A Zone Electrophoretic Study of Acid Mucopolysaccharides (AMPS) In Normal Human Serum.* (31201)

CLAES FRIMAN AND ROBERT BRUNISH (Introduced by G. Asboe-Hansen)
Connective Tissue Research Laboratory, Department of Dermatology, University of Copenhagen, Rigshospitalet, Copenhagen, Denmark

Investigations concerning the blood AMPS pattern have been relatively few, probably owing to low AMPS concentration.

An average AMPS uronic acid level of 206 μg% in normal human plasma has been reported(1), whereas an average concentration of 277 μg% was observed in normal serum (2). On paper chromatography of an AMPS fraction isolated from serum, 2 components, both of which were labile to testicular hyaluronidase, were observed(2). The chromatographic characteristics of one component resembled that of chondroitin sulfate (ChS). Schiller(3) presented conclusive evidence for ChS-A being the main AMPS of normal human plasma. Starting from several liters of human plasma she obtained 2 AMPS fractions. The nature of the electrophoretically slower fraction remained unresolved. Apparently it was heterogeneous and it probably contained hyaluronic acid (HA) as one component. In the serum of 2 patients with reticulum cell sarcoma and neuroblastoma, respectively, the presence of HA has been demonstrated(4).

Zone electrophoresis on cellulose acetate of AMPS mixtures has proved to be a sensitive analytical tool providing good separations (5,6). To the best of the authors' knowledge this method has not been applied to the analysis of blood AMPS.

Materials and methods. In an investigation undertaken to study the AMPS pattern of normal human serum and serum in various disease states, the AMPS fraction from 10-15 ml of serum obtained from healthy blood donors was isolated by a modification of the procedure of Bollet *et al*(2). The final precipitation step with protamine was replaced by an alcohol precipitation using 4.5 vol. of 96% ethanol saturated with sodium chloride in the presence of 0.5 N acetic acid. The precipitate was taken up in 2 ml of 0.05 N NaOH, 0.5 ml of which was dialyzed for about 72 hours against running tap water and then evaporated to dryness under reduced pressure. The residue was extracted with a few μl of 0.05 N NaOH, 1-2 μl of which were applied to the electrophoresis strip. Electrophoresis on cellulose acetate strips and staining with alcian blue was performed according to Brunish and Asboe-Hansen(6). As reference standards HA,† ChS (C.C.B.R., from cartilage) and heparin (Leo) were used. For detection of protein, strips were stained overnight with 0.0025% nigrosin in 2% acetic acid and rinsed with distilled water. To obtain an estimate of the relative concentrations of the different alcian blue staining components photoscanning was performed with the Joyce Chromoscan.

Results. Fig. 1 shows some representative serum AMPS patterns together with a typical standard pattern. In accordance with earlier reports(3,2), a component with the mobility of standard ChS (mostly A) was regularly encountered. On the basis of scanning, this component represented 44-100% of the total alcian blue staining material. It must be borne in mind, however, that the stoichiometry of the alcian blue-AMPS reaction is different for the individual AMPS. A component with similar mobility to HA can be seen in Pattern 1. In most samples an intermediate component situated between HA and ChS was observed. A fourth component slower than HA can be seen in Patterns 2 and 3. Hitherto all four components have not been demonstrated with certainty in the same serum sample. In the region of the 3 fastest alcian blue staining components no protein could be visualized by nigrosin staining. Protein was regularly seen at the origin

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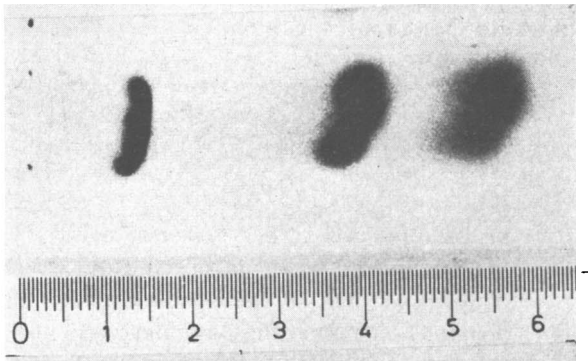
† Generously supplied by Dr. Bertil Högberg of the Leo Co., Hälsingborg, Sweden.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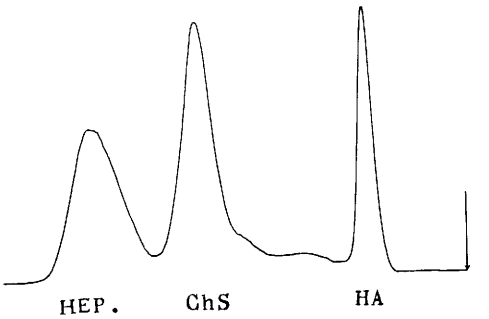
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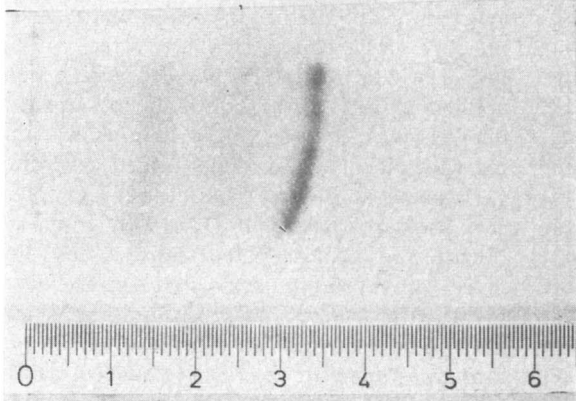
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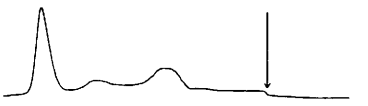

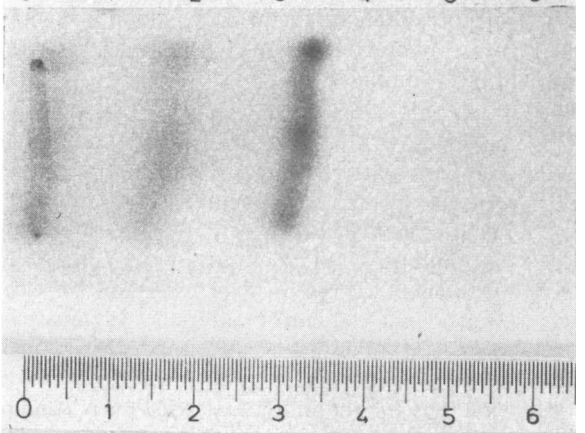
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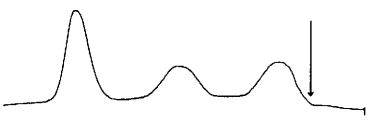

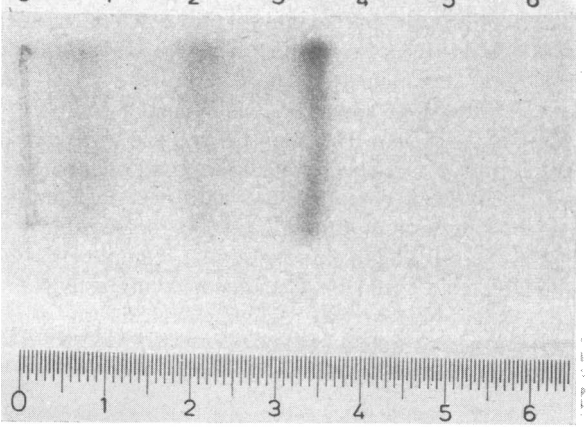
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FIG. 1. Electrophoretic patterns are situated on the left and the corresponding scan tracings on the right. Arrow on tracings indicates starting-point of the run. Uppermost separation obtained with a standard mixture (see text). Below, serum patterns 1-3 in descending order.

and in some instances partly overlapping the slowest alcian blue staining component.

Evidently, the exact chemical nature of the demonstrated alcian blue staining components cannot be determined solely on the basis of electrophoretic mobility. This study shows, however, that the AMPS pattern of normal human serum is more complex than previous studies indicate, since components are demonstrated in addition to those described by other workers. Work is in progress for the closer characterization of the serum AMPS components observed.

As the AMPS fraction electrophoresed in this study is derived from approximately 3-4 ml of serum it is obvious that the variability of the individual serum AMPS pattern in health and disease is open for detailed investigation.

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Comparison of Antisera to Various Gonadotropins as They Effect The Mouse Vaginal Cycle.* (31202)

CHARLES A. ELY, ROSEMARIE TUERCKE, AND BEI-LOO CHEN†
Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York City

Inhibition of endogenous pituitary gonadotropins by antiserum to heterologous gonadotropins has been reported since the earliest studies of antihormones began after 1930(1). Particularly convincing were studies by Meyer and his associates(2,3,4,5). However, since these studies involved the use of crude glandular extracts, they were open to the obvious criticism that antigonadotropic effects were due to non-specific factors or to antibodies to pituitary hormones other than those with gonadotropic activity. The fact that many workers failed to demonstrate cross-reactions between pituitary extracts from various animal sources, or to relate antihormone activity to precipitins *in vitro*, or to find evidence of endogenous hormone inactivity led to confusion, and there was some reluctance to accept the possibility that endogenous pituitary hormones of laboratory animals could be inhibited by antisera to pituitary hormones from other species. It was postulated that the use of more highly purified preparations would do away with the refractory state(6). Such doubts no longer appear to be justified.

With the exception of reports by Henry and van Dyke(7) and Gold *et al*(8), it has been demonstrated that antiserum to purified sheep luteinizing hormone cross-reacts with endogenous pituitary hormones of mice (9), rats(10-14) and rabbits(15), and antiserum to bovine thyrotropin was shown to inhibit the corresponding endogenous hormone in mice, rats, and guinea pigs(16). Conversely, antiserum to mouse thyrotropic hormone cross-reacts, though weakly, with beef thyrotropin(17). Among these studies reference to cross-reaction between endogenous gonadotropins and antiserum to follicle-stimulating hormone is missing. The present

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† Research Associate, Dept. of Anatomy.