Effect of different concentrations of the active principle extracted from *Caesulia axillaris* on germination of seeds of test weeds and of the test crop

Concentration of active fraction (mg/l)	Inhibition of germination (%)			
	Test crop	Test weeds		
	Oryza sativa	*Ocimum* sp.	*Ammannia* sp.	*Fimbrystylis* sp.
0 (control)	Zero	Zero	Zero	Zero
500	Zero	82.5	72.5	67.5
750	Zero	100.0	92.5	92.5
1000	Zero	100.0	100.0	100.0

with the chemical compound extracted from the wild plant, *Caesulia axillaris*, and the catechol obtained from a chemical firm. The concentrations used were 500, 750, and 1000 mg/l in distilled water. 100 seeds each of *O. sativa* and the test weeds were washed thoroughly with distilled water and were kept in petri-dishes (8.5 cm in diameter) on 1 layer of filter paper soaked with 5.0 ml solution of different concentrations. The petri dishes were kept in dark in an incubator at 32 °C for 36 h. Three replicates of each treatment were maintained. The untreated (control) seeds were provided with the same amount of distilled water. The seeds containing emerged radicles were counted for germination. It was found that a concentration of 500 mg/l of catechol inhibited germination of the test weeds by about 67%, while the other 2 concentrations (750 and 1000 mg/l) inhibited up to 100%; no inhibition, however, was noted in seeds of *O. sativa* at these concentrations (table). Similar results were obtained with the phenolic extract.

Discussion. Plant parts are known to exhibit biological activity on account of the presence of some active principles[7-10]. In the present investigation the leaves and stems of *Caesulia axillaris* exhibited more herbicidal activity than its roots. The active principle, identified as 1,2-dihydroxybenzene, is a well known bacteriostatic and bactericidal agent. Its selective herbicidal property has been established in the present investigation when it was found to be phytotoxic to *Ocimum* sp., *Ammannia* sp., and *Fimbrystylis* sp. but non-phytotoxic to *O. sativa* in the paddy fields. The present findings are significant from the point of view of exploitation of catechol as a herbicide against weeds of *O. sativa*.

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Fibrin and collagen deposition and fibroblasts proliferation in granuloma of murine leprosy. Comparison of two mouse strains with different immune reactions[1]

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Summary. Comparative immunofluorescence study with murine lepromas induced in C57BL/6NJcl (immunologically high responder) and CBA/N (low responder) mouse strains revealed that fibrin formation was associated with cell-mediated immune resistance against invasive bacilli. Histochemistry on paraffin sections further elucidated fibroblast proliferation and formation of collagen fibers following fibrin deposition only in murine lepromas with positive host reactions.

In murine leprosy, Poulter and Lefford[2] demonstrated that lymphocytic and monocytic infiltration against the bacilli is mediated by transferable cells but not by serum. The potential immunological reactivity which are genetically determined for each mouse strain induced murine lepromas which varied in their cellular composition. Kawaguchi[3] and Closs and Haugen[4] reported that a polar type of murine lepromas induced in C57BL/6 strain was shown to consist of phagocytic macrophages as well as non-phagocytizing monocytes, epithelioid cells, fibroblasts, and lymphocytes. Consequently, this type of murine lepromas with positive immune reaction was localized at the site of inoculation and occasionally showed spontaneous healing after ulceration. The other polar type of lesion without host reaction was shown to contain only macrophages ingesting bacilli but no lymphocytes or other reactive cells. The murine lepromas were continuously developed and finally showed dissemination of lepromatous lesions to the liver and spleen[5].

Activation of the coagulation system has been demonstrat-

ed during the development of chronic granulomatous inflammation[6]. Furthermore, earlier investigations suggested that tissue repair is triggered by fibrin formation in tissue. Experiments with implanted fibrin clots by Kwaan and Astrup[7] revealed that fibroblasts and endothelial cells infiltrated into the fibrin matrix after acute stage of inflammation with neutrophils. Fibrosis took place when the fibrin persisted longer after anti-fibrinolytic treatment[8]. The present study investigates kinetic changes in the pattern of fibrin deposition in different types of murine lepromas with and without immune reactions. Fibroblasts proliferation and collagen fiber deposition were studied to be correlated with the tissue fibrin.

Materials and methods. A suspension of 2×10^8 *Mycobacterium lepraemurium* was injected s.c. into the sternal area of 69 C57BL/6NJcl (high responder), and 38 CBA/N (low responder) male mice. At various weeks after inoculation, lesions were removed, weighed, frozen in liquid-nitrogen and stored at −20 °C. Immunofluorescence technique to detect fibrin deposition were employed on frozen tissue sections. Frozen sections (6 µm) were fixed in 95% ethanol for 2 min, and reacted with rabbit anti-mouse fibrin/fibrinogen serum, diluted 1/10, from the same lot previously used[6]. The reactivity of anti-mouse fibrin/fibrinogen serum was tested before use against citrated plasma from mice of each mouse strain. Serially diluted plasma were placed on agar plate and reacted with 1/10 diluted anti-mouse fibrin/fibrinogen serum by Ouchterlony's double diffusion method[9]. Single precipitin line was detected for both plasma diluted in a range of 1/1-1/32. The precipitin line showed complete fusion between strains, showing the identical antigenecity. The anti-serum treated sections were washed in 0.02 M phosphate buffer, pH 7.5 containing 0.14 M sodium chloride and stained with FITC labeled goat anti-rabbit IgG. The sections were observed and photographed with a fluorescence microscope. Immunological specificity was confirmed by use of normal rabbit serum and rabbit anti-mouse IgG. Adjacent sections were stained with hematoxylin and eosin (HE) and Ziehl-Neelsen (ZN) methods. Paraffin sections of 4 µm thick were stained with HE, ZN and elastica van Gieson (EvG) methods to demonstrate collagen fibers. Masson trichrome staining was also performed.

Results. Both strains of mice developed 2 different forms of murine lepromas respectively. Macroscopically the C57BL/6NJcl mice produced a firm s.c. nodule at the site of inoculation 4–5 weeks after infection. This showed cutaneous ulceration 9 weeks after infection and remained at the same size thereafter without systemic infection. CBA/N mice induced a soft and diffuse leproma which increased in weight exponentially from 11 weeks until 28 weeks after infection. Dissemination of lepromatous lesions took place after 30 weeks. HE and ZN preprations showed that the lesions in the C57BL/6NJcl mice consisted of phagocytic macrophages in the center and non-phagocytizing cells in the peripheral area, while in CBA/N strain mice the lesions predominantly consisted of phagocytic murine leprosy cells without lymphocytes. These observations established that typical polar types of murine lepromas were developed in both strains respectively. Changes in the pattern of fibrin deposition and increase of collagen fibers were compared between the 2 mouse strains.

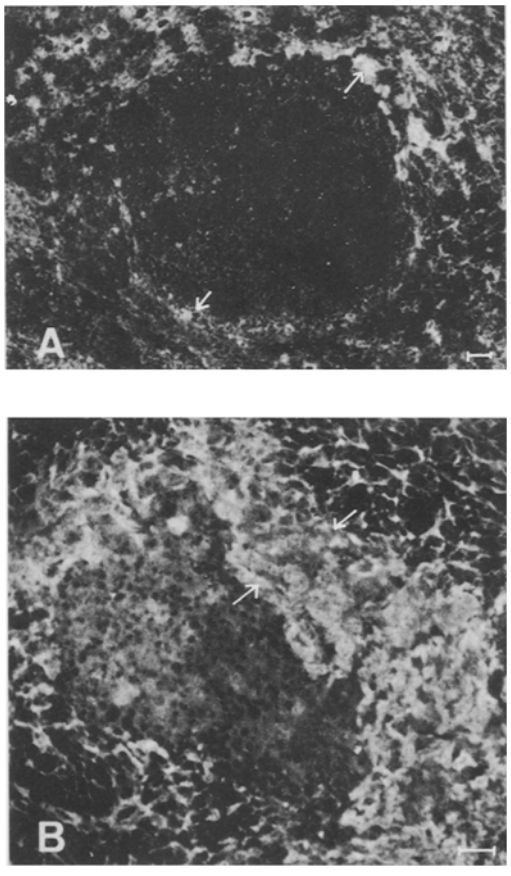

Figure 1. Fibrin deposition in C57BL/6NJcl mice. *A* 5 weeks after infection. The fluorescence was revealed in the peripheral area (arrow) but not in the center. *B* 24 weeks after infection. The area of fibrin deposition (arrow) increased mainly over the cells surrounding phagocytic macrophages. Bars: 40 µm.

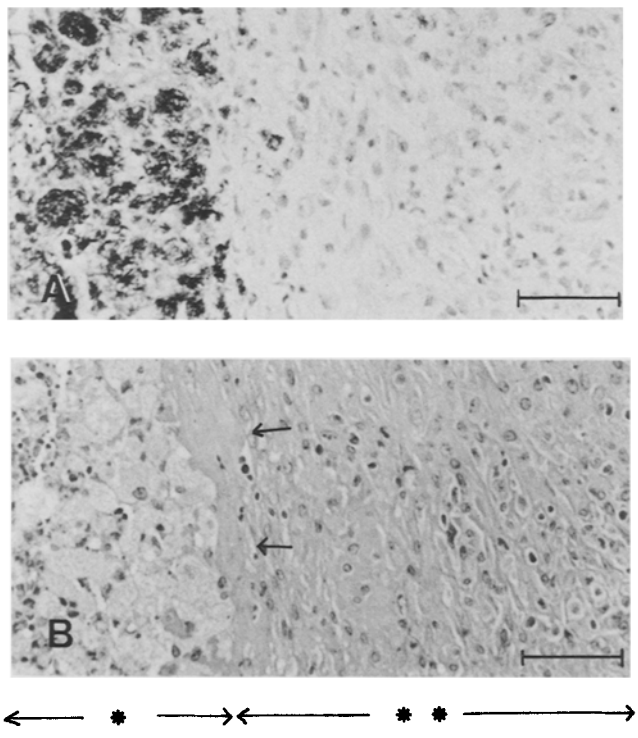

Figure 2. Histopathology of C57BL/6NJcl mice, 15 weeks after infection. *A* Ziehl-Neelsen. Phagocytic macrophages and the acid-fast bacilli are shown in a localized area (on the left ← * →) which was surrounded by reactive cells (right ← * * →). *B* Elastica van Gieson. Abundant collagen fibers (arrow) are shown to separate the bacilli-containing area (left) from outer mononuclear cell zone (right). Bars: 40 µm.

In C57BL/6NJcl strain mice after 4–5 weeks of inoculation the reactivity with anti-fibrin/fibrinogen serum was revealed on the peripheral area of each murine lepromas (fig. 1, A). It was found by comparing with HE and ZN stained sections that the site of fibrin deposition coincided with the area which exhibited mononuclear cell infiltration around the dilated vessels, whereas the central part of the lesions where phagocytic macrophages accumulated did not show the fluorescence. The area of fibrin deposition was shown to increase with time. Dense fluorescence was obtained after 15, 20 and 24 weeks after infection surrounding the phagocytic cell layer (fig. 1, B). Fibroblasts were observed after 5 weeks of inoculation in the outer layer. They were increased in number after 15–30 weeks of infection. Collagen fibers were coincidentally observed after 5 weeks and increased with the development of lepromas. After 15–30 weeks, a packed layer of the collagen fibers were demonstrated to separate the bacilli-containing area from the outer mononuclear cell zone (fig. 2, B). The collagen fibers, furthermore, were increased in the outer cell zone between epithelioid, fibroblastic and monocytic cells, forming multiple annular rings.

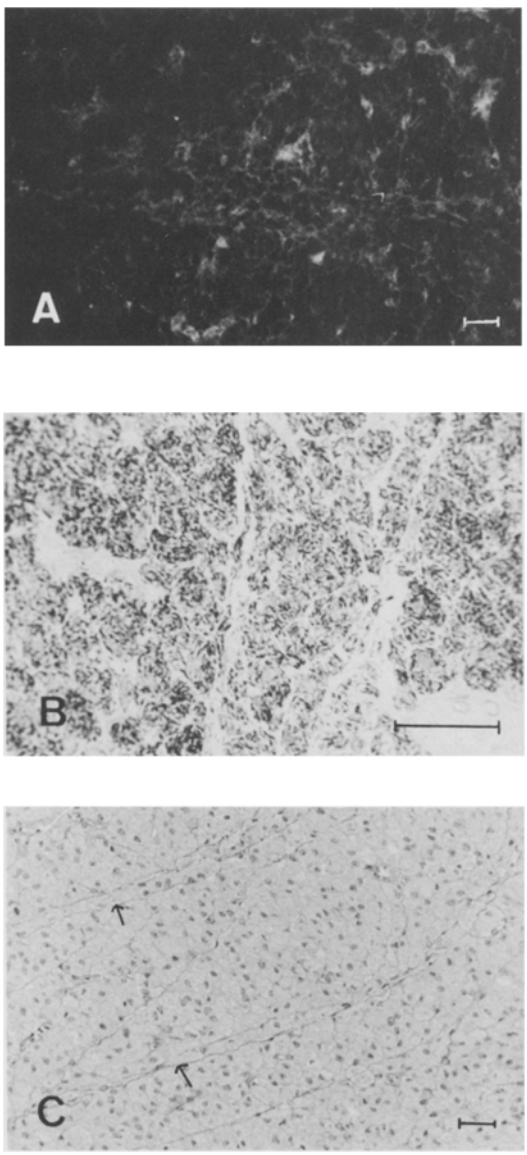

Figure 3. Fibrin deposition and histopathology in CBA/N mice, 20 weeks after infection. *A* Immunofluorescence. A small amount of fibrin was observed over the lesion. *B* Ziehl-Neelsen. Murine leprosy cells ingesting numerous bacilli were the major cell population and no other inflammatory cells were seen. *C* Elastica van Gieson. Collagen fibers were finely observed in the inter-cellular spaces but fibers did not increase in the periphery. Bars: 40 μm.

In CBA/N strain mice, threads of fibrin were observed in linear and meshlike pattern over the lesions after 11 weeks. After 15–30 weeks of inoculation, deposited fibrin did not increase in the reactivity both in the center and periphery, but showed identical pattern over the lesions (fig. 3, A). In these lesions, only small numbers of fibroblasts were observed between phagocytic murine leprosy cells (fig. 3, B) until 30 weeks after inoculation. Fine collagen fibers were observed in the inter-cellular spaces between phagocytic macrophages (fig. 3, C) after 11–30 weeks of infection. During this period collagen fibers did not increase. No boundary of collagen fibers to separate the murine leprosy cells was shown in this strain of mice.

Discussion. Demonstration of fibrin in the murine lepromas with positive host reactions accounts for coagulation in hypersensitivity. Activation of coagulation plays a role in pathogenesis of Masugi nephritis[10], cutaneous delayed hypersensitivity reactions[11], and macrophage disappearance phenomenon[12]. The finding that only a trace amount of fibrin was revealed in the immunologically non-reacting mice clearly indicates that the fibrin formation was primarily involved in resistance to murine leprosy. Colvin and Dvorak[11] have suggested that deposited fibrin is the basis of the induration of inflamed tissue in classical delayed hypersensitivity. In addition to the fibrin deposition the present study demonstrated that fibroblasts proliferated and formed numerous collagen fibers only in murine lepromas with hypersensitivity reactions, but not in the non-reactive murine lepromas. The collagen in lesions is apparently the reason for the firm s.c. murine lepromas. We suggest that the localizing effect of lepromatous lesions in the high responders may be induced by deposited tissue fibrin and subsequent collagen formation in the peripheral area of murine lepromas. The nonreactive, but invasive, murine lepromas in the CBA/N mice showed soft and diffuse texture without remarkable fibrin and collagen in the lepromas.

A possible cellular mechanism of activation of coagulation by stimulated monocytic cells is suggested by the present finding at the site of fibrin deposition. This view is supported by recent studies which have shown that monocytes secrete thromboplastin-like tissue factors after exposure to endotoxin[13]. In addition, recent cell culture experiments by Johnson and Ziff[14] clarified that lymphokine-rich preparations from phytochemaglutinin-stimulated mononuclear cells enhanced collagen synthesis of human fibroblasts. Further studies are needed to establish the relationship between formation of fibrin and collagen fibers in various infectious diseases.

The previous study on murine schistosomiasis[6] has shown fibrin deposition with the development of hypersensitivity granulomas in thymus-intact mice, whereas the athymic mice also produced granulomas consisting of fewer epithelioid cells and multinucleated giant cells. Fibrin was observed in the immature epithelioid cell granulomas in both athymic and the thymus-intact mice. These results imply that the activation of clotting is interrelated with cellular activity of monocytic cells but may not be directly mediated by T-lymphocytes.

Our recent biochemical studies[15] demonstrated that plasminogen activator and the regulating inhibitor of plasminogen activator occur in lesions during the development of immunoreactive murine lepromas. Coagulation and fibrinolysis seem to be actively involved in hypersensitive granulomatous tissue reactions.

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Serodiagnosis of viral hepatitis A: Rise in antibody titre and evaluation of three methods for detecting early and late antibodies

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Summary. A serological investigation was made on patients with viral hepatitis A and individuals with a past history of this disease. Titration of antibody in sequential samples was found to be of no help in diagnosis. Separation of early (IgM) from late (IgG) antibodies by protein A or by 2-mercaptoethanol did not prove to be convenient for the serodiagnosis. A chromatographic separation of late and early antibody was found to be satisfactory, and equivalent to a radioimmunoassay for IgM-antibodies.

Since hepatitis A virus (HAV) was discovered by Feinstone et al.[2], various methods have been described for the detection of anti-HAV antibodies: for instance, immune electron microscopy[2], immune adherence haemagglutination[3,4], complement fixation[5], radioimmunoassay[6,7], and more recently the enzyme-linked-immuno-sorbent assay[8,9].

Anti-HAV antibodies persist indefinitely. In our region, they were shown to be detectable in the serum of about half the normal adult population[10]. For diagnostic purposes, it became necessary to distinguish such late antibodies (IgG) from those produced at the onset of disease (IgM). Therefore, in addition to measuring the rise in titre between 2 early serum samples, several authors have used methods for the specific detection of IgM. These included: 1. inactivation of IgM with 2-mercaptoethanol (2-ME)[11], 2. removal of IgG by binding to staphylococci[12], 3. separation of immunoglobulin classes by ultracentrifugation[13], and 4. detection of IgM alone using anti-IgM specific antibody[9,14].

The aim of the present study was to compare 4 different procedures used to assess the acute phase nature of anti-HAV antibody production: 1. Serial samples of serum taken after onset of disease were titrated in a commercial radioimmunoassay (RIA) to look for a possible rise in titre. To distinguish IgM from IgG activity, samples were assayed in the same RIA, 2. before and after inactivating IgM with 2-ME, 3. before and after complexing IgG with staphylococcal protein A, and 4. before and after separating IgM by chromatography.

Materials and methods. 14 patients (6 ♂, 8 ♀) with acute viral hepatitis demonstrated by clinical evidence and rise in transaminase levels were studied. The markers of hepatitis B virus infection were absent in all cases. Sera were obtained at the onset of the disease and at weekly intervals until the 2nd or 3rd month. Sera taken after 2 months were considered as 'late' samples, sera taken before day 15 as 'early' samples. Seven healthy individuals with anti-HAV antibody and a past history of acute hepatitis A, 6 months to 5 years previously, also provided serum samples for the study of late antibodies.

Titration of anti-HAV antibody. A commercial RIA was used throughout (HAVAB™ kit, Abbott GmbH, Diagnostics Division, FRG). Its principle is a competition between the patient's antibody (any class of immunoglobulin) and I^{125}-labeled-antibody for a solid phase-bound antigen. Titration was performed by diluting the samples from $^1/_2$ to $^1/_{3200}$ in PBS. The measurements were made in duplicate on sequential serum samples. The titre was the last dilution below a cutoff value, calculated from the values of a positive and a negative control.

IgM inactivation by 2-mercaptoethanol (2-ME). 200 µl of

Table 1. Effect of protein A treatment, 2-ME treatment and chromatographic separation on the anti-HAV titre of serum from 7 healthy individuals who had recovered from acute hepatitis A infection

Case No.	Titre of anti-HAV antibody			
	Nontreated	After 2-ME treatment	After protein A treatment	After chromatographic separation (IgM fraction)
1	100*	100	20	neg.
2	1600	1600	200	neg.
3	200	200	50	neg.
4	100	100	20	neg.
5	1600	1600	400	neg.
6	100	100	20	neg.
7	20	20	5	neg.

*Reciprocal dilution.