maximum beneficial effect. In preliminary experiments a dose of 2000 mg/kg/day of terramycin (10 times the highest level used in this study) was tried but the preparation was so strongly acid that in many instances it caused perforation of the stomach and had to be abandoned(11).

*Summary.* Rats were given varying doses of terramycin hydrochloride beginning 72 hours and ending 1 to 4 hours prior to x-irradiation. The highest dosage level (200 mg/rat/day) was the most effective in lowering mortality. It is probable that larger doses of antibiotics are required in the x-irradiation syndrome than are required for the treatment of other diseases.

1. Gustafson, G. E., and Koletsky, S., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 489.

2. Koletsky, S., and Christie, J. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 363.

3. Miller, C. P., Hammond, C. W., Tompkins, M., and Shorter, G., *J. Lab. and Clin. Med.*, 1952, v39, 462.

4. Miller, C. P., Hammond, C. W., and Tompkins, M., *Science*, 1950, v111, 719.

5. Hammond, C. W., and Miller, C. P., *Ann. N. Y. Acad. Sci.*, 1950, v53, 303.

6. Miller, C. P., and Hammond, C. W., *Trans. Assn. Am. Phys.*, 1950, v63, 155.

7. Furth, F. W., Coulter, M. P., and Howland, J. W., UR-158, Univ. of Rochester.

8. ———, *Am. J. Path.*, 1952, v28, 25.

9. Coulter, M. P., Furth, F. W., and Howland, J. W., *Am. J. Path.*, 1952, v28, 875.

10. Smith, W. W., Smith, F., Ruth, H. J., Canter, H. Y., and Grenan, M. M., *Am. J. Physiol.*, 1953, v172, 351.

11. Unpublished data of authors

Received June 26, 1953. P.S.E.B.M., 1953, v84.

## Interference in Cortisone-Treated Hosts.* (20614)

JACOB FONG AND ROBERT LOUIE.

*From the Department of Bacteriology, University of California, Berkeley.*

Cortisone has been shown to suppress inflammation(1) and antibody formation(2), to induce lymphocytolysis and dissolution of lymphoid tissue(3), to enhance viral multiplication(4), and to increase the severity of infections produced by a wide variety of organisms(5-8). It is evident that a multitude of effects may be attributed to the action of cortisone. The present paper reports the results of studies of the interference phenomenon in cortisone-treated hosts.

*Materials and methods. Virus.* An egg-adapted strain of influenza A (PR8) virus was used and prepared by allantoic inoculation of 11-day embryos with 0.05 ml of a $10^{-6}$ dilution of infected allantoic fluid; after incubation of eggs at 36°C for 42-48 hours, the fluids were collected and concentrated by the method of red cell adsorption and elution. A final concentration of 5% chicken red cells was used for adsorption of virus; after 45 minutes in the cold, the supernatant fluid was removed and the agglutinated cells resuspended in a small volume of 0.2 M phosphate buffer, pH 7.4. Elution of virus was carried out in a 37°C water bath for 4 hours. The red cells were sedimented by brief centrifugation and the supernatant fluid used as a source of virus after adjustment of the hemagglutinin titer to 2560.

*Preparation of interfering virus.* Virus prepared in the above manner was exposed to 5 x $10^{-3}$ M sulfur mustard, [bis ($\beta$-chloroethyl) sulfide], for 3 hours at room temperature. The viral suspension was then centrifuged briefly to remove precipitated material and the supernatant fluid used as an interfering suspension after testing for inactivation of virus. The advantages of a sulfur mustard-inactivated interfering suspension will be described elsewhere(9). *Tests of inactivation of virus.* 11-day embryos were inoculated allantoically with 0.5 or 0.05 ml of undiluted suspension, and 0.05 ml of serial 10-fold dilutions

* This work was aided by a grant from the Research Committee of the University of California.

TABLE I. Results of Interference Tests in Cortisone-Treated Eggs.

| Pretreatment of host (ml) | Interfering virus (AD)* | Challenge virus ($ID_{50}$) | Individual HA titers | Geom. mean titers† |
|---|---|---|---|---|
| .2 water | 512 | 400 | <10, 10, 20, 20, 20, 20 | <16 |
| " | 256 | " | <10, <10, 10, 10, 20, 20 | <13 |
| " | 128 | " | 10, 20, 20, 40, 80, 80 | 32 |
| .2 cortisone | 512 | " | 10, 20, 20, 20, 20 | 17 |
| " | 256 | " | 40, 80, 160, 160, 160, 320 | 127 |
| " | 128 | " | 80, 160, 160, 320, 320, 640 | 226 |
| .2 cortone vehicle | 256 | " | 10, 20, 20, 20, 20, 40 | 20 |
| .2 water | 0 | " | 1280, 1280, 1280, 1280, 1280, 2560 | 1437 |

Time between each inj. = 30 min. (at 36°C).

* Refers to No. of agglutinating doses of interfering virus injected into eggs.

† Individual hemagglutinin titers of less than 10 were arbitrarily taken as 10 in calculation of geometric mean titers.

of virus. Four eggs were used for each dilution. After incubation of embryos for 42-48 hours at 36°C, the individual fluids were tested for hemagglutinins by mixing 0.1 ml of fluid with 0.25 ml of 1% cells. The test was read by the pattern method(10) after 30 minutes at room temperature.

*Infectivity titrations of challenge virus.* Serial 10-fold dilutions of virus were made in beef-heart infusion broth and 0.05 ml of dilution inoculated allantoically into each of four 11-day embryos. After the usual incubation, the fluids were tested for development of hemagglutinins as described above. The $ID_{50}$ was calculated by the method of Reed and Muench(11). *Interference tests.* Groups of six 11-day embryos were inoculated allantoically with varying amounts of inactivated virus; after 30 minutes at 36°C, a challenge dose of active homologous virus was given. The individual allantoic fluids of live embryos (following incubation at 36°C for 42-48 hours) were collected and titrated for their content of hemagglutinins. Chemical treatment of the host consisted of allantoic injections of commercial cortisone acetate (Merck: 25 mg/cc) given 30 minutes before introduction of interfering virus into the host. Controls were injected in a similar manner with the cortone vehicle (0.9% benzyl alcohol, 0.9% sodium chloride, 0.4% polyoxyethylene sorbitan monooleate, 0.5% sodium carboxymethylcellulose, distilled water, q. s. ad 100%). *Hemagglutinin titration.* Serial 2-fold dilutions of virus were made in saline and 0.5 ml of viral dilution was mixed with 0.5 ml of 1% chicken red cells. The test was read after 30 minutes at room temperature by the pattern method. The final dilution of virus in the last tube showing complete agglutination was taken as the end point, and the titer expressed as the reciprocal of the dilution of virus in this tube.

*Results. Increased yield of virus in cortisone-treated eggs receiving minimal completely interfering doses of inactivated virus.* The results of interference tests in untreated and cortisone-treated embryos are shown in Table I. In the interpretation of results, hemagglutinin titers of 20 or less were accepted as complete interference with propagation of challenge virus since titers of residual interfering virus may equal but seldom exceed this value.

It is evident that introduction of 5 mg of cortisone into eggs 30 minutes before injection of interfering virus resulted in higher yields of viral hemagglutinins. Comparison of the mean hemagglutinin titers of control and experimental series given 400 $ID_{50}$ of challenge virus 30 minutes after interfering virus revealed 7- to 10-fold higher titers of virus in cortisone-treated eggs. This effect was noticeable, however, only when a minimal completely interfering dose (256 AD in the experiment shown in Table I) or less of inactivated virus was employed. Despite prior injections of cortisone, an increased yield of virus was not demonstrable in eggs receiving 512 agglutinating doses of interfering virus.

Similar findings were obtained in other tests using 40,000 $ID_{50}$ of challenge virus (results not shown); while injections of this amount of challenge virus may result in higher mean

titers of hemagglutinins in both control and experimental eggs, the increase in yield of virus in cortisone-treated hosts was approximately of the same order of magnitude as that observed with 400 $ID_{50}$ of challenge virus.

The effect produced by injections of cortisone was not ascribable to the action of substances present in the cortone vehicle, for prior treatment of embryos with 0.2 ml of the latter failed to cause any significant increase in final yield of viral hemagglutinins.

In other interference tests in which 11-day embryos were given 2.5, 5 and 7.5 mg of cortisone 30 minutes before interfering virus, it was found that all 3 concentrations of cortisone were equally effective in promoting an increased yield of viral hemagglutinins (results not shown) in the presence of a minimal completely interfering dose of inactivated virus. This was attested by the more or less identical yields of viral hemagglutinins in all three groups of cortisone-treated eggs.

*Discussion and summary.* The results reported herein have shown that prior injections of cortisone in embryonated eggs subsequently given minimal completely interfering doses of inactivated virus followed by challenge virus resulted in yields of viral hemagglutinins which were 7- to 10-fold greater than that found in untreated eggs. All 3 concentrations of cortisone (2.5, 5 and 7.5 mg) utilized in these experiments appeared to be equally effective in this respect.

An enhanced growth of influenza viruses in cortisone-treated eggs has been described previously(4), and the viral concentration of pooled allantoic fluids from cortisone-treated eggs was shown to be 123 to 155% of the viral concentration of control eggs (for influenza A virus). In the interference experiments reported in this paper, the yield of virus in cortisone-treated eggs was 700 to 1000% of the yield in untreated embryos. In view of this discrepancy in the increased yields of virus in the two reports, it would appear that enhancement of reproduction of challenge virus in residual free cells not blocked by interfering virus was not the sole determining factor in the increased yield of virus reported in this paper.

1. Dougherty, T. F., and Schneebeli, G. L., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 854.

2. Germuth, F. G., and Ottinger, B., *ibid.*, 1950, v74, 815.

3. Dougherty, T. F., White, A., and Chase, J. H., *ibid.*, 1944, v56, 28.

4. Kilbourne, E. D., and Horsfall, F. L., Jr., *ibid*, 1951, v76, 116.

5. Schmidt, L. H., and Squires, W. L., *J. Exp. Med.*, 1951, v94, 501.

6. Kligman, A. M., Baldridge, G. D., Rebell, G., and Pillsbury, D. M., *J. Lab. and Clin. Med.*, 1951, v37, 615.

7. Mogabgab, W. J., and Thomas, L., *ibid.*, 1952, v39, 271.

8. Schwartzman, G., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 835.

9. Fong, J., *J. Immunol.*, in press.

10. Salk, J. E., *ibid.*, 1944, v49, 87.

11. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

Received July 1, 1953. P.S.E.B.M., 1953, v84.

## Histopathology of Amino Acid Deficiencies. II. Threonine.* (20615)

EARL B. SCOTT AND CHARLES SCHWARTZ.† (Introduced by Walter L. Hard.)

*From the Department of Anatomy, School of Medicine, University of South Dakota, Vermillion, S. D.*

The unfavorable effect of protein deficiency on body economy has been frequently described. Whether or not there is a specificity of tissue response to protein inadequacy, or more particularly to specific amino acid deficiency, is not well established. A previous report of phenylalanine deficiency(1) described alteration of the anterior pituitary, gonads, accessory sex glands, bone and adrenal glands. Investigations of threonine reported

* This investigation was supported by a grant from the National Institutes of Health.

† Present address: 718 Dearborn St., Iowa City, Iowa.