NOTES

INHIBITORY EFFECT OF SODIUM BICARBONATE AND SODIUM CARBONATE ON SPORE GERMINATION OF *BACILLUS SUBTILIS*

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In the previous reports (Hachisuka *et al.*, J. Bacteriol., **69,** 339 and **69,** 407, 1955) the effect of various nitrogen sources on spore germination of *Bacillus subtilis* (PCI 219) was examined. It was found that some amino acids had a stimulative effect on the germination in media composed of caramel from dextrose and inorganic phosphates. In this work, to adjust pH of media, sodium bicarbonate was used. Since then it has been noticed that this substance and also sodium carbonate have a marked inhibitory effect on spore germination of *B. subtilis*. The results are shown in tables 1 and 2. From these it is obvious that sodium bicarbonate and sodium carbonate inhibit germination. In this case, it may be possible to say that HCO_3 and CO_3 act as an inhibitor to the germination. The mechanism of the inhibition is not yet decided.

From the above facts it has been clarified that the previous report (Hachisuka *et al.*, J. Bacteriol., **69,** 399, 1955) contained some errors. Among amino acids examined in that

TABLE 1

Inhibitory effect of sodium bicarbonate on spore germination of Bacillus subtilis

Concentration of Sodium Bicarbonate	Rate of Germination after Incubation for 2 Hr
M	%
1:20	11
1:40	15
1:80	18
0	98
Control without caramel from glucose	4

The basal medium was composed of 1 per cent caramel from glucose, 0.5 per cent L-asparagine and M/100 phosphate buffer. Since the pH of medium rose above 7.2 after the addition of sodium bicarbonate, buffers were used to maintain the pH at 7.2. For example, in the case of M/40 sodium bicarbonate, the phosphate buffer consisted of 1 part of M/100 Na_2HPO_4 and 9 parts of M/100 KH_2PO_4.

TABLE 2

Inhibitory effect of sodium carbonate on spore germination of Bacillus subtilis

Concentration of Sodium Carbonate	Rate of Germination after Incubation for 2 Hr
M	%
1:50	5
1:100	40
0	94

The basal medium and method of adjustment of pH were same as in table 1.

TABLE 3

Effect of some amino acids on spore germination of Bacillus subtilis (Comparison between use of sodium hydroxide and sodium bicarbonate as neutralizing substance to adjust pH of media.)

Kind of Amino Acid	Rate of Germination after Incubation for 2 Hr	
	Without caramel from glucose	With caramel from glucose
	%	%
L-Glutamic acid	6 (6)	15 (15)
DL-Aspartic acid	5 (5)	67 (13)
L-Arginine (hydrochloride)	0 (0)	60 (4)
DL-Lysine (dihydrochloride)	0 (0)	10 (0)
L-Histidine (hydrochloride)	0 (0)	44 (6)
DL-Ornithine (hydrochloride)	0 (0)	75 (5)

Numbers in parentheses show those reported in the previous report (Hachisuka *et al.*, J. Bacteriol., **69,** 399, 1955).

The basal medium was composed of 1% caramel from glucose and M/100 phosphate buffer. Each amino acid was added in concentration of 0.5%. The pH of media was each time adjusted to 7.2 with sodium hydroxide.

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report there were some which needed adjustment of pH to the optimal value (7.2) with sodium bicarbonate after being added into the basal medium. These were L-glutamic acid, DL-aspartic acid, L-arginine hydrochloride, DL-lysine dihydrochloride, L-histidine hydrochloride and DL-ornithine hydrochloride. To correct the errors the experiments have been done using sodium hydroxide as a neutralizing substance in place of sodium bicarbonate. The results are shown in table 3. In each case the rate of the germination in the media containing caramel from glucose is higher than that reported in the previous report except in the case of L-glutamic acid. Hence conclusions mentioned in the previous report have partially to be corrected. DL-Aspartic acid, L-arginine and DL-ornithine may belong to the group which has a stimulative effect on both the growth and the germination.

A SELECTIVE MEDIUM FOR THE ISOLATION OF *VIBRIO FETUS* AND RELATED VIBRIOS[1]

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The isolation of *Vibrio fetus* from aborted bovine and ovine fetuses, placental fluids and bull semen is particularly problematic since *Vibrio fetus* is most easily cultivated in a semisolid type medium. In such media most bacterial contaminants multiply more rapidly than do the vibrio and pure culture isolations seldom succeed. Ryff and Lee (Am. J. Vet. Research, **6,** 149, 1945), Prier (Vet. Med., **46,** 358, 1951), Plastridge and Esterbrooks (Am. J. Vet. Research, **13,** 145, 1952) and Rolle and Mundt (Zentr. Vet. Med., **1,** 759, 1954) determined the sensitivity of *V. fetus* to various dyes, antibiotics and sulfa drugs. Their findings might be adapted to therapeutic uses or for development of selective media for the isolation of the agent. Hughes and Gilman (Cornell Vet., **43,** 463, 1953) reported a technique employing the principle of stab cultures to isolate *V. fetus*, while Schneider and Morse (Cornell Vet., **45,** 84, 1955) suggested that the incorporation of ox bile into a medium might facilitate the isolation of the vibrios from contaminated materials.

A medium has been devised which will inhibit most troublesome bacterial contaminants. Unfortunately species of the genera *Pseudomonas* and *Proteus* are not inhibited. The formula and preparation of the medium is as follows:

Albimi brucella broth	28 g
Agar agar	5 g
Ox bile (fresh)	40 ml
Ethyl violet[3] to give final concentration	1:800,000
Distilled water	1,000 ml

Autoclave at 121 C for 20 min. Then aseptically add:

Bacitracin (R)	25,000 units
Polymyxin B Sulfate (R)	5,000 units
Actidione (R)	100 mg

Dispense aseptically in screw cap tubes to give a column of medium 3 to 4 centimeters in depth.

Inoculate the tubes with the material by stabbing deeply with either several loopfuls or about 0.1 ml by means of a capillary pipette. *V. fetus* growth appears after 4 to 10 days at 37 C incubation.

This medium has allowed the growth of 40 strains of *V. fetus* and a number of related pathogenic vibrios of animal origin. Eight strains of the saprophytic *V. fetus*-like organisms (Price *et al.*, Am. J. Vet. Research, **58,** 164, 1955) failed to grow in the medium. This selective action may

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[3] Ethyl violet (Ethyl Purple 6B, Hartman-Leddon Co.).