## NOTES

## EFFECT OF COBALT ON POPULATION CHANGES IN $BRUCELLA\ ABORTUS$

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Waring et al. (J. Bacteriol., 66, 82-91, 1953) have observed that growth of Brucella suis in an iron-deficient medium was moderately reduced but that population changes were markedly suppressed. Recent data (Altenbern et al., J. Bacteriol., 73, 697-702, 1957) have implicated oxygen tension and azide or cyanide inhibited respiration as major factors influencing population changes in static cultures of originally smooth Brucella abortus strain 19. Cobalt is known to inhibit hemoglobin synthesis (Laforet and Donnall, J. Biol. Chem., 218, 595-598, 1956), to produce filamentous cells of Clostridium perfringens exhibiting homolactic glucose fermentation similar to iron-deficient cells (Shankar and Bard, J. Bacteriol., 69, 444-448, 1955), and to suppress toxin formation and heme synthesis by Corynebacterium diphtheriae (Clarke, J. Gen. Microbiol., 18, 708-719, 1958). It was suspected that cobalt might suppress population changes in Brucella cultures by inhibiting synthesis of a cyanidesensitive respiratory enzyme.

Tubes of Gerhardt-Wilson synthetic medium (Gerhardt and Wilson, J. Bacteriol., **56**, 17–24, 1948) or Albimi broth containing various amounts of cobalt were inoculated with suspensions of several smooth isolates of B. abortus strain 19. Following 14 days of incubation at 37 C, colonial variants were detected and counted by the methods described by Braun (J. Bacteriol., **51**, 327–349, 1946). The results in table 1 show that  $5 \mu g$  of cobaltous nitrate per ml almost completely suppressed population changes in the defined

TABLE 1
Suppression by cobaltous nitrate of population changes in cultures of various smooth isolates of Brucella abortus strain 19

Co(NO <sub>3</sub> ) <sub>2</sub> per ml of Medium	Per Cent Nonsmooth Variants in				
	Gerhardt-Wilson medium				Albimi broth
	2583S	A19-3S	1407-1S	1407-3S	1407-S3
μg					
0	26	16	39	42	94
1	10	7	22	36	
5	0	< 0.1	0	<1	89
10					76
20	Reduced growth				54

Cultures in Gerhardt-Wilson medium were incubated for 14 days at 37 C. Albimi broth cultures were incubated for 10 days at 37 C. All results are averages of triplicate determinations.

medium whereas significant suppression of population changes in Albimi broth was evident only at 20  $\mu$ g of cobalt per ml. When either smooth or derived nonsmooth cells were grown in broth (Albimi) containing 50 to 75  $\mu$ g of cobalt per ml, cyanide-sensitive respiration (Q<sub>02</sub>(N) with lactic acid as substrate) was significantly depressed while cyanide-insensitive respiration remained unaltered. These results again suggest a relationship between population changes in Brucella cultures and the synthesis of heme enzymes involved in terminal oxidation.