precipitates with excess antigen.

1. Weigle, W. O., and Maurer, P. H., *J. Immunol.*, 1957, v79, —.

2. Dean, H. R., *J. Hyg.*, 1912, v12, 259.

3. Hill, B. M., and Osler, A. G., *J. Immunol.*, 1955, v75, 146.

4. Morton, J. I., and Deutsch, H. F., *Arch. Biochem. and Biophys.*, 1956, v64, 26.

5. Weigle, W. O., and Maurer, P. H., *J. Immunol.*, 1957, v79,

6. Kekwick, R. A., and Cannan, R. K., *Biochem. J.*, 1936, v36, 227.

7. Talmage, D. W., Dixon, F. J., Bukantz, S. C., and Dammin, G. J., *J. Immunol.*, 1951, v67, 243.

8. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, v62, 697.

9. Bier, O. G., Leyton, G., Mayer, M. M., and Heidelberger, M., *ibid.*, 1945, v81, 449.

10. Kabat, E. A., and Mayer M. M., *Experimental Immunochemistry*, 1948, C. C. Thomas, Springfield, Ill.

11. Bukantz, S. C., Rein, C. R., and Kent, J. F., *J. Lab. Clin. Med.*, 1946, v31, 394.

12. Markham, R., *Biochem. J.*, 1942, v36, 790.

13. Levine, L., Cowan, K. M., Osler, A. G., and Mayer, M. M., *J. Immunol.*, 1953, v71, 359.

14. ———, *ibid.*, 1953, v71, 367.

15. Levine, L., Osler, A. G., and Mayer, M. M., *ibid.*, 1953, v71, 374.

16. Mayer, M. M., Levine, L., Rapp, J. J., and Marucci, A. A., *ibid.*, 1954, v73, 443.

17. Mayer, M. M., and Levine, L., *ibid.*, 1954, v72, 516.

18. Levine, L., and Mayer, M. M., *ibid.*, 1954, v73, 426.

19. Laporte, R., Harde DeLooze, L., and Sillard, R., *Ann. Past. Inst.*, 1955, v89, 16.

20. Neff, J. C., and Becker, E. L., *J. Immunol.*, 1954, v73, 286.

21. Heidelberger, M., *J. Exp. Med.*, 1941, v73, 681.

22. Heidelberger, M., and Mayer, M. M., *Advances in Enzymology*, Interscience Publishers, Inc., N. Y., 1948, v8, 71.

23. Maurer, P. H., and Talmage, D. W., *J. Immunol.*, 1953, v70, 135.

24. Boyd, W. C., *ibid.*, 1940, v38, 143.

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## Hyperreactivity of *Coxiella burnetii* Infected Guinea Pigs to Subsequent Injections of Bacterial Endotoxins. (23482)

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Following Shwartzman's report(1) of a necropurpurogenic factor in meningococci, many workers noted that skin tissue could be prepared for the reaction by local infection with a number of microorganisms. Thus, *Bacillus anthracis*(2), *Mycobacterium tuberculosis*(3), *Haemophilus influenzae*(4), hemolytic streptococci(5), and even the virus of vaccinia(2,6) have been used to prepare animals for the local Shwartzman reaction. When animals with such skin infections were injected intravenously with a "provoking" dose of a culture filtrate from certain Gram-negative microorganisms capable of eliciting the Shwartzman reaction, hemorrhagic necrosis occurred at the infected skin site within a few hours. A generalized Shwartzman reaction has been produced in animals with systemic infections of *Vibrio cholera*(7), *Corynebacteria* and *Mycobacterium tuberculosis*(8), and Group A streptococci(9). The generalized Shwartzman reaction can also be consistently evoked by the intravenous injection of two doses of endotoxin from a number of Gram-negative bacteria. The most prominent pathological alteration seen in the classical generalized Shwartzman reaction has been reported to be bilateral renal cortical necrosis (5,10,11). While studying the interference of Brucella endotoxin on the course of a *Coxiella burnetii* infection in guinea pigs, a phenomenon was observed which appeared analogous to the generalized Shwartzman reaction. Guinea pigs infected with the rick-

ettsiae for 3 days developed an acute sensitivity when injected intracardially with *Brucella suis* endotoxin. Lipopolysaccharides extracted from two other Gram-negative bacteria could also be used to provoke animals similarly prepared. This paper presents data concerning the production of this hyperreactivity in guinea pigs infected with *C. burnetii.*

*Materials and methods. C. burnetii,* AD strain, was stored in a quick-frozen state as a 10% yolk sac suspension in heart infusion broth (Difco). Guinea pigs were infected by the intraperitoneal (IP) route with 1 ml of inoculum appropriately diluted in heart infusion broth. *B. suis* endotoxin was prepared essentially by the method of Boivin, as described by Kabat and Mayer(12). The IP $LD_{50}$ of this preparation for 20 g albino mice was approximately 0.5 mg. A lipopolysaccharide extracted and purified from *Salmonella typhosa* was kindly supplied by Dr. M. Landy of the National Cancer Institute; a report from his laboratory has characterized this material(13). A similar preparation from *Escherichia coli* was obtained through the courtesy of Dr. D. Rowley of the Wright-Fleming Institute, London, England; this lipopolysaccharide also has been described in the literature(14). The provoking dose of endotoxin was invariably contained in 0.5 ml saline injected intracardially into etherized animals. A suspension of inactivated *C. burnetii* was used in certain studies as a substitute for the active rickettsiae. This material was prepared as follows: an emulsion containing $10^9$ guinea pig IP $ID_{50}$ of the rickettsiae per ml was treated with 0.2% formalin and incubated for 24 hours on a rotary shaker at 37°C. One ml of this preparation diluted 1:10 in heart infusion broth was injected intraperitoneally into guinea pigs as a preparative dose. In related studies, treatments with serum and antibiotic started on the day of infection and continued for 3 days thereafter were used to suppress the rickettsial infection. Guinea pigs received intramuscularly 1 mg tetracycline hydrochloride (Tetracyn, Chas. Pfizer and Co.), in 0.1 ml of distilled water twice daily. Pooled, filtered homologous antiserum was also employed which was obtained from guinea pigs that had survived for 28 days an infection produced with $10^8$ guinea pig IP $ID_{50}$ of *C. burnetii.* One ml of immune serum was injected intraperitoneally per animal per day.

TABLE I. Mortalities of *Coxiella burnetii* Infected Guinea Pigs Treated with Brucella Endotoxin. 10 animals/series.

| Treatment schedule* (days) | No. of animals dead: Days after infection | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 8 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| −1 | | | | | | 1 | 1 | 1 | | | 2 | 1 | 1 |
| +3 | | | | 7 | | | | | 1 | | 1 | | |
| +8 | | | | | 1 | | 1 | 5 | | | | | |
| −1, +3, +8 | | | | 9 | | | | | | | 1 | | |
| Untreated | | | | | | 1 | | 1 | 2 | 1 | 2 | 1 | |
| −1, +3, +8 (uninfected) | | | | | | | | | | | | | |

* 100 μg Brucella endotoxin in 0.5 ml saline inj. intracardially per animal. Schedule of treatment is relative to the time of infection (0 day) with $10^3$ guinea pig IP $ID_{50}$ of *C. burnetii.*

*Results.* Table I presents the data of a typical experiment in which *B. suis* endotoxin was administered to guinea pigs infected with *C. burnetii.* After the rickettsial infection was established for 3 days, animals (treatments +3 and −1, +3, +8) were extremely sensitive to injections of 100 μg of the endotoxin, deaths occurring within 6-24 hours after treatment. Normal uninfected animals appeared refractory to multiple injections of Brucella endotoxin, and the susceptibility to *C. burnetii* of infected guinea pigs which survived treatment with Brucella endotoxin did not appear to change.

The comparative effects of varied amounts of 3 bacterial endotoxins on guinea pigs infected 3 days previously with *C. burnetii* are presented in Table II. While responses to *E. coli* and *S. typhosa* lipopolysaccharides were obtained with 10 μg amounts which were smaller than the 25 μg of Brucella endotoxin used, the relative purity of the 3 compounds must not be considered identical. The *E. coli* and *S. typhosa* lipopolysaccharides represented more highly purified materials than the Brucella endotoxin prepared for these studies.

Guinea pigs infected with *C. burnetii* demonstrated a maximum sensitivity to *E. coli*

TABLE II. Comparative Effects of Selected Bacterial Endotoxins on Guinea Pigs Infected with *Coxiella burnetii.*

| Source of endotoxin | Endotoxin* ($\mu$g) | Mortalities,† dead/total |
|---|---|---|
| *B. suis* | 100 | 4/4 |
| | 50 | 2/4 |
| | 25 | 1/4 |
| | 0 | 0/5 |
| *E. coli* | 50 | 5/5 |
| | 10 | 5/5 |
| | 1 | 2/5 |
| *S. typhosa* | 50 | 4/5 |
| | 10 | 2/4 |
| | 1 | 0/5 |

All animals infected 3 days previously with $10^8$ guinea pig IP $ID_{50}$ of *C. burnetii.*

* Contained in 0.5 ml saline inj. intracardially.

† All deaths occurred within 24 hr of endotoxin treatment. Uninfected animals survived injections of 100 $\mu$g of the bacterial endotoxins.

lipopolysaccharide when they were treated 48-72 hours post infection (Table III). The intensity of the response decreased when longer periods of time elapsed between the preparative rickettsial infection and the provoking treatment, although experimentally some animals were provoked as late as 8 days after infection.

An experiment designed to show the importance of the infecting dose of *C. burnetii* when animals were subsequently treated with Brucella endotoxin demonstrated that dosages as low as $10^6$ guinea pig IP $ID_{50}$ could be used to prepare animals suitably for this reaction. The responses of guinea pigs prepared with $10^6$, $10^7$, or $10^8$ doses were identical when the provoking injection of 100 $\mu$g Brucella endotoxin was administered 3 days later. Those animals receiving $10^5$ guinea pig IP $ID_{50}$ or less did not respond to the provoking challenge of endotoxin. This appeared in agreement with Landy's observation that no clear-cut dose/effect relationship exists in the demonstration of this hyperreactive response(15).

Additional studies, summarized in Table IV, were performed to determine if an active infection of *C. burnetii* was required to prepare guinea pigs for this reaction. Guinea pigs which received $10^8$ guinea pig IP $ID_{50}$ of formalin inactivated rickettsiae were unaffected by a provoking dose of 100 $\mu$g Brucella endotoxin administered 3 days later. It was interesting to note also that when infected animals were treated daily with either homologous antiserum or Tetracyn for the 3-day interval between infection and the time of challenge they appeared quite resistant to provoking injections of Brucella endotoxin. The success of these therapeutic treatments was indicated by the fact that all treated guinea pigs survived the rickettsial infection which normally would have killed 70% of the animals.

Further experiments failed to demonstrate any increase of rickettsiae in either the blood

TABLE III. Significance of Time in Provoking the Hyperreactive Response in *Coxiella burnetii* Infected Guinea Pigs Treated with *Escherichia coli* Lipopolysaccharide. 5 animals/series.

| Treatment schedule* | Mortalities,† dead/total |
|---|---|
| 0 day | 0/5 |
| 1 | 3/5 |
| 2 | 5/5 |
| 3 | 5/5 |
| 4 | 3/5 |
| 5 | 0/5 |

* 100 $\mu$g lipopolysaccharide in 0.5 ml saline inj. intracardially per animal after their infection on 0 day with $10^8$ guinea pig IP $ID_{50}$ of *C. burnetii.*

† All deaths occurred within 24 hr of treatment. No uninfected animals died which received lipopolysaccharide alone.

TABLE IV. Effects of Various Preparative Injections and Subsequent Treatments on the Hyperreactivity Response in Guinea Pigs Provoked with Brucella Endotoxin.

| Preparative inj.* | Treatment (for 3 days) | Provoking dose (intracardially) | Mortalities,§ dead/total |
|---|---|---|---|
| *C. burnetii* | None | 100 $\mu$g *B. suis* endotoxin | 5/5 |
| | Homologous antiserum† | *Idem* | 1/5 |
| | Tetracyn‡ | " | 0/5 |
| | None | None | 0/5 |
| Formalin inactivated *C. burnetii* | None | 100 $\mu$g *B. suis* endotoxin | 0/5 |
| | " | None | 0/5 |

* $10^8$ guinea pig IP $ID_{50}$ inj. IP/animal. † 1 ml IP/animal/day. ‡ 1 mg intramusc. twice daily/animal. § All deaths occurred within 24 hr of endotoxin inj.

or the spleen of infected guinea pigs after treatment with Brucella endotoxin. Because of this, and because deaths occurred so quickly—in many cases as early as 6 hours after treatment with bacterial endotoxins—this phenomenon was considered to be a hyperreactive response, perhaps analogous to the generalized Shwartzman reaction.

In an effort to determine if the observed hyperreactivity was a manifestation of the generalized Shwartzman reaction, observations were made of the gross pathology of 30 necropsied guinea pigs which had succumbed to provoking doses of bacterial endotoxins 3 days after their infection with *C. burnetii*. Hyperemia of the adrenals and a variable amount of congestion of the mesenteric vessels were consistent findings, together with changes in the spleen typical of an early rickettsial infection. In those infected animals surviving the provoking dose of endotoxin some indication of adrenal hyperemia was present although the reaction was not as marked. Congestion of the mesenteric vessels of the animals in this group was extremely variable, both in degree and in occurrence.

The following organs were routinely removed for histological studies: liver, spleen, heart, lungs, kidneys, adrenals, mesenteric lymph nodes and sections of the intestine. Microscopic examination of tissues from 7 infected animals that died of the provoking challenge disclosed hemorrhage, hyperemia, some leucocytic infiltration into the adrenal cortex, and hyperemia of the vessels in the adrenal medulla. It should be noted that one animal failed to develop this pathology. Indications of an early granulomatous-like reaction were found in the spleen and liver of all the animals in this group. Tissues from 8 of 11 infected animals which survived the provoking dose of endotoxin showed a similar pathological response, but the changes were less prominent, and hemorrhage was occasionally absent. The remaining 3 guinea pigs in this group showed only microscopic evidence of the rickettsial infection. There was no indication of bilateral renal cortical necrosis in any of the animals examined.

In a limited number of uninfected guinea pigs treated with bacterial endotoxin no gross or microscopic pathology was noted.

In discussing the significance of these findings, two observations are worthy of note. The first is the consistent presence of adrenal pathology in *C. burnetii* infected guinea pigs subsequently treated with endotoxin preparations. The second is the complete absence of bilateral renal cortical necrosis in this same experimental group. Gronchi and Carnielli (16) have reported histological studies on the adrenals of guinea pigs which received 2 intracardial injections of a broth filtrate of *E. coli*. With this regimen which would elicit the generalized Shwartzman reaction they noted essentially the same pathology as described here. As an explanation for the absence of renal cortical necrosis reported to be characteristic of the generalized Shwartzman reaction, it is possible that although this lesion is a constant finding in the rabbit, the usual laboratory animal for such studies, it does not occur in guinea pigs.

Since the hyperreactivity elicited in *C. burnetii* infected guinea pigs by subsequent injections of bacterial endotoxins conforms generally in character to the generalized Shwartzman reaction, and because the pathology noted is in keeping with observations of others who have studied this reaction in guinea pigs, it is suggested that the hyperreactivity noted here is a manifestation of the generalized Shwartzman reaction.

*Summary.* Guinea pigs infected for 3 days with *C. burnetii* demonstrated a hyperreactivity to injections of bacterial endotoxins, with deaths occurring 6-24 hours after treatment. The preparative infectious dose of *C. burnetii* could be as low as $10^6$ guinea pig IP $ID_{50}$. The reaction has been provoked with 25 $\mu$g of Brucella endotoxin while 10 $\mu$g of purer preparations from *E. coli* and *S. typhosa* have produced a similar response. An optimal period of reactivity existed from 48-72 hours post infection. Formalin inactivated rickettsiae did not prepare animals for this reaction and infected guinea pigs treated with either rickettsial antiserum or Tetracyn could not be provoked by subsequent injections of endotoxin. The gross and microscopic studies

of selected tissues taken from hyperreactive animals consistently revealed hyperemia and hemorrhage of the adrenals. The described hyperreactivity appears to be a manifestation of the generalized Shwartzman reaction.

1. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1929, v26, 207.

2. Gratia, A., and Linz, R., *Compt. rend. Soc. biol.*, 1931, v107, 1579.

3. Debonera, G., Tzortzakis, N., and Falcketti, E., *ibid.*, 1932, v109, 24.

4. Witebsky, E., and Salm, H., *J. Exp. Med.*, 1937, v65, 43.

5. Shwartzman, G., *Phenomenon of Local Tissue Reactivity*, Paul B. Hoeber, Inc., N. Y., 1937.

6. Koplik, L. H., *Am. J. Path.*, 1935, v11, 842.

7. Sanarelli, G., *Ann. Inst. Pasteur*, 1924, v38, 11.

8. Bordet, P., *ibid.*, 1936, v56, 325.

9. Thomas, L., Denny, F. W., and Floyd, Joan, *J. Exp. Med.*, 1953, v97, 751.

10. Black-Schaffer, B., Hiebert, T. G., and Kerby, G. P., *Arch. Path.*, 1947, v43, 28.

11. Thomas, L., and Good, R. A., *J. Exp. Med.*, 1952, v96, 605.

12. Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, 1st Edit., Charles C. Thomas, Springfield, Ill., 1948, p514.

13. Webster, M. E., Sagin, J. F., Landy, M., and Johnson, A. G., *J. Immunol.*, 1955, v74, 455.

14. Rowley, D., *Brit. J. Exp. Path.*, 1956, v37, 223.

15. Landy, M., and Johnson, A. G., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 57.

16. Gronchi, V., and Carnielli, P., *Atti Soc. Med.-Chir. Padova*, 1934, v12, 433. (Biol. Abstr., 1936, v10, No. 6109).

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## Action of Sodium Desoxycholate on Arthropod-Borne Viruses. (23483)

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As a by-product of studies on the course of infection of monkeys with virulent yellow fever virus(1), it was discovered that bile from normal rhesus monkeys had a marked virucidal action on the virus. Bile in a dilution of 1:50 was shown to inactivate from 3.0 to 5.0 logs of several strains of yellow fever. In extending the experiments to determine the action of bile on other agents, it was discovered that viruses could be divided into two groups—one resistant and the other susceptible to the action of bile. Viruses which were resistant were the Lansing and $MEF_1$ strains of poliomyelitis, the GD VII strain of mouse encephalomyelitis and the Mengo strain of encephalo-myocarditis. Agents found to be susceptible were yellow fever, Uganda S, Ntaya, West Nile, dengue 1, dengue 2, St. Louis, Japanese B, Ilheus, EEE, Bwamba, Bunyamwera and Anopheles A. The method of determining the degree of virus inactivation was to make parallel titrations of the virus in the presence or absence of a 1:50 dilution of bile. A summary of such an experiment appears in Table I. The diluent used in these experiments was either 10% normal monkey serum in saline or a 0.2 solution of bovine albumen (bpa) in saline.

In the course of these experiments it was found that samples of bile from different normal rhesus monkeys varied in their capacity to inactivate a bile susceptible virus. This observation led to the search for a more clearly defined chemical substance. The use of sodium desoxycholate (DCA) was a logical choice as this salt had been used extensively in the study of the bile solubility of the pneumococcus. The results of a preliminary experiment to determine the action of DCA are shown in Table II. In this experiment, the action of DCA in two concentrations (1:1000 and 1:10,000) was tested on yellow fever virus and the higher concentration on GD VII. In addition, the yellow fever virus was titrated in the presence of a 1:50 dilution of bile. It will be observed that the 1:1000 dilution of DCA inactivated the yellow fever virus to approximately the same extent as the 1:50 dilution of bile, whereas this concentration of DCA had no action on the GD VII virus. The