mixture and corresponding amounts of benzoquinone caused a progressive decrease in the *Lactobacillus acidophilus* counts in samples withdrawn at hourly intervals over a 4-hour incubation period. The final count was reduced to about one-third the original number of organisms while that of untreated controls increased by about 30%.

Several of the naphthoquinones mentioned above have been found to inhibit in high dilution the growth of staphylococcus, streptococcus, and pneumococcus in Gladstone's medium and in broth. Quantitative data as to the bacteriostatic and lethal concentrations required against these organisms in several media are now being obtained.

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Growth of *Brucella* in a Simple Chemically Defined Medium.

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Zobell and Meyer[1] studied the metabolism of the *Brucella* in simplified media. In the absence of knowledge of the accessory growth factors required by this group only slight growth was obtained when such media were used. They were able however to show that certain strains of *Brucella* could utilize the ammonium ion as a source of nitrogen and various organic acids as a source of carbon and energy. They did not find that the simple sugars, including glucose, could serve as the sole source of carbon and energy. McNutt and Purwin[2] found that slight growth of some strains occurred with arabinose or xylose as the sole source of carbon. It is worthy of note that in these instances ingredients were used which are now known to carry various accessory growth factor substances as contaminants.

Koser, Breslove and Dorfman[3] were the first workers to obtain luxuriant growth of the *Brucella* in a chemically defined medium by the addition of known accessory growth factor substances. Their medium consisted of 17 amino acids, glucose, inorganic salts, and the appropriate accessory factors. Following the elucidation of at least some of the accessory growth factor requirements of this group it has become possible to obtain abundant growth of some strains of *Brucella* in a very simple chemically defined medium.

Eight strains of *Brucella*, 3 each of *Br. abortus* and *Br. suis*, and 2 of *Br. melitensis*, were used in this preliminary work. Five of these were stock laboratory strains obtained originally from Dr. I. F. Huddleson and from the State Department of Health, Austin, Texas. Two of the strains of *Br. abortus* were recent isolations from Texas dairy cattle, and one strain of *Br. suis* was an isolation from a human case of undulant fever one week prior to its use in this study. All strains grew well in a normal atmosphere.

The inorganic salts used were of C.P. grade. The glucose was the grade usually used in

[1] Zobell, C. E., and Meyer, K. F., *Science*, 1930, **72**, 176; *J. Infect. Dis.*, 1932, **51**, 344.

[2] McNutt, S. H., and Purwin, P., *J. Infect. Dis.*, 1931, **48**, 292.

[3] Koser, S. A., Breslove, B. B., and Dorfman, A., *J. Infect. Dis.*, 1941, **69**, 114.

bacterial fermentation tests. The accessory factors were pure crystalline preparations. The biotin was a solution of crystalline biotin (free acid) obtained from the S.M.A. corporation. The various ingredients were dissolved in redistilled water and sterilized by filtration through a Seitz filter. Growth tests were performed as described in a previous paper.[4] For initial seedings the inocula ranged between 4,000 and 25,000 cells per cc as judged by plate counts.

After preliminary experiments to determine the optimum amounts of the various ingredients the following medium was devised:

Ammonium Sulphate	0.5	g
Sodium Chloride	7.5	"
Dipotassium Phosphate	1.0	"
Magnesium Sulphate	0.1	"
Sodium Thiosulphate	0.1	"
Glucose	1.0	"
Thiamine Chloride	200	μg
Nicotinic Acid	200	"
Calcium Pantothenate	40	"
Biotin	1.0	"

Redistilled water to make 1,000 cc.
pH adjusted to 6.8 to 7.2.

Of the 8 strains used 7 grew well in this medium; although growth was slower the maximum amount of growth obtained was comparable to that obtained in Tryptose broth. With the stated seeding growth usually appeared within 42 to 60 hrs and became maximal within 6 to 7 days. One strain of *Br. melitensis* failed to grow in this medium even when large seedings were used. It is of interest that this strain likewise failed to grow in Koser's amino acid medium. The 7 strains that grew successfully were carried through 10 serial transplants. Excellent growth was maintained throughout the series. They were then examined with the usual morphological, biochemical and immunological tests for conformity to the characteristics of the respective species. All presented typical smooth colonies and all could be readily typed by means of the tests used. For the immunological tests absorbed typing sera were used. These sera reacted to full titer with the appropriate strains.

Other sources of ammonium ion are satisfactory; however when potassium nitrate was used as sole source of nitrogen no growth of any strain occurred. This may possibly be due to the effect of this compound on the Eh of the medium. In the absence of sodium thiosulphate only slight irregular growth of a few strains occurred. Many other sources of carbon and energy may be used to replace glucose. A subsequent paper will deal with this question. The medium may be sterilized in the autoclave at 15 lbs for 15 min without impairing its growth promoting properties.

This medium is inexpensive and lends itself readily for the further investigation of accessory growth factors required by strains of this group as well as to other physiological problems concerning the *Brucella*. Utilization of carbon compounds can be judged by success or failure to obtain growth rather than by less critical methods which must necessarily be employed when complex media are used.

[4] McCullough, N. B., and Dick, L. A., *J. Infect. Dis.*, 1942, **71**, 193.