

Specific reaction of aloe extract with serum proteins of various animals

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Summary. We found that aloe extract contains a lectin-like substance which reacts with serum proteins of various animals. Furthermore, in human serum 2 proteins, α_2 -macroglobulin and α_1 -antitrypsin, were shown to be reactive with aloe extract.

Aloe (*Aloe arborescens* Mill var. *natalensis* Berger) has been used in folklore medicine for centuries. Among the components of aloe, the low mol. wt components such as aloemodin, aloin and aloesin etc. have been well studied and used as purgatives. Also, there have been a few reports¹⁻³ on the pharmacognosical characteristics of aloe extract, probably the components of high mol. wt, which are effective for the treatment of radiation injury and burns, as well as anti-inflammatory. 2 kg of the fresh leaves of aloe were homogenized by Polytron and filtered through Whatman GF/A paper. Then the filtrate was dialyzed and concentrated by the Amicon hollow fibre dialyzer concentrator DC-2 and the concentrate containing components with mol. wt higher than 10,000 were lyophilized (the average yield; 691 mg). The lyophilized powder was used for immunodiffusion and immunoelectrophoresis studies. The monospecific antisera, rabbit antihuman α_2 -macroglobulin and rabbit antihuman α_1 -antitrypsin were purchased from Behringwerke AG, Marburg (Federal Repub-

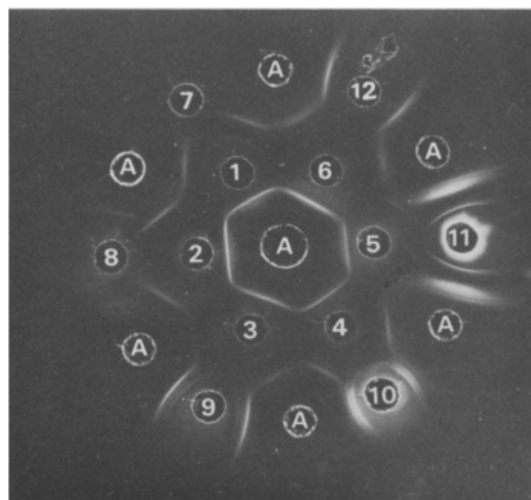
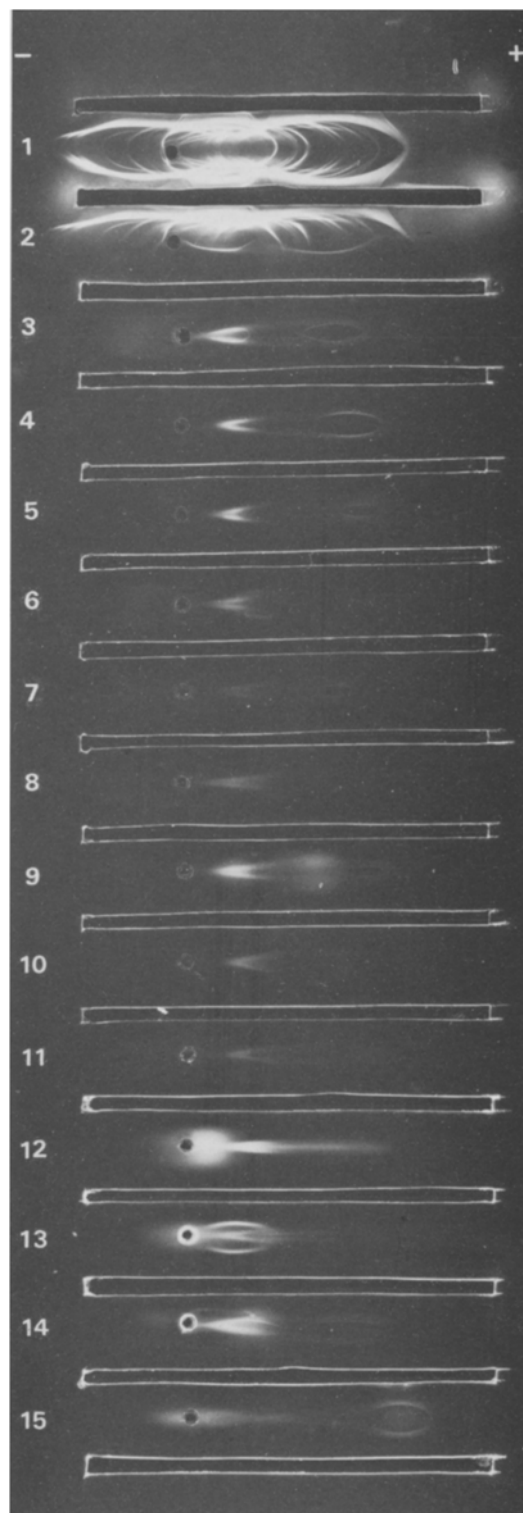


Fig. 1. Precipitin lines that appeared in agarose gel by reaction of aloe extract with serum proteins of various animals. Ouchterlony double diffusion in 1.0% agarose and phosphate buffer, pH 7.6. All the sera described below were diluted with 5fold phosphate buffered saline. All volumes of wells, approximately 0.05 ml. A, aloe extract, 10 mg/ml; 1, human serum; 2, rabbit serum; 3, rat serum; 4, mouse serum; 5, bovine serum; 6, cat serum; 7, egg white diluted with 4fold phosphate buffered saline; 8, carp serum; 9, frog serum; 10, egg yolk diluted with 4fold phosphate buffered saline; 11, snake serum; and 12, dog serum.

Fig. 2. Photograph of an immunoelectrophoresis run. Buffer, veronal buffer (pH 8.6, $\mu = 0.05$); —, cathode; +, anode; All volumes of wells, approximately 2 μ l; 1, 2, and 3, human serum; 4, dog serum; 5, cat serum; 6, pig serum; 7, horse serum; 8, bovine serum; 9, sheep serum; 10, rabbit serum; 11, rat serum; 12, mouse serum; 13, snake serum; 14, frog serum; 15, carp serum. The 1st and 2nd troughs contain antihuman serum and the others contain aloe extract (10 mg/ml); all volumes of troughs, approximately 0.15 ml.



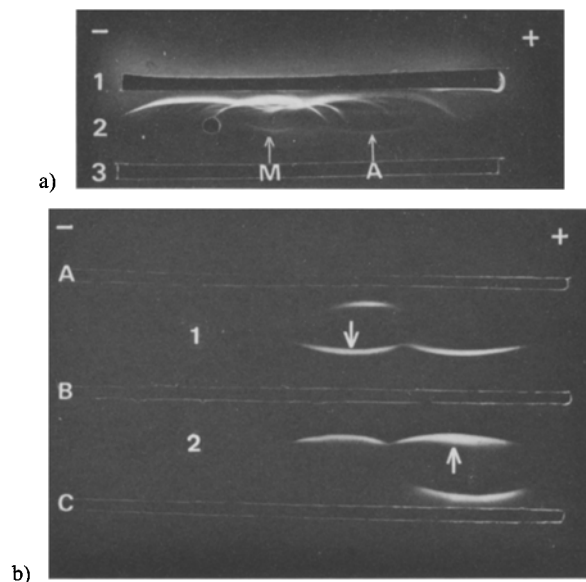


Fig. 3. Photograph of an immunoelectrophoresis run. The conditions of electrophoresis are the same as those of figure 2. *a*: 2 precipitin lines M and A correspond with those of α_2 -macroglobulin and α_1 -antitrypsin respectively. 1, Rabbit antihuman serum; 2, human serum; 3, crude extract of aloe. *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 μ l 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 nigromaculata*), 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 Boyd⁴ 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 demonstrated³, 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