Studies on DNAase-Sensitive Antigens of *Brucella abortus* by Complement-Fixation.* (26818)

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Phillips, Braun, and Plescia reported that the sera of rabbits injected intravenously with a DNA-rich preparation from *Brucella abortus*, extracted by 0.5% phenol in citrate-saline, contained antibodies against DNAase-sensitive antigens(1). In addition, these sera, tested either by the Ouchterlony gel-diffusion technic(2) or the quantitative precipitin method of Heidelberger and Kendall(3), contained antibodies against other antigens(4). In a continuing study, over 100 rabbits were immunized with DNA-rich preparations obtained by the procedure described by Braun *et al.*(5). All of the immune sera contained precipitins and it was assumed, as before, that some of these precipitins were directed against DNAase-sensitive antigens. However, on further examination of these sera it was found that less than 5% of them had the desired antibodies. The irregularity with which antisera containing antibodies against DNAase-sensitive antigens were produced and its dwindling supply necessitated the use of a method requiring far less serum than the quantitative precipitin method. Consequently, the use of complement-fixation was explored and the results are summarized in this paper.

Materials and methods. Sheep blood was obtained at a local slaughter house, and sensitized sheep cells were prepared according to the procedure described by Plescia *et al.*(6). Unless otherwise indicated, veronal-saline buffer with optimal concentrations of Ca^{++} and Mg^{++}(7) was used for all washings and dilutions. Whole guinea pig serum was used as a source of complement. Individual sera were pooled, divided into small portions and stored at –45°C.

Rabbits were immunized at regular intervals over a 4-week period with different amounts of DNA-rich preparations(5), employing different routes. Those receiving intravenously 100 mg in terms of DNA gave best results.

Complement-fixation tests were done in duplicate with the reactants added in the following order: 0.2 ml of antiserum or buffer, 0.1 ml of guinea pig complement diluted 1/40 and 0.2 ml of antigen or buffer. The reaction mixture was incubated for 1 hour at room temperature and kept overnight at 4°C before adding 1×10^8 sensitized cells to each tube and placing it in a waterbath at 37°C for 1 hr with mixing to keep the cells suspended. Then 2.0 ml of buffer were added to each tube, and the mixtures were centrifuged in a Clay-Adams clinical centrifuge at 2,000 r.p.m. for 6 minutes. Optical densities of the supernatants were determined at 541 mμ, using a model B Beckman spectrophotometer.

Precipitation analysis in agar gel was done according to the procedure of Ouchterlony (2). Noble Agar (Difco) was used at a concentration of 0.85% in physiological saline. Merthiolate (0.01%) was added as a preservative. The agar gel plates were prepared and stored at 4°C about a week before using. The wells were then filled with the appropriate reactants, and plates kept at 26°C for a week to 10 days before final readings were made.

Worthington 1x crystallized pancreatic DNAase was used for enzymatic digestion of DNA with a ratio of substrate to enzyme of 10:1 and $MgSO_4$ to give a final concentration of 0.01 *M*. Reactions were carried out at 37°C for 24 hrs.

Results. Titration of guinea pig complement. The complement-fixation test was carried out with the minimum amount of guinea pig serum giving complete lysis of sensitized sheep erythrocytes. To determine this amount, different amounts of guinea pig serum were used giving extents of hemolysis ranging from 0-100%. Such a titration (Fig.

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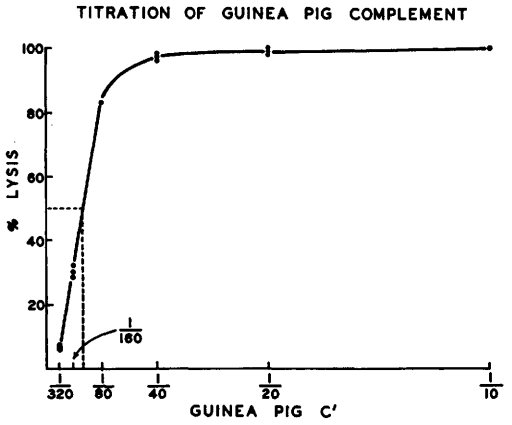

FIG. 1. Titration of guinea pig complement. Each determination was carried out in triplicate.

1) also indicated the sensitivity of the method and degree of reproducibility. On the basis of this titration, a dilution of 1:40 of guinea pig serum was used routinely. Results were reproducible throughout the entire range with a mean deviation of ± 2%. Sensitivity was uniform and greatest from 20-80%.

Analysis of antiserum for antibodies against DNAase-sensitive antigens. As an example of the antigen-antibody reactions studied, data obtained with antiserum #51 and DNA preparation #20 may be cited. The results of a gel-diffusion analysis are shown in Fig. 2. Whereas the DNAase itself showed no reaction with the antiserum, the control preparations, preincubated for 24 hours at 4°C or 37°C, showed several apparently similar bands of precipitation. Of these, the band closest to the antigen well was absent in the reaction between the antiserum and the preparation treated with DNAase at 37°C for 24 hours, indicating that some of the antibodies were directed against DNAase sensitive antigens. The reaction between antiserum #51 and DNA preparation #20 was also analyzed by complement-fixation (Table I). The antiserum was used at dilutions of

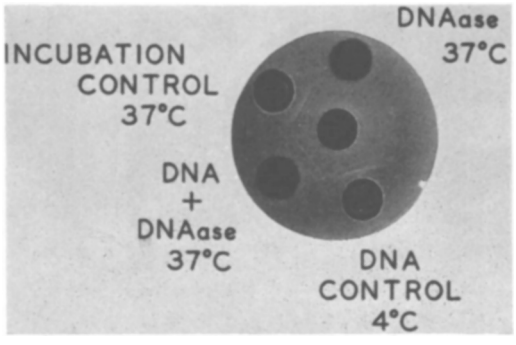

FIG. 2. Gel-diffusion analysis of rabbit anti-Brucella DNA serum #51 for antibodies against DNAase-sensitive antigens. Undiluted antiserum was placed in center well, different antigen preparations in outer well. Concentration of original DNA was .7 mg/ml.

TABLE I. Effect of DNAase on Antigens of *Brucella abortus* Tested by Complement-Fixation.

Antigen,* μg DNA	#20 preparation, 4°C Antiserum #51 1:1000	1:100	Buffer
	%		
.02	102†	99	100
.2	100	80	100
2.0	36	1	98
Buffer	100	100	100

* U.V. absorption at 260 mμ and based on purified calf thymus DNA as standard.

† Represents % cells lysed. Extent of complement fixation is inversely proportional to % of cells lysed.

Antigen, μg DNA	#20 preparation, 37°C, 24 hr Antiserum #51 1:1000	1:100	Buffer
	%		
.02	101	102	100
.2	100	74	101
2.0	51	0	99
Buffer	100	100	100

Antigen, μg DNA	#20 preparation, DNAase, 37°C, 24 hr Antiserum #51 1:1000	1:100	Buffer
	%		
.02	99	100	102
.2	102	98	101
2.0	98	1	103
Buffer	101	100	100

1:100 and 1:1000. At the higher concentration of antiserum considerably more fixation of complement occurred than at 1:1000, and DNAase treatment of the antigen appeared to have no effect. With antiserum diluted 1:1000 there was somewhat less fixation of complement but the effects of DNAase now became apparent because antibodies to antigens other than the DNAase-sensitive ones

TABLE II. Effect of DNAase on DNA Preparation #34 of *Brucella abortus* Tested by Complement-Fixation with Standard Antiserum #51.

#34 preparation, 4°C		
Antigen,* μg DNA	Antiserum #51 1:500	Buffer
	%	%
.12	100†	100
.6	99	100
1.1	71	103
2.2	33	99
Buffer	99	100

* Absorption at 260 mμ and based on purified calf thymus DNA as standard.
† % cells lysed.

#34 preparation, DNAase, 37°C, 24 hr		
Antigen, μg DNA	Antiserum #51 1:500	Buffer
	%	%
.12	100	100
.6	97	99
1.1	85	102
2.2	42	100
Buffer	100	100

had apparently been diluted out. Thus antibodies to DNAase-sensitive antigens in serum #51 were demonstrated by both precipitation in agar gel and complement-fixation.

Analysis of DNA preparations for DNAase-sensitive antigens by complement-fixation. Antiserum #51, having been shown to contain antibodies against DNAase-sensitive antigens, was used for detection of DNAase-sensitive antigens in different lots of Brucella DNA preparations. Table II shows the results with DNA preparation #34. Antiserum was diluted 1:500 instead of 1:1000 because there was essentially no fixation at 1:1000, presumably because this preparation did not contain DNAase-sensitive antigens. This was indeed the case, because at the dilution of 1:500 complement was fixed but pretreatment of the DNA preparation with DNAase had no effect. Similarly, no DNAase-sensitive antigens could be detected in this preparation by means of precipitation in agar gel.

Table III gives the results of a similar analysis employing DNA preparation 1C and antiserum #51. As in the analysis of preparation #34, the antiserum was diluted 1:500. Considerable fixation of complement occurred, and treatment of the antigen preparation with DNAase had a significant effect indicating the presence of DNAase-sensitive antigens.

Analysis of antisera for antibodies against DNAase-sensitive antigens by complement-fixation. DNA preparation 1C, shown to contain DNAase-sensitive antigens, was next used as a standard for detection of antibodies against DNAase-sensitive antigens. Two antisera, #88 and S-12, were examined. The results are shown in Tables IV and V. With antiserum #88, pre-treatment of the DNA with DNAase had little, if any, effect, in contrast to the results with antiserum S-12.

Discussion. The foregoing results clearly indicate that not all the antibodies in a given antiserum against a DNA-rich preparation are directed against DNAase-sensitive antigens. Such antibodies, when present, have been shown to be detectable by complement-fixation provided their concentrations are sufficiently great to permit the diluting out of antibodies directed against other antigens. In sera in which the fraction of antibodies against DNAase-sensitive antigens is small,

TABLE III. Effect of DNAase on DNA Preparation #1C of *Brucella abortus* Tested by Complement-Fixation.

#1C preparation, 4°C		
Antigen,* μg DNA	Antiserum #51 1:500	Buffer
	%	%
.05	99†	100
.1	95	101
.5	81	101
1.0	32	102
2.0	18	102
Buffer	100	100

* U.V. absorption at 260 mμ and based on purified calf thymus DNA as standard.
† % cell lysis.

#1C preparation, DNAase, 37°C, 24 hr		
Antigen, μg DNA	Antiserum #51 1:500	Buffer
	%	%
.05	101	101
.1	98	102
.5	97	103
1.0	88	102
2.0	60	102
Buffer	100	100

complement-fixation may still be used; however, it would be necessary first to absorb the antiserum with antigens other than those sensitive to DNAase. Such tests are now in progress.

The foregoing results also demonstrate the existence of considerable variation among different DNA preparations and among different antisera produced against such preparations. Having obtained antiserum #51, shown to contain antibodies against DNAase-sensitive antigens, it became possible to pretest DNA preparations for presence of DNAase-sensitive antigens. This resulted in the selection of an immunizing preparation containing an appreciable amount of DNAase-sensitive antigens, and when rabbits were injected with this material, over 50% of them produced antibodies against DNAase-sensitive antigens. Previously, when immunizing preparations were not selected for DNAase-sensitive antigens, less than 5% of the animals produced sera that contained the desired antibodies. However, additional controlled experiments will be needed before it can be concluded that production of antibodies to DNAase-sensitive antigens depends upon the detectable presence of such antigens in the immunizing preparation.

TABLE IV. Testing of Antiserum for Antibodies against DNAase-Sensitive Antigens by Complement-Fixation with Preparation #1C Known to Contain DNAase-Sensitive Antigens.

#1C preparation, 4°C

Antigen,* μg DNA	Antiserum #88 1:500	Buffer
	%	%
.05	99†	102
.1	97	100
.5	88	101
1.0	76	101
2.0	65	101
4.0	36	102
8.0	47	102
Buffer	99	100

* U.V. absorption at 260 mμ and based on purified calf thymus DNA as standard.
† % lysis.

#1C preparation, DNAase, 37°C, 24 hr

Antigen, μg DNA	Antiserum #88 1:500	Buffer
	%	%
.05	99	102
.1	100	100
.5	86	98
1.0	75	102
2.0	54	102
4.0	42	102
8.0	54	102
Buffer	100	100

TABLE V. Testing of Antiserum for Antibodies against DNAase-Sensitive Antigens by Complement Fixation with Preparation #1C Known to Contain DNAase-Sensitive Antigens.

#1C preparation, 4°C

Antigen,* μg DNA	Antiserum S-12 1:1000	1:500	Buffer
	%		
.5	72†	58	102
1.0	43	13	102
2.0	21	0	99
Buffer	100	100	100

* U.V. absorption at 260 mμ and based on purified calf thymus DNA as standard.
† % cells lysed.

#1C preparation, DNAase, 37°C, 24 hr

Antigen, μg DNA	Antiserum S-12 1:1000	1:500	Buffer
	%		
.5	93	96	100
1.0	89	90	102
2.0	85	24	100
Buffer	100	100	100

Summary. Complement-fixation has proved to be a rapid, sensitive method of testing DNA preparations obtained from *Brucella abortus* for DNAase-sensitive antigens, and similarly, for analyzing antisera for possible antibodies against DNAase-sensitive antigens. Considerable variation in such antigens has been found among various DNA-rich preparations isolated from the same *Brucella abortus* strain and among antisera prepared against such preparations. The percentage of rabbits that yielded antibodies against DNAase-sensitive antigens following immunization with a preparation selected for DNAase-sensitive antigens was greater than that obtained in prior studies with unselected immunizing preparations.

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A Modified Total Pancreatic Fistula.* (26819)

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The difficulty of maintaining dogs with a total pancreatic fistula in good electrolytic and nutritional balance is well-known(1,2). During our experiments a total fistula was required for various 24-hour collection periods and for spot testing. However, at other times there was no contraindication to returning the pancreatic juice to the animal and thus circumventing these difficulties. After the following preparation is functioning, no intravenous fluids or oral pancreas are required for maintenance:

The pancreatic ducts are located and all except the accessory duct are ligated and transected. The duodenum is transected 2-3 cm on either side of the accessory duct. A steel cannula is inserted into the duodenum containing the duct and this pancreatico-duodenal pouch is then closed. The transected duodenum is now anastomosed in continuity. A second cannula is placed in the distal duodenum, wrapped with omentum and brought through the body wall to the outside about 6 cm from the cannula of the pancreatico-duodenal pouch. A stiff-walled rubber tube is then used several days later to connect the 2 cannulae (Fig. 1).

Obstruction of the duodenal cannula by food can be controlled by doing a gastro-jejunostomy, inserting the cannula into the ileum, or constructing a one-way rubber flutter valve from a Penrose drain. The external portion of the fistula is protected by a cloth bag to prevent the dog chewing the tube.

If the pancreatico-duodenal fistula and gastro-jejunostomy are to be done, operative mortality can be reduced by doing the operation in 2 stages.

During collection periods a balloon or foot-ball-bladder on a threaded connecting tube is screwed into the cannula of the pancreatico-duodenal pouch. The duodenal cannula is blocked by a threaded plug.

Dogs can be maintained without difficulty for several months (or longer) on routine kennel diet and care. Occasional inspections must be made to remove concretions of precipitated salts from the lumen of the steel cannula. The

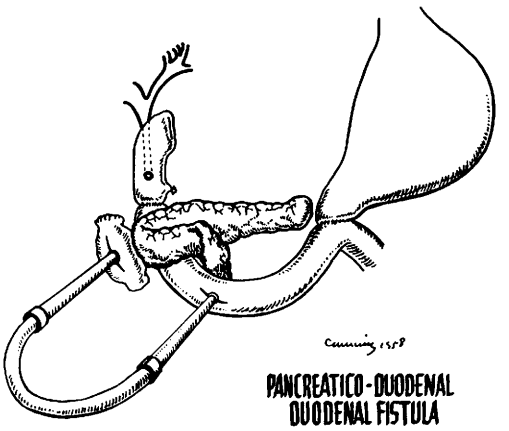

FIG. 1. A diagrammatic view of the preparation. During collection periods a balloon is attached to the pancreatic cannula and the duodenal cannula is capped.

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