SEROLOGICAL GROUPING OF THE PATHOGENIC CLOSTRIDIA

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ABSTRACT

ELLNER, PAUL D. (University of Vermont, Burlington), AND STANLEY S. GREEN. Serological grouping of the pathogenic clostridia. J. Bacteriol. **86**:1098–1100. 1963.—By use of the soluble antigens of ten species of pathogenic clostridia and specific globulins, it has been possible to divide these organisms into a number of groups based upon the presence or absence of precipitin lines in agar gel. A scheme for such a grouping is presented, and the utility of this method is discussed.

A previous study of the soluble antigens of the ten species of clostridia most frequently involved in human infections (Ellner and Green, 1963) disclosed that, although extreme heterogeneity occurs, a number of these species possess specific antigens, when examined by agar gel diffusion. Although cross-reactions among these species are frequent, subsequent investigations with specifically absorbed globulins in some cases have demonstrated that it is possible to identify the soluble antigens of these organisms.

In this paper, we describe a scheme by means of which these ten species may be grouped and identified by use of their soluble antigens in agar gel.

MATERIALS AND METHODS

The soluble antigens employed were the same as were used in the previous study (Ellner and Green, 1963). All methods pertaining to cultures, antigen production, immunization of animals, and agar gel diffusion have been described.

Absorption of globulins. Hydroxylapatite was prepared according to the method of Tiselius, Hjerten, and Levin (1956), and was suspended in sodium phosphate buffer (pH 6.8; $\mu = 0.01$) to about 35% (v/v). A 0.5-ml portion of this suspension was centrifuged, and the supernatant fluid was discarded. A 0.5-ml portion of soluble antigen, containing 10 to 20 mg of protein per ml was added to the packed hydroxylapatite, mixed, and placed at 5 C for 30 min. The chilled suspension was recentrifuged, and the supernatant fluid was discarded and replaced with 0.5 ml of the globulin to be absorbed. The hydroxylapatite was resuspended in the globulin by mixing, and the suspension was incubated at 37 C for 30 min. At this time, hydroxylapatite was removed from the globulin by centrifugation. In most cases, a single absorption sufficed. When necessary, however, the above process was repeated a second time.

RESULTS

Globulin prepared against *Clostridium histolyticum* reacted with the soluble antigens of all the clostridia studied and did not react with any of the soluble antigens of *Bacillus* species tested.

By use of globulin prepared against *C. novyi* type A, it was possible to separate the ten species into two groups: group I, which gave a positive precipitin reaction with *C. novyi* type A globulin, consisting of *C. sporogenes*, *C. novyi* types A and B, *C. bifermentans*, and *C. sordellii*; and group II, which failed to react with *C. novyi* type A globulin (Fig. 1).

Group I species could be further subdivided by their reaction with *C. sporogenes* globulin into subgroup IA which gave a positive reaction and consisted of *C. sporogenes* and *C. novyi* types A and B, and subgroup IB which failed to react with *C. sporogenes* globulin.

The individual members of subgroup IA could be identified by their reactions with *C. sordellii* and *C. tetani* globulins as indicated in Fig. 1.

Group II could likewise be subdivided, by the use of *C. septicum* globulin, into subgroup IIA which gave a positive reaction and consisted of *C. histolyticum*, *C. tetani*, *C. fallax*, and *C. sep-*

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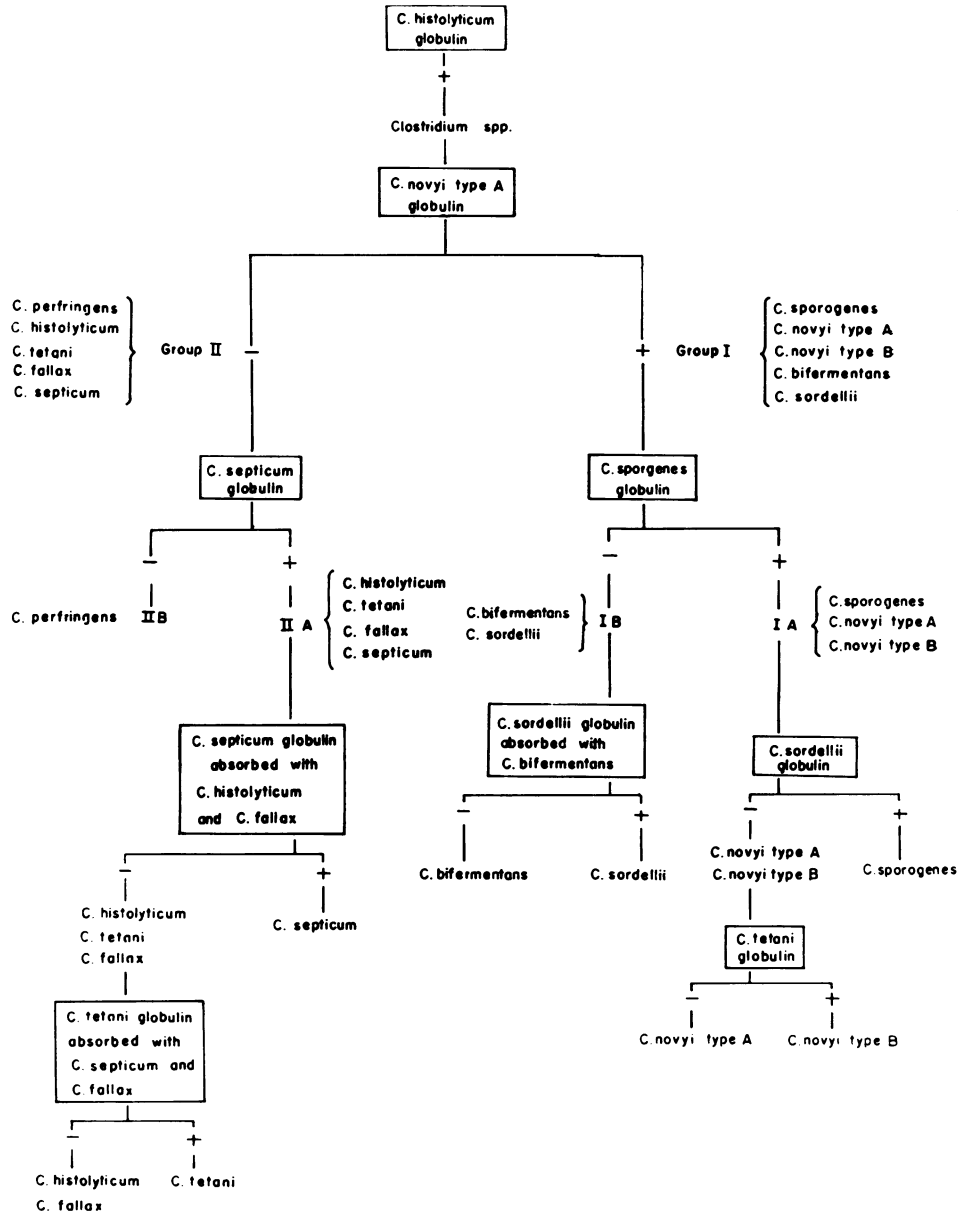

FIG. 1. *Scheme for the identification of the pathogenic clostridia. Presence of precipitin lines in agar gel is designated by +.*

ticum, and subgroup IIB consisting only of *C. perfringens* which failed to react.

The individual members of group IIA could be identified by the use of *C. septicum* globulin absorbed with *C. fallax* and *C. histolyticum*, and *C. tetani* globulin absorbed with *C. fallax* and *C. septicum* as shown in Fig. 1. It has not been possible to distinguish between *C. histolyticum* and *C. fallax*.

Discussion

As a result of these studies, it has been possible to subdivide ten species of clostridia involved in human infections into a number of groups based

upon the precipitin reaction of the soluble antigens of these organisms with specific globulins.

At the present time, the utility of such a serological grouping and identification for the general diagnostic laboratory is questionable, since the time involved in the preparation of soluble antigens and antisera is considerable, and would far exceed the time required for identification by conventional biochemical means. It may be possible, however, to utilize this scheme for the rapid identification of clostridia in wound, uterine, or other exudates if sufficient soluble antigen is present in these materials. This remains to be determined by future studies.

LITERATURE CITED

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