The results of this experiment and reëvaluation of the earlier roentgenograms seem to justify the conclusion that the lines of increased density which we observed in the roentgenograms at the site of repair of fractures, osteotomies or bone defects simply represented formations of cortical bone and had no definite relation to the phosphorus the patients were receiving. They were not growth or growth arrest lines.

Conclusions. 1. In the experimental animals, the feeding of inorganic phosphorus in doses large enough to cause transverse metaphyseal lines, has no demonstrable effect on the roentgenographic or microscopic appearance of the healing of fractures. 2. Transverse bands of increased density which may be produced at the juxta-epiphyseal region of growing children or animals by the feeding of phosphorus are probably "growth arrest lines" similar in their etiology to those produced by acute diseases such as scarlet fever.

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Increase of Bactericidins in the Serum of Cattle Following Recovery from Infection with *Brucella abortus.**

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Results given previously[1] have shown that bovine serum displayed a greater bactericidal activity against *Brucella abortus* than did a comparable amount of whole blood. Trials have accordingly been made to determine whether an increase in the titer of the serum bactericidins was to be found in individuals which had recovered from an infection with this organism.

The technic employed was essentially the same as that previously described.[1, 2] The serum was taken from (a) cows which had previously been artificially infected with *Br. abortus*, and from whose blood practically all agglutinins for the organisms had disappeared (*i.e.*, "recovered") and (b) from other animals which, insofar as

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[1] Irwin, M. R., Beach, B. A., and Bell, F. N., *J. Inf. Dis.*, 1936, **58**, 15.

[2] Shrigley, E. W., and Irwin, M. R., *J. Immunol.*, 1937, **32**, 281.

was known, had had no contact with the organism (*i.e.*, "normal").

A uniform quantity (0.3 cc) of serum, or of dilutions of serum, from an individual was added to each of a series of small tubes into which had been placed 0.05 cc of decimal dilutions of a 36- or 48-hour culture of a standardized saline suspension of *Br. abortus*. To each of the tubes containing diluted serum was then added 0.2 cc of complement of beef serum. (The complement was obtained by absorbing beef serum, with heat-killed cells of *Br. abortus*, at 0°C for 20-30 minutes.) Following incubation for 22-24 hours, the contents of each tube were poured into a petri dish with fluid pork-infusion agar, and incubated 5 or 6 days. For the bacterial control, 0.05 cc of each of the different dilutions was mixed with the fluid medium and poured into petri dishes. In these tests the number of organisms in the final dilution (−7) was generally between 10 and 50. Heated serum (0.3 cc), complement (0.3 cc), and the combination of heated serum (0.3 cc) and complement (0.2 cc), respectively, were treated in the same manner as the serum and diluted serum above. The results of this set-up, in contrast to the bactericidal activity of undiluted serum, provided a check on the efficiency of the absorption-technic.

Heated serum alone showed no bactericidal action, nor did complement by itself. Heated serum plus complement, however, was practically equal in activity to the undiluted, untreated serum, from "normal" or "recovered" animals, which allowed growth only in the −2 or −3 dilution and beyond representing the destruction of from 10^4 to 10^5 organisms. Some activity was usually displayed by the serum of "normal" animals at a dilution of 1 in 25, rarely at the next dilution, 1:125. In contrast, however, serum from "recovered" animals regularly caused a reaction in dilutions of 1 in 625, occasionally at higher dilutions. These latter animals had been artificially infected[2] (by way of the eye) 18 to 20 months before these tests were made, hence the possibility of a higher titer at an earlier time, in relation to the infection, cannot be excluded.

These findings indicate the presence of "immune" bactericidins, following an infection, in the absence of an appreciable titer of agglutinins. Further tests on these, and on the fluctuations of the titer of bactericidins in the serum of normal individuals from time to time will be presented later, with a discussion of their possible significance and their relation to the immune opsonins reported by Huddleson.[3]

[3] Huddleson, I. F., Johnson, H. W., and Meyer, D. B., Tech. Bull. No. 149, Mich. Agric. Exp. Sta., 1936.