THE EFFECTS OF TEMPERATURE ON E, COLI, FAECAL STREPTOCCOCCI AND BIFIDOBACTERIA AS FAECAL POLLUTION INDICATORS

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ABSTRACT

The experimented survival in water of thermotolerant coliforms, faecal streptococci and bifidobacteria (as T90), reveals that faecal streptococci may survive best at temperature between 10 and 25. The survival of thermotolerant coliforms was clearly better than survival of the other bacteria at 4 and 30; but the difference in survival of thermotolerant coliforms and faecal streptococci is not statistically significant at 30. Survival of bifidobacteria at the six temperatures used in this study was not as good as that of the coliforms or faecal streptococci. As a result, a bifidobacteria index in tropical waters (where temperatures of 30 or more are easily attained) may be routinely applied for detecting only very recent pollution.

MATERIALS AND METHODS

River Kubanni water samples were collected in keeping with Report 71 (1983) and analysed by membrane filtration. Samples were transported under ice in a picnic box and analysed within 2hrs of collection. Recommended procedures in Report 71 (1983), were followed thoroughout.

After mixing the sample by shaking several times, IL was put in each of six pyrex glass beakers, which were covered with aluminium foil. Each beaker was incubated in Gallenkamp type 2 incubators set at different temperatures. The incubators were set at 4, 10, 15, 20, 25 and 300°C respectively.

The original sample was thoroughly mixed and 10ml portions of it removed, serially diluted and filtered in duplicates through 0.45micron pore size cellulose acetate membrane filter from Oxoid. This was to determine the initial populations of the indicators in the water.

Each day for seven days, 10ml portions of water in each beaker at the respective incubator temperatures were withdrawn after mixing, serially diluted and filtered in duplicates as previously described.

INCUBATION

To isolate bifids, the filters were rolled unto the surface of YN - 17 of Oragui (1982), and incubated anaerobically in Oxoid HP11 anaerobic jars. The lids of the plastic petri - dishes had holes in them to permit circulation of gases through them during incubation. Anaerobiosis was achieved by means of Oxoid BR55 gas generating envelopes, and checked with Oxoid BR38 anaerobic indicators. The plates were incubated upside down for 48hr.

Faecal streptococci were isolated on Slanetz and Bartley medium from Oxoid. Duplicate filters were rolled unto the surface of the medium in petri-dishes which were wrapped in cellophane to prevent drying; and incubated upside down for 48hr. at 44 - 45°C.

Thermotolerant faecal coliforms were isolated on membrane Enriched Teepol Broth (METB) from Oxoid. Incubating absorbent pads (also from Oxoid) were saturated with METB in petri-dishes, and membrane filters were carefully placed on them in duplicates. The petri-dishes were wrapped in cellophane to prevent drying, and incubated upside down for 4hr at 35°C followed by 16 to 20hr at 44.5°C.

INTRODUCTION

The genus Bifidobacterium is potentially valuable as a bacterial indicator of faecal pollution (Morgan, 1978; Oragui, 1982). Bifidobacteria (bifids) are known to be the predominant species in the human intestine (Peach et al. 1955). In addition, some authorities (Gyllenberg et al. 1960) state that bifids can survive as well as, if not better than coliforms, in water.

In general, the survival rates of bacteria tend to be low when they occur outside their usual habitats. This is attributable to the antagonism and unfavourable condition prevalent in such environments. These factors do not usually operate in isolation, and observed reactions by bacteria to an environment are the result of the cumulative effects of all the factors taken together.

In the field situation, it is difficult, if not impossible, to control or monitor all the factors which might affect the survival of indicator bacteria. So, a comprehensive series of investigations are needed to compare the survival of indicator bacteria under different controlled conditions in any laboratory.

In this study, the effects of different temperatures - 4°C, 10°C, 15°C, 20°C, 25°C, and 30°C on the T90 of E. coli, faecal streptococci and bifidobacteria were investigated.

RESULT

Figures 1 to 6 are plots of the logs of indicator bacteria densities against time, at different temperatures. Apart from the 4°C, plot, E. coli always showed a tendency to increase in numbers during the first 24hr before the densities dropped over time. Both faecal streptococci and bifidobacterial did not demonstrate this tendency at any temperature except at 3 and 25°C when there was a slight increase in the number of faecal streptococci during the first 24hr.

At 10°C, the decline E. coli and bifids densities paralleled each other initially but thereafter, there was a similarity in the decline of both faecal streptococci and bifids (see Figure 1). This trend is also evident at other temperatures as shown in the subsequent Figures 2 to 6.

Generally, although initial bifid numbers were always higher than those of the other two indicators, bifids were always the least numerous at the end of the experiments, while E. coli were always most numerous.

Between 20°C and 30°C, bifids disappeared from the samples within 5 days (at 20°C) and 4 days (25°C and 30°C). E.coli persisted for all of the seven days even at 30°C while faecal streptococci could not be detected after the sixth day at 30 (see Figures 4 to 6). The T90 represents the time for 90% of bacteria to die in water.

When the survival rates (as T90) of the three bacteria were compared (Figure 7), it became evident that they all had an enhanced survival at 15°C.

The recorded T90 for E. coli at 10°C is aberrant and must have arisen from some experimental error, probably improper dispersion of cells in the water (see Fig. 7)

From Figure 7, it is noted that faecal streptococci had the highest survival rate of the three indicators at 10°C, 15°C and 20°C. Only at 5°C and 30°C did E. coli record the highest survival rate.

T90 for bifids were the lowest at all temperature except 10°C when E. coli was the least survivor as previously observed.

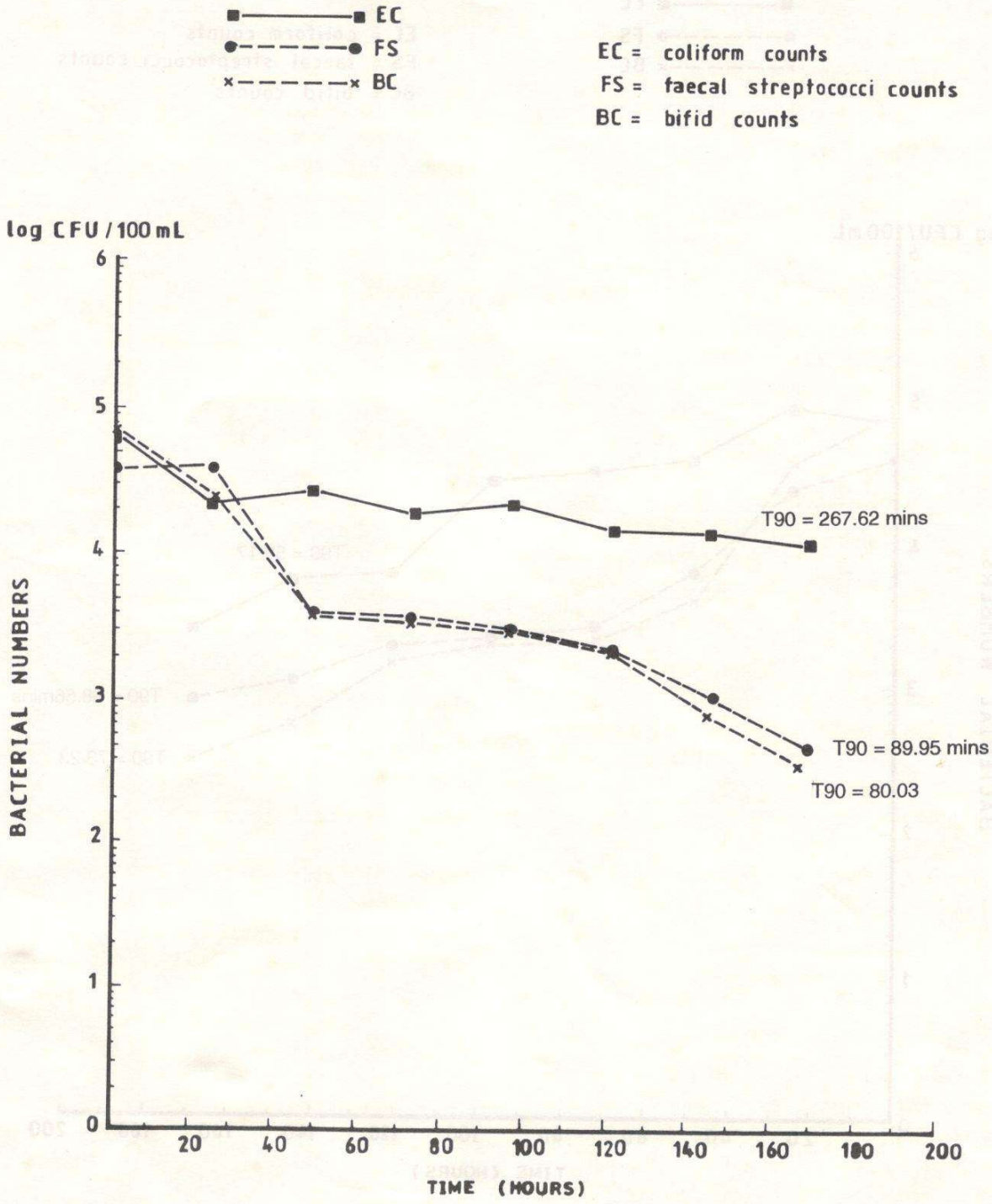


Fig. 1 Survival of indicators at 4 °C.

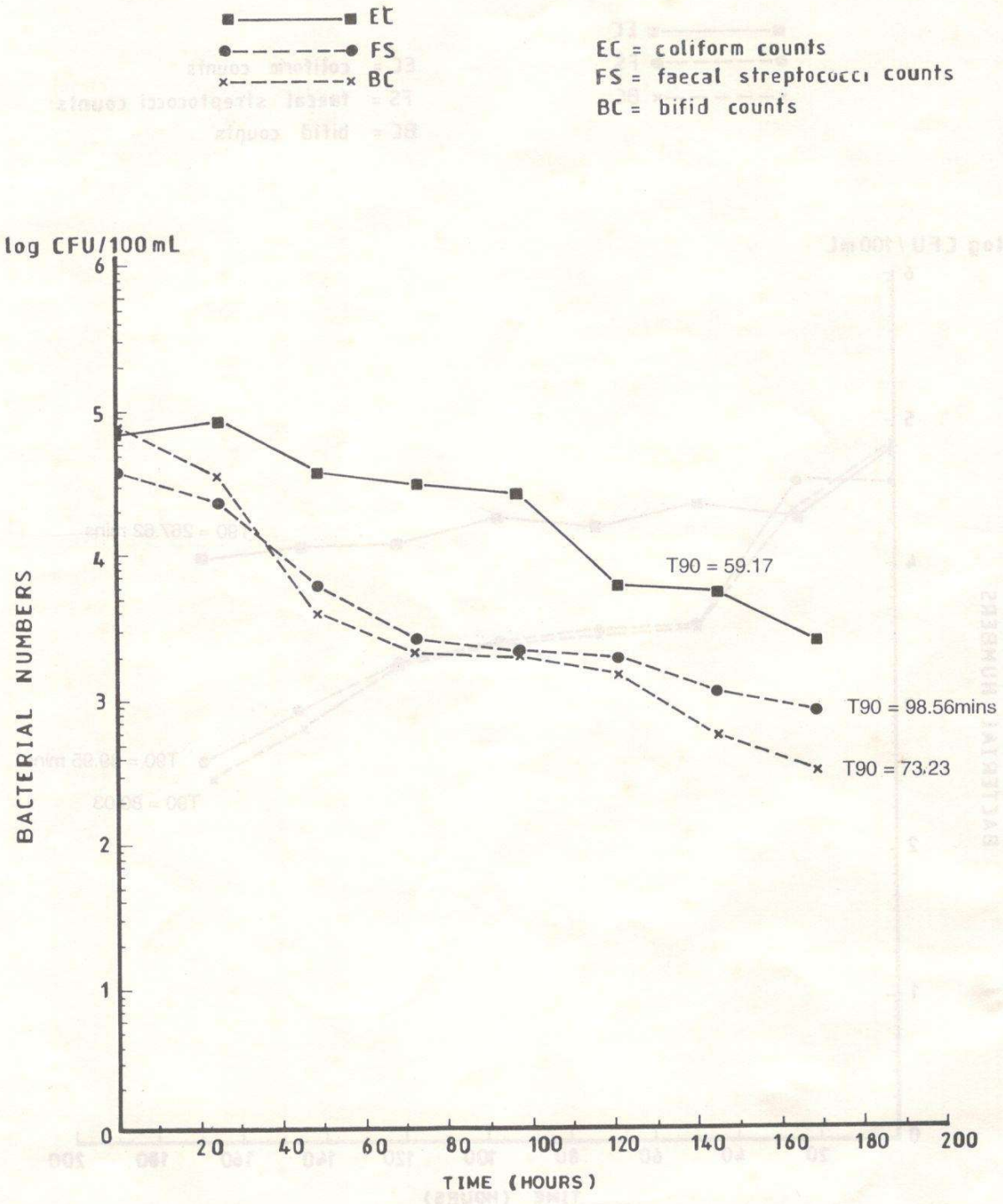


Fig. 2 Survival of indicators at 10°C

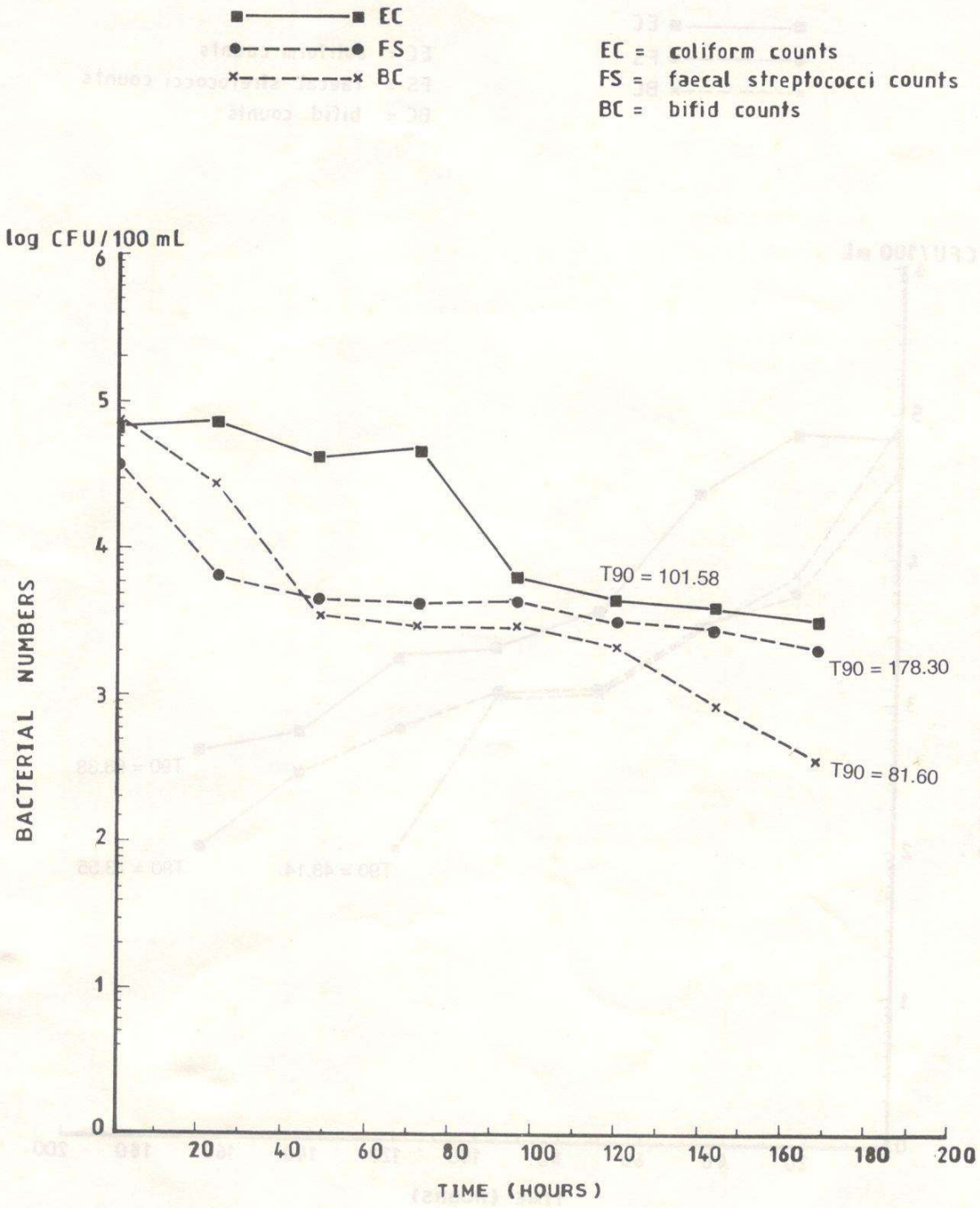


Fig. 3 Survival of indicators at 15 °C

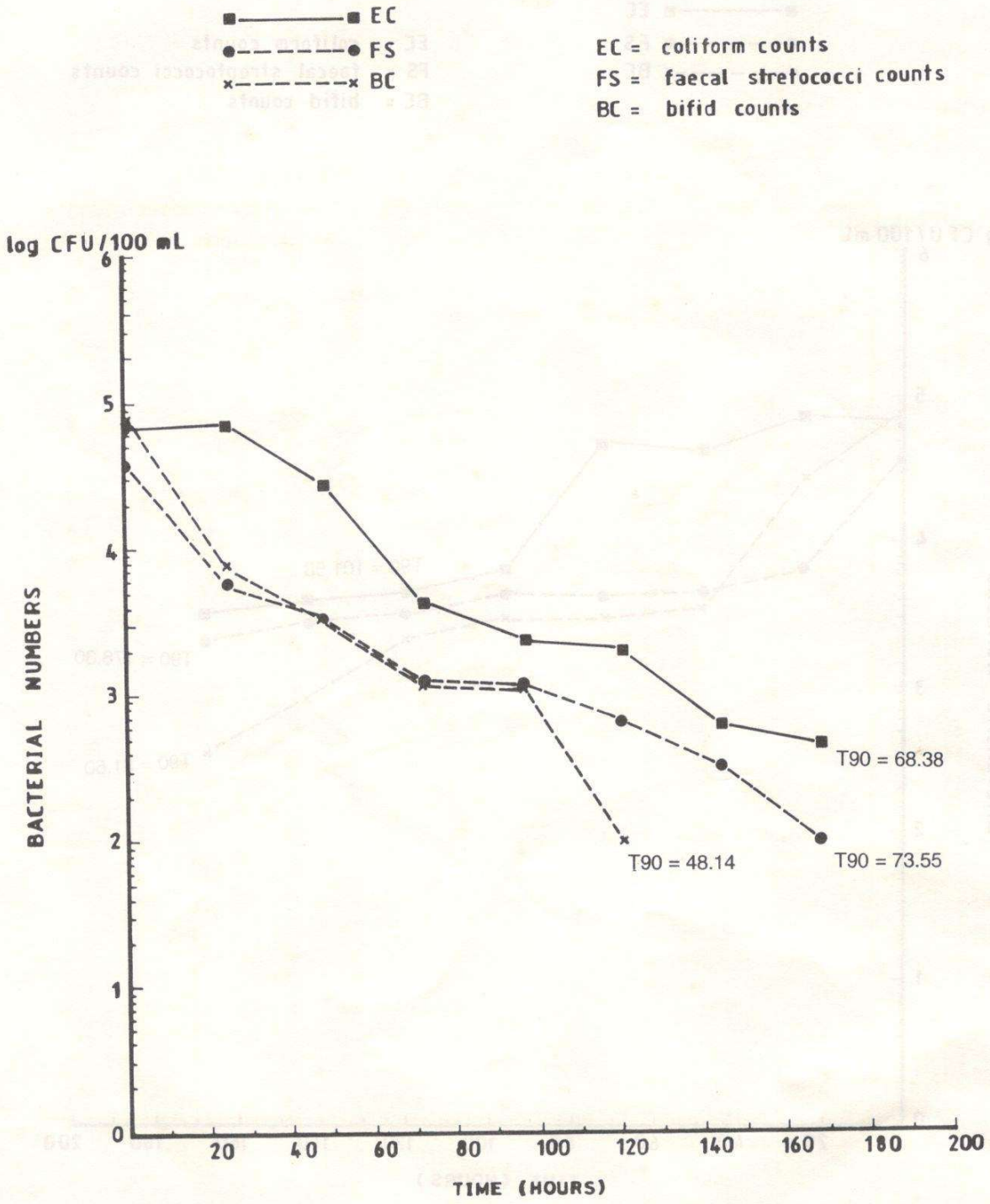


Fig. 4 Survival of indicators at 20 °C .

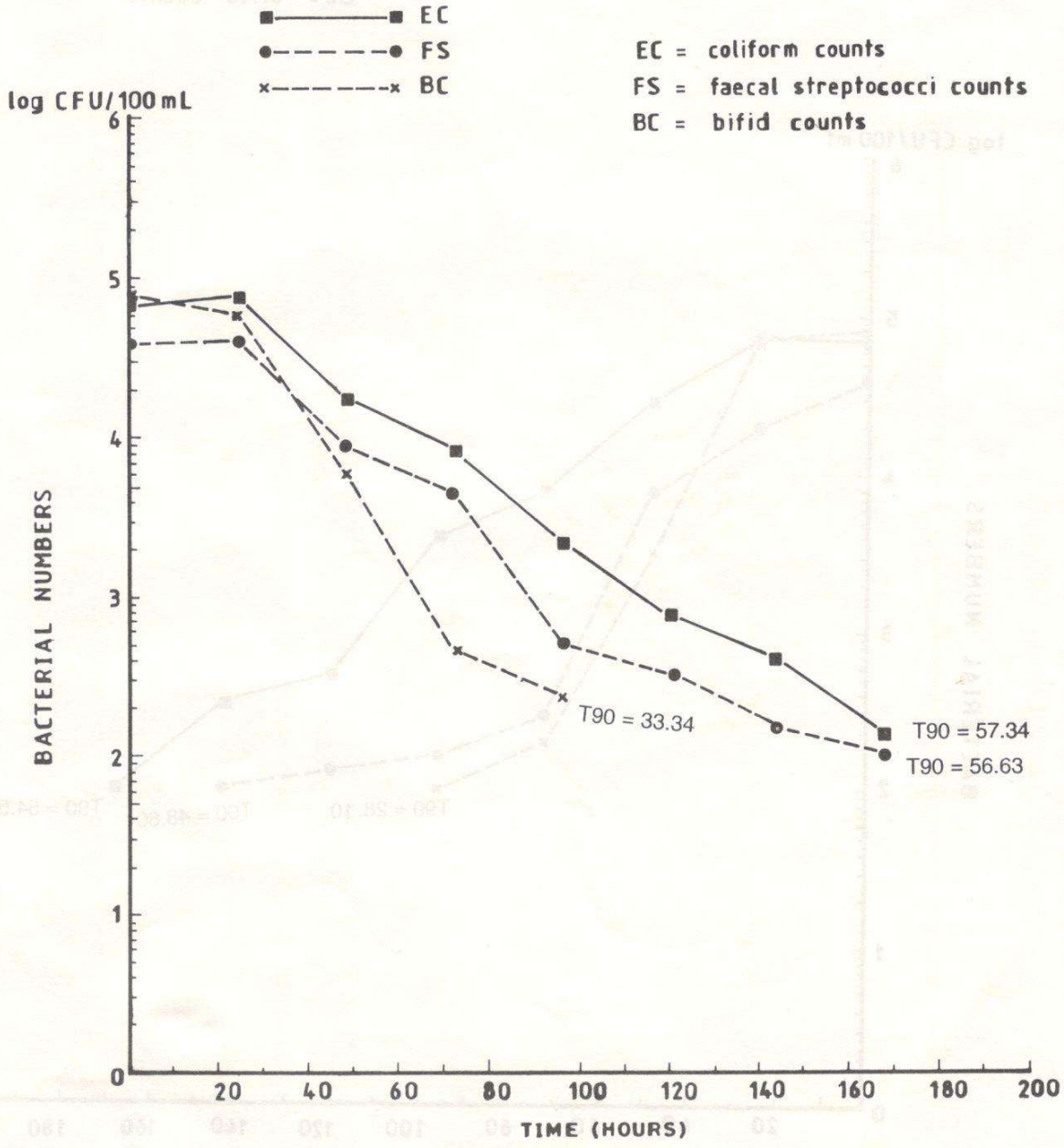


Fig. 5 Survival of indicators at 25 °C .

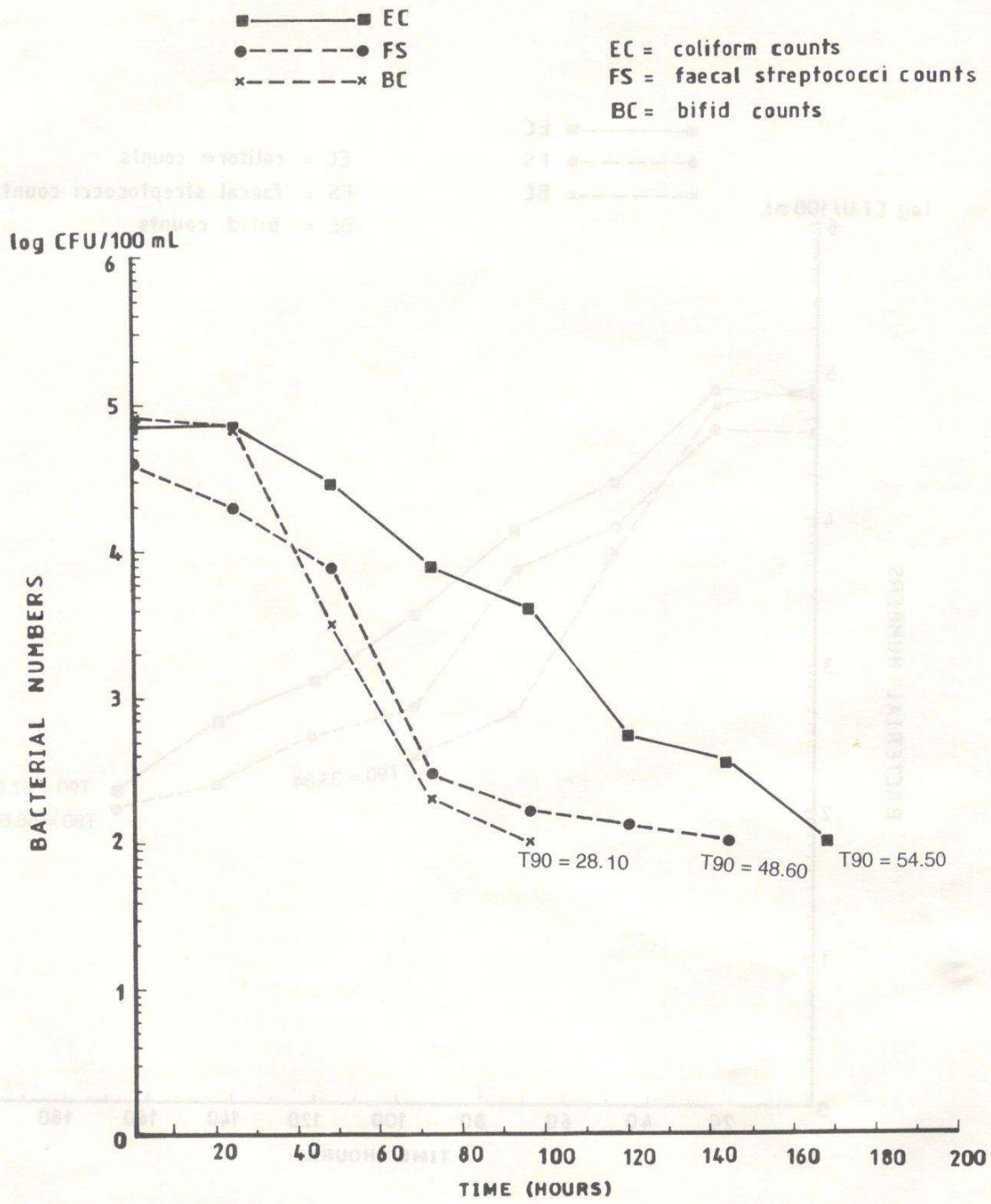


Fig. 6 Survival of indicators at 30 °C .

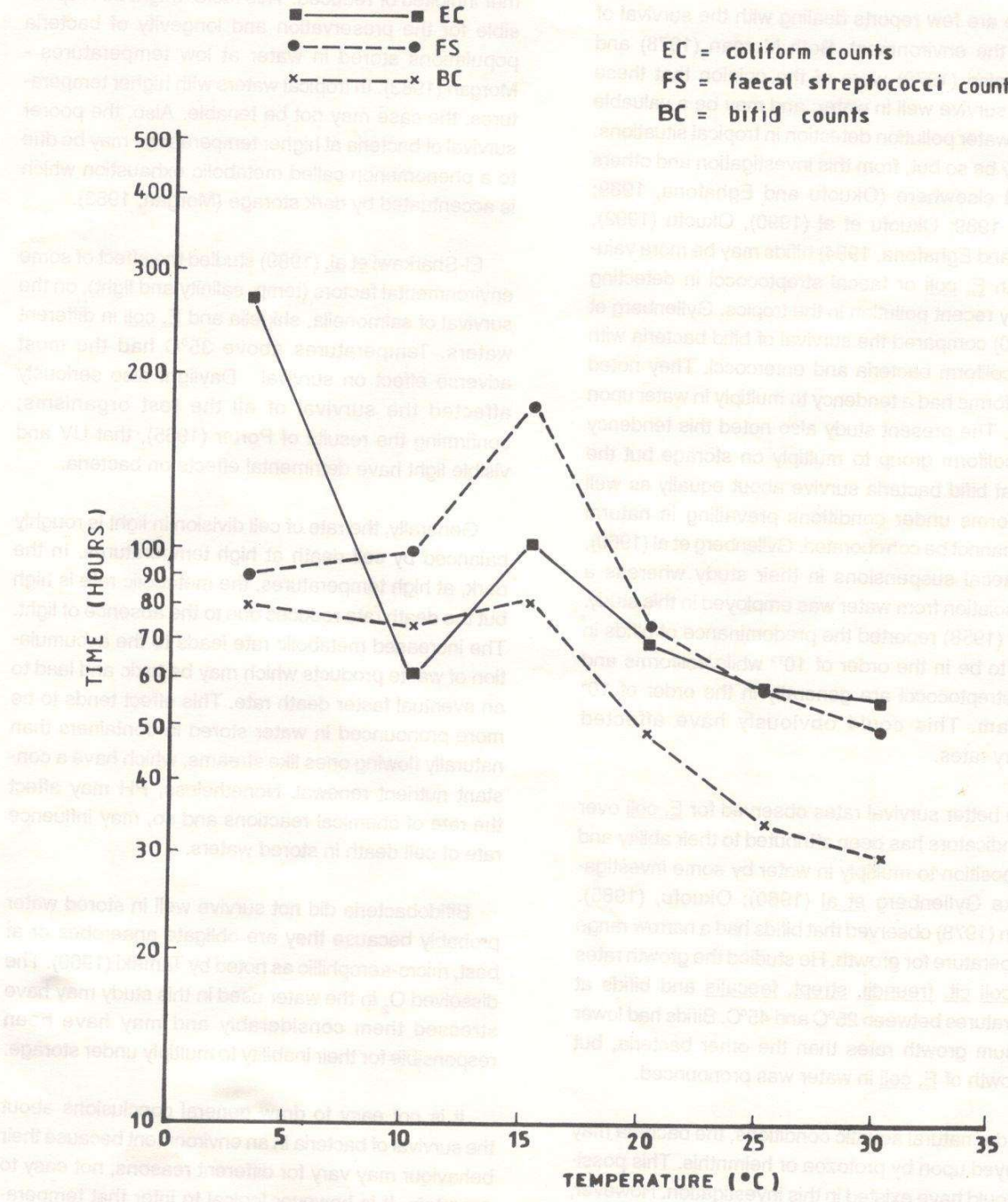


Fig. 7 Survival of indicators (T_{90}) at different temperatures.

DISCUSSION

There are few reports dealing with the survival of bifids in the environment. Both Morgan (1978) and Bhoonchaisri (1979) were of the opinion that these bacteria survive well in water; and may be a valuable index in water pollution detection in tropical situations. This may be so but, from this investigation and others reported elsewhere (Okuofu and Eghafona, 1989; Okuofu, 1989; Okuofu et al (1990), Okuofu (1992), Okuofu and Eghafona, 1994) bifids may be more valuable than E. coli or faecal streptococci in detecting only very recent pollution in the tropics. Gyllenberg et al. (1960) compared the survival of bifid bacteria with that of coliform bacteria and entercocci. They noted that coliforms had a tendency to multiply in water upon storage. The present study also noted this tendency of the coliform group to multiply on storage but the view that bifid bacteria survive about equally as well as coliforms under conditions prevailing in natural waters cannot be corroborated. Gyllenberg et al (1960), used faecal suspensions in their study whereas a direct isolation from water was employed in this study. Mossel (1958) reported the predominance of bifids in faeces to be in the order of 10^{19} while coliforms and faecal streptococci are generally in the order of 10^{8} per gram. This could obviously have affected recovery rates.

The better survival rates observed for E. coli over other indicators has been attributed to their ability and predisposition to multiply in water by some investigators like Gyllenberg et al (1960); Okuofu, (1985). Morgan (1978) observed that bifids had a narrow range of temperature for growth. He studied the growth rates of E. coli cit. freundii, strept. faecalis and bifids at temperatures between 25°C and 45°C. Bifids had lower maximum growth rates than the other bacteria, but the growth of E. coli in water was pronounced.

Under natural aquatic conditions, the bacteria may be preyed upon by protozoa or helmnthis. This possibility could have existed in this investigation. However, it is unlikely that such a phenomeon would have accounted for the better survival of one species of bacteria over another, unless the predation was selective.

At low temperatures, the activity of protozoa is either inhibited or reduced. This factor might be responsible for the preservation and longevity of bacteria populations stored in water at low temperatures - Morgan (1983). In tropical waters with higher temperatures, the case may not be tenable. Also, the poorer survival of bacteria at higher temperatures may be due to a phenomenon called metabolic exhaustion which is accentuated by dark storage (Morgan, 1983).

El-Sharkawi et al. (1989) studied the effect of some environmental factors (temp, salinity and light), on the survival of salmonella, shigella and E. coli in different waters. Temperatures above 35°C had the most adverse effect on survival. Daylight also seriously affected the survival of all the test organisms; confirming the results of Porter (1965), that UV and visible light have detrimental effects on bacteria.

Generally, the rate of cell division in light is roughly balanced by cell death at high temperatures. In the dark, at high temperatures, the metabolic rate is high but the death rate reduces due to the absence of light. The increased metabolic rate leads to the accumulation of waste products which may be toxic and lead to an eventual faster death rate. This effect tends to be more pronounced in water stored in containers than naturally flowing ones like streams, which have a constant nutrient renewal. Nonetheless, PH may affect the rate of chemical reactions and so, may influence rate of cell death in stored waters.

Bifidobacteria did not survive well in stored water probably because they are obligate anaerobes or at best, micro-aerophillic as noted by Tamaki (1960). The dissolved O_2 in the water used in this study may have stressed them considerably and may have been responsible for their inability to multiply under storage.

It is not easy to draw general conclusions about the survival of bacteria in an environment because their behaviour may vary for different reasons, not easy to ascertain. It is however logical to infer that temperature plays a major role in determining the survival rates of bacteria (El-sharkawi et al. 1989).

In concluding, a bifid index should be limited in use to detecting only recent pollution in hot climates. Also, it seems (from the results presented here), that faecal streptococci could be better pollution indicators in warm climates. Beyond 25°C, a combination of both the thermotolerant coliforms and faecal streptococci indices may be better.

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