CO_2 and 0.0590 gm. H_2O.
0.1000 " " required (Kjeldahl) 5.25 cc. 0.1 N acid.

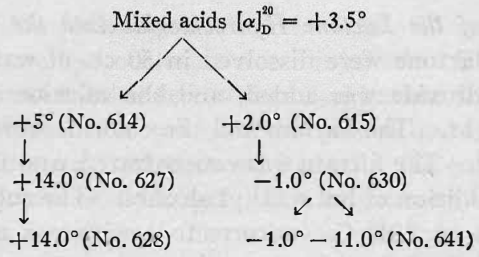
$C_6H_{13}NO_5$.	Calculated.	C	36.92,	H	6.66,	N 7.18.
	Found.	"	36.92,	"	6.60,	" 7.35.

The rotation of the substance was

Initial. Equilibrium.

$$[\alpha]_D^{20} = \frac{-0.22 \times 100}{1 \times 2} = -11.0° \qquad [\alpha]_D^{20} = \frac{-0.63 \times 100}{1 \times 2} = -31.5°$$

The progress of the fractionation of the epimers is given in the following chart:



The substance with rotation $[\alpha]_D^{20} = +5°$ was dissolved in a minimum amount of boiling water, allowed to stand 30 minutes, and while still warm was filtered. The precipitate thus formed had a rotation of $[\alpha]_D^{20} = +14.0°$. The yield of the substance was 87 gm. This was dissolved in 400 cc. of boiling water. On cooling, a precipitate was formed which was removed by filtration. The mother liquor was concentrated to dryness under diminished pressure. The residue was taken up in a minimum amount of water, and hot methyl alcohol was added to opalescence. The crystallization began immediately. The optical rotation of both substances was identical, $[\alpha]_D^{20} = +14.0°$.

d-Dextro-Xylohexosaminic Lactone Hydrochloride.—10 gm. of amino-acid were suspended in 100 cc. of 99.5 per cent ethyl alcohol into which hydrochloric acid gas was passed, without cooling, until the alcohol was saturated. At first the substance passed into solution and immediately afterwards a small amount of a white precipitate began to form. This in turn redissolved as more hydrochloric acid was passed in. Finally, after the solution had become saturated, crystallization of the lactone began and was complete after the solution had stood in the cold for a few hours. The crystals were then filtered off and washed with absolute alcohol. The yield was practically quantitative. The substance crystallizes in compact aggregates of prismatic needles grouped together in the form of spheres. It was recrystallized from an excess of methyl alcohol. The substance had a melting point of 205°C. (uncorrected) with gas evolution. The composition was as follows:

0.1000 gm. substance: 0.1234 gm. CO_2 and 0.0504 gm. H_2O.
0.2000 " " required (Kjeldahl) 9.31 cc. 0.1 N acid.
0.1000 " " " (Volhard) 4.5 cc. 0.1 N $AgNO_3$.

$C_6H_{12}NO_5Cl$.	Calculated.	C	33.71,	H	5.67,	N	6.55,	Cl 16.25.
	Found.	"	33.65,	"	5.64,	"	6.52,	" 16.00.

Conversion of the Lactone Hydrochloride into the Parent Acid.—5 gm. of the lactone were dissolved in 50 cc. of water. An excess of barium hydroxide was added, and the solution was allowed to stand over night. The barium and the chlorine were then removed quantitatively. The filtrate was concentrated, and the acid crystallized on the addition of hot methyl alcohol. The substance had the melting point of 224° C. (uncorrected) with gas evolution. The substance had the following composition:

0.1070 gm. substance: 0.1372 gm. CO_2 and 0.0620 gm. H_2O.
0.010 " " : 1.27 cc. N_2 in the Van Slyke micro apparatus at 26°C. and 759 mm.

$C_6H_{13}NO_5$. Calculated. C 36.92, H 6.66, N 7.18.
Found. " 37.04, " 6.86, " 6.99.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.28 \times 100}{1 \times 2} = +14.0°$$

Monobenzal-d-Levo-Xylohexosaminic Lactone Hydrochloride.—2.5 gm. of the *d*-levo-hexosaminic acid were taken up in a solution of 15 cc. of 99.5 per cent alcohol and 3 cc. of benzaldehyde. Hydrochloric acid gas was passed through the solution. The amino-acid went gradually into solution and after some time the entire solution solidified instantaneously. After 1 hour 2 cc. of ether (dried over sodium) were added and the material was allowed to stand an additional 2 hours. The lactone was then filtered and recrystallized out of methyl alcohol. The melting point was 206° C. (uncorrected) with gas evolution.

0.1023 gm. substance: 0.1938 gm. CO_2 and 0.0508 gm. H_2O.
0.1000 " " required (Kjeldahl) 3.34 cc. 0.1 N acid.

$C_{13}H_{15}NO_5HCl$ (molecular wt. 301.6). Calculated. C 51.72, H 5.35, N 4.69.
Found. " 51.81, " 5.57, " 4.68.

The optical rotation of the substance in 50 per cent alcohol was

$$[\alpha]_D^{20} = \frac{-1.21 \times 100}{1 \times 2} = -60.5°$$

Conversion of the Lactone into the Free Acid.—10 gm. of the dry monobenzal lactone hydrochloride were dissolved in 50 cc. of water, and to the solution were added 12.5 gm. of barium hydroxide containing 5 gm. of Ba. The solution was placed on a boiling water bath. It was soon noticed that on warming the solution turned yellow and ammonia was formed. The experiment was therefore interrupted after 7 minutes. The solution was then rendered acid with sulfuric acid. The filtrate from the barium sulfate precipitate was extracted repeatedly with ether, and the aqueous solution was freed quantitatively from sulfuric and hydrochloric acids. The filtrate was concentrated and the amino-acid crystallized on addition of hot methyl alcohol. The melting point of the acid was 200°C. (uncorrected) with gas evolution. The substance had the following composition:

0.1000 gm. substance: 0.1354 gm. CO_2 and 0.6000 gm. H_2O.
0.0100 " " : 1.36 cc. N_2 in the Van Slyke micro apparatus at 35°C. and 762 mm.

$C_6H_{13}NO_6$.	Calculated.	C	36.92,	H	6.66,	N 7.18.
	Found.	"	36.98,	"	6.71,	" 7.16.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.22 \times 100}{1 \times 2} = -11.0° \quad \text{Initial.}$$

$$[\alpha]_D^{20} = \frac{-0.60 \times 100}{1 \times 2} = -30.0° \quad \text{Equilibrium.}$$

c. Ribohexosaminic Acids.

The optical rotation of the mixed acids was (Levene and Clark, 1921)

$$[\alpha]_D^{20} = \frac{-0.16 \times 100}{1 \times 2} = -8.0°$$

Separation of the Two Acids.

Levo-d-Ribohexosaminic Acid.—This is the more insoluble form and is prepared without much difficulty. Two experiments are here reported.

Experiment 1.—57 gm. of the mixed acids were dissolved in 70 cc. of boiling water and allowed to crystallize over night. The yield

of the crystalline deposit was 22 gm. This substance had the following optical rotation (No. $638\frac{19}{20}$):

$$[\alpha]_D^{20} = \frac{-0.52 \times 100}{1 \times 2} = -26°$$

This rotation indicates that the substance was the pure levo form. On recrystallization from water two fractions were obtained (Nos. 640 and $641\frac{19}{20}$), each having the same rotation, and, as will be shown later, the substance prepared from the pure crystalline lactone possesses the same optical rotation.

Experiment 2.—142 gm. of the mixed acids were dissolved in 300 cc. of boiling water. 35 gm. of the levo form crystallized over night. The rotation of the substance was as follows (No. $88\frac{20}{21}$):

$$[\alpha]_D^{20} = \frac{-0.52 \times 100}{1 \times 2} = -26.0°$$

On further recrystallization the rotation of the substance did not change.

Properties of the Levo-d-Ribohexosaminic Acid.—The substance crystallizes in thin plates resembling those of cholesterol. It is soluble in water and insoluble in the usual organic solvents. The melting point of the substance is 212°C. (uncorrected) with decomposition.

Lactone Hydrochloride of the Levo-d-Ribohexosaminic Acid.—12 gm. of the acid carefully dried and pulverized were suspended in 600 cc. of alcohol (99.5 per cent) and dry hydrochloric acid gas was passed in for 15 minutes. The acid dissolved almost immediately. The solution was concentrated under diminished pressure at room temperature until crystallization took place in the distillation flask. The contents were then transferred to an evaporating dish and allowed to stand over night in a desiccator over sulfuric acid. The yield of the lactone was 12 gm. The melting point of the substance was 188°C. (uncorrected). It had the following composition (No. $24\frac{20}{21}$):

0.1076 gm. substance: 0.1304 gm. CO_2 and 0.0592 gm. H_2O.
0.2000 " " required (Kjeldahl) 8.74 cc. 0.1 N acid.
0.2000 " " " (Volhard) 9.00 " 0.1 N $AgNO_3$.

$C_6H_{12}NO_6Cl$.	Calculated.	C	33.71,	H	5.67,	N	6.55,	Cl 16.25.
	Found.	"	33.05,	"	6.17,	"	6.12,	" 16.00.

The substance still contained 0.46 per cent of mineral impurity. The peculiarity of this lactone was that by the Van Slyke method only 4.71 per cent of nitrogen was obtained, even when the reaction was allowed to proceed for 30 minutes.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.22 \times 100}{1 \times 2} = -11.0°$$

Conversion of the Lactone into Free Acid.—5 gm. of the lactone hydrochloride were dissolved in 50 cc. of water. The solution was treated with an excess of barium hydroxide and allowed to stand over night. Barium and chlorine were removed from the solution and the free acid was crystallized on concentration of the aqueous solution. For analysis it was recrystallized out of water on addition of a little alcohol. After three recrystallizations the substance was analyzed. The melting point was 212°C. (uncorrected). It analyzed as follows:

0.1010 gm. substance: 0.1364 gm. CO_2 and 0.1010 gm. H_2O.
0.1990 " " required (Kjeldahl) 10.10 cc. 0.1 N acid.

$C_6H_{13}NO_6$.	Calculated.	C	36.92,	H	6.66,	N 7.18.
	Found.	"	36.83,	"	7.04,	" 7.10.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.52 \times 100}{1 \times 2} = -26°$$

Dextro-d-Ribohexosaminic Acid.—The separation of the dextro form was found more difficult than that of its epimer, and was accompanied with considerable loss of material. The following procedure was finally adopted. All fractions with the optical rotation above 0.0° were combined, dissolved in about 5 to 8 volumes of hot water, and methyl alcohol was added to initial opalescence. The solution was then placed on a boiling water bath and allowed to crystallize. The crystalline deposit was filtered off while the mother liquor was still hot. The operation was repeated until a constant rotation was obtained. This was found to be

$$[\alpha]_D^{20} = \frac{+0.25 \times 100}{1 \times 2} = +12.5°$$

After three recrystallizations the rotation remained unchanged. As will be seen later the substance obtained from the lactone possessed the same optical activity. The substance had a melting point of 186°C. (uncorrected) and analyzed as follows:

0.1057 gm. substance: 0.1422 gm. CO_2 and 0.0626 gm. H_2O.
0.2000 " " required (Kjeldahl) 10.20 cc. 0.1 N acid.

$C_6H_{13}NO_5$. Calculated. C 36.92, H 6.66, N 7.18.
Found. " 36.70, " 6.63, " 7.14.

Lactone Hydrochloride of Dextro-d-Ribohexosaminic Acid.—5 gm. of the acid, carefully dried under diminished pressure at 50°C. and pulverized, were suspended in 300 cc. of absolute alcohol (99.5 per cent), and dry hydrochloric acid gas was passed through the alcohol. Solution was accomplished almost immediately. The gas was passed for 7 minutes. The solution was then concentrated under diminished pressure until a considerable sediment began to form in the distilling flask. The material was then transferred to an Erlenmeyer flask. The sediment on standing increased in volume, but was found to be amorphous. On heating, however, the sediment redissolved and on prolonged standing on the water bath with stirring a sediment of heavy crystals of the lactone settled out. The substance had a melting point of 150°C. (uncorrected) and analyzed as follows:

0.0977 gm. substance: 0.1212 gm. CO_2 and 0.0546 gm. H_2O.
0.1872 " " required (Kjeldahl) 8.85 cc. 0.1 N acid.

$C_6H_{12}NO_5Cl$. Calculated. C 33.71, H 5.67, N 6.55.
Found. " 33.83, " 6.25, " 6.62.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.43 \times 100}{1 \times 2} = +21.5°$$

Conversion of the Lactone into the Dextro-d-Ribohexosaminic Acid.—3 gm. of the lactone hydrochloride were dissolved in 25 cc. of water. The solution was rendered alkaline by means of barium oxide and allowed to stand over night. The barium and the hydrochloric acid were then removed and the remaining aqueous solution was concentrated to a small volume. To the concentrated solution

alcohol was added to opalescence, and the solution was allowed to digest on a boiling water bath until a heavy crystalline deposit formed. The yield of this substance was 2 gm. The melting point of the substance was 186°C. (uncorrected).

The rotation was

$$[\alpha]_D^{20} = \frac{+0.25 \times 100}{1 \times 2} = +12.5°$$

Thus the melting point and the optical rotation of the substance purified through conversion into its lactone and reconversion of this into the acid remained identical with those of the original material.

Dibenzal-Dextro-d-Ribohexosaminic Ethyl Ester Hydrochloride.—The dextro form differed from its epimer in that it formed the above compound under the same condition in which the levo form gave rise to its lactone hydrochloride. 2 gm. of the carefully dried and pulverized acid were suspended in 20 cc. of absolute alcohol (99.5 per cent) to which 2 cc. of redistilled benzaldehyde were added, and dry hydrochloric acid gas was passed through the solution. The acid dissolved rapidly, but the treatment with acid was continued for 7 minutes. The slightly turbid solution was allowed to stand over night. A crystalline deposit consisting microscopically of long needles was formed. It was filtered off, washed with alcohol and ether, dried, and analyzed. The substance (No. 232) had a melting point of 221°C. (uncorrected) and the following composition:

0.1069 gm. substance: 0.3270 gm. CO_2 and 0.0602 gm. H_2O.
0.1978 " " required (Kjeldahl) 4.60 cc. 0.1 N acid.

$C_{22}H_{26}NO_8Cl$.	Calculated.	C	60.65,	H	6.34,	N 3.20.
	Found.	"	60.48,	"	6.31,	" 3.25.

The rotation of the substance dissolved in methyl alcohol was

$$[\alpha]_D^{20} = \frac{-0.26 \times 100}{1 \times 1} = -26°$$

From the mother liquor of the dibenzal derivative on standing a second crop of crystals formed, which once recrystallized had the composition and the physical properties of the lactone hydrochloride of the dextro-*d*-ribohexosaminic acid. This observation is important

inasmuch as it offers additional evidence of the purity of the dextro-*d*-ribohexosaminic acid. The substance analyzed as follows:

0.0942 gm. substance: 0.1174 gm. CO_2 and 0.0512 gm. H_2O.
0.0918 " " required (Kjeldahl) 4.5 cc. 0.1 N acid.

$C_6H_{12}NO_5Cl$. Calculated. C 33.71, H 5.67, N 6.55.
Found. " 33.99, " 6.08. " 6.86.

The optical rotation of the substance in 2.5 per cent hydrochloric acid was

$$[\alpha]_D^{20} = \frac{+0.43 \times 100}{1 \times 2} = +21.5°$$

3. SYNTHESIS OF HEXOSAMINES.

a. Chitosamine.

For the preparation of all lactones of hexosaminic acids it is important to start with very pure material. The glucosaminic acid used in these experiments was recrystallized several times out of water. 5 gm. lots of the acid were taken up in 50 cc. of 99.5 per cent alcohol. Dry hydrochloric acid gas was passed in, in a lively stream. The acid soon dissolved. After a while a flocculent precipitate began to appear. If the solution was actively shaken during this phase nearly all the flocculent precipitate of the esters disappeared. For a while the solution remained clear and then turned quite opaque. At this stage it was found advisable to immerse the flask in a cooling mixture and to continue passing the gas. Soon a precipitate of the lactone appeared. This was allowed to stand over night in the refrigerator. One can wash the lactone by decantation with 99.5 per cent alcohol, and with dry ether, and finally filter the lactone and dry it in a vacuum desiccator. It is then possible to continue the work on the pure lactone. However, it was found that this mode of procedure had no advantage over the less troublesome method of Fischer and Leuchs. In most of the experiments the latter method was followed (Levene, 1916, *c*, *d*; 1917, *a*).

Isolation of Glucosamine Hydrochloride.—5 gm. of the lactone hydrochloride were reduced with 40 gm. of potassium amalgam (2 per cent), hydrochloric acid being used for neutralization. The

reduction was carried out in a vessel immersed in a cooling mixture and provided with a mechanical stirrer. At the end of the experiment the solution tested with Fehling's reagent showed a reduction equivalent to 1.9 gm. of glucose. The reaction product was concentrated nearly to dryness. The residue was taken up in about 30 cc. of methyl alcohol, and concentrated hydrochloric acid was added drop by drop. The flask was carefully warmed over a flame until the syrupy material was all in solution and the salt had the appearance of a white crystalline powder. The salt was filtered. It showed the presence of considerable sugar, judging by its reducing power towards Fehling's solution.

The mother liquor on standing in the refrigerator formed a precipitate of practically pure glucosamine hydrochloride.

0.1030 gm. substance: 0.1246 gm CO_2, 0.0600 gm. H_2O, and 0.0026 gm. ash (2.55 per cent).

$C_6H_{13}NO_5HCl$.	Calculated.	C	33.40,	H	6.54.
	Found.	"	33.85,	"	6.69.

In a few other experiments the sugar contained more salt. This experiment shows that favorable conditions can be found for the direct isolation of glucosamine hydrochloride from the reaction product.

Preparation of the Pentabenzoyl Derivative.—The material used for this preparation was obtained from several reduction experiments. Potassium and sodium amalgam were used for reduction, and hydrochloric acid for neutralization. The reaction product was treated as in the above experiment. The fraction containing only sugar and salt was used for benzoylation.

6.5 gm. of the sugar (estimated by means of Fehling's solution) were taken up in 140 cc. of water, 25 cc. of benzoyl chloride, and 40 cc. of a 50 per cent sodium hydroxide solution. It was shaken for about $1\frac{1}{2}$ hours with constant cooling, and then placed in a shaking machine over night. The benzoyl derivative, which had the appearance of dry white balls, was washed with water, then dissolved in chloroform, and shaken with water in a separatory funnel. Finally the chloroform solution was dried over sodium sulfate, filtered, and concentrated to dryness. The residue was recrystallized out of 4

liters of 98 per cent alcohol. On cooling, the substance crystallized in long colorless needles. The mother liquor on concentration gave a second precipitate. The substance melted at 216°C. and had the following composition:

0.1012 gm. substance: 0.2606 gm. CO_2 and 0.0453 gm. H_2O.
0.2530 " " required (Kjeldahl) 5.05 cc. 0.1 N acid.

$C_6H_8NO_5(C_6H_5CO)_5$.	Calculated.	C	70.38,	H	4.73,	N 2.00.
	Found.	"	70.23,	"	5.00,	" 1.99.

The optical rotation of the substance in pyridine was

$$[\alpha]_D^{20} = \frac{+0.58 \times 1.9992}{0.5 \times 0.0522} = +44°$$

The benzoyl derivative had been converted into the hydrochloride of glucosamine by previous workers; hence it was not considered important to repeat it on this occasion.

b. Chondrosamine.

15 gm. of lyxohexosaminic acid were taken up in three small flasks, each containing 5 gm. of the acid and 50 cc. of 99.5 per cent alcohol (Levene, 1916, *c*; 1917, *a*). Into each a lively stream of dry hydrochloric acid gas was passed. The formation of the lactone proceeded in the same way as with the glucosaminic acid. The flasks were allowed to stand over night, and the mixture was then transferred to a distilling flask by the aid of distilled water. 150 gm. of 2.5 per cent sodium amalgam were used for reduction. At the end of the experiment the solution tested with Fehling's reagent showed a reduction equivalent to 6.5 gm. of glucose.

The product of reaction was concentrated under diminished pressure nearly to dryness. Care was taken to keep the temperature of the water bath at 40°C. The residue was taken up in 75 cc. of methyl alcohol, and concentrated hydrochloric acid added drop by drop until all the syrup dissolved and sodium chloride appeared in the form of white crystalline powder. This first precipitate showed no reducing power.

The filtrate was again concentrated nearly to dryness and taken up in 99.5 per cent ethyl alcohol and hydrochloric acid as before.

On standing, an oily mass separated out. In the course of the night this turned to a crystalline mass. The supernatant liquor was decanted, and the crystalline mass triturated with methyl alcohol. It consisted of long heavy prisms with needle-shaped points. The substance burned as carbohydrates do and left behind a small amount of ash. The liquid which had been decanted off the crystalline mass on standing began to form on the walls and on the bottom of the flask a crystalline deposit consisting of long prisms of the same appearance as those of the crystalline mass. On burning, it left practically no ash.

The substance was redissolved in a little water, and 99.5 per cent alcohol saturated with hydrochloric acid gas was added in small portions until on scratching the sugar began to crystallize. The crystals had the same appearance as on first crystallization. The substance melted at 185°C. (corrected).

The rotation was

Initial. Equilibrium.

$$[\alpha]_D^{25} = \frac{+1.50 \times 2.0392}{1.0 \times 0.0616} = +59.3° \qquad [\alpha]_D^{25} = \frac{+2.18 \times 2.0392}{1.0 \times 0.0516} = +98.8°$$

0.1050 gm. substance dried in a vacuum desiccator over sulfuric acid at 25°C.: 0.1284 gm. CO_2 and 0.0604 gm. H_2O.

0.0244 gm. substance: 2.94 cc. N_2 at 22° and 762 mm.

0.0820 " " required 3.7 cc. 0.1 N $AgNO_3$ solution.

$C_{12}H_{13}O_5N \cdot HCl$.	Calculated.	C	33.40,	H	6.54,	N	6.51,	Cl 16.45.
	Found.	"	33.35,	"	6.39,	"	6.74,	" 16.00.

35 gm. of the synthetic sugar were prepared.

The mother liquor of the crystalline mass was concentrated under diminished pressure. It contained 3 gm. of sugar and was taken up in 400 cc. of water; 8 gm. of phenylhydrazine dissolved in glacial acetic acid were added, and the flask containing the solution was immersed in a boiling water bath for 4 hours. The osazone began forming while the solution was still heated. On cooling, a voluminous precipitate of osazone deposited. The osazone was taken up in 400 cc. of boiling water which was kept boiling while pyridine was added gradually until the solution was complete. After three recrystallizations microscopic slides showed perfectly formed

elongated plates with pointed ends, and an absolute absence of oily droplets. The osazone was then filtered on a suction funnel, transferred into a mixture of equal parts of alcohol and ether, and filtered. It then had the appearance of light orange glistening plates.

It melted at 201°C. and decomposed at 202° (corrected). Mixed with osazone from chondrosamine and with galactosazone it showed the same melting and decomposition points.

0.1006 gm. substance: 14.2 cc. N_2 at 29°C. and 757 mm.

$C_{18}H_{24}N_4O_4$.	Calculated.	H 15.64.
	Found.	" 15.8.

For the rotation of the substance 0.05 gm. was dissolved in 5 cc. of Neuberg's pyridine and alcohol mixture. The initial rotation was + 0.36°; after 24 hours, + 0.03°. (It was not followed further.)

α and β Forms of Chondrosamine Hydrochloride.—A sample of chondrosamine recrystallized out of water and ethyl alcohol saturated with hydrochloric acid.

Initial. Equilibrium.

$$[\alpha]_D^\circ = \frac{+1.54 \times 2.0385}{1 \times 0.0516} = +53.1^\circ \qquad [\alpha]_D^{25} = \frac{+2.28 \times 2.0392}{1 \times 0.0514} = +90.4^\circ$$

A sample was dissolved in a minimum amount of water, and glacial acetic acid was added until the substance began to crystallize, the mixture was brought to a boil, and filtered.

Initial. Equilibrium.

$$[\alpha]_D^\circ = \frac{+3.04 \times 2.5}{1 \times 0.125} = +60.4^\circ \qquad [\alpha]_D^{25} = \frac{+4.5 \times 2.5}{1 \times 0.125} = +90.0^\circ$$

A sample crystallized out of a minimum amount of aqueous hydrochloric acid.

Initial. Equilibrium.

$$[\alpha]_D^\circ = \frac{+2.60 \times 2.5}{1 \times 0.1272} = +57.10^\circ \qquad [\alpha]_D^{25} = \frac{+4.80 \times 2.5}{1 \times 0.1272} = +94.20^\circ$$

No attempt was made to explain the slight discrepancies in the solutions since the principal object was to determine conditions controlling the formation of either one of the forms. As all attempts to obtain a sample with the original rotation failed, the rotation of the original material was redetermined.[3]

[3] Dr. J. López-Suárez and Dr. G. M. Meyer controlled the reading.

Initial. Equilibrium.

$$[\alpha]_D^* = \frac{+2.50 \times 2.5}{1 \times 0.0500} = +125° \qquad [\alpha]_D^{25} = \frac{+1.90 \times 2.0}{1 \times 0.0500} = +95°$$

Calculating on the basis of Hudson's formula, the molecular rotation of the end carbon atom $= \frac{(125.0 - 515.0)}{2} 215.5 = 8{,}400.$ The value found by Hudson for hexoses was in the neighborhood of 8,000. The original form is to be regarded as the α and the new as the β form.

Pentacetyl Derivative of the Synthetic Chondrosamine.—The substance was prepared from the natural chondrosamine by Hudson and Dale. Practically the same conditions were followed in this work.

5 gm. of zinc chloride were dissolved in 30 cc. of acetic anhydride. To this solution 5 gm. of the hydrochloride were added, and the mixture was warmed gently until a lively reaction developed. The reaction was kept up for 2 minutes, then the reaction product was poured into 100 cc. of water cooled to 0°C. The mixture was neutralized with potassium bicarbonate, transferred to a separatory funnel, and extracted with chloroform. The chloroform extract was washed with water. Over the chloroform a layer of crystals appeared which were insoluble in water. The crystals consisted of the more insoluble fraction of the pentacetates. The chloroform extract was evaporated to dryness under diminished pressure, the residue was recrystallized out of alcohol, and from the mother liquor a second crop of crystals was obtained. The top fraction consisted of the pure α form, while the more soluble form was practically the pure β form. The α form turned slightly brown at 232° and melted with decomposition at 237°C. (corrected). The optical rotation in chloroform solution was

$$[\alpha]_D^{20} = \frac{+0.07 \times 20.0}{2 \times 0.0802} = +8.7°$$

A similar fraction from natural chondrosamine had a melting point of 235°C. The optical rotation was

$$[\alpha]_D^{20} = \frac{+0.09 \times 20.0}{2 \times 0.075} = +12°$$

Hudson and Dale found for their β form the melting point 235° (with decomposition) $[\alpha]_D^{20} = +11.00°$.

The β form was apparently not quite pure, but taking into consideration the small quantity of starting material it is rather surprising that each form could be separated with so little difficulty. The melting point of the β form was very sharp at 197°C. The optical rotation in chloroform solution was

$$[\alpha]_D^{20} = \frac{+0.90 \times 20.0}{2 \times 1.000} = +90°$$

Hudson and Dale found for the α form the melting point 182–183°C. and $[\alpha]_D^{20} = 101.3°$. The composition of the pentacetyl derivative was the following:

0.1014 gm. substance: 0.1834 gm. CO_2 and 0.0560 gm. H_2O.

$C_6H_8NO_5(CH_3CO)_5$.	Calculated.	C 49.49,	H 5.96.
	Found.	" 49.32,	" 6.18.

c. Epichitosamine.

The reduction of the lactone into the sugar was carried out in the same manner as on previous occasions (Levene, 1919). Lots of 10 gm. of lactone hydrochloride were taken up in 60 cc. of water and reduced with 125 gm. of a 2 per cent sodium amalgam. The reaction of the solution was kept on the acid side by adding at short intervals small quantities of hydrochloric acid.

The filtrate from the mercury is concentrated at diminished pressure and at room temperature. Care has to be taken to carry out the concentration in such a manner that the material remains colorless. The sodium chloride is separated by fractional precipitation first with methyl alcohol and subsequently with ethyl alcohol. After the product was obtained in such a state of purity that on ignition no ash was visible, the sugar was recrystallized once or twice out of water.

The analysis of the hydrochloride gave the following results:

0.1042 gm. substance: 0.1298 gm. CO_2 and 0.0614 gm. H_2O.

0.0100 " " in Van Slyke's micro apparatus: 1.18 cc. N_2 at 21°C. and 765 mm.

0.0200 gm. substance in Van Slyke's micro apparatus, in 5 minutes: 2.43 cc. N_2 at 25°C. and 764 mm.

The melting point of the substance was 187°C. (corrected).

$C_6H_{13}NO_5HCl$.	Calculated.	C	33.40,	H	6.54,	N	6.51,	Cl 16.45.
Found.	I. (No. $328\frac{1}{1}\frac{7}{8}$)					"	6.73	
"	II. (" $575\frac{1}{1}\frac{7}{8}$)	C	33.97,	H	6.61,			
"	III. (" $111\frac{1}{1}\frac{8}{9}$)							Cl 6.78

The rotation of the substance in 5 per cent HCl solution was

Initial.	Equilibrium.
$[\alpha]_D^{20} = \frac{-0.47 \times 100}{1 \times 10} = -4.7°$	$[\alpha]_D^{20} = \frac{-0.47 \times 100}{1 \times 10} = -4.7°$

Preparation of the Osazone.— 4.6 gm. of the sugar hydrochloride were taken up in 200 cc. of water, the solution was neutralized with sodium acetate, and a solution of 8 gm. of phenylhydrazine in 5 cc. of glacial acetic acid was added and the entire solution allowed to stand on the boiling water bath for 1 hour. An osazone formed which was recrystallized from methyl alcohol. The melting point at rapid heating was 205°C. It had the following composition:

0.1000 gm. substance: 0.2202 gm. CO_2 and 0.5800 gm. H_2O.

$C_{18}H_{24}N_4O_4$.	Calculated.	C	60.33,	H	6.14.
	Found.	"	60.05,	"	6.49.

0.1000 gm. substance dissolved in 10 cc. Neuberg's alcohol-pyridine solution, had an initial rotation, −0.31° and equilibrium −0.15°, which is in accord with the rotation found for glucosazone.

d. Dextro-Xylohexosamine.

The lactone was prepared in the same manner as that of chitosaminic acid (Levene, 1916, *d*). Also in the reduction the general plan was followed. However, all the attempts to obtain a fraction free from the lactone were for the present unsuccessful. When hydrochloric acid was used for neutralization the salt always separated out free from sugar, and when sulfuric acid was employed for the same purpose, salt, lactone, and sugar crystallized in the same fraction.

A solution containing about 6 gm. of the amino sugar and 1 gm. of amino nitrogen was taken up in 500 cc. of water to which 80 cc.

of benzoyl chloride and 110 cc. of a 50 per cent sodium hydroxide solution were added. The benzoylation proceeded as in the glucosamine experiment. The final product was taken up in methyl alcohol, and ether was added as long as it caused the precipitation of an oil. The supernatant liquid was decanted and allowed to evaporate spontaneously. A precipitate of white needles formed. It was filtered off and recrystallized once out of 99.5 per cent alcohol. It melted at 162°C. and had the following composition:

0.1012 gm. substance: 0.2632 gm. CO_2 and 0.0416 gm. H_2O.

$C_6H_8NO_5(C_6H_5CO)_5$.	Calculated.	C	70.38,	H	4.73.
	Found.	"	70.93,	"	4.60.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{+1.0 \times 1.9422}{0.5 \times 0.0550} = +77.6°$$

Osazone of Xylohexosamine.—A solution containing 2.5 gm. of the amino sugar was taken up in 250 cc. of water, and 6 gm. of phenylhydrazine dissolved in glacial acetic acid were added. The flask containing the solution was then placed in a boiling water bath for 4 hours. On cooling, the osazone settled out. It was recrystallized 4 times out of water and pyridine. It then consisted uniformly of curved needles. The substance was taken up in a very little alcohol and ether. It dissolved and crystallized on evaporation of the solvent in the form of a bright lemon-yellow precipitate. It melted at 173° (corrected) and decomposed at 185°C. The substance had the following composition:

0.1000 gm. substance: 0.2208 gm. CO_2 and 0.0560 gm. H_2O.

$C_{18}H_{24}N_4O_4$.	Calculated.	C	60.33,	H	6.14.
	Found.	"	60.21,	"	6.22.

The rotation of the substance was as follows:

0.1000 gm. substance dissolved in 5.0 cc. Neuberg's alcohol-pyridine solution had an initial rotation of $\alpha = +0.07°$, and after 40 hours $\alpha = +0.45°$.

4. 2-5-ANHYDROPENTOXYCAPROIC ACIDS.

a. 2-5-Anhydromannonic and Anhydrogluconic Acids.

Of the series only four had been prepared. Two, chitonic and chitaric, were prepared by Tiemann and by Fischer. It will be seen from the following chapter that chitonic is oxidized into 2-5-anhydromannosaccharic and chitaric into 2-5-anhydrosaccharic; hence chitonic has the configuration of the anhydromannonic, and chitaric of the anhydrogluconic. On the basis of the experience on chitosamine, epichitosamine and epichitosaminic acid should form 2-5-anhydrogluconic and 2-5-anhydromannonic acid respectively. The expectation regarding the epichitosaminic was realized. It formed on deamination anhydromannonic acid. Contrary to expectation the lactone also gave rise to the anhydromannonic acid.

Deamination of Epichitosaminic Acid (Levene, 1918, b).—The mother liquor after deamination was reduced by means of aluminum amalgam according to the method of Levene and Meyer, and the resulting solution neutralized with calcium carbonate, and concentrated under diminished pressure to a small volume. The composition of the Ca salt was the following:

0.1020 gm. substance: 0.1244 gm. CO_2, 0.0482 gm. H_2O, and 0.0134 gm. CaO.

$(C_6H_9O_6)_2Ca+2H_2O$.	Calculated.	C	33.49,	H	5.12,	Ca 9.30.
	Found.	"	33.26,	"	5.07,	" 9.38.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.71 \times 100}{1 \times 2} = +35.5°$$

Under similar conditions the lactone gave a salt of the following composition:

0.1030 gm. substance: 0.1282 gm. CO_2, 0.0484 gm. H_2O, and 0.0138 gm. CaO.

$(C_6H_9O_6)_2Ca+2H_2O$.	Calculated.	C	33.49,	H	5.12,	Ca 9.30.
	Found.	"	33.94,	"	5.26,	" 9.56.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.71 \times 100}{1 \times 2} = +35.5°$$

b. 2-5-Anhydrotalonic and Anhydrogalactonic Acids. Prepared only in the Form of Their Brucine Salts.

2-5-Anhydrotalonic acid was obtained from chondrosamine and from epichondrosaminic acid (Levene, 1918, *b*). The brucine salt was prepared in larger quantities from the natural sugar and from the sugar obtained from lyxohexosaminic acid. Lots of 30 gm. of chondrosamine hydrochloride were dissolved in 150 cc. of water. To the solution 30 gm. of silver nitrite and a few drops of hydrochloric acid were added. The mixture was allowed to react for 6 hours. The silver chloride was then removed by filtration, the filtrate was placed on a boiling water bath for 5 minutes, then treated with a slight excess of hydrochloric acid, and again filtered. To the filtrate 65 gm. of bromine were added and the mixture was allowed to stand at room temperature for 3 days. The remaining traces of bromine were removed by shaking the solution with mercury; and the hydrobromic acid, by means of lead carbonate and silver carbonate. Finally the nitric acid still present in the solution was removed by the aluminum amalgam method. The resulting solution was transformed into the brucine salt in the usual manner. The solution of the brucine salt was concentrated to a small volume when, on cooling, the brucine salt was crystallized out. When first obtained, the substance was readily soluble in methyl alcohol. However, on repeated recrystallization, its solubility diminished, so that towards the end it dissolved only in a large volume of boiling methyl alcohol. The salt then crystallized in large polygonal prisms. The melting point was 218°C.

The optical rotation was

$$[\alpha]_D^{20} = \frac{-0.63 \times 100}{2 \times 2.522} = -12.4°$$

The analysis of the substance gave the following results:

0.1063 gm. substance, on drying under diminished pressure at the temperature of xylene vapor, lost 0.003 gm. H_2O.

$C_{29}H_{36}N_2O_{10} + H_2O$.	Calculated.	H_2O	3.11.
	Found.	"	3.58.

0.1025 gm. dry substance: 0.2284 gm. CO_2 and 0.0569 gm. H_2O.

$C_{29}H_{36}N_2O_{10}$.	Calculated.	C	60.80,	H	6.10.
	Found.	"	60.78,	"	6.15.

From the synthetic epichondrosaminic acid the substance was prepared in the following manner. 30 gm. of the acid were dissolved in a solution of 200 cc. of water and 40 cc. of 10 per cent hydrochloric acid. 40 gm. of silver nitrite were added. The following morning 10 gm. of silver nitrite and 10 cc. of 10 per cent hydrochloric acid were added. After 30 hours from the beginning of the experiment the silver chloride was removed by filtration, and the other remaining silver by hydrogen sulfide. From the filtrate the nitric and nitrous acids were removed by the aluminum amalgam method. The substance had the following composition:

0.1012 gm. substance, on drying in a zylene bath under diminished pressure lost 0.0038 gm. H_2O.

0.0974 gm. substance: 0.2158 gm. CO_2 and 0.0562 gm. H_2O.

$C_{29}H_{36}N_2O_{10}H_2O$.	Calculated.	H_2O	3.11.		
	Found.	"	3.75.		
$C_{29}H_{36}N_2O_{10}$.	Calculated.	C	60.80,	H	6.10.
	Found.	"	60.42,	"	6.45.

The substance had a melting point of 218°C. and the rotation was

$$[\alpha]_D^{20} = \frac{-0.65 \times 100}{2 \times 2.66} = -12.3°$$

In order to prove its identity with 2-5-anhydrotalonic acid it was oxidized by means of nitric acid into 2-5-anhydrotalomucic acid. 21 gm. of the brucine salt were freed from brucine by means of barium hydroxide and chloroform. After the removal of barium, the solution was concentrated to 40 cc., an equal volume of concentrated nitric acid was added, and the solution was boiled over a free flame until a lively evolution of red fumes set in. The solution was then transferred to a large clock glass and evaporated to dryness. The residue was dissolved in water and again evaporated to dryness. This operation was repeated. The reaction product was converted into the calcium salt. For purification this calcium salt was decomposed by a little less than the calculated amount of oxalic acid and then reconverted with the calcium salt. This operation was repeated twice.

0.1008 gm. air-dry substance, on drying in a xylene bath under diminished pressure, lost 0.0130 gm. H_2O.

$C_6H_8O_7Ca + 3H_2O$. Calculated. H_2O 12.68.
Found. " 12.90.

0.0878 gm. substance: 0.0936 gm. CO_2 and 0.0274 gm. H_2O.
0.0896 " " : 0.0206 gm. CaO.

$C_6H_8O_7Ca + H_2O$. Calculated. C 29.03, H 3.22, Ca 22.58.
Found. " 29.07, " 3.49, " 22.99.

The optical rotation of the substance in a 10 per cent solution of HCl was

$$[\alpha]_D^{20} = \frac{-0.30 \times 100}{1 \times 0.4} = -7.5°$$

Thus the anhydrohexonic acid obtained from lyxohexosamine yields on further oxidation an optically active anhydrotetrahydroxyadipic acid; hence it possesses the structure of anhydrotalonic acid.

2-5-Anhydrogalactonic Acid.—This acid (Levene, 1917, *a*) was obtained from chondrosaminic acid. 10 gm. lots of chondrosaminic acid were treated in the same manner as epichondrosaminic acid in the above experiment. The brucine salt obtained in this manner melted at 244°.

The optical rotation was

$$[\alpha]_D^{30} = \frac{-0.47 \times 100}{2 \times 2.508} = -9.37°$$

The composition of the substance was the following:

0.0988 gm. substance, dried in a xylene bath: 0.2196 gm. CO_2 and 0.0576 gm. H_2O.

$C_{29}H_{26}N_2O_{10}$. Calculated. C 60.80, H 6.10.
Found. " 60.73, " 6.53.

10 gm. of the brucine salt were freed from brucine as in the above experiment. The brucine-free solution was evaporated to 25 cc., diluted with an equal volume of concentrated nitric acid, and boiled over a free flame until the volume was reduced to about 20 cc.; then 10 cc. of nitric acid were again added, and the solution was

again boiled over free flame. When the solution was concentrated to 20 cc. it was transferred to a clock glass and concentrated on a boiling water bath to dryness. The substance immediately crystallized. It was redissolved in water and again evaporated. The operation was repeated once more. The final crystalline residue was dissolved in hot acetone and a very little ether was added, when a small amorphous precipitate formed. This was removed by filtration, and the filtrate was allowed to crystallize. The crystals were filtered, redissolved in hot acetone, and allowed to crystallize. The final product melted at 205°C. 0.1600 gm. of the substance dissolved in 2.5 cc. of water showed no optical activity.

5. 2-5-ANHYDROTETROXYADIPIC ACIDS.

a. 2-5-Anhydrosaccharic Acid (Levene and La Forge, 1915, a).

Acid Potassium Salt.—10 gm. of chitosaminic acid were deaminized with silver nitrite according to Fischer and Tiemann, the resulting solution concentrated to about 25 cc. and an equal volume of nitric acid added in the cold. Oxidation was carried out under the same conditions as already described for chondrosaminic acid. The syrupy product was dissolved in 10 cc. of water, neutralized with strong potassium hydroxide solution, and an equal volume of glacial acetic acid added. Upon dilution to 50 cc. with alcohol the acid potassium salt of the dibasic acid soon began to crystallize, and after standing one-half hour in the refrigerator the yield of the substance washed and dried amounted to about 4 gm. For analysis it was recrystallized from about 2 parts of hot water. The product contains 0.5 of a molecule of crystal water, which can be removed in vacuum at 115°.

0.1868 gm. substance: 0.0058 gm. H_2O.
0.1811 " dried substance: 0.0511 gm. K_2CO_3.

$C_6H_9O_8K + 0.5\,H_2O$.	Calculated.	H_2O	3.7.
	Found.	"	3.10.
$C_6H_9O_8K$.	Calculated.	K	15.73.
	Found.	"	15.94.

Lead Salt.—2.5 gm. of the acid potassium salt were dissolved in about 100 cc. of water and an excess of neutral acetate solution was

slowly added in the cold. Crystallization began after a few minutes and was complete after one-half hour. The lead salt crystallizes under these conditions in large nearly square plates which are very difficultly soluble in water. The yield was practically quantitative. The salt contained 2 molecules of crystal water, which could be removed by heating in vacuum at 150°. The dried preparation analyzed for a neutral lead salt of an anhydrodicarboxylic hexonic acid.

0.2418 gm. substance: 0.0204 gm. H_2O.

$C_6H_8O_8Pb + 2H_2O$.	Calculated.	H_2O	7.99.
$C_6H_6O_7Pb + 2H_2O$.	"	"	8.32.
	Found.	"	8.40.

0.2214 gm. dried substance: 0.1690 gm. $PbSO_4$.

$C_6H_8O_8Pb$.	Calculated.	Pb	49.88.
$C_6H_6O_7Pb$.	"	"	52.18.
	Found.	"	52.10.

Free Acid.—7.8 gm. of the lead salt were suspended in 150 cc. of water, and slightly less than the calculated amount of sulfuric acid was added. The suspension was warmed on the water bath for 1 hour and then diluted with an equal volume of alcohol, filtered, and the filtrate concentrated in vacuum to a thick syrup. This was dissolved in acetone, filtered, and concentrated on the water bath. The resulting syrup crystallized in the desiccator to a semisolid cake. The crude product was extracted with a small amount of a mixture of 1 part amyl alcohol and 2 parts ether, filtered, and washed with the same solvent mixture and finally with dry ether. It may be recrystallized by dissolving in a small amount of acetone and allowing the solution to evaporate nearly to dryness in the air. It crystallizes in aggregates of large plates, the edges of which are usually rounded off by the solvent action of the ether used for washing. The acid is extremely soluble in the usual reagents, with the exception of cold amyl alcohol and ether. The yield was about 2 gm. of pure substance. The product contains 1 molecule of crystal water which can be removed by heating under diminished pressure at 78°. The dry substance melts at 160° (uncorrected).

0.1278 gm. substance: 0.0107 gm. H_2O.

$C_6H_8O_7 + H_2O$.	Calculated.	H_2O	8.57.
	Found.	"	8.38.

0.1159 gm. substance: 0.1598 gm. CO_2 and 0.0467 gm. H_2O.

$C_6H_8O_7$.	Calculated.	C	37.50,	H	4.28.
	Found.	"	37.57,	"	4.47.

0.0898 gm. substance containing crystal water required for neutralization 8.6 cc. 0.1 N NaOH (calculated: 8.4 cc.).

The specific rotation in water was

$$[\alpha]_D^{20} = \frac{+3.12 \times 2.1478}{1.033 \times 0.1637} = +39.7°$$

2-5-Anhydrosaccharic acid should also be formed on oxidation of epichitosamine. In its place, however, saccharic acid was formed.

b. 2-5-Anhydromannosaccharic Acid.

This acid (Levene, 1918, *b*) was previously obtained by Tiemann on oxidation of chitosamine. In view of the fact that epichitosaminic acid and its lactone on deamination gave rise to chitonic acids they should give on oxidation 2-5-anhydromannonic acid. In this instance the acid and its lactone gave rise to the same anhydro acid. The Walden inversion took place possibly in both.

c. 2-5-Anhydro-l-Saccharic Acid.

This acid (Levene and La Forge, 1915, *b*) is formed on oxidation of dextro-*d*-xylohexosaminic lactone and of levo-*d*-xylohexosaminic acid, thus showing that in one of the two the Walden inversion took place, probably in the acid.

3.5 gm. of the hydrochloride of xylohexosaminic acid lactone were dissolved in 50 cc. of water and deaminized with 4 gm. of silver nitrite. The reaction mixture was kept at 0° for the first 5 hours and then allowed to stand over night at room temperature. If silver was present in the solution it was removed with a few drops of hydrochloric acid, the silver chloride filtered off, and the filtrate concentrated in vacuum to about 15 cc. An equal volume of concentrated

nitric acid was then added and the solution boiled over a small flame for about 12 minutes. It was then evaporated in a flat dish on the water bath to a syrup which was freed from most of the nitric acid by repeating the evaporation after adding a small amount of water. The reaction product was taken up in about 6 cc. of water, neutralized with a strong solution of potassium hydroxide, and allowed to stand for 15 minutes at room temperature, after which an equal volume of glacial acetic acid and about 3 volumes of absolute alcohol were added. Upon standing in the refrigerator from 4 to 5 hours, crystallization of the acid potassium salt was complete. The yield was 1.6 gm. It was recrystallized from 2 parts of hot water.

0.2314 gm. substance: 0.0797 gm. K_2SO_4.

$C_6H_7O_7K + H_2O$.	Calculated.	K	15.70.
	Found.	"	15.45.

The rotation of the air-dried substance was

$$[\alpha]_D^{28} = \frac{-2.54 \times 2.1114}{1 \times 0.1400} = -38.1°$$

4 gm. of the acid potassium salt were dissolved in about 150 cc. of water and the calculated amount of a 5 per cent solution of lead acetate was added. Crystallization of the lead salt began at once and was complete after about 1 hour. The lead salt was filtered off with suction, washed with water, and dried at 100°. The yield was 6 gm. The lead salt was suspended in about 100 cc. of water, and slightly less than the calculated amount of sulfuric acid added. After standing about 1 hour on the water bath the lead sulfate was filtered off and the filtrate concentrated in vacuum to a thick syrup which was dissolved in dry acetone and again evaporated to dryness. The syrupy residue, which contained lead salts, was extracted with dry acetone, and the colorless filtrate evaporated in a dish on the water bath to a thick syrup. By repeating the evaporation with acetone a few times the syrup crystallized on cooling without crystal water. It was freed from a small amount of adhering syrup by washing with a mixture of 1 part of amyl alcohol and 2 parts of dry ether. The yield was 1.5 gm. It may be recrystallized by dissolving in a

small amount of dry acetone and allowing the solution to evaporate in the air. The substance melts at 163°.

0.1092 gm. substance: 0.1514 gm. CO_2 and 0.0410 gm. H_2O.

$C_6H_8O_7$.	Calculated.	C	37.50,	H	4.20.
	Found.	"	37.80,	"	4.20.

The rotation of the substance was

$$[\alpha]_D^{21} = \frac{-3.08 \times 2.1547}{1.034 \times 0.1657} = -38.80°$$

Deamination of d-Levo-Xylohexosaminic Acid (Levene, 1918, b).— 7 gm. of the acid were deaminized in the way described above. The reaction product was brought, by distillation under diminished pressure, to a volume of 30 cc. An equal volume of nitric acid was added. The solution was heated over a free flame until the beginning of the evolution of fumes; it was then gently heated for 7 minutes after which the solution was transferred to a clock glass, and evaporated on a water bath to dryness. The residue was again dissolved in a solution consisting of 5 cc. of nitric acid and 5 cc. of water. The final product was converted into the acid potassium salt in the manner described by Levene and LaForge. The yield of the crude product was 3.05 gm. The salt was recrystallized three times and then had the following composition:

0.1000 gm. substance: 0.0366 gm. K_2SO_4.

$C_6H_7O_7K + H_2O$.	Calculated.	K	15.70.
	Found.	"	16.03.

The optical rotation was

$$[\alpha]_D^{20} = \frac{-0.75 \times 100}{1 \times 2} = -37.5°$$

The fact that this acid was the antipodal form to the acid obtained from chitosaminic brought out the configuration of the corresponding acids as anhydrogulonic and anhydrogluconic respectively, and therefore of their epimers as anhydromannonic and anhydroidonic acids.

d. 2-5-Anhydroidosaccharic Acid.

This acid (Levene and LaForge, 1915, *b*) was obtained on oxidation of dextro-*d*-xylohexosaminic acid.

10 gm. of pure dextro-*d*-xylohexosaminic acid were dissolved in 100 cc. of water containing 3 per cent of hydrochloric acid, and 10 gm. of silver nitrate were added. Twice in the course of 24 hours, portions of 2 gm. of silver nitrite and 4 cc. of 10 per cent hydrochloric acid were added. The excess of hydrochloric acid was removed quantitatively by means of silver nitrate. The filtrate was then concentrated to 20 cc., an equal volume of nitric acid was added, and the solution was kept boiling for 12 minutes. The solution was then evaporated on a clock glass. Immediately on evaporation the substance crystallized. It was taken up in a minimum amount of water and several volumes of acetone were added. On standing, the substance crystallized. The yield of recrystallized substance was 4.8 gm. The composition of the substance was the following:

0.1128 gm. substance lost on drying 0.0186 gm.

$C_6H_8O_7 + 2H_2O$.	Calculated.	H_2O	15.80.
	Found.	"	16.49.

0.0942 gm. dry substance on combustion: 0.1282 gm. CO_2 and 0.0362 gm. H_2O.

$C_6H_8O_7$.	Calculated.	C	37.50,	H	4.20.
	Found.	"	37.11,	"	4.30.

The rotation of the subject was

$$[\alpha]_D^{20} = \frac{-1.56 \times 100}{1 \times 2} = -78°$$

or calculated for the dry substance = − 93.4°.

The same substance was obtained by Levene and LaForge on oxidation of the mixed xylohexosaminic acids.

e. 2-5-Anhydromucic Acid.

This acid was obtained originally from the natural chitosaminic acid by Levene and LaForge and subsequently from the synthetic levo-*d*-lyxohexosaminic acid.

9 gm. of lyxohexosaminic acid were dissolved in 100 cc. of 3 per cent hydrochloric acid, and 15 gm. of silver nitrite added in portions over a period of about 4 hours. A few cc. of 10 per cent hydrochloric acid were then added and the reaction was allowed to proceed over night at room temperature. The silver chloride was filtered off and all but a slight excess of the hydrochloric acid removed from the filtrate with silver nitrate. The clear solution was concentrated under diminished pressure to about 25 cc., 30 cc. of concentrated nitric acid were added, and the solution was warmed in an Erlenmeyer flask over a small flame until a vigorous evolution of red fumes began. The flame was then removed and the reaction allowed to proceed with occasional warming. After 12 minutes the solution was poured into two glass dishes and evaporated to a thick syrup on the water bath. The contents of the dishes were then diluted with a little water, combined, and again concentrated. After evaporating once more with water, the syrup on cooling solidified to a mass of white crystals which appeared under the microscope as white oblong plates. After standing in the refrigerator for a time the mass was triturated with a few cc. of a mixture of dry acetone and ether, and filtered on a Büchner funnel. The process was repeated once more to remove the adhering syrup. The crude product after drying weighed about 2 gm., although more crystallized from the acetone-ether mixture used for drying. For analysis it was recrystallized by dissolving in about 100 parts of boiling dry acetone, and filtering and concentrating the solution to about one-eighth of its volume. Anhydromucic acid crystallizes from acetone without crystal water in long prisms which melt at 203–204° (corrected).

0.1040 gm. substance required 10.7 cc. 0.1 N KOH; calculated, 10.8 cc.
0.1563 " " in 3 cc. H_2O showed no rotation with D-light in a 1 dm. tube, where a rotation of 0.02° would not have escaped detection.
0.1424 gm. substance: 0.1946 gm. CO_2 and 0.0534 gm. H_2O.

$C_6H_8O_7$.	Calculated.	C 37.50,	H 4.20.
	Found.	" 37.27,	" 4.17.

f. 2-5-Anhydro-d-Talomucic Acid.

This acid was obtained first by Levene and LaForge on oxidation of natural chondrosamine (1918, *a*). Later it was obtained from

the synthetic chondrosamine (Ledderhose, 1880), from synthetic dextro-*d*-lyxohexosaminic acid (Levene, 1918, *b*) (epichondrosaminic acid) and from dextro-*d*-ribohexosaminic acids (1921).

From Synthetic Sugar.—6 gm. of the synthetic chondrosamine hydrochloride were dissolved in 30 cc. of water containing 1 cc. of hydrochloric acid. 8 gm. of silver nitrite were added and the mixture was allowed to stand for 6 hours. It was then filtered. The silver was removed from the filtrate by means of hydrogen sulfide. The clear filtrate from the silver sulfide was concentrated to 20 cc., cooled to 0°C., and diluted with 20 cc. of nitric acid also cooled at 0°C. The solution was allowed to stand over night.. It was then boiled over a free flame until a lively evolution of yellow fumes set in. The solution was then transferred to a clock glass, evaporated on a water bath, and the calcium salt was then prepared in the usual way. The salt was reprecipitated twice and had the following composition:

0.1054 gm. substance, on drying in a xylene bath, lost 0.0130 gm. H_2O.

$C_6H_8O_8Ca + 2H_2O$.	Calculated.	H_2O 12.68.
	Found.	" 12.3.

0.0924 gm. dry substance, on combustion: 0.0982 gm. CO_2, 0.0298 gm. H_2O, and 0.0924 gm. CaO.

$C_6H_6O_8Ca + H_2O$.	Calculated.	C 29.03, H 3.22, Ca 22.58.
	Found.	" 28.98, " 3.60, " 23.05.

The optical rotation of the substance in 10 per cent HCl was

Initial.	Equilibrium.
$[\alpha]_D^{20} = \frac{-0.32 \times 100}{1 \times 4} = -8.0°$	$[\alpha]_D^{20} = \frac{-0.32 \times 100}{1 \times 4} = -8.0°$

Hence the substance is 2-5-anhydrotalomucic acid. It is identical with the substance previously obtained from natural chondrosamine.

From Dextro-d-Lyxohexosaminic Acid.—The solution obtained on deamination of 6 gm. of the amino-acid was concentrated to 35 cc., and an equal volume of concentrated nitric acid was added, the solution heated over a flame until the evolution of red fumes, and then the heating maintained for 7 minutes. The reaction product

was rapidly evaporated with constant stirring on a clock glass placed on a boiling water bath. The residue was redissolved in a solution of 5 cc. of water and 5 cc. of nitric acid, and again evaporated to dryness. The product was then evaporated twice with water in order to remove the adhering nitric acid. Finally the aqueous solution was converted into the calcium salt. The yield was 3.5 gm. of the crude calcium salt. The crude material was purified by removing the calcium by means of oxalic acid and reconverting the filtrate into the calcium salt. The dry substance had the following composition:

0.0988 gm. substance: 0.1042 gm. CO_2, 0.0302 gm. H_2O, and 0.0222 gm. CaO.

($C_6H_8O_8Ca$).	Calculated.	C	29.03,	H	3.22,	CaO 22.58.
	Found.	"	28.76,	"	3.44,	" 22.47.

The rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.45 \times 100}{1 \times 5} = -9.0°$$

Under the same conditions of oxidation, chondrosaminic acid yields the inactive anhydromucic acid.

2-5-Anhydrotalomucic Acid From Dextro-d-Ribohexosaminic Acid.— 5 gm. of the substance were deaminized in the same manner as the levo form. For oxidation the solution was diluted to 60 cc., to which an equal volume of nitric acid was added. The resulting solution was heated over a free flame for 13 minutes. The product of the reaction was transferred to clock glasses and rapidly concentrated nearly to dryness. The residue was reoxidized once more with a solution of equal parts of water and nitric acid, and finally evaporated with water to remove nitric acid. The residue was then converted into Ca salt. The yield was 2.8 gm. For purification the salt was reconverted into free acid by means of oxalic acid and reconverted into the Ca salt. The pure salt crystallized partly in plates and partly in prismatic needles. Heated under diminished pressure at a temperature of xylene vapor, it lost 2 molecules of water. The salt was levorotatory. It analyzed as follows:

0.1216 gm. substance on drying lost 0.0160 gm.

$C_6H_8O_8Ca + 2H_2O$.	Calculated.	H_2O 12.68
	Found.	" 13.16

0.1056 gm. substance: 0.1120 gm. CO_2, 0.0302 gm. H_2O, and 0.0242 gm. CaO.

$C_6H_8O_8Ca + H_2O$.	Calculated.	C	29.03,	H	3.22,	CaO	22.58.
	Found.	"	28.98,	"	3.20,	"	22.92.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{-0.18 \times 100}{1 \times 2} = -9.0°$$

Free Acid.—6.8 gm. of the calcium salt prepared from the natural sugar were introduced in portions into 250 cc. of boiling water containing 3.2 gm. of oxalic acid. After boiling for one-half hour, the solution was treated with a little animal charcoal and filtered; the filtrate was concentrated to a syrup which was dissolved in about 30 cc. of acetone, filtered, and again concentrated to a syrup on the water bath. On standing over night, large colorless prisms had separated out. Upon completion of crystallization, the adhering syrup was dissolved from the crystals with a few cc. of a solution of equal parts of acetone and ether. The crystals were then filtered off with suction and washed with a small amount of acetone-ether solution. The crystals consisted of large parallelopipeds which melted at 179–181° (uncorrected), and at this temperature slowly decomposed with gas evolution. The pure substance is rather difficultly soluble in acetone (about 1 to 40 at the boiling point of the solvent). For analysis it was dissolved in acetone, which was allowed to evaporate partially. The melting point of the recrystallized product did not differ from that of the first. The substance is easily soluble in water and alcohol, but nearly insoluble in ether. The yield amounted to 2.2 gm.

0.1094 gm. substance: 0.1480 gm. CO_2 and 0.0404 gm. H_2O.

$C_6H_8O_7$.	Calculated.	C	37.50,	H	4.20.
	Found.	"	37.11,	"	4.15.

1500 gm. substance required 15.9 cc. 0.1 N NaOH (calculated: 15.6 cc.).

$$[\alpha]_D^{28} = \frac{-1.07 \times 3.3839}{1 \times 0.2056 \times 1.0262} = -16.56°$$

g. 2-5-Anhydroallomucic Acid.

This acid (Levene and Clark) was prepared from levo-*d*-ribohexosaminic acid. Two 5 gm. portions of the amino-acid were dissolved each in 35 cc. of water; to the solution 15 cc. of 10 per cent hydrochloric acid and 5 gm. of silver nitrite were added. The mixture was allowed to react over night. Twice during the reaction 2 gm. portions of the nitrite and 2 cc. of hydrochloric acid were added. The reaction product was freed from excess of silver and the filtrate reduced to a volume of 75 cc. by distillation under diminished pressure at 40–50°C. of the water bath. To this solution 50 cc. of nitric acid were added, and the resulting solution was heated over a flame for 20 minutes and then rapidly concentrated to a thick mass on a water bath. The thick residue was dissolved in 10 cc. of a solution consisting of equal parts of water and concentrated nitric acid, then evaporated once with water to remove nitric acid. The final residue was then converted into the calcium salt of 2-5-anhydroallomucic acid. For final analysis the salt was suspended in water and its calcium removed by boiling in hot water containing a slight excess over the required amount of oxalic acid.

The calcium salt of 2-5-anhydroallomucic differs from that of 2-5-anhydrotalomucic, first by being optically inactive and second by the difference in behavior on heating. Both salts crystallize with 3 molecules of crystal water. Heated under diminished pressure at the temperature of xylene vapor, anhydroallomucic salt loses all its 3 molecules of crystal water, whereas the corresponding anhydrotalomucic salt loses only 2, retaining the third one. The Ca salt of anhydroallomucic acid analyzed as follows:

0.1228 gm. substance on drying lost 0.0244 gm.

$C_6H_6O_7Ca + 3H_2O$.	Calculated.	H_2O 19.02.
	Found.	" 19.87.

0.0984 gm. substance on combustion: 0.1100 gm. CO_2, 0.0270 gm. H_2O, and 0.0984 gm. CaO.

$C_6H_6O_7Ca$.	Calculated.	C	31.30,	H	2.61,	CaO	24.35.
	Found.	"	30.99,	"	3.07,	"	25.00.

6. CHITOSE AND EPICHITOSE.

Chitose was obtained first by Ledderhose (1880) and by Tiemann. Fischer and Andrea have recognized the substance as 2-5-anhydrohexose. In the light of the fact that on further oxidation the substance is converted into 2-5-anhydromannosaccharic acid, it is evident that it has the structure of anhydromannose. Epichitose, being the epimer of chitose, has the configuration of 2-5-anhydroglucose. The substance was prepared in the following way.

Epichitose (Levene, 1919).—15 gm. of ash-free epichitosamine hydrochloride were taken up in 250 cc. of water and 80 gm. of mercuric oxide, and heated on a boiling water bath for 30 minutes, at the end of which time the greater part of the oxide turned from orange to gray. Through the filtrate from the oxide, hydrogen sulfide gas was passed and the filtrate from the sulfide was concentrated to a very thick syrup. This was taken up with a little methyl alcohol. On standing over night a crystalline deposit formed. It was nitrogen-free, it reduced Fehling's solution on heating, and melted at 240° (corrected) with decomposition. The analysis of the substance gave the following results:

0.1031 gm. substance: 0.1670 gm. CO_2 and 0.0570 gm. H_2O.

$C_6H_{10}O_5$	Calculated.	C	44.44,	H	6.17.
	Found.	"	44.18,	"	6.18.

The optical rotation of the substance was

Initial. Equilibrium.

$$[\alpha]_D^{15} = \frac{-1.92 \times 100}{1 \times 2} = -96° \qquad [\alpha]_D^{25} = \frac{-1.92 \times 100}{1 \times 2} = -96°$$

7. 3-AMINOHEPTONIC ACIDS.

3-Aminoheptonic acids were prepared in the form of their amorphous copper salts by Neuberg, and Neuberg and Wold. These were not pure.

a. Chitosaminoheptonic Acids.

Preparation of Chitosaminoheptonic Acids (Levene, 1916, a; Levene and Matsuo).—The conditions for the preparation o these acids are

more uncertain than of any other substance of this group. At one time (1915) good yields of the crystalline substance were obtained in the following way. 50 gm. of chitosamine hydrochloride were taken up in 100 cc. of water, 18 cc. of an 80 per cent aqueous prussic acid and 25 cc. of ammonium hydroxide were added, and the solution was allowed to stand from 24 to 48 hours. The product was then transferred to water containing about 150 gm. of barium hydroxide, and the solution was boiled over free flame as long as ammonia was still evolved (about 48 hours). The barium and hydrochloric acid were removed in the usual way and the final solution was concentrated under diminished pressure to a thick syrup. This was taken up in a little water, hot 95 per cent alcohol was added as long as a gummy precipitate formed, and the supernatant liquid was decanted and allowed to stand over night at 0°C. A crystalline deposit formed, and from the mother liquor, on concentration and repeated treatment with alcohol, etc., a second crop of crystals was generally obtained. The total yield was about 30 per cent of the employed chitosamine. This material was slightly levorotatory.

In 1916 the laboratories were transferred to a new building, and all attempts to prepare the material by apparently the same process failed. After many experiments it was finally possible again to obtain the substance under the following conditions. 35 gm. of chitosamine hydrochloride were dissolved in 100 cc. of water; 35 gm. of aqueous 80 per cent prussic acid and 20 cc. of ammonia were added. The mixture was warmed to 30°C. and then allowed to stand at room temperature for 1 hour. The temperature generally remained constant and all the sugar was dissolved in the course of that time. The solution was then transferred to an aqueous solution of barium hydroxide and the further treatment proceeded as above. The final solution was concentrated to a small volume and hot methyl alcohol was added to slight opalescence. The solution was then placed on a hot water bath until a crystalline deposit began to form. The yield varied, the maximum being 15 per cent of the employed chitosamine hydrochloride. The specific rotation of this substance was $[\alpha]_D^{20} = + 4.0°$.

Dextro-d-Chitosaminoheptonic Acid.—130 gm. of chitosaminoheptonic acid were dissolved in 4 parts of hot water. The original material had the following rotation in 2.5 per cent HCl solution:

Initial. Equilibrium.

No. 225

$$[\alpha]_D^{20} = \frac{+0.16 \times 100}{1 \times 4} = +4° \qquad [\alpha]_D^{20} = \frac{-0.04 \times 100}{1 \times 4} = -1°$$

After standing over night no crystals separated, hence the solution was concentrated under diminished pressure until a crystalline deposit began to form and then allowed to stand over night. 74 gm. of crystals were obtained which had the following rotation in 2.5 per cent HCl solution.

Initial.

No. 236

$$[\alpha]_D^{25} = \frac{+0.23 \times 100}{1 \times 4} = +5.7°$$

These 74 gm. of heptonic acid were dissolved in 150 cc. of hot water; 43 gm. of crystals settled out which had the following rotation in 2.5 per cent HCl solution.

Initial. Final (24 hrs.).

No. 246

$$[\alpha]_D^{25} = \frac{+0.26 \times 100}{1 \times 4} = +6.5° \qquad [\alpha]_D^{25} = \frac{+0.11 \times 100}{1 \times 4} = +2.7°$$

These crystals were dissolved again in 86 cc. of hot water and 35 gm. of crystals were obtained which had the following rotation in 2.5 per cent HCl solution.

Initial.

No. 247

$$[\alpha]_D^{25} = \frac{+0.26 \times 100}{1 \times 4} = +6.5°$$

These 35 gm. were again dissolved in 70 cc. of hot water, and there were obtained 28 gm. of crystals which had the following rotation in 2.5 per cent HCl solution.

Initial.

$$[\alpha]_D^{25} = \frac{+0.26 \times 100}{1 \times 4} = +6.5°$$

From the mother liquor 37 gm. of the same substance were obtained; the total yield was 65 gm. The substance crystallized out of water in heavy prisms. Melting point was 192°C. with decomposition.

Levo-d-Chitosaminoheptonic Acid.—To the mother liquor of the first precipitate, hot methyl alcohol was added to a slight opalescence and the solution was allowed to stand for 4 hours. A precipitate then separated and was filtered off; the mother liquor was concentrated under diminished pressure and hot alcohol was added. A precipitate was again formed and filtered off, and to the mother liquor more alcohol was added. 5 gm. of crystals separated which had the following rotation in 2.5 per cent HCl solution.

Initial.

No. 307

$$[\alpha]_D^{25} = \frac{-0.16 \times 100}{1 \times 4} = -4°$$

These 5 gm. were dissolved in 5 cc. of hot water. After long standing there were obtained 1.85 gm. of crystals which had the following rotation in 2.5 per cent HCl solution.

Initial.

No. 309

$$[\alpha]_D^{25} = \frac{-0.13 \times 100}{1 \times 2} = -6.5°$$

The mother liquor of No. 307 was allowed to stand. On long standing, there crystallized 4.75 gm. of a substance with the following rotation in 2.5 per cent HCl solution.

Initial.

No. 261

$$[\alpha]_D^{25} = \frac{-0.11 \times 100}{1 \times 2} = -5.5°$$

These crystals were dissolved in 5 cc. of hot water and gave 1.6 gm. of crystals which had the following rotation.

Initial.

No. 358

$$[\alpha]_D^{25} = \frac{-0.15 \times 100}{1 \times 2} = -7.5°$$

Nos. 309 and 358 were combined and dissolved in 4 cc. of hot water. 1.5 gm. of crystals were obtained which had the following rotation in 2.5 per cent HCl solution.

Initial. Final (24 hrs.).

No. 380

$$[\alpha]_D^{25} = \frac{-0.15 \times 100}{1 \times 2} = -7.5° \qquad [\alpha]_D^{25} = \frac{-0.24 \times 100}{1 \times 2} = -12°$$

The 1.5 gm. were dissolved again in 1.5 cc. of hot water and gave 1.2 gm. of crystals which had the following rotation in 2.5 per cent HCl solution.

Initial.

No. 381

$$[\alpha]_D^{25} = \frac{-0.15 \times 100}{1 \times 2} = -7.5°$$

It crystallized out of 25 per cent alcohol solution in long prismatic needles. The melting point was 139°C. (corrected) with decomposition.

b. Chondrosaminoheptonic Acid.

Preparation of Chondrosaminoheptonic Acid (Levene, 1916, c; Levene and Matsuo).—35 gm. of chondrosamine hydrochloride were taken up in 70 cc. of water; 35 cc. of 80 per cent aqueous solution of hydrocyanic acid and 22 cc. of strong ammonia water were added. This mixture was warmed at 42–45° for about 15 minutes. The solution turned dark in color and became viscous. It was cooled in an ice-alcohol bath until the temperature of solution came down to 0°C., when it was transferred to an aqueous solution of 200 gm. of barium hydroxide. The solution was allowed to stand about 1 hour, then boiled on free flame for about 3 hours and distilled under diminished pressure to dryness. The residue was dissolved in 100 cc. of water and the solution was again distilled to dryness; this operation was repeated 10 times. By this procedure all ammonia was driven off.

Barium was removed by means of a small excess of sulfuric acid, and the excess of this was removed by means of lead carbonate. The hydrochloric acid was removed by means of $AgCO_3$. The excess of lead and silver was removed by means of hydrogen sulfide gas. The filtrate from silver sulfide was concentrated under diminished pressure to a syrup, and seeded with chondrosaminoheptonic acid. Hot methyl alcohol was added very cautiously until slight opalescence, and the solution was allowed to stand for about 72 hours at

room temperature. (Sometimes the substance crystallized out of aqueous solution.)

The crystalline deposit was filtered, washed with 50 per cent alcohol, 95 per cent alcohol, and absolute alcohol successively, and finally with ether. The best yield was 11 gm. In this manner 443 gm. of the substance were prepared.

Levo-d-Chondrosaminoheptonic Acid.—440 gm. of chondrosaminoheptonic acid, which had the following rotation in 2.5 per cent HCl solution,

Initial.

$$[\alpha]_D^{25} = \frac{-0.14 \times 100}{1 \times 4} = -3.5°$$

were dissolved in 800 cc. of hot water. On standing over night crystals separated out, which had the following rotation:

Initial. Equilibrium (24 hrs.).

$$[\alpha]_D^{26} = \frac{-0.30 \times 100}{1 \times 4} = -7.5° \qquad [\alpha]_D^{25} = \frac{-0.48 \times 100}{1 \times 4} = -12°$$

The yield was 344 gm.

The 344 gm. of heptonic acid were dissolved in 800 cc. of hot water, and 391 gm. of crystals were obtained.

The rotation in 2.5 per cent HCl solution was

Initial. Final (24 hrs.).

$$[\alpha]_D^{25} = \frac{-0.33 \times 100}{1 \times 4} = -8° \qquad [\alpha]_D^{26} = \frac{-0.49 \times 100}{1 \times 4} = -12°$$

These crystals were dissolved again in 800 cc. of hot water, and 265 gm. of crystals were obtained which had the same rotation in 2.5 per cent HCl solution.

Initial. Final (24 hrs.).

$$[\alpha]_D^{25} = \frac{-0.33 \times 100}{1 \times 4} = -8° \qquad [\alpha]_D^{25} = \frac{-0.52 \times 100}{1 \times 4} = -13°$$

In the mother liquor a further deposit of the same substance settled out. The total yield of levo-*d*-chondrosaminoheptonic acid was 313 gm. It crystallized out of water in elongated prisms. The melting point was 139°C. (corrected) with decomposition.

Dextro-d-Chondrosaminoheptonic Acid.—The mother liquor of the first crystallization was concentrated under diminished pressure and a precipitate formed which was filtered off. To the filtrate an equal volume of 95 per cent alcohol was added and the solution was allowed to stand for 24 hours. A second precipitate formed and was filtered off again and the solution was poured into absolute alcohol under constant stirring. A gummy precipitate formed which was removed and the solution was concentrated to a syrup under diminished pressure at room temperature. Alcohol was then added and the solution allowed to stand for several days.

Crystals separated which had the following rotation in 2.5 per cent HCl solution:

	Initial.	Final (24 hrs.).
No. 351	$[\alpha]_D^{25} = \frac{+0.64 \times 100}{1 \times 4} = +16°$	$[\alpha]_D^{25} = \frac{+1.02 \times 100}{1 \times 4} = +25°$

The yield was 1.9 gm.

In the mother liquor a second deposit of 2.3 gm. of crystals formed which had the following rotation in 2.5 per cent HCl solution:

	Initial.	Final (24 hrs.).
No. 311	$[\alpha]_D^{25} = \frac{+0.50 \times 100}{1 \times 2} = +25°$	$[\alpha]_D^{25} = \frac{+0.85 \times 100}{1 \times 2} = +42°$

In the mother liquor a further deposit of crystals formed which had the following rotation in 2.5 per cent HCl solution:

	Initial.	Final (24 hrs.).
No. 352	$[\alpha]_D^{25} = \frac{+0.85 \times 100}{1 \times 2} = +42°$	$[\alpha]_D^{25} = \frac{+1.43 \times 100}{1 \times 2} = +71°$

From the mother liquor of this substance there was obtained on further crystallization a substance with the following specific rotation in 2.5 per cent HCl solution:

	Initial.	Final (24 hrs.).
No. 407	$[\alpha]_D^{25} = \frac{+0.85 \times 100}{1 \times 2} = +42°$	$[\alpha]_D^{25} = \frac{+1.30 \times 100}{1 \times 2} = +65°$

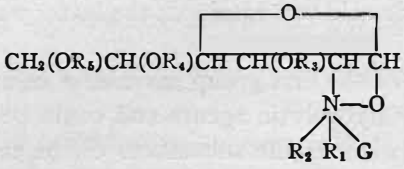
PART II.

MUCINS AND MUCOIDS.

THEORETICAL.

1. GENERAL CONSIDERATIONS.

The structure of mucin and allied substances has remained in obscurity until recent years, although these substances interested both the chemist and the physiologist. Knowledge was lacking, particularly regarding the mode of union between the sugar and the rest of the molecule. The information given in text-books was generally limited to that of the solubilities and some other physical properties. The substances were classified in three groups: mucins, mucoids, and glucoproteins. More recently Irvine and Hynd (1913) have suggested the following structure of this group of substances:



According to this conception, the amino group of the nitrogenous sugars serves as a bridge for the linking of amino-acids to the sugar, in a peptide as well as in a glycosidic union. The hypothesis suggested itself to Irvine and Hynd on the ground of their work on the glycosides of chitosamine, and was based entirely on speculative evidence. Irvine expressed the view rather tentatively, realizing the need of experimental proof.

On the other hand, evidence was accumulating tending to show that all substances allied to mucins contained in their molecule a complex acid which in its structure bore no similarity or relationship to peptides.

For many years it was known that an acid of the type of conjugated sulfuric acid existed in the molecule of chondromucoid (Mörner). Later its existence was revealed also in tendomucoid. The acid

under the name of chondroitin sulfuric acid was discovered by Mörner, and received further attention through the efforts of Schmiedeberg at a time when methods applied to the study of carbohydrates were very imperfect. Later, in the course of the work it was found that the acid could not be isolated with equal facility from every mucin or mucoid (Levene, 1900–01; López-Suárez). In many instances the removal of the protein radicle was accomplished only after numerous operations, and in the course of these operations the conjugated sulfuric acid suffered partial decomposition. It is still not very clear whether the differences of individual mucins lay in the protein or in the acid radicles.

Finally a chemical distinction was discovered between the conjugated sulfuric acids of different origin (Mandel and Levene, Alzona, Levene and López-Suárez, 1916, *a*). The difference was in the components. Whereas one contained in its molecule the nitrogenous hexose, chondrosamine, the other had in its place chitosamine. Müller had previously shown that this sugar could be obtained on hydrolysis of salivary mucin. For the former the name chondroitin sulfuric acid was retained; the latter was named mucoitin sulfuric acid.

The substances of the first group revealed a comparatively greater resistance towards hydrolytic agents and could be obtained in a fair degree of purity, whereas the substances of the second group, when prepared free from protein, always appeared in the form of a mixture of mucoitin sulfuric acid with mucoitin. Also the intermediary substances showed a difference in their resistance towards hydrolytic agents, so that chondrosin could be prepared in a practically pure state whereas mucosin was found so labile that, under the conditions which permitted the preparation of the former, the latter was nearly completely hydrolyzed. On the other hand, when mucoitin sulfuric acid was subjected to milder hydrolysis a mucosin resulted which still contained undecomposed mucoitin.

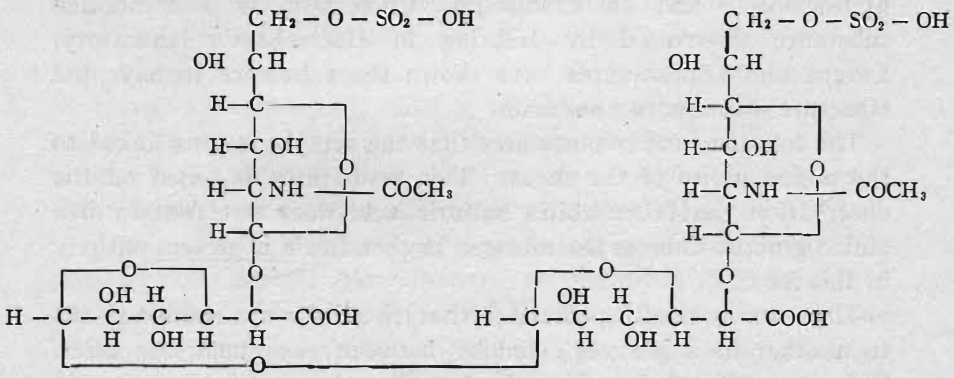
Because of this instability of mucoitin sulfuric acid, the acid itself and the products of its partial hydrolysis were not obtained in the same degree of purity as chondroitin sulfuric acid and the corresponding products derived from it.

Furthermore, some differences were observed between various members of the mucoitin sulfuric acid group. It is true that, since the individual members were not always obtained in absolutely pure state, there is also the possibility that these differences were brought about by impurities. However, the impression still remains that the acids of this group belong to two types. A representative of one type is the substance derived from funis mucin. The acid of this type is characterized by its gelatinous nature when precipitated by glacial acetic acid, by the comparatively small quantity of glacial acetic acid required for its precipitation, and by the readiness with which it forms an insoluble barium salt. The barium salt is very sparingly soluble in water, but is readily dissolved in the presence of acetates.

The second type of mucoitin sulfuric acid is characterized by the greater solubility of the barium salt, by the fact that the substance is precipitated by glacial acetic acid in the form of a flocculent precipitate, and by the fact that a large excess of the acid is required in order to bring about the precipitation. A representative member of this type is the substance obtained from gastric mucus.

2. STRUCTURE OF CHONDROITIN SULFURIC ACID.

The structure of chondroitin sulfuric acid is most satisfactorily expressed by the following graphic formula:



In both formulas the position of the sulfuric acid radicle, the place of union of sugar and glucuronic acid, and the position of the amino groups are arbitrary. To the sulfuric acid is assigned a different position in the two substances in order to indicate the existence of a difference in their respective behavior towards hydrolytic agents.

The data which led to the formula were obtained by Levene and La Forge, (1913, 1914*a*, 1915*a*) and by Levene. The formula postulates that chondroitin sulfuric acid contains 4 components in equimolecular proportion: chondrosaminic, glucuronic, acetic and sulfuric acids. Each one of these components has been isolated and identified.

The order of linking as expressed in this formula is the following: Chondrosamine and glucuronic acid are linked into a disaccharide. This was made evident by the discovery of Schmiedeberg, that on partial hydrolysis of chondroitin sulfuric acid a disaccharide chondrosin is formed. The linking of the disaccharide is as expressed in the formula and not as assumed by Schmiedeberg; namely, carbon atom 1 of chondrosamine is combined in glucosidic linking with one of the hydroxyls of the glucuronic acid. The experimental facts leading to this conclusion are the following:

1. In chondrosin the carboxyl of glucuronic acid is free.

2. When chondrosin is oxidized by bromine and subsequently cleaved, saccharic acid is formed.

3. In chondrosin the nitrogen is present in the form of a primary amino group. This observation was made on amorphous chondrosin hydrochloride and on chondridin. Chondridin is a crystalline substance discovered by Hebting in Hofmeister's laboratory. Levene and López-Suárez have shown the substance to have the structure of anhydrochondrosin.

The formula further postulates that the acetyl group is linked to the amino group of the sugar. This assumption is based on the observation that chondroitin sulfuric acid does not contain free amino groups, whereas the nitrogen in chondrosin is present entirely in this form.

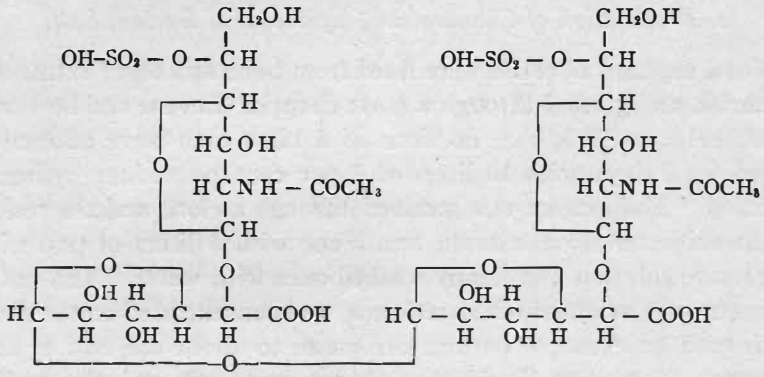
The formula finally postulates that two units are combined one to another in a glucosidic linking between the glucuronic acids. This assumption is based on the fact that chondroitin sulfuric acid does not reduce Fehling's solution, whereas chondrosin does.

3. STRUCTURE OF MUCOITIN SULFURIC ACID.

Concerning the structure of the acids of this group, it seems permissible to assume the same hypothesis as was formulated for chondroitin sulfuric acid. On removal of sulfuric acid a substance is formed which is analogous to chondroitin. It is non-reducing, and does not contain free amino groups. On hydrolysis it forms mucosin, which is a disaccharide composed of glucuronic acid and chitosamine. Mucoitin, similarly to chondroitin, contains one acetyl group to each molecule of chitosamine, glucuronic, and sulfuric acids.

The glucuronic acid was identified in all the acids of the mucoitin sulfuric acid group but not with the same degree of exactness. Whereas in the acids of the funis mucin type the glucuronic acid was identified by the formation of furfural, by the analysis of the phenylhydrazine derivatives, and by the analysis of the acid potassium salt of saccharic acid formed on oxidation with nitric acid. In the acids of the second group it was demonstrated only by the furfural distillation and by the formation of the phenylhydrazine, which, however, was obtained in a quantity too small for purification and analysis.

As regards the details of the structure, all the arguments which suggested the structural formula of chondroitin sulfuric acid may be repeated in the formulation for mucoitin sulfuric acid. This is as follows:



Several details of the graphic formula are entirely arbitrary. They are the position of the sulfuric acid radicle, the carbon atom in glucuronic acid which is combined with chondrosamine, and the allocation of the amino group in the carbon atom 2 of the sugar.

4. DISTRIBUTION OF THE ACIDS OF VARIOUS TYPES.

The distribution of the acids in various organs and tissues was found to be the following:

I. Chondroitin sulfuric acid.
- 1 Cartilage.
- 2 Tendons.
- 3 Aorta.
- 4 Sclera.

II. Mucoitin sulfuric acid.
- A. 1 Funis mucin
- 2 Humor vitreous.
- 3 Cornea.
- B. 1 Mucin of gastric mucosa.
- 2 Serum mucoid.
- 3 Ovomucoid.
- 4 Ovarian cysts.

Table IX contains a summary of the analytical data obtained on the individual substances.

EXPERIMENTAL.

1. GROUP I. CHONDROITIN SULFURIC ACID.

I. From Chondromucoid.

a. Preparation of Chondroitin Sulfuric Acid Barium Salt.

Nasal septums of cattle were freed from bone and other extraneous material, and ground through a meat chopper (Levene and La Forge, 1913; 1914, *a;* 1915, *a*). Portions of 5 kilos each were allowed to stand for 2 days with 10 liters of 2 per cent potassium hydroxide solution. The extract was strained through a cloth and the residue again subjected to the same treatment with 5 liters of potassium hydroxide solution and finally washed once with water. The united extracts were acidified with acetic acid and concentrated on the steam bath with an excess of barium carbonate to about one-half of their volume. The clear liquid was then poured off and the residue thrown on a folded filter and allowed to drain off. This filtrate was united with the decanted liquid and the whole acidified with acetic acid and evaporated as before with barium carbonate to about 2 liters.

TABLE IX.

		Theory.	Group I.				Group II.				
							A.			B.	
			Cartilage.	Tendons.	Aorta.	Sclera.	Navel cord.	Vitreous humor.	Cornea.	Gastric mucus.	Serum mucoid.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
	C	27.80		25.13		34.27	33.39	34.39	37.83		
	H	3.48		3.88		4.86	5.38	5.72	6.22		
	N	2.32	2.14	2.11	2.54	5.66	4.03	4.96	4.62	3.47	0.25
$C_{25}H_{44}O_{29}N_2S_2Ba_2$	S	5.30	3.72	4.26	2.40	4.57	4.07	3.63	3.10	1.48	1.96
Mucoitin, chondroitin, sulfuric acids.	Base.	22.70		18.35		28.83		21.50	14.74		29.14
	Furfural.	33.0					17.5	23.0		13.0	
	Acetyl.	10.0					9.4			7.8	
	$[\alpha]_D$						−45.6			-	
Glucuronic acid derivatives.			+	+	−	−	+			+	
	C	36.9	37.3	35.8			38.25				
	H	5.64	5.66	6.0			5.80				
$C_{13}H_{21}O_{11}N \cdot HCl$	N	3.58	3.45	3.45	4.34		−				
Chondrosin or mucosin.	NH_2N	3.58	3.45	3.45	3.41		3.24			2.12	
	$[\alpha]_D$		+43.4	+41.5	+42.0		+25.5			+25.7	
Sugar.			Chondrosamine.				Glucosamine.				

The separated protein and barium carbonate were removed by centrifugalization and the clear yellow liquid dropped into 8 times its volume of glacial acetic acid and kept agitated by a turbine. The acid potassium salt thus obtained was filtered by suction, washed with glacial acetic acid, and finally with alcohol and ether.

200 gm. of this product, which gave a slight biuret test, were dissolved in 10 liters of water and while the solution was kept stirred with a turbine, a solution of basic lead acetate was dropped in until complete precipitation had taken place. The lead salt, after having been washed several times by grinding in a mortar with water and filtering with suction, was suspended in 5 liters of water containing 100 gm. of barium acetate and 50 cc. of acetic acid and decomposed by long treatment with hydrogen sulfide with constant stirring. After standing for 12 hours the lead sulfide was filtered off and the slightly turbid solution of the barium salt precipitated by the addition of about one-third of its volume of 95 per cent alcohol. After filtering and washing with 50 per cent alcohol, then with 95 per cent alcohol, absolute alcohol, and finally with ether, the product after drying was a pure white powder, showing no trace of biuret.

This product is a mixture of the barium salts of chondroitin and chondroitin sulfuric acid. It showed no reduction of Fehling's solution, and in the apparatus of Van Slyke no amino nitrogen.

0.5070 gm. substance: 7.75 cc. 0.1 N NH_3.
0.4650 " " : 7.40 " 0.1 N NH_3.
0.4166 " " : 0.1125 gm. $BaSO_4$.

$C_{18}H_{27}NSO_{17}$. Calculated. N 2.01, S 4.60.
Found. " 2.14, 2.23, " 3.72.

A simpler way for the preparation of free chondroitin sulfuric acid is to dissolve the lead salt in a 10 per cent hydrochloric acid. The filtrate from lead chloride is then treated with glacial acetic acid until all the chondroitin sulfuric acid is precipitated. This is then washed with glacial acetic acid, alcohol, and finally with ether.

b. Estimation of Acetyl Groups.

25 gm. of the barium salt of chondroitin sulfuric acid were repurified by dissolving in 2 liters of distilled water with the addition

of 10 gm. of pure barium chloride, and precipitating by the addition of 1 liter of 95 per cent alcohol. The precipitate was washed chlorine-free with 50 per cent alcohol, absolute alcohol, and finally with ether, and dried in vacuum. About 10 gm. of this material were dissolved in 10 cc. of water.

The acetic acid determinations were carried out in the following manner. The solution of the chondroitin sulfuric acid barium salt was subjected to simultaneous hydrolysis with 25 per cent sulfuric acid and distillation at atmospheric pressure. The distillate was collected in a receiver cooled with ice, and protected from the atmospheric carbon dioxide by soda lime, while the volume of the solution in the reaction flask was kept constant by the addition of water through a dropping funnel.

5 cc. solution: 7.40 cc. 0.1 N NH_3 (Kjeldahl).
5 " " : 7.60 " 0.1 N acetic acid.
5 " " : 7.80 " 0.1 N " "
5 " " : 7.55 " 0.1 N " "

5 cc. of another solution, which corresponded to 8.50 cc. of 0.1 N NH_3 (Kjeldahl) were hydrolyzed in the same apparatus with 20 per cent barium hydroxide until no more ammonia was given off. The solution was then acidified with sulfuric acid, and the acetic acid determination carried out as above.

5 cc. solution: 9.75 cc. 0.1 N acetic acid.

c. Preparation of Chondrosin.

50 gm. of the barium salt of chondroitin sulfuric acid were dissolved in 150 cc. of equal parts of concentrated hydrochloric acid and water and heated for 1 hour on the water bath. Barium sulfate began to separate at once, and after 1 hour the solution, which was only slightly colored, showed its maximum reduction of Fehling's solution, and all the nitrogen was present as amino nitrogen. The filtered solution was evaporated in vacuum to a very thick syrup and this was taken up in about 40 cc. of hot water and poured into 500 cc. of absolute alcohol. Partial precipitation of the chondrosin hydrochloric acid salt as a nearly colorless flocculent precipitate takes place. After standing over night, 2 volumes of absolute ether were added and

the precipitate was filtered with suction, and again thoroughly washed with absolute ether. For a final purification the product thus obtained is dissolved in about its own weight of water, and precipitated and washed again as above described. It was a quite colorless powder, which when properly washed is not hygroscopic. The yield of the first product, dried over calcium chloride for 2 days in vacuum, was 27 gm.

0.1966 gm. substance, dried to constant weight at 100°: 12.7 cc. amino N at 21°, 764 mm. N = 3.67 per cent.

0.3319 gm. chondroitin sulfuric acid barium salt: 5.8 cc. 0.1 N amino N = 2.44 per cent.

0.5044 gm. substance hydrolyzed for 1 hr. with 1 part HCl and one part H_2O: 18.3 cc. N at 16°, 760 mm. (Van Slyke). N = 2.11 per cent.

d. Preparation of Chondridin.

Mode of Preparation of the Substance.—This differed in its details from that of Hebting. The analytical data published by this author for chondridin seemed to agree with those required by theory for chondrosin oxalate. At the outset of the work it was planned to test this possibility. Chondrosin chlorohydrate was prepared following the conditions employed in the earlier work by Levene and La Forge. The chondrosin chlorohydrate obtained in this manner was freed from hydrochloric acid. To the aqueous solution of chondrosin a slight excess over 1 equivalent of oxalic acid was added, and to the resulting solution alcohol was added to opalescence. On standing there was no evidence of crystallization. However, when the solution was allowed to stand on a boiling water bath for 1 hour prior to the addition of alcohol, crystallization did take place. Alcohol was added to marked opalescence and the solution was allowed to remain on the water bath until it clarified. On scratching along the walls of the beaker a crystalline deposit soon began to form. The substance was recrystallized by dissolving in hot water, adding to the aqueous solution 99.5 per cent alcohol to opalescence, and boiling the solution until it clarified. After 3 or 4 recrystallizations the substance contained only traces of mineral impurities.

In later experiments the preparation of the substance was somewhat simplified; namely, no attempt was made to isolate the chon-

drosin hydrochloride before the digestion with oxalic acid. The procedure was as follows: Portions of 50 gm. of the barium salt of chondroitin sulfuric acid were hydrolyzed by heating for 1 hour on the water bath in a solution of 150 cc. of 20 per cent hydrochloric acid. From the product of reaction, barium and hydrochloric acid were removed and the solution was concentrated to a small volume under diminished pressure. The subsequent treatment was as above described.

Properties of the Substance.—The lactone is a white crystalline powder. It does not melt, but contracts and turns dark at 200°C. The analysis of the substance was as follows:

No. 43. 0.1176 gm. substance, dried to constant weight at temperature of water vapor and under diminished pressure, lost 0.0078 gm.

No. 81. 0.1130 gm. substance under the same conditions, lost 0.0075 gm.

$C_{12}H_{21}NO_{11} + 1\frac{1}{2}H_2O$.	Calculated.	H_2O	7.20.
	Found. 1.	"	6.63.
	2.	"	6.63.

No. 43. 0.1098 gm. of the above substance on combustion: 0.1626 gm. CO_2 and 0.0580 gm. H_2O.

0.1867 gm. substance used for Kjeldahl nitrogen estimation, required for neutralization 5.30 cc. of 0.1 N acid.

0.0500 gm. substance for the amino estimation was dissolved in 5 cc. of water. 2 cc. solution in the Van Slyke micro apparatus: 1.36 cc. N_2 at 20°C., and 764 mm.

No. 81. 0.1055 gm. substance: 0.1564 gm. CO_2 and 0.0568 gm. H_2O.

$C_{12}H_{19}NO_{10} + H_2O$.	Calculated.	C	40.54,	H	5.96,	N	3.94,	NH_2-N 3.94.
	Found. 1.	C	40.43,	H	5.98.			
	2.	"	40.11,	"	5.92,	N	3.97,	NH_2-N 3.89.

The rotation of the air-dry substance was

$$[\alpha]_D^{20} = \frac{+0.97 \times 100}{1.6 \times 1} = +61°$$

Titration of the Substance with Alkali.— 0.1000 gm. of the substance was dissolved in 25 cc. of water and titrated with 0.1 N sodium hydroxide. Alizarin was used as indicator. After the addition of the first drop, the solution reacted neutral. 0.1000 gm. of the substance was dissolved in 25 cc. of water. 15 cc. of 0.1 N alkali were

added and the solution was allowed to stand over night. It required 12.45 cc. of 0.1 N acid to titrate the solution to neutral.

$C_{12}H_{19}NO_{10} + 2\frac{1}{2}H_2O$.	Calculated.	Molecular weight	382.17.
	Found.	" "	392.

Furfural Estimation.—0.2000 gm. of the substance was distilled in the usual way with hydrochloric acid having a specific gravity of 1.06. The yield of the phloroglucide was 0.0248, which corresponds to 0.00744 gm. of glucuronic acid.

$C_{12}H_{19}NO_{10} + 2\frac{1}{2}H_2O$.	Calculated.	Glucuronic acid	50.77.
	Found.	" "	37.2.

Taking into consideration the limit of error of the method, the result is not unsatisfactory.

e. Preparation of Desaminochondrosin.

3 gm. of chondrosin hydrochloride in 50 cc. of water were treated with the calculated amount of silver nitrite (1.1 gm.). After standing for several hours at room temperature the reaction mixture was warmed on the water bath with occasional shaking. After the solution had been allowed to stand over night at room temperature it was again warmed on the water bath for about 2 hours, after addition of 0.3 gm. of silver nitrite and about 5 cc. of diluted hydrochloric acid. The excess of silver was then removed with a slight excess of hydrochloric acid and the solution evaporated in vacuum to a syrup which was taken up in a very small quantity of water and poured into dry acetone. The gummy precipitate hardened quickly, and was then ground with more dry acetone and washed with ether. The product was a white amorphous powder resembling chondrosin in all its physical properties and in its power to reduce Fehling's solution, and gave the same amount of furfural.

0.3710 gm. substance, dried at 100° in vacuum: 0.0575 gm. phloroglucoside corresponding to 0.1725 gm. glucuronic acid.

f. Identification of Glucuronic Acid.

Cleavage of Chondrosin with Sodium Amalgam.—12 gm. of chondrosin hydrochloride in 100 cc. of water were allowed to stand with

100 gm. of 2.5 per cent sodium amalgam. After about 20 minutes at room temperature the solution took on a bright yellow color and at the same time evolution of ammonia began. The solution is then neutralized with sulfuric acid, and 100 gm. of sodium amalgam were again added, the temperature always being kept at about 25°. After about 1 hour the solution was again acidified with sulfuric acid and allowed to stand over night, after the addition of a third 100 gm. portion of amalgam. The solution is then separated from the mercury and filtered from the sodium sulfate with the addition of some animal charcoal.

Preparation of the Phenylhydrazine Compound.—The solution obtained by the above treatment was diluted to about 200 cc. and after the addition of 15 gm. of phenylhydrazine in 50 per cent acetic acid allowed to stand on the water bath. After about 20 to 30 minutes a dark tarry material separates together with a small amount of solid material. At this point the solution is quickly filtered with suction on a hot funnel into a hot flask, and the filtrate allowed to stand from 2 to 3 hours on the water bath. After this time the solution was filled with long yellow needles to which very little of the light-colored oil adhered. The crystals were filtered and washed with warm water and then with cold absolute alcohol until no more oil drops could be discerned under the microscope. When dried in vacuum the product melts with decomposition at about 115°C. Attempts to recrystallize did not effect a purification, and therefore the first product was used for the analysis.

0.1188 gm. substance: 0.2484 gm. CO_2 and 0.0634 gm. H_2O.
0.1278 " " : 19.2 cc. N_2, 17°, 758 mm.

$C_{24}H_{26}N_6O_4 + 1\frac{1}{2} H_2O$.	Calculated.	C	58.93,	H	5.93,	N 17.17.
	Found.	"	58.80,	"	6.12,	" 17.3.

0.0599 gm. substance in 5 cc. pyridine-alcohol mixture rotated in a 0.5 dm. tube with D-light − 0.32°.

Phenylhydrazine Compound from Glucuron.—1 gm. of glucuron was warmed on the water bath for 2 hours with a little more than the required amount of normal sodium hydroxide. The solution was neutralized with acetic acid, and 4 gm. of phenylhydrazine in 50 per cent acetic acid and 4 gm. of sodium acetate were added. After a

short time crystallization of the phenylhydrazine compound in long yellow needles began, and after 3 hours their amount had reached 1.6 gm. The material was purified by washing with cold alcohol and ether. It decomposed at about 115°C.

0.0598 gm. substance in 5 cc. pyridine-alcohol mixture rotated in a 0.5 dm. tube with D-light − 0.32°.

By prolonged heating in vacuum at 100° the substance lost weight, but before becoming constant, decomposition sets in, while at lower temperatures no loss of weight was observed.

Parabromophenylhydrazine Compound from Chondrosin.—20 gm. of chondrosin hydrochloride were treated in the usual way with sodium amalgam, and the resulting solution, after acidifying with acetic acid, was heated on the water bath with 4 gm. of parabromophenylhydrazine hydrochloride. After about 1 hour the solution was filtered from the separated tarry material and allowed to stand for 3 hours longer on the water bath. The impure phenylhydrazine compound obtained was washed with alcohol until the impurities had been removed, and then with ether. The substance may be recrystallized by dissolving it in as small a quantity as possible of a mixture of 1 part 50 per cent acetic acid and 1 part alcohol, and then precipitating by the addition of 2 parts of hot water.

0.0568 gm. substance in 5 cc. pyridine-alcohol mixture rotated with D-light in a 0.5 dm. tube − 0.8°.

0.0614 gm. substance twice recrystallized under the same conditions, rotated −0.75°.

0.1454 gm. substance: 12.5 cc. N_2, 22°, 762 mm.
0.1126 " " : 0.0118 gm. AgBr.

Calculated.	Br	28.95,	N	10.15.
Found.	"	27.0,	"	9.7.

Parabromophenylhydrazine Compound from Glucuron.—1 gm. of glucuron in 100 cc. of water was heated on the water bath with 2.5 gm. of parabromophenylhydrazine hydrochloric acid salt, which had been purified by twice recrystallizing from dilute hydrochloric acid and washing with ether, and 2.5 gm. of sodium acetate. After about 1 hour 0.3 gm. of a yellow crystalline substance had separated. The mother liquor filtered from the first crystallization gave upon

further heating 0.2 gm. more of the same substance. After recrystallization from 50 per cent acetic acid and alcohol it had the following composition:

0.1436 gm. substance: 13 cc. N_2 at 22°, 758 mm.
0.1268 " " : 0.0824 gm. AgBr.
0.1338 " " : 0.0124 " Na_2SO_4.

$Br_2C_{18}H_{17}O_8N_4Na(C_{12}H_{17}N_2O_7Br)$.

Calculated.	Br	28.95,	Na	4.17,	N	10.15.
Found.	"	27.65,	"	3.01,	"	10.20.

0.0653 gm. substance in 5 cc. pyridine-alcohol mixture rotated in a 0.5 dm. tube with D-light – 0.90°.

g. Nitric Acid Oxidation of the Products of Hydrolysis of Chondrosin.

25 gm. of chondrosin hydrochloride were treated with sodium amalgam in exactly the same manner as described in the previous experiment. The solution, after having been freed from inorganic salts by precipitation with alcohol, was evaporated to a syrup. This syrup was quickly evaporated in a flat dish with dilute nitric acid composed of 1 part of nitric acid, specific gravity of 1.42, and 1 part of water. The residue was then evaporated several times with water, and finally taken up in 15 cc. of water and neutralized with potassium hydroxide. Upon addition of glacial acetic acid the crystallization of the acid potassium saccharate began after a short time. After 2 days the yield amounted to 1.1 gm. For analysis it was recrystallized from water.

0.1253 gm. substance: 0.0427 gm. K_2SO_4.

Calculated.	K	15.72.
Found.	"	15.32.

h. Nitric Acid Oxidation of Chondrosin and Subsequent Hydrolysis.

10 gm. of chondrosin hydrochloride were evaporated in a flat dish on a water bath with 10 cc. of nitric acid and 10 cc. of water. The residue was dissolved in 10 cc. of water and 5 cc. of nitric acid and again evaporated to dryness. The final residue was then dissolved in 10 cc. of water and the solution divided into 2 parts of 7 and 3 cc. each and neutralized in the cold with potassium hydroxide.

The larger portion, after addition of 2 cc. of 50 per cent potassium hydroxide, was allowed to stand for 2 hours on the water bath and then acidified with acetic acid. After several hours the acid potassium saccharate began to separate. The yield amounted to 0.5 gm. after 2 days. From the smaller portion, after addition of acetic acid, only a trace of the same substance separated after long standing.

0.1276 gm. substance: 0.0440 gm. K_2SO_4.

Calculated.	K	15.72.
Found.	"	15.46.

Brom Oxidation of the Products of Hydrolysis of Chondrosin.—A solution of 25 gm. of chondrosin hydrochloride was treated in the usual way with 2.5 per cent sodium amalgam. The solution was acidified with hydrochloric acid and allowed to stand for 5 days at ordinary temperature with an excess of bromine. It was then concentrated in vacuum to about 100 cc. and the principal amount of the salt separated by pouring the substance into hot absolute alcohol. The alcoholic solution was concentrated in vacuum to a syrup, taken up in water, and the halogen determined in an aliquot part. The requisite amount of lead acetate was then added to the remainder of the solution and the lead chloride and bromide were removed by filtration. The excess of lead was then removed by hydrogen sulfide and the solution evaporated in vacuum to about 30 cc. It was then neutralized with potassium hydroxide and after the addition of 10 cc. of glacial acetic acid allowed to stand for 2 days in the refrigerator. The separated crystals were filtered by suction and the product recrystallized from water. After drying it amounted to 1.6 gm.

0.1209 gm. substance: 0.0419 gm. K_2SO_4.
0.1210 " " : 0.0466 " H_2O and 0.1242 gm. CO_2.

$C_6H_9O_8K$.	Calculated.	H	3.65,	C	27.90,*	K	15.72.
	Found.	"	4.28,	"	27.98,	"	15.55.

* Considering that 1 atom of carbon is contained in the ash as K_2CO_3.

Furfural from Chondrosin after Oxidation with Nitric Acid.—0.4219 gm. of chondrosin (calculated from the nitrogen content) was evaporated to dryness with 5 cc. of concentrated nitric acid and

5 cc. of water. After repeated evaporation with water the solution of the residue was distilled in the usual way with hydrochloric acid of specific gravity 1.06 until no more furfural was given off. Upon addition of 0.1 gm. of phloroglucin 0.0076 gm. of phloroglucoside was obtained, corresponding to 0.0218 gm. of glucuronic acid, or about one-tenth of the amount present in chondrosin.

i. Identification of Chondrosamine.

The aminohexose of chondroitin sulfuric acid was identified as 2-amino-*d*-lyxohexose by the identity of the physical constants of the natural sugar with those of the synthetic prepared from amino-lyxoside, by the identity of the phenylosazone with the phenyl-galactosazone, and by the fact that on oxidation the sugar gives rise to chondrosaminic acid which is identical with the synthetic levo-*d*-lyxohexosaminic acid.

The amino sugar on deamination and subsequent oxidation gave 2-5-anhydrotalonic and 2-5-anhydrotalomucic acids. As was to be expected, 2-5-anhydrotalomucic acid gave anhydromucic and pyromucic acids.

Preparation of Chondrosamine from Chondroitin Sulfuric Acid.— 75 gm. of chondroitin sulfuric acid barium salt were hydrolyzed for about 7½ hours with 400 cc. of 20 per cent hydrochloric acid, with the addition of 15 gm. of stannous chloride. Barium sulfate began to separate at once, and the solution soon began to take on a yellow color which passed rapidly through brown to black with the separation of dark particles due to decomposition of glucuronic acid. Upon completion of the reaction the solution was diluted with twice its volume of warm water, and, without filtering, the tin removed with hydrogen sulfide. The sulfides of tin were separated by filtration with suction, leaving a clear, almost colorless filtrate which, without further treatment, was concentrated in vacuum to about 35 cc. This syrup-like residue was at once taken up in 75 to 80 cc.[4] of absolute alcohol, poured into a beaker, and the hydrochloride of the amino hexose caused to crystallize by adding about 100 cc. of absolute ether slowly in portions of about 10 cc. with constant

[4] The presence of too much alcohol or water causes the product to separate oily at first.

scratching of the sides of the vessel. The deposit of long white prismatic needles thus obtained was filtered with suction, and washed with absolute alcohol and ether. The first yield usually amounts to about 16 gm. while, upon addition of 50 cc. more of ether to the first filtrate, about 4 gm. more of equally pure product are obtained. The total yield corresponds to about 90 per cent of the theory. Upon recrystallization under the above conditions or on long keeping in a desiccator, the product tends to lose a small amount of hydrochloric acid, since an analysis of a product twice so treated gave the following figures:

0.1794 gm. substance required 14.6 cc. $AgNO_3$ solution (1 cc. = 0.00186 gm. Cl).
0.1701 " " : 0.0977 gm. H_2O and 0.2113 gm. CO_2.

$C_6H_{13}O_5N \cdot HCl$.	Calculated.	C 33.40,	H 6.54,	Cl 16.45.
	Found.	" 33.93,	" 6.55,	" 15.15.

The optical measurement was carried out at 0°. All apparatus was cooled to this temperature.

0.3000 gm. substance in 3 cc. H_2O, total weight of solution 3.2871 gm. specific gravity 1.0352, had the following rotation:

After 5 min. $+ 6.10°$: $[\alpha]_D^{20} = 129.°$
" 24 hrs. $+ 4.44°$: $[\alpha]_D^{20} = 94.°$

The activity of pure glucosamine was determined for comparison under the same condition.

0.3000 gm. substance in 3 cc. H_2O, weight of solution 3.2876 gm., specific gravity 1.0327, rotated

After 5 min. $+ 4.79°$: $[\alpha]_D^{20} = 102.°$
" 24 hrs. $+ 3.50°$: $[\alpha]_D^{20} = 74.°$

Later the process was simplified as follows: 400 gm. of the dry lead salt were taken up in 1,600 cc. of a 20 per cent solution of hydrochloric acid, 40 gm. of stannous chloride and 100 gm. of barium chloride added, and all was heated with return condenser over a free flame for 12 hours. When the hydrolysis was completed the reaction product was filtered. The filtrate was concentrated under diminished pressure to a thick syrup. This was taken up in 800 cc. of water and the barium removed by means of sulfuric acid. The final filtrate was concentrated under diminished pressure to a thick

syrup. This was taken up in about 100 cc. of methyl alcohol, and ether was added very cautiously until the sugar began to crystallize. The crystallization progressed continuously for about 24 hours. The yield was about 35 to 40 gm. of chondrosamine hydrochloride.

j. Phenylosazone of Chondrosamine.

Osazone of Chondrosamine.—4 gm. of chondrosamine hydrochloride were dissolved in 400 cc. of water, and enough sodium acetate was added to neutralize the hydrochloric acid. To this solution were added 10 gm. of phenylhydrazine dissolved in glacial acetic acid. The solution was then immersed for 5 hours in a boiling water bath. A considerable precipitate of osazone appeared during the process of heating. A voluminous precipitate formed on cooling over night. The precipitate was then filtered, washed, and suspended in 500 cc. of hot water. The flask was placed over a flame, and pyridine added in small portions until osazone dissolved. The operation was repeated once. Under the microscope the osazone consisted of long plates with pointed ends. No impurity could be noticed on the microscopic slide. The precipitate was filtered on a suction funnel, washed with a very small portion of alcohol, and then transferred into a solution of equal parts of alcohol and ether, allowed to stand for several hours in the refrigerator, and filtered. It had the appearance of very light orange glistening plates. It melted at 201° and decomposed at 202°C. A mixed melting point with galactosazone was identical with the original. Also a mixed melting point with the osazone obtained from synthetic lyxohexosamine was identical.

For the optical rotation only 0.0500 gm. were used, since it was found that by employing 0.100 gm. in the Neuberg pyridine-alcohol solution one was not certain of accomplishing rapidly a complete solution. A parallel experiment was made with the osazone obtained from the synthetic lyxohexosamine.

0.0500 gm. substance in a 0.5 dm. tube had an initial rotation of $\alpha = +0.36°$

After 24 hrs.	0.00°
" 40 "	−0.10°
" 80 "	−0.25°
" 96 "	−0.30°

Observations were interrupted at this point.

k. Preparation of Chondrosaminic Acid from Chondrosamine.

Chondrosaminic Acid from Natural and Synthetic Sugars.—This acid was obtained from both the natural and synthetic sugar by oxidation with mercuric oxide. The conditions given by Pringsheim and Ruschmann for oxidation of glucosamine had to be modified.

4 gm. of sugar were dissolved in 62 cc. of water; 20 gm. of mercuric oxide were added, and the mixture was warmed on the water bath for 6 minutes. The reaction product was filtered immediately, the filtrate was freed from mercury by means of hydrogen sulfide, the filtrate from the sulfide concentrated to a small volume, under diminished pressure, when the acid crystallized in the distilling flask. The substance was recrystallized once. It did not melt, but turned light brown at 190°, like the acid obtained from the natural sugar. A parallel measurement of the rotation of each product gave the following results:

Natural product.

Initial (in 2.5 per cent HCl). Equilibrium.

$$[\alpha]_D^{25} = \frac{-0.90 \times 2.5}{1 \times 0.1254} = -18° \qquad [\alpha]_D^{25} = \frac{-1.60 \times 2.5}{1 \times 0.1254} = -32°$$

Synthetic.

Initial (in 2.5 per cent HCl). Equilibrium.

$$[\alpha]_D^{25} = \frac{-0.94 \times 2.5}{1 \times 0.1256} = -18° \qquad [\alpha]_D^{25} = \frac{-1.60 \times 2.5}{1 \times 0.1256} = -32°$$

The analysis of the acid from the natural sugar was:

0.0992 gm. substance: 0.1326 gm. CO_2 and 0.0568 gm. H_2O.
0.1396 gm. " : (Van Slyke) 17.3 cc. N_2 at 22°, 760 mm.

$C_6H_{13}O_6N$. Calculated. C 36.92, H 6.66, N 7.18.
Found. " 36.61, " 6.44, " 7.02.

The analysis of the synthetic acid gave the following results:

0.1050 gm. substance: 0.1426 gm. CO_2 and 0.0618 gm. H_2O.

$C_6H_{13}NO_6$. Calculated. C 36.92, H 6.66.
Found. " 37.03. " 6.58.

Thus the identity of the two chondrosaminic acids is established.

*l. Preparation of 2-5-Anhydrotalonic Acid.**

*m. Oxidation of 2-5-Anhydrotalonic to 2-5-Anhydrotalomucic Acid.***

n. Oxidation of Chondrosamine to 2-5-Anhydrotalomucic Acid.

9 gm. of chondrosamine hydrochloride were deaminized with silver nitrite, the resulting solution was concentrated to about 20 cc., mixed with an equal volume of concentrated nitric acid, and allowed to stand over night at room temperature. It was then rapidly evaporated in a shallow dish on a water bath and the syrup, after having been again evaporated with water, was diluted to 250 cc., and boiled with calcium carbonate until neutral (one-half hour). The filtrate, upon standing for 2 days, deposited white prisms of the calcium salt of a dibasic hexonic acid. The yield did not exceed 25 per cent of the theory. For analysis it was recrystallized by dissolving in 50 parts of boiling water containing slightly over the theoretical amount of oxalic acid and again transformed into the calcium salt by boiling with calcium carbonate. The compound contained 2 molecules of crystal water which can be removed in vacuum at 108°. By heating for 16 hours at 138° no further appreciable loss of weight was observed.

The dried substance analyzes best for the calcium salt of a normal dibasic hexonic acid.

0.1504 gm. air-dried substance: 0.0200 gm. H_2O (108°).
0.1508 " " " : 0.0208 " " (108°).
0.1509 " " " : 0.0198 " " (108°).
0.1670 " " " : 0.0232 " " (140°).

$C_6H_8O_8Ca + 2H_2O$. Calculated. H_2O 12.68.
Found. I. " 13.3.
" II. " 13.
" III. " 13.1.
" IV. " 13.3.

0.1304 gm. dried substance: 0.0344 gm. H_2O, 0.1376 gm. CO_2, and 0.0302 gm. CaO.

0.1312 gm. dried substance: 0.0380 gm. H_2O, 0.1380 gm. CO_2, and 0.0302 gm. CaO.

0.1030 gm. dried substance: 0.0296 gm. H_2O, 0.1083 gm. CO_2, and 0.0243 gm. CaO.

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* See page 40.
** See page 41.

$C_6H_8O_6Ca$.	Calculated.	C	29.03,	H	3.22,	CaO	22.6.
	Found. I.	"	28.78,	"	2.95,	"	23.4.
	" II.	"	28.68,	"	3.24,	"	21.8.
	" III.	"	28.67,	"	3.22,	"	23.2.
	" IV.					"	22.5.

0.1020 gm. substance, in 2 cc. 10 per cent HCl, rotated in 1 dm. tube:

After 10 min., −0.45°.
" 18 hrs., −0.37°.

*o. Preparation of 2-5-Anhydrogalactonic Acid.**

p. Conversion of 2-5-Anhydrotalomucic into Anhydromucic Acid.

1 gm. of 2-5-anhydrotalomucic acid in 1 cc. of concentrated hydrochloric acid plus 1 cc. of concentrated hydrobromic acid heated in a sealed tube at 150°C. for 8 hours, according to Fischer gave 0.2 gm. of anhydromucic acid.

0.1002 gm. substance: 0.1684 gm. CO_2 and 0.0276 gm. H_2O.

$C_6H_4O_5$.	Calculated.	C	46.16,	H	2.57.
	Found.	"	45.84,	"	3.08.

q. Conversion of 2-5-Anhydrotalomucic into Pyromucic Acid.

1 gm. of 2-5-anhydrotalomucic acid was heated at about 200°C. in a test-tube in an atmosphere of carbon dioxide for one-half hour. The substance sublimed and separated out on the cooler parts of the tube from which it was afterwards removed, extracted with ether, and recrystallized by dissolving in a large amount of ether and allowing the solution to evaporate. It melted at 135°C.

0.1000 gm. substance: 0.1964 gm. CO_2 and 0.0370 gm. H_2O.

$C_5H_4O_3$.	Calculated.	C	53.57,	H	3.57.
	Found.	"	53.56,	"	4.14.

II. From Tendomucoid.

The presence of a conjugated sulfuric acid in the molecule of tendomucoid was first discovered by Levene. Later Mandel and Levene showed the presence of a similar acid in many tissues and even in leucocytes.

* See page 42.

a. Preparation of Tendomucoid and Chondroitin Sulfuric Acid Contained in It.

Owing to the nature of the combined protein the method of preparing chondroitin sulfuric acid from tendons differs somewhat from its preparation from cartilage (Levene, 1914, *b*). Portions of 50 achilles tendons from cattle were cleaned, passed through a hashing machine and allowed to stand over night with 20 liters of two-thirds saturated lime water. The liquid was strained off and the process repeated once again on the residue. The combined filtrates were just acidified with hydrochloric acid which produced a flocculent precipitate of tendomucoid. The supernatant liquid is then siphoned off and after addition of an equal volume of 95 per cent alcohol the mucoid was filtered off on a folded filter. The moist product was agitated for some time with 1.5 liters of a 2 per cent potassium hydroxide solution. After standing over night the turbid brown solution was acidified with acetic acid and the separated protein removed by filtration on a folded filter. The filtrates from two such experiments were neutralized with sodium hydroxide and the chondroitin sulfuric acid was precipitated by a solution of basic lead acetate. The lead precipitate was repeatedly washed by triturating in a mortar with distilled water and filtering with suction. The washed product was suspended in about 2 liters of water; 10 cc. of glacial acetic acid and 20 gm. of barium acetate were added and decomposition was effected by passing in hydrogen sulfide with constant stirring. The lead sulfide was filtered off with suction, the filtrate concentrated to about 350 cc., and the barium salt precipitated by the addition of about 250 cc. of alcohol. It was then filtered with suction, washed, first with 50 per cent, then with 95 per cent, with absolute alcohol, and finally with ether. The yield amounts to about 12 to 15 gm.

0.2220 gm. substance: 0.2043 gm. CO_2 and 0.0775 gm. H_2O.
0.6136 " " : 9.25 cc. 0.1 N NH_3 (Kjeldahl).
0.6141 " " : 9.50 " 0.1 N acetic acid.
0.7002 " " : 0.2170 gm. $BaSO_4$.

$C_{28}H_{44}N_2S_2O_{29}$. Calculated. C 27.80, H 3.48, N 2.32, S 5.30, Ba 22.70.
Found. " 25.13, " 3.88, " 2.11, " 4.26, " 18.35.
N:C = 1:13.89.

b. Chondrosin.

Chondrosin Hydrochloride.—This substance was prepared exactly as described in a previous section (Levene and La Forge, 1913). It analyzed as follows:

0.1258 gm. substance: 0.1653 gm. CO_2 and 0.0685 gm. H_2O.
0.2238 " " : 13.6 cc. N_2 (Van Slyke) at 20°, 763 mm.
0.4024 " " : in 3 cc. water, weight of solution 3.3792 gm., rotated in a 0.5 dm. tube at 20° with D-light, + 2.46°.

Chondrosin Hydrochloride from Cartilage.

0.1584 gm. substance: 0.2167 gm. CO_2 and 0.0806 gm. H_2O.
0.2085 " " : 12.5 cc. amino N at 18°, 765 mm. (Van Slyke).
0.6543 " " : in 3 " H_2O, weight of solution 3.6481 gm., rotated in a 0.5 dm. tube with D-light, + 3.90°.

$C_{12}H_{21}NO_{11}HCl$(390.5). Calculated. C 36.9, H 5.64, N 3.58.
Found. (From tendons.) " 35.8, " 6.00, " 3.45.
N:C = 1:12.1. $[\alpha]_D^{20} = +41.5°$.
Found. (From cartilage.) C 37.3, H 5.66, N 3.45.
N:C = 1:12.6. $[\alpha]_D^{20} = +43.4°$.

c. Chondrosamine Hydrochloride.

This substance was prepared in exactly the same manner as described in a previous section (Levene and La Forge, 1914, *a*).

From 18 gm. of the barium salt 3.5 gm. of amino hexose were obtained. For analysis it was dissolved in 3 parts of water with the addition of a few drops of hydrochloric acid, allowed to crystallize by evaporation and dried in a desiccator. The melting point was 180°.

0.1596 gm. substance: 0.1930 gm. CO_2 and 0.0945 gm. H_2O.
0.1516 " " : 17.6 cc. N_2 (Van Slyke) at 19°, 774 mm.
0.1932 " " : in 2 cc. of water, weight of solution 2.1904 gm., rotated in a 1 dm. tube at 20° with D-light:

After about 15 min., +10.75°
" 24 hrs., + 8.5°

$C_6H_{13}O_5NHCl$. Calculated. C 33.40, H 6.54, N 6.51.
Found. " 33.01, " 6.57, " 6.79.

Equilibrium (without consideration of specific gravity).

$[\alpha]_D^{20} = +96.4°$.

d. Glucuronic Acid Osazone Hydrazide.

This substance was prepared exactly as described in a previous section (Levene and La Forge, 1913).

From 4 gm. of chondrosin hydrochloride 0.1 gm. of the substance was obtained. The melting point was exactly the same as the product from glucuronic acid cartilage chondrosin, 122°.

0.0805 gm. substance: 12.1 cc. N_2 (Dumas) at 24°, 766 mm.

$C_{24}H_{26}N_6O_4 + 1\frac{1}{2}\ H_2O$. Calculated. N 17.17.
Found. " 16.9.

III. From Aorta Mucoid.

a. Preparation of Chondroitin Sulfuric Acid.

100 pounds of aorta freed from extraneous tissue were put through a hashing machine, taken up in 20 liters of a 2 per cent solution of sodium hydroxide, and allowed to stand for 36 hours (Levene and López-Suárez, 1918). The extract was decanted and the residue again extracted for another 36 hours. The combined solutions were strained, neutralized, concentrated with an excess of barium carbonate, filtered, and finally precipitated with glacial acetic acid. The precipitate had the appearance of chondroitin sulfuric acid. The precipitate was washed with glacial acetic acid, then with alcohol, and dried. The yield was 40 gm. Of these, 8 gm. were used for hydrolysis and the remaining material was purified in the following manner.

The material was again dissolved in water and precipitated with glacial acetic acid. The precipitate was washed with glacial acetic acid and then with alcohol. The dry precipitate was dissolved with the aid of potassium hydroxide. To this solution a slight excess over the required amount of barium chloride was added, followed by the addition of an equal volume of 95 per cent alcohol. The precipitate was washed by decantation with 50 per cent alcohol until free from barium chloride. The washing was then continued with alcohol of progressively increasing strength, finally with ether, and dried. The yield of this material was 25 gm. The substance analyzed as follows:

0.2000 gm. substance required for neutralization 3.63 cc. 0.1 N acid.
0.2000 " " on fusion: 0.0354 gm. $BaSO_4$.
0.0778 " " " combustion: 0.0818 gm. CO_2 and 0.0386 gm. H_2O.

$C_{26}H_{44}O_{29}N_2S_2Ba_2$.	Calculated.	C	27.8,	H	3.48,	N	2.32,	S 5.30.
	Found.	"	28.7,	"	3.35,	"	2.54,	" 2.4.

b. Preparation of Chondrosamine.

8 gm. of the substance with 60 cc. of 20 per cent hydrochloric acid, together with 1.5 gm. of barium chloride and 1.5 gm. of stannous chloride, were hydrolyzed with a reflux condenser for 12 hours. The product of hydrolysis was freed from barium and tin, and the filtrate, concentrated under diminished pressure, warmed to about 50°C. in a water bath. On concentration the sugar crystallized in long, microscopic, prismatic needles. These were transferred to a flask by means of alcohol containing hydrochloric acid. The flask was allowed to stand over night and the precipitate was then filtered and washed with alcohol and ether. The yield was about 1 gm. The melting point was 183° (uncorrected).

0.0200 gm. substance in the Van Slyke apparatus: 2.30 cc. N_2 at 25°C. and 762.7 mm.

$C_6H_{13}O_6N \cdot HCl$.	Calculated.	N	6.51.
	Found.	"	6.40.

The rotation of the substance was

Initial.	Equilibrium.
$[\alpha]_D^{25} = \frac{+1.64 \times 2.0593}{1 \times 0.0508} = +66°$	$\frac{+2.27 \times 2.0593}{1 \times 0.0508} = +91°$

c. Preparation of Chondrosin.

20 gm. of chondroitin sulfuric acid were hydrolyzed for 1 hour in a boiling water bath with 60 cc. of 20 per cent hydrochloric acid. The reaction product was filtered, concentrated under diminished pressure, and precipitated by means of alcohol and ether. The yield was 4 gm. The analysis of the substance gave the following results:

0.1000 gm. substance required for neutralization 3.10 cc. 0.1 N acid.
0.0200 " " in the Van Slyke micro apparatus: 1.2 cc. N_2 at 25°C. and 759.6 mm.

$C_{12}H_{21}O_{11}N \cdot HCl$.	Calculated.	Total N	3.58,	NH_2-N	3.58.
	Found.	"	4.34,	"	3.41.

The rotation of the substance was

$$[\alpha]_D^{25} = \frac{+1.04 \times 2.0026}{1 \times 0.0496} = +42°$$

IV. From Sclera Mucoid.

a. Preparation of Chondroitin Sulfuric Acid.

For the preparation of the substance (Levene and López-Suarez, 1918), originally the sclera and cornea were worked up in one. The hydrolysis of the substance, however, revealed the presence of two sugars, chitosamine and chondrosamine. Because of this, in later experiments the cornea was dissected out. The sample used for ultimate analysis was prepared from sclera and cornea combined. But the sugar fraction still contained some glucosamine. On the other hand, the mucoid from the cornea contained only glucosamine. The sclera was the only tissue which yielded mucoid containing both sugars, and the possibility is not excluded that one of them (chitosamine) is derived from adhering extraneous tissues.

The procedure for the preparation of the conjugated sulfuric acid was the following. The eyes were freed from adhering muscle and connective tissue, then the humor vitreous, lens, and retina were removed. Finally, the cornea and sclera were washed with running water from all extraneous material. After this the corneas were carefully dissected out, and the scleras were minced in the hashing machine, and placed in a large volume of 3 per cent sodium hydroxide. For the corneas of 1,000 eyes 20 liters of sodium hydroxide were used. The extraction was continued 3 days, at the end of which time the solution was strained through cheese-cloth and neutralized with acetic acid. Barium carbonate was then added in excess and the mixture was concentrated on a water bath to a small volume. The product of the reaction was filtered on a suction funnel and then

converted into the lead salt. The lead salt was treated in the usual way. It was converted into the sodium salt for the analysis of conjugated sulfuric acid. For the isolation of the sugar it was hydrolyzed directly. The sodium salt analyzed as follows:

0.1903 gm. substance required for neutralization 7.69 cc. 0.1 N acid.
0.2855 " " : 0.0950 gm. $BaSO_4$.
0.0986 " " : 0.1239 " CO_2 and 0.0428 gm. H_2O.

$C_{26}H_{44}O_{29}N_2S_2Ba_2$.	Calculated.	C	27.8,	H	3.48,	N	2.32,	S	5.30,	Ba 22.70.
	Found.	"	34.3,	"	4.86,	"	5.66,	"	4.57,	" 23.83.

b. Preparation of Chondrosamine.

12 gm. of the lead salt with 2 gm. stannous chloride, 2 gm. of barium chloride, and 60 gm. of 20 per cent hydrochloric acid were heated with a reflux condenser for 10 hours over a free flame. The reaction product was freed from lead, tin, and barium, and then concentrated to syrup. This was dissolved in a minimal amount of hot methyl alcohol. Soon crystals appeared which had the typical appearance of chitosamine. These were filtered off and the mother liquor was allowed to stand; from time to time a few drops of ether were added. At the end of a week the maximum amount of chondrosamine hydrochloride settled out. The best yield for 1 kg. was 0.5 gm. of the sugar.

The melting point of this was 182° (corrected). The analysis of the substance was as follows:

0.0200 gm. substance: 2.39 cc. N_2 at 19°C. and 745 mm.
0.0523 " " required for titration of the HCl 2.39 cc. $AgNO_3$(1 cc. = 0.003546 gm.).
0.1036 gm. substance: 0.1224 gm. CO_2 and 0.0602 gm. H_2O.

$C_6H_{13}O_5N \cdot HCl$.	Calculated.	C	33.40,	H	6.54,	N	6.57,	Cl	16.45.	
	Found.	"	33.18,	"	6.90,	"	6.38,	"	16.2.	

The rotation of the substance was

Initial.	Equilibrium.
$[\alpha]_D^{25} = \frac{+1.42 \times 2.0408}{1 \times 0.0510} = +57°$	$\frac{+2.39 \times 2.0408}{1 \times 0.0510} = +96°$

2. GROUP II A. MUCOITIN SULFURIC ACIDS.

I. From Funis Mucin.

a. Preparation of Mucoitin Sulfuric Acid.

For the preparation of the substance (Levene and López-Suárez, 1918; 1916, *b*), it is essential to free the cords from all adhering blood clots and blood vessels, as otherwise the resulting substance is contaminated with nucleic acid. The separation of the two is very troublesome. The purification, however, was accomplished by vigorous treatment with glacial acetic acid. The treatment with lead acetate is a convenient step in order to free the substance from adhering salt as well as from other impurities.

In removing the lead by means of hydrogen sulfide one has to bear in mind the insolubility of the acid in water. Because of this, it is necessary to carry out the separation of lead in a slightly alkaline solution.

The details of the process as carried out at present are as follows: About 100 cords, freed from blood vessels, either shredded or chopped in a hashing machine, were taken up in 6 liters of 72 per cent NaOH and allowed to stand for 3 days, then acidulated and centrifugalized to remove the precipitate. The supernatant liquid was concentrated with an excess of barium carbonate on a water bath. This operation was continued for 24 hours and the product centrifugalized. The supernatant liquid was allowed to stand on a hot water bath after a second addition of barium carbonate. Water was added from time to time. The operation was continued for about 2 days. The resulting material was then centrifugalized, and the supernatant liquid allowed to stand until part of the barium acetate had crystallized out. The material was again centrifugalized and the clear supernatant solution precipitated with glacial acetic acid. The precipitate was redissolved in water on addition of barium acetate; the substance was reprecipitated out of this solution with glacial acetic acid. The crude material was washed with 95 per cent alcohol to remove the excess of acetic acid. The material was dissolved in water, and the solution was neutralized with a solution of barium hydroxide until it reacted neutral to litmus.

To the final solution enough 95 per cent alcohol was added to precipitate the crude barium salt. This was washed first with 50 per cent alcohol to remove adhering barium acetate, then with alcohol of increasing concentration, and with 99.5 per cent alcohol and then with ether. The final product analyzed as follows:

0.1000 gm. substance required for neutralization 2.88 cc. 0.1 N acid.
0.2000 " " on fusion: 0.0592 gm. $BaSO_4$.
0.0936 " " " combustion: 0.0450 gm. H_2O and 0.1146 gm. CO_2.

$C_{26}H_{44}O_{29}N_2S_2Ba_2$. Calculated. C 27.8, H 3.48, N 2.32, S 5.30.
Found. " 33.4, " 5.38, " 4.03, " 4.07.

The optical rotation of the substance was

$$[\alpha]_D^{25} = \frac{-0.50 \times 5.0641}{1 \times 0.0555} = -46°$$

b. Preparation of Mucosin.

An attempt was made to prepare mucosin under the same conditions of hydrolysis as employed for the preparation of chondrosin. However, the largest part of the substance underwent complete hydrolysis, with the formation of free chitosamine, which was identified in the usual way. It was found subsequently, that a substance analogous to chondrosin could be obtained under the following conditions.

4.5 gm. of the barium salt were dissolved in 100 cc. of 10 per cent hydrochloric acid and heated on a water bath for $\frac{1}{2}$ hour. The solution then contained all its nitrogen in form of primary amino nitrogen, and showed a reduction of Fehling's solution equivalent to 1.12 gm. of glucose; the theory requires 1.26 gm. The solution was freed quantitatively from barium, and concentrated to 3 cc. under diminished pressure at a temperature of water bath not exceeding 45°C. This was then gradually poured into 200 cc. of alcohol, to which 400 cc. of dry ether had been added. A white flocculent precipitate then formed. It was allowed to stand over night, then filtered, and dried. The yield was 1.5 gm.

0.0188 gm. substance, in the Van Slyke micro apparatus: 1 cc. N_2 at 27° and 759.3 mm. pressure.

0.0918 gm. substance: 0.1288 gm. CO_2 and 0.0476 gm. H_2O.

$C_{12}H_{21}O_{11}N \cdot HCl$. Calculated. C 36.9, H 5.64, NH_2-N 3.58.
Found. " 38.25, " 5.8, " 3.2.

$$[\alpha]_D^{20} = \frac{+0.66 \times 2.0287}{1 \times 0.0524} = +26°$$

Later it was found possible to prepare mucosin by hydrolyzing the barium salt for one-half hour on a water bath in an aqueous solution of 1 per cent sulfuric acid. The excess of acid was then removed by means of barium hydroxide and the filtrate concentrated.

0.1004 gm. substance: 0.1312 gm. CO_2 and 0.0478 gm. H_2O.
0.1371 " " : 3.50 cc. 0.1 N acid (Kjeldahl).
0.1247 gm. substance on fusion: 0.0214 gm. $BaSO_4$.
0.1315 " " : 0.0410 gm. $BaSO_4$.

$C_{28}H_{42}N_2S_2O_{29}Ba_2$. Calculated. C 27.80, H 3.48, N 2.32, S 5.30, Ba 22.70.
Found. " 35.61, " 5.30, " 3.57, " 2.35, " 18.3.

c. Preparation of Chitosamine.

The material obtained for this experiment was prepared by the first of the two procedures described above. 50 cords were passed through a meat chopper, and then extracted with 1,500 cc. of a 3 per cent solution of sodium hydroxide. The solution was treated exactly as the first sample. The yield was 15 gm. of the lead salt.

These 15 gm. were taken up in 100 cc. of 20 per cent hydrochloric acid solution, 1.5 gm. stannous chloride were added, and the mixture was heated with a reflux condenser for 10 hours. The barium sulfate and pigment were removed by filtration, and the remaining clear solution was concentrated under diminished pressure to small volume. The residue was dissolved in 100 cc. of distilled water, and lead and tin were removed by means of hydrogen sulfide. The filtrate from the sulfide was freed from barium quantitatively and the clear solution was concentrated under diminished pressure to a syrup. A perfectly white crystalline deposit formed in the distilling flask. This was transferred to a suction funnel by means of methyl alcohol. It consisted of perfectly uniform crystals, in appearance unusual for glucosamine hydrochloride. Once recrystallized out of dilute alcohol it assumed the crystal form typical of chitosamine hydrochloride. Heated in a sealed capillary to a tem-

perature of 220°C. it contracted slightly, turned dark, but did not melt. Dried over sulfuric acid under diminished pressure the substance gave the following analytical results:

0.0314 gm. substance in Van Slyke micro apparatus: 3.80 cc. N_2 at 25° and 765 mm.

0.1577 gm. substance required 7.30 cc. 0.1 N $AgNO_3$.

$C_6H_{13}NO_5HCl$.	Calculated.	N	6.50,	Cl	16.45.
	Found.	"	6.45,	"	16.4.

The rotation of the substance was

Initial.

$$[\alpha]_D^{25} = \frac{+4.08 \times 2.1459}{0.5 \times 0.2008 \times 1.04} = 85°$$

Equilibrium.

$$[\alpha]_D^{25} = \frac{+3.5 \times 2.1459}{0.5 \times 0.2008 \times 1.04} = 72°$$

d. Identification of Glucuronic Acid.

The presence of glucuronic acid was demonstrated by the formation of furfural on distillation of the mucoitin sulfuric acid with hydrochloric acid, by the phenylhydrazine derivative of glucuronic acid after the hydrolysis of mucosin with sodium amalgam, and finally by the isolation of the acid potassium salt of saccharic acid on oxidation of mucosin with nitric acid.

The estimation of the yield of furfural phloroglucide also permitted an approximate estimate of the proportion of glucuronic in the molecule of the mucoitin sulfuric acid.

Distillation with Hydrochloric Acid.—1.5 gm. of barium salt were distilled over a flame in 250 cc. of HCl (specific gravity 1.06) until the distillate no longer gave a test with aniline acetate. To the distillate 0.3 gm. of phloroglucide was added and the solution allowed to stand over night. The phloroglucide was filtered over a Gooch crucible. The yield was 0.0870 gm., which corresponds to 0.2610 gm. of glucuronic acid. The theory requires 0.5000 gm.

Hydrolysis by Means of Sodium Amalgam.—3.5 gm. of mucosin hydrochloride were dissolved in 50 cc., and 150 gm. of 2 per cent sodium amalgam were added in 25 gm. portions. After each addition the solution was neutralized with sulfuric acid. After the last portion of amalgam had been added the flask was placed in a shaking machine for 5 hours and then allowed to stand over night. The

following day the solution was filtered and neutralized with sulfuric acid. 5 gm. of phenylhydrazine dissolved in 5 cc. of glacial acetic acid were then added and the solution was warmed on a boiling water bath for 30 minutes with a reflux condenser. On cooling over night a crystalline deposit formed. This was filtered, suspended in water, again filtered, and suspended in 99.5 per cent alcohol, filtered, and dried in a vacuum desiccator over sulfuric acid.

The melting point of the substance was 125°C. and decomposition with effervescence took place at 132°C. (corrected). A sample prepared from chondrosin had exactly the same melting point. In the communication of Levene and La Forge the melting point was given at 115°. The manner of purification of the substance as carried out at the later date was more rigorous, and the melting point of 125°C. with decomposition at 132° is to be regarded as the correct one.

0.0632 gm. substance on combustion: 9.4 cc. N_2 at 28°C. and 767.5 mm.

$C_{24}H_{26}O_4N_6+1\frac{1}{2}H_2O$.	Calculated.	N	17.17.
	Found.	"	16.9.

e. Oxidation with Nitric Acid with Subsequent Hydrolysis.

10 gm. of mucosin hydrochloride were dissolved in 10 cc. of distilled water to which 10 cc. of nitric acid (specific gravity 1.40) were added, and the solution was heated over a free flame until the evolution of nitrous acid fumes became very lively. The solution was immediately transferred to a clock glass and evaporated with constant stirring. The subsequent treatment was as usual. The final solution was made up to 10 cc. Of this 1 cc. was used as control, and 9 cc. were allowed to digest with 2 cc. of a 50 per cent solution of potassium hydroxide on a boiling water bath for 2 hours. The solution was then made acid with acetic acid and the acid potassium salt was allowed to crystallize. The crude salt on fractionation out of water yielded a sample of the salt which analyzed as follows:

0.1000 gm. salt: 0.0356 gm. K_2SO_4.

$C_6H_9O_7K$.	Calculated.	K	15.70.
	Found.	"	15.95.

f. Estimation of the Number of Acetyl Groups.

2 gm. of the barium salt of mucoitin sulfuric acid were dissolved in 200 cc. of water containing 15 gm. of barium hydroxide, and hydrolyzed on a water bath for 5 hours. The product of the reaction was rendered acid to Congo red by means of sulfuric acid, and filtered. The solution was then distilled guarding the original volume (600 cc.). The distillate was received in a measured volume of 0.1 N sodium hydroxide. 31.3 cc. of 0.1 N alkali were neutralized by the distillate. Calculated for acetic acid the yield was 0.1878 gm. The theory for one acetyl group requires 0.1944 gm.

Identification of Acetic Acid.—The entire distillate was concentrated under diminished pressure to 8 cc., rendered acid with sulfuric acid, and extracted with ether. To the ethereal extract a few drops of aqueous ammonia were added and the ether was allowed to evaporate spontaneously. The residue was converted into the silver salt.

0.1052 gm. dry substance: 0.0681 gm. Ag.

$C_2H_3O_2Ag$.	Calculated.	Ag	64.26.
	Found.	"	64.73.

II. From Vitreous Mucoid.

a. Preparation of Mucoitin Sulfuric Acid.

To vitreous humor of 1,000 eyes enough of a 50 per cent sodium hydroxide solution was added to make the concentration of alkali 3 per cent (Levene and López-Suárez, 1918). The material was allowed to stand 3 days; it was then acidulated and concentrated on a water bath after addition of an excess of barium carbonate. The final product was filtered on suction. To the filtrate enough of basic lead acetate solution was added to precipitate all of the acid. The crude lead salt was washed by decantation, then filtered, and the precipitate was washed once with glacial acetic acid. The precipitate was then filtered and washed with alcohol. After this the substance was taken up in water, the mixture rendered slightly alkaline by means of a solution of potassium hydroxide, and the lead salt was decomposed by hydrogen sulfide. From the

filtrate, hydrogen sulfide was removed by aeration and the solution was poured into 2 liters of alcohol. A precipitate thus formed was washed with alcohol and ether. The substance analyzed as follows:

0.1000 gm. substance required for neutralization 3.54 cc. 0.1 N acid.
0.2000 " " : 0.0528 gm. $BaSO_4$.
0.0958 " Ba salt: 0.1208 gm. CO_2 and 0.0490 gm. H_2O.

$C_{26}H_{44}O_{28}N_2S_2Ba_2$. Calculated. C 27.8, H 3.48, N 2.32, S 5.3, Base 22.7.
Found. " 34.4, " 5.72, " 4.96, " 3.6, " 21.5.

b. Preparation of Chitosamine.

6 gm. of the substance were dissolved in 30 cc. of 20 per cent HCl + 1 gm. of stannous chloride + 1 gm. of barium chloride heated with a reflux condenser for 8 hours over a Babo funnel. The solution was filtered, decomposed with hydrogen sulfide, and freed from barium quantitatively. The final solution was concentrated under diminished pressure to about 5 cc. The sugar began to crystallize in the distilling flask. The entire residue was taken up in methyl alcohol and allowed to crystallize at room temperature. The yield of the substance was 1 gm.

0.0302 gm. substance: 3.47 cc. N_2 at 19°C. and 745 mm.

$C_6H_{13}O_6N \cdot HCl$. Calculated. N 6.51.
Found. " 6.45.

The rotation of the substance was

Initial. Equilibrium.

$$[\alpha]_D^{20} = \frac{+4.60 \times 2.1248}{1 \times 0.1010 \times 1.0018} = +97° \qquad \frac{+3.43 \times 2.1248}{1 \times 0.1010 \times 1.0018} = +72°$$

c. Furfural Distillation.

1.5 gm. of the barium salt were distilled with 250 cc. of HCl (1.06) as long as distillate showed the presence of furfural. Further treatment was carried out as above, and 0.3 gm. of phloroglucine was added. The yield was 0.1165 gm., which corresponds to 0.3495 gm. of glucuronic acid. The theory required 0.500 gm.

III. From Cornea Mucoid.

a. Preparation of Mucoitin Sulfuric Acid.

The corneas of 1,000 beef eyes were mechanically separated from the sclera and placed in 1,500 cc. of 3 per cent sodium hydroxide solution and allowed to stand for 3 days, then strained through cheese-cloth and acidulated with acetic acid (Levene and López-Suárez, 1918). Barium carbonate was added in excess and the mixture concentrated on a water bath to a thick syrupy mass containing the coagulated protein and the barium carbonate.

The mass was filtered on suction, and to the filtrate sufficient lead carbonate was added to precipitate all the acid. The purification of the lead salt was carried out in the manner described above. The final substance had the following composition.

0.1000 gm. substance required for neutralization 2.42 cc. 0.1 N acid.
0.2000 " " : 0.0330 gm. $BaSO_4$.

A second precipitate was prepared as follows: The corneas were treated with alkali in the same manner as in the former experiment. The acidulated solution was concentrated in the presence of barium carbonate, and the filtrate poured into an excess of glacial acetic acid. The precipitate was washed repeatedly with glacial acetic acid, then with alcohol. The dry substance was then redissolved in a little water with the aid of potassium hydroxide. The solution was poured into a large excess of 99.5 per cent alcohol. The potassium salt obtained in this manner was dried and analyzed.

0.2000 gm. substance required for neutralization 6.90 cc. 0.1 N acid.
0.2000 " " on fusion: 0.0452 gm. $BaSO_4$.
0.0950 " potassium salt of substance: 0.1318 gm. CO_2 and 0.0528 gm. H_2O.

$C_{26}H_{44}O_{29}N_2S_2Ba_2$. Calculated. C 27.8, H 3.48, N 2.32, S 5.30, Base 22.70.
Found. I. " 3.93, " 2.27.
" II. C 37.83, H 6.32, " 4.62, " 3.10, Base 14.74.

b. Preparation of Chitosamine.

4.5 gm. of the substance were hydrolyzed in 60 cc. of 20 per cent hydrochloric acid, together with 1 gm. of barium chloride and 1 gm. of stannous chloride, and the solution was heated with a reflux

condenser for 12 hours over a Babo funnel. The solution as usual turned dark brown. It was diluted with an equal volume of water and was then freed from tin by means of hydrogen sulfide, and from barium by means of sulfuric acid. The solution was concentrated to a thick syrup. Chitosamine crystallized in the distilling flask. It was taken up in methyl alcohol and kept at room temperature in order to complete the crystallization. The yield was 0.520 gm. The appearance of the crystals under the microscope was typical for glucosamine. The substance turned brown at about 200°C., and black at 220°C. It did not melt.

0.0200 gm. substance in the Van Slyke apparatus: 2.41 cc. of N_2 at 27°C. and 756.8 mm.

$C_6H_{13}O_5N \cdot HCl$. Calculated. N 6.51.
Found. " 6.80.

The rotation of the substance was

Initial.	Equilibrium.
$[\alpha]_D^{20} = \frac{+2.27 \times 2.0390}{1 \times 0.0485} = +95°$	$\frac{+1.71 \times 2.0390}{1 \times 0.485} = +72°$

3. GROUP II B. MUCOITIN SULFURIC ACIDS.

I. From Mucin of the Gastric Mucosa.

a. Preparation of Mucoitin Sulfuric Acid.

Mucus was removed from the gastric wall mechanically and a concentrated solution of barium hydroxide was added to make the total solution contain 3 per cent of the hydroxide (Levene and López-Suárez, 1916, *a;* 1918). The solution was allowed to stand for 3 days at room temperature. At the end of this time the solution was rendered acid to Congo red by means of sulfuric acid, then centrifugalized. The supernatant liquid was neutralized with a solution of barium hydroxide until neutral to Congo red, but still acid to litmus, and finally neutralized to litmus by means of barium carbonate, then boiled for about 3 hours, and filtered. To the filtrate again barium carbonate was added and the mixture was allowed to stand on a water bath from 2 to 3 days until a sample of the filtrate showed a negative biuret test. This was centrifugalized and the supernatant

liquid precipitated by means of glacial acetic acid. The precipitate was redissolved in water and reprecipitated by means of glacial acetic acid. The precipitate thus formed was repeatedly washed with 95 per cent alcohol until most of the glacial acetic acid was removed. This material was then dissolved in a minimum amount of water, the solution was exactly neutralized with a solution of barium hydroxide, and the barium salt of mucoitin sulfuric acid precipitated by means of alcohol. The crude salt was repeatedly washed with a 50 per cent solution of alcohol until most of the inorganic impurities were removed, then with alcohol of progressively increasing concentration. This salt was a mixture of mucoitin, sulfuric, and nucleic acids. To separate the two, the mixture was taken up in water and centrifugalized. The salt of the nucleic acid, being insoluble, was removed in this manner. To complete separation it was necessary to repeat the operation several times. Finally the clear solution was poured into an excess of alcohol, giving a precipitate of the barium salt of the mucoitin sulfuric acid. A sample of the material prepared in this manner had the following composition:

0.1000 gm. substance required for neutralization 2.48 cc. 0.1 N acid.
0.1500 " " : 0.0162 gm. $BaSO_4$.

$C_{26}H_{44}O_{29}N_2S_2Ba_2$	Calculated.	N	2.32,	S	5.30.
	Found.	"	3.47,	"	1.48

$$[\alpha]_D^{20} = \frac{-0.19 \times 5.3035}{1 \times 0.0447} = -23°$$

b. Preparation of Chitosamine.

20 gm. of the partially purified substance, taken up with 100 cc. of 20 per cent hydrochloric acid and 4 gm. of stannous chloride, were heated with a return condenser over flame for $7\frac{1}{2}$ hours. The solution was diluted with an equal volume of water, hydrogen sulfide passed through the solution, and the filtrate concentrated under diminished pressure (approximately 15 mm.) nearly to dryness. A crystalline sediment formed in the flask. This was transferred to a beaker by means of methyl alcohol. The substance was recrystallized out of dilute methyl alcohol. Unlike chondrosamine hydro-

chloride, the substance was insoluble in methyl alcohol, and crystallized in plates resembling those of chitosamine hydrochloride. The substance did not melt. It reduced Fehling's solution, and formed a glucosazone. For analysis it was dried in a vacuum desiccator.

0.0200 gm. substance in the Van Slyke amino apparatus: 2.38 cc. N at 25° and 757 mm.

0.1500 gm. substance by the Volhard method required 6.86 cc. 0.1 N $AgNO_3$.

$C_6H_{13}O_5NHCl$.	Calculated.	N	6.51,	Cl	16.45.
	Found.	"	6.57,	"	16.23.

The rotation of the substance was

Initial. Equilibrium.

$$[\alpha]_D^{20} = \frac{+4.30 \times 2.1862}{0.5 \times 0.2008 \times 1.039} = +90.1° \qquad [\alpha]_D^{20} = \frac{+3.37 \times 2.1862}{0.5 \times 0.2008 \times 1.039} = +71°$$

c. Oxidation of the Chitosamine.

6 gm. of the sugar were dissolved in 25 cc. of water, 6 gm. of silver nitrite were added, and the mixture was allowed to stand 6 hours; then another portion of 3 gm. of silver nitrite and the equivalent quantity of a 10 per cent hydrochloric acid solution were added. The mixture was allowed to stand over night. The solution then contained 0.0027 gm. of amino nitrogen. The excess of silver was removed by means of hydrogen sulfide. To the solution 15 gm. of bromine were added and allowed to stand for 48 hours. The calcium salt of chitonic acid was then prepared in the usual way.

For analysis the calcium salt was dried in a vacuum desiccator at the temperature of water vapor.

0.0994 gm. substance: 0.1212 gm. CO_2, 0.0472 gm. H_2O, and 0.0134 gm. CaO.

$(C_6H_9O_6)Ca + 2H_2O$.	Calculated.	C	33.47,	H	5.11,	CaO 13.07.
	Found.	"	33.25,	"	5.27,	" 13.6.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+1.63 \times 2.149}{1 \times 1.000 \times 1.010} = +34.7°$$

Fischer gives for the same substance

$$[\alpha]_D^{10} = +32.8°$$

d. Preparation of Mucosin.

For the preparation of mucosin 14 gm. of the barium salt were dissolved in 100 cc. of water and 15 cc. of concentrated hydrochloric acid, and allowed to stand on the boiling water bath for 20 minutes. The solution was concentrated under diminished pressure (the temperature of the bath not exceeding 40°C.) to a volume of 5 cc. The solution was poured into 2 liters of a 50 per cent mixture of alcohol and ether. A precipitate formed which was removed by filtration. The precipitate was then dissolved in about 3 cc. of water and precipitated by 400 cc. of 99.5 per cent alcohol. To the filtrate, an equal volume of ether was added and thus a second precipitate was formed which reduced Fehling's solution, but was not yet free from mucoitin.

0.0100 gm. in the Van Slyke apparatus: 0.38 cc. N at 22° and 753.7 mm.

$C_{12}H_{21}O_{11}N \cdot HCl$.	Calculated.	NH_2-N	3.58.
	Found.	"	2.12.

The optical rotation of the substance was

$$[\alpha]_D^{20} = \frac{+0.23 \times 5.8780}{1 \times 0.0525} = +26°$$

e. Furfural Distillation.

The presence of glucuronic acid was demonstrated by furfural distillation. 0.0065 gm. were distilled with 250 cc. of hydrochloric acid (specific gravity 1.06). The yield of phloroglucine was 0.0440 gm. The theory requires 0.100 gm.

f. Hydrolysis by Sodium Amalgam.

7 gm. of mucosin prepared as above were dissolved in 100 cc. of water, and 200 gm. of a 2 per cent amalgam were added in portions of 25 gm. at intervals. The entire operation lasted 24 hours. Before each new addition of amalgam the solution was neutralized with sulfuric acid. The final product was separated from mercury and filtered. To this solution were added 7 cc. of phenylhydrazine dissolved in 7 cc. of glacial acetic acid, and the entire solution was allowed to stand for 30 minutes with a reflux condenser on a boiling

water bath. The reaction product was filtered from tar, and allowed to stand at 0°C. over night. A small crystalline deposit formed. There was not sufficient material for purification or analysis.

g. Acetyl Estimation.

2 gm. of the barium salt with 300 cc. of water and 15 gm. of barium hydroxide, were allowed to hydrolyze for 5 hours, then neutralized, and the acetic acid was distilled into a 0.1 N solution of sodium hydroxide. The acid neutralized 26 cc. of the 0.1 N alkali, which corresponds to 0.156 gm. of acetic acid. The theory for one acetyl group requires 0.194 gm. The distillate was concentrated to a very small volume. This was acidulated with sulfuric acid and extracted with ether. From this the silver salt was obtained. It analyzed as follows:

0.1032 gm. substance: 0.0662 gm. Ag.

$C_2H_4O_2Ag$.	Calculated.	Ag	64.14.
	Found.	"	64.3.

II. From Serum Mucoid.

a. Preparation of Mucoitin Sulfuric Acid.

The mucoitin sulfuric acid from this mucoid (Levene and López-Suárez, 1918) was prepared on one occasion by treatment of the entire serum, and on the other, by treatment of the protein obtained from the serum on coagulation by boiling.

The first sample was prepared in the following way. To 12.5 liters of the serum a 50 per cent solution of NaOH was added until the solution contained 3 per cent of alkali. It was allowed to stand for 3 days at 40°C., then rendered acid by means of acetic acid, and concentrated on a water bath in the presence of excess of $BaCO_3$. The filtrate was converted into the lead salt. This was treated with glacial acetic acid, dried with alcohol, freed from lead, and again reprecipitated with lead acetate. The lead salt was repeatedly washed in a mortar with glacial acetic acid. Finally it was washed with alcohol and dried. The yield of the dry substance was 14 gm.

The substance contained 5.10 per cent nitrogen. This sample was used for hydrolysis.

The second sample was prepared from the coagulum obtained from 12.5 liters of beef serum. The process of preparation was exactly as in the above experiment. The lead salt was converted into the barium salt. The lead salt was suspended in water, and an excess of barium carbonate was added, and hydrogen sulfide gas passed until all lead separated out. From the filtrate the hydrogen sulfide was removed by aeration. The solution was finally precipitated by means of 99.5 per cent alcohol. The precipitate was then dissolved in a little water and the mixture was centrifugalized to remove all insoluble Ba salts. This operation was repeated several times. Finally the solution was precipitated by means of 99.5 per cent alcohol. This substance was dried and yielded 1.5 gm. Analysis gave the following values:

0.1000 gm. substance required for neutralization 3.75 cc. 0.1 N acid.
0.1961 " " on fusion: 0.0279 gm. $BaSO_4$.
0.1008 " " " combustion: 0.1098 gm. CO_2 and 0.0400 gm. H_2O.
0.1961 " " treated with sulfuric acid: 0.0971 gm. $BaSO_4$.

$C_{26}H_{44}O_{29}N_2S_2Ba$.	Calculated.	C	27.80,	H	3.48,	N	2.32,	S	5.30,	Ba 22.74.
	Found.	"	29.71,	"	4.44,	"	5.25,	"	1.96,	" 29.14.

b. Preparation of Chitosamine.

13 gm. of the first sample were taken up in 80 cc. of a 20 per cent solution of hydrochloric acid. 2 gm. of barium chloride and 2 gm. of stannous chloride were added. The solution was heated with a reflux condenser for 13 hours. The dark brown solution was filtered from the melanin and diluted with an equal volume of water. The solution was freed from lead and tin by means of hydrogen sulfide and from barium by sulfuric acid. The solution was concentrated under diminished pressure nearly to dryness. The residue was taken up in methyl alcohol and allowed to stand. Typical crystals of glucosamine hydrochloride separated out.

0.0200 gm. in the Van Slyke apparatus: 2.23 cc. N at 25°C. and 759.6 mm.

$C_6H_{13}O_5N \cdot HCl$.	Calculated.	N	6.51.
	Found.	"	6.19.

Initial. Equilibrium.

$$[\alpha]_D^{25} = \frac{+1.78 \times 2.0355}{1 \times 0.0405} = +89° \qquad [\alpha]_D^{25} = \frac{+1.42 \times 2.0355}{1 \times 0.0405} = +71°$$

The substance began to turn brown at 200°C. and turned black at 220°C. It did not melt.

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