CHAPTER 9

SUMMARY

The reduction in dental caries in the industrialized countries has become increasingly obvious over the last decades. A better understanding of why and how dental caries occurs, has made significant reductions in the disease possible through preventive programmes. The increased knowledge has, however, also emphasized the complex nature of the caries process. Unraveling the aetiology is a prime requisite if the choice of the most efficient, and appropriate preventive measure for the scala of clinical conditions is to be made. The necessity of carrying out more studies into the causes of dental caries was thus clear. These studies can, in general, be carried out in two ways: in vitro, as a laboratory experiment or in vivo as a clinical study. An in-vitro experimental set-up, which closely mirrors normal oral conditions was chosen for the present study. The basic idea for an experimental design was mooted in the 1950's. The name "artificial mouth" was first used by Pigman and co-workers in 1952. An artificial mouth is a system in which bacteria, inoculated for example on enamel slabs, can be grown under standardized conditions in terms of liquids and gas supply for a fixed period of time. Over the years several research groups have contributed to the development of this concept and its realization.

These developments are described in chapter 2, up to and including the design, development and testing of the apparatus used in this project. The requirements listed by Dibdin and co-workers in 1976 were used as the starting point. These are:
1) The apparatus must be simple, easy to sterilize and capable of sustaining experiments over long periods of time.

2) Recharging of supply reservoirs must be possible under sterile conditions.

3) It should be possible to separately inoculate and maintain several samples at the time.

4) Saliva and gas supply must be maintained under controlled conditions, whilst the introduction of other substances should be controlled by a programmed supply system.

To attain the first goal, namely that of controlled conditions and experimental reproducibility, the criteria formulated by Dibdin et al. were more precisely formulated:

a. The apparatus should be built up and used in a laminar flow cabinet. Infection from the surrounding air is thus prevented.

b. Supply and waste container capacity must be sufficient for the full experimental period without changing any connectors.

Furthermore a new combined gas and liquid inlet system was developed. Infection and bacterial growth into the supply tubes were prevented by the downward gas flow.

Experiments with this modified culture-vessel set-up resulted in plaque growth and lesion development in the enamel slabs. No significant technical problems occurred and the apparatus fulfilled all the criteria.

Now that it had been established that the project was technically feasible further development of the apparatus followed.

The sample capacity, in particular, had to be increased without affecting its simplicity and manageability. The compact rotating sample holder design, described in chapter 3 achieved this by providing spaces for 12 enamel samples whilst still using the same auxiliary equipment. Once an experimental run had been started the system only needed to be opened if samples were to be removed. Tests with both single strain inoculation of Streptococcus mutans and inoculation of mixed Streptococcus mutans and Veillonella al-
Semnlc handler rennndrreihle nlanrre accumulation and lesion formation occurred. The first goal of the project had thus been attained and the possibility of carrying out a series of specific bacteriological experiments created. The first of these experiments into the development and metabolic activity of a S. mutans plaque is described in chapter 4. The plaque which developed after 1, 2 and 4 days was examined by removing the enamel samples from the apparatus and preparing them for examination in the Scanning Electron Microscope (SEM). This showed that the plaque developed from randomly spread small groups, or single bacteria, on day 1 to multilayered colonies on day 4. Viable cell counts (CFU) and measurement of the total cellmass (DNA) present a picture of initial plaque increase finally resulting in an equilibrium between plaque formation and degradation during the experimental period.

Lactic acid proved to be the predominant metabolic end product in the plaque when sucrose was supplied. In the presence of only low amounts of carbohydrate the plaque metabolism changed accordingly. These results are in agreement with earlier in vivo results in animals and man. The degree of similarity between the oral milieu and that of the artificial mouth was thus demonstrated. The enamel lesions formed under these experimental conditions also showed strong similarities with those found in vivo i.e. the "classical picture" of mineral loss in the surface enamel after one week gradually changing to the formation of a surface layer and subsurface lesion after 3 weeks. This development was measured using both microhardness and micro-radiographical techniques. S.E.M. studies were also used to illustrate the progress of the demineralization process. This showed enlarged interprismatic spaces, which, in all probability, form the pathways for mineral loss from the deeper enamel layers.

The results of experiments with S. mutans and V. alcalescens...
are described in chapter 6. A foodchain exists between these microorganisms. Lactic acid which is a fermentation product of S. mutans metabolism forms the substrate for V. alcalescens. From the caries standpoint this is an interesting feature since lactic acid is converted to weaker acids which may have a smaller capacity to demineralize enamel. The results, however, presented a different picture. Early plaque development in the mixed plaques was quite similar to that of mono, S. mutans plaques. The amounts of both viable cells and total cell mass, however, were significantly higher in the mixed than in the single S. mutans plaque. The amounts of acid detected were also higher and lactic acid, in particular, was the most abundant. No decisive role could be found for acetic, formic and propionic acid. Lesion formation was more pronounced and lesions progressed more quickly in enamel samples incubated with the mixed bacterial plaque. A reduction in caries activity as a result of the food chain S. mutans - V. alcalescens did not occur.

Whether this phenomenon would be found with a different bacterial combination is studied in chapter 7. A monobacterial plaque of Actinomyces naeslundii was compared to a mixed plaque of Actinomyces naeslundii and Veillonella alcalescens. Here it was also found that the mixed plaques developed more rapidly and produced more acid. Again the bacterial combination appeared to have a stimulating effect. Lactic acid was present in larger amounts in the mono Actinomyces plaque, then in the mixed plaques. There, acetic, formic, succinic and propionic acid were found in the largest quantities. However, enamel demineralization was significantly greater in the samples exposed to the mixed bacterial plaques. This demonstrates that weaker acids have the capacity to cause distinct mineral loss.

Finally, in chapter 8 the place of the artificial mouth in caries research is discussed on the basis of the experimental results presented, and the direction future studies should take, is given.