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# STUDY OF BRUCELLA POLYSACCHARID ANTIGENES

## II. IMMUNOLOGICAL BEHAVIOR*

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The antigenic constitution and the immunological properties of isolated components of microorganisms of the genus Brucella have been investigated by various authors. An examination of the various descriptions in the literature on the subject, however, shows disagreements, which indicates that the subject is still in the developmental phase.

Wilson & Miles (1932) postulated the presence of two antigens in each one of the three species (Brucella abortus, Brucella melitenis, and Brucella suis), designating them as A and M. Olitzki and Gurevitch (1933) indicated two specific antigens, A for Br. abortus and M for Br. melitensis, and one non-specific antigene G common to all species. Renoux and Mahaffey (1955) describe a more complex antigenic structure and cite four antigens: A, M, Z, and r present in the smooth specimens of the various species. In a recent revision of a series of experiments, Olitzki (1960) cites among other facts, one obtained by precipitation tests in gel, by which extracts of brucella obtained by ultra-sonic methods formed regularly, when tested against immune serum, six lines of precipitation, one additional line (7 or D) appearing in the presence of hyperimmune serum. Furthermore, with sera obtained after injection of antigen emulsified in Freund adjuvant, at least ten lines of precipitation could be differentiated. It was observed, in the meantime, that the antigens were not species-specific, being absorbed, therefore, by anti-sera of three species. R. J. and S. A. Carrere (1958) obtained with "endo-antigens" isolated ultrasonically or by trituration of

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melitensis serum and only one of the specimens produced a precipitation reaction with anti-sera of the three species.

Mosimann (1949) prepared with Br. abortus an aqueous extract and various fractions (polysaccharidic, glycolipidic, nucleoproteinaceous, and polypeptidic) that were investigated as regards toxicity, antigenicity, and sensitizing properties. They found it to be the atoxic polysacchardidic fraction, behaving immunologically as a hapten and suitable for the investigation of allergic states.

Braude (1948) observed that a fraction of hydrocarbonate nature and a complete antigen caused in humans the immediate appearance of an allergic reaction, the reaction being conditioned by the presence of precipitation in the serum. This type of reaction could be transferred passively by non-allergic persons by means of the Praunitz-Kustner technique.

Fust et al. (1949) and Godluck (1954) also prepared polysaccharids that were used in tests for allergy.

Parnas and Mierzejewski (1957) and Parnas et al. (1959) isolated from Brucella various fractions, fraction III being of a polysaccharidic nature. This fraction occurs as a hapten, causing in tests for allergy less intensity in the reaction than other fractions that were studied.

Gary et al. (1958), using ethylene, ether, and sodium hydroxide, isolated a polysaccharid that had the immunological properties of a hapten.

Barber et al. (1962), using phenol and sodium desoxicolate, isolated a polysaccharidic fraction linked to nucleoprotein that would correspond to the Wilson and Miles antigen (1932). This substance causes a single precipitation line in agar gel, having, however, hapten characteristics and allergic properties.

In the present work, we analyse the polysaccharid isolated by the Fuller technique starting with Br. abortus B99, in a smooth state, as to its immunclogical behavior in vivo and in vitro, making use among other methods of precipitation in agar gel, immunoelectrophoresis, and passive cutaneous anaphylaxia.

These data are also compared with those previously observed in relation to the tubercle polysaccharid isolated by the same technique (M. A. Fuks, 1959), because of the occurrence of cross reactions between the two groups of microorganisms (Serpa and Fuks, 1960).

Polysaccharidic antigen: Isolated by the Fuller technique as referred to in a previous work (Serpa and Fuks, 1960).

Sora employed: (a) hyperimmune bovine sera and from infected cattle in which the possibility of the occurrence of a tuberculous disease was excluded; (b) immune sora from rabbits obtained after immunization with Br. abortus B99 #2 of McFarland, killed by heat, making use of the veins, subcutaneously and intracutaneously to a total of ten injections in each case. The animals were bled after a lapse of ten days after the last injection.

Immunological behavior "in vivo": (a) Immunization of animals: rabbits were injected intradermically with an isotonic solution of polysaccharidic antigen in doses of 0.05, 0.1, 0.3, 0.6, and 1.0 milligrams at intervals of three days between each dose. After a lapse of seven days, the animals were bled and injected anew with doses of 1.0 and 2.0 milligrams

of the polysaccharidic antigen emulsified in incomplete Freund adjuvant. At the end of seven days, the animals were bled again.

During the immunization, determinations were made of the temperature, weight, leucocytic formula, electrophoretic profile, and of the occurrence of any local reaction. (b) Sensitivity reactions: The reaction of passive cutaneous anaphylaxia was made according to the technique described by Ovary (1959) using, however, 0.2 milliliters of hyperimmune serum and 2.0 milligrams of polysaccharidic antigen in aqueous solution.

The allergic reactions were observed making use of polysacchardic antigen at 1:1000 (0.1 milliliters intradermically).

Immunological behavior "in vitro": (a) Precipitation in a capillary tube: Using the sera described above, precipitation reactions were carried out according to the Uhlenhuth technique; (b) Precipitation in agar gel: Using bovine hyperimmune bovine serum and a polysaccharidic antigen solution of Brucella at 0.1%, according to the technique of Ouchterlony; (c) Immunoelectrophoresis in agar gel: Carried out with the use of a tampon of veronal of pH 8.6, mu = 0.1, and a current of 0.8 milliamperes per centimeter. After a 12-hour migration period, the hyperimmune bovine serum was added, the reading being taken after the lapse of 24 hours.

## RESULTS

Analysing the data obtained, it may be noted that in precipitation tests made in a capillary tube with hyperimmune bovine serum, the polysaccharid reacts strongly up to a dilution of 1:1 million, weaker reactions being obtained with higher dilutions.

Fig. 1 - Precipitation reaction in agar gel using the homologous polysaccharide system of Brucella and hyperimmune bovine sera. (1) isotonic saline solution; (2) and (3) hyperimmune bovine sera; (4) normal bovine serum; (5) polysaccharidic antigen of Brucella 0.1%.

By means of precipitation tests in agar gel using the Ouchterlony technique, a single precipitation line was obtained (Fig. 1), which demonstrates the homogeneity of the polysaccharidic fraction, a fact that is confirmed by immunoelectrophoresis.

In the tests made with immunized rabbit sera with a polysaccharidic solution or after emulsion in Freund adjuvant, we did not obtain precipitation reactions in any of the dilutions that were used. Positive reactions were obtained, however, with immunized animal sera with the Brucella suspension.

We found also in the animals injected with a polysaccharidic fraction the absence of local reaction or substantial alterations of temperature, weight leucocytic formula, or electrophoretic profile of the sera.

Fig. 2 - Reaction of passive cutaneous anaphylaxia in guinea pigs using the homologous polysaccharidic system of Brucella and hyperimmune bovine serum.

In the animals previously immunized with Brucella, we did not obtain reactions of an allergic type when they were injected intradermically with a polysaccharidic antigen. On the other hand, making use of passive cutaneous anaphylaxis in guinea pigs, we found that the polysaccharide produced positive reactions (Fig. 2).

## DISCUSSION

As was noted in the introductory portion of this work, the antigenic constitution of Brucella still presents controversial points.

In relation to the polysaccharidic fractions, it is noted that, in general, these fractions are sorologically active in precipitation tests (Favilli and Biancalani, 1932, 1934; Topping, 1934; Higginbotham and Healtman, 1936; Libby and Joyner, 1941; Morales Otero and Pomales-Lebron, 1943; Leon and Cano, 1958; Barber et al., 1962). Our observations confirm these data, since the hydrocarbonate fraction in the experiments was found to be highly active in tests of this nature.

As for the behavior in the test animals, most authors maintain that the purified substance is incapable of stimulating the formation of antibodies, being, however, a hapten. At the same time, some of the investigators were able to isolate polysaccharidic fractions of Brucella with the characteristics of complete antigens (Libby and Joyner, 1941; Braude, 1948). Leon and Cano (1958) observed that the polysaccharidic fraction isolated by the "biodialase" method came, when absorbed to aluminum phosphate, to show the behavior of a complete antigen. In regard to this aspect, our results allowed the conclusion that the isolated substance is a complex hapten.

The utilization of polysaccharidic antigens of Brucella in the investigation of reactions of an allergic nature, cutaneous, has been referred to by various authors (Libby and Joyner, 1941; Graude, 1948; Fust, 1949; Mosimann, 1949; Godluck, 1954; Parnas and Mierzejewski, 1957; Parnas et al, 1959). It was not possible for us, however, to detect any reaction of this type in the animals immunized with brucela.

Considering that the antigens form intracellular complexes, it would not be unreasonable to admit that in the experiments in which the immunological activity has been related to fractions of a non-hydrocarbonate nature (Huston et al., 1934; Morales Otero and Gonzales, 1938; Pennel and Huddleson, 1938, a, b), there may be contamination of the material by polysaccharidic antigens that do not reveal themselves through conventional methods and that may be responsible for the occurrence of antigen-antibody reactions once the polysaccharid isolated by us as well as those described in other investigations present high sorological activity, as already referred to.

Braude (1948) observed also that the transfer is possible of circulating antibodies in bruceloses lending themselves to the Prausnitz-Kustner test. As was seen, the polysaccharid under discussion at present showed itself to be active in tests of passive cutaneous anaphylaxia in guinea pigs. Taking into account the sensitivity of the test of passive cutaneous anaphylaxis, Braude's discovery is confirmed indirectly, demonstrating once more in this fashion the sorological reactivity of polysaccharidic antigens extracted from Brucella.

Finally, comparing the results just obtained with those previously observed by one of us (Fuks, 1959) in relation to the polysaccharidic antigen isolated by the same technique, starting with Mycobacterium bovis, sample BCG, the following common points are found: they are immunologically active in precipitation tests, they show the behavior characteristics of a complex hapten, they are free from toxicity, and they are incapable of revealing allergic states.

SUMMARY

In the present investigation, observations were carried out on the immunological behavior in vivo and in vitro of the polysaccharidic fraction isolated by the Fuller technique, starting with Br. abortus B99.

It was found that the fraction being investigated was a complex hapten, atoxic to rabbits and sorologically active in precipitation tests. In animals that had been previously innoculated with Brucella, the injection of the polysaccharidic antigen did not induce reactions of an allergic nature, although it did give a passive cutaneous anaphylaxis test. At the same time, its innoculation did not produce any change of temperature, weight, leucocytic formula, or electrophoretic profile of the sera of the animals.

The data obtained are compared with those previously referred to by one of the authors for a polysaccharidic fraction of the tubercle bacillus, once the cross reactions between the two antigens have been described.

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## UTILIZATION OF PRECIPITATION IN GEL AND PASSIVE CUTANEOUS ANAPHYLAXIA IN THE INVESTIGATION OF CROSS REACTIONS BETWEEN POLYSACCHARIDIC ANTIGENS OF BRUCELA AND THE TUBERCLE BACILLUS.

In a previous work (Serpa and Fuks, 1960), an investigation was made of the occurrence of cross reactions between hydrocarbonate antigens of Brucella and of Mycobacterium tuberculosis. At that time, tests were made of passive hemagglutination, the occurrence of such reactions having been observed.

In the present work, an attempt was made to study the phenomenon, utilizing methods of immunoprecipitation in gel and passive cutaneous anaphylaxia, once it had also been observed that the polysaccharidic antigens under investigation contained common "oses" (galactose and glycosamine), which may perhaps explain the reason for the occurrence of cross reactions in the two reacting fractions.

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