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Monovalent and Polyvalent Antigens for Use in the Diagnosis of Bang's Disease.*

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The agglutinability of 54 strains of *Bact. abortus* prepared as monovalent antigens and one polyvalent antigen has been studied. Bovine sera showing a large variation in agglutinin content have been employed.

In these studies there was no significant variation in the agglutinability of the monovalent antigens. Any of the cultures would have been suitable for the production of antigen for use in the diagnosis of Bang's disease. There was no advantage or disadvantage in favor of any of the monovalent antigens as compared with the polyvalent antigen. The one polyvalent antigen used was prepared from 8 strains of *Bact. abortus,* all of which were isolated from cattle. The 54 cultures varied in age of cultivation on artificial media from 13 years to a few days. Thirty-nine were isolated at the Minnesota Experiment Station. Four originated in Pennsylvania. Four were received from Connecticut, 3 from New Zealand, one (No. 80) was isolated in California, and one more was received from California that had been isolated in Oregon but cultivated in California for several years, one from New York, and one from Oregon. Of the 39 isolated at Minnesota, 32 were from cattle, 5 from horses, and 2 from swine. Those from cattle were isolated from whole milk, colostrum, placenta, vaginal discharges, foetal lung, foetal stomach contents and from enlarged joints. In one case 4 cultures were isolated from the same animal but from different tissues or fluids. One of the cultures isolated from a bovine was classified by Huddleson as *Para-abortus.*

In preliminary studies, sera from 97 cattle were used, 14 of which showed high agglutinin content, 15 showed medium agglutinin content, 24 had a very low titre, and 44 negative sera with no agglutinin content. In the later studies all of the antigens were tested with the sera of 19 cattle. Of these, one showed a high titre (above 1:5000), one had a titre of 1:1000, fifteen showed low to medium titre (1:25 to 1:250) and 2 were negative. The titre of all of these sera remained fairly constant throughout the course of the experiment with the exception of the serum that was highest in

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agglutinin content. This one showed a steady decline in its maximum titre.

Technic: In these studies all antigens were made from 4-day cultures that were grown in a 10% CO_2 atmosphere on horse serum agar standardized to pH 7.2 and washed in sterile physiological saline solution containing 0.5% phenol. All antigens were standardized to the same concentration by use of the centrifuge and nepholometer. All tests were set up within 48 hours after the antigens were prepared. Groups of 10 monovalent antigens with the stock polyvalent antigen were set up at one time. Fresh sera were always used. Observations were made at 24, 48, 72, 96, and 120 hour intervals. The tests were incubated at 37.5° C.

Results: With High Agglutinin Content Sera (Titre above 1:1000). There was considerable variation in the maximum agglutinability of the monovalent antigens, *e. g.,* occasionally a serum would agglutinate one antigen up to 1:10,000 and another antigen only 1:4000. In no case was an antigen found which failed to be agglutinated in 1:1000 dilution with high titre serum. There was no apparent relationship between speed of agglutination and maximum agglutinability of the monovalent antigens. That is, a slow agglutinating antigen often showed a higher titre after 72 to 96 hours than another antigen which agglutinated more rapidly in the lower dilutions. The maximum titre was usually not reached until the 96 hour reading, but there was practically no change between the 96 and 120 hour reading.

With Low to Medium Agglutinin Content Sera (Titre 1:25 *to* 1:500). No significant variations in agglutinability of the antigens with this group of sera were encountered. The slight variations that were recorded were not consistent and were within the limits of error of technic and observation. In no case would the diagnosis have been changed by these variations. The maximum titre was usually reached at the end of 48 hours but occasionally a slightly higher titre was observed at the 72 hour reading.

With Negative Sera. None of the antigens used in these studies showed agglutination with negative sera except in the preliminary studies where the sera from 76 supposedly negative cattle were used. In this group there were 26 sera which did show some agglutination in the 1:25 and 1:50 dilutions. Such sera were not selective but showed agglutination in these dilutions with practically all of the antigens. The slight variations were not consistent and were within the limits of error. Agglutination in these cases may or may not have been due to specific agglutinins in the sera.

Other Observations. Cattle tested with monovalent antigens, prepared from cultures that were isolated from their own tissues, did not show any difference in titre than when other antigens were used. This has been noted with 3 animals. Three monovalent antigens became contaminated with other bacteria. At this time they gave a much higher titre with all types of sera than other pure antigens. When antigens were prepared from cultures of these strains that were not contaminated, this discrepancy was not in evidence.