and Congo red can detoxify the toxin, then evidence of reticulocytogenic action, or of stimulation of bone marrow, could not be expected in a normal animal, where the toxin is not acting. It is possible that the toxin of pernicious anemia is not as readily detoxified by Congo red as are certain poisons and bacterial toxins.[11] The interesting fact remains that Congo red can act beneficially in a pathological state of the blood (pernicious anemia), and obviously non-specifically. Barker's recent negative report[12] is not final without more crucial and exhaustive tests.

Conclusions. 1. The variation of reticulocytes in normal pigeons has been determined. 2. Concentrated liver extract, injected intramuscularly, produced the usual rise in reticulocytes in normal pigeons, while Congo red, injected intravenously in the same and other pigeons for days, did not. This does not mean that Congo red can not act beneficially in pernicious anemia. The difficulties of bioassaying antipernicious anemia agents in normal animals are real and not always appreciated.

9587 P

Sensitization of Guinea Pigs to Cyclic Compounds and Effect on the Hematopoietic System.

ALFRED GOLDEN AND ALEXANDER SILVERGLADE. (Introduced by S. H. Gray.)

From the Snodgras Laboratories, City Hospital No. 1, St. Louis, Mo.

In recent years much experimental investigation of agranulocytosis has given rise to the view that "chemicals containing the benzene ring, may so depress the bone marrow that leucopenia results, and bacterial infection of the mouth may develop in consequence."[1] It has been the contention of Madison and Squier[2] that agranulocytosis is the result of drug *hypersensitivity* (to amidopyrine).

Landsteiner and Jacobs,[3] reported sensitization of guinea pigs to simple benzene ring compounds injected into, or spread on the skin of those animals for a number of consecutive days. Among those

11 Hanzlik and Butt, *J. Pharm. Exp. Therap.*, 1928, **33**, 260.

12 Barker, W. H., *Am. J. Med. Sci.*, 1937, **194**, 293.

1 Boyd, W., *The Pathology of Internal Diseases*, 2nd Ed., Lea & Febiger, Phila., 1935, p. 621.

2 Squier, T. L., and Madison, F. W., *Wis. Med. J.*, 1935, **34**, 175.

3 Landsteiner, K., and Jacobs, J., *J. Exp. Med.*, 1935, **61**, 643.

was 2:4 dinitro parachlor benzene. Accordingly, this procedure was repeated here, and blood studies made.

An alcoholic stock solution of 2:4 dinitro parachlor benzene was made, which when diluted with normal saline solution contained 1/500 mg. of the compound per 0.1 cc. Blood counts (r.b.c., w.b.c., and differentials) were made on all guinea pigs before, during, and after the injection period. Eight animals were used, 4 of which remained alive throughout the entire experimental period. Ffteen daily injections of 0.1 cc. each were made intracutaneously. The compound was found to be a slight irritant. Skin reactions, however, were read at all times by comparison with a previously uninjected albino guinea pig control. One month after the beginning of the injections each animal received 5 separate intracutaneous doses of 0.1 cc. each, and an albino control was similarly treated. The previously injected animals responded with marked zones of inflammatory edema at each injection site, a slight eosinophile rise, no significant change in the white blood cell count, no change in the red cell count, an increase in the lymphocyte percentage in three of the injected animals, and a decrease in the polymorphonuclear percentage in the same animals. The control animal's counts remained unchanged. Two weeks later the procedure was repeated using another albino control. The differential count in this instance was comparable to the level of these animals prior to the sensitizing procedure, with the exception of a slight eosinophilia. The red, and white cell counts remained unchanged. Seventeen days later each animal, and an albino control received 0.1 cc. of the solution intracutaneously. The procedure was repeated in 3 days, and blood counts were taken 6 days later. There was a slight decrease in the polymorphonuclear percentage and an increase in the lymphocyte percentage with no appreciable change in the red, or white cell counts. In each of the above instances the sensitized animals responded with marked zones of inflammation while the control showed no reaction, or one less than 2 mm. in diameter.

The route of injection was then altered. Nineteen days after the last injection each animal, and an albino control received 0.1 cc. of the diluted stock solution intracutaneously, 0.1 cc. subcutaneously, and 0.1 cc. intraperitoneally. No significant hematologic changes were observed.

The animals were observed for one month. Then each received intraperitoneally 1.5-2.0 cc. of an 18-hr. broth culture of an hemolytic *Staph. albus* in one instance; and a pneumococcus at another time. Both organisms were freshly isolated from human infections. After each inoculation each animal, and an albino control received

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.

9588

Ascorbic Acid Stimulation of Specific Antibody Production.*

R. R. MADISON† AND W. H. MANWARING.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

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.

† Eli Lilly and Co. Research Associate in Bacteriology, Stanford University, California.

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