

reported as the dominant finding in a newly described disease, neutrophilic urticaria, also construed to represent urticaria induced by physical stimuli (16).

Delayed pressure urticaria provides an expanded awareness of IL-1 mediated disease. Our patient's episodic attacks enabled us to detect IL-1 through its systemic effects and then to trace the source of this "cell-injury hormone" to pressure-injured skin. IL-1 may well be implicated in the pathogenesis of other types of urticaria as well as other types of vesiculobullous disease, such as allergic contact dermatitis.

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## Analysis of Increased Urinary Acid Glycosaminoglycans in a Patient with Relapsing Polychondritis

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Tadaki T, Aiba S, Igarashi M, Tagami H. Analysis of increased urinary acid glycosaminoglycans in a patient with relapsing polychondritis. *Acta Derm Venereol (Stockh)* 1987; 67: 441-445.

We analysed the composition of glycosaminoglycans (GAGs) found in an increased amount in the urine from a patient with relapsing polychondritis (RP) by means of electrophoresis.

since to our knowledge no analytical studies on the composition of GAGs in the urine from the patients with RP have been performed. Unexpectedly, the major GAGs detected in increased amount in the urine were found to be dermatan sulfate (DS) and hyaluronic acid (HA), the excretion of which correlated with the activity of the disease. Both DS and HA are the major components not of the cartilage but of the skin. Therefore, our findings suggest that the increased GAGs in RP are derived from skin or other tissues containing these GAGs rather than from the cartilage, unless there is an extensive cartilage destruction. *Key words: Electrophoresis; Dermatan sulfate; Hyaluronic acid.* (Received December 15, 1986.)

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Relapsing polychondritis (RP) is a systemic inflammatory disorder involving of the cartilaginous tissues throughout the body accompanied by ocular inflammation, vestibular damage and occasional erythematous skin lesions (1). Several investigators have reported an increase in glycosaminoglycans (GAGs) in the urine from the patients with RP (2, 3, 4, 5) suggesting that an increase in GAGs in the urine from patients with RP might be related to the cartilage destruction and might appear helpful for the diagnosis of RP.

In cartilage, major components of GAGs are chondroitin 4-sulfate (Ch-4s) and chondroitin 6-sulfate (Ch-6s), whereas minor components are hyaluronic acid (HA) and chondroitin sulfate-D (CHs-D). If the GAGs in the urine from the patients with RP are derived from cartilage, they should mainly be Ch-4s and Ch-6s. However, no active analysis of the components of GAGs in the urine of RP has been performed.

Here we report the case of a patient with RP in whom we analysed the composition of GAGs in the urine by electrophoresis before and after treatment. Unexpectedly, the results suggested that his urinary GAGs was derived from the skin rather than from the cartilage.

## REPORT OF A CASE

A 55-year-old Japanese man who had a four-month history of recurrent edematous swelling and several subcutaneous nodules on his legs was first seen in February 1984, because of a sudden onset of red swelling with severe tenderness in his ears.

Physical examination revealed swelling and erythema in his both auricles except for the part of the lobulus and several subcutaneous nodules including some cord-like indurations, 1 to 2 cm in diameter and suggestive of thrombophlebitis in his extremities.

Routine laboratory investigations were normal, except for elevated erythrocyte sedimentation rate, 82 mm/h, and positive C-reactive protein. No circulating antibodies against bovine type-II collagen was demonstrated.

A skin biopsy specimen taken from the right auricle showed an inflammatory infiltration composed of neutrophils and lymphocytes in the deep dermis and around the cartilage, accompanying partial destruction of the cartilage.

Systemic prednisone 20 mg/day given under the diagnosis of RP was effective to suppress all the symptoms but later trials to taper the dosage were always encountered by recurrence of indurated erythematous plaques, which histologically showed a feature of vasculitis surrounded by dense dermal neutrophilic infiltrate. Eventually he developed neurosensory hearing loss, tinnitus and vertigo one year later.

## INVESTIGATION

### *Separation of GAGs from the urine*

Urine of the patient was collected for 24 h before and after treatment. Urine was also collected from a healthy 58-year-old Japanese male and a 64-year-old Japanese male, as a control. GAGs were precipitated from the urine according to the method described by Endo et al. (6).

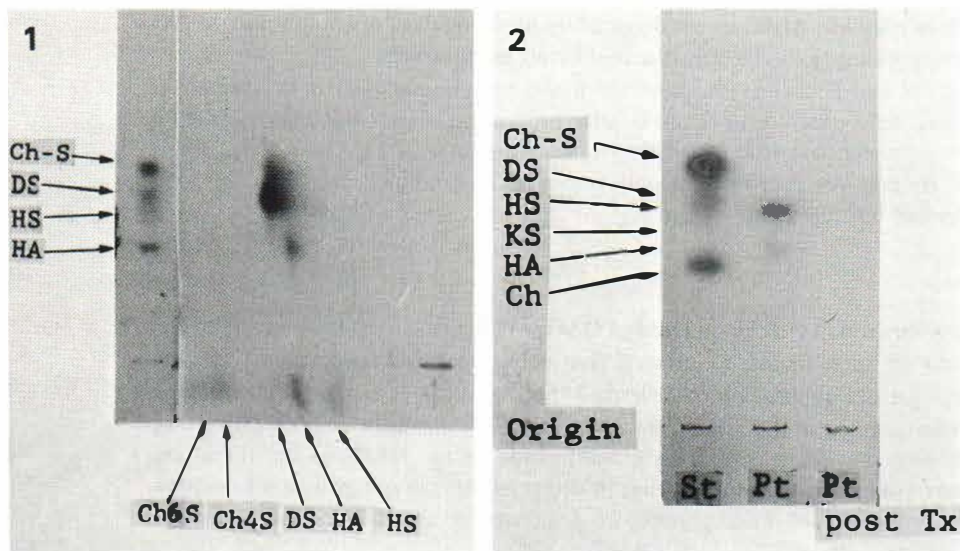


Fig. 1. Two-dimensional electrophoretogram of acid GAGs isolated from urine before treatment. Arrows indicate the mobility of standard GAGs.

Fig. 2. One-dimensional electrophoretogram of acid GAGs isolated from urine before treatment (second lane) and after treatment (third lane). Arrows indicate the mobility of standard GAGs (first lane).

#### Electrophoresis of GAGs from urine

(i) *Two-dimensional electrophoresis.* GAGs from the urine obtained before treatment which were dissolved in 20  $\mu$ l water containing 0.5 g/l phenol red were loaded on a cellulose acetate strip (70 $\times$ 60 mm) in 0.1 M pyridine formic acid at 0.5 mA/cm for 50 min and then loaded in 0.1 M barium acetate buffer (pH 8.0) at 1 mA/cm for 3 h. The cellulose acetate strip was stained in a 0.25% w/v aqueous alcian blue solution for 10 min and destained in 1% acetic acid. Thereafter the electrophoretic pattern was compared with those of standard GAGs, i.e. hyaluronic acid (HA, human umbilical cord), chondroitin 4-sulfate (Ch-4s, whale cartilage), chondroitin 6-sulfate (Ch-6s, shark cartilage), chondroitin (Ch), dermatan sulfate (DS, pig skin), heparan sulfate (HS, bovine kidney) and keratan sulfate (KS, bovine cornea).

(ii) *One-dimensional electrophoresis.* GAGs from the same amount of urine collected before and after treatment were loaded on a cellulose acetate strip (80 $\times$ 55 mm) in 0.1 M pyridine formic acid (pH 3.2) at 0.5 mA/cm for one hour. The cellulose acetate strip was stained in alcian blue and destained in acetic acid. Then the spots on the strip were scanned at 570 nm by means of an autoscanner FLUR-VIS densitometer (Helena Lab., Co., Italy).

#### Enzymatic degradation of GAGs from urine

GAGs from the urine of the patients were digested by streptomyces hyaluronidase (Seikagaku Kogyo Co., Tokyo), chondroitinase AC or chondroitinase ABS (Seikagaku Kogyo Co., Tokyo). After digestion they were loaded on a cellulose acetate strip for one-dimensional electrophoresis.

## RESULTS

In two-dimensional electrophoretogram the GAGs separated from the urine of normal controls showed trace spots corresponding with those of standard Ch-6s, Ch-4s and HS. On the other hand GAGs separated from the urine of the patient collected before treatment showed only two spots accorded with those of standard DS and HA, no spots corresponding with those of Ch-6s, Ch-4s or HS were found (Fig. 1). The spot according with DS was

digested by chondroitinase ABC but not digested by hyaluronidase or chondroitinase AC, while that corresponding with HA was digested by all these enzymes.

The spots of DS and HA were also detected in one-dimensional electrophoretogram of the urine obtained before treatment. Their relative proportion in the composition of GAGs estimated from densitometric scan of the electrophoretogram were 73% and 27%, respectively (Fig. 2). By contrast they became only traces in the urine collected after improvement of the disease by treatment.

## DISCUSSION

Despite the previous reports of the increase of GAGs in the urine from the patients of RP, the composition of such increased urinary GAGs has to our knowledge never been analysed except for one report, which described that low sulfated chondroitin sulfate or glycoprotein was detected in the urine from a case of RP by means of column chromatography (7). Although we did not perform quantification of the GAGs in the urine, our qualitative study confirmed that the increase in urinary GAGs in our patient was demonstrable only before treatment. Furthermore, we demonstrated that DS and HA was major GAGs in such urine.

Increases in the amount of urinary GAGs are found in several dermatoses. Increased DS have been reported in mucopolysaccharidosis (8) and HA in epidermolysis bullosa dystrophica (9). In collagen disease urinary GAGs are also increased, whose major GAGs are HS (10, 11). Although HA is observed in the urine of normal healthy individuals, its quantity is very low. Therefore, DS and HA, particularly the former, as major components of urinary GAGs in our case of RP, are distinct from those reported in other diseases.

It is reasonable to presume that the increased urinary GAGs noticed in this disease are induced by cartilage destruction (2, 3, 4). However the detected GAGs in the urine of our case suggest that they are derived from the cutaneous tissue rather than from cartilage, because both DS and HA are contained in large quantities in deep dermis (12), while Ch-6s and Ch-4s were predominant GAGs in the cartilage. We think that the increased DS and HA in the urine noted with the exacerbation of RP in our case are dermal DS and HA released due to skin tissue destruction caused by inflammation. But this does not rule out the possibility that they are also derived from other connective tissues containing these GAGs.

It is possible that, although there occurred destruction of cartilage, the released amount of Ch-6s and Ch-4s in the urine might not be large enough to be detected. Kaplan (13) has shown that when Ch-6s and Ch-4s were injected into the circulation of dogs, no significant increase in their amount occurred in urine, while DS was detected in urine after the injection. Another possibility is that the extent of the cartilage destruction in our case was unusually small compared with skin involvements as a case of RP.

In conclusion, despite the prominent inflammatory changes in the cartilage, the results of our studies suggest that skin involvement with destruction of the connective tissue also constitutes a major facet in our case of RP. To assert that urinary GAGs derived from dermal connective tissue becomes a reliable marker for disease activity of RP, further data collected from other patients with RP are required, particularly those obtained by using much more quantitative methods.

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## Experimental Folliculitis with *Pityrosporum orbiculare*: The Influence of Host Response

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Goodfield MJD, Saihan EM, Crowley J. Experimental folliculitis with *Pityrosporum orbiculare*: The influence of host response. *Acta Derm Venereol (Stockh)* 1987; 67: 445-447.

The aetiology of the folliculitis associated with seborrhoeic eczema is unclear, though the yeast, *Pityrosporum orbiculare* has been implicated. *P. orbiculare* was applied under occlusion to normal forearm skin of patients with seborrhoeic eczema (SE), seborrhoeic eczema and folliculitis (SEF), and normal controls. There were significant differences in response to occlusion between the three groups. Those patients with previous clinical evidence of folliculitis (SEF) developed folliculitis at the site of occlusion more frequently than either of the other two groups ( $p < 0.001$ ), in whom only one patient developed skin changes. This difference was not explained by the response to occlusion alone, nor by natural carriage of yeasts. These results suggest that the yeast *P. orbiculare* is necessary for the development of folliculitis, but that the nature of the host response determines those patients prone to follicular inflammation. (Received February 19, 1987.)

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Seborrhoeic eczema (SE), and the folliculitis associated with it (SEF), are common disorders of uncertain aetiology. The role of the yeast, *Pityrosporum orbiculare*, has been much discussed in this context (1). *P. orbiculare* is a lipophilic yeast which exists as a commensal in 90% of normal individuals (2), but in SE and SEF yeast overgrowth has been demonstrated (1, 3), though mycelia are rarely found, in contrast to other yeast diseases (4). This aetiology is further supported by the response to topical and oral antifungal therapy (3, 5 and 6). Despite the proposed common aetiology, folliculitis is not found in all patients with SE, however, and there must be other factors determining those prone to folliculitis.

In a series of experimental infections, Faergemann showed that following application and occlusion of pityrosporal yeasts, those people with previous pityriasis versicolor