The accentuation of previously existing changes, as illustrated by Figure 3, could be better explained if penicillamine acted by reducing residual –S–S– bonds holding together the fragments resulting from previous proteolysis, as suggested in relation to papain digestion of IgG[19].

The high susceptibility of IgG3 proteins to penicillamine treatment and to denaturation during storage could be a result of a loose tertiary structure. Under those circumstances the protein molecules would be more easily unfoldable and would become more readily susceptible to degradation.

Penicillamine treatment, by revealing these differences, appears as a promising system for the identification of IgG3 monoclonal proteins[20,21].

Résumé. Il est bien connu que les immunoglobulines IgG maintenues longtemps à des températures voisines de 0 °C sont dénaturées. L'addition de pénicillamine au sérum peut accentuer cette dénaturation. Dans ces conditions, les immunoglobulines du type IgG3 sont les plus sensibles et le traitement par la pénicillamine peut être utilisé pour les identifier.

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Immunological Adjuvants IV. Relationship between Adjuvant Activity and Antigenicity in Mycobacterial Adjuvant

BRAUN et al.[1,2] postulate that an antigenicity of the lipopolysaccharide (endotoxin) of Gram-negative bacteria may play an important role in its adjuvant activity. They also inferred a similar mechanism operating in relation to mycobacterial adjuvants[2]. SCHIERMAN and MCBRIDE[3] reported the necessity of antigenicity for an adjuvant located in a chicken erythrocytes.

Lipopolysaccharide of tubercle bacilli designated as wax D[4] behaves as an adjuvant[5]. Endotoxin and wax D have many properties in common[4,6]. We now report, however, that antigenicity of wax D appears not to be correlated with its adjuvant activity.

Materials and methods. We have recently isolated a 'pure' wax D containing little or no tuberculin-sensitizing antigen from H37Ra strain cultivated 4 weeks on Sauton medium. A subfraction AD6 was prepared by acetylation from the 'pure' wax D as described previously[7].

Guinea-pigs were sensitized with a single injection into the right hind foot pad of 0.2 ml of the water-in-oil emulsion containing 1 mg of usual wax D isolated from the strain H37Ra with or without 1 mg of twice-recrystallized ovalbumin (Sigma Chemical Co. USA) or 3 mg of the AD6 subfraction with or without 1 mg of the ovalbumin. The emulsion was prepared by mixing 0.7 ml of a phosphate buffered saline solution, 0.1 ml of Arlacel A and 0.6 ml of Drackeol. Zero, 5, 10, 15, 21 and 28 days after the sensitization, animals were bled for the assay of antibody titers and 35 days after the sensitization skin and corneal tests were performed. Antibody titers were measured by passive hemagglutinations using sheep erythrocytes sensitized either by ovalbumin or tuberculo-protein π^8 according to Persellin's technique[9], or by the water-soluble portion of wax D, as described previously[10]. Figure 2 shows data obtained on day 35 only.

Results and discussion. As shown in Figures 1, 2 and Table, the group I which had received usual wax D alone showed positive corneal and skin reactions to the tuberculo-protein and produced antibodies against the tuberculo-protein as well as to the water-soluble portion of wax D, indicating that usual wax D has antigenicities. When the antigen ovalbumin was added to this wax D in the sensitizing emulsion, animals exhibited high levels of corneal and delayed type skin reactions to the tuberculo-protein and ovalbumin and produced high titers of antibodies to ovalbumin, as well as to tuberculo-protein and the water-soluble portion (group II).

On the contrary, as shown in Figures 1, 2 and Table, corneal and skin reactions of the group III injected with only AD6 were completely negative and no antibody production occurred. Addition of ovalbumin to this AD6 in the sensitizing emulsion resulted in a marked antibody production to ovalbumin and in high levels of corneal and delayed type skin reactions to ovalbumin (group IV). Again, no immunological response was detected to the antigens associated with the usual wax D or tubercle bacilli. Guinea-pigs in group V which had received ovalbumin alone exhibited the positive but only immediate skin reaction.

In other experiments guinea-pigs which had received even greater amounts of AD6 (5 and 10 mg per animal)

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Corneal reaction of each group as tested by ovalbumin and tuberculo-protein π

Group	Test antigens	
	Ovalbumin[a]	Tuberculo-protein π[b]
I (Wax D)	—, —, —, —, —	+, +, +, +, —
II (Wax D + Ovalbumin)	+++, +++, ++	+, +, +
III (AD6)	—, —, —, —, —	—, —, —, —, —
IV (AD6 + Ovalbumin)	+++, +++, ++, ++, ++, +	—, —, —, —, —, —
V (Ovalbumin)	—, —, —, —	—, —, —, —

[a] Phosphate buffered saline (pH 7.2) solution (20 mg/ml) was used. [b] Phosphate buffered saline (pH 7.2) solution (4 mg/ml) was used.

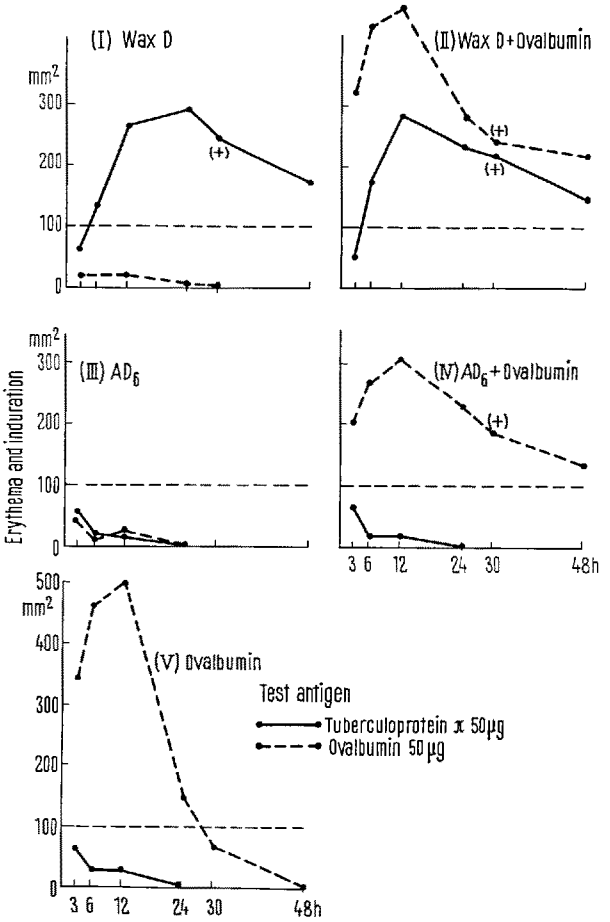

Fig. 1. Skin reactions to ovalbumin and to tuberculo-protein π ead in the course of time. (+) indicates the presence of induration.

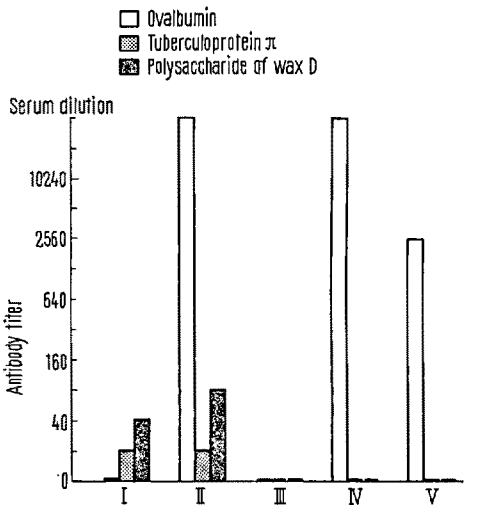

Fig. 2. The levels of serum antibody to ovalbumin, to tuberculo-protein π and to the polysaccharide portion of wax D assayed on the 35th day following sensitization.

or similar acetylated subfraction of the 'pure' wax D, hexane-insoluble AD[7] (20 mg per animal), in a water-in-oil emulsion showed no immunological response to the tuberculo-protein and to the polysaccharide antigenic determinants. Injection of acetylated wax D into rabbits caused no antibody formation which agglutinate wax D or acetylated wax D particles, suggesting that acetylated wax D may contain no other antigenic materials causing antibody formation in a significant amount[11]. That AD6 in the doses of 0.2–10 mg possesses adjuvant activity was repeatedly confirmed in several different test systems[11,12].

These data indicate that the usual wax D possesses adjuvant activity and at least two kinds of antigenicity, while AD6 of the 'pure' wax D possesses the adjuvant activity but lacks the antigenicity.

The data also suggest that there may be no relationship in wax D between the antigenicity and adjuvant activity and, therefore, that the mechanism of the adjuvant action of wax D may be different from that of endotoxin, though a possible presence of a very small amount of other unknown antigenic material (s) cannot be completely excluded[13].

Zusammenfassung. Während sich bei Endotoxinen der Adjuvanseffekt proportional zur Antigenität verhält, ist diese Beziehung bei dem aus Tuberkelbazillen isolierten Wachs D nicht der Fall.

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