($C_{15}H_{10}N_2OCl$) and 241 ($C_{14}H_{10}N_2Cl$) fragments are related to the benzodiazepinone moiety. The peak at *m/e* 341 consists of the oxazepam portion with one TMS group but without the acetal oxygen atom at C-3. The TMS group is most likely located at the N-1 position, which indicates that N-1 is not linked to the glucuronic acid portion. Thus, this peak at *m/e* 341 is an important fragment ion which proves that the link with the glucuronic acid is formed through the C-3 acetal oxygen rather than the N-1 nitrogen. The *O*-glucuronide structure is in agreement with the observation that oxazepam glucuronide is readily cleaved by β-glucuronidase[4].

The above fragments are characteristic of the two moieties of the molecule and on EI no ions indicative of the presence of intact conjugate were observed. The CI-spectra of the metabolite, on the contrary, exhibited an intense MH^+ ion at *m/e* 823 as shown in the Figure. This is the mass expected from the oxazepam glucuronide which has been penta-silylated. This finding verifies the oxazepam-glucuronic acid conjugation. The peak at *m/e* 751 is the MH^+ ion of the tetra-silylated glucuronide due to incomplete silylation; different silylating conditions change the relative intensity of this peak. It is interesting to note that the fragmentation of the silylated glucuronic acid portion is much simpler in the CI-spectrum. The peak at *m/e* 375 is the only major ion related to the silylated glucuronic acid portion, i.e., the characteristic EI fragment ions at *m/e* 217 and 204 are conspicuously absent in the CI-spectrum. All other major peaks, including 431, 359, 341, 287 and 269, show the chlorine isotopic peak, therefore are related to the oxazepam moiety. No metastable ions were found to substantiate any fragmentation pathway. Since no important metabolite-related ions were observed below *m/e* 200 from both EI- and CI-spectra, they not shown in the Figure.

Similarly, observation of the MH^+ ion of a penta-silylated product at *m/e* 857 verified the glucuronide conjugation of lorazepam. Partial silylation of lorazepam glucuronide also yielded peaks at *m/e* 713 and 785 due to its tri-silylated and tetra-silylated products, respectively. Again, *m/e* 375 is the only ion related to the silylated glucuronic acid portion.

The striking difference between the EI and CI results clearly demonstrates the advantage of CI mass spectrometry. CI is highly sensitive, yields intense MH^+ ions and is also relatively unaffected by sample purify. This technique undoubtedly will be employed with increasing frequency in various metabolic studies especially those involving metabolites previously undetectable by EI-mass spectrometry.

Zusammenfassung. Die Glucuronide des Oxazepams und Lorazepams wurden aus dem Harn von Zwergschweinen, denen die entsprechenden Benzodiazepine verabreicht wurden, isoliert. Die Metaboliten wurden durch Analyse der Spaltprodukte gekennzeichnet. Massenspektrometrie der Trimethylsilylderavite war für die Struktur der *O*-Glucuronide beweisend.

T. T. L. Chang, Ch. F. Kuhlman, R. T. Schillings, S. F. Sisenwine, C. O. Tio and H. W. Ruelius

Wyeth Laboratories, Inc., Research Division, P.O. Box 8299, Philadelphia (Pennsylvania 19101, USA),
12 January 1973.

Occurrence of Heparin or its Related Acidic Glucosaminoglycan in Human Aortic Tissue

Analytical studies of human aortic connective tissue established that the aortic acidic glycosaminoglycans (AGAG) consist of hyaluronic acid, chondroitin sulfate isomers and heparan sulfates[1,2]. However, there is no agreement about heparin in human aortic tissue, because there is only a trace of heparin, if any, in the tissue[1-4]. Since these arterial AGAG as well as heparin function physiologically as anti-coagulants[5], anti-lipemics[6] and anti-thrombogenics[7], it should be important to study the presence of heparin in aortic tissue. An appreciable number of mast cells as a source of heparin has been reported in the arterial connective tissues. In 1937, Jorpes[8] first described the presence of mast cells in pig arterial tissue from which he extracted heparin. In human arterial tissue, several investigators reported the existence of mast cells in relation to the pathogenesis of atherosclerosis[9-11]. The present paper reports the occurrence of heparin or its related glucosaminoglycan in human aortic tissue.

Materials and methods. Freshly obtained, pooled, human aortic samples from subjects ranging in age from 40 to 77 years were subjected to the preparation of aortic AGAG by the method previously described[12,13]. Human aortic intima and media layers were carefully separated from the adventitia and homogenized in a grinder. After the lipids were repeatedly extracted with methanol-chloroform (1:2, v/v) from the samples, proteolytic

Table I. Fraction pattern of acidic glycosaminoglycans in human aorta on a Dowex 1-X2 column by stepwise elution with increased NaCl concentration

Molarity of NaCl	0.5	1.25	1.75	3.0	Total
Acidic glycosaminoglycans					
as glucuronic acid (mg)	0.73	6.60	15.67	2.41	25.41
Percentage	2.9	26.0	61.6	9.5	100.0

The yield of applied acidic glycosaminoglycans was 29.6 mg as glucuronic acid.

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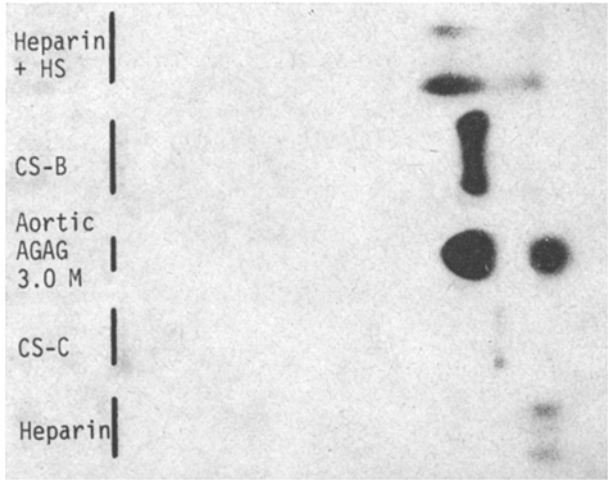

Fig. 1. Electrophoretic separation of aortic acidic glycosaminoglycans eluted at the 3.0 *M* NaCl from a Dowex 1-X2 column followed by the digestion with chondroitinase-ABC. Approximately 15 μg of sample (as glucuronic acid) were applied. Note the faster migration spot resembled that of standard heparin. HS, heparan sulfate; CS-B and CS-C, chondroitin sulfate B and C.

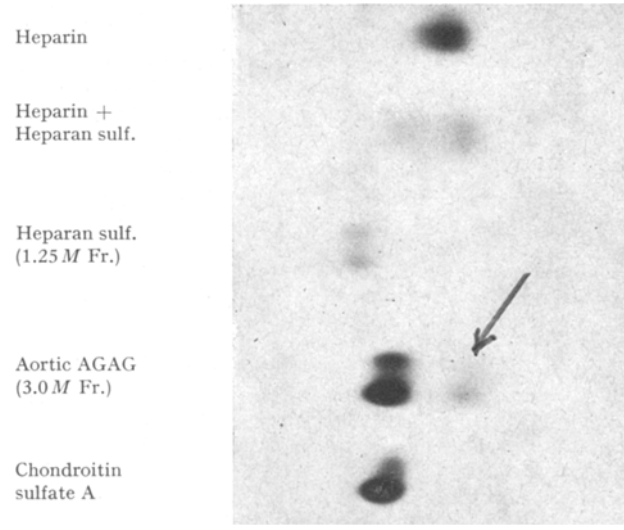

Fig. 2. Two spots corresponding to heparin and chondroitin sulfate A were detected in the Figure. The faster moving spot of aortic AGAG eluted at 3.0 *M* NaCl prior to the treatment with chondroitinase-ABC was stained much less than that of Figure 1 which was treated with the enzyme.

digestion with pronase-P was carried out[14], followed by a treatment of 0.5 *N* NaOH at 4 °C overnight. Trichloroacetic acid was then added at the final concentration of 10%, kept at 4 °C overnight and centrifuged. The supernatants were subjected to dialysis against running tap water and the undialyzable AGAG were concentrated by a rotatory flash evaporator. Crude AGAG were precipitated by addition of 4 volumes of ethanol saturated with sodium acetate and kept at 4 °C overnight. Following centrifugation, aortic AGAG were dried in vaccum over P_2O_5. The yield of AGAG was determined by the carbazole reaction as glucuronic acid[15].

The preparation of aortic AGAG thus obtained were fractionated on a Dowex 1-X2 column (chloride form, 200–400 mesh) by stepwise elution with increasing molarities of NaCl as shown in Table I. The effluents in each tube were pooled at the same salt concentrations and dialyzed against distilled water overnight and concentrated. Electrophoretic identification of aortic AGAG was made using a 0.1 *M* veronal-phosphate buffer (pH 8.6) on a cellulose acetate strip at 0.5 mA/cm for 60 min, followed by staining in 0.05% toluidine blue in 2% acetic acid. Standard heparin and heparan sulfates were gifts from Upjohn Co., Michigan, and chondroitin sulfates were supplied from Seikagaku Kogyo Co., Tokyo.

Enzymatic assay for aortic AGAG eluted at the 3.0 *M* NaCl was carried out at the disaccharide subunit with chondroitinase-ABC as described in previous papers[13,14,16]. The chondroitin sulfates of the AGAG at this fraction were digested with chondroitinase-ABC in order to demonstrate the clear spot of heparin by electrophoresis. For identification of the component of hexosamine moiety, the AGAG were hydrolyzed in 4 *N* HCl for 18 h at 100 °C and analyzed on an automatic aminoanalyzer (Hitachi KLA-3B).

Results and discussion. Approximately 85% recovery was obtained by the glucuronic acid determination, after the crude aortic AGAG (29.6 mg) were applied on a Dowex 1-X2 column followed by collection and dialysis procedure. The fractionation pattern of aortic AGAG on a Dowex 1-X2 column, as illustrated in Table I, indicated that the majority of aortic AGAG was eluted at the1.25 *M* and 1.75 *M* NaCl fractions and only 9.5% of AGAG consisted of that of the 3.0 *M* eluate.

Electrophoretic separation of aortic AGAG at the 3.0 *M* NaCl fraction demonstrated 2 spots corresponding to those of standard chondroitin sulfate isomers (B and C types) and heparin. The partial enzymatic degradation of AGAG in the fraction with chondroitinase-ABC resulted in a clear demonstration of the spot identical with heparin (Figure 1 and 2). This faster moving spot was stained metachromatically more reddish than the spot moving slowly. No spot corresponding to heparin appeared at a lower concentration than 3.0 *M* NaCl.

Table II illustrates the result of enzymatic assay of aortic AGAG at the 3.0 *M* NaCl with chondroitinase-ABC. It shows that 3 unsaturated disaccharides degraded

Table II. The distribution of unsaturated disaccharides of aortic acidic glycosaminoglycans eluted at 3.0 *M* NaCl from a Dowex 1-X2 column followed by digestion with chondroitinase-ABC

Unsaturated disaccharide	Yield (μg)	Percentage
4-sulfated	25.0	36.3
6-sulfated	24.6	35.8
Di-sulfated	19.2	27.9
Total	68.8	100.0
Undigested glycosaminoglycans	48.6	41.4%/total

The yield of acidic glycosaminoglycans was 145 μg. 1 unit of enzyme was used.

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with the enzyme appeared in similar quantities, indicating that oversulfated chondroitin sulfate consisted of such a high quantity of approximately 1/3 of the total chondroitin sulfate isomers. Because chondroitinase-ABC digests chondroitin monosulfates (A, B, C isomers) but does not digest completely oversulfated ones eluted at a high salt concentration, a possible presence of the oversulfated chondroitin sulfate in the undigested AGAG, which include heparin or the related substance, cannot be dismissed.

The aminoanalytical result of the hydrolyzates of the undigested aortic AGAG with chondroitinase-ABC at the 3.0 *M* fraction showed 2 main peaks corresponding to glucosamine and galactosamine moieties. The ratio of the glucosamine to galactosamine was approximately 1:8 which was proportional to the ratio of heparin or its related glucosaminoglycan to chondroitin sulfates when electrophoretically separated. Since no anthrone positive substance appeared in the fraction, the presence of keratan sulfate should be ruled out. Insufficient substance prevented further identification of aortic AGAG, heparin in particular. Nevertheless, the present findings suggest the occurrence of heparin and/or the related glucosaminoglycan in human aorta, in addition to the major constituents of chondroitin sulfate isomers, including oversulfated ones[17].

Zusammenfassung. Heparin und Glucosaminoglycan wurde in kleinen Mengen in der menschlichen Aorta mit Elektrophorese festgestellt.

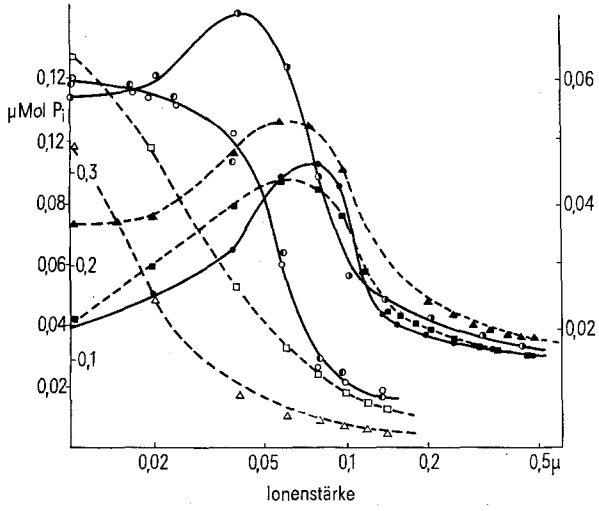
K. Izuka and K. Murata

Department of Medicine and Physical Therapy, University of Tokyo, Faculty of Medicine, Hongo, Bunkyo-ku, Tokyo, Japan, 28 December 1972.

[17] Acknowledgments. We are grateful to Prof. Y. Horiuchi for his interest in this study. We are indebted to Dr. K. Nakazawa for preparing human aortae and to Miss H. Kojima for skilful assistance. The present study was supported by grants in aid for scientific research from the Education Ministry and from Mitsui Life Insurance in Japan.

Die Abhängigkeit der Magnesium-'aktivierten Inosin' Triphosphatase-Aktivität des Aktomyosins von der Ionenstärke

Die kontraktile Aktivität der Myofibrille wird durch den Troponinkomplex reguliert. Dieser steht mit dem Aktin in Wechselwirkung und hemmt bei Calciumionenkonzentrationen unter etwa 10^{-7} M die Aktin-Myosin-Interaktion[1]. Da jedoch der Troponinkomplex nicht direkt mit dem Aktin reagiert und nur auf jedes siebte Aktinmonomer ein Troponinmolekül kommt[2], müsste deshalb eine indirekte Wirkung des Troponinkomplexes über das Tropomyosinmolekül postuliert werden. Diese Annahme scheint auch deshalb plausibel, weil das zwischen den beiden helical umeinandergewundenen Aktindoppelsträngen verlaufende, langgestreckte Tropomyosinmolekül[3,4] bei Aktivierung des Muskels im Filament seine Position[5] ändert. Daher wäre es interessant zu wissen, ob das Tropomyosin B – zumindesten unter bestimmten Versuchsbedingungen – die Aktin-Myosin-Interaktion hemmen kann (vgl. auch Schaub[6]).



Einfluss der Ionenstärke auf die Magnesium-aktivierten NTPase-Aktivitäten von «natürlichem» und «desensibilisiertem» Aktomyosin von Herz- und Skelettmuskel. Testansatz siehe Methodik. ---□---, NAM Herz; ---△---, DAM Herz; —○—, NAM Skelett; —◐—, DAM Skelett ATP; —◑—, DAM Skelett ITP; offene Symbole, Magnesium-ATP; ausgefüllte Symbole, Magnesium-ITP; NTPase-Aktivität in µMol/min/mg Eiweiss. Rechte Ordinate, ATPase- und ITPase-Aktivität des Herzmuskelaktomyosin; Linke Ordinate, ATPase (Innenseite) und ITPase-Aktivität (Aussenseite) des Skelettmuskelaktomyosins.

Eine solche Versuchsbedigung scheint bei der Abhängigkeit der Mg-aktivierten Inosintriphosphatase Aktivität des Aktomyosin zu bestehen. Es wird daher im Folgenden über Versuche berichtet, die eine Hemmwirkung des Tropomyosin B auf die Interaktion zwischen Aktin und Myosin – gemessen als ITP-ase-Aktivität – zeigen.

Methodik. Natürliches Aktomyosin (enthält Troponinkomplex und Tropomyosin B) bzw. «desensibilisiertes Aktomyosin» (enthält weder Troponinkomplex noch Tropomyosin B) wurde nach den Methoden von Jenny[7] bzw. Schaub et al.[8,9] präpariert. Tropomyosin B wurde wie bei Schaub und Perry[10] beschrieben präpariert und gelelektrophoretisch geprüft.

Der Ansatz zur NTPase-Aktivitätsbestimmung enthielt 25 m*M* *Tris*-HCl (pH 7.6), 10^{-5} *M* Ca^{++}, 2.5 m*M* $MgCl_2$, 2.5 m*M* Adenosintriphosphat (ATP) oder Inosintriphosphat (ITP). Die Temperatur war 25 °C, die gewünschte Ionenstärke wird mit KCl eingestellt.

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