# Susceptibility of Hamsters to Peripherally Inoculated Japanese B and St. Louis Viruses Following Cortisone, X-Ray, Trauma.* (23102)

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In making cross immunization studies with certain of the better known Group B encephalitis viruses, Hammon and Sather(1) employed the Syrian hamster. These animals were effectively challenged by a peripheral route with West Nile (WN) virus and with Murray Valley (MVE) encephalitis viruses, since both of these agents produced fatal infections through high virus dilutions when inoculated either subcutaneously or intraperitoneally. In these experiments previous immunization had been carried out through inapparent infection with St. Louis (SLE) and Japanese B (JBE) viruses. Similar challenge could not be made, however, in the other direction using JBE or SLE viruses after immunization with WN, MVE or other Group B viruses. An adequately susceptible experimental animal for peripheral challenge with JBE and SLE viruses was much desired, but none was currently available. Among the possible uses for an animal highly susceptible to quantitative peripheral challenge were: (1) a more suitable potency test for specific formalin inactivated virus vaccines; (2) a means for testing for immunity induced by previous infection with an immunologically related virus. Our most immediate interest in finding such an animal was to test for immunity against JBE and SLE viruses following experimental infection by a related avirulent living virus immunizing infection to be supplemented by one or more injections of specific formalin inactivated virus vaccine as discussed by Hammon and Sather(1) and Hammon, Sather, Lennette and Reeves(2). Use of an avirulent WN virus infection had been specifically suggested to precede the use of a JBE inactivated virus vaccine, since use of the latter alone had not appeared to give adequate protection in adult, military usage (3). Flexner and Amoss(4) reported that "normal monkey or horse serum, isotonic salt solution, and Ringer's and Locke's solution when injected into the meninges promote infection with the virus of poliomyelitis introduced into the blood, the nose, or the subcutaneous tissues." Zwick, Seifried and Witte (5) found that cutaneous inoculation of rabbits with Borna virus succeeds in producing disease in exceptional instances only, but that disease is easily brought about by such inoculation if an injury is produced at the same time in the central nervous system by injection of non-specific fluids (normal rabbit serum, normal horse serum, or isotonic chloride solution). Working with mice, Sawyer and Lloyd(6) showed that intraperitoneal inoculation of yellow fever virus, which was without pathological effect in the intact adult animal, produced encephalitis if the brain was injured. They used 2% starch in 0.9% NaCl solution, and inoculated the animal with 0.03 cc intracerebrally. Simple trauma to the brain with a needle also had a provocative effect. Later, Webster and Clow(7) indicated a similar facilitating effect of brain injury with the virus of SLE. Burnet and Lush(8) found that brain injury enhanced the effect of louping ill virus. On the other hand, Olitsky, Cox and Syverton(9) were unable to induce disease with vesicular stomatitis virus injected intraperitoneally even though the brain was simultaneously traumatized. In Eastern equine encephalitis virus infection, King(10) reported that traumatization of the brain by simple needling had insignificant effect. However, he found that a non-specific irritation by injection of glycerin into the peritoneal cavity increased incidence of dis-

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ease in mice to subcutaneous inoculation of this virus. Marie(11) reported a similar action with rabies virus after intraperitoneal injection of India ink. It has been demonstrated that cortisone produces a highly significant enhancement of experimental intracerebral infection with the Lansing strain of poliovirus in the hamster(12,13). Adult mice can be lethally infected with Coxsackie virus if cortisone is administered in a single dose prior to the inoculation of the virus(14). Susceptibility of such adult mice is reported to be fully as great as that of infant mice. The only work with cortisone which we have found reported to date using any of the Group B encephalitis viruses is that by Volmer and Hurlbut(15) with JBE virus. They attempted to use cortisone as a therapeutic agent in mice. Instead of reducing mortality of mice inoculated subcutaneously, they found that this product increased it. The effect, however, was not sufficiently marked to encourage us to employ this animal in our work, at least not until hamsters had been tried. It would appear from the studies of a number of investigators(16-22) that cortisone most probably acts through inhibition of assimilation of antigen and of the synthesis of antibody globulin. Evidence of the effect of X-irradiation on immunity is found in animals irradiated shortly before immunization with non-living antigens. The effects produced by X-ray on innate and acquired immunity of the intact animal have been reviewed by Taliaferro(23). More recent reports(24-27) have shown that prior exposure of the whole animal to X-ray increases its susceptibility to selected viral agents. This effect may be due to an impairment of the antibody response. Syverton, *et al.*(24) showed that when both X-ray and cortisone were employed a markedly synergistic effect could be demonstrated. Adult mice normally not susceptible to poliovirus, Coxsackie virus, and certain other agents developed rapidly progressive lethal infection following this combination treatment.

*Materials and methods.* Syrian hamsters obtained from the Lakeview Hamster Colony, were used in all the experiments here reported. They were about 8 weeks old, of both sexes. A frozen pool of JBE Nakayama strain virus, mouse passage 45, hamster passage 3, with an $LD_{50}$ of $10^{-7.5}$ in mice was used throughout. St. Louis Webster strain of virus as a frozen mouse brain suspension with an infecting titer in mice of $10^{-7.8}$ was employed when this agent was used. The susceptibility of each hamster to either of these viruses was tested by inoculating it subcutaneously with 0.1 cc of a suitable dilution of virus. The cortisone used was manufactured by Sharp and Dohme. Before starting an experiment with cortisone, each hamster was weighed and the hamsters were put in groups having as nearly as possible the same weight. It was usually possible to use only one sex of animals in any one experiment. However, from experiment to experiment, no sex differences in susceptibility were ever detected. The total dose of cortisone in milligrams was calculated on the basis of body weight of the hamster in grams. The total dose of cortisone was given in 4 daily injections. On the first day of the experiment, the hamster received its first dose of cortisone. On the second day, following the cortisone, virus was injected by the subcutaneous route. Approximately 35% of the total cortisone dosage was given the first day, 25% on the second, and 20% on each of the last 2 days. Roentgen rays‡ were generated by a 4 milliampere current operating at 100 kilovolts and were filtered through 1.0 mm of aluminum (HVL 1.1 mm AL). The size of the field was 20 cm square; individual readings taken at selected points in the target area with a Victoreen r meter varied from 4.65 to 4.90 roentgens in air per minute. A single massive dose of radiation was administered to the whole body of the animal at a skin target distance of 78 cm at an average rate of 4.7 r per minute. The hamsters were put in a wire basket in a manner which allowed them to sleep side by side without too much movement. The basket was covered with a used X-ray film which was fastened to the basket by adhesive tape.

‡ The work with X-rays was done with the advice, assistance and equipment of Dr. F. S. Cheever, a member of this Department.

TABLE I. Effect of Cortisone on Susceptibility of Hamsters to Subcutaneous Inoculation of JBE Virus.

| Test | Cortisone,* mg | Virus inoc.† | Mortality ratio | Mortality % |
|---|---|---|---|---|
| I | .05 | Virus | 5/15 | 33.3 |
| | " | None | 0/15 | .0 |
| | None | Virus | 1/15 | 6.6 |
| II | .1 | Virus | 5/ 6 | 83.0 |
| | " | None | 0/ 6 | .0 |
| | None | Virus | 1/ 6 | 16.6 |
| III | .2 | Virus | 10/10 | 100.0 |
| | " | None | 0/10 | .0 |
| | None | Virus | 0/10 | .0 |
| IV | .3 | Virus | 15/15 | 100.0 |
| | " | None | 1/15 | 6.6 |

* Cortisone by g body wt.
† 0.1 cc of $10^{-1}$ mouse brain suspension.

*Results. Exp. 1 and 2. Brain irritation and intraperitoneal glycerin with JBE virus.* Cerebral irritation with 2% starch and peritoneal trauma with 0.5% glycerin following subcutaneous inoculation of a $10^{-1}$ dilution of JBE virus were both tried. Mortality changed from 10% in the controls to only 30% and 40% respectively. Since this was not an adequate mortality for our purposes this line of investigation was not followed further.

*Exp. 3 and 4. Various dosages of cortisone and a $10^{-1}$ dilution of JBE.* In Exp. 3 four tests were performed using different total doses of cortisone. In the first a total dose of 0.05 mg/g body weight was employed; in the second 0.1 mg; in the third 0.2 mg; in the fourth 0.3 mg. In each test, with one exception, there were 3 basic groups of hamsters: (1) treated with cortisone and injected with the virus; (2) treated with cortisone only; and (3) injected with virus only. In the fourth test, Group 3, the virus controls were omitted. Hamsters in all tests were watched for a period of 3 weeks. Those which died or were moribund during this period were autopsied and the liver, spleen, and blood were cultured on blood agar. Deaths caused by excess cortisone could be detected by this means, since cultures from organs of these animals showed heavy bacterial growth. It can be seen from Table I that the dose of 0.05 mg of cortisone/g body weight plus virus gave 33% mortality; the dose of 0.1 mg plus virus gave 83% mortality; and the doses of 0.2 and 0.3 mg gave 100% mortality. No deaths occurred in the cortisone control groups except in the last test, in which the 0.3 mg dosage was used. Pathogenic bacteria were found only in certain of the autopsied hamsters of this last group, those receiving the greatest amount of cortisone. Only 6.6 to 16.6% of the hamsters died in the virus control groups. From these results it appeared that a total dose of 0.2 mg cortisone/g body weight was the most promising one. Therefore, a confirming experiment (No. 4) was performed, using this dose of cortisone and virus again. Eight hamsters were used in each group. Results of this experiment confirmed the findings of the previous one. There was 100% mortality in those receiving cortisone and virus, 12.5% in those receiving only virus, and 0% in those receiving only cortisone.

*Exp. 5. Titration of JBE in cortisone-treated hamsters.* An experiment was then performed in the manner of a virus titration. A 10-fold serial dilution of virus was inoculated subcutaneously in groups of hamsters, each treated with a total dose of 0.2 mg cortisone/g body weight in the way described above. Five hamsters were used for each virus dilution. Results of this titration are shown in Table II. The $LD_{50}$ titer of JBE virus as determined by subcutaneous inoculation was greater than $10^{-6}$, while in normal hamsters it is regularly less than $10^{-1}$.

*Exp. 6. Cortisone and a $10^{-1}$ dilution of SLE.* In this experiment hamsters were treated with cortisone in exactly the same manner as in the JBE experiments using a total of 0.2 mg/g body weight. On the second day of the experiment the hamsters were inoculated subcutaneously with 0.1 cc of a $10^{-1}$ dilution of SLE virus. Results are shown in Table III. Cortisone increased the mortality of hamsters to subcutaneous inocu-

TABLE II. Titration of JBE Virus Subcut. in Hamsters Treated with Cortisone.

| | Virus dilution and mortality ratio | | | | | |
|---|---|---|---|---|---|---|
| Cortisone* | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| .2 mg | 5/5 | 5/5 | 4/5 | 5/5 | 4/5 | 4/5 |
| None | 0/5 | 0/5 | 0/5 | ——not done—— | | |

* Cortisone by g body wt.

TABLE III. Effect of Cortisone on Susceptibility of Hamsters to Subcutaneous Inoculation of SLE Virus.

| Cortisone,* mg | Virus inoc.† | Days of death after virus inoc. | Mortality ratio | Mortality % |
|---|---|---|---|---|
| .2 | Virus | 7, 9, 11, 14 | 4/5 | 80 |
| " | None | ‡ | 0/5 | 0 |
| None | Virus | ‡ | 0/5 | 0 |

* Cortisone by g body wt.
† .1 cc of $10^{-1}$ mouse brain suspension.
‡ No deaths.

lation of a $10^{-1}$ dilution of SLE virus from 0% to 80%.

*Exp. 7. Titration of SLE in cortisone-treated hamsters.* In this experiment the hamsters were divided into 2 groups: (1) 40 hamsters treated with 0.2 mg cortisone/g body weight and inoculated with serial 10-fold dilutions of SLE virus (5 with each dilution) as shown in Table IV; (2) 15 hamsters inoculated (5 each) with $10^{-1}$ through $10^{-3}$ dilutions of the virus only. The mortality of those hamsters given cortisone with different dilutions of the virus was fairly constant at 3 or 4 out of 5 through the $10^{-7}$ dilution, but was never 100%. Because of this an $LD_{50}$ cannot be calculated. No hamsters died in the non-cortisone treated groups. In this experiment no cortisone control group was used; however, all moribund hamsters were autopsied and cultures made without detecting any evidence of death due to bacterial infection from excessive cortisone.

*Exp. 8 and 9. X-ray with a $10^{-1}$ dilution of JBE.* In 2 essentially identical experiments, test hamsters each received a dose of 450 r of X-ray on the first day, and on the second day each was given 0.1 cc of a $10^{-1}$ dilution of virus subcutaneously. Groups of virus control and X-ray control hamsters were included in each of the experiments. In the first, 15 hamsters were used in each group, in the second 8 were used. In Experiments 8

TABLE IV. Titration of SLE Virus Subcut. in Hamsters Treated with Cortisone.

| | Virus dilution and mortality ratio | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Cortisone* | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ | $10^{-7}$ | $10^{-8}$ |
| .2 mg | 3/5 | 4/5 | 3/5 | 4/5 | 3/5 | 4/5 | 4/5 | 2/5 |
| None | 0/5 | 0/5 | 0/5 | ——— | not done | ——— | | |

* Cortisone by g body wt.

TABLE V. Effect of 450 r X-ray on Susceptibility of Hamsters to Subcutaneous Inoculation of JBE Virus.

| Exp. | Treatment | Virus inoc.* | Mortality Ratio | Mortality % |
|---|---|---|---|---|
| 8 | 450 r | Virus | 12/14 | 85.7 |
| | " | None | 1/15 | 6.6 |
| | None | Virus | 1/15 | 6.6 |
| 9 | 450 r | Virus | 7/ 8 | 87.5 |
| | " | None | 1/ 8 | 12.5 |
| | None | Virus | 1/ 8 | 12.5 |

* .1 cc of $10^{-1}$ mouse brain suspension.

and 9, 450 r of X-ray increased susceptibility of hamsters to subcutaneous inoculation of JBE virus (Table V). A mortality of about 85% was observed in the test groups, and of only 6.6% to 12.5% in the X-ray control groups.

*Exp. 10. X-ray and cortisone with a $10^{-1}$ dilution of JBE.* X-ray was administered as described above, but in doses of 150, 300 and 400 r. Cortisone was administered in doses of 0.10, 0.15 and 0.20 mg/g body weight. Various combinations of cortisone and X-ray were tried, hoping to find a more effective method of increasing susceptibility of hamsters to subcutaneous inoculation of JBE virus.

Seven groups of hamsters were used in this experiment (Table VI). Each of the first 4 groups was composed of 16 animals and each of the fifth and sixth groups was composed of 14 animals. Each of these groups was divided into 2 subgroups; one subgroup received X-ray and cortisone plus virus; the other subgroup acted as a control for the X-ray and cortisone without virus. The seventh group, of 8 hamsters, was inoculated with the virus only and acted as a virus control group. The total dose of X-ray was given on the first day of the experiment, and cortisone was given over a period of 4 days beginning on the day X-ray was administered. Virus in a $10^{-1}$ dilution was given on the second day of the experiment. The various combinations of X-ray and cortisone used gave a 100% mortality rate in all the test groups which received virus (Table VI). Also, there were deaths in the control treatment groups except in that receiving the smallest dose of both X-ray and cortisone. Deaths in the virus control group

TABLE VI. Effect of Cortisone and X-ray on Susceptibility of Hamsters to Subcutaneous Inoculation of JBE Virus.

| | Cortisone in mg/g body wt | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | .1 | | | .15 | | | .2 | | |
| X-ray in r units | Virus inoc. | Mortality Ratio | % | Virus inoc. | Mortality Ratio | % | Virus inoc. | Mortality Ratio | % |
| 150 | 0 | 0/8 | 0 | 0 | 1/8 | 12.5 | 0 | 1/8 | 12.5 |
| | + | 8/8 | 100 | + | 8/8 | 100 | + | 8/8 | 100 |
| 300 | 0 | 1/8 | 12.5 | 0 | 1/7 | 14.2 | | | |
| | + | 8/8 | 100 | + | 7/7 | 100 | | | |
| 450 | 0 | 2/7 | 28.5 | | | | | | |
| | + | 7/7 | 100 | | | | | | |

Mortality ratio in 8 hamsters inoculated with the virus only was 1/8 or 12.5%.

were 12.5%. It can be concluded that a combination of 150 r X-ray plus a total dose of cortisone of 0.1 mg/g body weight did not produce deaths due to over-dosage but did increase the susceptibility of the animals to death as a result of peripheral inoculation of virus. Larger doses led to deaths in the controls.

*Exp. 11. Titration of JBE in X-ray plus cortisone-treated hamsters.* In this experiment serial 10-fold dilutions of virus were inoculated subcutaneously in groups of 5 hamsters treated with 150 r of X-ray plus 0.15 mg cortisone/g body weight, a dosage decided upon following Exp. 10. For comparison of the effect of 0.2 mg of cortisone alone, shown to be non-toxic but effective in previous experiments, similar groups of virus-injected hamsters were treated in this manner. Virus controls were also included.

The $LD_{50}$ titer of JBE virus in the subcutaneously inoculated hamsters given X-ray and cortisone was between $10^{-4}$ and $10^{-5}$ (Table VII). Cortisone in a 0.2 mg dose without X-ray, however, gave an $LD_{50}$ of $>10^{-6}$ in the virus titration.

*Discussion.* Hamsters, with few exceptions, do not become sick or die from subcutaneous inoculation of JBE or SLE virus(28). From the work of previous investigators it was found that brain irritation or peritoneal irritation may enhance the susceptibility of some animals to peripheral inoculation of certain viruses. Since we were interested in finding an animal susceptible to subcutaneous inoculation of these viruses, these 2 methods were tried on hamsters, but without too much success. Since the adult mouse is slightly more susceptible to peripheral injection of JBE virus than the hamster treated by either of these 2 methods, this did not appear to be worthy of further testing for our purposes. The possibility was considered that the poor effect of brain irritation and intraperitoneal glycerin on susceptibility of hamsters to subcutaneous inoculation of JBE virus might be due to the fact that neither of these 2 methods had an inhibitory effect on development of specific neutralizing antibodies which might be playing an important role in protection of the hamsters. Other methods which might have such an effect were selected for subsequent experiments.

Whether or not the results were due to immunologic factors, it is obvious that a suitably administered regime of cortisone greatly enhanced susceptibility of the hamsters to peripheral injection of these viruses. The susceptibility to JBE, in fact, practically equalled that which occurs when the virus is given intracerebrally. By maximum irradiation with X-rays compatible with survival of most of the controls the susceptibility of hamsters to subcutaneous inoculation of JBE virus increased from 6.6% or 12.6% in non-irradiated hamsters to about 85%. Further

TABLE VII. Titration of JBE Virus Subcut. in Hamsters Treated with Cortisone Alone or with Combination of Cortisone and X-ray.

| | Virus dilution and mortality ratio | | | | | |
|---|---|---|---|---|---|---|
| Treatment | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | $10^{-5}$ | $10^{-6}$ |
| .2 mg cortisone | 5/5 | 5/5 | 4/5 | 5/5 | 4/5 | 4/5 |
| 150 r X-ray + .15 mg cortisone | 5/5 | 5/5 | 5/5 | 5/5 | 0/5 | 2/5 |
| None | 0/5 | 0/5 | 0/5 | —not done— | | |

alteration of time and dosage factors might have improved this.

Treatment of hamsters with X-ray plus cortisone in the maximum combination dose tolerated rendered hamsters more highly susceptible to JBE virus than maximum doses of X-ray alone. This effect might be due to a synergistic action between X-ray and cortisone as suggested by Syverton, *et al.*(24). However, since a simultaneous comparison of the effect of cortisone alone with the best combination treatment tested showed the former to be superior to the combination, and since the use of cortisone alone was a simpler procedure to employ and beset with fewer variables, the combination treatment was not investigated further. Subsequent work on immunologic response of the inoculated animals, with and without cortisone, and on evaluation of immunization procedures reported in subsequent papers was carried out using cortisone alone.

*Summary.* 1) Brain irritation and intraperitoneal glycerin were tried to enhance susceptibility of hamsters to subcutaneous inoculation of JBE virus. Both of these methods have a slight enhansive effect, but not of the degree desired for the purposes of this study. 2) A total dose of 0.2 mg cortisone/g body weight, when given to hamsters as described, rendered them highly susceptible to subcutaneous inoculation of JBE virus and to only a slightly less degree to SLE virus. 3) It was found that a dose of 450 r of X-ray detectably increased susceptibility of hamsters to subcutaneous inoculation of JBE virus. This was the maximum dose of X-ray tolerated, and increase in susceptibility observed was less marked than with cortisone. 4) Combined treatment of hamsters with the maximum tolerated combined doses of X-ray and cortisone (150 r of X-ray and 0.15 mg cortisone) rendered them much more highly susceptible than X-ray alone; however, the combination tested was not as effective as cortisone alone in a subtoxic dose. Cortisone at a dosage of 0.2 mg/g body weight was adopted as a standard procedure for subsequent work. The increase in susceptibility to JBE virus was in excess of 100,000 fold, rendering the animals almost as susceptible to peripheral inoculation as to intracerebral.

1. Hammon, W. McD., and Sather, G. E., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 521.

2. Hammon, W. McD., Sather, G. E., Lennette, E. H., and Reeves, W. C., *ibid.*, 1956, v91, 517.

3. 406th Med. Gen. Lab. Ann. Historical Rept., Professional Sect., 1952, p43.

4. Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1917, v25, 525.

5. Zwick, W., Seifried, O., and Witte, J., *Arch. f. Wissensch. U. Prakt. Tierh.*, 1929, v59, 511.

6. Sawyer, W. A., and Lloyd, W., *J. Exp. Med.*, 1931, v54, 533.

7. Webster, L. T., and Clow, A. D., *ibid.*, 1936, v63, 443.

8. Burnet, F. M., and Lush, D., *Austral. J. Exp. Biol. and Med. Sci.*, 1938, v16, 233.

9. Olitsky, P. K., Cox, H. R., and Syverton, J. T, *J. Exp. Med.*, 1934, v59, 159.

10. King, L. S., *ibid.*, 1940, v71, 95.

11. Marie, A. C., *Ann. Inst. Pasteur*, 1934, v52, 481.

12. Shwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

13. ———, *Am. J. Path.*, 1951, v27, 714.

14. Kilbourne, E. D., and Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 135.

15. Vollmer, E. P., and Hurlbut, H. S., *J. Infect. Dis.*, 1951, v89, 103.

16. Bjorneboe, M., Fishel, E. E., and Stoerk, H. C., *J. Exp. Med.*, 1951, v93, 37.

17. Bjorneboe, M., and Gormsen, H., *Acta Path. et Microbiol. Scand.*, 1943, v20, 649.

18. Fishel, E. E., Stoerk, H. C., Bjorneboe, M., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 111.

19. Marshall, L. M., and Friedberg, F., *Endocrinol.*, 1951, v48, 113.

20. Engel, F. L., *N. Y. Acad. Med., Sect. Microbiol.*, 1952, Chap. 2.

21. Moeschlin, S., Baguena, R., and Baguena, J., *Bull. l'Acad. Suisse des Sciences Med.*, 1952, v8, 153.

22. Kilbourne, E. D., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 685.

23. Taliaferro, W. H., and Taliaferro, L. G., *J. Immunol.*, 1951, v66, 181.

24. Syverton, J. T., Werder, A. A., Friedman, J., Roth, F. J., Jr., Graham, A. B., and Mira, O. J. PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 123.

25. Bentler, E., and Gezon, H. M., *J. Immunol.*, 1952, v68, 227.

26. Blumenthal, H. T., Greiff, D., Chiga, M., and Pinkerton, H., *J. Exp. Med.*, 1953, v97, 135.

27. Cheever, F. S., *J. Immunol.*, 1953, v71, 431.

28. Lennette, E. H., PROC. SOC. EXP. BIOL. AND MED., 1941, v47, 178.

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