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Correspondence

TO THE EDITOR, *British Journal of Venereal Diseases*

Lack of effect of bicarbonate on the survival of *Treponema pallidum* (Nichols) in vitro

Sir,

No published studies have reported the effect of bicarbonate (HCO_3^-) on the survival of *Treponema pallidum* in vitro.

Carbon dioxide (CO_2) has been shown to be beneficial to *Neisseria gonorrhoeae, N meningitidis,* some stapylococci,[1] and other bacteria.[2 3] CO_2 serves as a carboxylating agent[4] and as an essential precursor for the synthesis of certain amino acids.[5] It is also required for fatty acid synthesis,[1 6] biosynthesis of purine ribonucleotides, which are precursors of DNA and RNA and are an integral part of many coenzymes.[6]

Different concentrations of sodium bicarbonate ($NaHCO_3$) were used in both a cell-free and cell-culture system to determine whether bicarbonate had any beneficial effect on the survival of *T pallidum* in vitro.

The medium in the cell-free system[7] was slightly modified by replacing the sodium thioglycollate with 2 mmol/l dithiothreitol (DTT). In the cell-culture system, Eagle's minimal essential medium with 10% heat-inactivated fetal calf serum, 0·5 mmol/l DTT, and 20 mmol/l N-2-hydroxyethyl-piperazine-N-2-ethane-sulphonic-acid (HEPES) was used.

T pallidum was harvested from infected rabbit testes.[7] Anaerobic incubation has been described.[7] For microaerophilic incubation, tubes with cottonwool plugs were equilibrated with 3% O_2[7] for two days before inoculation with treponemes. Treponemes were then added to an approximate final density of 5×10^6/ml, followed by HCO_3^- to give a final concentration of from 10^{-5} to 10^{-2} mol/l. The cottonwool plugs were immediately changed to rubber stoppers to prevent escape of CO_2. The pH remained at 7·3±0·2 in the cell-free system. The incubation temperature was 34°C.

The isolation and subculture of the rabbit testicular cell lines have been described.[8]

Viability of *T pallidum* in vitro was determined by the percentage motility under darkfield microscopy and their virulence in rabbits.[7] In the cell-culture system, attached treponemes were separated from tissue cells[8] before virulence was tested.

Ten experiments were performed in a cell-free medium under anaerobic conditions; a representative experiment is shown in the figure. There was no enhancement by HCO_3^- (10^{-2} to 10^{-5} mol/l) on the retention of motility of *T pallidum* (figure; *a*); the percentage motility in the presence of 10^{-3} and 10^{-2} mol/l HCO_3^- was consistently lower than the control. Similarly, HCO_3^- (10^{-2} to 10^{-5} mol/l) had no beneficial effect on the retention of virulence of *T pallidum* in vitro (figure; *b*).

Bicarbonate (10^{-5} to 10^{-2} mol/l) showed no enhancement of treponemal survival in two experiments under microaerophilic conditions with 3% O_2. The percentage motility (figure; *c*) and latent periods (figure; *d*) were virtually the same at all concentrations tested and were not appreciably different from the control tubes lacking bicarbonate.

In three experiments in cell-cultures, the pH of the medium dropped to 6·8 at day 4 in the control tubes while remaining at 7·2 in the presence of HCO_3^-/CO_2. When the pH of the medium was regularly adjusted, however, HCO_3^- (10^{-4} to 10^{-2} mol/l) had no enhancing effect on the retention of treponemal motility (figure, *e*) or virulence (figure, *f*) in the presence of tissue culture cells. Thus, in the media tested, HCO_3^-/CO_2 may serve mainly as a buffering system rather than as a reactant in any essential metabolic process.

Possible reasons why HCO_3^-/CO_2 had no enhancing effect on treponemal survival in vitro may be: (a) CO_2 requirements were satisfied by its endogenous metabolism, such as degradation of pyruvate and glucose.[9 10] (b) *T pallidum* under conditions which do not allow multiplication may have no capacity to use CO_2 even if it normally utilises it. (c) Even if *T pallidum* had the capacity to incorporate CO_2, it may be unnecessary. Since the medium was extremely rich, the presence of these nutrients may have shut down certain biosynthetic pathways. The incorporation of $NaH^{14}CO_3$ was insignificant when compared to total glucose incorporated.[10] (d) CO_2 would not be used for fatty acid synthesis since *T pallidum,* like other treponemes, cannot synthesise fatty acids.[11] (e) HCO_3^-/CO_2 may serve as a buffer in a closed system, but with HEPES (20 mmol/l) serving this purpose HCO_3^-/CO_2 was unnecessary in both the cell-free and cell-culture systems.

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Yours faithfully,

G H W Wong
B M Steiner
S R Graves

Department of Microbiology,
Monash University,
Victoria,
Australia.

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