portal count rose to 12,000 *B. prodigiosus* per cc. by the end of 15 minutes. The most rapid absorption thus far recorded by us, however, was during acute anaphylactic shock (horse proteins). In one test, for example, the portal count rose within 10 minutes to over 1,000,000 viable microorganisms per cc. with over 100,000,000 colonies (agar plate) per gm. of liver emulsion at the end of the test (30 minutes).

This finding suggests a hitherto unemphasized factor in the clinical picture of anaphylaxis and related shock conditions. It also suggests a conceivable source of error in the production of specific antisera, any shock condition during immunization conceivably vitiating serum specificity.

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Reduction of Nitrates and Nitrites by Representatives of the Brucella Group.

C. E. ZOBELL AND K. F. MEYER.

From the George Williams Hooper Foundation, University of California, San Francisco, California.

The Brucella group is generally believed to lack the ability to reduce nitrates. Bergey[1] characterizes both *Br. abortus* and *Br. melitensis* as being non-reducers. Duncan and Whitby[2] record similar findings. Evans,[3] Topley and Wilson[4] and Lustig and Vernoni[5] report that nitrates are occasionally reduced.

An investigation was made to determine the factors which influence nitrate reduction and the cause of reported irregularities. A medium of the following composition was used: Peptone 2 gm., beef extract 1 gm.; NaCl 3 gm.; agar 2 gm.; H_2O 1000 cc. The reaction was adjusted to pH 6.8. The semisolid consistency favors the growth of the Brucella and accentuates the zone phenomenon, particularly when inoculated with a dilute suspension of cells. The majority of the Brucella grow in limited zones 2 to 6 mm. below

[1] Bergey, "Determinative Bacteriology," 1930, **3**, 366.

[2] Duncan and Whitby, "A System of Bacteriology," 1930, **5**, 395.

[3] Evans, *J. Infect. Dis.*, 1918, **22**, 580.

[4] Topley and Wilson, "Principles of Bacteriology and Immunity," 1929, **1** 508.

[5] Lustig and Vernoni, *Handb. d. pathogenen Microorganismen*, 1928, **4**, 520.

the surface. The *abortus* type* is usually the deepest, the *suis** are nearest the top and the *melitensis** are intermediate. Upon the addition of 0.2% KNO_3 to the medium, a reversal of positions is observed. Now the *suis* types multiply throughout the medium in the form of a pseudo-anaerobic growth, the *abortus* do so to a less extent but the growth zone of the *melitensis* is confined to a few mm. below the surface. The two former varieties are apparently able to obtain their oxygen requirements from the disintegration of nitrates.

Every strain of a collection of 425 Brucella tested was found to reduce nitrates vigorously. The presence of nitrites, the disappearance of nitrates and the formation of gas were observed as criteria of the reduction. Frequently 0.1% KNO_3 is quantitatively reduced in 2 days. There is very little tendency for nitrites to accumulate because they are simultaneously reduced, which may account for the apparently negative results reported in the literature. Many strains destroy 0.2% KNO_3 as well as the intermediate nitrites in 4 days. Every strain tested was able to cause the disappearence of 0.01% KNO_2 in 5 days; some destroyed 10 times as much during the same interval. A higher concentration of nitrite has a differential toxic effect. The *abortus* and the *melitensis* types are inhibited by 0.2% KNO_2 while the *suis* continue to multiply in this concentration. The latter variety is the most active nitrate and nitrite reducer. The *melitensis* types reduce nitrites more rapidly than the *abortus*.

Semisolid media expedite nitrate reduction. The beneficial effect is attributed to the improved growth and the favorable O/R potential which results from the partial exclusion of atmospheric oxygen. Nitrate reduction is more rapid in semisolid media than in broth or on agar slants. The multiplication of Brucella in semisolid peptone media normally causes the O/R potential to drop to near rH 11.5 at pH 6.8. The presence of nitrates retards this drop until their reduction is complete. The stabilizing influence on the O/R potential indicates an activation of nitrate oxygen.

Succinic, lactic and citric acids, glucose, xylose, galactose and arabinose accelerate nitrate reduction. Under these conditions nitrogen gas is liberated by some strains. Most of the *suis* and *abortus* produce gas but the *melitensis* types were found to be non-gas-producers under identical conditions. The influence of these carbon compounds on nitrate reduction offers further proof that they are attacked by the Brucella. Quantitative tests show that the presence of

* The types have been determined by extensive dye tolerance and H_2S tests, the results of which are now in press.

nitrates materially increases the utilization of glucose by all varieties.

Studies in synthetic media gave no evidence that nitrates can supply the nitrogen requirements. Nor did the addition of increasing amounts of utilizable nitrogen compounds effect nitrate reduction any more than could be accounted for by the multiplication rate. Compounds containing reduceable sulphur retard nitrate reduction. Cystine and thiosulfates in concentrations which are ordinarily tolerated become toxic in conjunction with nitrates.

Iodides exert an antagonistic or synergetic inimical action. Nitrate reduction is facilitated by 0.2% KI. However, 1.0% KI in media with 0.2% KNO_3 inhibits multiplication although 2.0% KI is tolerated in the absence of nitrates. The *abortus* and *melitensis* are more sensitive in this respect than are the *suis* types. The toxic effect is thought to be due to an accumulation of nitrites from the rapid reduction of nitrates. In media containing 0.2% each of KNO_3 and KI the nitrates quickly disappear. The test is of some differential value. Every *suis* type produced an abundance of nitrogen gas to the exclusion of the other 2 varieties when grown in such a medium.

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Cross Immunization Experiments with the Poliomyelitis Virus and that of Encephalomyelitis in Horses.

B. HOWITT. (Introduced by K. F. Meyer.)

From the George Williams Hooper Foundation, University of California, San Francisco, California.

During the summer and fall of 1930 and 1931, an epizootic of an acute encephalomyelitis broke out among the horses and mules in the rural districts of California.[1] Meyer, Haring and Howitt[2] report the recovery of a filterable virus as the causative agent and describe hemorrhage, oedema and acute inflammatory lesions in the brain with slight involvement of the cord. Since there were certain similarities between the equine malady and human poliomyelitis and since outbreaks of both forms chanced to occur simultaneously dur-

[1] Haring, C. M., Howarth, J. A., and Meyer, K. F., Univ. Calif. Agri. Exp. Station, 1931, Circular 322, 1.

[2] Meyer, K. F., Haring, C. M., and Howitt, B., *Science*, 1931, **74**, 227.