0.1 cc. of the solution of the chemical intracutaneously. The only response was a temporary leucocytosis. The animals were observed for another 2 weeks before concluding the experiment. No changes were observed.

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Ascorbic Acid Stimulation of Specific Antibody Production.*

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Jusatz[1] reported that oral administration of vitamin A, B, C, or D is without appreciable effect on the bactericidal titer of the blood serum of rabbits or on specific-antibody production in this animal species. However, intravenous injection of a massive dose of vitamin C (sodium salt of ascorbic acid) increased the bactericidal index about two-fold and specific-precipitin production about five-fold. We have attempted to repeat his experiments with ascorbic acid and to extend his antibody-stimulating studies to include other enzyme-activators, other animal species and other types of antigens. The present paper summarizes our initial confirmatory results with ascorbic acid introduced parenterally into rabbits during the process of active immunization against horse-serum proteins.

A total of twenty 2000 gm. control rabbits were each injected intravenously with 0.5 cc. of horse serum. An equal number of rabbits of the same size and weight were each injected intravenously with 0.5 cc. of horse serum plus 100 mg. of crystalline synthetic ascorbic acid (Merck). Each injected animal was bled from the ear vein at frequent intervals during the next 50 days and the resulting antiserums were titrated for antihorse precipitins. Composite data from the two groups are recorded in Fig. 1.

Ascorbic acid plus horse-serum proteins caused a prompter formation of specific precipitins than occurred in the control group injected with undenatured horse serum. The antibody-stimulating

* Work supported in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

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1 Jusatz, H. J., *Z. f. Immunitätsforsch.*, 1936, **88**, 483.

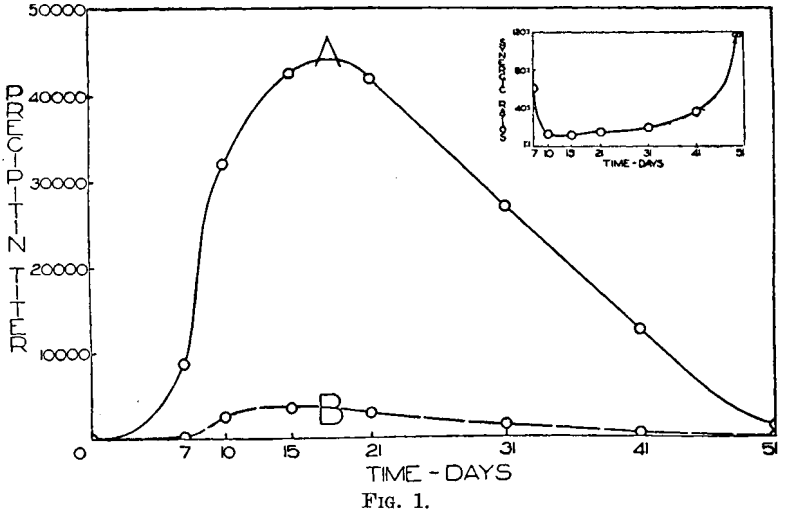

FIG. 1.
Vitamin C Stimulation of Specific Precipitin Production.

Precipitin-titers were determined as follows: 0.2 cc. of undiluted antiserum was overlaid with suitable dilutions of the antigen (horse serum). The tubes were then placed in the water bath at 37.5°C for 30 minutes and ring formation was observed. The tubes were then shaken and incubated for an additional period of 2 hours and then placed in the ice chest (4°C) overnight. Final readings were made the next morning. This is substantially the Lancefield technic.[2] The composite data were determined by averaging the ring-test titer and overnight readings and calculating a general average for the entire group of 20 animals. Recorded titers are expressed as precipitin-units per cc. of antiserum.

A. Composite data from twenty 2000 gm. rabbits, each injected intravenously with 0.5 cc. of horse serum mixed with 100 mg. of crystalline ascorbic acid (Merck).

B. Composite data from twenty 2000 gm. control rabbits each injected intravenously with 0.5 cc. of horse serum.

ratio for ascorbic acid when thus used is about 30:1 during the earlier stages of active immunization, falling to about a 12:1 ratio during the height of active immunity (14-21 days). Specific precipitins disappear from the control or non-vitaminized group by the fiftieth day, at which time the vitaminized group has a residual titer nearly equal to that of the control group at the height of active immunity.

In so far as the above tests were made with a purely arbitrary dose of ascorbic acid, an attempt was made to determine the optimal antibody-stimulating dose of this vitamin. Twenty-one 2000 gm. rabbits were divided into 7 groups of 3 each. Each group was injected intravenously with 0.5 cc. of horse serum plus varying amounts of ascorbic acid. The average 14- to 21-day precipitin-titers of the 7 groups are recorded in Fig. 2.

From this figure it is evident that massive intravenous doses of

[2] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

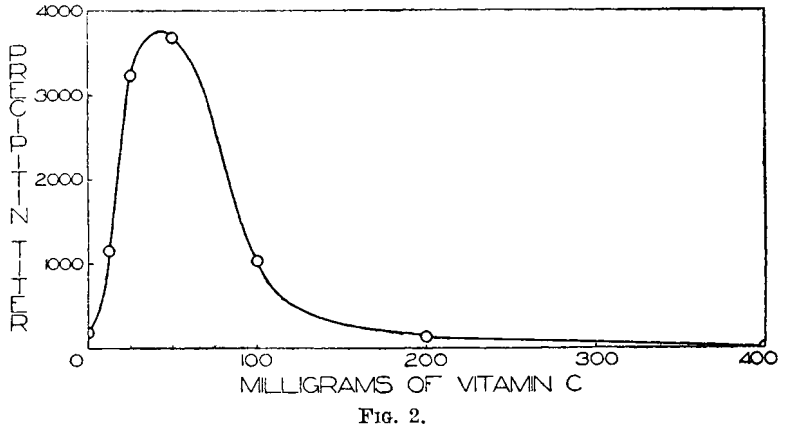

FIG. 2.

Determination of Optimal Antibody-stimulating Dose of Ascorbic Acid.

The curve shows changes in the average 14 to 21-day precipitin-titer in 7 groups of rabbits injected with varying doses of ascorbic acid mixed with 0.5 cc. of horse serum. Titers determined as in Fig. 1. The optimal antibody-stimulating dose is apparently midway between 25 and 50 mg. of ascorbic acid injected intravenously.

unneutralized ascorbic acid are toxic for rabbits, 400 mg., for example, completely suppressing specific-precipitin production. The optimal antibody-stimulating dose is apparently in the neighborhood of 37.5 mg. of ascorbic acid given intravenously. With this dose the average 14- to 21-day titer is about 20 times that of the control group.

The above result recalls the well-known differences between the antibody-stimulating and antibody-inhibiting doses of certain metallic salts reported by Walbum, *et al.*,[3] and of the alleged optimal prophylactic dose of vitamin C reported by Jungeblut and his associates in their studies of vitamin-C therapy in diphtheria[4] and poliomyelitis.[5]

To test the effect of variations in the site and method of injection of ascorbic acid, groups of about 4 rabbits were injected intra-abdominally, subcutaneously, or endermally with arbitrary doses of horse serum or with the same dose plus arbitrary amounts of ascorbic acid. Several other small groups were given multiple in-

[3] Walbum, E. L., *Comp. rend. Soc. Biol.*, 1921, **85**, 761; *Z. f. Immunitätsforsch.*, 1926, **47**, 213; *Z. Tuberk.*, 1927, **48**, 193; *ibid.*, 1928, **51**, 209; *ibid.*, 1929, **53**, 292; *Z. f. Immunitätsforsch.*, 1929, **61**, 146; Walbum, E. L., and Mörch., J. R., *Ann. Inst. Pasteur*, 1923, **37**, 396; Madsen, T., and Mörch, J. R., *Acta Tuberc. Scand.*, 1926, **2**, 99; *Z. f. Hyg.*, 1928, **109**, 224.

[4] Jungeblut, C. W., and Zwemer, R. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1229.

[5] Jungeblut, C. W., *J. Exp. Med.*, 1937, **65**, 127.

jections with vitaminized or non-vitaminized horse serum. In all cases stimulation of precipitin-production was noted, the contrast being practically identical with those recorded in Fig. 1.

In other groups the method of injection was varied by injecting the horse serum and ascorbic acid separately, such as at different times in the same ear vein, or in different veins, or by giving horse serum intravenously and vitamin C intraäbdominally. Stimulation of specific-precipitin production was noted by all of these technics, confirming the conclusions of Burky[6] and of Swift and Schultz[7] in their studies of the immuno-"synergic"‡ effects of staphylococcal toxin. The antibody-stimulation, however, was less pronounced in these separate injections than those previously obtained by mixing the horse serum and ascorbic acid before injection.

The relative efficiency of ascorbic acid and its sodium salt was also compared in small groups of animals. Sodium ascorbate prepared by Sollmann's technic[9] was found to be but about half as effective as unneutralized ascorbic acid. Sodium ascorbate, however, is apparently unstable, a commercial preparation tested on a small group of rabbits being without demonstrable antibody-stimulating effect.

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Treatment of Human Pellagra with Nicotinic Acid.

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Pellagrins can be cured while on a maize diet by the oral administration of a filtrate of liver which contains the so-called "filtrate factor" but which is free from riboflavin and rat antidermatitis factor.[1]

[6] Burky, E. L., *J. Allergy*, 1934, **5**, 466.

[7] Swift, H. F., and Schultz, M. P., *J. Exp. Med.*, 1936, **63**, 703, 725.

‡ On presentation of this paper before the Pacific Coast Branch, Oct. 16, 1937, Dr. Swift's use of the word "synergic" was criticized by Dr. Tainter and other attending pharmacologists. In their opinion some variant of the word "potentiation" would be more nearly in accord with accepted usage.[8]

[8] Sollmann, T., *A Manual of Pharmacology*, W. B. Saunders Co., 5th Ed., 1936, p. 80.

[9] Sollmann, T., *ibid.*, p. 115.

[1] Fouts, P. J., Lepkovsky, S., Helmer, O. M., and Jukes, T. H., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 245.