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Production of Antibody Against Conjugated Dipicolinic Acid (2,6 Pyridine Dicarboxylic Acid)

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An immunological precipitation system, with rabbit dipicolinyl antibody, was developed for the purpose of evaluating the role of spore dipicolinic acid as a specific haptenic determinant.

Dipicolinic acid (DPA), a major constituent of bacterial spores, was isolated and identified by Powell (3). Numerous investigations have involved the role of DPA in the heat resistance and dormancy of bacterial spores. However, no data have been reported concerning the possible role of DPA as a specific haptenic determinant in the production of antiserum against bacterial spores. Evaluation of this possibility required the production of antibody against DPA and the development of an immunological precipitation system for the assay of this antibody. The production of antiserum immunologically specific for the hapten, DPA, was reported by J. R. Lowell, Jr., Aerojet-General Corp., El Monte, Calif. (*personal communication*). This antiserum was obtained from rabbits immunized with a conjugate of azo-coupled DPA and human gamma globulin.

The DPA immunizing antigen reported in this note was prepared by conjugating bovine serum albumin (BSA) with DPA, by use of 1-ethyl-3-(3-dimethyl-amino propyl) carbodiimide hydrochloride, by the method of Sheehan and Hess (6). The conjugate (120 mg) was administered intramuscularly to a rabbit during a 9-week period. Antigen for the beginning three weekly injections was emulsified in an equal volume of Freund's complete adjuvant (Difco).

Antigen for the assay system was prepared by conjugating DPA with a heterologous protein, bovine gamma globulin (BGG), by the mixed anhydride synthesis of Vaughn and Osato (7). The successful coupling of DPA to BSA and BGG was demonstrated by comparison of the ultraviolet absorption spectra of conjugated and native carrier proteins.

Rabbit antiserum against DPA-BSA gave a positive ring-precipitin test with DPA-BGG antigen. The specificity of this reaction is shown by the results in Table 1. There was no precipitation with BGG, but a heavy precipitate formed when antiserum was combined with BSA alone or BSA conjugated with DPA. A moderate amount of precipitate was formed with the heterologous antigen, DPA-BGG, indicating the presence of specific antibody against DPA. Further support for the specificity of dipicolinyl antibody is shown in Table 2. These results were obtained by the rapid turbidimetric assay for precipitating antibody (W. F. Vincent, *unpublished*). DPA strongly inhibited the precipitation, whereas 2,5 pyridine dicarboxylic acid and *Bacillus anthracis* spore extract gave 34 and 78% inhibition,

TABLE 1. *Specificity of antibody against DPA*[a]

Antigen	Antiserum to DPA-BSA
BSA	++++[b]
BGG	0
DPA-BSA	++++
DPA-BGG	++
DPA	0

[a] With pre-immune serum, there was no precipitation with any of the antigens.

[b] Symbols: 0, no precipitation; ++, moderate precipitation; ++++, heavy precipitation.

TABLE 2. *Inhibition of precipitation of antibody against DPA by various inhibitors*

Inhibitor[a]	Per cent inhibition
2,6 Pyridine dicarboxylic acid (DPA)	90
2,5 Pyridine dicarboxylic acid	34
Aqueous extract of *B. anthracis* spores	78

[a] Inhibitor concentration was 1.25 μmoles per ml of reaction mixture. Extract of *B. anthracis* spores was prepared by heating washed spores for 15 min in a boiling-water bath. Based on ultraviolet absorption data, the spore extract was adjusted to contain an equivalent amount of DPA.

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respectively. No immunological precipitate was formed in the qualitative ring-precipitin test when DPA-BGG was combined with rabbit antiserum against washed, autoclaved, whole *B. anthracis* spores. Stimulation of antibody to spore DPA would require that DPA be attached to an immunogenic macromolecule. Autoclaved *B. anthracis* spores used for immunization might lack sufficient bound DPA to produce a significant immune response in the rabbit. DPA is released from spores during germination (4) or when autoclaved (2). Rode and Foster (5) showed significant differences in the rate at which DPA was released from spores at various temperatures. El-Bisi et al. (1) suggested the existence of DPA in more than one structural form. In the spore, it may be loosely bound and easily released by mild heating, or it may be strongly bound and require extreme heat to be released.

Our results demonstrated the production of specific dipicolinyl antibody in the rabbit after immunization with a DPA-BSA conjugate. Dipicolinyl antibody was not detected by the ring-precipitin method in sera from rabbits immunized with autoclaved *B. anthracis* spores.

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