Acid Mucopolysaccharides in Rat Skin. An Electrophoretic Study of Dermal and Subcutaneous Tissue.* (30377)

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Acid mucopolysaccharides are normal components of the ground substance of connective tissues of mammals. Mast cells contain granules staining metachromatically. In the rat they are believed to synthesize, store and release heparin(1-3), and probably also hyaluronate(4). Histamine, too, is considered a constituent of the mast cells(5).

One has to consider that rat skin, when studied histologically, is not a uniform structure. The dermis has a specific structure and build-up, characterized by its dense structure of collagen fibrils, its ectodermal appendages and a relatively small amount of homogeneous ground substance. Subcutaneous tissue, on the other hand, is a loose, areolar connective tissue with fat cells and a layer of striated muscle called the *panniculus carnosus*. It might, therefore, be expected that the chemistry of these two connective tissue layers of the skin is different.

The present paper reports on electrophoretic separation and characterization of acid mucopolysaccharides in alkaline extracts of the isolated dermis and the isolated subcutaneous tissue in rats. Besides, histologic examinations of rat skin, especially with regard to metachromasia of ground substance and mast cell granules, were performed.

Material and methods. Four male Wistar rats (State Serum Institute, Copenhagen) weighing approximately 180 g were injected intraperitoneally with radioactively labelled inorganic sodium sulfate, corresponding to 1 mC ^{35}S. Forty-eight hours later the animals were killed by ether. At this time, according to Boström and Gardell(6), practically all demonstrable ^{35}S in skin is incorporated in sulfated mucopolycaccharides. Only a negligible proportion is due to free sulfate(6) or to cystine(7).

The skin was shaved with an electrical clipper and a sharp knife. From symmetrical areas of the back distal to the costal arch, circular skin samples with a diameter of 2 cm were removed down to the muscular fascia. Upon removal of rat skin the natural cleavage zone is the loose connective tissue layer between the *panniculus carnosus* and the skeletal muscles of the trunk. Subcutaneous tissue, including the subcutaneous striated muscle, was carefully dissected off the dermis proper. Minced samples were pooled and lyophilized for 48 hours over phosphopentoxide at room temperature under a pressure of 2 mm Hg. Defatting was carried out by mechanical shaking through one hour in acetone, one hour in ether and another hour in acetone. Finally, the samples were lyophilized and ground in a Wiley mill through a 40 gauge sieve.

Mucopolysaccharides in samples of dermis (479.9 mg) and subcutaneous tissue (502.4 mg) were extracted with 5 ml of 0.5 N sodium hydroxide for 24 hours in a cool room. After centrifugation the supernatant was removed for electrophoretic studies.

Electrophoresis. Five microliters of the supernatant were applied to cellulose-acetate strips (5 × 36 cm), and electrophoresis was carried out for 5 hours in lithium acetate buffer (pH 7.5) with a running voltage of 100. After drying the strips, mucopolysaccharides were stained with Alcian blue according to Foster and Pearce(8). Standards were run simultaneously with the tissue samples. The hyaluronate standard was prepared from bovine vitreous body(9), chondroitin sulfate from bovine nasal septa (California, Biochemical Research, Lot 109970). Heparin was a commercial, bovine preparation (LEO, 133 units per mg). The standards were dissolved in 0.05 N sodium hydroxide

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before being submitted to electrophoresis. The strips were scanned in the Joyce-Loebl Chromoscan (Gateshead, England), employing a red filter and reflected light. Other strips were stained for protein with amido-black (0.5% in 7.5% acetic acid).

Autoradiography. The strips were placed in the dark under Kodirex (Kodak) X-ray film with an exposure time of 2 months. The blackening of the film, representing radiosulfate was recorded by scanning the autoradiogram by the Chromoscan, using reflected light.

For *histologic studies* biopsies of skin samples were fixed in a 4% aqueous solution of lead subacetate for 24 hours, embedded in paraffin, sectioned and stained with a 0.5% aqueous solution of toluidine blue, which, rather selectively, stains acid mucopolysaccharides metachromatically.

Results. The *electrophoretic patterns* obtained are shown in Fig. 1 and 2. In the

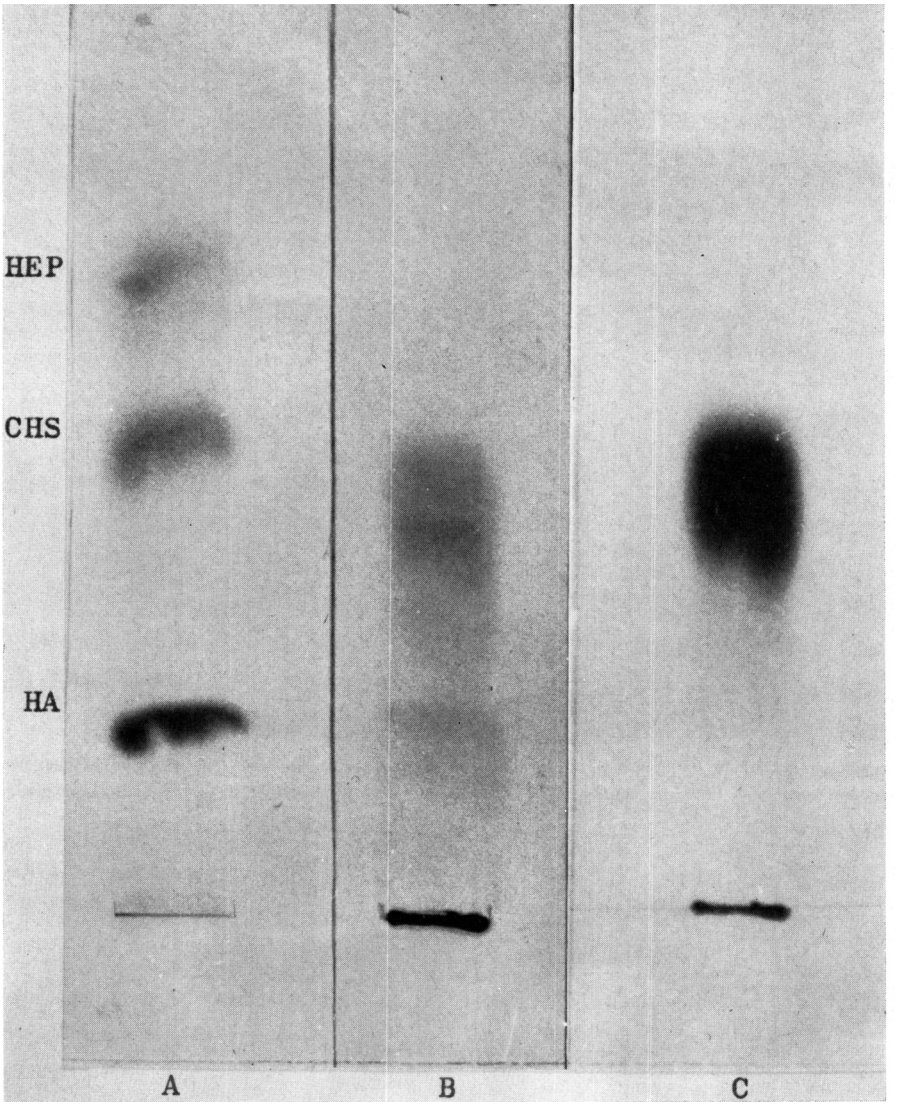

FIG. 1. Electrophoretic separation of acid mucopolysaccharides in extract of rat dermis. A. References. HA: hyaluronate. CHS: chondroitin sulfate. HEP: heparin. Stain: Alcian blue, pH 3. B. Electrophoretic pattern of acid mucopolysaccharides in tissue extract. Stain: Alcian blue, pH 3. C. Autoradiogram of B, showing location of ^{35}S.

dermal samples, bands with a mobility like hyaluronate and chondroitin sulfate references were demonstrated. Besides, one band with a mobility slightly slower than the chondroitin sulfate standard was constantly observed. In the subcutaneous tissue samples, one additional band with a mobility like the heparin standard was found. Some mucopolysaccharide material was retained at the origin. In the dermal samples the staining intensity of the unidentified mucopolysaccharide lying between the chondroitin sulfate standard and hyaluronate standard was larger than that of chondroitin sulfate as measured by scanning, while in the subcutaneous tissue samples the reverse was found.

Autoradiograms of the strips are shown in Fig. 1 and 2. Comparing the curves obtained

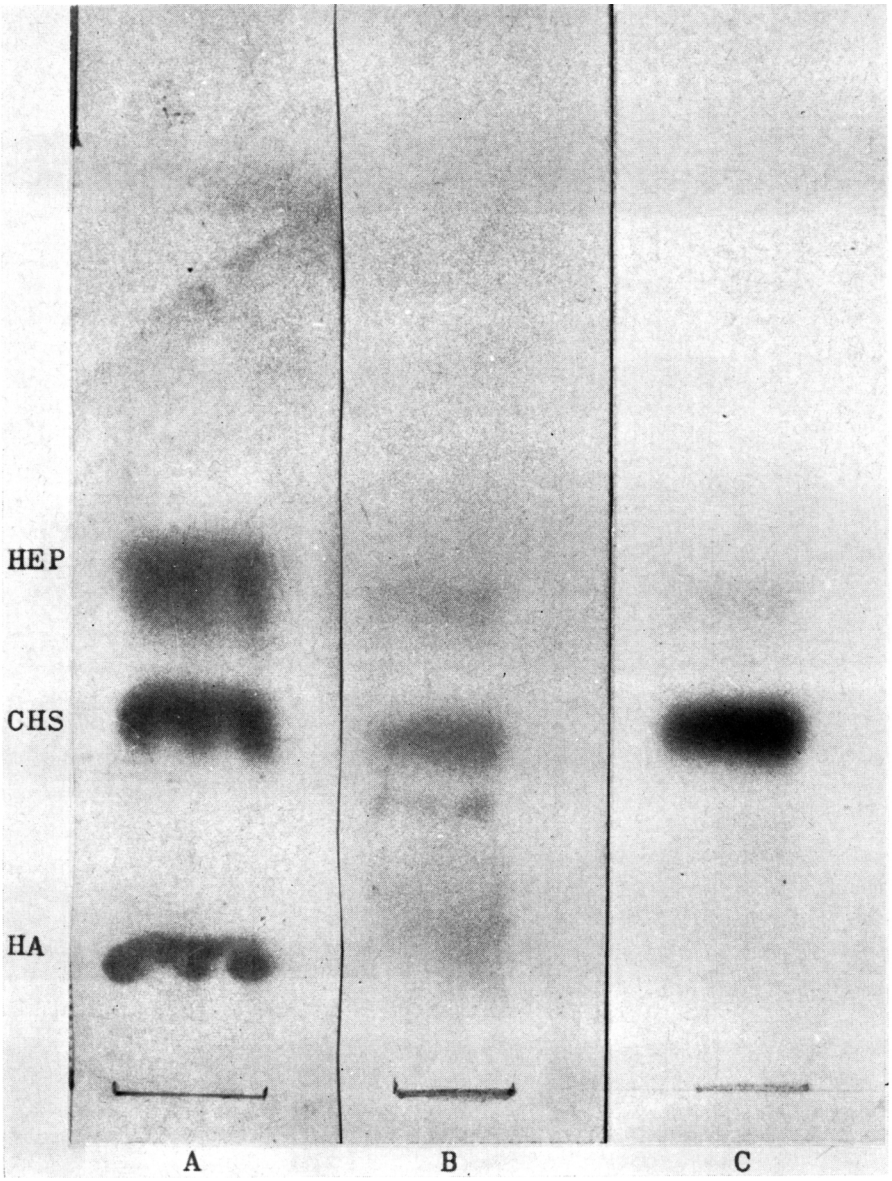

FIG. 2. Electrophoretic separation of acid mucopolysaccharides in extract of rat subcutaneous tissue. Symbols same as in Fig. 1.

by scanning of the Alcian blue stained strips and the corresponding autoradiograms, it was found that all bands except the one with a mobility like the hyaluronate reference contained radioactive sulfur.

Strips stained with amido-black revealed protein largely retained at the starting line. Presumably, the Alcian blue stained material found here is due to acid mucopolysaccharides still bound to protein. No trace of protein was found corresponding to the bands of the acid mucopolysaccharides that actually migrated.

Histologic examination. In Fig. 3, a section of the skin region under study is shown. The line of separation when removing the skin is pointed out. The metachromatic staining was mainly restricted to heavily granulated mast cells. They were rather sparse in the dermis with increased tendency to accu-

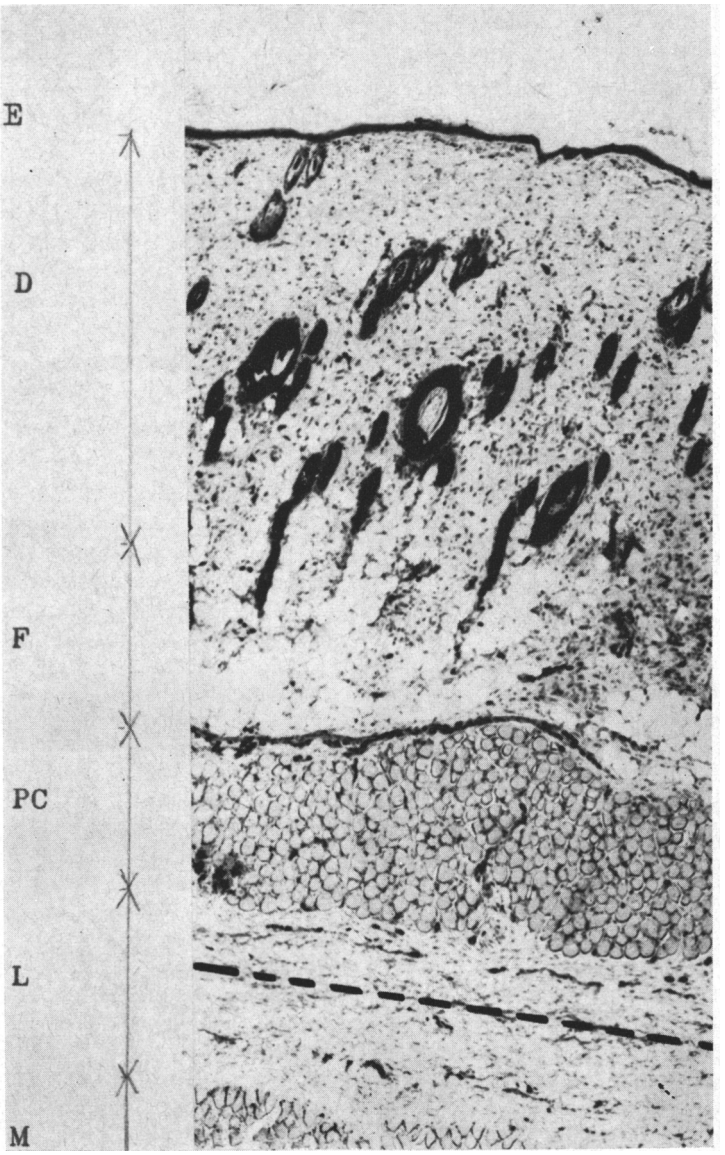

FIG. 3. Section of skin region under study. Magn. 32 ×. Stain: toluidine blue, 0.5% aqueous solution. E: epidermis. D: dermis. F: fatty layer. PC: *Panniculus carnosus.* L: loose areolar connective tissue. Dotted line indicates separation zone. M: abdominal muscle.

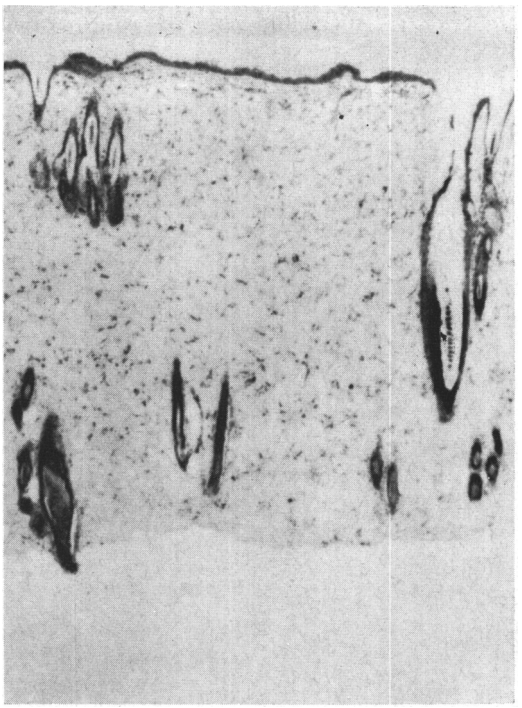

FIG. 4. Section of skin after removal of subcutaneous tissue. Magn. 32 ×. Stain: toluidine blue, 0.5% aqueous solution.

mulate in the deeper parts. In the subcutaneous layer, on the other hand, many mast cells were found, especially concentrated on both sides of the *panniculus carnosus*. In Fig. 4 the dermis proper, after removal of subcutaneous tissue, is presented.

Discussion. Extraction with 0.5 N sodium hydroxide should yield heparin equally well from dermis and from subcutaneous tissue, even without proteolytic digestion, since alkaline extraction is used for isolation of heparin from a variety of tissues(10,11). Heparin has been demonstrated as the only acid mucopolysaccharide in mast cells obtained from peritoneal washings of rat(2,3). It is worth speculating whether the content of heparin in subcutaneous tissue is a chemical reflection of the high concentration of tissue mast cells found there. Thus, the 2 layers of rat skin seem to differ in respect to acid mucopolysaccharides, not only histologically, but chemically as well. Also the concentration of histamine reflects a difference. In unpublished studies on the histamine content in rat skin, Marckmann and Zachariae found approximately twice as much histamine in the subcutaneous tissue as in the isolated dermis. The histamine values were calculated per gram dry defatted weight.

Any study of the influence of hormones, drugs, age, etc., on the function, metabolism, and concentration of acid mucopolysaccharides in rat skin presupposes a clear definition of what is included in the samples under study. Is it dermis *or* dermis together with adjacent subcutaneous tissue? Furthermore, the data presented here indicate that rat subcutaneous tissue is a suitable material for studies on heparin.

Summary. Acid mucopolysaccharides of rat skin were separated by electrophoresis. In the dermis, hyaluronate, chondroitin sulfate and a non-identified acid sulfomucopolysaccharide were demonstrated. In subcutaneous tissue, in addition to these, heparin was found. It is suggested that in respect to acid mucopolysaccharides, dermal and subcutaneous tissues are strictly different. Studies on acid mucopolysaccharides in rat skin require a clear definition of what is understood by skin.

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