RELATIONSHIPS BETWEEN SELECTED INTRA-ORAL CHARACTERISTICS
ENVIRONMENTAL VARIABLES AND CARIES EXPERIENCE
OF PRIMITIVE PEOPLES IN NEW GUINEA

VOLUME I

Thesis submitted to the Faculty of Dentistry of the University of Sydney for examination for the degree of Doctor of Dental Science

August, 1974. R.G. Schamschule
# TABLE OF CONTENTS

## VOLUME I

<table>
<thead>
<tr>
<th>Acknowledgements</th>
<th>vii</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of tables</td>
<td>ix</td>
</tr>
<tr>
<td>List of figures</td>
<td>xii</td>
</tr>
</tbody>
</table>

### CHAPTER 1. INTRODUCTION

1.1. Rationale.                                      1

1.2. Aims of study.
   - 1.2.1. General aims.                             4
   - 1.2.2. Specific aims.                            5

1.3. Reviews and support.                            6

1.4. Responsibilities of the author regarding the initiation, conduct and direction of the work. (Stated in accordance with the by-laws of the Faculty of Dentistry, University of Sydney, Chapter XV, 32.(2)). 11

1.5. Statement regarding the originality of the work presented. (In accordance with the by-laws of the Faculty of Dentistry, University of Sydney, Chapter XV, 32.(1)). 14

### CHAPTER 2. BACKGROUND. THE STUDY ENVIRONMENT.

2.1. General considerations.                         16

2.2. Dental aspects.                                 18

*End*
CHAPTER 3. METHODS.

3.1. Sample design and study parameters.
   3.1.1. Sampling strategy.
   3.1.2. Study parameters

3.2. Field methods.
   3.2.1. Introduction.
   3.2.2. Preparations.
   3.2.3. April – May, 1972, Field visit.
      3.2.3.1. The human sample.
      3.2.3.2. General field procedure.
      3.2.3.3. Screening and assignment of subjects to project
      3.2.3.4. Saliva collection.
      3.2.3.5. Collection of pooled plaque.
      3.2.3.6. Plaque activity test in-situ (UL2 plaque).
      3.2.3.7. Collection of UL2 plaque for bacteriological examination.
      3.2.3.8. Cleaning of teeth.
      3.2.3.9. Dental examination.
         (A) Dental caries.
         (B) Hypoplasia.
      3.2.3.10. Clinical test for dissolution rate of surface enamel in-vivo; sampling for fluoride determinations.
      3.2.3.11. Examination for enamel surface wear and anterior tooth enamel defects.
      3.2.3.12. Enamel sampling for chemical analysis.
      3.2.3.13. Tooth colour classification.
3.2.3.14. Impressions for cusp height and cusp angle measurements. 78

3.2.4. March - April, 1973, Field visit. 78

3.2.4.1. Saliva collection for chemical analyses. 79
3.2.4.2. Collection of pooled plaque; sample (2). 80
3.2.4.3. Measurement of mesio-distal crown diameter of upper anterior teeth. 81
3.2.4.4. Recording of the frequency of betel-chewing. 83

3.2.5. July, 1973, Field visit. 84

3.2.5.1. Collection of saliva. 84
3.2.5.2. Viscosity of saliva. 85
3.2.5.3. Buffering capacity and bicarbonate content of saliva. 86

3.3. Laboratory methods. 90

3.3.1. General comments on methods employed. 90

3.3.1.1. Selection and basic principles of methods used for the determination of inorganic constituents of surface enamel and activated whole saliva. 90

3.3.1.2. Microbiological methods. 106.

3.3.1.3. Determination of selected inorganic constituents of water samples. 108

3.3.2. Details of methods. 109

3.3.2.1. Determination of selected inorganic constituents and dissolution rate of surface enamel. 109

3.3.2.1.1. Dispensing and storage of sample dilutions for Ca, Mg, Sr, K, Ba, Zn and P determinations. 109

3.3.2.1.2. Calcium determinations. 116
3.3.2.1.3. Magnesium determinations. 120

Ctd...
3.3.2.1.4. Strontium determinations. 124
3.3.2.1.5. Potassium determinations. 131
3.3.2.1.6. Barium determinations. 137
3.3.2.1.7. Zinc determinations. 147
3.3.2.1.8. Phosphorus determinations. 152
3.3.2.1.9. Determination of dissolution rate and fluoride content of surface enamel. 156

3.3.2.2. Preparation and analyses of saliva specimens. 161
3.3.2.2.1. Volume estimation and colour grading. 161
3.3.2.2.2. Fluoride determinations. 164
3.3.2.2.3. Calcium determinations. 168

3.3.2.3. Determination of the dry weight of pooled plaque samples (1) and (2). 172

3.3.2.4. Determination of the total number and the proportions of predominating colony types of cultivable streptococci in dental plaque. 173

3.3.2.5. Water analyses. 196
3.3.2.5.1. Calcium determinations. 198
3.3.2.5.2. Strontium determinations. 199
3.3.2.5.3. Fluoride determinations. 200

3.4. Statistical methods. 203
3.4.1. Introduction. 203
3.4.2. Data summary. 204
3.4.3. Correlation matrix. 204
3.4.4. Stepwise multiple linear regression analysis. 207
3.4.5. Discriminant function analysis. 209

Ctd...
CHAPTER 4. RESULTS AND DISCUSSION.

4.1. Introduction.

4.2. Data summary.

4.2.1. Dental caries (Categories 10, 12, 14 and 229).

4.2.2. Tooth colour and enamel surface wear (Categories 7 and 9).

4.2.3. Plaque quantity (Categories 16, 19 and 203).

4.2.4. Dissolution rate of surface enamel (Category 22).

4.2.5. Plaque pH and activity measurements (Categories 23, 24, 25 and 26).

4.2.6. Streptococci in plaque (Categories 29 - 104).

4.2.7. Hypoplasia index, mean score (Category 201).

4.2.8. Mesio-distal crown diameters of upper anterior teeth (Categories 205 - 207).

4.2.9. Variables related to the betel-chewing habit (Categories 208, 209 and 213).

4.2.10. Viscosity of saliva (Category 214).

4.2.11. Bicarbonate content and buffering capacity of saliva (Categories 215, 216, 217, 218 and 219).


4.2.13. The calcium content of saliva (Categories 239 and 240).

4.2.14. The fluoride content of saliva (Category 243).

4.2.15. The fluoride, calcium and strontium content of household water.

4.3. Correlation matrix.

4.4. Stepwise multiple linear regression analyses.
4.5. Discriminant function analyses.  330

CHAPTER 5. CONCLUSIONS AND SUMMARY

5.1. Precis of study.  340
5.2. Conclusions.  341
5.3. Summary.  354

Bibliography.  356 - 417
ACKNOWLEDGEMENTS.

I am grateful to my esteemed co-workers and friends for their contributions to this investigation. Thanks are expressed to:

Dr. D.E. Barmes, for introducing me to the Papua New Guinea scene many years ago, and for his unfailing trust, support and encouragement.

Professor B.L. Adkins, for several consultations concerning the design and statistical evaluation of the study.

Dr. G. Charlton and Mrs. Barbara Blainey, for their co-operation in the field and in the laboratory throughout the investigation, and for their constructive criticism of selected parts of the manuscript.

Dr. Josie Tabua, for assistance in the field.

Mrs. Helen Agus, for laboratory assistance, for the preparation of figures, for her help with the organization of the manuscript and for valuable editorial contributions.

Dr. B.G. Davey, for providing geological background information.

Mr. D.E. Hart and Mrs. Christa Lossin, for their help with logistic organization.

Mr. B.M. Bycroft, Mr. C. Eichten and Miss Suzanne Schmocker, for laboratory assistance.
Mr. W. Gulbinat and Mrs. Jennifer Sardo-Infirri, for computer programming and data organization.

Mrs. Beulah Symmons, for assistance with literature research.

Dr. B.R.D. Gillings, for supplying extracted teeth, for reading the manuscript and for his valuable editorial comments.

Mr. P. Ward and Miss Barbel Bischoff, for preparing the photographic illustrations.

Mrs. Judith Ritter, who typed most of the initial draft of the manuscript and Miss Maureen Campbell, Mrs. Ethel Roseman, and Mrs. Joyce Ford, who typed parts of it.

Mrs. Judith Rostron, for final typing of the text.

Financial support was provided by the National Institute of Dental Research, Bethesda, Md., U.S.A. and by the World Health Organization, Geneva, Switzerland. The computer facilities of the World Health Organization were made available for data processing.

The Department of Public Health and the Department of District Administration of Papua New Guinea assisted with auxiliary personnel and field equipment.
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Abbreviated designation</th>
<th>Page Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>List of variables examined</td>
<td>7 - 10</td>
</tr>
<tr>
<td>2</td>
<td>Dilution sequences used for UL2 and pooled plaque cultures</td>
<td>175</td>
</tr>
<tr>
<td>3</td>
<td>The relationship between mean streptococcal counts in plaque and the time of plaque collection</td>
<td>244</td>
</tr>
<tr>
<td>4</td>
<td>The relationship between the percentage of streptococcal colony types in plaque and the time of plaque collection</td>
<td>246</td>
</tr>
<tr>
<td>5</td>
<td>The mean caries experience of subjects stratified according to the time of plaque collection</td>
<td>249</td>
</tr>
<tr>
<td>6</td>
<td>The fluoride, strontium and calcium contents of river water samples</td>
<td>285</td>
</tr>
<tr>
<td>7</td>
<td>Inter-study comparison of the calcium, strontium and fluoride contents of river water samples</td>
<td>288</td>
</tr>
<tr>
<td>8</td>
<td>Comparison of caries experience with the mean concentration of fluoride in river water, enamel and saliva</td>
<td>290</td>
</tr>
</tbody>
</table>

Ctd...
Correlation coefficients between the mean fluoride contents of water (5 rivers), enamel, saliva and mean caries experience 292

Correlation coefficients between the fluoride contents of river water, enamel and saliva, and caries experience 293

Comparison of caries experience with the mean concentrations of strontium in river water and enamel. 298

Correlation coefficients between the mean strontium contents of water (5 rivers) and enamel, and mean caries experience 299

Correlation coefficients between the strontium contents of river water, enamel, and caries experience 300

Correlation coefficients between caries experience and various expressions of streptococci isolated from pooled and UL2 plaque 313

Correlations obtained between caries experience and three sets of independent variables representing properties of plaque, enamel and saliva (all subjects) 317

Ctd...
16 Correlations between ten selected variables representing properties of plaque, enamel and saliva (all subjects) 320

17 Correlations between selected variables and the severity score of carious lesions (carious subjects only) 323

18 Summary of results of discriminant function analyses 336
<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Abbreviated designation</th>
<th>Page Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Outline map of New Guinea</td>
<td>26</td>
</tr>
<tr>
<td>2</td>
<td>The human sample</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>Reduction of mean wet weight of plaque samples with time</td>
<td>53</td>
</tr>
<tr>
<td>4</td>
<td>Plaque activity measurement <em>in-situ</em></td>
<td>57</td>
</tr>
<tr>
<td>5</td>
<td>Recorder tracing of plaque activity</td>
<td>59</td>
</tr>
<tr>
<td>6</td>
<td>Tooth colour range</td>
<td>77</td>
</tr>
<tr>
<td>7</td>
<td>Kit for measurement of viscosity of saliva</td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>Optimum and non-optimum 'dry' conditions (flameless atomic absorption spectroscopy)</td>
<td>97-99</td>
</tr>
<tr>
<td>9</td>
<td>Optimum and non-optimum 'ash' conditions (as above)</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>Optimum and non-optimum 'atomize' conditions (as above)</td>
<td>101,102</td>
</tr>
<tr>
<td>11</td>
<td>Typical calibration graph for calcium determination in enamel</td>
<td>119</td>
</tr>
<tr>
<td>12</td>
<td>Typical calibration graph for magnesium determination in enamel</td>
<td>123</td>
</tr>
<tr>
<td>13</td>
<td>Typical calibration graph for strontium determination in enamel</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>Ctd...</td>
<td></td>
</tr>
<tr>
<td>Figure No.</td>
<td>Abbreviated designation</td>
<td>Page Nos.</td>
</tr>
<tr>
<td>-----------</td>
<td>----------------------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>14</td>
<td>Typical calibration graph for potassium determination in enamel</td>
<td>134</td>
</tr>
<tr>
<td>15</td>
<td>Establishment of the barium content of the enamel internal standard using the multiple additions method</td>
<td>142</td>
</tr>
<tr>
<td>16</td>
<td>Typical calibration graph for zinc determination in enamel</td>
<td>150</td>
</tr>
<tr>
<td>17</td>
<td>Typical calibration graph for phosphorus determination in enamel</td>
<td>155</td>
</tr>
<tr>
<td>18</td>
<td>Calibration graph for fluoride determination in enamel</td>
<td>159</td>
</tr>
<tr>
<td>19</td>
<td>Saliva colour standards</td>
<td>163</td>
</tr>
<tr>
<td>20</td>
<td>Calibration graph and time response plot for fluoride determination in saliva</td>
<td>166</td>
</tr>
<tr>
<td>21</td>
<td>Typical calibration graph for calcium determination in saliva</td>
<td>171</td>
</tr>
<tr>
<td>22</td>
<td>Streptococcal colony types</td>
<td>178-182</td>
</tr>
<tr>
<td>23</td>
<td>Typical calibration graph for fluoride determination in water</td>
<td>202</td>
</tr>
<tr>
<td>24</td>
<td>Two-dimensional representation of the basic principle of discriminant function analysis</td>
<td>211</td>
</tr>
<tr>
<td></td>
<td>Ctd...</td>
<td></td>
</tr>
<tr>
<td>Figure No.</td>
<td>Abbreviated designation</td>
<td>Page Nos.</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>25</td>
<td>Distribution of mean caries experience (DMFT) between villages</td>
<td>217</td>
</tr>
<tr>
<td>26</td>
<td>Scatter diagram of plaque weights</td>
<td>226</td>
</tr>
</tbody>
</table>

A reduced copy of the dental examination chart, Form 1, used in this investigation appears on Page 34.
CHAPTER 1.

INTRODUCTION.

1.1. Rationale.

A recent technical report of the World Health Organization (1) indicated virtually universal acceptance of the concept that dental caries is a disease of multi-factorial aetiology.

Animal studies gave strong support to the contention that the development and progress of carious lesions depend upon the interaction of numerous factors. (2)(3)(4)(5) Keyes and Fitzgerald (4) proposed the existence of three major aetiological subdivisions, into one of which all the operating factors can be classed, by suggesting that carious lesions cannot occur, unless susceptible tooth surfaces are colonized by bacteria, which are supplied with a suitable substrate. In considering interrelationships of the determinants of the carious process, Keyes (6) stated: "Since there are numerous variables in each of the three groupings, the intensity of the contribution from a single one is always difficult to assess" and ".... it is often exceedingly difficult to know whether one contributory group or more is responsible for the result."

The complexity of factors influencing caries resistance was recognised by Davies (7) who asserted that ".... resistance to caries depends not only on intrinsic factors affecting the teeth themselves; it depends also on extrinsic factors in the immediate environment of the teeth." and that ".... since multiple factors are involved, complex statistical techniques are required to
assess the relative importance of each."

In full agreement with both statements, the author of this thesis would go even further and propose that, unless information is available regarding all the operating determinants of the caries process, or alternatively, those which were not measured were known to be constant, it is presumptuous to attribute variation in caries to the determinant or determinants measured.

This statement may seem inconsistent with the generally accepted finding that the presence of appropriate levels of fluoride in the drinking water confers protection against dental caries in man, because the protective effect of fluoride was recognised without examining in depth other parameters that may have influenced susceptibility to the disease. However, a selection of pertinent Public Health Service papers (8) indicate that this recognition stemmed from clinical observations and epidemiological studies conducted in areas where the effect of fluoride was so overwhelming that, in comparison, the contributions made by other variables were negligible and for all practical purposes amounted to a constant. Furthermore, it is well recognised that the protection conferred by fluoride is neither absolute nor uniform for all tooth surfaces and that it varies from individual to individual and from population to population exposed to the same level of fluoride. Indeed, these latter facts emphasize the importance of other determinants and indicate the need to consider them even in the presence of apparently dominant influencing factors.

Under these circumstances, multi-directional approaches to
caries aetiology studies appear desirable, yet most epidemiological investigations have been restricted to the examination of associations between one or a few recognised or suspected influencing factors, usually confined to one of the three major aetiological areas, and the prevalence or incidence of the disease. Such approaches have made substantial contributions to our knowledge of caries aetiology. However, in the absence of an overwhelming effect on the part of the variable or variables studied, their capacity to predict individual variation in caries in terms of variation of the determinants examined has been usually low, in spite of the demonstration of statistically significant associations. This is not surprising if one considers that, in most cases, the great majority of the operating factors were assumed to be constant or were simply ignored.

The fact that it is not possible to measure all variables that influence caries susceptibility, particularly because some of them are almost certainly unknown, is not a valid reason for abandoning attempts to explain individual variation in caries experience to the fullest possible extent, using the means afforded by our present knowledge.

It could be argued, that caries studies may be more profitably conducted on animal models, where a larger proportion of the variables can be kept constant than in a human sample. In animal experiments it is generally possible to minimize or eliminate variation in the areas of genetics, nutrition, diet and bacterial flora. However, the oral environment of experimental animals is materially different from the oral environment of man and inter-species comparability tends to decrease with
phylogenetic distance. The species used as animal models rarely or never suffer from caries in their natural state and experimental odontolysis frequently has to be induced under extreme and artificial dieto-bacterial pressures. Under these circumstances, extrapolation of the results obtained to man can be formidably difficult. While the author does not deny the usefulness or validity of animal experiments for studying certain aspects of dental caries, it is his firm belief that, as far as possible, determinants influencing individual susceptibility or resistance to dental caries in man should be studied where the disease occurs, in the human oral cavity.

1.2. Aims of study.

1.2.1. General aims.

The thesis presented deals with the design, execution and results of a multi-directional investigation of caries aetiology, built on the foundations of several years of method development and field and laboratory research involving primitive populations.

The basic purpose of the study is to contribute to present knowledge concerning the aetiology of dental caries, with the ultimate aim of utilizing our understanding of the disease for the development of comprehensive preventive measures.

Data presented in this work relate to a specific population in New Guinea. Although aetiological and preventive implications of the findings concern primarily the same population, they are by no means limited to it or to similar primitive groups. In
fact, it is expected that the majority of findings will have universal relevance to the aetiology and prevention of the disease.

1.2.2. **Specific aims.**

The specific aims of the investigation are:

1) To establish mean values and individual variation for selected intra-oral variables, known or suspected to influence susceptibility or resistance to caries, in a primitive population exhibiting contrasts in caries prevalence.

2) To confirm or reject the hypothesis that sets of the intra-oral variables measured have a significant capacity to 'explain' variation in individual caries experience.

3) To examine the extent to which individuals with materially different caries experience can be classified as such, on the basis of the information contained in the independent variables alone.

Several comments are appropriate in respect of these aims:

With reference to the first aim, the variables included in the investigation are listed in Table 1, pages 7 - 10. The variables were selected according to relevance as indicated by previous studies, by current concepts of caries aetiology, and according to feasibility in terms of techniques, prevailing field
conditions and cost.

The second aim implies 'explanation' in a statistical sense, for which a test or tests of statistical significance will be carried out. The author feels, however, that in such cases a judgement regarding the practical significance of the result is of no less importance and this matter is considered when the results are discussed.

In respect of the third aim, it is to be noted that throughout this text, expressions of caries experience are considered as dependent variables and all other parameters are termed independent variables.

1.3. Reviews and support.

The study presented in this thesis was executed within the framework of a wider investigation which included concurrent examination of the major and trace element composition of soil, food and water, and surveys of nutrition, diet and genetic markers for the same population. The project was reviewed and approved in respect of scientific merit, and the protection of human subjects participating in biomedical research, by the appropriate committees of the World Health Organization, Geneva, Switzerland and the National Institute of Dental Research, Bethesda, Maryland, U.S.A. Financial support was awarded by both organizations.
### TABLE 1.

**List of Variables Examined.**

<table>
<thead>
<tr>
<th>Variable (Category) Number</th>
<th>Description</th>
<th>Units of Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Age</td>
<td>years</td>
</tr>
<tr>
<td>7</td>
<td>Tooth colour</td>
<td>6 categories</td>
</tr>
<tr>
<td>9</td>
<td>Enamel surface wear</td>
<td>7 categories</td>
</tr>
<tr>
<td>10</td>
<td>DMFT (decayed, missing, filled teeth)</td>
<td>--</td>
</tr>
<tr>
<td>12</td>
<td>DMFS (decayed, missing, filled surfaces)</td>
<td>--</td>
</tr>
<tr>
<td>14</td>
<td>Severity score of carious lesions</td>
<td>--</td>
</tr>
<tr>
<td>16</td>
<td>Pooled plaque quantity (1)</td>
<td>mg dry wt</td>
</tr>
<tr>
<td>19</td>
<td>Plaque quantity, upper left permanent lateral incisor tooth, labial surface, marked area. (Hereafter UL2 plaque quantity)</td>
<td>mg wet wt</td>
</tr>
<tr>
<td>22</td>
<td>Dissolution rate of surface enamel</td>
<td>µg Ca</td>
</tr>
<tr>
<td>23</td>
<td>Plaque activity: resting pH; before adding substrate</td>
<td>pH units</td>
</tr>
<tr>
<td>24</td>
<td>Plaque activity: pH 20 seconds after adding substrate</td>
<td>pH units</td>
</tr>
<tr>
<td>25</td>
<td>Plaque activity: pH 80 seconds after adding substrate</td>
<td>pH units</td>
</tr>
<tr>
<td>26</td>
<td>Plaque activity: rate of change of hydrogen ion activity 20-80 seconds</td>
<td>H⁺ activity/minute</td>
</tr>
<tr>
<td>29</td>
<td><em>Streptococcus</em> spp. in pooled plaque: total number x 10⁸ of colony forming units per mg wet weight plaque processed.</td>
<td>colonies/ mg wet wt</td>
</tr>
</tbody>
</table>
### TABLE 1 (Continued)

<p>| 30 | Streptococcus spp. in pooled plaque: total number ( \times 10^8 ) of colony forming units ( \times ) wet weight plaque collected. | colonies ( \times ) mg wet wt |
| 31 | Streptococcus spp. in pooled plaque: Colony types 1-12 respectively, as a proportion of the total number of colony forming units per mg wet weight plaque processed. (Variable No. 31 = Colony type 1, 32=2, 33=3, 34=4, 35=5, 36=6, 37=7, 38=8, 39=9, 40=10, 41=11, 42=12.) | per cent |
| 43 | Streptococcus spp. in pooled plaque: Colony types 1-12 respectively, as total number ( \times 10^8 ) of colony forming units per mg wet weight plaque processed. (Variable No. 43 = Colony type 1, 44=2, 45=3, 46=4, 47=5, 48=6, 49=7, 50=8, 51=9, 52=10, 53=11, 54=12.) | colonies/ mg wet wt |
| 55 | Streptococcus spp. in pooled plaque: Colony types 1-12 respectively, as total number ( \times 10^8 ) of colony forming units ( \times ) mg wet weight plaque collected. (Variable No. 55 = Colony type 1, 56=2, 57=3, 58=4, 59=5, 60=6, 61=7, 62=8, 63=9, 64=10, 65=11, 66=12.) | colonies ( \times ) mg wet wt |
| 67 | Streptococcus spp. in UL2 plaque: total number ( \times 10^8 ) of colony forming units per mg wet weight plaque processed. | colonies/ mg wet wt |
| 68 | Streptococcus spp. in UL2 plaque: total number ( \times 10^8 ) of colony forming units ( \times ) mg wet weight plaque collected | colonies ( \times ) mg wet wt |</p>
<table>
<thead>
<tr>
<th>69 - 80</th>
<th><strong>Streptococcus spp.</strong> in UL2 plaque:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colony types 1-12 respectively, as</td>
</tr>
<tr>
<td></td>
<td>a proportion of the total number of</td>
</tr>
<tr>
<td></td>
<td>colony forming units per mg wet</td>
</tr>
<tr>
<td></td>
<td>weight plaque processed.</td>
</tr>
<tr>
<td></td>
<td>(Variable No. 69 = Colony type 1,</td>
</tr>
<tr>
<td></td>
<td>70=2, 71=3, 72=4, 73=5, 74=6, 75=7,</td>
</tr>
<tr>
<td></td>
<td>76=8, 77=9, 78=10, 79=11, 80=12.)</td>
</tr>
<tr>
<td></td>
<td>per cent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>81 - 92</th>
<th><strong>Streptococcus spp.</strong> in UL2 plaque:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colony types 1-12 respectively, as</td>
</tr>
<tr>
<td></td>
<td>total number x 10^8 of colony</td>
</tr>
<tr>
<td></td>
<td>forming units per mg wet weight</td>
</tr>
<tr>
<td></td>
<td>plaque processed.</td>
</tr>
<tr>
<td></td>
<td>(Variable No. 81 = Colony type 1,</td>
</tr>
<tr>
<td></td>
<td>82=2, 83=3, 84=4, 85=5, 86=6, 87=7,</td>
</tr>
<tr>
<td></td>
<td>88=8, 89=9, 90=10, 91=11, 92=12.)</td>
</tr>
<tr>
<td></td>
<td>colonies/mg wet wt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>93 - 104</th>
<th><strong>Streptococcus spp.</strong> in UL2 plaque:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Colony types 1-12 respectively, as</td>
</tr>
<tr>
<td></td>
<td>total number x 10^8 of colony</td>
</tr>
<tr>
<td></td>
<td>forming units x mg wet weight</td>
</tr>
<tr>
<td></td>
<td>plaque collected.</td>
</tr>
<tr>
<td></td>
<td>(Variable No. 93 = Colony type 1,</td>
</tr>
<tr>
<td></td>
<td>94=2, 95=3, 96=4, 97=5, 98=6, 99=7,</td>
</tr>
<tr>
<td></td>
<td>100=8, 101=9, 102=10, 103=11, 104=12.)</td>
</tr>
<tr>
<td></td>
<td>colonies x mg wet wt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>201</th>
<th>Hypoplasia index, mean score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>--</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>203</th>
<th>Pooled plaque quantity (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg dry wt</td>
</tr>
</tbody>
</table>

<p>| 205     | Mesio-distal crown diameter of upper |</p>
<table>
<thead>
<tr>
<th></th>
<th>permanent central incisor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
</tr>
</tbody>
</table>

<p>| 206     | Mesio-distal crown diameter of upper |</p>
<table>
<thead>
<tr>
<th></th>
<th>permanent lateral incisor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
</tr>
</tbody>
</table>

<p>| 207     | Mesio-distal crown diameter of upper |</p>
<table>
<thead>
<tr>
<th></th>
<th>permanent canine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mm</td>
</tr>
<tr>
<td>208</td>
<td>Number of Areca nuts chewed per day</td>
</tr>
<tr>
<td>209</td>
<td>Time elapsed since last betel-chewing</td>
</tr>
<tr>
<td>213</td>
<td>Colour of saliva</td>
</tr>
<tr>
<td>214</td>
<td>Viscosity of saliva</td>
</tr>
<tr>
<td>215</td>
<td>Bicarbonate content of saliva</td>
</tr>
<tr>
<td>217</td>
<td>Buffering capacity of saliva: initial pH</td>
</tr>
<tr>
<td>218</td>
<td>Buffering capacity of saliva: first parameter</td>
</tr>
<tr>
<td>219</td>
<td>Buffering capacity of saliva: second parameter</td>
</tr>
<tr>
<td>220</td>
<td>Calcium content of enamel biopsy specimen mg</td>
</tr>
<tr>
<td>229</td>
<td>Severity score of carious lesions/DMFS</td>
</tr>
<tr>
<td>230</td>
<td>Magnesium content of surface enamel</td>
</tr>
<tr>
<td>231</td>
<td>Potassium content of surface enamel</td>
</tr>
<tr>
<td>232</td>
<td>Phosphorus content of surface enamel</td>
</tr>
<tr>
<td>233</td>
<td>Barium content of surface enamel</td>
</tr>
<tr>
<td>234</td>
<td>Strontium content of surface enamel</td>
</tr>
<tr>
<td>235</td>
<td>Zinc content of surface enamel</td>
</tr>
<tr>
<td>236</td>
<td>Fluoride content of surface enamel</td>
</tr>
<tr>
<td>239</td>
<td>Calcium content of saliva</td>
</tr>
<tr>
<td>240</td>
<td>Calcium content of saliva</td>
</tr>
<tr>
<td>243</td>
<td>Fluoride content of saliva</td>
</tr>
</tbody>
</table>
1.4. Responsibilities of the author regarding the initiation, conduct and direction of the work.  (Stated in accordance with the by-laws of the Faculty of Dentistry, University of Sydney, Chapter XV, 32. (2)).

The study presented in this thesis has taken four years to accomplish and could not have been executed single-handedly. The diversity and volume of the work have made it mandatory to enlist assistance. It is obvious, however, that particularly for the purposes of this report, the importance of conceptual, developmental and interpretive contributions is considerably greater than that of routine field or technical assistance. As most of the work contributed by others falls into the latter category and was executed under his personal direction and supervision, the author considers himself responsible for not less than 80 per cent of the work presented. For complete clarity, the author's involvement in various phases of the project is set out under appropriate headings.

(a) Initiation of work and study design.

The author's interest in caries studies involving primitive peoples was kindled by Dr. D.E. Barmes's* discovery of a caries free population in the Sepik District of New Guinea and by participation in a subsequent investigation conducted by Barmes. (10)

The present project was initiated in the fiscal context as the outcome of a meeting at the National Institute of Dental

Research, Bethesda, Maryland, U.S.A., where results of past research in Papua New Guinea were discussed. The author presented available and potential techniques for further investigation and was appointed Senior Scientist of the proposed project. In this capacity, the author had major responsibility for drawing up the project design, which, in the conceptual sense, was dominated by his rationale outlined in Section 1.1. In the technical sense, the project design was accomplished entirely by the author, based on his own experiences and on consultations at an international level.

Background information obtained by the author over a period of seven years in individual and joint investigations\(^\text{(11)(12)(13)(14)(15)(16)(17)(18)(19)}\) had a dominating influence on the choice of setting for the study, while the feasibility to carry it out rested almost entirely on his personal effort and familiarity with the environment.

The methods used in the investigation were derived in part from techniques employed in a multi-factorial longitudinal study of caries aetiology which involved 400 New South Wales school children.\(^\text{(20)(21)(22)}\) The latter study, most of which is yet unpublished, was a jointly-developed concept of Professor G. Charlton* and the author who participated as co-principal investigator over its whole duration of three years. Other aspects of the methodology employed were developed or modified specifically for this project in the author's laboratory.

* Associate Professor, Dental Bacteriology, Faculty of Dentistry University of Sydney.
(b) **Field investigations.**

The author was in charge of all field investigations and all procedures were carried out under his personal supervision. Examinations for dental caries and hypoplasia, the collection of plaque specimens and measurements of dental morphology were performed exclusively by the author. The greater part of field technical procedures pertaining to plaque microbiology and to the properties of saliva were also carried out by the author who, at one time or another, as dictated by the daily requirements of work, personally performed every type of field test, technical task or data collection that appears in this text.

(c) **Laboratory work.**

With the exception of part of the fluoride estimations, all laboratory work, development or routine, was carried out in the author's laboratory by the author, or by graduate or technical assistants under his direction and personal supervision. In particular, all developmental work concerning the determination of trace element content of surface enamel, using flameless atomic absorption spectroscopy, and all routine estimations of strontium, potassium and zinc were performed exclusively by the author. In order to avoid person to person variation and possible bias in the classification of colony types of *Streptococcus* species (sub-section 3.2.2.4.), colony classifications were carried out independently by a graduate assistant, trained in this field by the author, who at the time was unaware of the caries distribution in the human sample.
(d) **Result processing.**

The statistical methodology used in the study was developed as a result of several discussions with Professor B.L. Adkins.*

The results were hand tabulated, coded and organized for computer data processing under the author's instructions. Computer processing was carried out according to the author's requests at the World Health Organization, Geneva, Switzerland, using standard biomedical programmes.

Examination of details of the data, and statistical tests other than those presented as computer outputs, were carried out by the author.

(e) **Presentation of work.**

The results were evaluated and this presentation was written exclusively by the author.

1.5. **Statement regarding the originality of the work presented.**

*(Stated in accordance with the by-laws of the Faculty of Dentistry, University of Sydney, Chapter XV, 32, (1)).*

All work presented in this thesis is original. Moreover, the material is unique in many respects for the following reasons:

(a) There is no precedent in the literature for a multi-directional study of caries aetiology which is comparable to the one presented, either in magnitude or in approach.

* Head, Department of Mathematics, University of Queensland, St. Lucia, Qld.
(b) The setting of the study in a primitive environment in itself makes the work unique.

(c) Method development by the author led to the application of flameless atomic absorption spectroscopy for the determination of the trace element content of surface enamel for the first time. The resultant information is the first of its kind for the elements investigated.
CHAPTER 2.

BACKGROUND, THE STUDY ENVIRONMENT.

2.1 General considerations.

Papua New Guinea is a basically primitive country occupying some 183,000 square miles of land, distributed between a main island mass and numerous smaller islands, situated in the tropics between $0^\circ 42'$ and $11^\circ 38'$ latitudes S and $140^\circ 47'$ and $156^\circ 03'$ longitudes E. The backbone of the main island consists of massive mountain ranges, surrounded by narrow coastal strips, which expand into extensive plains and swampy lowlands associated with two major rivers, the Sepik and the Fly.

In larger urban centres, mainly on the coast, amenities of civilization are available but the great majority of the 2.5 million inhabitants of the country have largely retained their primitive mode of life, and first contacts are still made with people in remote areas. With minor exceptions, Papuans and New Guineans are Melanesian in origin and most of them live in village communities of some 100-200 persons. In outback villages life still follows ancestral patterns, and in the absence of a cash economy subsistence is based on the produce of the village gardens, gathering of uncultivated foods and on the round-the-year availability of a staple food, invariably of carbohydrate nature. Because of geographical and climatic conditions, a single staple usually predominates in large areas, although some overlaps occur. (23) The staple food, sago in the lowlands and foothills, sweet potato in the highlands, may account for as much as 90 per cent of the total calorie intake over long periods of
time, and dominates the diet at all times.

Communications are generally difficult and many villages can be contacted by foot or river patrols only. Until recently, geographical obstacles and the fierce disposition of the people have contributed considerably to the isolation of villages. In turn, isolation led to considerable inbreeding and has resulted in the development of some 700 languages in the country. Even today, intermarriage between members of different village (language) groups is virtually unheard of in rural communities.

Available health services are based ultimately on large district hospitals, where practically all types of routine treatment practised in modern medicine and dentistry can be extended to those in need. When warranted, patients reach these hospitals through a chain of referrals, within a network of lesser establishments which have progressively decreasing treatment capabilities towards the outback. In truly remote areas it may take some days' travel on foot or by canoe to reach even the nearest aidpost. As a result of tradition, distance and apathy, the use of native medicines administered by the local medicine-man, in preference to modern treatment, is the rule rather than the exception in remote villages. Malaria, leprosy and tuberculosis are still endemic in many areas and diseases practically extinct in modern communities, such as yaws and elephantiasis, are relatively common.

A school dental service administers incremental dental care to schoolchildren of up to 15 years of age, but as yet the coverage is far from complete even for children who do attend
school. For all practical purposes no dental treatment, even of an emergency nature, is readily available in the outback.

Educational facilities are being actively developed, but the network of government and mission schools becomes rather thin as more remote parts of the country are approached. An estimated 50 per cent of all primary school age children attend schools and this proportion decreases, sometimes to nil, with increasing distance from the main centers.

2.2. Dental aspects.

Early clinical observations in the few accessible areas led to the publication of descriptive accounts of dental diseases in Papua New Guinea. (24)(25)(26) Extensive epidemiological studies, (23)(27(28)(29)(30) involving samples from most regions of the country, carried out between 1956 and 1970, established baseline values and ranges of variation in the prevalence of dental caries. Generally, the prevalence of the disease was found to be considerably lower than in highly developed communities. A rough estimation based on one of the studies (23) gave an overall country mean of approximately 3.5 decayed, missing and filled teeth (DMFT) per person. Of major interest was the finding of several distinct levels of caries prevalence, including caries-free communities, and the existence of sharp contrasts in the prevalence of the disease between population groups which were living under comparable primitive conditions and were consuming the same staple diet.

The potential of the situation for aetiological research
was recognized and several investigations were developed with the aims to explore, describe and compare aspects of the environment of caries-free and caries-active groups. As a result of these investigations, the following associations with caries prevalence, pertinent to the present report, were defined:

a) Inverse associations with the concentrations of alkali and alkaline earth elements in village garden soils, especially barium, calcium, lithium, magnesium, potassium and strontium. (10)(13)

b) Direct associations with the prevalence and proportionate numbers of certain *Streptococcus* species in dental plaque. (12)

c) Direct associations with the width of mandibular and maxillary dental arches and with the bucco-lingual diameters of upper and lower second permanent molar teeth. (14)

d) A weak but persistent direct association with oral debris. (10)

In addition, numerous observations were made, some of which were difficult or impossible to translate into figures or precise statements, yet had a strong bearing on the conception and design of the present study. Thus, it became apparent that generally caries prevalence was low in villages situated on alluvial soil and higher where the clay content of the soil was high. Caries prevalence also appeared to change with topography, increasing
directly with the height of the river banks and with the vicinity of the foothills and mountains.

The clinical impression was gained that in some populations most carious lesions were associated with malformed (dysplastic-hypoplastic) teeth. Similar associations observed in Polynesians by Baume and Vulliémoz\(^{(31)}\) were attributed to nutritional factors.\(^{(32)}\)

It has been reported by Davies\(^{(33)}\) that the high prevalence of a peculiar type of odontolysis affecting the deciduous dentition ("Odontoclasia", Jones, Larsen and Pritchard\(^{(34)}\) of Polynesian children examined at Pukapuka Atoll, was associated with structural defects characterized by defective matrix formation and calcification. The author drew attention to the possibility that dietary deficiency in Vitamin A, and possibly low Vitamin C and protein intake could predispose to this condition.

In a subsequent discourse, Davies\(^{(35)}\) further emphasized the possible importance of nutritional inadequacies during tooth development in relation to hypoplasia, but concluded that available information was insufficient to identify the specific nutrients involved. Similar conclusions were reached in a dental and nutrition survey in New Guinea by Sinclair, Cameron and Goldsworthy\(^{(26)}\) who did not find consistent associations between the availability or intake of macronutrients, and tooth defects or caries.

Extreme differences in the progressiveness and destructiveness of lesions were noted between populations. On the whole, carious lesions appeared to be less progressive than in civilized
societies and arrested lesions were seen frequently. In some groups, however, progressive, highly destructive, and even truly rampant caries were prevalent.

The rates of attrition observed also varied from area to area, but were always higher than in civilized societies and not infrequently led to occlusal exposure of the pulp chamber in older persons.

A considerable amount of information was collected concerning cultural and social traditions. Particular attention was paid to oral hygiene methods, if any, practised by the people. Observations of the betel-chewing custom and the resultant stain and deposits on teeth gave rise to much thought concerning the possible effect of the practice on caries.

Most importantly, food cultivating and gathering practices were observed and the preparation of foods and variations in nutritional status and dietary patterns received close attention. With respect to the diet, it was clear that in many outback areas Western-type processed or refined foods were either virtually unused or have made insufficient impact to affect dental health noticeably.

Consideration of the available information led to the conclusion that pursuing the investigation reported in this volume in a suitably selected remote area of New Guinea could afford the following unique advantages:

a) Substantial populations of caries-free people are available to define, in chosen parameters, a completely
caries-inactive oral environment.

b) Population groups, exhibiting caries experiences ranging from zero to high, and living under comparable conditions may be selected for study.

c) The dietary and eating habits of people consuming a given staple within the same geographical area are uniform, simple and almost totally dependent on the immediate environment. Thus, the risk incurred through substrate supply to the plaque flora, a variable which is virtually impossible to estimate in civilized populations, is minimized and may even approach a constant.

d) Lack of dental treatment permits undisturbed development of carious lesions to the limits imposed by environmental conditions alone.
CHAPTER 3.

METHODS.

3.1. Sample design and study parameters.

3.1.1. Sampling strategy.

The investigation described in this text was not conceived as an isolated study. Several aspects of the environment and some aspects of the oral biology of the people, which are outside the scope of this report, were studied. Moreover, even in the early planning stages, consideration was given to the possibility of extension and future follow-up work. These facts were kept in mind when the attributes that would characterize an 'ideal' study population were considered. The following conclusions were reached:

a) The sample should exhibit marked contrasts, as well as intermediate levels of caries prevalence. The range should include caries-free individuals and persons who experience destructive caries.

b) The population should be situated in remote areas, should conduct a primitive mode of life and have a negligible migration rate both within and outside their native land. Individuals included in the sample should be life-long residents of their village.

c) The villages from which the sample is drawn should exhibit geographical continuity.
d) The staple diet should be the same throughout the sample and the consumption of Western-type foods should be insignificant.

e) Individuals included in the sample should not have received dental treatment at any time during their lives.

f) A sufficient number of individuals, aged between 14 - 18 years and equally distributed between the sexes, should be available for examination.

The most stringent requirements in determining the sample size for comparing two groups of people with different levels of caries experiences were considered to be in the areas of measuring the dissolution rate of surface enamel, fluoride content of surface enamel, plaque activity and buffer capacity of saliva. (The variables included in the investigation are listed in Table 1, pages 7-10.) For these determinations, preliminary work by the author had shown that approximately 40 subjects per group were needed to enable statistically significant differences of the order of 10 per cent or less of the mean value to be detected. To permit replacement of samples that proved unsuitable or became damaged in transit or in processing, the number was increased to 50.

The relatively narrow age range of 14-18 years was considered desirable to restrict variation within parameters dependent on tooth age.

Previous experience and results of epidemiological studies indicated the existence of populations that would satisfy some
or several of the criteria listed for an 'ideal' sample, but experience also suggested that it was most unlikely that all the criteria could be met by a single population.

To explore the best possible combination of caries contrasts and environmental conditions, extensive reconnaissance visits were conducted in the Western District of Papua and the Eastern and Western Highlands and the Sepik District of New Guinea, where the major contrasts in caries prevalence were known to exist. Following consideration of the advantages and disadvantages associated with the groups examined, inhabitants of villages situated on the Wogupmeri-Karawari-Blackwater-Korosameri river system, tributary to the Sepik River, were chosen for study (see Figure 1, page 26).

This population satisfied the first five requirements listed for an ideal sample, and had the additional unexpected advantage of being situated within a rectangle of 20 x 40 miles on a single river system, with villages extending from the plain of the Sepik River to the foothills of the Highlands. A comparatively small disadvantage was that census or village records were not available at the time, and because of low water levels, efforts to visit the villages on the higher reaches of the Wogupmeri River failed. As a consequence, the exact size of the proposed study population and age and sex distribution could not be determined in advance. The staple food in the area was sago.

Based on preliminary estimation of caries prevalence levels it was expected that the villages would fall into three strata, and the following tentative sampling strategy was drawn up:
FIGURE 1: OUTLINE MAP OF PAPUA NEW GUINEA
SHOWING RECONNAISSANCE VISIT SITES
Group 1. It was known that a number of villages existed in the area of the Karawari River and its tributaries in the Sepik District of New Guinea, in which the mean caries prevalence in the age-group of 14-18 years was approximately 7 decayed permanent teeth (D.T.) per person, with a range from 0 to more than 15 D.T. per person.

All subjects aged 14-18 years were to be examined for caries prevalence in several of these villages. The number of villages included in the sampling was to depend on reaching 50 subjects for each of the categories detailed below:

a) 0-2 D.T. per person,
b) 3-5 D.T. per person,
c) 6+ D.T. per person,

giving a total of 150 subjects for Group 1.

Group 2. It was known that other villages existed in the same area, in which the mean caries prevalence between the ages of 14-18 years was approximately 2.5 D.T. per person. In each such village, there was a substantial number of caries-free people and the expected range of caries experience was from 0 to approximately 8 D.T. per person. One hundred subjects were to be selected within the 14-18 age group in several such villages until 50 caries-free subjects and 50 subjects with 3 or more D.T. per person were obtained.

Group 3. It was known that villages also existed in the same area, wherein all people subjected to clinical examination were found to be free of caries. A total of 50 subjects, in the
age group of 14-18 years, were to be selected for the sample from two or more such villages.

The ratio of 150 : 100 : 50 subjects in the three groups was decided upon to make possible statistical examination of the results on the basis of caries contrasts within Groups 1 and 2, without overloading the sample with caries-free subjects.

Subjects were to be matched by sex where possible within Groups 1, 2 and 3, and within each caries experience class.

Because the exact number of subjects available and the details of caries distribution were unknown, flexibility was proposed for subject selection in Groups 1 and 2. As caries will have been recorded for all individuals aged 14-18 years, if the distribution of individuals with given numbers of carious teeth, and matching difficulties indicated a change in design in favour of collecting continuous data, such a change would be possible. In addition, the possible need to make decisions in the field to extend the age range, to include outlying villages, or to reduce the total proposed sample size was recognized.

Where a number of villages would be required to reach the stated sample size, all subjects of suitable age were to be taken from all but the last village, in which a sampling fraction was to be used. Attempts were to be made to include people in the sample who were absent briefly from the village, but not those on extended absence.

Sampling was to be restricted to life-long residents
of the villages and every effort was to be made to identify, classify according to true residence, or, if indicated, exclude from the sample, itinerant persons if such were present in the villages. To facilitate identification and to permit examination of associations with familial patterns, if the latter were warranted by the data, records of family relations were to be collected.

Data were to be collected in respect of the variables listed in Table 1, pages 7 - 10, from all individuals included in the sample, using the procedures described under Field and Laboratory methods. (Sections 3.2. and 3.3.).

The sampling strategy design was of necessity tentative and subject to modifications at the time of the field study. Modifications made are described in the appropriate parts of Section 3.2.1., Field methods.

3.1.2. Study parameters.

The variables dealt with in this report and units of measurements are listed in Table 1, pages 7 - 10. Variable numbers which are missing from the consecutive order were either of a classificatory nature, such as subject, sex or village identification numbers, or were assigned to transformations of the variables listed, which were examined but not used in the final data processing.

Examination of Table 1 will show the extensive range of
variables included in the investigation. Most of these have not been studied otherwise than in populations of very limited size, few of them have been investigated together in the same sample, and some of them have never been examined at all.

As the relevance of most of the parameters investigated has been universally recognized, detailed justification would be superfluous. In some instances, however, comments will be made concerning special characteristics of the study population, which are relevant to the variables examined. Similarly, observations of the author and associates made in comparable or contrasting populations, will be mentioned in connection with the appropriate variables where the collection of the data is described. These observations and previous findings listed in Section 2.2. received careful consideration in the selection of variables.

In general, the variables included in the report relate to two of the three major aetiological areas of Keyes and Fitzgerald, the microbial flora of plaque and host resistance or susceptibility. An endeavour was made to include variables that reflected details within these areas, such as the types of streptococci present in plaque and aspects of surface enamel composition, as well as others which could be regarded as indicators, in a wider sense, of the status of the aetiological area. Plaque activity and enamel dissolution rate are examples of the latter categories.

Consideration was given to obtaining individual data for several additional parameters within the two aetiological areas, of which three deserve special mention. Extension of plaque microbiology to the whole cultivable flora was thought to be
desirable, but it could not be included because the enormous resources required were not available. Provision was made, in the author's laboratory, for the examination of the major and trace mineral content of plaque collected within this project, but the full data will not be available for approximately two years. Finally, aspects of the aggregating and adherence-inhibiting capacity of saliva (36)(37)(38)(39) will be examined using a limited subsample with contrasting caries prevalence. These facts were mentioned to indicate that a) eventually, data relating to the latter parameters will be evaluated together with those listed in Table 1, although in no way do they form part of this thesis, and b) consideration was given to studying a still wider range of parameters but this was precluded by limitations of available techniques and resources.

The third aetiological area, substrate supply to the plaque flora, is an important case in point. No reliable means, compatible with available resources, could be found to obtain individual data on aspects like the frequency of food intake. However, observations of the diet at the village and family level confirmed beyond doubt that all villagers depended on sago for their staple food, and no major differences in food intake pattern were noted.

These observations point towards a much lesser and certainly more uniform risk incurred in the substrate supply area by the population studied than by most advanced societies. While one could assume from the findings that variation in substrate supply contributed relatively little to variation in caries experience, any attempt to estimate the extent of contribution would be
conjectural.

The content of selected minerals was determined in samples of household water collected in each village. As these variables were related to villages rather than to individuals, they were not included in Table 1 and these data were processed independently from the rest.

3.2. Field methods.

3.2.1. Introduction.

Preparatory work and the procedures used for the collection of biological materials and data, together with details of clinical tests completed in the field, are described in this Section. (For details of laboratory and/or analytical procedures see Section 3.3. Laboratory methods).

A short account of organization and preparation is given in sub-section 3.2.2.

It proved impossible to conduct all clinical examinations and tests and to collect oral biological materials during one field visit. Most data and materials were collected during the main April - May, 1972 patrol. The procedure and methods followed on that occasion are set out in sub-section 3.2.3.

Two further field visits were made, in March - April and in July, 1973. The methods used during the latter two visits are given in sub-sections 3.2.4. and 3.2.5. respectively.
3.2.2. Preparations.

(a) Technical aspects.

(i) Dental examinations. Following field examinations during the reconnaissance visit to Papua New Guinea, basic criteria were laid down for dental examinations and a draft of the examination form was prepared. (See Form 1, page 34).

(ii) Microbiology. A previously employed method for measuring plaque volume required considerable experience, and apart from inherent variability, the technique was subject to considerable variation between operators. An improved method of quantitation by weighing was developed.

Trials concerned with the preservation and transport of plaque samples for subsequent streptococcal counts were conducted during January - March, 1972. Previously used methods could not be employed under Papua New Guinea field conditions and communications systems. Transport and storage techniques in common use were either impractical for our purposes or failed to maintain trial specimens without material changes in viable counts. Eventually a method involving dehydration of cells in a medium of high osmotic pressure, followed by preservation and storage in liquid nitrogen was developed. Details of these methods are given in sub-section 3.2.3.5.

(iii) Plaque activity test. The method used by Charlton, Blainey and Schamschula in their N.S.W. studies, was modified to use an isolated specimen of plaque in-situ to permit later evaluation of the acid-producing capacity of the
### Dental Examination Sheet

**WHO Project NIH-NIDR-72-2003**

**Form 1. Dental examination**

<table>
<thead>
<tr>
<th>Name</th>
<th>Date of birth</th>
<th>3-5 Age</th>
<th>6-9 Individual No.</th>
</tr>
</thead>
</table>

**10. Sex:**

<table>
<thead>
<tr>
<th>11-13 Relationship</th>
<th>Date of examination</th>
<th>14-15 Village:</th>
</tr>
</thead>
</table>

#### Maxilla

<table>
<thead>
<tr>
<th>Left</th>
<th>OCC. *</th>
<th>BUC.</th>
<th>LING.</th>
<th>DIS. MES.</th>
<th>Mandible</th>
<th>Left</th>
<th>OCC. **</th>
<th>BUC.</th>
<th>LING.</th>
<th>DIS. MES.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxilla ATP.</td>
<td>P. S.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maxilla ATP.</td>
<td>P. S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>8</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>7</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63</td>
<td>6</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79</td>
<td>5</td>
<td>64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>47</td>
<td>3</td>
<td>32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>63</td>
<td>2</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79</td>
<td>2</td>
<td>1</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

#### Mandible

<table>
<thead>
<tr>
<th>Right</th>
<th>OCC. *</th>
<th>BUC.</th>
<th>LING.</th>
<th>MES. DIS.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxilla ATP.</td>
<td>P. S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>64</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Remarks:
- * For molar teeth only, occlusal is divided into distal and mesial
- ** For premolar teeth only, occlusal is divided into mesial and distal
specimen against its mass and microbial content. (Sub-section 3.2.3.6.)

(iv) **Test for surface enamel dissolution rate.** The relative merits and disadvantages, and the results obtained by the techniques of Charlton, Blainey and Schamschula\(^{(21)}\), and that of Schamschula and Blainey\(^{(44)}\) were examined. The latter technique using a different system of etch fluid distribution was adopted. (Sub-section 3.2.3.10.)

(v) **Buffering capacity and bicarbonate content of saliva.** Ericsson’s method\(^{(45)}\) for measuring the buffering capacity of saliva was modified for use under the field conditions expected. In addition, a technique using stepwise titration for establishing two measures of buffering capacity, which could be combined with a modification of Segal’s method\(^{(46)}\) for the estimation of the bicarbonate content of saliva, was developed. (Sub-section 3.2.5.3.)

(vi) **Viscosity of saliva.** A simple viscometer suitable for field use was made and tested.

(vii) **Chemical analyses.** Preparatory work was conducted along two main lines: a) sampling methods, and b) preservation, transport and storage of biological materials.

Enamel biopsy techniques were examined and the analytical requirements were reviewed. Preliminary experiments were carried out on a large number of extracted teeth. These included examination of enamel thickness on different tooth
surfaces, determination of the amount of enamel obtained using various biopsy methods, microscopic examination of surface effect, sectioning and staining for free calcium. It was decided to use a mineral acid biopsy method involving the labial surfaces of canine teeth. (Sub-section 3.2.3.12.)

Consideration was given to the plaque and saliva collection methods. Stainless steel chisels were chosen in preference to plastic implements for plaque collection.

To obtain activated saliva, it was decided to use soft vinyl tubing for masticatory stimulation.

In preserving the enamel samples the main considerations were to avoid change of volume and contamination. Both were achieved by heat-sealing the samples in polyethylene tubing as described in sub-section 3.2.3.12.

The main problems in the preservation of saliva samples were avoiding contamination and the strong likelihood of mould growth, either of which would make subsequent quantitation and handling of samples difficult. In view of the contamination risk involved with chemical preservatives, it was decided to sterilize the samples by autoclaving.

The following procedures were employed to decontaminate containers and materials used for obtaining and storing biological samples.

The polyurethane discs used for etching were soaked in 5 volumes of approximately 0.2 M Aristar nitric acid (B.D.H. Chemicals Ltd., Poole, Dorset, U.K.) for 24 hours and after
decanting the acid were compressed and washed repeatedly in approximately 100 volumes of distilled deionized water.

The polyethylene tubing used for storage of the enamel samples was washed in a two per cent solution of combined anionic-cationic detergent (Detex 11, Borer Chemie, Solothurn, Switzerland), followed by rinsing in three changes of distilled water, six changes of deionized water and two changes of glass-distilled, deionized water.

The vinyl tubing used for the collection of saliva specimens was washed in Detex 11, and rinsed in a large excess of distilled, deionized and glass-distilled, deionized water.

The saliva containers were washed in dilute nitric acid and were rinsed in distilled, deionized water.

It was shown by the satisfactory blank levels in later analyses that the decontamination procedures were successful.

(b) Logistics.

The survey area is remote and most villages can be approached by river only. The upper reaches of the Wogupmeri River are hazardous to approach at any time and extremely difficult or impossible to navigate at low river levels.

Under these conditions replacement of equipment or materials would have been either most time-consuming or completely impractical. Therefore, as far as possible, critical equipment and materials were duplicated and repair kits were carried.

Dugout canoes, speedboats and occasionally jetboats were
used for local transport.

A chain of transport consisting of canoe, chartered aircraft and regular airlines was established for the transport of viable cultures in liquid nitrogen from the field to the laboratory.

For communications, two radio sets were obtained by the courtesy of the Department of District Administration, Wewak, Papua New Guinea.

The Department of Public Health, Konedobu, Papua New Guinea, provided portable dental chairs and engines and assisted with local personnel and camping equipment.

Permits to import biological materials to Australia were obtained from the Quarantine Section, Commonwealth Department of Health, Canberra, A.C.T.

3.2.3. April-May, 1972, Field Visit.

3.2.3.1. The human sample.

On arrival in the field in April, 1972, examination of village records indicated that the population of villages along the Wogupmeri and upper reaches of Karawari Rivers was smaller than expected.

It was considered undesirable to include in the study villages outside this river system, and highly desirable to maintain an initial sample size of approximately 300 subjects, distributed between high, intermediate and zero levels of caries
prevalence in the approximate ratio of 3 : 2 : 1. Therefore, the originally intended age group of 14-18 years was extended to 12-24 years. The additional provision was made that, at the lower end of the age group only subjects who had all permanent second molar and canine teeth fully erupted would be included in the sample.

Because of the limitation imposed by the size of the population, the alternative of collecting continuous rather than discontinuous data in respect of caries prevalence, already provided for in the sample design, was adopted.

Population characteristics made it impossible to obtain complete balance of age and sex between strata of caries prevalence, but the departures were relatively minor and were catered for in the statistical examination of the results.

The human sample consisted of 301 subjects, 154 males and 147 females distributed between villages as shown in Figure 2, page 40.

3.2.3.2. General field procedure.

Where possible, notice was sent to villages informing them in advance of the pending arrival of a 'health' survey team. On arrival, men of prestige in the village such as the Luluai, Tultul or elders were contacted, and the purpose, details and voluntary nature of the project were explained to them. At the same time, teenagers and young adults were pointed out amongst the bystanders, to indicate the age group desired for examinations and tests. The people were asked not to make material changes in their
pattern of life during the team's stay in the village and in particular, not to change their eating and oral hygiene habits or practices before or during examinations.

A list of prospective subjects was prepared from the Government census books carried by the survey team. The subjects who, according to their birth records or appearance, fell into the age group of 12-24 years, were asked to participate in the survey, and invariably consented.

As the survey team usually stayed in a village or locality for several days, subjects temporarily absent in the fields, bush, or sago camps were usually seen on another day. Thus, apart from the occasional person away at a great distance or for a long term, such as plantation workers or a few teenagers at distant mission schools, practically all persons in the selected age group were examined in all villages except in Village 13, Sangriman. Here, the number of persons in the age group was about 30 per cent greater than the number required for the survey and the selection was made on a 'first come included' basis, with simultaneous consideration given to age and sex balance.

The equipment and materials required for each day's work were set up under a tarpaulin in a suitable central area. The examinations and tests were carried out according to a predetermined routine, which was perfected during the first few days of the field investigation. In developing the routine, consideration was given to avoiding untoward inter-effects of tests and to smooth flow of subjects. Tests took about 40 minutes to complete for each subject, with short periods of waiting in-between stages.
As a rule, subjects did not leave the examination area between tests. Samples collected were removed from the examination area and placed into suitable storage, as soon as possible after collection.

The order of examinations, tests and collection of specimens is shown below:

- Screening and assignment of subjects to project.
  - Establishment of residence qualifications and family affiliations.
  - Estimation of age.
  - Registration of basic identification on forms.

Saliva collection.

Collection of pooled plaque.
  - Aliquot removed for microbiology.
  - Bulk preserved for chemical analyses.

Plaque activity test in-situ (upper left permanent lateral incisor, labial surface – UL2 plaque).
  - Initial pH before adding substrate.
  - pH 20 seconds after adding substrate.
  - pH after 80 seconds.

Collection of UL2 plaque for bacteriological examination.

Cleaning of teeth.

Dental examination.
  - Dental caries.
  - Hypoplasia.

Test for surface enamel dissolution rate and sampling for fluoride content estimation.
Examination for enamel wear and anterior tooth defects.

Enamel sampling for chemical analyses.

Tooth colour classification.

Impressions for cusp height and cusp inclination measurements.

Saliva collection.

After the examinations participants received rewards which usually consisted of either cash or goods such as soap, canned food, tobacco, kerosene, etc. The excellent cooperation extended to the team on this patrol and on several return visits since, by subjects and village people in general, was further enhanced by giving emergency dental and medical treatment, dressing wounds, distributing medicines and counselling and aiding people as far as possible where advice or help was sought.

The duties in the field were arduous because after the clinical examinations extensive technical tasks had to be completed in connection with microbiological work, preservation and storage of samples, maintenance of equipment, revision of forms and logistic arrangements.

Every effort was made, however, to collect information concerning pertinent aspects of the environment and economy such as the main sources of food and drinking water, the existence, location and size of gardens, the extent of food gathering areas, the availability of trade goods and particularly of Western-type
foods. Similar interest was taken in the people, whose diet and eating habits, history, traditions, customs, daily activities, tribal relationships, language and tribal boundaries, area of movement, general health status and level of sophistication were noted. The information obtained will not be detailed in this report, except for the following remarks about oral hygiene.

Oral hygiene practices known to the people ranged from rinsing the mouth with river water, the use of sharpened twigs to dislodge debris, rubbing tooth surfaces with tree-bark or vegetable material such as the husk of green Areca nut, to the use of river sand and kaolin-like soil for polishing teeth. Actual signs of these practices were seen infrequently and even then cleaning was usually restricted to visible tooth surfaces. The impression was gained that females' teeth were somewhat cleaner than males' teeth but, on the whole, very few 'clean mouths' were seen. Of the latter, Village 11, Kundiman, appeared to have more than its fair share, but even after repeated questioning Kundiman subjects remained adamant that they did not clean their teeth in anticipation of the dental examination. For all practical purposes, toothbrush and dentifrice were unknown.

3.2.3.3. Screening and assignment of subjects to project.

(a) Establishment of residence qualifications and family affiliations.

To avoid the introduction of unknown variables, the sample design required that a person included in the sample should have lived in one village or in a defined locality preferably for the whole of his or her lifetime. Because of pre-natal effects of
the environment on tooth development, the subject's mother should have lived in the same village or locality during the period of pregnancy.

The project design also made it mandatory that the family affiliations of subjects should be known.

To establish eligibility, prospective subjects were identified by their own name given at birth, (people in this area are usually known by one name only at any one time, but change their name quite frequently) and by their father's and mother's name, and the information was checked against the census book records. The subjects were then questioned along the following lines:

Question 1: - Has the person lived in one family since birth?
Answer 1:    - (a) Yes - go to question 2.
              (b) No - refer to R.G. Schamschula (R.G.S.) for instructions.

Question 2: - Has the person lived in this village since birth?
Answer 2:    - (a) Yes - register on forms and go to question 4.
              (b) No - go to question 3.

Question 3: - For what periods has the person been absent from the village except for local absences such as at a nearby village school?
Answer 3:    - (a) Less than 2 years ) Register on forms
              Up to 2 years ) and go to question 4.
              (b) More than 2 years - reject.
              (c) Doubtful - check with R.G.S.

Question 4: - Are mother and/or father immigrants?
Answer 4:    - (a) No - fully acceptable for village.
(b) Yes - interview parents and record on dental form for child.

(c) Doubtful - check with R.G.S.

As seen from the information given above, the residence qualifications were eased to the extent that absence for two years was considered permissible.

With the exception of one person, whose child was rejected from the sample on this ground, the few other immigrant parents were previously resident in one of the villages of the same village (language) group, and the mother was pregnant with the child in her present village.

Eligible subjects were given a number which they held until all tests were completed.

In establishing family affiliations, no attempt was made to trace relationships of subjects further than to true parents. History of inbreeding within villages suggests, however, that most people in a given village were not too distantly related to each other.

The average coefficient of consanguinity, \( F \), in small populations, at equilibrium, is given by the formula:

\[
F = \frac{1}{4Nm+1}
\]

where \( N \) is the number of persons in the community, and \( m \) is the migration rate. Thus, in a village of 100 persons with three immigrants, \((m=0.03)\) \( F \) is of the order of 8 per cent, indicating that, on the average, individuals are related at least as closely as first cousins.
The coding detailed below was entered on Form 1 and was newly commenced in each village. The first digit of the code refers to the interrelationships of siblings and parents and the second and third digit identifies the family.

Example for family unit 03

Brothers and/or sisters:
(a) Same father and mother
   - first digits are 0, family digits are identical 003
(b) Different father
   - first digits are 1, family digits are identical 103
(c) Different mother
   - first digits are 2, family digits are identical 203

Actual examples of further combinations that occurred:
Three brothers and/or sisters, all from the same father,
one from different mother, Village 13: subjects 215 001
                                               223 001
                                               216 201

Four brothers and/or sisters all from the same father,
two from mother A and two from mother B, Village 16:
subjects 246 004
                                               293 004
                                               262 304
                                               292 304

(b) Estimation of age.

The ages of most subjects had been recorded in the Government census books. Apart from a few instances when entries were based on reports made by missionaries, Infant Welfare Sisters
or Department of Public Health personnel at or soon after birth, the age records were made by officers of the Department of District Administration, during visits to the villages at intervals of two to three years.

The recorded ages corresponded reasonably closely to age estimations made routinely by ourselves. Where possible, our estimations were based on the status of the dentition. When this was not possible, personal appearance and information obtained from relatives and elders were used as guides.

Where there was a marked conflict between recorded age and our estimation, the latter was entered on Form 1 to the nearest year. Age was designated Variable 2.

(c) Registration of basic identification on forms.

The name, individual number, estimated year of birth, age, sex, family code number, village number and the date of examination were entered on the examination forms prior to the tests.

3.2.3.4. Saliva collection.

Two specimens of activated whole saliva were collected from each subject during this field visit. The first specimen was obtained prior to all examinations and before cleaning the subject's teeth. The second specimen was collected as the last step in the clinical field procedure.

The samples were used for purposes outside the scope of this report. However, collection of the specimens is reported, because it formed part of the sequence of field tests. In
addition, observations of differences between the colours and viscosities of the samples resulted in including these variables in the range of parameters examined.

The method of collecting the specimens on which the results presented in this report are based is described in subsection 3.2.4.1.

3.2.3.5. Collection of pooled plaque.

In preparation for plaque collection, obvious food particles, vegetable fibres, Areca nut debris and similar superficial material of short standing, were removed from around the teeth with an explorer or cotton wool swabs. Sections of the dental arches were isolated and excess moisture was removed from the coronal surfaces with absorbent cotton wool.

Remaining soft deposits were then collected from all accessible tooth surfaces, with the exception of the labial surface of the upper left permanent lateral incisor, using stainless steel chisels remodelled for this purpose from dental hand instruments (Ash No. PFI-179 and PFI-4, Amalgamated Dental Trades Distributors Ltd., London, U.K.). The movements of the chisel were limited apically to the outlines of the free margins of the gingiva to avoid plaque from the gingival crevice. Plaque overlying calculus extending beyond the gingival crevice was collected with less chisel pressure than from enamel surfaces, to avoid including calcified material in the sample.

As it was collected, the plaque was pooled in the lid of
a polypropylene container (5-P, Disposable Products, Adelaide, S.A.) the rim of which was trimmed to facilitate access, and was mixed with a 1 mm diameter borosilicate glass rod until it appeared homogenous. When necessary, the moisture content was adjusted by removing excess moisture with filter paper strips (Whatman No. 541, 0.008 per cent ash) or by adding traces of glass-distilled, deionized water with a clean glass rod, to match the consistency of a standard, made up of linseed oil and whiting. This method was found rather unsatisfactory under field conditions, and after the first 23 subjects it was substituted by drying the plaque in-situ to a standard degree, previously indicated by the linseed oil-whiting mixture, as judged by the consistency and appearance of the plaque material itself.

(a) Subsampling of pooled plaque for bacteriological examination.

A small, representative aliquot of the mixed pooled plaque, approximately 0.2 - 0.5 mg wet weight, was placed on a pre-weighed plastic disc held ready by an assistant, and was weighed immediately to the nearest 0.001 mg on a torsion balance, the chamber of which was lined with wet cotton wool to reduce evaporation rate (Model 420-A, Sauter, A.G., Ebingen, West Germany).

The disc holding the plaque sample was dropped into 2.5 ml VMG II transport medium (40) contained in a polypropylene vial (5-P, Disposable Products, Adelaide, S.A.). The transport medium was modified by omitting charcoal and by adding sucrose (A.R. Grade, B.D.H. Ltd., Poole, Dorset, U.K.) to a final concentration
of 10 per cent.

After removal of the plastic disc which floated to the surface of the medium, the plaque suspension was transferred into a 2.5 ml capacity glass tissue homogenizer with a teflon pestle of 0.1 mm clearance (Bio-Tech Pty. Ltd., Guildford, N.S.W.) and homogenized using 20 uniform strokes with three complete twists following the downward stroke and interposed by a re-transfer into the original vial after 10 strokes.

Previous work using Gram stained homogenized plaque specimens showed that this method produced approximately 50 per cent single cells, 25 per cent diplococcal forms, 20 per cent short chains or small clumps (estimated to have consisted of 3-6 cells) and 5 per cent long chains or large clumps (of over 6 cells) of recognizable Gram positive cocci.

At the end of the day's work, 0.5 ml of the homogenized suspension was transferred into polyethylene tubing ('Portex' PP 355, bore 3.5 mm, external diameter 4.5 mm, Boots Pure Drug Co., Hythe, Kent, U.K.) with a standard tuberculin syringe fitted with flexible polyethylene tubing over an 18 gauge stainless steel needle.

The homogenizers and syringes were sterilized by boiling between samples. The polyethylene 'straws' containing the samples were heat-sealed with artery forceps over a portable Bunsen burner, followed by snap freezing, storage and air transport to the laboratory, in stainless steel vacuum storage vessels containing liquid nitrogen at approximately -196°C.

As bacterial counts, described under laboratory methods, were based on the wet weight of plaque, the variability introduced by variations in the moisture content of the plaque samples at the time of collection, and loss of water before completion of weighing were considered to be major sources of experimental error.

To minimize variability arising from the latter source, the weighing procedure was standardized rigidly and streamlined to enable establishment of the wet weight within the shortest possible time. Timing of the process had shown that the average time from the completion of the plaque collection to the reading out of the wet weight value was 31 seconds (S.D. = ± 7.3 seconds). The rate of loss of weight due to evaporation was tested by observing the weight change with time of 14 plaque samples, covering the range of wet weights of samples obtained in the field. The mean loss of weight, expressed as a percentage of weight at 30 seconds, over a period of 5 minutes is shown in Figure 3, page 53, which also illustrates the estimation of error in wet weight due to evaporation, found to be within ± 4.2 per cent for 95 per cent of the samples. (For the purpose of this estimation the relevant part of the percentage weight-time curve was taken as a straight line.)

To estimate the error due to initial differences between the moisture content of individual plaque specimens, the same 14 samples were dried to constant weights, the differences between
REDUCTION OF MEAN WET WEIGHT OF 1/4 PLQUE SAMPLES WITH TIME, IN RELATION TO VARIATION IN MEAN TIME TAKEN TO ESTABLISH WET WEIGHT

ERROR IN WET WEIGHT DUE TO EVAPORATION
±4.2% FOR 95% OF SAMPLES
the wet and dry weights representing the amount of unbound water in each sample. The mean water content was 82.4 per cent of the 30 second wet weight (S.D. = ± 3.2 per cent).

Bacteriological examination of the pooled plaque specimens, on which Variables 29-66 were based, is described in sub-section 3.3.2.4., Laboratory methods.

(b) Preservation of pooled plaque.

After removing the aliquot for bacteriological examination, the bulk of the pooled plaque sample was sterilized by autoclaving at 14 p.s.i. for 10 minutes in a pressure cooker (Model 7, Namco Overseas Corporation of Australia Ltd., Sydney, N.S.W.). The samples were kept in closed polypropylene vials (5-P, Disposable Products, Adelaide, S.A.) at ambient temperature until transported to the laboratory by air.

Weighing of these samples, on which Variable 16, pooled plaque quantity (1), is based, is described in Laboratory methods, sub-section 3.3.2.3.

3.2.3.6. Plaque activity test in-situ (UL2 plaque).

For the purpose of this study, plaque activity has been defined as the mean rate of change in hydrogen ion concentration produced in-situ by an isolated specimen of plaque of known quantity over the period of one minute, beginning 20 seconds after the application of 5 µl of a 20 per cent aqueous sucrose solution.

The resting pH and the pH 20 seconds after applying the sucrose
solution to the specimen were recorded as accessory measurements. The time of day and the time elapsed since the last meal were noted in respect of each plaque activity determination, for future evaluation of the relationships between these factors and plaque pH values.

It was considered important that undisturbed and well consolidated plaque of long standing should be used for the plaque activity test in-situ, and for the bacteriological examination of the same isolated plaque material following its removal from the enamel surface. The apical quarter of the labial surface of the upper left permanent lateral incisor tooth was selected as the test site, because of its well sheltered position created by the palatal inclination of the neck of the tooth in the majority of subjects, and by the prominences of the adjoining central and canine teeth. Good accessibility was also necessary for the delicate instrumentation.

An area of plaque, immediately incisal to the free margin of the gingiva, in the midline of the labial surface of the tooth, was demarcated. This was achieved using a stainless steel instrument machined for this purpose to form three sides of a hollow square with 1.5 mm long sides and sharp edges. The fourth side of the square was formed by the gingival margin. The plaque inside the area marked was isolated from surrounding soft deposits by removing immediately adjacent material from the enamel surface with fine stainless steel chisels. (Previous work\(^{20}\) had shown, that smooth enamel surfaces from which plaque has been carefully removed by the same method, were not visibly stained by a disclosing
solution of 0.1 per cent aqueous basic fuchsin.)

The pH measurements were made using a battery-operated portable pH meter (Keithley 602 solid state electrometer, Keithley Instruments Inc., Cleveland, Ohio, U.S.A.) with a standard calomel reference electrode, and specially made, flexibly mounted, glass measuring electrodes of approximately 75 µm diameter, based on a silver-silver chloride internal reference cell. The instrument was standardized before and after measurements by immersion in buffer solutions at pH 4.0 and pH 7.0 ('Soloid' buffer solution tablets, Burroughs Wellcome & Co., London, U.K.) at 37°C in a water bath. The pH was recorded continuously on a portable potentiometric recorder (Minigor Type RD 501, Goertz Electro G.m.b.H., Wien, Austria) for the duration of each test.

For pH measurements, the reference electrode was placed in contact with the soft tissues of the labial sulcus opposite to the apex of the root, and the measuring electrode was held in contact with the plaque (Figure 4, page 57). As soon as a stable reading was obtained, 5 μl of a 20 per cent aqueous solution of sucrose (A.R. Grade, B.D.H. Ltd., Poole, Dorset, U.K.) was placed on the plaque with a repeating pipette (Eppendorf Gerätebau, Netheler, G.m.b.H., Hamburg, West Germany), and the recorder chart marked. The pH values immediately preceding and 20 seconds after this point were taken as the resting pH and the initial pH after adding the substrate respectively.

Plaque activity was determined by calculating the change in hydrogen ion concentration during a test interval of one minute.
FIGURE 4

PLAQUE ACTIVITY MEASUREMENT IN-SITU
The recorded pH values were converted into hydrogen ion concentration and were plotted against time. The line of best fit between 20 seconds and 80 seconds after the addition of substrate was used to determine the rate of change of hydrogen ion concentration per minute.

A recorder tracing, the calculation of plaque activity and the variable numbers associated with these measurements are shown in Figure 5, page 59.

Because of breakdown of the measuring equipment, caused by excessive humidity, no plaque activity measurements were carried out for subjects 1 - 66 and for the 13 subjects in Village 15, Mumeri.

3.2.3.7. Collection of UL2 plaque for bacteriological examination.

After completion of the plaque activity measurements, excess moisture was absorbed from the isolated plaque specimen with a fine filter paper strip (Whatman No. 541, 0.008 per cent ash) and the whole specimen was removed, weighed, homogenized, preserved and transported to the laboratory for bacteriological analysis using the procedures described for pooled plaque.

As the original intention was to examine the relationship between the activity and aspects of microbial composition of the same plaque specimen, no samples were collected from subjects 1 - 66 and from the 13 subjects in Mumeri village for whom no plaque activity measurements were made.

3.2.3.8. Cleaning of teeth.

Apart from food debris, plaque and calculus, a tenacious
a) Recorder Tracing of Plaque Activity
   1. Resting pH = 5.20 (Variable 23)
   2. pH at 20 seconds after supply of sucrose = 5.35 (Variable 24)
   3. " 80 " " " " = 4.60 (Variable 25)

b) The change of hydrogen ion concentration with time
   
   Hydrogen ion concentration at 20 seconds = 45 x 10^{-7}M
   " " " 80 " = 251 x 10^{-7}M

   Rate of change of hydrogen ion concentration = 206 x 10^{-7}M/min.
   (Variable 26)
and strongly adherent film of brown-black colour was present on many tooth surfaces. The material was found to be structureless and non-cellular on microscopical examination, appeared to be in direct contact with the enamel, deep to the cellular and much softer plaque material. It was most abundant on open smooth surfaces, which were frequently completely covered by it. There is little doubt that the presence of this film was associated with betel-chewing.

Usually no surface deposits other than calculus or stain were left on the teeth after plaque collection. These were removed to the extent required for dental examination. Special attention was given to the labial surfaces of the upper permanent canine teeth, which were cleaned with the aid of a prophylaxis paste made up of silicon carbide (1200 mesh, Naxos Products Pty. Ltd., Sydney, N.S.W.) and glycerol (A.R. Grade, B.D.H. Ltd., Poole, Dorset, U.K.) in a 1:1 (w/w) ratio. The paste was applied with a rotating rubber cup, driven by a portable dental engine. In some cases the 'betel stain' was best removed with stainless steel chisels. After cleaning, the subjects rinsed their mouths with their usual drinking water, until all visible traces of the prophylactic paste were removed.

3.2.3.9. Dental examination.

(A) Dental caries.

Determination of caries prevalence was based on the condition of permanent teeth only. The basic criteria used for a carious lesion were those described in the World Health Organization dental epidemiology manual, Oral Health Surveys: Basic Methods.
However, in addition to the standard DMFT measure, provisions were made in the experimental design to record the following details:

1. **Type of surface involvement.**

Carious lesions were recorded according to the initial surface involvement. This provision has made the classification of lesions into pit, fissure, proximal smooth surface, open smooth surface, cemental and atypical caries possible.

Where carious lesions extended from the point or area of their origin to one or more other surfaces, only the surface of origin, as judged clinically, was classed as carious, except when additional surfaces had collapsed to an extent where such distinction was not possible. In the latter case, all collapsed surfaces were classed as deep caries.

2. **Subclassification of lesions according to penetration and severity.**

(i) Doubtful or reversible lesions: suspected pit or fissure caries which could not be proved by clinical means, or subsurface decalcification of smooth surfaces without detectable structural weakening of the surface enamel.

(ii) Initial caries: where the lesion passed the standard criteria, but there was no clinical evidence of penetration into dentine.

(iii) Dentinal caries: where softened dentine could be observed or detected on instrumentation, but penetration beyond the dentino-enamel junction was observed or judged to be no more than approximately 1 mm.
(iv) Deep caries: where cavitation extending into the dentine more than approximately 1 mm, up to and including pulp exposure, and/or partial or complete collapse of the crown was found.

3. **Subclassification of lesions into:**

   (i) Conventional

   (ii) Associated with hypoplastic areas

   (iii) Apparently arrested

Of the details described above, the type of surface involvement was recorded to allow for the well recognized possibility that different environmental associations may apply to different types of carious lesions. Subclassifications 2 and 3 were included as safeguards to allow superimposition of qualitative information, such as the severity of lesions, on the basic quantitative assessment of caries prevalence, and to facilitate detection and evaluation of peculiar manifestations of the disease, which may develop under various and possibly unique sets of environmental conditions. For example, in some New Guinea populations the attrition rate is so high that slowly progressing or arrested lesions, mainly of the pit or fissure type, may disappear in time. In another group, it was observed that approximately 50 per cent of carious lesions were initiated within hypoplastic areas at sites usually not susceptible to the disease. Clearly, lesions of these types could not be evaluated in the same aetiological context as conventional caries.

The scoring system used is shown below:

00 = unerupted

01 = sound
02 = subsurface decalcification (smooth surface) or doubtful lesion (pit or fissure)
03 = initial caries
04 = dentinal caries
05 = open cavitation
06 = restoration
07 = missing because of caries
08 = unable to classify

12, 13, 14, 15, 16; as 2 - 6 above, but within hypoplastic area.
24, 25; arrested lesions, corresponding to 4 and 5 above.
34, 35; arrested lesions within hypoplastic area corresponding to 4 and 5 above.

Dental examinations were performed immediately after collection of saliva and plaque. These procedures resulted in cleaning all accessible tooth surfaces in most instances. Where necessary, remaining debris and calculus that may have interfered with proper assessment of the condition of the tooth surfaces were removed prior to examination.

The subjects were seated in a portable dental chair and examined using a mouth mirror and sickle explorer in natural daylight. All dental examinations were carried out by the author. The scores called out were recorded immediately by a graduate assistant, Dr. Josie Tabua (J.T.). During the first two days of the survey, duplicate examinations were carried out by Dr. D.E. Barmes, Chief, Dental Health, W.H.O. Details of criteria were reviewed, discussed and finalised with reference
to specific lesions during this period. The lesser score was assigned whenever there was doubt regarding the existence or extent of a lesion.

In villages where subjects with carious teeth were found, approximately ten per cent of the subjects were re-examined at the end of the day. Scores that were re-classified on the second examination, amounted to approximately three per cent of the total, and were mostly in the subsurface decalcification or doubtful pit and fissure lesion category.

In order to correct doubtful entries and avoid omissions, the dental charts were revised as soon as practicable after a set of examinations.

Caries experience was expressed in four different ways:

a) DMFT, (Variable 10) was calculated as the number of teeth of an individual with one or more surfaces classified as 03, 04, 05, 06 or 07. It should be noted that scores of 02 were disregarded for DMFT and DMFS measures because of their doubtful nature and because of the recognised possibility of recalcification at that stage. Scores of 12 - 16, 24 - 25 and 34 - 35 were also not considered, because different aetiological circumstances apply to these lesions than to conventional caries. The prevalence of the latter categories was small.

b) DMFS, (Variable 12) was calculated as the number of surfaces classified as 03, 04, 05, 06 or 07. In calculating the DMFS measure, special provision had to be made for the M component of the score. The total number of D surfaces and the total number of D teeth were found for each village and the
ratio of D surfaces to D teeth was calculated. An estimate of the number of missing surfaces for an individual in that village was obtained by multiplying the number of missing teeth for the person by that ratio. Thus the DMFS figure is not necessarily an integer for individuals with missing teeth.

Example of calculation:

<table>
<thead>
<tr>
<th>Village</th>
<th>Subject</th>
<th>DT</th>
<th>MT</th>
<th>DS</th>
<th>FS</th>
<th>MS</th>
<th>DMFS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>5</td>
<td>0</td>
<td>2.7</td>
<td>7.7</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>5</td>
<td>1</td>
<td>7</td>
<td>0</td>
<td>1.35</td>
<td>8.35</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>6</td>
<td>3</td>
<td>9</td>
<td>0</td>
<td>4.05</td>
<td>13.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>17</td>
<td>0</td>
<td>4.05</td>
<td></td>
</tr>
</tbody>
</table>

DS/DT = 23/17 = 1.35

c) Severity score of carious lesions. (Variable 14). This experimental measure of caries experience was designed by the author using a system of weighting DMF surfaces in relation to the severity of the lesions. The basic reasons for devising this system were previous observations of marked variations in the severity of lesions in similar populations and the fact that apart from isolated instances (50)(51)(52) the severity as opposed to the number of lesions received little attention in epidemiological surveys. It was considered of interest to examine the relationship between severity score and conventional measures of caries prevalence and the effect on correlations with independent variables when the severity score was substituted for DMFT or DMFS as the dependent variable.
All categories of conventional caries, including 02 were used in this system. The weights assigned were chosen arbitrarily, but the amount of tooth material destroyed was kept in mind. The weight applied and formula used to find the severity score for individuals is shown below:

<table>
<thead>
<tr>
<th>Number of surfaces</th>
<th>Coded</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ &quot; &quot;</td>
<td>01 x 0</td>
</tr>
<tr>
<td>+ &quot; &quot;</td>
<td>02 x 2</td>
</tr>
<tr>
<td>+ &quot; &quot;</td>
<td>03 x 4</td>
</tr>
<tr>
<td>+ &quot; &quot;</td>
<td>04 x 6</td>
</tr>
<tr>
<td>+ &quot; &quot;</td>
<td>06 x 6</td>
</tr>
<tr>
<td>+ &quot; &quot;</td>
<td>05 x 12</td>
</tr>
<tr>
<td>+ &quot; missing surfaces as per b)&quot;</td>
<td>x 14</td>
</tr>
</tbody>
</table>

d) Severity score of carious lesions/DMFS. (Variable 229). The severity score is influenced by the number of permanent teeth standing. Because third molar teeth have increased importance with respect to total caries experience where caries prevalence is low, it was desirable to neutralize the dependence of this measure on the number of erupted teeth. Variable 229 eliminates this objection and converts severity into an average intensity for those surfaces which have been attacked.

(B) Hypoplasia.

The index developed by Davies, Losee, Cadell, Kean and Ludwig (53) was used to assess the prevalence of enamel hypoplasia in all permanent teeth.

The criteria set for the index are reproduced below:
EXAMPLES:

DESCRIPTION: Part or whole of the enamel surface is rough but there is no evidence of pitting.

The enamel surface is rough and there is pitting which involves less than 50 per cent of the coronal surface area.

The enamel surface is rough and there is pitting which involves more than 50 per cent of the coronal surface area.

SCORE: 1 2 3

The examinations were carried out following dental caries examinations and under the same conditions as for caries scoring.

The mean hypoplasia score was calculated by dividing the sum of hypoplasia scores of a subject by the number of teeth standing (Variable 201).

3.2.3.10. Clinical test for dissolution rate of surface enamel in-vivo; sampling for fluoride determinations.

The fluoride content of surface enamel was determined using the etched material obtained by testing the dissolution rate of enamel, described in the following paragraphs.

In view of site-to-site variations in the fluoride content of enamel, which is dependent on a variety of factors, the use of a standard test site was an indispensable requirement.

An area on the midline of the labial surface of the upper left permanent lateral incisor tooth, centered approximately 2 - 3 mm incisally to the gingival margin, was selected for the
following reasons: The use of an anterior surface was desirable for accessibility and instrumentation. A smooth surface was necessary for uniform contact of the solubility etch fluid. Open smooth surfaces were reported to receive preferential protection from fluoride after eruption. By using practically the same test site for enamel dissolution rate and fluoride content determinations, as for plaque activity and bacterial composition studies, provision was made for future examination of the interrelationships of these variables.

After collection of the specimen used for the plaque activity test, remaining minor stains or debris were removed from the surface. Where possible, prophylaxis was performed with hand instruments or with an engine-driven, soft, rotating rubber cup, without the use of abrasives. The test site was inspected for the presence of extraneous material after adding a drop of disclosing solution, 0.1 per cent aqueous basic fuchsin (16379, G.T. Gurr, London, U.K.), and was accepted as clean when the solution failed to stain the surface. The tooth was then isolated, wiped with cotton pellets moistened with deionized, distilled water and dried with absorbent cotton wool. The enamel was carefully examined for signs of decalcification or caries and for the presence of defects. If any one of these was present, the test was carried out on the same tooth on the opposite side. Recent findings concerning the comparability of the fluoride content of contralateral teeth of an individual, support the validity of the procedure adopted. For three subjects, who had both teeth affected, the test was omitted.

A mask of adhesive tape (No. 1222, Minnesota Mining and
Manufacturing Co., St. Paul, Minnesota, U.S.A.) was attached to the enamel with a 2 mm diameter circular cutout centered at the test side. One microlitre of 1.0 M hydrochloric acid (A.R. Grade, Ajax Chemicals, Sydney, N.S.W.) was dispensed with a microlitre syringe (Type 1/B, Scientific Glass Engineering Pty. Ltd., Melbourne, Vic.) onto the isolated enamel test site. After exactly 15 seconds, the etch fluid was drawn up into a graduated polyethylene capillary tube, the percentage recovery was noted, and the fluid was discharged and rinsed into 200 μl of 0.23 M sodium formate buffer, pH 4.6 (formic acid: A.R. Grade, B.D.H. Ltd., Poole, Dorset, U.K.; sodium hydroxide: 'Volucon', May and Baker Ltd., Dagenham, Essex, U.K.), held in a stoppered container made of polyethylene tubing ('Portex' PP 390, Boots Pure Drug Co., Hythe, Kent, U.K.). Traces of acid remaining on the enamel surface were wiped off with moist cotton wool, the adhesive tape was removed, the tooth was thoroughly rinsed with water and the surface was lightly polished.

The containers holding the samples were heat-sealed with artery forceps over a portable Bunsen burner and were stored and transported to the laboratory at ambient temperature in an airtight container in the presence of moist cotton wool.

The dissolution rate of surface enamel was expressed as micrograms of calcium removed by the standard etch (Variable 22) and the fluoride content of surface enamel as ppm (Variable 236). Laboratory procedures in respect of these variables are described in sub-section 3.3.2.1.9.
3.2.3.11. Examination for enamel surface wear and anterior tooth enamel defects.

The fluoride content of enamel is known to decrease progressively (but not evenly) from the surface towards the dentino-enamel junction\(^{(59)(60)}\) and wear results in the removal of fluoride-rich surface enamel.\(^{(61)}\) Both solubility and fluoride content may be influenced by structural variations.\(^{(21)(62)(63)}\) Therefore, the wear status of the test surface and the presence of visible structural variations in the enamel of the upper anterior teeth were noted after close examination of the dried surfaces under 2 x magnification in natural daylight.

Four primary categories of wear of enamel were further subdivided on the basis of the associations with firmly adherent, dark-stained, extrinsic deposits at the biopsy site and on adjacent tooth surfaces. This subdivision produced a total of seven categories which represented increasing degrees of wear. The categories were:

1) Perichymata prominent at biopsy site; no surface deposit present.

2) Perichymata visible at biopsy site but not prominent because of loss of surface structure through abrasion; surface deposit present.

3) As for 2) above, but no surface deposit present.

4) Perichymata not visible at biopsy site; surface deposit present.

5) As for 4) above; no surface deposit present.
6) As for 5) above; heavy deposit on most areas of the tooth crown except the labial surface and particularly on the lingual surfaces.

7) Marked wear on the whole labial surface with no surface deposit present.

Enamel surface wear is represented by Variable 9.

The presence of the following structural features (not necessarily defects) were noted whenever they were visible on any of the anterior teeth: gross hypoplasia, hypoplastic cusp tips, pits, circumferential grooves, flecks and surface whiteness. The latter is a white lacy pattern within the enamel, most prominent in the highest contours of the perichymata; it appears to be a property of well formed teeth, decreases and disappears with wear and is not related to surface or subsurface decalcification. Diefenbach, Nevitt and Frankel\(^{64}\) described a similar feature in subjects exposed to optimum levels of fluoride during tooth development.

These structural features were exhibited by relatively few individuals. Because of the difficulty involved in categorizing such features without a great deal of subjectivity, they were not included in the list of variables. However, this information, together with clinical observations, has been utilized already (sub-section 4.2.15) and will be examined in the future in relation to enamel solubility rate and composition.

3.2.3.12. Enamel sampling for chemical analyses.

The following requirements were considered in relation to clinical aspects of enamel sampling:
The biopsy must not lead to actual or potential damage, such as increased susceptibility to caries, or disfigurement of the enamel.

The procedure should yield sufficient material for the chemical analyses planned.

The sampling should be distributed over the largest possible area, (a) to minimize depth, (b) to avoid compositional bias that could arise when a limited area is sampled, and (c) because surface or near surface enamel composition is of primary interest in the context of caries aetiology.

The depth of the etch should be known, to permit adjustments of analytical results in relation to the distribution pattern of those constituents which are known to vary in concentration with the depth of enamel.\(^{(59)(65)(66)(67)(68)(69)}\) Increment with age in the zinc, lead, copper, manganese, tin and aluminum content of enamel has been recognized by Little and Steadman\(^{(70)}\) who state, however, that no marked increment is found from the surface inward in completely sound enamel.

The site should afford good visibility, free access for instrumentation, and an even surface for sampling.

Berman and Slack\(^{(71)}\) reported that the pattern of caries attack of tooth types was independent of the caries susceptibility of the individual, and canine teeth were subject to the lowest order of attack rate. After detailed examination of sectioned, extracted teeth (sub-section 3.2.2.) the incisal halves of the
labial surfaces of the upper permanent canine teeth were selected for test sites, because in the absence of defects these areas are virtually immune to caries, and offer a large surface over the thickest layer of enamel in anterior teeth.

Consideration of methods intended for or capable of modification for use for enamel sampling and the analytical requirements suggested the use of a mineral acid etch.

After extensive trials using extracted teeth, the following procedure was adopted:

The test surfaces were cleaned as described previously (subsection 3.2.3.7.) until a disclosing solution, 0.1 per cent aqueous basic fuchsin (16379, G.T. Gurr, London, U.K.) failed to stain the surface. The tooth was then isolated, wiped with cotton pellets moistened with glass-distilled, deionized water, and dried with absorbent cotton wool. The sampling area was carefully examined for signs of defects, decalcification or caries. No caries or decalcification which originated within the biopsy area on the labial surface was seen in any of the subjects. However, on occasions defects, surface irregularities or undermining encroachment by proximal lesions were noted. In these cases biopsy was not carried out on the affected site.

A mask of adhesive tape (No. 1222, Minnesota Mining and Manufacturing Co., St. Paul, Minnesota, U.S.A.) with a 4.8 mm diameter circular cutout was attached to the distal plane of the labial surface of the upper left permanent canine tooth, as near to
the incisal edge as practical, without involving the incisal edge.

Five microlitres of 3.9 M nitric acid (Aristar Grade, B.D.H.
Chemicals Ltd., Poole, Dorset, U.K.), dispensed with a repeating
pipette (Eppendorf Gerätebau Netheler G.m.b.H., Hamburg, West
Germany), was absorbed into a 1 mm thick 4.8 mm diameter poly-
urethane foam disc (Foam Division, Cable Makers of Australia Pty.
Ltd., Fairfield, N.S.W.), held on a piece of clear polyethylene
sheet. The disc was placed in the tape cutout with acid-resistant
tweezers (No. 92-00-13, Orion Research Inc., Mass., U.S.) and was
lightly touched at one-second intervals for 15 seconds, when it was
transferred into a stoppered container made of polyethylene tubing
of acid left on the tooth were wiped off immediately with
absorbent cotton wool, the site was rinsed thoroughly with glass-
distilled, deionized water and polished with silicon carbide-
glycerol prophylactic paste (1200 mesh silicon carbide, Naxos
Products Pty. Ltd., Sydney, N.S.W.; A.R. Grade glycerol, B.D.H.
Chemicals, Ltd., Poole, Dorset, U.K., in 1 : 1 w/w ratio), using
an engine-driven, rotating, rubber cup.

The same procedure was carried out on the mesial plane of
the labial surface of the tooth, where the disc was placed in a
diagonally offset position to avoid or minimize overlap. Two
similar biopsies were made on the upper right permanent canine.
No biopsies were made on 2 subjects and 2 and 3 biopsies were
made on 8 and 6 subjects respectively, because of the presence
of surface imperfections or proximal caries. The four biopsy
discs, each containing 5 µl of etch fluid, were pooled in the
one container which was then heat-sealed with artery forceps
over a portable Bunsen burner and kept in an airtight container in the presence of moist cotton wool at ambient temperature until required for analysis.

One blank sample, containing all reagents and materials except the enamel, was prepared using an identical procedure, for each 25 samples.

Previous experiments carried out on extracted teeth had shown that the weight of the enamel removed under the conditions described was of the order of 0.75 - 0.95 mg per etch. Assuming uniform etching for the area (18.1 mm²) and taking 2.95, the value given by Karlström (87) as the density of enamel, the expected depth range was 14.0 - 17.8 μm.

\[
\text{Depth (μm)} = \frac{\text{Weight (mg) } \times 1000}{\text{Area (mm}^2\text{) } \times \text{density}}
\]

Variables 220, 230, 231, 232, 233, 234 and 235 were based on analyses of the biopsy specimen, which are described in 3.3. Laboratory methods.

3.2.3.13. Tooth colour classification.

As early as in 1915, Black and McKay (88) reported colour and opacity changes in teeth which are now known to have been associated with the fluoride content of the drinking water. Diefenbach et alii (64) found that the enamel had a bluish-white appearance when developed in the presence of optimum fluoride. A variety of other elements or compounds are known or suspected to affect the intrinsic colour of teeth. Kempf and McKay (89) attributed the brown discoloration of mottled enamel to manganese.
Diffusely discoloured sound enamel was found to have much higher zinc, lead, copper, manganese, tin, aluminum and fluorine content than non-discoloured enamel from the same tooth, and the darkening of enamel appeared to be directly related to its lead and zinc content.\(^{(70)}\) Shwachmann and Schuster\(^{(90)}\) suggested that tetracycline could be a cause of colour change in teeth, a fact later proved by Wallman and Hilton.\(^{(91)}\) In a recent study Brearley and Porteous\(^{(92)}\) have demonstrated that children with tetracycline-affected dentitions had significantly lower caries experience than appropriate controls.

Unpublished data from a study of schoolchildren in N.S.W.\(^{(20)}\) indicate possible interrelationships between tooth colour, enamel fluoride content and solubility. Posen\(^{(62)}\) reported that enamel from brown and white spots was consistently and significantly less soluble than adjacent normal enamel.

The colour of clean upper right permanent canine teeth was assigned to one of six categories by matching the middle third of the labial surface of the tooth to the closest shade on an artificial tooth shade guide ('Dentron', Dentsplay Pty. Ltd., Preston, Vic.) in natural daylight (Figure 6, page 77.). The upper lip was retracted but the tooth was not dried for colour matching. Flecks or opacities were disregarded.

To facilitate data processing, the manufacturers' numbers 2, 11, 5, 7, 10 and 9 were replaced by categories 1, 2, 3, 4, 5 and 6 respectively, representing increasing colour intensity of the "natural" cream-yellow colour. All teeth exhibiting distinct greyish-blue colour tone were classed in category 5, regardless
FIGURE 6

TOOTH COLOUR RANGE
of colour intensity.

Tooth colour appears as Variable 7 in the statistical analyses.

3.2.3.14. Impressions for cusp height and angle measurement.

Reported associations between morphological characteristics of teeth and the trace element content of soils, food and water (14)(93)(94)(95)(96)(97)(98)(99)(100) suggested that some measure of morphological variation should be established in this study. The height and inclination of the cusps of selected teeth were considered pertinent parameters because of previously observed trends indicating possible associations between caries experience and cusp height in a neighbouring group of indigenous people. (101)

Because of the presence of detectable wear of cusps in some 70 per cent of the subjects, and technical difficulties in relation to cusp angle measurements, data collected in respect of these variables were rejected.

3.2.4. March - April, 1973 Field Visit.

This visit was conducted primarily to collect saliva for calcium and fluoride content estimations. However, the opportunity was taken to carry out the following additional procedures:

Further plaque collection for a second estimate of pooled plaque quantity and future chemical analyses.

The measurement of mesio-distal crown diameter of upper anterior teeth to replace rejected morphological parameters.

The recording of the frequency of betel-chewing.