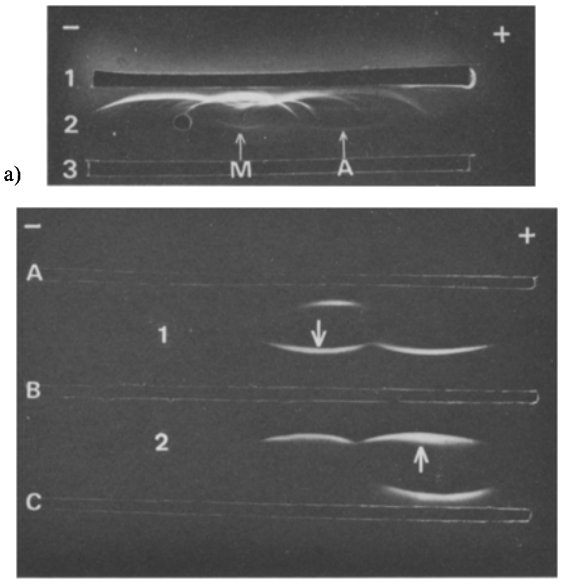

Fig. 3. Photograph of an immunoelectrophoresis run. The conditions of electrophoresis are the same as those of figure 2. *a:* 2 precipitin lines M and A correspond with those of α_2-macroglobulin and α_1-antitrypsin respectively. 1, Rabbit antihuman serum; 2, human serum; 3, crude extract of aloe. *b:* Precipitin reactions were developed against rabbit antihuman α_2-macroglobulin, against rabbit antihuman α_1-antitrypsin and against aloe extract. The electrophoretic mobilities of the precipitin lines (arrow) of human serum proteins against aloe extract coincide to that of α_2-macroglobulin and α_1-antitrypsin. Well 1 and 2, 4 µl of human serum; trough A, rabbit antihuman α_2-macroglobulin; trough B, aloe extract (10 mg/ml); trough C, rabbit antihuman α_1-antitrypsin; all volumes of troughs, approximately 0.15 ml.

lic of Germany). Sera of 13 animals, namely, human, rabbit *(Orytolagus cuniculus)*, sheep *(Ovis aries)*, dog *(Canis familiaris)*, cat *(Felis catus)*, horse *(Equus caballus)*, pig *(Sus scrofa)*, rat *(Rattus norvegicus)*, bovine *(Bos taurus)*, mouse *(Mus musculus)*, carp *(Cyprinus carpio)*, snake *(Elaphe climacophara)*, and frog *(Rana nigromuculata)*, were tested. Of these, the precipitin reactions of 10 sera and egg yolk and white of chicken *(Gallus domesticus)* were demonstrated by a specially designed immunodiffusion plate (figure 1). The extract of aloe reacted not only with mammalian sera but also with fish, reptile, amphibia as well as with egg yolk, but egg white was not reactive. In almost all sera and egg yolk, 2 or more precipitin lines could be detected. To determine serum proteins that react with the extract of aloe, the reactions with electrophoresed serum proteins of various animals were studied, as shown in figure 2. Analogous patterns of precipitin lines were seen in mammalian sera. The nature of the proteins of animal serum reacting with the aloe extract remains to be studied further, but especially with human serum, 2 clear precipitin lines (arrow) were detected as shown in figure 2. As suspects implied from the patterns of the 2 precipitin lines, α_2-macroglobulin, haptoglobulin, α_1-antitrypsin, and α_1-acid glycoprotein were tested. The results shown in the upper part of figure 3 suggested that α_2-macroglobulin and α_1-antitrypsin may react with aloe extract. Furthermore, in the experiment of the lower part of figure 3, definitive proof was obtained by using each of the monospecific antibodies, rabbit antihuman α_2-macroglobulin and rabbit antihuman α_1-antitrypsin instead of rabbit antihuman whole serum. Lectin proposed by Boyd[4] is the term for proteins that possess the ability to agglutinate erythrocytes and have been found mostly in the seeds of plants. It has already been well established that both agglutination and precipitation can be caused by the same mechanism of antigen-antibody reaction. The representative lectin, concanavalin A, can agglutinate erythrocytes as well as precipitate serum proteins[5,6]. The present experiment indicated that some biologically active lectin-like proteins are contained in the leaves of aloe. Moreover, they may be implicated in the possible anti-inflammatory action and the therapeutic effects for burns, as serum proteins reacting with aloe extract were α_2-macroglobulin and α_1-antitrypsin which are known to be most representative protease inhibitors, since repair and remodeling of the connective tissue and regulation of the vascular tone are examples of metabolic processes in which proteolytic enzymes occupy a key position, and crystalline trypsin has been used in surgery for the treatment of these conditions.

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Effect of papain on experimental amyloidosis

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Summary. Experimental amyloidosis was induced in mice by repeated injections of complete Freund's adjuvant (CFA) reinforced with a bacterial vaccine. Papain was administered i.p. at various time intervals during the treatment with CFA. Amyloidosis was found only in the spleen and the liver. No statistically significant differences were found between the papain-treated and the control groups. It is assumed that, although papain released the polysaccharide moiety from the polysaccharide protein complex, the released polysaccharides were most probably bound by electrostatic forces to the amyloid fibres, and did not interfere with amyloidogenesis.

Acid mucopolysaccharides (AMPS) consist mainly of heparin sulfate, with chondroitin sulfate in smaller quantities[1,2]. The fibrillar structure of amyloid and its glycoprotein character have been demonstrated[3], but the role of AMPS and their relationship to amyloid fibres is still open to discussion. Histochemical studies have proved that heparin sulfate and, in smaller quantities, chondroitin sulfate are present in amyloid-laden organs. It has been proposed that AMPS contribute to amyloid fibre formation in a way similar to the fibrillar protein formation in

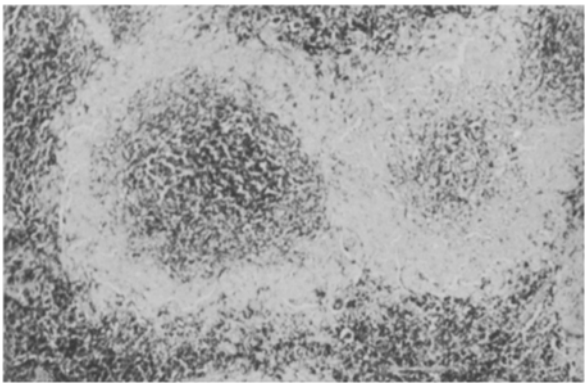

Fig. 1. Amyloid deposition in the spleen. Note perifollicular and follicular replacement by amyloid. H and E.

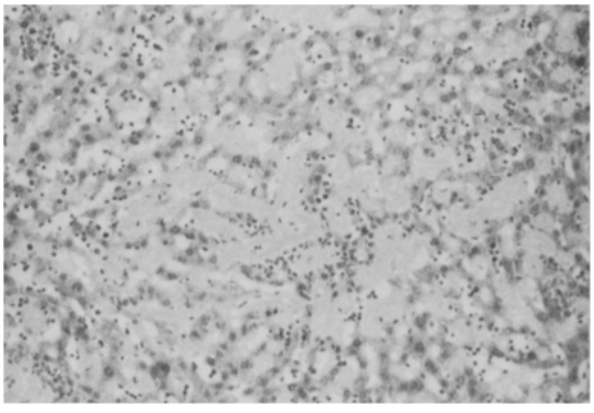

Fig. 2. Amyloid deposits in liver. Note trebedular liver atrophy. H and E.

collagen[4]. It has also been suggested that the AMPS found in amyloid deposits may originate from the connective tissue ground substance in which the amyloid fibres are embedded[5]. Decrease and/or derangement in the synthesis of AMPS with a parallel decrease in the incidence of experimentally induced amyloidosis have been described[6,7]. As papain is a potent proteolytic enzyme which can separate the polysaccharide moiety from the protein core[8], we considered it worthwhile to investigate its effect on amyloidogenesis. In this study we report the effect of papain on amyloidosis induced in mice with complete Freund's adjuvant (CFA).

Materials and methods. 170 male white mice of the Hebrew University strain, each weighing 30–40 g, were divided into 7 groups, as described below. The animals were injected i.p. with 1 ml of 0.3% papain in saline and/or s.c. with 0.3 ml of a mixture of equal parts of CFA and saline, reinforced with dried *Mycobacterium tuberculosis*, 0.1 mg/ml, according to the protocol described below. Unless otherwise stated, the animals were killed 7 days after the administration of the last injection.

Group 1 (15 mice): CFA, 1 injection per week for 6 weeks, was administered to each of these mice. This group served as the control group.

Group 2 (35 mice): Over a 6-week period during which the animals were given CFA in a manner similar to that for group 1 mice, papain was administered every 48 h, until the 6th CFA injection.

Group 3 (30 mice): The animals were injected once a week with papain and once a week with CFA for 6 weeks. Each CFA injection was given 24 h after a papain injection.

Group 4 (30 mice): These mice were given the same CFA regimen as the group 1 animals. Thereafter each mouse received 4 papain injections at 48-h intervals. The animals were killed 2 days after the last papain injection.

Group 5 (15 mice): Each animal was given 1 CFA injection per week for 4 weeks. After 8 days, each mouse received 2 more injections of CFA. These animals served as the control group for groups 4 and 6.

Group 6 (30 mice): The regimen was the same as that for the group 5 animals, except that during the 8-day period between the 4 and 5 CFA injections, each animal was given an injection of papain every 48 h.

Group 7 (15 mice): Papain was administered every 48 h for 6 weeks, and the mice were killed 2 days after the last injection.

The animals were killed by cervical dislocation, and small portions were taken from the spleen, liver, heart and kidney and fixed in 4% formalin for histological examination. Sections were stained with hematoxylin and eosin, Congo red, methyl violet and colloidal iron.

Results. Spleen, liver, heart and kidney were examined histologically. Amyloid was found only in the spleen and the liver: there were no amyloid deposits in the kidney or the heart in any of the groups studied. In the spleen, the amyloid deposits were perifollicular and the follicles were compressed and/or completely replaced by amyloid, according to the extent of amyloid involvement (figure 1). Large areas of the splenic tissue, particularly the red pulp, were replaced by amyloid deposits. In the liver, amyloid was deposited along the sinusoids, and the amyloid deposits caused atrophy of the liver cords and formed confluent masses, according to the severity of the involvement (figure 2). Amyloid deposition occurred in vessel walls particularly in the portal spaces. The liver was found to be less affected than the spleen.

Table 1

Group No.	No. of animals	Amyloid in spleen		Amyloid in liver		Death	
1*	15	11	73.3%	9	60%	0	0 %
2	35	14	40%	10	28.5%	13	33.5%
3	30	7	23.3%	5	16.6%	18	60 %
4	30	15	50%	11	36.6%	9	30 %

*Control group.

Table 2

Group No.	No. of animals	Amyloid in spleen		Amyloid in liver		Deaths	
5*	15	11	73.3%	7	46.6%	0	0%
6	30	9	30%	8	26.6%	18	60%
7	15	0	0	0	0		

*Control group.

No differences were found in staining properties for AMPS by colloidal iron among the different experimental groups. Colloidal-iron-stained areas were bluish, whereas the amyloid areas stained by methyl violet were red. As indicated in table 1, the incidence of amyloid in the spleen in control group 1 was 73% and that in the liver was 60%. In the animals given papain and CFA (groups 2 and 3) the incidence of amyloid in the spleen ranged from 23 to 50%, and that in the liver from 16 to 36%. In the animals treated with papain alone, there were no amyloid deposits and no other pathology (group 7).

Table 2 summarizes the incidence of amyloid in spleen and liver in a control group (group 5) and in the 2 papain-treated groups (groups 4 and 6) which received papain over an 8-day period. The administration of papain during the period of CFA administration decreased the incidence of splenic and hepatic amyloidosis. While the incidence of splenic amyloidosis was 73% and that of hepatic amyloidosis was 46% in control group 5, the incidence of amyloidosis in the papain-treated animals was 30 and 26%, respectively (table 2).

A high percentage, between 30 and 60%, of the papain-treated animals died. None of the mice in the CFA-treated groups (groups 1 and 5) died. The incidence of hepatic and splenic amyloidosis was lower in the papain-treated than in the control animals, but this difference was not statistically significant (by the χ^2 test).

Discussion. It has previously been shown that amyloid-laden organs contain AMPS in higher concentrations than normal organs[1,2]. There are many contradictions regarding the presence and/or role of AMPS in amyloid deposits. Glenner et al. related the presence of AMPS in the amyloid deposits to the AMPS which are normally found in the ground substance in which the amyloid is deposited[5]. On the other hand, the presence of AMPS in amyloid deposits may indicate that the AMPS play a role in the formation of amyloid fibres. It is possible that the formation of amyloid fibres is analogous to the formation of collagen fibres[4]. Pras et al. discussed the role of AMPS in the regulation of amyloid fibre formation and rigidity[9]. The fact that the decrease in AMPS synthesis in diabetes parallels a decrease in the incidence of amyloidosis also suggests that AMPS may play a role in amyloid fibre formation[6].

The AMPS consist of a linear carbohydrate chain, carrying negatively charged carboxy and sulfate groups, covalently linked to a protein core[10]. Papain is a potent proteolytic enzyme which can liberate the polysaccharides from the protein core. In papain-treated mice, the AMPS normally found in the liver cell membrane were no longer present[11]. We therefore used papain to digest the AMPS and to study the influence of the lysed polysaccharides on experimental amyloidosis. No statistically significant differences were found in the incidence of amyloidosis among the papain-treated and control groups. This can be explained by the fact that, although papain released the polysaccharides from the protein polysaccharide complex, the released polysaccharides were immediately bound to the amyloid fibres by electrostatic forces. As has been shown by Pras et al., amyloid fibre subunits are highly charged molecules containing both positively and negatively charged groups[12]. The AMPS with their negatively charged groups are linked by electrostatic forces to the amyloid fibres, and in this way stabilize and confer rigidity on the amyloid fibres, but do not interfere with amyloidogenesis. These findings support the opinion of researchers that AMPS present in the amyloid originate in the ground substance and become bound to the amyloid fibres.

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Neutral proteases in the guinea-pig lymphocytes[1]

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Summary. Partial purification of neutral proteolytic enzymes in guinea-pig lymphocytes yielded 2 enzymes. Both enzymes were heat-labile and inhibited by thiol reagents. The molecular weights were more than 200,000 and 150,000–200,000, and optimal pH around 9 and 8, respectively.

The whole question of mediators of delayed hypersensitivity reaction (DHR) and the delineation of a molecular basis for cellular immune responses is an active area of investigation. While lymphokines are thought to participate in the expression of DHR[2,3], the exact role of the lymphokines in the pathogenesis of DHR is still unclear.

The present communication describes attempts to isolate, partially purify and characterize the neutral proteases of lymphocytes (NPLs), and discuss the role of NPLs in inflammatory changes.

Materials and methods. Hartley guinea-pigs, 300–500 g, were sensitized by injecting into 4 footpads 10 µg of bovine γ-globulin (Armour, Kankakee, USA) emulsified in complete Freund's adjuvant. 9 days later, lymph node cells were teased out in Hanks solution from the regional lymph nodes of the animals. More than 90% of the cells were lymphocytes by Giemsa stain and viability (85–90%) was determined by trypan blue dye exclusion. The cells were homogenized in 0.34 M sucrose and extracted over night with 67 mM phosphate buffer, pH 7.4. Proteolytic activity