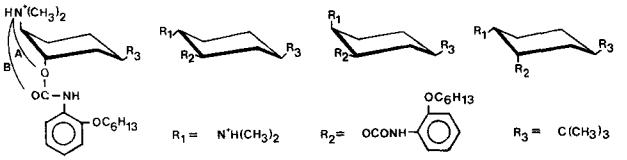
Conformationally fixed diastereoisomeric pentacaine derivatives*

Isomer No.*	1	2	3	4
				
A (10^{-10} m)**	3.7	≥2.9	≤2.9	≤2.9
B (10^{-10} m)**	3.2-5.6	≥1.0-5.2	≤1.0-5.2	≤1.0-5.2
m.p. °C				
Oxalate	128-130	50	-	-
Hydrochloride	169-172	72-78	163-166	95-97

* All compounds are racemic isomers, hence, the structures do not show absolute configurations. ** The distances were measured on Dreiding stereomodels.

(fig. 1,B) and in the time course of action potential recovery during drug wash-out (fig. 1,C). The same drug sequence has been found also in an inhibitory effect of the isomers (10^{-6}-10^{-4} moles/l) on both phasic and tonic contractions of guinea-pig ileum induced by transmural electrical stimulation (fig. 2) or by acetylcholine or norepinephrine (5×10^{-6} moles/l). Obviously, the type of nerve fibers and/or excitable membrane does not play any essential role in the observed differences in the biological effects of the isomers.

Büchi[16] pointed to the importance of distance between positively charged nitrogen of amino group and carbon of carbonyl group in local anesthetics with classical structure[17] in their interaction with membrane binding sites.

In the 1st approximation, we may say that the activity of the studied diastereoisomers, which might be considered to be fixed conformers of mobile cyclohexane derivatives, increases with decreasing distance between the N and O atoms (1 < 2 < 4 < 3). As known, in vicinal di-substituted cyclohexanes the distance between diequatorial (trans) substituents is somewhat greater than between the axial-equitorial (cis) ones. Thus, in the isomer No. 2 the distance is greater than in the cis isomers Nos 3 and 4. As the results of syn-axial interactions the bulky (solvated) axial ammonium group in isomer No. 3 is more pressed towards the oxygen function than is the sterically less demanding oxygen in the isomer No. 4, making thus the distance shorter in the isomer No. 3 than in No. 4.

Another factor affecting the activity might be basicity (and hence the lipophilicity) of the studied compounds, which is likely to vary a little with the spatial relationship of the neighbouring groups.

The results indicate that the biological activity of the studied type of derivatives depends not only on configuration but also on the actual conformation in which the given compound exists.

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An immunohistochemical study on the distribution of histiocytes containing S-100 protein-like antigen in cutaneous T-cell lymphoma/leukemia; a preliminary report[1]

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The immunohistochemical distribution of histiocytes containing S-100 protein-like antigen in the skin lesions of cutaneous T-cell lymphoma/leukemia is investigated. Marked hyperplasia of these histiocytes is found in two cases of mycosis fungoides.

S-100 protein[2] is generally considered to be a specific protein of the nervous system. However, many authors have recently demonstrated that several nonneuroectodermal cells contained S-100 protein-like antigen which could not be distinguished immunologically from S-100 protein of the nervous system[3-5]. In human lymphoid tissues, S-100 protein-like antigen is present in interditating reticulum cells (IDC)[6] and in Langerhans cells[5]. These types of histiocytes are closely similar in fine structure[7] and several authors suggest that they are of the same origin and play an important role in the maturation of T-lymphocytes[7-9]. Although these special types of histiocytes could usually be recognized by electron microscopic observations[10], the immunohistochemical staining using antibody against S-100 protein makes it possible to identify them easily at the light microscope level. In the present study, we investigated the

distribution of histiocytes containing S-100 protein-like antigen in the skin lesions of cutaneous T-cell lymphoma/leukemia.

Materials and methods. The preparation of antibody against S-100 protein and its specificity were previously described[3]. A modified immunoperoxidase procedure of PAP method by Sternberger et al.[11] was also described previously[6]. The skin lesions of 7 patients with mycosis fungoides and of a patient with Sézary syndrome were examined (table).

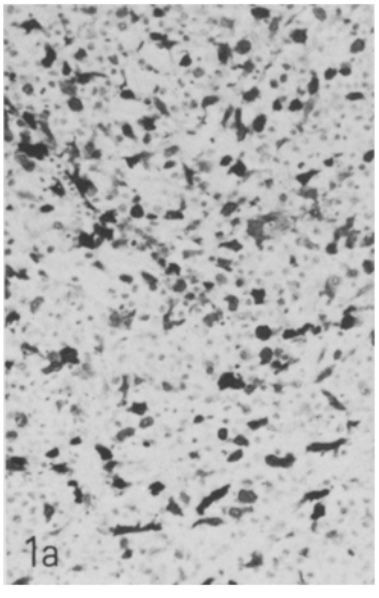
Results. The skin lesions in 2 out of 7 cases of mycosis fungoides, which corresponded to erythematous and plaque stages respectively, showed remarkable hyperplasia of histiocytes containing S-100 protein-like antigen (fig. 1a and table). The positive cells possessed irregularly shaped, euchromatin-rich nuclei and abundant cytoplasm with a few cytoplasmic projections (fig. 1b). Neoplastic lymphocytes with convoluted or bizzare, heterochromatin-rich nuclei were negative for S-100 protein-like antigen. In addition, there were many plasma cells, granulocytes and histiocytes without S-100 protein-like like antigen. The cutaneous lesion of 5 other patients with mycosis fungoides, which corresponded to the tumor stage, and of a patient with Sézary syndrome contained none or only small numbers of histiocytes S-100 protein-like antigen (fig. 2 and table). In these cases, the majority of infiltrating cells was composed of atypical lymphocytes with convoluted, heterochromatin-rich nuclei.

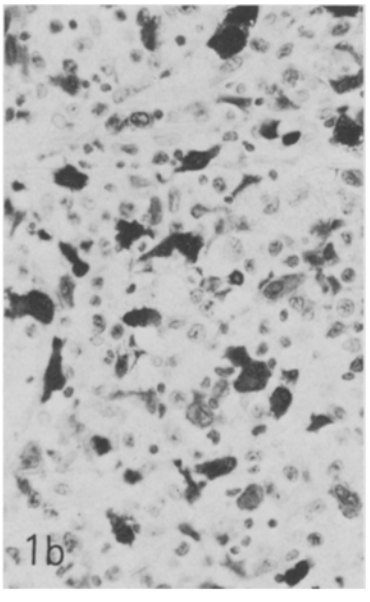
Discussion. The light microscopic features of histiocytes containing S-100 protein-like antigen in the present cases showed the distinctive characteristics of IDC[7]. IDC have been demonstrated in the skin lesions of mycosis fungoides[12]. Furthermore, extremely large numbers of histiocytes strongly positive for human Ia-like antigen, which were considered to be IDC, were found in the cutaneous lesion of mycosis fungoides[13]. As far as we know, such remarkable hyperplasia of histiocytes containing S-100 protein-like antigen is only found in dermatopathic lymphadenitis[6] and in histiocytosis X (T. Nakajima, unpublished data). The main morphologic feature of dermatopathic lymphadenitis is marked accumulation of IDC in paracortical regions of the lymph node[7], and it is of interest that dermatopathic lymphadenitis is often accompanied by mycosis fungoides of the early and plaque stages. The skin lesions of the tumor stage of mycosis fungoides, however, contain only small numbers of histiocytes with S-100 protein-like antigen. Judging from these data, it may be concluded that histiocytes containing S-100 protein-like antigen, which are probably IDC, remarkably increase in number as a kind of host reaction of the early and plaque stages of mycosis fungoides, and decrease in number in the tumor stage. The question whether S-100 protein-like antigen in these special types of human histiocytes is biochemically identical to S-100 protein of the nervous system must be settled by further investigations. Finally, it is further suggested that the immunohistochemical staining using antibody against S-100 protein is very useful to investigate the distribution and behaviors of IDC and Langerhans cells in human lymphoma/leukemia.

The skin lesions from patients with cutaneous T-cell lymphoma/leukemia examined by the PAP method using antibody against S-100 protein

	Case No.	Age	Sex	Histological stage	Histiocytes with S-100 protein-like antigen
Mycosis fungoides	1	64	M	P	+ + +
	2	74	M	T	+
	3	68	F	T	+
	4	81	M	T	+
	5	81	F	T	−
	6	66	F	T	−
	7	54	M	E	+ + +
Sézary syndrome	8	83	M		−

E, Erythematous stage; P, plaque stage; T, tumor stage; + + +, marked hyperplasia of positive cells; +, small numbers of positive cells; −, negative.





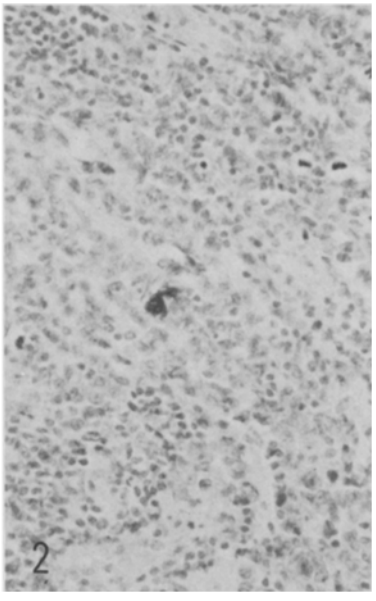


Figure 1. *a* Marked hyperplasia of histiocytes containing S-100 protein-like antigen in the skin lesion of a patient with mycosis fungoides of the plaque stage (case 1). Anti-S-100 and hematoxylin, × 90. *b* A close-up view of the histiocytes containing S-100 protein-like antigen with deeply indented nuclei and abundant cytoplasm extending a few delicate cytoplasmic processes (case 1). Anti-S-100 and hematoxylin, × 300.

Figure 2. Only small numbers of histiocytes containing S-100 protein-like antigen are present in a case of the tumor stage of mycosis fungoides (case 3). Anti-S-100 and hematoxylin, × 200.

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Specific interaction between soybean agglutinin and lipid bilayers containing the GM_1 ganglioside

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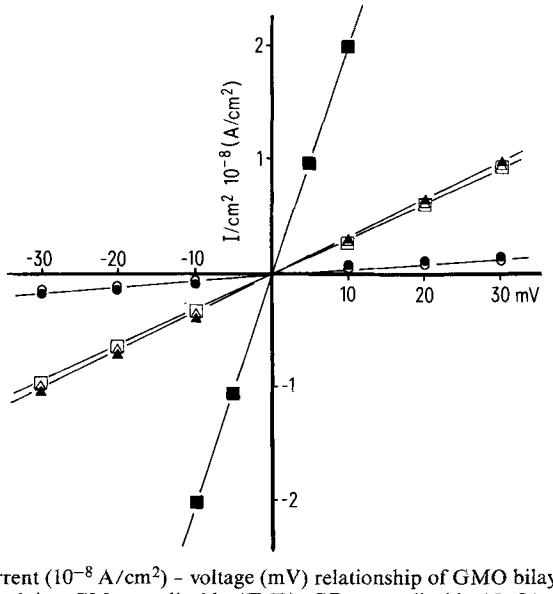
Summary. A specific interaction was demonstrated between glycerolmonoleate bilayer membranes containing the GM_1 ganglioside and soybean agglutinin. Electrical conductance changes are discussed in terms of ganglioside clustering in the bilayer.

Plant lectins agglutinate cells by binding to the plasma membrane and forming cross-bridges between cells[2,3]. Significant progress has been made in recent years in the isolation of glycoproteins containing receptors for sugar-specific plant lectins. However, it has recently been suggested that glycolipids could be implicated in this recognition process[4-8]. We demonstrate here that soybean agglutinin interacts specifically with GM_1 ganglioside incorporated into a planar lipid bilayer by measuring the electrical conductance changes observed after addition of the lectin in the bathing solution. This permeability change may initiate membrane events (activation processes) by providing hydrophilic pores for the influx of cations.

Material and methods. Soybean agglutinin (SBA) (type VI), glycerolmonoleate (GMO) were purchased from Sigma Chemical Co. GM_1 ganglioside (galactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide), GD_{1a} ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl)-galactosylglucosylceramide) and GT_1 ganglioside (N-acetylneuraminylgalactosyl-N-acetylgalactosaminyl-(N-acetylneuraminyl-N acetylneuraminyl)-galactosylglucosylceramide were Supelco products. The mixtures of GMO-gangliosides (98/2 molar ratio) were dissolved in $CHCl_3$-methanol-decane (30/5/65). The bilayers were formed on a 1.3 mm diameter aperture in a Teflon cell separating 2 aqueous phases (2.5 cm^3), as described elsewhere[9-11]. The membrane conductances were determined by measuring the specific current (I) as a function of imposed potential differences (V). The aqueous phase contained $CaCl_2$ 1 mM, Tris-HCl 10^{-2} at pH 7.2.

Results and discussion. The figure shows a marked change in lipid membrane conductance before and after addition of soybean agglutinin in the aqueous phase bathing the bilayer containing GM_1 ganglioside. No conductance changes were observed when GD_{1a} and GT_1 were incorporated into the bilayer. This effect is not the consequence of a nonspecific penetration of the agglutinin into the lipid bilayer. Indeed, soybean agglutinin does not affect the conductance of a pure GMO bilayer.

It has been shown[7] that agglutination of liposomes containing erythrocyte lipids is inhibited in the presence of N-acetylgalactosamine. Our results indicate that the neuraminic acid attached to the terminal galactose strongly inhibits the recognition of the N-acetylgalactosamine present in the GT_1 and GD_{1a} gangliosides. A similar observation was made several years ago by Novogrodsky et al.[12] with cells treated with neuraminidase. Indeed, after neuraminidase treatment, they had an increased number of receptors. A possible explanation of this conductance change is that the ganglioside-lectin interaction modifies the lectin conformation and induces its penetration into the lipid bilayer. It



Current (10^{-8} A/cm^2) - voltage (mV) relationship of GMO bilayers containing GM_1 ganglioside (□ ■), GD_{1a} ganglioside (○ ●) and GT_1 ganglioside (△ ▲), in the absence (open symbols) or in the presence (filled symbols) of soybean agglutinin (200 µg/ml).