as the rats in the control group which received an equal amount of untreated vitamin. Our data therefore indicate that if there were a simultaneous destruction of APF activity, the degree of destruction could not be as extensive as that of the microbiological activity.

Summary and conclusion. When a solution of crystalline vit. B_{12} was subjected to a series of reducing agents between pH 4 and 7, at which range this vitamin is stable, a marked loss of the microbiological activity occurred. This loss of microbiological activity can be attributed to the reducing power of the agents, but is not necessarily related to the disappearance of the intensity of the red color. Our preliminary data also demonstrate that the destruction of the microbiological activity was not accompanied by the destruction to an equal extent of the APF activity.

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Failure to Isolate Brucella from Prostatic Tissue of Individuals Living in an Endemic Area.* (18093)

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Brucella have been recovered from the fallopian tubes, appendix, gall bladder, ovaries, serous surfaces of the intestines, joint fluid, feces and urine, and from extirpated human tonsils(1-4). Parsons and Poston(5) cultured brucella from the lymph nodes of 10 out of 19 cases of Hodgkins' disease, whereas brucella were recovered from the nodes of only one of 67 apparently normal individuals. Forbus and Gunter(6) reported that in 5 cases of Hodgkins' disease, *Brucella suis* or *Brucella melitensis* was isolated from one or more of the following: lymph nodes, liver, spleen, testis, bile, kidneys, and rib marrow. More recently, McVay(7) cultured prostatic tissue obtained from 34 apparently healthy individuals. *Brucella abortus* was isolated from the glands of 2 men, and *Br. melitensis* from a third. This report has prompted a similar study at the University of Minnesota Hospitals. The majority of the patients entering the hospitals are referred from rural areas, where brucellosis is endemic. In a recent epidemiologic survey of bacteriologic proved brucellosis in Minnesota, it was observed that the disease not only occurred more frequently in a rural population, but also, that brucellosis was recognized most frequently in adult males(8). Through the cooperation and help of Dr. C. D. Creevy, Director of the Division of Urological Surgery at the University of Minnesota Hospitals, patients presenting themselves for resection of the prostate gland were selected for study.

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Materials and methods. Specimens of prostate gland removed by a transurethral approach and collected in sterile Petri dishes were ground in sterile saline solution. Three-fourths ml of the saline suspension of tissue was spread over the surface of plates of trypticase soy agar,† which contained gentian violet in a dilution of 1:700,000(9). The inoculated plates were incubated at 37°C in a jar containing 10% carbon dioxide, and observed for bacterial growth for 15 days before being discarded. Slide agglutination tests were carried out with suspected colonies and rabbit antibrucella serum. A potent antibrucella rabbit serum was prepared by immunizing with a strain of *Br. abortus* (Lynch 524). Identification of the bacteria, other than for brucella, was not attempted, except for studying smears prepared with Gram's stain. Two large loopfuls of the saline suspension of ground prostate gland were also inoculated into 2 ml of tryptose phosphate broth, and incubated at 37°C for 18 hours in a jar containing 10% carbon dioxide. If growth appeared, the organisms were exposed to the antibrucella rabbit serum for evidence of agglutination. Control cultural and serologic studies of the foregoing type were carried out simultaneously with a culture of *Br. abortus*. Suspensions of prostatic tissue were also inoculated into the yolk sacs of living chick embryos by a method utilized in this laboratory for culturing brucella and described elsewhere(10). This is a reliable method for recovering small numbers of brucella. Two-tenths ml of tissue suspension was injected into 5 day chick embryos. The eggs were incubated at 37°C for 2 to 7 days, and then loopfuls of yolk were transferred directly to plates of trypticase soy agar containing gentian violet, and the plates were placed in a jar containing 10% carbon dioxide and incubation was carried out at 37°C for 15 to 20 days. Control experiments indicated that an inoculum of as few as 150 colonies of *Br. abortus* in prostatic tissues could be recovered from the eggs. Brucella were separated from other bacterial contaminants by the method of Amoss(2).

As an indication of the exposure of the patients to brucellosis, intradermal tests were carried out with brucellergen(9). A positive test represented a local reaction with 1 cm or more of edema 48 hours after injecting the antigen. Agglutination tests on the serum of the patients were performed with an antigen of *Br. abortus* obtained from the Bureau of Animal Industry, of the United States Department of Agriculture. The tube agglutination test was done with falling concentrations of serum, and evidence of agglutination was noted at the end of 24 and 48 hours of incubation at 37°C in a water bath.

Results. Prostatic tissue was obtained from 100 males. The ages of the patients varied from 50 to 90 years. The vast majority were farmers, and 10 stated that they had been exposed to Bangs' disease in their cattle. In 85 patients, the histologic diagnosis of the excised prostatic tissue was benign prostatic hypertrophy. The remaining 15 patients had carcinoma of the prostate. Except for the first 16 patients, suspensions of prostatic tissue were injected into chick embryos, and in not a single instance was brucella recovered. Prostatic tissue from every patient was cultured for brucella in tryptose phosphate broth and the tissues were also inoculated on plates of trypticase soy-gentian violet agar. Brucella were not isolated from any of the material. That many of the patients had been exposed to brucellosis was revealed by the fact that 29 patients or almost one-third of those tested had positive skins for brucellergen. Furthermore, 38 or approximately one-third of the patients had agglutinins for brucella in their sera. In 24 of these patients, the agglutinin titer was 1:80 or less. In 14 patients the titer was 1:160 or over. Two individuals had titers of 1:1280. While this information corroborated a history of exposure to the disease, in no instance was there definite evidence of an active infection being present.

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Summary. 1. Prostatic tissue obtained surgically from 100 consecutive males, who had lived for many years in an endemic area of brucellosis in Minnesota, were cultured for brucella and in no instance were brucella isolated. 2. On the basis of historical evidence, as well as positive intradermal tests and the presence of brucella agglutinins, many of the individuals had been exposed to brucellosis.

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Reversal of Aminopterin Inhibition in the Chick Embryo with the *Leuconostoc citrovorum* Factor.* (18094)

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In an earlier study(1), minute amounts of aminopterin were shown to inhibit development of the chick embryo. Under the conditions tested, this inhibition could be partially alleviated by thymidine, and more effectively by thymidine plus hypoxanthine desoxyriboside but not by folic acid, vitamin B_{12} or several other substances. The conclusion was drawn that aminopterin inhibited synthesis of thymidine and one or more of the purine desoxyribosides by the embryo, and that by eliminating the need for these synthetic reactions during the early stages of embryonic development, added desoxyribosides counteracted the inhibitory action of aminopterin. Since aminopterin appears to act as an anti-folic acid, the direct or indirect participation of folic acid in synthesis of these desoxyribosides was indicated. Recent evidence(2-6) indicates that the *Leuconostoc citrovorum* factor (CF) is a metabolically active form of folic acid, and that it can be formed synthetically from folic acid(5,6). Concentrates of this substance counteract aminopterin inhibition of *Leuconostoc citrovorum*(4). Amounts of CF previously available for testing failed to alleviate aminopterin inhibition of the chick embryo; it was pointed out, however, that larger amounts might prove effective(1). It is shown below that adequate amounts of CF partially counteract aminopterin inhibition of the chick embryo under conditions where folic acid is ineffective.

Experimental. Procedures used in injecting solutions and incubating eggs were exactly similar to those described previously(1,7). All injections made prior to incubation totaled 0.1 ml. Those made at 3 days totaled 0.2 ml.

Results. Table I presents the results obtained when aminopterin was injected alone or together with CF concentrate, folinic acid (6), or folic acid just prior to incubation. A pronounced effect of CF in reversing the inhibitory effect of aminopterin is evidenced not only by the increased hatch obtained in lots receiving adequate amounts of this substance, but by the greatly decreased number of embryos that died early in the incubation period. Larger amounts of the concentrate are required for this purpose in those lots receiving 20 μg than in those receiving 10

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