JOURNAL OF BACTERIOLOGY, May 1968, p. 1580-1586
Vol. 95, No. 5

Passive Cutaneous Anaphylaxis with Antigens from *Coxiella burneti*

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Received for publication 11 January 1968

Passive cutaneous anaphylaxis (PCA) was produced in guinea pigs sensitized with guinea pig *Coxiella burneti* phase I–II antiserum and challenged with dimethylsulfoxide- or trichloroacetic acid-soluble extracts from phase I cells. The PCA reaction could not be induced by whole or mechanically disrupted phase I or phase II *C. burneti* cells or by extracted cells or extracts of phase II cells. The antibody responsible for PCA was in the $7S\gamma_1$ (fast γ) globulin. Sensitization of the skin by $7S\gamma_1$ antibody could be blocked nonspecifically by $7S\gamma_1$ globulin from normal serum or from phase II antiserum. The $7S\gamma_2$ (slow γ) globulin antibody inhibited the reaction specifically. Some antiserum pools containing high agglutinin and complement-fixing titers to phase I *C. burneti* cells failed to initiate the PCA reaction, perhaps due to an imbalanced ratio of γ_1 to γ_2 specific globulins or to an imbalance in the ratio of specific to nonspecific γ_1 globulins. Agglutinins to phase I cells were found in both γ_1 and γ_2 antibody globulins. Complement-fixing antibodies were found in the γ_2 globulin fraction.

Passive cutaneous anaphylaxis (PCA) has been reported mainly in systems involving soluble antigens (10). However, Ovary (10) and Owen et al. (12) showed that injection of some particulate antigens can also produce a PCA reaction. We have not been able to produce PCA reactions in guinea pigs with a challenge of either whole or mechanically broken *Coxiella burneti* cells. The extraction of soluble antigen from phase I *C. burneti* with dimethylsulfoxide (DMSO; 6) or trichloroacetic acid (2) furnished soluble antigens which, when used as challenge substances, produced PCA in guinea pigs sensitized with antisera to phase I *C. burneti*.

Factors which affect the induction of this PCA reaction in guinea pigs and the distribution of PCA antibodies, complement-fixing (CF) antibodies, and agglutinins in the immune globulins were studied. In addition, the influence of rickettsial phase (13) on some serological and immunological reactions is reported.

MATERIALS AND METHODS

Preparation of soluble antigen. Either live or Formalin-killed, purified suspensions of *C. burneti* (fifth egg passage of Ohio 314 strain, or second egg passage of the Nine Mile strain) were employed. The rickettsial cells were purified by methods previously described (5, 7). Purified suspensions were centrifuged at 20,000 × *g* for 20 min, resuspended to a concentration of 1 mg per ml in DMSO (Crown Zellerbach Corp., San Francisco, Calif.), and extracted at 50 C with constant agitation for 24 hr. After centrifugation at 20,000 × *g* for 20 min in glass centrifuge tubes, the extract was dialyzed against running tap water for 24 hr and finally against distilled water or saline for 24 hr. The dialysis tubing was washed with 50% DMSO in distilled water for several hours and was rinsed thoroughly with distilled water prior to use. Trichloroacetic acid extracts were prepared by methods previously described (2). Routine preparations of extracts were pervaporated to desired concentrations calculated from dry weight determinations on small samples of each preparation. Agar-gel immunodiffusion, performed by the method of Ouchterlony (8), with these extracts and various antisera containing phase I antibodies [pool 5 (phase I–II) and Q62–67 (phase I–II)] gave lines of identity, and there was no precipitin reaction with phase II antisera (Fig. 1). The trichloroacetic acid extract usually gave two bands with phase I–II antiserum, as shown by Anacker et al. (1).

PCA. The guinea pig antisera used were prepared by procedures indicated in Table 1. The test for PCA was performed by the method of Ovary (9, 10). White guinea pigs (250 to 300 g, Hartley strain) were injected intradermally (id), on a shaved area of the back, with 0.1-ml volumes of antibody fractions. The challenge was given at various times after id inoculation by injection into the saphenous vein of 1 ml of 0.15 M NaCl containing about 1 mg of soluble antigen and 0.5% Evans blue dye. The reactions were read 30 min to 1 hr after challenge.

Immunoelectrophoresis. Immunoelectrophoresis was performed as described by Grabar and Williams (4), and fractions were obtained as described by Ovary et

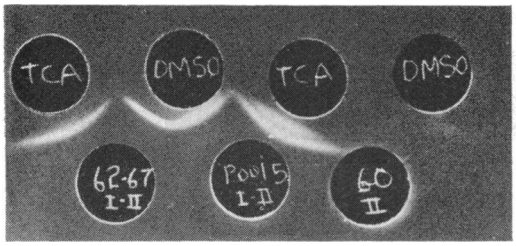

FIG. 1. *Zones of precipitate produced by DMSO or tricholoroacetic acid extract (500 μg/ml) of phase I Coxiella burneti and 1:2 dilution of hyperimmune guinea pig serum. Q62–67, phase I–II; pool 5, phase I–II; and Q60, phase II.*

al. (11). A layer of approximately 2.5 mm of 1.5% Ionagar #2 (Oxoid) in Veronal buffer (*p*H 8.2 and 0.05 ionic strength) containing 1:10,000 Merthiolate was poured on an 8 × 10 cm glass lantern slide. After the agar solidified, a rectangular cut, 3 × 84 mm, was made parallel to the long side of the slide 1 cm from the edge, but the agar was not removed until after electrophoresis. In the center of the slide, a trough (3 × 47 mm) perpendicular to the outside cut was made and was filled with the desired serum, mixed with an equal part of 3% Ionagar in buffer at 50 C. Electrophoresis was accomplished by applying 20 v across each plate for 4 hr. At the completion of electrophoresis, a 33 × 84 mm section was cut from the middle of the plate, and, beginning at the cathodal end, this section was subdivided into 12 transverse strips 5 mm wide and 1 strip 24 mm wide (Fig. 2). Each section was placed in 2 ml of physiological saline, extracted overnight at 4 C, quickly frozen and thawed several times, and finally centrifuged; the supernatant fluid was harvested. These extracts were tested for the ability to produce PCA, agglutinate phase I *C. burneti* cells, and fix complement. The remaining portion of the agar plate was developed by placing rabbit anti-guinea pig serum in the trough and incubating the plate at room temperature in a moist atmosphere overnight. By this procedure, the antibody activity of the extracts could be correlated with the precipitin arcs.

CF tests were performed by the method of Welsh et al. (15), and agglutination tests were performed by the micromethod of Fiset et al. (*in preparation*).

RESULTS

Chemical analyses of whole cells and of the various extracts are given in Table 2. From 1 mg of purified cells extracted with DMSO, 0.19 to 0.35 mg of soluble material was obtained. Solubility of DMSO and trichloroacetic acid extracts was impaired approximately 50% after lyophilization, but their antigenicity remained unchanged.

The first successful attempt to produce PCA in the guinea pig with soluble antigens was accomplished with Q59 antiserum pool (*see* Table 1) and a DMSO extract of Ohio Q, phase I. Antisera Q59, Q60, and pool 5 were used as sensitizing materials in dilutions of 1:2, 1:5, and 1:50. Samples of each of these dilutions (0.1 ml) were injected intracutaneously into 12 guinea pigs. Ten sites were sensitized per guinea pig. At 4, 24, and 48 hr postsensitization, groups of four guinea pigs were challenged intravenously with 0.84 mg of DMSO extract in an aqueous solution of 0.5% Evans blue dye. PCA was produced with Q59 antiserum (phase I–II), but not with pool 5 (phase I–II) and Q60 (phase II) antisera (Fig. 3).

TABLE 1. *Description of guinea pig antisera used*

Serum pool	Strain of *Coxiella burneti* used for infection or vaccination	Reciprocal complement fixation titer		Reciprocal microagglutination titer		Days post-infection or postvaccination when pool made	Method of[a] immunization
		Phase I	Phase II	Phase I	Phase II		
Q60	Ohio	0	1,024	2	1,024	21	Infected
Q59	Nine Mile/Ohio	512	128	512	128	140	Vaccinated, boosted
Q62–67	Ohio	2,048	1,024	512	512	91	Vaccinated, boosted
Pool 5	Ohio/California	2,048	1,536	512	1,024	62	Vaccinated, challenged, boosted
Q38–39	California	1,024	2,048	128	256	205	Infected, boosted
Q43	Henzerling/California	768	1,024	128	512	75	Vaccinated, challenged, boosted
Q47	Ohio	512	3,072	512	2,048	75	Infected, boosted
Q49	California	1,536	1,024	512	128	160	Infected, boosted
Q52	Nine Mile	4,096	4,096	512	2,048	75	Vaccinated, challenged, boosted

[a] Guinea pigs infected or challenged with 10,000 ID_{50} (guinea pig doses). Vaccination dose varied from 0.04 to 40 μg injected subcutaneously, without adjuvants. Booster dose was usually 100 μg of purified organisms injected intraperitoneally.

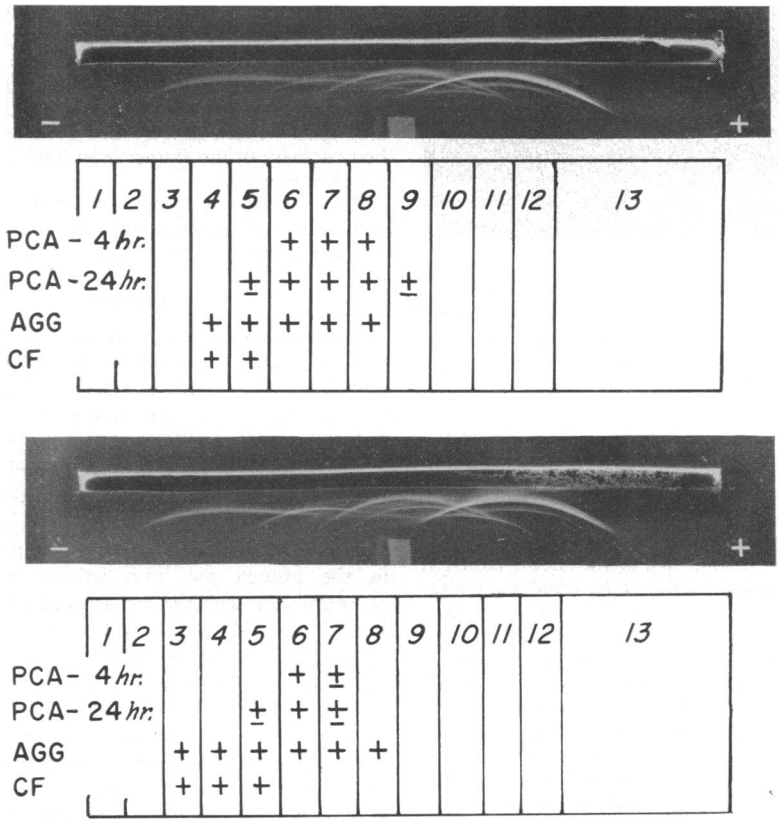

FIG. 2. *Distribution of three antigen/antibody reactions obtained with eluates of transverse agar strips after electrophoresis of Q fever antisera (top, Q59 antiserum; bottom, Q62–67 antiserum) from guinea pigs. Correlating immunoelectrophoretic patterns, as developed with rabbit anti-guinea pig serum, are shown in each case above the results obtained with eluates of numbered strips. Passive cutaneous anaphylactic (PCA) activity was tested in guinea pigs at 4 and 24 hr postsensitization. Phase I agglutination (AGG) and phase I complement fixation (CF) with undiluted eluates are also shown.*

PCA reactions were weak when challenge was done at 4 hr after sensitization; they were stronger when 24 hr had elapsed and were strongest after 48 hr. Similar tests, which utilized trichloroacetic acid extracts (1 mg) of Nine Mile or Ohio phase I *C. burneti*, gave optimal PCA reactions at 24 hr postsensitization with Q59 antiserum, but failed to produce PCA when Q60 or pool 5 antisera were used. Increasing the amount or altering the kind of soluble extract or increasing the period between sensitization and challenge did not produce PCA reactions with Q60 or pool 5 antisera. Figure 4 shows the PCA reactions when either DMSO or trichloroacetic acid extracts were used as challenge material.

Various whole phase I–II antisera were tested for the ability to elicit the PCA reaction in guinea pigs (Fig. 5). Pools Q49, Q38–39, Q59, and Q62–67 were capable of producing PCA when the guinea pigs were challenged at 24 hr postsensitization. Pool Q59 was the only whole antiserum tested which produced PCA after only a 4-hr latent period before challenge. Other antisera tested were not capable of producing PCA under the same conditions.

Fractionation of antisera by electrophoresis. Four antisera (pool 5, Q59, Q60, and Q62–67) and a normal guinea pig serum pool were concentrated fivefold and fractionated by electrophoresis. The resulting fractions were tested for PCA activity and for phase I CF and agglutinating antibodies. The PCA activity in Q59 serum was in the $7S\gamma_1$, whereas the phase I CF activity was mainly in the $7S\gamma_2$. Phase I agglutinins were in both fast and slow γ-globulin fractions (Fig. 2). Similar observations have been previously made by Ovary et al. (11) and Bloch et al. (3) with other antigen-antibody systems. The PCA reactions produced by the fast γ fraction did not require a prolonged latent period, since

TABLE 2. *Chemical composition of preparations of Coxiella burneti, Ohio Q 5EP*

Prepn	Nitrogen	Total carbohydrate	Fatty acid	Phosphorous	Hexose	Hexosamine
	%	%	%	%	%	%
Whole cells[a]	11.1	7.5	27.2	2.52	—	—
Pool C whole cells	11.3	8.85	30.2	2.09	2.17	2.67
Trichloroacetic acid extract[a]	4.52	25.2	24.8	1.78	—	—
DMSO extract (Q63)	3.00	10.5	24.6	1.19	2.51	1.50

[a] Values in table from Anacker et al. (1).

strong reactions were obtained when animals were challenged 4 hr after sensitization. Only slightly better reactions were obtained when the challenge was done 24 hr after sensitization. The fast γ portion of Q62–67 pool also produced good PCA reactions at the 4 hr challenge. The agglutination activity in this serum was also found in both fast and slow γ-globulin fractions. None of the pool 5 fractions produced PCA reactions, although CF against both phase I and phase II cells was found in the slow γ-globulin, and phase I agglutinating activity was found in both fast and slow γ-globulins. All electrophoretic fractions of serum Q60, a phase II serum, and normal guinea pig serum failed to elicit PCA when the guinea pigs were challenged with phase I soluble antigen.

Inhibition of PCA. The PCA activity of the active fractions from phase I–II (Q59 or Q62–67) antisera was markedly inhibited when they were mixed with the corresponding fractions from phase II (Q60) or with a normal guinea pig serum. However, comparable inhibition was caused by the addition of slow 7*S* γ-globulin fractions from normal guinea pig sera.

DISCUSSION

Our results demonstrated that DMSO- or trichloroacetic acid-extracted antigens from phase I *C. burneti* could provoke PCA reactions in guinea pigs sensitized with antisera to phase I cells. When either whole cells or mechanically disrupted cells were used as the challenging antigen, PCA reactions could not be produced. DMSO or trichloroacetic acid extracts of phase II cells or whole phase II cells also failed to provoke PCA in guinea pigs sensitized with either phase II or phase I–II antisera. Since PCA reactions were produced with soluble extracts of phase I cells and not with insoluble antigens, it

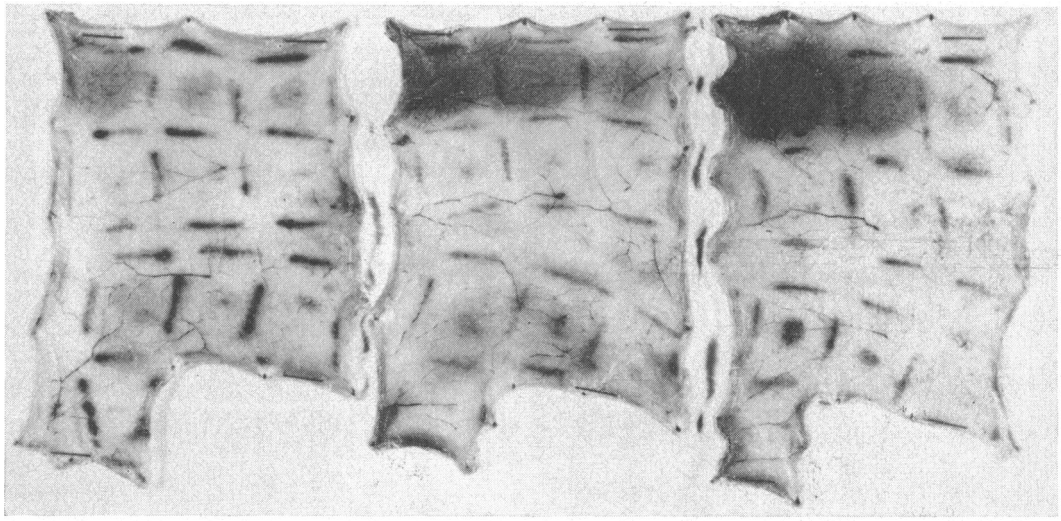

FIG. 3. *Passive cutaneous anaphylaxis (PCA) produced in the guinea pig skin by challenge with 0.84 mg of DMSO extract (Ohio strain of Coxiella burneti, phase I) in 0.5% Evans blue dye at 4 (left), 24 (center), and 48 (right) hr after sensitization with antiserum dilutions. Top of skin sections, left to right: 1:2, 1:5, and 1:50 dilution of Q59 (phase I–II). Remaining sites were sensitized with 1:2, 1:5, and 1:50 dilutions of Q60 (phase II), pool 5 (phase I–II), and saline. Pool 5 dilutions caused hemorrhage (non-PCA) reactions at injection sites.*

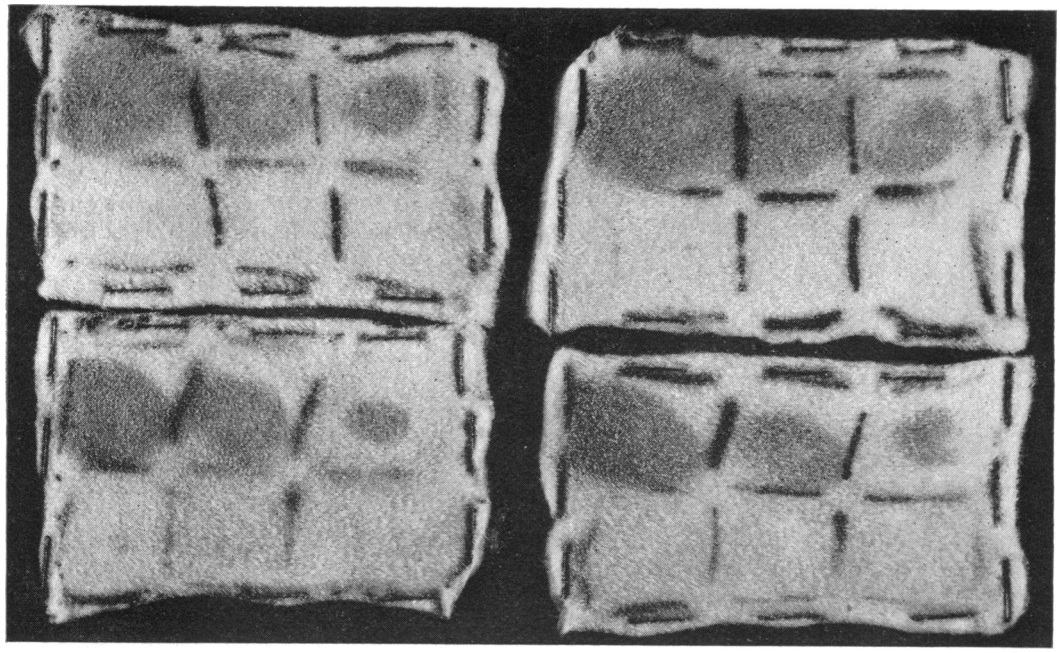

FIG. 4. *Comparison of passive cutaneous anaphylaxis reaction in guinea pigs when either DMSO (right) or trichloroacetic acid (left) extracts of Nine Mile, phase I Coxiella burneti were used for challenge. Guinea pigs were sensitized with 1:2, 1:5, and 1:50 dilution of (top to bottom) Q59 (phase I–II), Q60 (phase II), Q62–67 (phase I–II), and pool 5 (phase I–II). Animals were challenged 24 hr postsensitization with 1 mg/ml of extract in 0.5% Evans blue.*

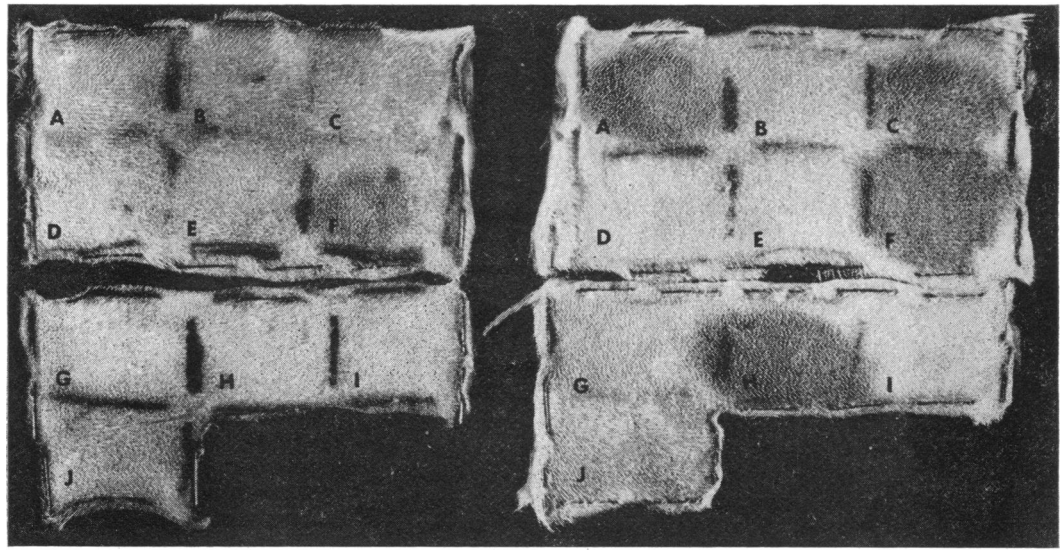

FIG. 5. *Passive cutaneous anaphylaxis in guinea pigs sensitized with 1:2 dilution of serum pools. (A) Q49, phase I–II; (B) Q43, phase I–II; (C) Q38–39, phase I–II; (D) Q52, phase I–II; (E) Q47, phase I–II; (F) Q59, phase I–II; (G) Q60, phase II; (H) Q62–67, phase I–II; (I) pool 5, phase I–II; and (J) saline. Challenged 4 (left) and 24 (right) hr postsensitization with 1 mg/ml of DMSO extract of Nine Mile Phase I in 0.5% Evans blue.*

seems that, with the present system, the provoking antigen has to be in a soluble form. Other investigators have been able to produce PCA reactions with systems involving particulate antigen, such as sheep erythrocytes (10) or bacterial cells (12), but in these cases the possibility of a release of soluble antigens from the challenge material is likely. *C. burneti* cells do not release any detectable amount of antigens when suspended in water or saline. Tissue fluids may solubilize some antigen, but not in the amounts necessary to elicit the PCA reaction in sensitized guinea pigs.

The antibodies responsible for PCA reactions in the guinea pig were in the electrophoretically fast moving $7S\gamma_1$ globulin. This confirms similar observations made with other antigen-antibody systems (3, 11). Recently, Strejan and Campbell (14), employing Ascaris antigens and their corresponding antibodies, obtained a modified PCA reaction with $7S\gamma_2$ antibodies as well. Under the conditions of our experiments, which were similar to those used by Ovary et al. (11), *C. burneti* phase I $7S\gamma_2$ antibodies did not induce PCA. As expected, both γ_1 and γ_2 antibody globulins were capable of agglutinating phase I *C. burneti* cells, and only the γ_2 fraction was able to fix complement.

The PCA reaction produced with γ_1 antibody globulin could be inhibited nonspecifically by the corresponding serum fraction obtained from either anti-phase II guinea pig antiserum or from normal guinea pig serum. These results confirm observations made by other investigators (11). Ovary et al. have shown that $7S\gamma_2$ antibodies in a proper concentration can specifically block PCA by combining with the antigen, but cannot inhibit the PCA reaction produced by a heterologous system (11). Although we obtained nonspecific inhibition with electrophoretic fractions from the slow γ region of normal guinea pig sera, these fractions may have been contaminated with γ_1 globulin, and this globulin, rather than γ_2, may have been responsible for the inhibition observed. Four of nine phase I–II antiserum pools were capable of producing PCA reactions when the challenge dose of DMSO or trichloroacetic acid extract of phase I *C. burneti* was given 24 hr after sensitization. One of these four was also positive at 4 hr after sensitization. When the γ_1 antibody in these sera was purified by electrophoresis, it was found that the latent period was not necessary. In light of the work of Ovary et al. (11), it would appear that the γ_2 antibody in antisera that produced PCA, when challenge was performed at 24 hr, produced a temporary, specific inhibition which disappeared when it diffused away from the skin site. Although this hypothesis explains the observation, it is not yet certain whether γ_1 and γ_2 antibodies diffuse from the skin at different rates. Strejan and Campbell (14) have found that purified γ_2 globulin fractions seem to "fix" to the skin and produce a modified PCA reaction in the guinea pig. Five of the nine pools of phase I-II antisera tested failed to initiate the PCA reaction in guinea pigs, even when the γ_1 fraction was isolated. It is possible that these latter sera contained a high proportion of nonspecific γ_1 antibody which nonspecifically inhibited the PCA reaction.

It is noteworthy that the antiserum pools made from sera of guinea pigs harvested 90 or more days after the initial antigen injection or infection caused the PCA reactions, whereas those harvested earlier did not.

The failure to produce PCA reactions with the phase II-anti-phase II *C. burneti* system must be due to the inability, thus far, to prepare the phase II antigen in a soluble form. When and if this is accomplished, it should be possible to produce PCA reactions with phase II $7S\gamma_1$.

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