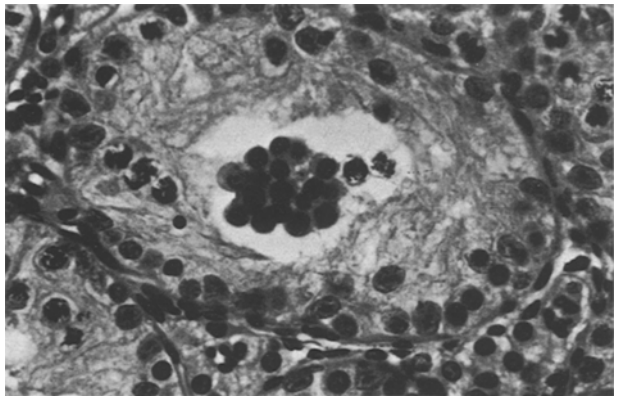

Fig. 1. Section of seminiferous tubules from testis of rabbit conventionally vasectomized 18 months previously (H and E, × 400).

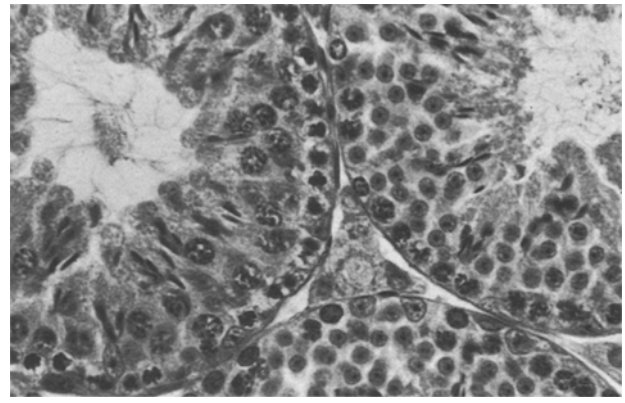

Fig. 2. Section of seminiferous tubules from testis of rabbit sham-vasectomized 18 months previously (H and E, × 400).

stained with haematoxylin and eosin and examined by light microscopy.

Results and discussion. CMI to spermatozoa in vitro was detected in the vasectomized and vas resected rabbits 6, 12 and 18 months after surgery while CMI was not detected in sham-vasectomized rabbit throughout the experimental period and in the vasectomized and vas resected animals 3 months after surgery (table). 18 months after surgery, the caudae epididymides from the vasectomized and vas resected rabbits appeared distended, without cysts and granulomata. The testes on the other hand were reduced in weight, were tender and pale whitish. By light microscopy, the caudae epididymides were aspermatogenic. A few macrophages were found in the yellowish fluid obtained from this part of the tract. In the testes, the seminiferous tubules were aspermatogenic, and desquamation was accompanied by inter- and intratubular mononuclear infiltration (figure 2). The epididymides and testes from the sham-vasectomized animal appeared normal. No differences in morphology could be found in the tracts of vas resected and conventionally vasectomized rabbits.

Detected CMI in vitro correlated with the histopathology of the testes 18 months after vasectomy although this autoimmunity was probably not the only cause of the observed orchitis since humoral antibodies and immune complexes have been shown to be elevated in the rabbit 6 months after vasectomy[2,3]. Due to the small number of the animals studied here, these observations need to be confirmed in larger numbers of rabbits. The role of each of the factors that have been identified by this and other studies in vivo, needs to be defined. Further, due to species response differences to vasectomy, care needs to be taken in efforts to relate animal model findings to the situation in man.

1 Acknowledgments. We acknowledge the financial support of the Australian Research Grants Committee and the Australian and Kenyan Governments for support through the Australian Development Assistance Bureau.

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Properties of vascular permeability factor in human sera for guinea-pig skin

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Summary. Properties of vascular permeability factor in native human sera (PF/Nat) showed close similarities with those of necrotizing factor. Time course studies revealed that skin necrosis could be initiated by enhanced vascular permeability.

Since the original description by Edler and Wilhelm in 1958[1] on PF/Nat in native human sera which induced enhanced vascular permeability (P) for guinea-pig skin, its properties and/or significance had been scarecely investigated. In this paper, the studies of the properties of PF/Nat or P factor are described, which show a close similarity with those of necrotizing(N) factor[2-9], inducing local inflammation up to necrosis in the guinea-pig skin after intradermal injections of the native sera. However, the differences between the P factor and the N factor in the initial phase of the activity and in the distribution among human sera might suggest that enhanced P activity initiated in the induction of skin necrosis.

Materials and methods. Sera: Sera were obtained from healthy individuals and patients with collagen diseases, and stored at −70 °C.

The P and N factor: 0.1 ml of serum specimens was injected in the shaved dorsal skin of Hartley strain guinea-pigs weighing 250–300 g and 1 ml of 0.5% Evans blue solution (Daiichi-kagaku Ltd, Tokyo) was injected i.v. 45 min after. For a time study, the specimens were injected at various intervals prior to the dye injection. The P activity

was observed 30 min after the dye injection and the N activity was observed 24 h after the injection of sera. The degree of the activities was divided into 4 grades: (III, II, I, 0) for P factor, and 5 grades (+++, ++, +, ±, −) for N factor.

Treatment of serum in vitro: Fresh sera were heated at 45 or 56 °C. Dialization of sera was performed against phosphate buffered saline (PBS), pH 7.2, at 4 °C for 16 h with several changes of the buffer. Absorptions of sera were performed with the nucleated cells of guinea-pigs and the erythrocytes of sheep and guinea-pigs. Nucleated cells were obtained by filtrating minced spleen or kidney through mesh wire screen (No. 50). More than 80% of the cells were viable as determined by trypan blue dye exclusion test. 1 vol. of packed cells and 4 vol of serum samples undiluted or diluted to 1:4 were mixed and incubated for 30 min at various temperatures; 0 °C, 25 °C, 37 °C and 56 °C, and the supernatant was separated by centrifugation; this procedure was repeated twice. To find the effects of pretreatment of sera with various drugs, the serum samples diluted to 1:2 were mixed at 37 °C for 30 min with an equal volume of various solutions of several drugs: 50 µg/ml and 300 µg/ml of diphenhydramin-HCl (Kowa Ltd, Osaka), 15 µg/ml and 60 µg/ml of soy bean trypsin inhibitor (SBTI) (Shionogi Ltd, Osaka), and 0.025 M, 0.1 M and 0.2 M of 2-mercaptoethanol (2-ME). Fractionation of the sera was performed by gel filtration through Sephadex G 200.

Results and discussions. Examinations of more than 50 sera showed that the P activity was demonstrated in all serum samples tested and the definite N activity was observed in high frequencies in collagen diseases sera and in lower frequencies in healthy sera. Results of the time studies on the P activity showed clearly its appearance in the early phase up to 45–60 min after the intradermal injections. In the pathologic serum, weak N activity was observed as early as 30 min and its intensity was increased gradually to necrosis after around 24 h. However, in the healthy serum, slight N activity was suspected only after 24 h. Skin lesions, especially in the central area affected by the N factor, did not show blueing even in the early phase. Dilution of the

Table 1. Vascular permeability (P) and necrotizing activity (N) of human sera for guinea-pig skin. Time course studies by a pathologic (Behçet's disease) and a healthy serum

Serum	Activity	Time (prior to the dye injection) 15 min	30 min	45 min	60 min	90 min	120 min	240 min	24 h
Pathologic serum	P	III* (14×13)**	III (13×13)	III (13×14)	I (12×12)	0	0	0	0
	N	−***	±	+	+	++	++	++	+++
Healthy serum	P	II (12×12)	II (13×14)	II (11×10)	I (4×5)	0	0	0	0
	N	−	−	−	−	−	−	−	±

* Intensity of blueings (III: strong, II: moderate, I: weak, 0: none). ** Diameter of blued spots in mm. *** Grading was followed according to the previous[7] described criteria, (+++, strongly positive, −, negative).

Table 2. Comparisons of the properties between vascular permeability (P) factor and necrotizing (N) factor for guinea-pig skin

	P factor	N factor
Distribution in:		
Healthy sera	II ~ III* (1)**	−~+* (4, 6, 7, 8)
Collagen diseases sera	III	++~+++
Effects of pretreatments in vitro with***:		
Heating for 30 min at:		
45 °C	III	+++ (8)
56 °C	0 ~ I	− (6, 7, 8)
Dialysis.	III	+++ (4, 7)
Dilutions, to 1:2-4	III	−~+ (8)
1:50	0 ~ I	−
Absorptions with:		
Nucleated cells of guinea-pigs at:		
37 °C	0	−~+ (5, 7, 8)
0 °C	II ~ III	+++ (4), −~+ (8)
Erythrocytes of sheep and guinea-pigs at:		
37 °C	0	
0 °C	II ~ III	+++ (4)
Various drugs:		
antihistaminics	III (1)	+++ (5, 6****)
SBTI	III	++~+++ (6, 8)
2-ME	0	− (9)
D-penicillamine	0 ~ I (9)	+ (9)
Activity in fraction*****	19S (+7S?)	19S (8)

* Gradings of the intensity of reactions; III (strongly positive) to 0 (negative) in P activity, and +++ (strongly positive) to − (negative) in N activity. ** Number of references in parentheses. *** Untreated samples used was graded as III or +++. **** In in vivo treatment. ***** Column chromatography on Sephadex G200 for P factor and on Sephadex G100 for N factor.

sera to 1:4 usually lost the activity for induction of congestions and hemorrhages, although the P activity was not affected.

Results obtained on the properties of P factor were summarized in table 2. Heating sera at 56 °C for 30 min abolished the activity, but no significant effect was obtained in heating sera at 45 °C for 30 min. Dialysis of the serum did not remove the activity. To discover whether the factor had affinity to cell surface membrane, absorption experiments were performed. The activity was completely removed by kidney cells and erythrocytes at 37 °C, but not significantly at 0 °C or 25 °C. Spleen cells also removed the activity significantly, but not completely at 37 °C. Heterophile antibodies to guinea-pig cells were removed by these absorptions, as reported by other reseachers[4]. Treatment of sera with 2-ME abolished the activity at a final concentration of 0.1 M. Diphenhydramin-HCl and SBTI had no effect on the activity, which was in agreement with the results obtained by Edler and Wilhelm[1]. Fractionation of the serum through Sephadex G 200 showed that the activity was demonstrated mainly in 19 S fraction. As seen in table 2, comparisons of the properties between the P and N factor obtained in our laboratory, and from the literature[1-9], indicated that these 2 factors were almost similar. Such results were speculated already by Edler et al.[1] and Klemperer et al.[6]. However, no attempts to prove the relationship between these had been performed. Time course studies revealed that these 2 factors acted in the different phases; the P factor acted in the early stage and the N factor acted in the later stage. Whenever the P activity was removed or abolished by the pretreatments, no N activity was observed. Therefore, it seemed to be plausible that the P factor of PF/Nat played as an initiator followed by the activation of some unknown factors to develop to skin necrosis.

The long-lasting permeability factor in sera of some patients with IgG monoclonal gammapathy was previously reported[10], and it was easily distinguished from PF/Nat by its long-lasting activity, resistance to heating at 56 °C and belonging to the IgG fraction.

* Acknowledgment. The authors wish to thank Prof. H. Hayashi, Kumamoto University for encouragement and discussions.

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Etude comparative de l'effet de la thymosine et d'un extrait de cultures cellulaires épithéliales thymiques sur la production d'anticorps

Comparative study of the effect of thymosin and supernatant from epithelial thymic cells on antibody production

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Summary. A thymic extract (TE) was preparated from supernatant of mice thymic epithelial cultures according to the purification of thymosin. TE and thymosin stimulated, in vitro, the immune response of mouse against sheep red blood cells.

Les cellules thymiques épithéliales sont généralement considérées comme la source des facteurs de différenciation des cellules souches hématopoiétiques en lymphocytes T. Cependant, la relation entre ces facteurs et des extraits de cultures cellulaires épithéliales reste imprécise.

Dans notre travail nous avons comparé le pouvoir de stimulation sur la production d'anticorps, in vitro et in vivo, de ces extraits et de la thymosine. Les extraits ont été préparés à partir de surnageants de cultures cellulaires épithéliales selon la technique employée pour l'isolement de la thymosine.

Matériels et méthodes. Cultures de cellules épithéliales thymiques. Les thymus prélevés sur des souris mâles Swiss agées de 30 jours sont traités par la collagénase (0,05%) puis par la trypsine (0,25%)[3]. Les fragments thymiques, débarrassés des lymphocytes, sont répartis à la surface de flacon de culture (Flacon Falcon, 30 ml) avec une fine pellicule de milieu de culture (milieu 199 + 10% de sérum de veau). Ce milieu est remplacé au bout de 48 h. Après 4 jours de culture nous observons un tapis cellulaire complet; nous remplaçons le milieu nutritif. Après 8 jours de culture à +37 °C nous raclons délicatement le fond du flacon; le milieu contenant les cellules en suspension est alors centrifugé (700 × g; 15 min), le surnageant est prélevé pour l'extraction et le culot contenant les cellules épithéliales est utilisé pour la vérification de la structure des cellules en microscopie électronique. Parallèlement aux cultures cellulaires épithéliales thymiques, nous réalisons dans les mêmes conditions des cultures de cellules de rein de souris.

Vérification de la structure cellulaire épithéliale en microscopie électronique. Le culot obtenu comme il a été décrit précédemment est traité pour l'étude en microscopie électronique (solution de glutaraldéhyde : acide osmique : résine). Après solidification, les coupes, faites à l'ultramicrotome Reichert, sont étudiées au microscope Siemens Elmiskop 1 A.

Préparation des extraits. Nous avons utilisé des thymus de veau; l'extraction et la purification de la thymosine sont faites selon la technique de Goldstein et coll.[4-6] résumée sur le tableau. La préparation des extraits à partir de surnageants de cultures cellulaires épithéliales thymiques est faite selon cette même technique. Le surnageant obtenu après centrifugation du milieu de culture est considéré comme la fraction I du tableau et nous permet d'obtenir