while the cytoplasm shows no tendency to develop azure granules and form platelets The pathologic alteration of the megakaryocytes in some pernicious anemia bone marrows may account for the decrease in platelets occasionally found in cases of pernicious anemia.

From these observations upon the qualitative alterations of the marrow cells one must conclude that the lack of anti-pernicious anemia principle produces a panmyelopathy, in that three distinct cell lines are effected. The normoblastic or definitive series is inhibited while the pathologic red cell series, the megaloblastic, proliferates. The neutrophilic series is effected to the extent that there is developing in the marrow a pathologic series which gives rise to the abnormal pernicious anemia neutrophils (macropolycytes) of the peripheral blood. Also, in some cases the megakaryocytes are pathologically altered so that they are not producing platelets. This interpretation of the panmyelopathy in pernicious anemia is different from Tempka and Braun's[2] in that we do not believe the qualitative alterations in the neutrophils and megakaryocytes represent degenerative manifestations. The very fact that all of these above mentioned alterations disappear under adequate therapy and the marrow returns to normal favors our interpretation.[4]

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The Isolation of *Brucella abortus* from the Blood-Stream of Cattle.*

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Soule[1] has perhaps done the most extensive work on this subject. His method consisted of adding 50 cc. of whole blood to 450 cc. of glycerol-infusion-broth and then incubating under 10% carbon dioxide. More than 5000 ordinary "herd-run" cattle were studied. Two series of tests were made on each animal at intervals of approximately 6 months. In the first series of tests agglutinins were present in the blood of 2,237 of the animals but only 299 gave positive blood-cultures. In the second series agglutinins were present

4 Tochowicz, L., *Folia Haematol.*, 1934, **53**, 16; Braun, B., *ibid.*, 1934, **53**, 27.

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1 Soule, M. H., *Premier Congrés International de Microbiologie*, 1930, **1**, 606.

in 2,607 cases with 206 positive blood-cultures. There were 40 positive blood-cultures without concomitant agglutinins.

This cultural method evidently requires extreme aseptic precautions in order to prevent contamination of the cultures with extraneous organisms. In our use of this technic, contaminations with molds, spore-forming bacteria, and gram-positive cocci occurred in nearly two-thirds of our cultures and we isolated *Brucella abortus* but once in 75 attempts.

Because of these discouraging results we studied 4 other methods, using the blood of cattle and more extensively the blood of guinea pigs. Rainsford's[2] method of removing all the serum from a clot of blood and then culturing the clot was used as well as the technic of Boez and Robin.[3] The latter authors maintain that the bactericidal action of the blood is destroyed by mixing it with an acid citrate which prevents clotting and reduces the pH to approximately 5.5. This acidity will not kill the bacteria but does not allow their multiplication. The acidity is counteracted by cultivation in an alkaline medium at pH 8.3. The mixed blood and medium attain a pH of approximately 7.5 which is suitable for the growth of the majority of bacteria. Haring[4] has had success drawing blood into sufficient sodium citrate to prevent clotting, heating to 56°C. for 15 minutes and inoculating blood agar slants with the heated mixture. Stewart *et al.*[5] recommend the method of Massa and Battistini[6]: The blood is collected in *liquoïde* "Roche" (sodium polyanetholsulphonate), which is anticoagulant and destructive of leukocytes but not bacteria. Ten cc. of blood are drawn into 2 cc. of 1% aqueous solution of *liquoïde*. The mixture is incubated for 10 days and then transplanted to agar slants. Continued incubation of the blood and *liquoïde* mixture may be allowed and subcultures made at intervals.

The blood of each of 12 cows with long-standing Bang's disease was tested at least once by the above 5 technics. *Brucella abortus* was isolated but once from 105 cultures; this isolation was made by the acid-citrate method.

A study was then made to determine if a bacteremia was present in the incubative stages of the disease. Two heifers in early pregnancy were artificially infected. Daily blood-cultures were made

2 Rainsford, S. G., *J. Roy. Nav. M. Serv.*, 1933, **19**, 1.

3 Boez, L., and Robin, L. H., *Compt. rend. Soc. de biol.*, 1929, **101**, 1009.

4 Haring, C. M., personal communication.

5 Stewart, B., *et al.*, *California and West. Med.*, 1935, **43**, No. 2.

6 Massa, M., and Battistini, G., *Ztschr. für Bakt.*, 1 Abt., Originale, 1934, **131**, 241.

from these animals using Haring's heated citrate method. Table I shows the results of this study.

TABLE I.

Animal No.	Type and date of infection	Agglutinative titre of 1:100	*Brucella abortus* isolated on following dates
342	*Per os*, 1-28-36	3-2-36	Feb. 26, Mar. 2, 7, 11, 18, 24, 27, May 1
349	Conjunctival, 2-13-36	2-24-36	Feb. 24, Mar. 3, 4, 11, 28, April 28, May 5

Lübke[7] found a bacteremia in 90.4% of 21 recently infected animals and 5.9% of 81 cases of old infections. These studies were made by inoculating guinea pigs with 5 cc. of citrated blood and later culturing the guinea pigs. Direct cultures from the blood were not attempted. This inoculative method did not yield as good results in our hands as cultural methods. Cultures and inoculations were made in duplicate in 40 cases. *Brucella abortus* was never isolated by the inoculative method but was isolated 7 times by culture. On several occasions only 1 or 2 colonies of *Brucella* were found, indicating that relatively few bacteria were present in the blood. The numbers were probably not sufficient to infect the guinea pigs.

Supplementary work on cultural methods was done on the blood of guinea pigs. It was determined that a bacteremia occurs regularly 24-48 hours after intra-abdominal inoculations with *Brucella abortus*. Cardiac punctures were made at this time on 33 of the guinea pigs. Six cc. of blood were drawn from the heart and used in 1 cc. amounts to compare the above technics. *Brucella abortus* was isolated from 27 of these samples by one or more methods. It was isolated 12 times from the clot, 15 times from the whole-blood infusion-broth, 17 times from the acid citrate, 18 times from the heated citrate, and 21 times from the *liquoïde* cultures.

These results indicate that a bacteremia is not common in cattle with long-standing Bang's disease, but may be easily detected in recently infected cattle; also that the acid citrate, the heated citrate and the *liquoïde* culture methods are efficient for detecting a bacteremia due to *Brucella abortus*.

[7] Lübke, A., *Ztschr. f. Infektionskr.*, 1935, **47**, 240.