

SYSTEMIC HYALINOSIS OR FIBROMATOSIS HYALINICA MULTIPLEX JUVENILIS AS A CONGENITAL SYNDROME

*A new entity based on the inborn error of the acid mucopolysaccharide
metabolism in connective tissue cells?*

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Abstract. Histochemical, biochemical and electronmicroscopic studies were carried out on a patient with a congenital connective tissue disorder consisting of joint contractures, cortical bone defects, gingival hypertrophy and skin and subcutaneous tumors. This case was previously reported by one of the authors under the title "systemic hyalinosi in connection with epidermolysis bullosa polydystrophica and hyalinosi cutis et mucosae". By cellulose acetate electrophoresis as well as by enzymatic digestion with chondroitinases, increased chondroitin 6-sulfate in the skin lesion of this patient was demonstrated. Moreover, electronmicroscopic study suggests its abnormal synthesis by the connective tissue cells. Chondroitin 6-sulfate is normally minimal in adult skin, but a primary constituent of bone and cartilage, and since it represents 20% of the acid glycosaminoglycans of embryonic pig skin, it may be assumed that in this syndrome the dermal connective tissue cells either failed to differentiate into those of normal postnatal skin or were transformed into those of bone or cartilage. Urinary acid glycosaminoglycans values were not elevated, which is in sharp contrast with the situation in mucopolysaccharidosis.

In 1964, one of the authors (6) reported with Hori on a 1-year-old boy suffering from an unknown syndrome, which consists of contractures of joints over the entire body, cortical defect of tibia, papules, nodes or tumors in the skin as well as in the subcutaneous tissue and hypertrophy of gingiva, sometimes with fever and hypoglycemic seizure. Similar cases were described by Purić et al. (9), Drescher et al. (3) (as "fibromatosis hyalinica multiplex juvenilis"), and also by Horio et al. (4). Purić's case is a 11-year-old boy; Drescher's, a boy and a girl aged 5 and 4 years, respectively; and Horio's, a 4-year-old girl. These cases present rather similar clinical findings: skin

lesion (papules on the nape, flat-elevated plaque on the buttocks and nodes or tumors on the head, back, elbow etc.), hypertrophy of gums, and in some cases, remarkable flexural contractures of joints and abnormal bone shadows or atrophy of muscles (Table I). The skin lesions develop soon after birth or in early ages and the patient's brother and sister suffers from the same illness.

In every case reported hitherto, including the author's, the characteristic microscopic findings are: a conspicuous homogenization (hyalinosi) of the affected connective tissue [skin (3, 4, 6, 9); oral mucous membrane (4, 6); articular capsule (6); bone (6)], and connective tissue cells "embedded" in homogenized areas and which tend to be round-formed and vacuolated. The homogenized tissue as well as vacuolated cells stain positively with PAS, in the present case metachromatic with toluidine blue, and in Drescher's, positive with astra blue.

The above-mentioned findings may suggest in the first place an abnormal mucopolysaccharide metabolism, but the exact nature of the ailment is still unknown. This report deals with histochemical, biochemical and electronmicroscopic studies on the skin lesion especially in respect to glycosaminoglycans. The urinary acid glycosaminoglycan of the patient was also examined in order to differentiate it from that of mucopolysaccharidosis.

REPORT OF A CASE

A Japanese boy, born in April 1958, developed skin nodules around the nose soon after birth. Contractures of joints as well as hypertrophy of gingiva and flat-elevated

Table 1. Cases reported hitherto

Reporter	Title of report of case	Case (sex, age)	Family history	Clinical findings				Physical development	Intelligence
				Skin	Oral cavity	Joints	Bone		
Puretić (1962)	A unique form of mesenchymal dysplasia	Boy aged 11 years	Brother and sister also affected?	Nodes, partly calcified, ulcerated. Developed already at 6 years of age	Hypertrophy of gums and oral mucosa	Flexural contractures of joints	Osteolysis of distal phalanges of fingers	Retarded	Good
Ishikawa (1964)	Systemic hyalinosis	Boy aged 1 year	Parents in consanguinity. Sister also affected?	Papules, flat plaques and nodes. Developed since a few months of age	Hypertrophy of gums	Pronounced flexural contractures of joints	Cortical defect in tibia (now pronounced osseous destruction)	Retarded	Good
Drescher (1967)	Fibromatosis hyalinica multiplex juvenilis	Girl aged 4 years and boy aged 5 years		Tumors, developed since the age of two	Hypertrophy of gums	Normal	Normal		
Horio (1968)	Atypical hyalinosis cutis et mucosae	Girl aged 4 years	Parents in consanguinity. Sister also affected	Papules, plaques and nodes. Appeared from the age of 5 months	Hypertrophy of gums	Normal	Bone-cyst on the skull	Normal	Normal

erythematous nodes were noted between 2 and 8 months of age. Thereafter, white semitransparent papules developed on the nape. His parents are consanguineous and his elder sister died in infancy with flexural contracture of elbows. The patient was first seen in the clinic of Tokyo University in 1959 at the age of 1 year and 8 months. He had flexural contractures of shoulders, elbows, knees and hips, firm papules as well as normal-colored or livid firm nodes, some of which, however, were soft and later became ulcerated, and also atrophy of muscles. Physically, he showed remarkable retardation, but his mental devel-

opment was good. Laboratory studies showed no remarkable abnormalities except for a cortical defect in tibia. (Clinical finding at the first visit was described in detail in the preceding report (6).) Later, the patient had an occasional attack of fever of unknown cause as well as of hypoglycemic seizure. Despite various kinds of therapy (corticosteroids, antibiotics, chloroquine etc.), the patient thereafter developed more and larger tumors, partly calcified, ulcerated and bleeding (Fig. 1). Furthermore,



Fig. 1. Skin lesions at the age of 12 years and 11 months.

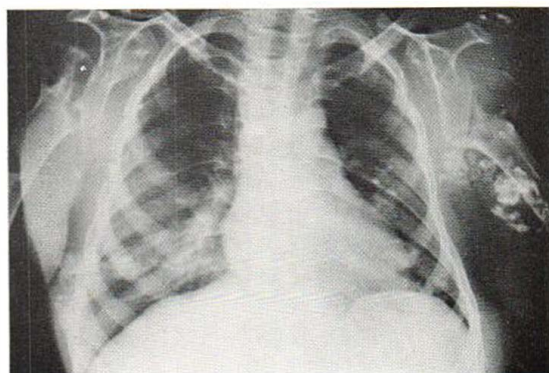


Fig. 2. X-ray photograph of the patient shows pronounced osseous cysts, and calcification inside the skin lesions.

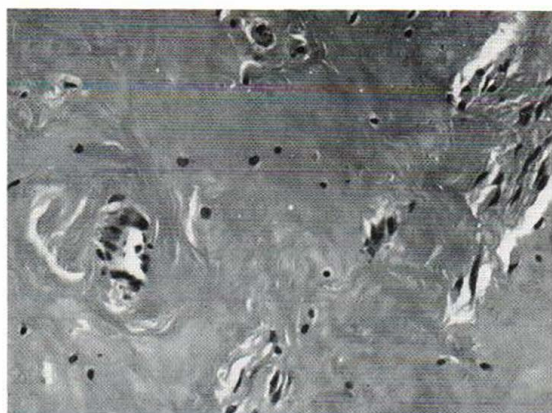


Fig. 3. Histopathology of the skin lesion. Conspicuous homogenization of the connective tissue and round cells with clear or vacuolated cytoplasm are characteristic of this disease. Hematoxylin-eosin stain, $\times 180$.

destruction of the osseous cortex has gradually developed in many bones especially in the bones of extremities (Fig. 2). The patient was admitted to the Kanto-Teishin Hospital in March, 1971 for examination and removal of tumors on the head. Laboratory studies disclosed this time the following abnormal findings: hypoglycemia, albuminuria (sometimes hematuria), decreased urinary excretion of 17-ketosteroids and 17-hydroxycorticosteroids, high E.S.R., delay in bleeding time as well as moderate increase of serum calcium value. (A more detailed report on the patient followed up for about 10 years will probably be published in the future.)

MATERIAL AND METHODS

The surgically resected occipital tumor was used for routine histological study as well as for alcian blue, colloidal iron or PAS staining. Stains for glycosaminoglycans were done, using the section fixed in formol-alcohol. Acid glycosaminoglycans were histochemically identified by incubation in streptomyces- or testis-hyaluronidase solution (100–150 units enzyme in 1 ml of physiological saline, incubated for 5–24 hours) as well as with chondroitinase ABC or AC (0.2–0.5 units enzyme in 0.05 ml of tris-HCl buffer solution (pH 8.0) incubated for 2 hours). Streptomyces-hyaluronidase digests only hyaluronic acid (HA), whereas testicular hyaluronidase can digest HA, chondroitin 4-sulfate (Ch 4-S) and chondroitin 6-sulfate (Ch 6-S). Chondroitinase ABC digests HA, Ch 4-S, Ch 6-S, dermatan sulfate (DS) and chondroitin. Chondroitinase AC is different from chondroitinase ABC in that DS is not digested by the former enzyme (13). Biochemical analysis of the skin glycosaminoglycans was done in the following manner: Crude glycosaminoglycans were isolated from the occipital skin tumor by the method of Schiller et al. (11) and dissolved in a small amount of water. With a part of it, electrophoresis on cellulose acetate (Oxoid) was performed in 0.1 M zinc acetate

at 0.5 mA/cm for 80 minutes. By this method, chondroitin sulfate, DS and HA were separated from each other, but not Ch 4-S from Ch 6-S. Furthermore, identification of bands was done by enzymatic digestion as carried out in histochemistry. After electrophoresis, the strips were stained in 0.5% alcian blue solution and the alcianophilia of bands was scanned with a densitometer at 610 nm. By comparing it with that of standard reagents, the percentage skin glycosaminoglycan distribution was determined (5). (Ch 4-S, Ch 6-S and DS were purchased from Seikagaku Kogyo, Tokyo; HA from Sigma). At the same time, enzymatic determination of the skin glycosaminoglycans was done with chondroitinases and chondrosulfatases by the procedure of Saito-Yamagata-Suzuki (10) (after their second method). As for the other part of crude glycosaminoglycan solution, total amount was estimated as hexuronic acid after a modified procedure of Dische (2). Urinary glycosaminoglycans were isolated by a modified procedure of Di Ferrante and Rich (12), and the total value-estimation as well as electrophoretic analysis was done in the same manner as in the case of the skin glycosaminoglycans. The material for electron-microscopic study was fixed in phosphate-buffered osmium tetroxide (pH 7.4) immediately after surgical removal, dehydrated in graded alcohols, embedded in epoxy resin, then fine-sectioned and double-stained with uranyl acetate and lead. The sections were examined with a Hidachi HS-7 type electron microscope operating at 50 kV.

RESULTS

Histopathology. In the moderately-homogenized areas of the skin, the connective tissue cells are increased, and are fusiform or round-formed. On the other hand, in the area of marked homogenization, the blood vessels are enlarged and a cleavage of the tissue is seen. The cells, often with pyknotic nucleus, are smaller and inclined to be more round (Fig. 3).



Fig. 4. Colloidal-iron positive cells are seen in hyalinized, partly colloidal-iron positive, partly PAS positive skin. Hale-PAS stain, $\times 580$.

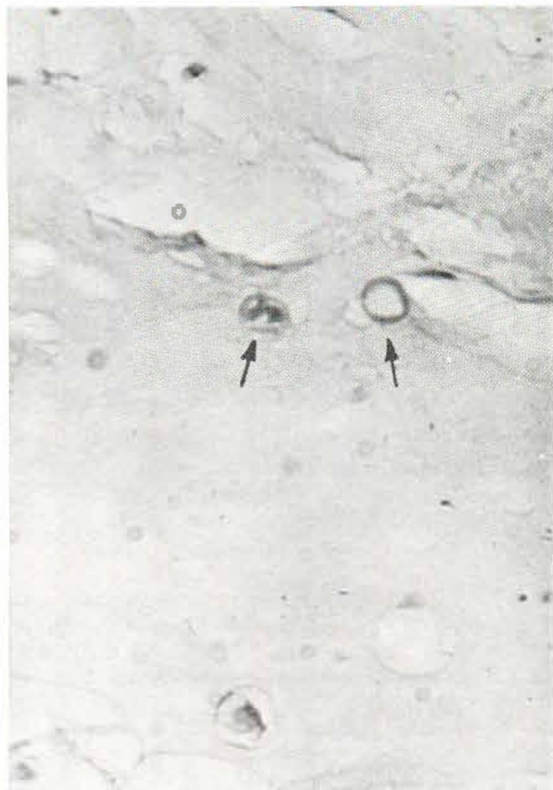
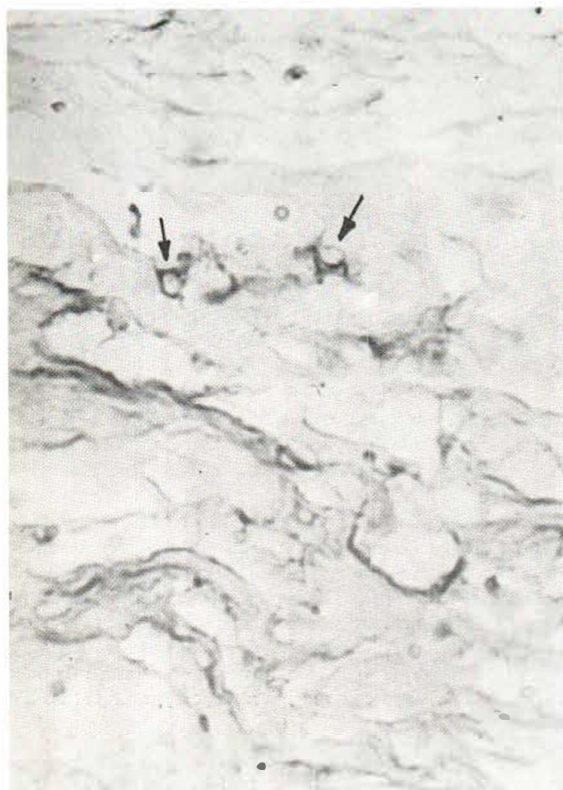


Fig. 5. Digestion of alcian blue stain by chondroitinase AC. (a) Control section, treated with tris-HCl buffer solution, pH 8.0 without enzyme. (b) Section digested with chondroitinase AC. Extracellular alcian blue positive re-

action is completely digested by chondroitinase AC. Intracellular stain (arrow) is remarkably decreased. Alcian blue stain. $\times 175$.

Histochemistry of skin glycosaminoglycans. Between PAS-positive homogenized areas there are colloidal iron or alcian blue positive fibrillar substances. In the cytoplasm of the cells a granular material, also stainable with colloidal iron or alcian blue, is seen (Fig. 4). Reaction for glycosaminoglycans is stronger in less intensely homogenized areas than in conspicuously homogenized areas, that is, the reaction is replaced by increased PAS-staining, and with development of histological hyalinosis.

The reaction for glycosaminoglycans persists after digestion with streptomyces-hyaluronidase, but is evidently decreased by incubation with testicular hyaluronidase or chondroitinase AC (Fig. 5). In conclusion, the histochemically stained acid glycosaminoglycan is, for the most part, chondroitin sulfate.

Biochemical analysis of skin glycosaminoglycans. Total glycosaminoglycans amount to 1.1 mg as

glucuronic acid per gram defatted dry weight, that is, no difference from the normal value in adults (1, 5). Distribution pattern: on electrophoresis in zinc acetate, chondroitin sulfate 61%, dermatan sulfate 24%, hyaluronic acid 15%; by the enzymatic digestion method, chondroitin 4-sulfate 3.8%, chondroitin 6-sulfate 56.6%, dermatan sulfate 31.4%, hyaluronic acid and other glycosaminoglycans 8.2% (Fig. 6). The two methods display fairly similar results. With the latter method, increased Ch 6-S is evident, whereas the normal dermis shows only dermatan sulfate and hyaluronic acid on electrophoresis (5).

Urinary acid glycosaminoglycans. The total value of urinary glycosaminoglycans was, on average, 2.4 mg/day as hexuronic acid. This value was not above normal. Nor were there any pathological findings in the electrophoretic pattern in zinc acetate.

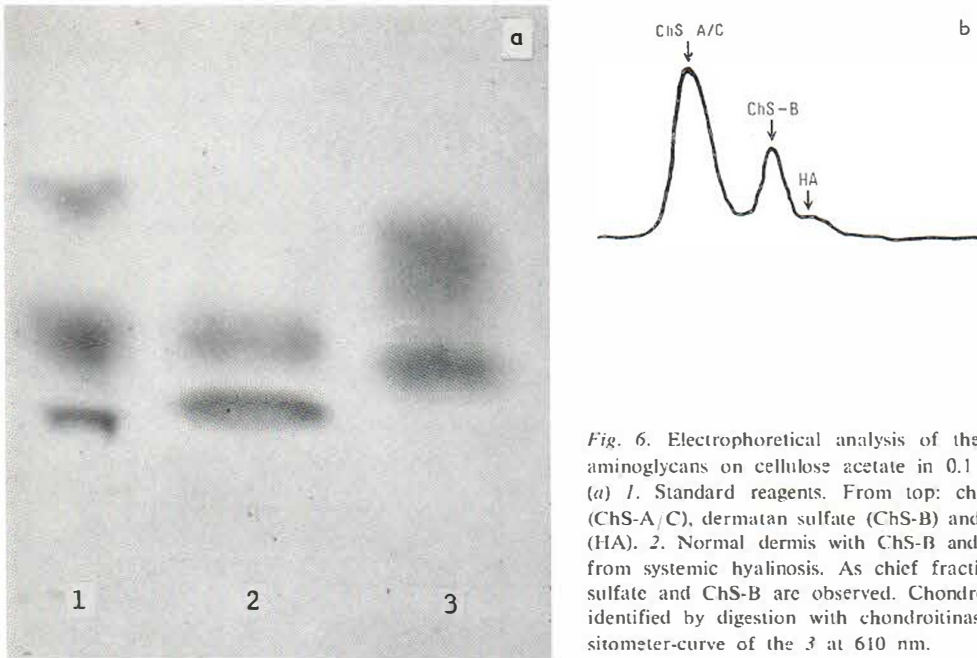


Fig. 6. Electrophoretal analysis of the dermal glycosaminoglycans on cellulose acetate in 0.1 M zinc acetate. (a) 1. Standard reagents. From top: chondroitin sulfate (ChS-A/C), dermatan sulfate (ChS-B) and hyaluronic acid (HA). 2. Normal dermis with ChS-B and HA. 3. Dermis from systemic hyalinos. As chief fractions, chondroitin sulfate and ChS-B are observed. Chondroitin sulfate was identified by digestion with chondroitinase AC. (b) Densitometer-curve of the 3 at 610 nm.



Fig. 7. Electromicroscopic finding. Connective tissue cells with enlarged vacuoles full of fine granular or filamentous materials. $\times 8\,700$.

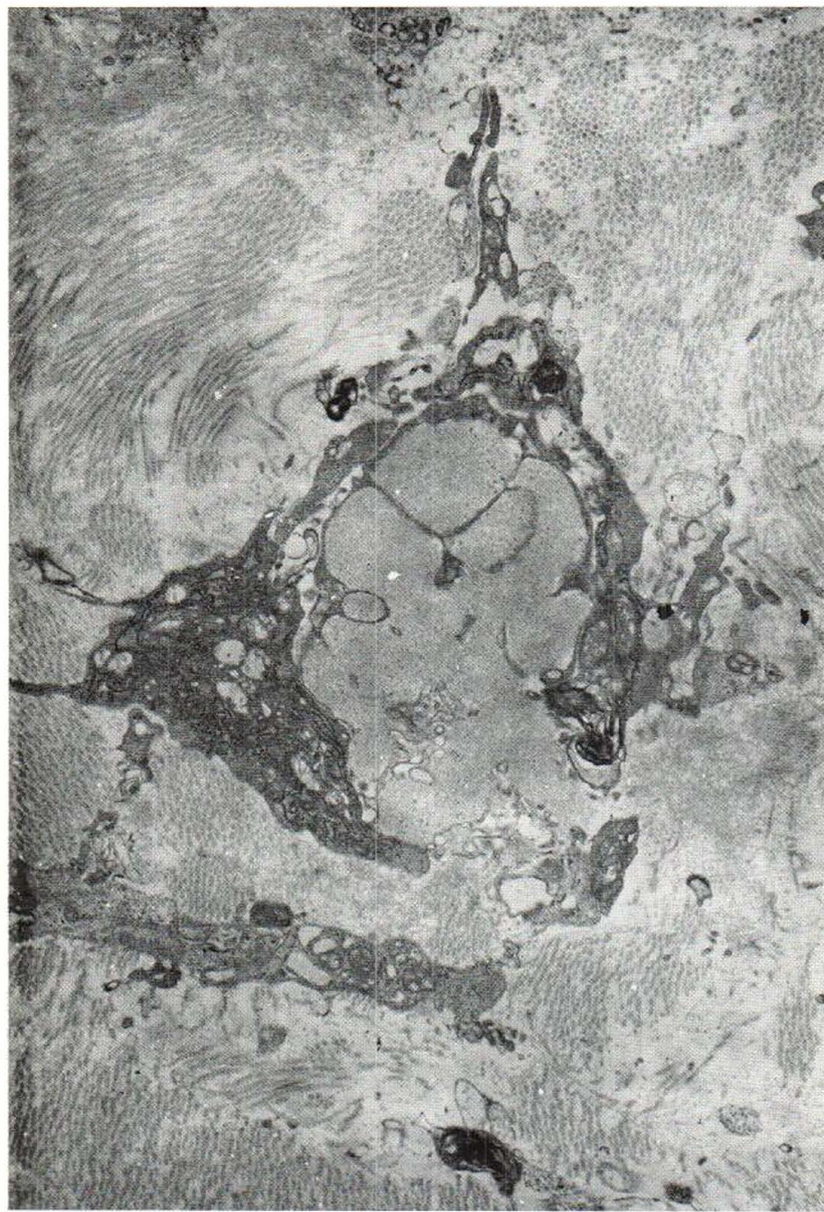


Fig. 8. Degenerative connective tissue cells with overflowing of the contents. $\times 8150$.

Electronmicroscopic findings. A characteristic picture is seen alike: In the protoplasm of connective tissue cells of the corium, there are various-sized vacuoles which are full of fine filamentous or granular materials (Fig. 7). In addition, masses of substance similar to the vacuole content are observed in the neighboring connective tissue. The vacuoles are bounded by a layer of membrane with and without ribosomes. The vacuoles with ribosome correspond to rough-surfaced endoplasmic reticulum, whereas those with-

out ribosome are possibly derived from Golgi apparatus. With reference to light microscopic findings, the content of the enlarged endoplasmic reticulum or Golgi apparatus is considered to be glycosaminoglycan. It is also noticeable that with enlargement of the vesicles the plasma membrane seems to rupture and the intravesicular substance floods the neighboring connective tissue, where it is deposited between collagen fibres (Fig. 8). These electronmicroscopic findings may account for increased synthesis of glycosaminoglycan by

the connective tissue cells, because according to modern views (8) the glycosaminoglycans are produced in the endoplasmic reticulum and Golgi apparatus. The collagen fibres show no change in band pattern or in diameter.

COMMENT

This histochemical, biochemical and electron-microscopic study of the skin lesion showed an increase in chondroitin 6-sulfate deposits, supposedly resulting from its abnormal synthesis by the connective tissue cells. Since in every case reported hitherto the sister or brother had suffered from the same illness, the above-mentioned abnormality of the cells is considered to be based on a genetic disturbance. Chondroitin 6-sulfate is normally minimal in adult skin, but a primary constituent of bone and cartilage (1). Loewi & Meyer (7) reported that in embryonic pig skin chondroitin 6-sulfate represented about 20% of the total acid glycosaminoglycans. It may therefore be assumed that in this syndrome the dermal connective tissue cells either failed to differentiate into those of the postnatal skin or were transformed into those of bone or cartilage. Nevertheless, further study is required, especially as regards glycosaminoglycan metabolism.

A possibility of known mucopolysaccharidosis with increased glycosaminoglycan excretion in the urine can be rejected in this work. Moreover, histochemical, biochemical and electronmicroscopic findings on the skin lesion of the patient were different from those of patients with mucopolysaccharidosis, including the case experienced by the present authors (unpublished).

Apart from the findings discussed, hyalinized areas show strongly positive PAS-stain and its relationship to the abnormal chondroitin sulfate deposits has not been explained in this work. However, from the fact that in less hyalinized areas a more marked reaction to glycosaminoglycans is observed, PAS-positive hyalinosis may be of secondary nature, consequent upon abnormal synthesis and deposits of chondroitin sulfate in the skin.

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