

Immune Mechanisms in Byssinosis*

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Serologic studies of potential immune mechanisms in byssinosis have been encumbered by cotton plant constituents that precipitate serum proteins in a nonimmunologic manner. Taylor et al¹ were the first to encounter this problem during the preparation of their "cotton antigen" from cotton bract material. They isolated this "antigen" to serologically titrate sera from byssinotic and nonbyssinotic cardroom workers and normal control subjects. Although they found significant differences in mean titers between cardroom workers and controls and between byssinotic and nonbyssinotic cardroom workers, all of their control sera showed some reaction with this antigen. Edwards and Jones² determined that this same interaction was nonimmunologic and applied the term "pseudo-immune" to it.

We report that cardroom cotton dust and cotton stem material contain a similar pseudoimmune precipitating agent and that elaborate chemical purification schemes are not necessary to demonstrate it. We also identify the serum proteins that react with this agent.

MATERIALS AND METHODS

Specimen Collection and Extract Preparation

Bract, carpels, stems, leaves, and immature cotton lint were collected manually from frost-killed field cotton near Lubbock, Tex. Cardroom cotton dust was collected at Khar-toum North, Sudan.

Preshift Monday serum samples were collected from 59 cotton textile workers and stored at -20°C . Sera were also collected from 35 nonexposed normal volunteers and stored similarly.

The individual parts of plants and the cardroom cotton dust were ground to 60 mesh. These particles were then extracted separately at 4°C in sterile, nonpyrogenic water (20 g/L). The supernatant fluids were filter-sterilized and concentrated 40-fold by pervaporation (4°C) and stored at -20°C .

Serologic Analyses

Worker and control serum samples were examined for evidence of immune and pseudoimmune precipitation by gel diffusion,³ counterimmunoelectrophoresis,⁴ and immunoelectrophoresis.³

One-ml portions of sera from normal human subjects, cotton textile workers and a commercial pooled serum preparation (which lacked β -lipoprotein) were each mixed with 0.5 ml aliquots of either the cardroom cotton dust or stem extract. These mixtures were incubated for 30 min at 37°C and finally centrifuged for 20 min at 10,000 g to remove the resultant precipitates. The supernatant sera were recovered and

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stored at -20°C .

Permanent records of precipitin arc patterns were made by staining the agarose slides with 0.15 percent Amido black 10 B (Biorad Laboratories) according to standard techniques.⁵ The lipoprotein pseudoimmune precipitates were detected using a Sudan black B (Polysciences, Inc) staining technique.⁶

Plant polyphenolic tannins were removed from the aqueous extracts before the concentration step by adsorption with insoluble polyvinylpyrrolidone^{7,8} (PVP; Calbiochem).

RESULTS

All control and worker sera were negative for precipitating antibodies against extracts of plant parts and cardroom cotton dust as determined by gel diffusion and counterimmunoelectrophoresis.

During the course of the sera examination for specific precipitating antibodies, it was noted that the extracts of stems and cardroom cotton dust precipitated certain serum proteins in a nonimmunologic reaction. Every control or worker serum tested showed the same precipitation pattern: one large, distinct band close to the serum well and one small, diffuse band close to the extract well.

Admixture of human sera with extracts of either cardroom cotton dust or stems resulted in the disappearance of the large, distinct band when the sera were examined by gel diffusion (Fig 1). A commercial reference serum that lacked β -lipoprotein did not demonstrate the large

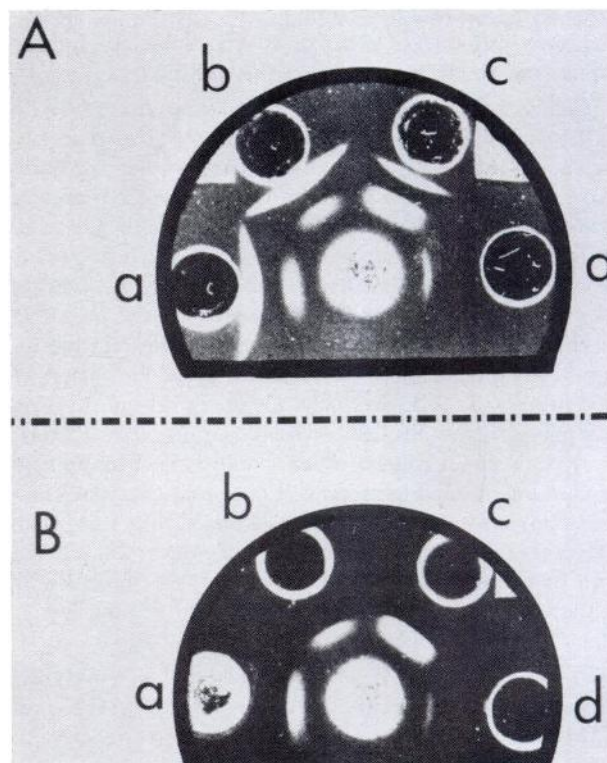


FIGURE 1. Gel diffusion of cotton stem extract (*center well*) with normal human serum (a), cotton textile workers' sera (b,c), and a commercial reference serum that lacked β -lipoprotein (d). A: precipitation pattern before admixture of sera with extract; B: after admixture. (Reprinted with permission.¹¹)

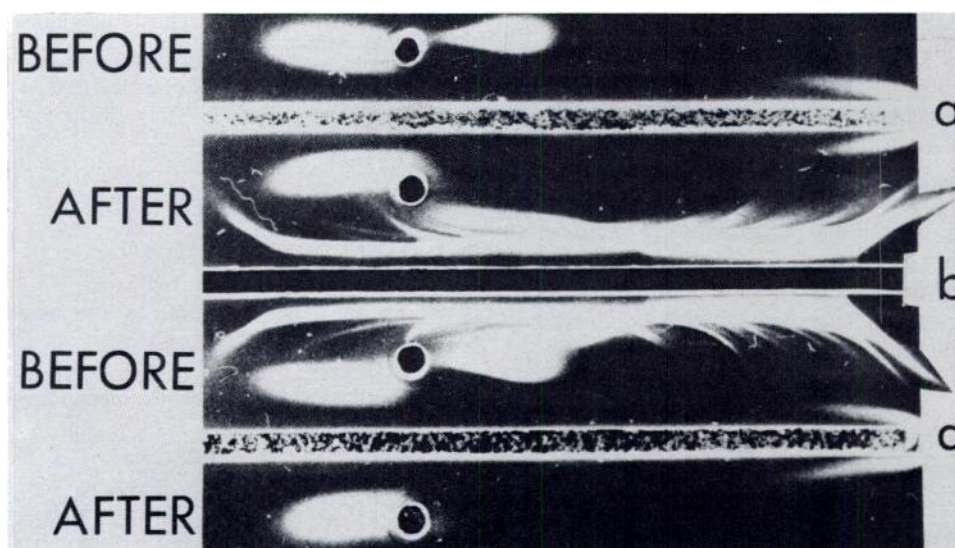


FIGURE 2. Immunoelectrophoresis of cotton textile worker serum before and after admixture with cotton stem extract. Slide developed with cotton stem extract (a) and antihuman serum (b). Anode is to right. (Reprinted with permission.¹¹)

distinct band after gel diffusion with either neat or after mixing with the extracts.

Immunoelectrophoresis of the control and worker sera resulted in two bands of precipitation when developed with the cotton dust or stem extracts. Prior mixing of the sera and precipitation with either extract removed the β -migrating band while the γ -migrating band was unaffected (Fig 2).

The precipitation bands observed in Figure 1 were uniformly positive when stained with the general protein stain, Amido black. Likewise, the large distinct band of precipitation which was closest to the serum wells in the gel diffusion plates (Figure 1A) stained positively for lipoprotein. The β -region band but not the γ -region band observed after immunoelectrophoresis (Fig 2) stained positively for lipoprotein when Sudan black B was used.

Gel diffusion of untreated human serum with anti-serum to human β -lipoprotein and with stem extract resulted in a line of identity which demonstrated that the large distinct band of precipitation contained human β -lipoprotein.

The immunoelectrophoretic band observed in the γ -region was not identifiable when reacted in gel with specific antibody against human IgG, IgA, or IgM (data not shown).

The plant agent responsible for precipitation of the β -lipoprotein and γ -globulin was suspected of being a cotton plant polyphenolic tannin. To test this hypothesis, identical extracts of cotton stems and cardroom cotton dust were prepared, but were first mixed with polyvinylpyrrolidone (PVP) to remove plant tannins before the extract was concentrated.^{7,8} Comparison of the PVP-treated and non-PVP-treated extracts by gel diffusion with normal sera from active cotton textile workers revealed that the PVP-treated extracts did not precipitate the two serum proteins in a pseudoimmune reaction.

DISCUSSION

We were unable to demonstrate true precipitating antibodies to extracts of cotton bract, carpels, stems, leaves, immature cotton lint, or cardroom cotton dust in the sera of 59 cotton textile workers.

The identification of the agent responsible for the pseudoimmune precipitation reaction was aided by the finding that an extract of tea leaves possessed the same characteristic. Tea and other plant leaves have been shown to contain large quantities of plant tannins, which are known to interact with and to complex proteins of several biochemical types.^{7,8} Polyvinylpyrrolidone (PVP) was used in this study because it is a specific insoluble adsorbent for polyphenolic tannins.⁷ After adsorption of the stem, cardroom cotton dust, and tea extracts with PVP, these extracts no longer precipitated serum proteins. From these observations, it was concluded that a plant polyphenolic tannin was probably the agent responsible for the pseudoimmune precipitation reaction. Whether this is a single tannin or a heterogeneous group of tannins is unknown.

Only serum proteins that migrated electrophoretically in the beta and gamma region were precipitated by the plant tannin, in a manner similar on electrophoresis to the precipitation of α -, β -, and γ -region serum proteins by phytohemagglutinin.⁹ We identified the β -region protein as human β -lipoprotein. However, we were unable to identify the specific γ -region proteins involved, even with the use of monospecific antisera. The most probable reason is that the plant tannin complexed with the immunoglobulins (perhaps with the Fc region) such that the immunoglobulin class-specific antigenic determinants were masked.

In conclusion, we identified the material in extracts of cotton stems and cardroom cotton dust that precipitate normal human β - and γ -globulins in a pseudoimmune fashion. We further identified the β -globulin as human β -lipoprotein. The possible role of the pseudo-

immune precipitation reaction in the pathogenesis of byssinosis is not defined by the present study, nor is it clear whether this reaction occurs *in vivo*. If it did occur, one could speculate that aggregation of γ -globulins, specifically or in a pseudoimmune manner, could initiate several biological reactions via complement activation.¹¹ Further examination of that reaction, as well as the role of β -lipoprotein in the pathogenesis of byssinosis, would be necessary to verify our suggestion. In any case, the *in vitro* observation of this pseudoimmune reaction emphasizes the problems associated with laboratory investigations of the immunologic aspects of byssinosis.

SUMMARY

The pathogenesis of byssinosis has been attributed to several different immunopathologic mechanisms, including a type III (immune complex) pulmonary injury. To further examine this type III theory, sera (Monday preshift) from 59 cotton textile workers were examined by gel diffusion and counterimmunoelectrophoresis for precipitating antibodies to aqueous extracts of cotton bract, carpels, stems, leaves, immature cotton lint, and cardroom cotton dust. Sera were also collected from 35 nonexposed normal volunteers and examined similarly. No true precipitating antibodies to these extracts could be detected in any of the control or worker serum samples.

The aqueous extracts of cardroom cotton dust and cotton stems were found to contain naturally occurring components that precipitated (in agarose gel) β -lipoprotein and γ -globulins (mostly IgG) in a nonimmunologic manner. Sera from normal human controls and cotton textile workers all produced identical patterns of reaction with these two extracts. Treatment of these extracts with polyvinylpyrrolidone, a specific insoluble adsorbent for polyphenolic tannins, eliminated this pseudoimmune reaction.

Although the role this pseudoimmune reaction may play in the pathogenesis of byssinosis is still unknown, it demonstrates the problems associated with laboratory-based investigations of the immunologic aspects of byssinosis.

The Relevance of Lacinilene C7-Methyl Ether to Byssinosis*

Experience with Natural Product from Bracts and Synthetic Chemical in Leukocyte Recruitment

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Cotton dust, when inhaled by textile workers, causes airway narrowing manifested by a reduction in FEV₁, chest tightness, shortness of breath,¹⁻³ peripheral leukocytosis consisting mainly of polymorphonuclear leukocytes, small elevations of temperature, and leukocyte recruitment to air passages.⁴ The time course of these phenomena suggests that they are related.

In hamsters, leukocytes are recruited to airways by cotton dust and extracts^{5,6} modeling faithfully in regard the time course, the response of worker panels in the model card room, or in the cross-sectional studies of "Monday morning asthma" in textile workers.¹ Fluorescent materials from such extracts attract leukocytes in modified Boyden chambers.⁷ They have been identified as lacinilene⁸ and synthesized.^{9,10} This article reports