tern of intracellular parasites reflects the exact internalization pattern of trypomastigotes in Mø. Considering that the parasites are free to move and randomly establish contacts and interactions with all the available Møs one would expect a random distribution of intracellular forms, best described by a Poisson model. Since this is not the case, various mechanisms can be advanced to account for the intracellular aggregation. The first one would be a non-random killing of intracellular forms in the sense that cells with few parasites would be less able to lyse them than cells with a large number of forms. This does not seem to be the case, since at late incubation times, when the number of intracellular forms begins to fall, the degree of clustering also decreases. As a matter of fact, our data is a strong indication that intracellular killing occurs at random. Another possibility is that a clustered or contagious pattern of penetration occurs. One could suggest that once a Mø is penetrated by a parasite it becomes more susceptible to subsequent penetration, or that it "turns-on" resistance to subsequent penetration in adjacent nonpenetrated cells. The former possibility may be due to membrane alterations following penetration or even to some sort of chemotactic stimulus released by a penetrated cell. The induced resistance could also be due to the release of a cell factor, as indeed has been shown to occur after *T. cruzi* infection[11]. Our results tend to favor the "facilitating" hypothesis. Both normal resistant (B10) and susceptible (A/J) strains of mice present the same number of infected macrophages at a given moment throughout the incubation period (figure 1). This indicates that the initial sorting of cells by parasites, in the same number of Mø increases sharply in the A/J but not in the B10 mice. Also, one can see that new clusters arise at a similar rate in cells from both strains. If a protective soluble factor were to be released by Mø one would expect a decrease in the number of clusters with incubation time, in the B10 strain. Finally, a clustered frequency distribution could be due to the existence of a Mø sub-population with a different phagocytic capacity or penetrability. This last assumption could certainly be due to the existence of macrophages bearing special receptors for *T. cruzi*. As a matter of fact Nogueira and Cohn[12] have postulated the existence of a macrophage receptor for culture forms of *T. cruzi*. Hyde and Dvorak[13] observed a negative binomial frequency distribution of the Ernestina strain of *T. cruzi* in secondary bovine embryo skeletal muscle cells. We now show it to occur with a different strain of parasite in a cell populatin that is not only actively penetrated by parasites but also able to phagocytize them. The phenomenon also occurs in different strains of mice and irrespective of the total number of internalized parasites. This fact certainly means that this specific frequency distribution is measuring a very basic and general phenomenon underlying *T. cruzi*/host interaction.

1 Reprint requests should be addressed to: Instituto de Biofísica - U.F.R.J., Centro de Ciências da Saúde - Bl.G, Cidade Universitária - ZC-32, 21.9.40 Rio de Janeiro, RJ (Brazil).
2 Acknowledgments. We thank Dr Sylvio T. Torres for providing the inbred strains of mice. The work was supported by the Brazilian Research Council (CNPq) and the Financiadora de Estudos e Projetos (FINEP).
3 T. Trischman, H. Tanowitz, M. Wittner and B. Bloom, Expl. Parasit. *45*, 160 (1978).
4 I.L. Pécora and M.A. Barcinski, An. Acad. bras. Cienc., in press (1980).
5 C.I. Bliss, Biometrics *9*, 197 (1953).
6 H.D. Crofton, Parasitology *62*, 343 (1971).
7 F.J. Anscombe, Biometrika *37*, 358 (1950).
8 H.D. Crofton, Parasitology *62*, 179 (1971).
9 F.N. David and P.G. Moore, Ann. Bot. *18*, 48 (1954).
10 J.A. Dvorak and T.P. Hyde, Expl. Parasit. *34*, 268 (1973).
11 N.S. Nogueira, S. Gordon and Z. Cohn, J. exp. Med. *146*, 157 (1977).
12 N.S. Nogueira and Z. Cohn, J. exp. Med. *143*, 1402 (1976).
13 T.P. Hyde and J.A. Dvorak, Expl. Parasit. *34*, 284 (1973).

Dextranase-producing organisms in dental plaque from caries-free and caries-active naval recruits[1]

B.L. Lamberts, I.L. Shklair and R.G. Walter

Naval Dental Research Institute, Naval Base, Building 1-H, Great Lakes (Illinois 60088, USA), 13 November 1979

Summary. Dental plaque samples from caries-free and caries-active naval recruits were assayed for the prevalence of dextranase-producing organisms. These organisms were found in the plaque of all of the subjects. Mean percentages of dextranase-producing organisms with respect to total colony count for the 2 groups of subjects were not significantly different.

The oral organism, *Streptococcus mutans*, produces extracellular glucans from sucrose which may promote plaque formation and dental caries by enabling masses of bacteria to adhere to teeth[2]. The glucans are composed of α-1, 6- and α-1, 3-linked glucosyl residues[3-5]. Certain plaque organisms elaborate dextranases which can hydrolyze the α-1, 6 linkages and thus may partially degrade the glucans[6,7]. If the dextranases from plaque organisms were to confer significant protection against dental caries through glucan degradation, a direct relationship between the prevalence of dextranase-producing organisms in plaque and caries resistance might be demonstrable. In this study we have compared the prevalence of these organisms in plaque samples from 2 groups of young men of widely differing caries experience.

Materials and methods. The subjects were 19 caries-free and 20 caries-active male US naval recruits, 17-25 years of age. Subjects designated caries-free had no evidence of active or past tooth decay, whereas each subject designated caries-active showed open lesions on at least 8 posterior teeth. Plaque samples were obtained on waxed dental floss by passing the floss between the contact points of the 1st and 2nd molar, and of the 1st molar and 2nd bicuspid of each dental quadrant. The 2 plaque samples from each quadrant were combined in thioglycolate-broth holding medium (minus carbohydrate and indicator, Difco Labs, Detroit, Mich.), and were sonicated. Additional details of these procedures have been described elsewhere[8]. The samples were diluted $1:10^3$ and $1:10^4$, and the dilutions were spread in duplicate onto heart infusion plates (Difco Labs) con-

taining 0.1% blue dextran (Pharmacia, Uppsala, Sweden). By this procedure one of the dilutions would ordinarily yield 30–300 colonies per plate. The plates were incubated at 37 °C for 5–7 days in a 95% N_2–5% CO_2 atmosphere and dextranase-producing colonies (DPC), identified by their haloes of degraded blue dextran[7], were counted along with total colonies for each plate. The percentage of DPC per quadrant was based on the overall count of DPC and total colonies for the 4 plates of each quadrant sample.

Results and discussion. All of the subjects showed DPC in the plaque samples from at least 1 of their quadrants and, for 28 subjects (14 from each group), these colonies were present in all of their quadrants. The majority of the DPC were *Actinomyces* species; most of the remaining DPC were strains of *Streptococcus mutans.* The data for the quadrants were evaluated statistically by a non-parametric (Kruskal-Wallis) test and by a weighted analysis of variance. The differences in mean DPC percentages among the quadrants within each group were determined, as well as the differences in mean DPC percentages between the corresponding quadrants of the 2 groups. None of the differences, either within groups or between groups, were significant at the 5% level.

In a separate comparison of the groups, each subject's total DPC and total colony count were used to calculate the overall DPC percentage for each subject. The table shows data for all subjects in order of increasing percentage ranges. Although the mean overall values for the groups were the same (3.15%), 13 of the 19 caries-free subjects showed percentages below 1.60 compared with only 5 of the 20 caries-active subjects. This trend is contrary to what might be expected if the DPC were significant anticaries factors for the caries-free subjects. One explanation for the results is that the caries-active recruits tend to be more infected by *S. mutans* than their caries-free counterparts[8], and thus probably have correspondingly higher levels of plaque glucans to promote the growth of dextranase-producing organisms. The prevalence of the organisms would then simply appear to reflect the availability of the glucan substrate.

It was concluded that this comparison of DPC levels between groups of caries-free and caries-active subjects did not provide sufficient evidence to support a caries-protective role for the DPC.

Percentage ranges of dextranase-producing colonies in plaque samples from caries-free and caries-active subjects

DPC percentage range	Number of subjects Caries-free (19)	Caries-active (20)
0.00–1.00	7	3
1.01–1.60	6	2
1.61–2.00	0	3
2.01–3.00	0	3
3.01–4.00	2	4
4.01–5.00	0	0
Above 5.00	4	5
Overall mean ± SD	3.15 ± 4.51	3.15 ± 2.29

1 The authors thank DTI Samuel Shelton and Mr Eric Mandel for technical assistance. The research was supported by Naval Medical Research and Development Command Project ZF58.524.012-0026, Bethesda, Maryland. The opinions expressed herein are those of the authors and are not to be construed as reflecting the views of the Navy Department or the Naval Service at large. The use of commercially available products does not imply endorsement of these products or preference to other similar products on the market.

2 R.J. Gibbons, Caries Res. *2*, 164 (1968).
3 P. Critchley, J.M. Wood, C.A. Saxton and S.A. Leach, Caries Res. *1*, 122 (1967).
4 B. Guggenheim, Helv. odont. Acta *14*, suppl. 5, 89 (1970).
5 P. Colson, H.C. Jarrell, B.L. Lamberts and I.C.P. Smith, Carbohyd. Res. *71*, 265 (1979).
6 M.D. Dewar and G.J. Walker, Caries Res. *9*, 21 (1975).
7 R.H. Staat, T.H. Gawronski and C.F. Schachtele, Infect. Immun. *8*, 1009 (1973).
8 I.L. Shklair, H.J. Keene and P. Cullen, Archs oral Biol. *19*, 199 (1974).

Evolutionary implications of ascorbic acid production in the Australian lungfish[1]

D.E. Dykhuizen[2], K.M. Harrison and B.J. Richardson

Research School of Biological Sciences, Australian National University, Canberra (A.C.T., Australia), 18 October 1979

Summary. It is shown that the Australian lungfish *(Neoceratodus forsteri)* synthesizes ascorbic acid in its kidney, suggesting that ascorbic acid synthesis started before the origin of the tetrapods.

The ability to synthesize ascorbic acid and the location of this ability in particular organs varies phylogenetically in vertebrates[3]. Amphibians, reptiles and egg-laying mammals (prototherians) produce ascorbic acid in their kidneys[3,4]. In the metatherian and eutherian mammals, ascorbic acid can be synthesized in the liver[3,4], but this ability has been lost in such divergent lines as those leading to the bats[5], anthropoid primates and guinea-pigs[3–5]. In birds, a similar evolutionary pattern has been found[6]. The bony fish are unable to synthesize ascorbic acid[7,8]. Thus Catterjee[3,8] has suggested that tetrapods developed the pathway for synthesizing ascorbic acid de novo. This suggestion cannot be true since the Australian lungfish synthesizes ascorbic acid in its kidney (table).

The Dipnoi, the sub-class to which the Australian lungfish belongs, the Rhipidista, the order from which the reptiles and amphibians arose, and the Achinopterygii, the class to which modern teleost fish belong, all seem to have arisen from a common ancestor some time before the middle Devonian (375 MYA)[9]. Since ascorbic acid synthesis is found in 2 of the 3 lines, it is likely that the common ancestor of all of these lines could synthesize ascorbic acid. Thus the lack of synthesis in the bony fish is likely to be a loss, as in the anthropoid primates, and the pattern of ascorbic acid synthesis in the primitive fish should be studied.

Ascorbic acid synthesis was determined using sodium-D-glucuronate as the substrate by the method of Chatterjee[10]. This method demands that all 3 of the enzymes required for the synthesis of ascorbic acid[11] be active. The table shows that a marsupial mouse synthesizes ascorbic acid in its liver. This agrees with the finding that other marsupials