EFFECT OF HEAVY METALS ON BACTERIAL ADHERENCE

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THE RELATIONSHIP between zinc, iron and bacterial infection remains obscure (Beisel, 1976). It is known that high concentrations of iron can promote infection, perhaps by favouring bacterial growth (Weinberg, 1978), and that serum concentrations of iron and zinc are frequently reduced during stressful situations including infection (Halsted and Smith, 1970; Beisel, 1976). Probably this is secondary to tissue sequestration (Weinberg, 1978), decreased gastrointestinal absorption (Beresford, Neale and Brooks, 1971; Beisel, 1976), and release of adrenocorticotrophic hormone (Falchuk, 1977). It is also known that adherence of bacteria to mucosal cells can precede colonisation and infection (Ward and Watt, 1972; Ellen and Gibbons, 1974; McNeish *et al.*, 1975; LaForce *et al.*, 1976). In the present study the relationship between heavy metals and bacterial adherence was investigated.

MATERIALS AND METHODS

Bacteria. Strains of Enterobacteriaceae, representing at least 50% of the total growth of sputum cultures, were obtained from specimens submitted to the University of Iowa Medical Microbiology Laboratory. They were stored in pure culture on nutrient-agar slants at 4°C and used within 30 days without passage. Chest radiographs and clinical histories from the patients gave no evidence of respiratory-tract infections. The bacteria were radiolabelled as previously described (Sugarman and Donta, 1979) with 25 µCi (methyl-³H)-thymidine (New England Nuclear) for 16 h in 5 ml of Eagle's Minimal Essential Medium (Gibco, Grand Island, New York). Before each assay the bacteria were centrifuged and washed thrice and resuspended in isotonic saline buffered with 0.05M Tris (Sigma) at pH 7.4; the bacterial concentrations were 1×10^8 /ml as determined by ³H-counts per minute (c.p.m.) and quantitative pour plates.

Buccal cells. Receptor buccal cells were collected by scraping the oral mucosal membrane of the investigator and resuspending the released cells in isotonic saline with 0.05M Tris at pH 7.4. The cell preparations were centrifuged and washed thrice and resuspended at a concentration of 1×10^5 /ml as determined by counts in a haemocytometer.

Radio-adherence assay. On three or more separate occasions, 1 ml each of bacteria and buccal-cell suspensions were incubated in duplicate at 37°C in the presence or absence of buffered commercially obtained heavy metal salts in a shaking apparatus for various periods of time. Control mixtures contained no buccal cells. Incubation mixtures were subsequently poured over 12-µm membrane filters (Nuclepore) and washed extensively. Remaining radioactivity on dried filters placed in toluene-based Omnifluor (New England Nuclear) was counted with a liquid scintillation counter. The counts (c.p.m.) so obtained, less control counts, were converted to bacterial numbers per cell.

Statistical methods. Standard *t*-test analyses were used. The ranges in the table and bars on the figure represent one standard deviation.

RESULTS

Effects of different metals

Results obtained with six strains of three genera of Enterobacteriaceae were similar. Iron

and zinc were the only two metals studied that increased adherence. Representative data are given in the table. The ferric and ferrous forms of iron were active in this regard. Experiments repeated with readily available sulphate salts of most of the metals yielded nearly identical results. To examine the effects of the buffer system and pH, the experiments were repeated with all cell washings and incubations performed separately in isotonic sodium chloride, isotonic Tris, and 0.05M Tris with sodium chloride at pH 6.0 and 8.0. The same adherence patterns were obtained, regardless of these amendments.

TABLE
Number of adherent bacteria per receptor cell after incubation of bacteria, buccal cells and 0.3 mmolar heavy metal salts for 30 min. at 37°C

Metal salt	Number of adherent bacteria of the stated species per buccal cell		
	<i>Klebsiella pneumoniae</i>	<i>Proteus mirabilis</i>	<i>Escherichia coli</i>
Sodium chloride	35 ± 5	17 ± 2	11 ± 2
Zinc chloride	80 ± 7*	40 ± 6*	32 ± 4*
Ferric chloride	69 ± 8*	50 ± 5*	37 ± 6*
Ferrous chloride	73 ± 10*	37 ± 4	34 ± 3*
Calcium chloride	28 ± 5	16 ± 2	8 ± 2
Lithium chloride	38 ± 6	20 ± 3	9 ± 2
Magnesium chloride	37 ± 5	19 ± 1	8 ± 1
Potassium chloride	34 ± 4	21 ± 4	11 ± 3

* = Significant ($p < 0.05$) increase over result obtained in control test with sodium chloride.

FIG. 1.—Effect of zinc concentration on adherence. Average number of adherent bacteria per buccal cell was determined after 60-min. incubation of 1×10^8 bacteria with 1×10^5 buccal cells in test mixtures containing a range of concentrations of zinc chloride.

FIG. 2.—Localisation of zinc activity. Preincubation of bacteria and buccal cells separately with buffered saline or 0.3 mmolar zinc chloride for 30 min. (1–4 below) preceded incubation of mixtures of 1×10^8 *Klebsiella pneumoniae* and 1×10^5 buccal cells for a further 30 min. 1 = Both with zinc; 2 = bacteria with zinc, buccal cells with buffer; 3 = bacteria with buffer, buccal cells with zinc; 4 = both with buffer; I = standard deviation.

Effects of heavy-metal concentration and period of incubation

With both strains of *Escherichia coli*, significant ($p < 0.05$) increases in adherence noted with 0.3 mmolar zinc and iron were detectable after 5 or more minutes of incubation and were maximal after 1–1.5 h. Data obtained for the other genera tested were similar. Zinc and iron concentrations of 2×10^{-5} M and 1×10^{-4} M respectively led to significant ($p < 0.05$) increases in adherence after incubation for 60 min. Adherence further increased with the addition of more metallic salts up to a concentration of 5×10^{-3} M, above which it remained stable. Representative data are illustrated in fig. 1.

Localisation of site of zinc activity

Preincubation for 30 min. of each strain of bacteria with the zinc salt, with subsequent removal of the salt by centrifugations and washings, reproduced the above effects. Preincubation of the metal salts with the receptor cells yielded attachment not significantly different from that obtained with the controls. The results obtained with a strain of *Klebsiella pneumoniae* are illustrated in fig. 2.

DISCUSSION

In the in-vitro system described, zinc and iron salts, but not salts of several other metals,

increased the adherence of Enterobacteriaceae to human buccal epithelial cells. Because adherence of bacteria to mucosal cells can precede colonisation and infection (Ward and Watt, 1972; Ellen and Gibbons, 1974; McNeish *et al.*, 1975; LaForce *et al.*, 1976), zinc and iron may be important in the colonisation of mucosal tracts with commensal bacteria, or with pathogenic bacteria before infection.

It has been suggested that high concentrations of iron promote infection by favouring bacterial growth (Weinberg, 1978), but this does not explain these experimental results. Bacterial multiplication would correspondingly decrease the amount of ^3H per bacterium, and multiplication of enterobacteria does not occur in buffered saline at 37°C for well over an hour when tested in this system.

Physiological concentrations of zinc salts seem to interact with the bacteria within a few minutes to cause increased adherence to buccal cells. The nature of the interaction remains to be determined.

SUMMARY

A preliminary examination has been made of the effects of salts of heavy metals on bacterial adherence. ^3H -thymidine labelled strains of Enterobacteriaceae isolated from sputa were incubated with human buccal cells and metallic salts. 10^{-4}M zinc or iron salts significantly increased adherence of Enterobacteriaceae to human buccal cells in an in-vitro system. These effects were not altered by variation of the buffer system used, and seem dependent upon interactions between metals and bacteria that occur within about 5 minutes.

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