of the injected animals was greater than was found in the corresponding guinea pigs of the first and second series which were subjected to half the dose extending over a longer period of time.

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A Rapid Method of Conferring Protection to the Peritoneum Against Experimental Peritonitis.*

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Goldblatt and I[1, 2, 3] demonstrated that by immunization with living or heat killed colon bacilli an immunity could be conferred upon dogs against experimental colon bacillus and fecal peritonitis. We found the immunity to be efficient 17 to 18 days after the first immunizing injection and that living organisms produced a greater protection than heat killed bacteria. Confirmatory evidence of an establishment of an immunity was supplied by Herrmann.[4]

The purpose of the work was to determine the shortest time in which an immunity could be conferred upon an animal so that it could survive an experimentally produced peritonitis. To determine the efficacy of different antigens, heat killed colon bacilli (culture 300) and a mixture of heat killed colon bacillus, streptococcus, *B. pyocyaneus, enterococcus, Bacillus mucosus capsulatus* and *B. welchii* was used. The bacteria composing the mixture were isolated from appendices with appendicitis and were of a determined marked virulence to animals. The second variable factor to be determined was the number of injections given to the animal. All the injections were administered intraperitoneally. The experimental peritonitis was produced either by injecting intraperitoneally into dogs the washings of 3 slants of live *B. coli* (300) suspended in 40 cc. of 2½% gum tragacanth or the washings of 3 slants of the bacterial mixture suspended in 40 cc. of a 2½% gum tragacanth.

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1 Steinberg, B., and Goldblatt, H., *Am. J. Path.*, 1927, **3**, 541.

2 Goldblatt, H., and Steinberg, B., *Arch. Int. Med.*, 1928, **41**, 42.

3 Steinberg, B., and Goldblatt, H., *Arch. Int. Med.*, 1928, **42**, 415.

4 Herrmann, S. F., *Arch. Surg.*, 1929, **18**, 2202.

A series of 4 groups of dogs were immunized with heat killed colon bacilli. One group (12 animals) had 1 injection of 1 billion organisms and on the following day was given *B. coli* peritonitis. Of the 12 animals, 10 died. The second group (10 animals) had 2 injections, the first of 1 billion bacteria and the second on the next day of 2 billion organisms. The day after the second injection, the animals were given colon bacillus peritonitis. Of the 10 dogs, 8 died. The third group (10 animals) had 3 injections of 1, 2 and 3 billion organisms respectively on successive days and *B. coli* peritonitis on the day after the third injection. Of the 10 animals, 9 died. The fourth group (28 animals in 3 different series) had 4 injections of 1, 2, 3 and 4 billion organisms respectively on 4 successive days and *B. coli* peritonitis on the day following the fourth injection. Of the 28 animals, 10 died. There was a survival of 65%. Considering the overwhelming peritonitis which was 3 to 5 times the lethal dose for an average dog (10 to 15 kilograms weight), the results of the 4 injections were considered satisfactory. With each group, normal control dogs were given simultaneously with the protected animals a colon bacillus peritonitis. The control dogs invariably died.

Since the maximum percentage of survivals was obtained with 4 successive immunizing doses with peritonitis on the day following the fourth injection, a similar procedure was employed with the bacterial mixture experiment. Twelve dogs were immunized with 1, 2, 3 and 4 billion bacteria (each organism forming one-sixth of the mixture) on 4 successive days and a colon bacillus peritonitis was given the day following the fourth injection. Of the 12 dogs, 9 died. A percentage survival of 25. All the accompanying normal control dogs with colon bacillus peritonitis died.

To determine the relative number of survivals of the colon bacillus and the bacterial mixture immunized dogs followed by a bacterial mixture peritonitis 2 groups of animals were used. One group of animals (10) was given 4 injections on successive days of heat killed colon bacilli and a bacterial mixture peritonitis on the day following the fourth injection. Of the 10 animals, 7 died. Another group of 9 animals was given 4 injections of a heat killed bacterial mixture on successive days and a bacterial mixture peritonitis on the day following the last injection. Of the 9 dogs, 7 died. The bacterial mixture immunized animals, even following a bacterial mixture peritonitis, showed a smaller percentage of survivals (22%) than the colon bacillus immunized group (30%). The colon bacillus (300) apparently is an excellent antigen in protecting the peri-

toneum against peritonitis. These experiments add to our previously expressed contention[5] that the protective process is not specific in nature. The serum and the peritoneal exudate of the protected animals with peritonitis did not contain humoral antibodies to account for their survival.

The principle of peritoneal protection used in the above experiments was applied to man, prior to abdominal operations requiring resection of bowel. The protecting material consists at present of 50 cc. of physiological salt solution in which are suspended 4 billion colon bacilli (culture 300) and is given in 4 successive daily injections of 5 cc., 10 cc., 15 cc., and 20 cc., respectively.

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Effect of Hyperleukocytosis (Hyperleukocytic Pre-Immunity) on Infection.*

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In the previous article[1] it was demonstrated that a single intraperitoneal injection of heat killed *B. coli* may prevent the death of a dog from an otherwise lethal *B. coli* peritoneal infection. The peritonitis was produced the day after the protecting injection. Four successive injections on successive days resulted in the survival of 65% of the animals. No humoral antibodies were found to account for this protection. In order to determine the rôle played by the cellular antibodies, cell counts of the peripheral blood and of the peritoneal exudate and peritoneal bacterial counts were made hourly during the course of a peritoneal infection in normal and protected dogs. Throughout these experiments, peritonitis was produced by the intraperitoneal introduction of 3 billion living *B. coli* suspended in 40 cc. of a 2½% gum tragacanth.

The peripheral leukocytes of normal dogs dropped rapidly and seldom exceeded the count prior to onset of infection. The leukocytes in the peritoneal exudate were polymorphonuclears and were

[5] Steinberg, B., and Snyder, D., *Arch. Path.*, 1929, **8**, 419.

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[1] Preceding article.