Vitamin A Adjuvant with *Arizona hinshawii* Bacterin

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Received for publication 27 July 1972

Vitamin A provided adjuvant effect for *Arizona hinshawii* bacterin in protection of *Coturnix* quail against severe challenge.

*Arizona hinshawii* bacterins have not been satisfactory for immunization of turkeys, even though a variety of adjuvants (including aluminum hydroxide, alginate, Freund incomplete, chrome alum, and aluminum alum) have been tried (3). In screening for the efficacy of the various adjuvants by using mouse protection tests, aluminum hydroxide was the most effective in preventing death. However, turkeys vaccinated with this adjuvant bacterin had higher susceptibility to challenge than did unimmunized controls. It was demonstrated that less protection was offered with greater amounts of endotoxin (3). Accordingly, the sensitization was attributed to the endotoxin of the organism. The most effective adjuvant in the bacterin for use in turkeys was provided with a slow chrome alum treatment of the organisms. This adjuvant probably prevented the release of endotoxin by binding the toxin through mordant activity.

Vitamin A is antagonistic to endotoxin (2). Rabbits inoculated intracutaneously with a lipopolysaccharide from *Salmonella abortus equi* had delayed and reduced reactions when pretreated with four doses of vitamin A. The vitamin also has adjuvant properties (1), as demonstrated with mice inoculated with bovine gamma globulin. However, in studies by Steinberg et al. (4), none of the depot-type or surface-active adjuvants, including vitamin A, increased the circulating antibody response of chickens to human serum albumin.

Results of several experiments in which turkeys received bacterins prepared with *Arizona hinshawii* and a series of different adjuvants, or vitamin A, were inconclusive. In contrast, preliminary studies with *Coturnix* quail (*Coturnix coturnix japonica*) as the test animal gave promising results. Therefore, an experiment was designed to measure the adjuvanticity of vitamin A with *A. hinshawii* bacterin in *Coturnix*.

Adult *Coturnix* quail were used in the experiment. Sixteen birds, in each of four groups, were inoculated subcutaneously with 0.5 ml of the various bacterins, with and without vitamin A, as indicated in Table 1. Those quail receiving the vitamin were inoculated with 10,000 units of A acetate at a different subcutaneous site (neck region). A fifth group of quail received vitamin A only, and a sixth served as controls. Eighteen days after vaccination the immunity of the quail was tested by intravenous challenge with $5 \times 10^4$ viable *A. hinshawii* organisms. The results of this challenge are tabulated in Fig. 1. At 24 hr postchallenge there was no significant difference in mortality among birds inoculated (immunized) with whole culture, with whole culture and vitamin A, or with “de-endotoxinated” culture and vitamin A. By the 7 days postchallenge, when there were no further clinical signs in the survivors, the groups vaccinated with vitamin A bacterin were significantly protected against challenge, compared to the controls. The initial delay of death with vitamin A alone was unexpected.

Vitamin A may function as an adjuvant through two possible mechanisms: One through the ingestion of organisms by a stimulated lysosome which prepares the antigen for antibody-producing cells, and the other (suggested by Dresser [1]) through damage to lysosome membranes which stimulates cell division and, therefore, provides more cells to act on the antigen. The delay in death provided (offered) by vitamin A alone could be the result of anti-endotoxic effects of the vitamin, as demonstrated by Heilmeyer (2) with salmonella endotoxin. A large amount of endotoxin was inoculated with the challenge dose. As soon as the bacterial effect was exerted, the death
TABLE 1. Method of bacterin preparation and schedule of immunization, with and without vitamin A

| Bacterin                  | Method prepn                                      | Vitamin A |
|---------------------------|--------------------------------------------------|-----------|
| De-endotoxinized          | 1.5 liters of culture was centrifuged, and the cells were suspended in 1.0 liter of 1 M sodium chloride and stirred at room temperature. The process was repeated three times and the cells were centrifuged and suspended in saline. | +         |
| De-endotoxinized          | 1.5 liters of culture was centrifuged, and the cells were suspended in 1.0 liter of 1 M sodium chloride and stirred at room temperature. The process was repeated three times and the cells were centrifuged and suspended in saline. | -         |
| Whole culture             | 18 hr culture in Trypticase soy broth             | +         |
| Whole culture             | 18 hr culture in Trypticase soy broth             | -         |

*All bacterins were propagated in Trypticase soy broth for 18 hr and heat treated at 56 C for 30 min and preserved with 0.3% formaldehyde. Bacterin preparation contained 10⁹ Arizona hinshawii/ml.

Fig. 1. Cumulative deaths of Coturnix quail after intravenous challenge with Arizona hinshawii following immunization with various homologous bacterins, with and without vitamin A.

The initial protective effect of the whole culture might be attributable to free endotoxin released from the organism by the heating process. Possibly there was enough endotoxin to act as an adjuvant. This point is emphasized in that the bacterin in which the endotoxin was removed provided much less protection than did the whole culture.

This investigation was supported by Public Health Service General Research Support grant GRS-72-134 and the California Turkey Industry Federation.

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