

beiden Extremen des Gels je ein Streifen von Filterpapier aufgelegt (wir benutzen Whatman 1), welche den Kontakt mit den Puffern in den Kammern herstellen.

Nach dem Erhalten des Stärkels wird darauf mit einer Rasierklinge ein ca. 4 cm langer Schlitz senkrecht zur Laufrichtung aufgebracht und mittels einer Pipette die Probe gleichmässig aufgetragen (20 λ einer 10prozentigen Proteinlösung). Man kann auch einen Papierstreifen, welcher vorher mit der Probe getränkt wurde, in den Schlitz einlegen. Selbstverständlich muss die Höhe ca. 4 mm sein. (Wir verwendeten z. B. 10 aneinanderliegende Whatman-1-Streifen 4 cm $\times$ 4 mm.) Durch leichtes Drücken des Stärkels von beiden Seiten wird der Schlitz wieder dicht gemacht. Hierbei tritt öfters, hauptsächlich, wenn man die Probe direkt (ohne Papier) einführt, ein Teil wieder heraus. Dieser wird mit einem Filterpapier vorsichtig abgetrocknet.

Die Glasplatte wird genau wie bei der Papierelektrophorese in die Elektrophoresekammern gelegt, wobei die Filterpapiere an den Seiten des Stärkels in dem Puffer der Kammer getränkt werden, um den Kontakt herzustellen.

Nach der Elektrophorese wird die Glasplatte in die Lösung des Färbemittels gelegt und genau wie ein Papierstreifen behandelt. Zur Anfärbung mit Amido-Schwarz genügt es, wenn man die Glasplatte mit dem Stärkel 30 sec in das Färbemittel legt. Schon bei dem zweiten Wechseln des Entfärbers löst sich das Stärkel von der Glasplatte und verliert nicht mehr seine Konsistenz.

Präparative Stärkel-Elektrophorese. Von 2 gleich behandelten Glasplatten wird eine zur Anfärbung und die andere zur Eluierung der Fraktionen benützt. Dabei soll folgendes beachtet werden: (1) Jeder Überschuss der

Probe (Heraustreten aus dem zusammengeschobenen Schlitz) muss vermieden werden; sonst zeigt sich nur auf der auf der Glasplatte aufliegenden Seite eine gute Trennung, während sich oben ein «Schwanz» bildet. (2) Bei der Anfärbung zieht sich das Stärkel zusammen. Die Lage der entsprechenden Fraktionen kann auf dem ungefärbten Streifen mit Hilfe eines Gummibandes festgestellt werden, auf das die Lage der Fraktionen von dem angefärbten Streifen übertragen wurde und das dann auf die Länge des ungefärbten Streifens ausgezogen wird.

Diese Methode bietet folgende Vorteile: (1) Kurze Dauer der Elektrophorese. Durch die geringe Schichtdicke wird die Dauer der Elektrophorese auf die der Papierelektrophorese reduziert. Für eine Trennung des Serums in 15 Fraktionen wird eine Laufzeit in Boratpuffer (nach SMITHIES) von 15 h bei 100 V benötigt. (2) Geringer Verbrauch von Stärke. (3) Bidimensionale Elektrophorese auf Stärkelglasplatten von 20 $\times$ 20 cm. (4) Keine Sonderapparaturen werden benötigt. (5) Es konnten gegenüber der Methode von SMITHIES keine Nachteile in bezug auf den Trenneffekt festgestellt werden. (6) Das komplizierte Halbieren des Stärkels entfällt.

Summary. A modification of SMITHIES starch gel electrophoresis is presented. Thin starch gel layers (4 mm thick) were prepared on glass plates and used for analytical or preparative electrophoresis.

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A Method for Turbidimetric Measurement of Heparin: Its Application to the Estimation of Heparin in Aortic Tissue

Our previous studies¹⁻³ suggested the possible role of sulfated polysaccharides in the metabolism of aortic tissue during the atherosclerotic process. The presence of hyaluronic acid, heparitin sulfate and chondroitin sulfates including β -heparin⁴ in aortic tissue is well established^{5,6}, but only a few reports^{7,8} have so far described the occurrence of heparin in the aortic walls. Since heparin is a trace material scattered through connective tissues, the question as to whether heparin is present in aortic tissue is still unanswered. A selective method for measurement of heparin, therefore, offers a possibility for estimation of heparin contents in connective tissues by use of the quaternary ammonium salt fractionation methods for acid mucopolysaccharides (AMPS)⁹. This paper deals with the quantitative measurement of heparin by a turbidimetric method and its application to the estimation of heparin in aortic tissue.

As standard AMPS, heparin and heparitin sulfate (Upjohn Co.) and hyaluronic acid and chondroitin sulfates (Sigma Chemical Co.) were purified through Dowex 1-X2 columns by the stepwise elution method of SCHILLER et al.¹⁰, followed by dialyzation and lyophilization. AMPS were extracted from the intima-media layers of 6 adult human thoracic aortae and 10 bovine thoracic

aortae according to the method of DYRBYE and KIRK¹¹; average AMPS yields of 0.19 g per 100 g of starting wet tissue weight were obtained in both species. The extracted AMPS were then passed through Dowex 50 columns.

Quantities of heparin ranging from 20 to 200 μ g per ml of 1 M acetate buffer (1 M sodium acetate-acetic acid, pH 5.2) were added to test tubes in 1 ml aliquots; 1 ml of acetate buffer was used as a blank. 4 ml of 1.6 M NaCl in 0.1% cetylpyridinium chloride (CPC) were added to each tube to bring to 5 ml the total volume (the final NaCl concentration was 1.28 M in 0.08% CPC). After mixing the solutions, the tubes were warmed in a water

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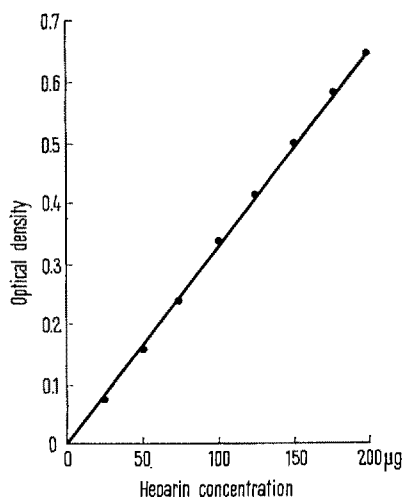
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bath at 37°C for 60 min. The resulting turbidity as measured by optical density was determined with a Beckman DU spectrophotometer against the blank at 500 m μ with a slit of 10 mm.

When the standard AMPS were added to the prescribed NaCl-CPC solution, it was found that (1) only heparin produced turbidity by the formation of heparin-CPC complex, whereas no turbidity was produced by hyaluronic acid, heparitin sulfate and chondroitin sulfates, and (2) within the range of heparin concentration studied, optical density of the heparin-CPC complex was proportional to the concentration of heparin (Figure). When 100 μ g of hyaluronic acid, heparitin sulfate and chondroitin sulfates were added individually or collectively to 100 μ g of heparin solution, the recoveries of heparin added to these AMPS ranged from 98 to 100%.

This procedure was applied to the estimation of heparin contents in aortic tissue; the AMPS extracted from human and bovine aortae were prepared in acetate buffer solutions and examined in the manner described above. The bovine aortic AMPS gave an average heparin yield of 0.75 mg (ranging from 0.4 to 1.3 mg) per 100 g of starting wet tissue weight. No detectable turbidity resulted, however, with any human aortic AMPS, even though amounts equivalent in weight to those of the examined bovine aortic AMPS were utilized.



Relation between the heparin concentration and the turbidity.

To the author's knowledge, a method for direct estimation of heparin which distinguishes heparin from other AMPS has not yet been reported. The present procedure is selective for heparin and has the advantage that the heparin is readily recoverable from the heparin-CPC complex and is available for further analyses; heparin is dissociated from the heparin-CPC complex by increasing the concentration of NaCl to 2.1 M or greater. Analyses of the recovered samples showed approximately 32% uronic acid¹² and 24% hexosamine¹³; paper chromatography of the hexosamine¹⁴ indicated it to be glucosamine. Insufficient material prevented further characterization.

In the last decade much attention has been called to the relationship between mast cells and AMPS in arterial walls, especially in respect to arteriosclerosis and the potent physiological functions of heparin and related substances as anticoagulant compounds and as stimulators for lipid clearing factor^{15,16}. As it is evident that mast cells which produce heparin¹⁷ are widely distributed in blood vessels of various species⁷, it is of interest to ascertain whether and to what extent heparin occurs in aortic walls. Present observation would indicate that appreciable traces of heparin or related substances are present in bovine aortae but not in human aortae. This result would support the early reports of JORPES et al.⁷ (1) that bovine aortic walls contain relatively more mast cells than aortic walls of other species, and (2) that crude heparin has been isolated from bovine aortae.

Zusammenfassung. Es wird eine vereinfachte Methode zur quantitativen Bestimmung des Heparins, bei Ausschliessung anderer Mucopolysaccharide, sowie Hyaluronsäure, Chondroitinschwefelsäure und Heparitinschwefelsäure, beschrieben. Es ergibt sich, dass das Aortagewebe des Rindes in kleinen Mengen Heparin enthält, während es in der menschlichen Aorta nicht gefunden werden konnte.

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PRO LABORATORIO

Apparatus for *in vitro* Studies of Mammalian Peripheral Nerves and Spinal Funiculi

In the apparatus developed by LEHMANN¹, and in its modification by RUDIN and EISENMAN², the temperature was controlled by immersing a watertight nerve chamber in a water bath. For the drainage of shunting fluids at the electrodes, the chamber and the input and output tubing for the gases had to be taken out of the bath and the chamber had to be opened. This type of apparatus was

obviously inconvenient to handle, and drainage led to a temporary disturbance of the equilibrium in the nerve chamber.

It was found possible to overcome these disadvantages by constructing the apparatus illustrated in Figures 1 and 2, which consists of a system for moistening and warming

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