AN INVESTIGATION INTO THE EFFECTS OF TWO BIOCERAMICS ON RAT MANDIBULAR BONE

James Fraser McCord

Thesis presented for the degree of Doctor of Dental Surgery of the University of Edinburgh

1986
# CONTENTS

<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>v</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ix</td>
</tr>
<tr>
<td>PREFACE</td>
<td>x</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>xi</td>
</tr>
<tr>
<td>GLOSSARY OF ABBREVIATIONS</td>
<td>xiii</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>CHAPTER 1 LITERATURE REVIEW</td>
<td>4</td>
</tr>
<tr>
<td>1. Historical Review</td>
<td>4</td>
</tr>
<tr>
<td>2. Classification of Dental Implants</td>
<td>6</td>
</tr>
<tr>
<td>3. Ideal Properties of Dental Implants</td>
<td>8</td>
</tr>
<tr>
<td>4. Implant Materials</td>
<td>10</td>
</tr>
<tr>
<td>4.1 Metals</td>
<td>10</td>
</tr>
<tr>
<td>4.2 Polymers</td>
<td>16</td>
</tr>
<tr>
<td>4.3 Ceramics</td>
<td>20</td>
</tr>
<tr>
<td>5. Implant:Tissue Interface</td>
<td>41</td>
</tr>
<tr>
<td>5.1 General Review</td>
<td>41</td>
</tr>
<tr>
<td>5.2 Tissue:Metal Implant Interface</td>
<td>44</td>
</tr>
<tr>
<td>5.3 Tissue:Polymer Implant Interface</td>
<td>50</td>
</tr>
<tr>
<td>5.4 Tissue:Ceramic Interface</td>
<td>55</td>
</tr>
<tr>
<td>6. Tumour Induction by Implant Materials</td>
<td>70</td>
</tr>
<tr>
<td>6.1 Animal Studies</td>
<td>70</td>
</tr>
<tr>
<td>6.2 Tumour Formation in Human Implant Sites</td>
<td>72</td>
</tr>
<tr>
<td>7. Summary</td>
<td>72</td>
</tr>
<tr>
<td>CHAPTER 2</td>
<td>CRYOSURGERY</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>CHAPTER 3</td>
<td>AIMS OF THE PRESENT STUDY</td>
</tr>
<tr>
<td>CHAPTER 4</td>
<td>MATERIALS AND METHODS</td>
</tr>
<tr>
<td><strong>Introduction</strong></td>
<td></td>
</tr>
<tr>
<td>1. Cytotoxicity and Biocompatibility Testing</td>
<td></td>
</tr>
<tr>
<td>2. Animal Experimental Method</td>
<td></td>
</tr>
<tr>
<td>2.1 Experimental Design</td>
<td></td>
</tr>
<tr>
<td>2.2 Surgical Technique</td>
<td></td>
</tr>
<tr>
<td>3. Fracture Strength Measuring</td>
<td></td>
</tr>
<tr>
<td>4. Preparation of Tissues for Histological Investigation</td>
<td></td>
</tr>
<tr>
<td>4.1 Decalcified Section Technique</td>
<td></td>
</tr>
<tr>
<td>CHAPTER 5</td>
<td>INVESTIGATION OF ERRORS</td>
</tr>
<tr>
<td>1. Measurement Errors</td>
<td></td>
</tr>
<tr>
<td>1.1 Errors in Weighing</td>
<td></td>
</tr>
<tr>
<td>1.2 Errors in Strength Testing</td>
<td></td>
</tr>
<tr>
<td>2. Random Errors</td>
<td></td>
</tr>
<tr>
<td>2.1 Operator-Induced Errors</td>
<td></td>
</tr>
<tr>
<td>2.2 Apparatus-Induced Random Errors</td>
<td></td>
</tr>
<tr>
<td>3. Conclusions</td>
<td></td>
</tr>
<tr>
<td>CHAPTER 6</td>
<td>STATISTICAL METHODS AND ANALYSES</td>
</tr>
<tr>
<td>1. Introduction</td>
<td></td>
</tr>
<tr>
<td>2. Symbols</td>
<td></td>
</tr>
<tr>
<td>3. Statistical Methods</td>
<td></td>
</tr>
<tr>
<td>4. Analyses of Data</td>
<td></td>
</tr>
<tr>
<td>5. Calculations</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 7 RESULTS

Introduction 111

1. In vitro Tests 111

2. In vivo Experiments 114

   2.1 Mechanical (Fracture Strength) Test Results 116

   2.2 Histological Findings 138

CHAPTER 8 DISCUSSION 167

BIBLIOGRAPHY 197
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>List of some physical properties of five implant materials and of cortical bone</td>
<td>14</td>
</tr>
<tr>
<td>4.1</td>
<td>Plan of experimental procedure, including a summary of surgical procedure(s) and sacrifice periods</td>
<td>85</td>
</tr>
<tr>
<td>5.1</td>
<td>Lists of intra and inter observer readings used to determine error of measurement</td>
<td>105</td>
</tr>
<tr>
<td>7.1</td>
<td>Recorded right and left fracture strengths of five male and five female rats (non-surgical controls)</td>
<td>117</td>
</tr>
<tr>
<td>7.2</td>
<td>Fracture strength results of study on unweakened bone</td>
<td>118</td>
</tr>
<tr>
<td>7.3(a)</td>
<td>'P' value of differences in left:right ratios of mean values between non-surgical and surgical controls (2 weeks)</td>
<td>123</td>
</tr>
<tr>
<td>7.3(b)</td>
<td>'P' values of differences in left:right mean values between surgical controls and groups where implants were placed on unweakened bone</td>
<td>123</td>
</tr>
<tr>
<td>7.4</td>
<td>Results of the effects of cryosurgery on the fracture strength of mandibular bone</td>
<td>124</td>
</tr>
<tr>
<td>7.5</td>
<td>'P' values of differences in left:right mean values between surgery controls and groups where implants were placed on bone subjected to cryosurgery</td>
<td>128</td>
</tr>
<tr>
<td>7.6</td>
<td>Results of the effects of cryosurgery plus dense hydroxyapatite (granules) on the fracture strength of bone</td>
<td>129</td>
</tr>
<tr>
<td>7.7</td>
<td>'P' values of differences in left:right mean values between cryosurgery controls and groups where implants were placed on bone subjected to cryosurgery</td>
<td>132</td>
</tr>
<tr>
<td>7.8</td>
<td>Results of the effects of cryosurgery plus β-tricalcium phosphate on the fracture strength of mandibular bone</td>
<td>133</td>
</tr>
<tr>
<td>7.9</td>
<td>Results of the effects of cryosurgery plus hydroxyapatite (blocks) on the fracture strength of mandibular bone</td>
<td>136</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Left masseter muscle exposed after submandibular incision. The associated anatomy and two tying-off ligatures (A&amp;B) are labelled on the overlay.</td>
<td>88</td>
</tr>
<tr>
<td>2</td>
<td>Left mandibular ramus exposed by reflection of left masseter muscle and its periosteum. An outline of the mandible is drawn on the overlay to facilitate orientation.</td>
<td>89</td>
</tr>
<tr>
<td>3</td>
<td>Rectangular block of dense hydroxyapatite placed on implant 'site'.</td>
<td>91</td>
</tr>
<tr>
<td>4</td>
<td>Speybly DFS 30 cryosurgery apparatus. The tonsillar probe and external thermocouple are indicated.</td>
<td>92</td>
</tr>
<tr>
<td>5a</td>
<td>Load cell of RDP Howden servo-hydraulic universal testing machine (Type U-M 5/2). The ascending ramp and the fixed point are indicated.</td>
<td>95</td>
</tr>
<tr>
<td>5b</td>
<td>Recording unit of RDP Howden universal testing machine. Electronic connections to other components are labelled.</td>
<td>96</td>
</tr>
<tr>
<td>6</td>
<td>Watanabe linear graphic servo-corder, Type S.R. 657.</td>
<td>98</td>
</tr>
<tr>
<td>7</td>
<td>Photograph of a typical graphic recording of a fracture test. The value recorded is one half of the actual fracture load, expressed in kiloNewtons.</td>
<td>99</td>
</tr>
<tr>
<td>8</td>
<td>Photograph of lateral aspect of a left mandible of a rat (x 2). Histological sections were taken at 6μ intervals from the area between the lines A-A₁ and B-B₁.</td>
<td>101</td>
</tr>
<tr>
<td>9</td>
<td>Granules of dense hydroxyapatite in tissue culture. Intimate contact between fibroblasts and granule demonstrated with no evidence of toxicity. Mag. x 300.</td>
<td>112</td>
</tr>
<tr>
<td>10</td>
<td>Granules of β-tricalcium phosphate in tissue culture. Obvious biocompatibility is demonstrated by close contact of cells and implant material. Mag. x 300.</td>
<td>113</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>11</td>
<td>Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of surgery alone, surgery plus granules of dense hydroxyapatite (HA) and surgery plus β tricalcium phosphate (TCP).</td>
<td>120</td>
</tr>
<tr>
<td>12</td>
<td>Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of cryosurgery alone, cryosurgery plus granules of dense hydroxyapatite (HA) and cryosurgery plus β tricalcium phosphate (TCP).</td>
<td>127</td>
</tr>
<tr>
<td>13</td>
<td>Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of cryosurgery plus dense hydroxyapatite (HA) granules and cryosurgery plus dense hydroxyapatite (HA) blocks.</td>
<td>137</td>
</tr>
<tr>
<td>14</td>
<td>Coronal section of the left mandibular ramus of a rat at the level of the lingula: two weeks after surgery only. (H &amp; E x 100).</td>
<td>140</td>
</tr>
<tr>
<td>15</td>
<td>Coronal section of the left mandibular ramus of a rat at the level of the lingula: eight weeks after surgery only. (H &amp; E x 100).</td>
<td>141</td>
</tr>
<tr>
<td>16</td>
<td>Coronal section of the left mandibular ramus of a rat at the level of the lingula: 26 weeks after surgery only. (H &amp; E x 100).</td>
<td>142</td>
</tr>
<tr>
<td>17</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of lingula. Granules of dense hydroxyapatite were added to unweakened bone: two weeks post-implantation. (H &amp; E x 100).</td>
<td>143</td>
</tr>
<tr>
<td>18</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite were added to unweakened bone: eight weeks post-implantation. (H &amp; E x 40).</td>
<td>144</td>
</tr>
<tr>
<td>19</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite were added to unweakened bone: 26 weeks post-implantation. (H &amp; E x 40).</td>
<td>145</td>
</tr>
<tr>
<td>20</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of β tricalcium phosphate were added to unweakened bone: two weeks post-implantation. (H &amp; E x 40).</td>
<td>147</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>21</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of β tricalcium phosphate were added to unweakened bone: eight weeks post-implantation (H &amp; E x 40).</td>
<td>148</td>
</tr>
<tr>
<td>22</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of β tricalcium phosphate were added to unweakened bone: 26 weeks post-implantation. (H &amp; E x 40).</td>
<td>149</td>
</tr>
<tr>
<td>23</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: two weeks post-cryosurgery. (H &amp; E x 100).</td>
<td>151</td>
</tr>
<tr>
<td>24</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: eight weeks post-cryosurgery. (H &amp; E x 100).</td>
<td>152</td>
</tr>
<tr>
<td>25</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: 26 weeks post-cryosurgery. (H &amp; E x 100).</td>
<td>153</td>
</tr>
<tr>
<td>26</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: two weeks post-implantation. (H &amp; E x 100).</td>
<td>154</td>
</tr>
<tr>
<td>27</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: eight weeks post-implantation. (H &amp; E x 100).</td>
<td>155</td>
</tr>
<tr>
<td>28</td>
<td>Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: 26 weeks post-implantation. (H &amp; E x 40).</td>
<td>156</td>
</tr>
<tr>
<td>29</td>
<td>Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: two weeks post-implantation. (H &amp; E x 100).</td>
<td>158</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>--------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>30</td>
<td>Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: eight weeks post-implantation. (H &amp; E x 40).</td>
<td>159</td>
</tr>
<tr>
<td>31</td>
<td>Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: 26 weeks post-implantation. (H &amp; E x 40).</td>
<td>160</td>
</tr>
<tr>
<td>32</td>
<td>Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: two weeks post-implantation. (H &amp; E x 100).</td>
<td>161</td>
</tr>
<tr>
<td>33</td>
<td>Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: eight weeks post-implantation. (H &amp; E x 40).</td>
<td>162</td>
</tr>
<tr>
<td>34</td>
<td>Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: 26 weeks post-implantation. (H &amp; E x 40).</td>
<td>163</td>
</tr>
<tr>
<td>35</td>
<td>Section through left mandibular ramus of a rat at the level of the lingula. Carbonised wood implant control added to unweakened bone: eight weeks post-implantation. (H &amp; E x 40). I S = Implant Space.</td>
<td>165</td>
</tr>
<tr>
<td>36</td>
<td>Section through left mandibular ramus of a rat at the level of the lingula. Implant control added to a cryolesion: eight weeks post-implantation. (H &amp; E x 40). I S = Implant Space.</td>
<td>166</td>
</tr>
</tbody>
</table>
ACKNOWLEDGEMENTS

I wish to acknowledge, with gratitude, the assistance I have received from various sources.

I am indebted to Mr. K.W. Tyson and staff of the Department of Prosthetic Dentistry, Edinburgh University for their active interest in this research and for their acceptance of extra clinical duties during the course of these studies.

I am grateful to Dr. C.P. Wallis, Department of Preventive Dentistry and Dr. A. McKendrick, Edinburgh Regional Computer Centre for statistical advice and to Dr. G.H. Moody, Department of Oral Medicine and Oral Pathology, for the interpretation of histological sections.

I wish to record my thanks to John Mitchell, George Christie and Bob Shields for their technical skills and to Robert Renton and Ian Goddard for their photographic skills. Thanks are also due to Dr. R.G. Dalton and his staff at the Medical Faculty Animal Area.

I am deeply indebted to Dr. David Wray, my adviser. His friendship, coupled to his attention to detail, professionalism and zeal were constant sources of inspiration.

My sincere thanks go to Miss Yvonne Stables for her patient acceptance of illegible drafts. Her typing skills are confirmed in this thesis.

Finally, heartfelt thanks go to my wife, Morag, and our three children, Jamie, George and Jennifer. Their encouragement, patience and understanding made the completion of this thesis possible.
The work for this thesis was undertaken in the Department of Prosthetic Dentistry, University of Edinburgh, during the period July 1984 to May 1986. The work was supported by a grant from the Lothian Health Board.

Record must be made of the unconditional provision of implant materials from Calcitec Inc., Ethicon Ltd. and Johnson & Johnson Ltd. Without the co-operation of these companies, the work would not have been possible.

Some of the techniques used in this thesis are modifications of previously published work and some are original techniques developed by the author in conjunction with laboratory staff of the Department of Oral and Maxillofacial Surgery.

The applications of these techniques as described in the present study, were undertaken by the author personally. Tissue microtomy and surgical assistance was carried out by technical staff under the direct supervision of the author except tissue culture which was supervised by Miss Yvonne Barlow. No portion of this thesis has been submitted in support of an application for another degree or qualification of this or another university or institute of learning.
The aim of this study was to evaluate the effects of both dense hydroxyapatite and β tricalcium phosphate on mandibular bone, in an attempt to offer guidelines for their use. The importance of these guidelines is that although both of these bioceramics have been used in clinical studies there are no established guidelines to indicate which clinical situation is best served by either dense hydroxyapatite or β tricalcium phosphate. This is evidenced by the fact that many materials have been used as dental implants and that many shapes and forms of implants have been used in an attempt either to stabilise dentures or to restore facial form.

In vitro and in vivo investigations are included in this study. The in vivo investigation, on 231 male Sprague-Dawley rats contained controlled studies on weakened and unweakened mandibular bone; cryosurgery was used to weaken bone in this investigation as previous studies demonstrated significant reduction in bone strength after cryosurgery. The in vivo studies were assessed in two ways; first, mechanical assessments of fracture strength were determined by three-point bending tests. Second, histological examination of mandibular bone was performed in parallel to the mechanical evaluation, in an attempt to interpret fracture test results from histological findings.

The investigations in this thesis indicated, for the first time, that both materials result in significant increased fracture strength of mandibular bone. The histological findings indicated that these increases in fracture strength were biologically significant.
The results presented in this thesis suggest that both materials are biocompatible and that β tricalcium phosphate is more ideally used in unweakened bone whereas dense hydroxyapatite, gives its optimal effect on weakened bone, although it gives more rapid early increases in strength than does β tricalcium phosphate. It is suggested that those biomaterials may be of benefit in those clinical situations where surgery on mandibular bone may render it prone to fracture.
GLOSSARY OF ABBREVIATIONS

B.S.  British Standard Specification
gm   Gram or gram-molecular weight
G.Pa  Giga (10^9) Pascals
kgmf/cm^2 Kilograms force per square centimetre
L.T.I. Low Temperature Isotope
M.  Molar solution
mg  Milligram (10^-3 gram)
ml  Millilitre (10^-3 litre)
mm  Millimetre (10^-3 metre)
mML^-1 Milli moles per litre
M.Pa  Mega (10^6) Pascals
N.I.D.R. National Institute for Dental Research
P.M.M.A. Poly(Methyl) Methacrylate
P.T.F.E. Poly Tetra Fluoroethylene
μ  One micron (10^-6 metre)
μg  One microgram (10^-6 gram)
U per ml Units per ml
U H M W Ultra high molecular weight
INTRODUCTION

This thesis is devoted to the study of two dental implant materials. As will be demonstrated in the literature review, dental implants have been extensively researched. The purpose of this introduction, in addition to outlining the layout and the objectives of this thesis, is to account for the necessity of dental implants.

If complete dentures are to be worn satisfactorily, then these dentures, especially the lower denture, should exhibit retention and stability. The lower denture is especially vulnerable as it lacks the bony support of the upper denture bearing area. In addition, an immeasurable degree of neuro-muscular control of these dentures must be learned by the patient. Moreover, the alveolar bone used to support the denture is undergoing progressive and irreversible resorption. Atwood (1979) termed this phenomenon "residual ridge resorption" and considered that the mean vertical loss of "healed ridges" was 0.5 mm per year. However this vertical bone loss is not even in both arches; Tallgren (1967) demonstrated that vertical bone loss in the region of mandibular incisors and canines was four times that of the corresponding maxillary segment. Although exceptions are found, one consequence of prolonged wearing of complete lower dentures is the atrophic mandibular ridge and in these patients denture wearing can be difficult if neuro-muscular co-ordination is poor.

In addition to pre-prosthetic surgery, where sulcus-deepening procedures may be used to increase stability, two other treatment means may be used to prevent or overcome the problems of atrophic mandibular ridges in particular.
First of all, residual ridge resorption may be prevented or, more accurately, reduced by the construction of overdentures. In this treatment, dentures are constructed over pre-prepared stumps of one, two or several teeth. Crum & Rooney (1978) demonstrated that in dentures constructed over two mandibular canine roots, bone loss under overdentures was one eighth that of a conventional denture over a five year period. Overdenture teeth may be root-treated or not, depending on the amount of secondary dentine present in the pulp chamber. One school of thought advocates the retention of vital roots "buried" beneath the gingivae (Casey & Lauciello 1980). Whatever the means used, overdentures, by maintaining fibres of the periodontal membrane, are able to maintain production of alveolar bone.

The second method concerns the many patients for whom overdentures are no longer possible; for the already edentulous patient who is unable to wear dentures dental implants may be considered.

In this thesis, a comprehensive review of dental implants is presented, including their historical development, classification and ideal properties. This review also includes a discussion on the physical and chemical characteristics of each implant material discussed, in addition to sections on tissue-interface reactions and implant-tumourigenesis.

The purpose of this thesis was to investigate the effect of two biomaterials on rat mandibular bone. The biomaterials to be investigated were dense, sintered hydroxyapatite and sintered \( \beta \) tricalcium phosphate. In \textit{vitro} and \textit{in vivo} testing of both materials were explored. The \textit{in vitro} experiment evaluated the non-toxicity and biocompatibility of both the materials on tissue
cultures of human fibroblasts. In vivo testing of both materials was performed on 231 adult male Sprague-Dawley rats using a carefully controlled surgical technique. The effects of both test materials on rat mandibular bone was evaluated on bone which was unweakened and also on bone which was previously subjected to cryosurgery. Cryosurgery has been demonstrated to significantly decrease bone strength (Fisher et al. 1977). In this controlled evaluation, it was possible, in addition, to study the effect of implant form on rat mandibular bone.

The aim of this controlled study was to test the Null Hypothesis that neither material in any way affected mandibular bone. To this end, mechanical and histological investigations were performed in parallel to each other.

The results are illustrated, where appropriate, in tabular or graphic form to demonstrate the findings of the mechanical testing, whereas histological photographs provide visible evidence of any changes in mandibular bone following the application of the implant materials.
1. **Historical Review**

Through the centuries, man has attempted to create a purposeful change in his appearance for cosmetic or functional reasons. Implants are one of the methods which have been used to effect these changes. An implant is anything which is fixed, instilled or engrafted (Oxford English Dictionary). By extension a dental implant can be considered any material which is fixed, instilled or engrafted into the oro-facial structures. One of the earliest implants, circa 800 A.D., was a dark coloured stone inserted into the anterior alveolus of a Mayan mandible. This implant had, presumably, been in place for some time as it had a covering of calculus connecting it to the adjacent teeth (Andrews 1893). In another example, from a similar period, three missing mandibular incisors were replaced by the shells of a bivalve mollusc (Bobbio 1972).

Maggiolo, in 1807, inserted gold roots to support pivot teeth. It would appear that implants did not gain favour in medical and dental circles until the late 19th Century, presumably as a result of improved industrial and technological techniques (Greenfield 1913). The consequence of these advances meant that metallic implants could be fabricated in such a way that the inherent strength of metals could be utilised to support prostheses (Lee 1970).
5.

In 1887, Harris implanted a lead covered platinum root onto which was affixed a porcelain crown. The lead was fashioned to fit the socket and also roughened for retention (Greenfield 1913). Bonwill, in 1895, implanted pins of gold or iridium, into the alveolar bone; by increasing the numbers he was able to support either a single tooth or a complete denture (Greenfield 1913). This was the first reference to a complete denture being stabilised by dental implants (Cutright 1983). Gramm, in 1898, also implanted lead roots after he had enlarged the apical regions of the socket, possibly in an attempt to gain retention for the implant (Cutright 1983). Payne, also in 1898, placed silver capsules into root sockets and porcelain crowns were attached to the silver capsules (Cutright 1983).

More elaborate techniques followed and fabricated cage-like artificial roots, which consisted of reinforced wire loops of gold, were used around 1910 (Herschfeld 1984).

With increasing knowledge of metallurgy in the 1930's, (Chattman 1970), greater varieties of shapes were used varying in design from open spiral and double helical spirals, to blade and blade-vent implants (Lee 1970; Manderson 1971). Advances in laboratory techniques, meant greater accuracy in casting cobalt chrome designs and cast subperiosteal implants came into prominence (Dahl 1943; Goldberg & Gershkof 1949). At the same time, greater knowledge in the field of chemistry brought about the use of polymers and ceramics as implant materials (Smith 1975). A large variety of materials are now used as implants: these materials are selected on the grounds of their biocompatibility, rigidity or resilience according to the demands of function to which they are
subjected.

Research in the definitive anatomy and physiology of edentulous ridges has resulted in the knowledge that if severe alveolar resorption or enlarged maxillary antrae are present, then subperiosteal implants are preferable to endosseous implants. If, however, alveolar bone remained, then subperiosteal implants would be contraindicated (Manski 1982).

Although the morbid anatomy of the oral structures has been known for centuries, accurate, radiographic techniques are, medically speaking, relatively recent.

The importance of awareness of the anatomical factors pertaining to the placement of dental implants is a factor expressed only recently in dental literature (Barker 1976; Denissen et al 1984).

2. Classification of Dental Implants

Despite the variety of materials and techniques employed there are only, basically, two categories of implants (Johns 1971; Adams & Williams 1985). These are labelled retentive implants and supportive implants.

Retentive implant systems, which are rarely used nowadays, are basically of two types:-

a) Mucosal inserts:

These metallic implants resemble collar studs and project out of the fitting surface of the upper denture and fit into surgically created pockets in the palatal mucosa. They rely on fibrosis for their retention (Lew & Kastenbaum 1953; Evasic 1983).
b) Magnetic implants:

These implants consisted of cobalt-platinum alloy magnets which were placed in a crypt in the alveolus and covered with tantalum gauze (Behrman & Egan 1953). A magnet of opposite polarity was placed in the denture base in opposition to the implanted magnet.

The supportive implant receives its support from bone by one of three methods:-

a) Intraosseous or endosseous implant:

This type of implant lies partly within bone, being inserted into either a fresh extraction socket or a surgically created socket.

b) Subperiosteal implant:

This implant form has a more extensive, cast, framework which is inserted over the bone but beneath the periosteum. A two-stage surgical technique is required for fabrication and insertion of the implant.

c) Totally buried implant:

This type of implant is employed in maxillofacial reconstruction and in the augmentation of alveolar ridges.

From this general classification of dental implants, many forms exist. The endosseous implants may take the form of pins, screws or blades (Mack 1974). Moreover, the screws may be open spirals, helical spirals, cylindrical or hollow vented cylinders; the blades may be vented or non-vented (Cutright 1983). With the exception of the implants which are totally buried, most implants share one common feature, namely a portion or strut which projects into the oral cavity. The terminology describing this
implant–epithelial interface is contentious, although the term per-perimucosal seal has been considered to give a good description of the relationship of the implant strut to the overlying soft tissue (McKinney et al 1984).

3. Ideal Properties of Dental Implants

Implant materials, themselves, should satisfy many criteria before they are considered suitable for implantation including the nature of the response of the host tissues to the implant and to the degradation products of the implant (Bienstock 1955; Williams 1971–6; Bagnall 1980; Hench & Ethridge 1982). Cohen (1967) in a review of orthopaedic materials, discussed tissue tolerance and Harris (1974) stated that dental implants should have adequate strength to permit oral functions and should be acceptable immunologically to the host tissues. Garrington & Lightbody (1972) have listed the following five requirements for dental implants.

a) Bio-compatibility

The implants should be compatible with the host tissues and should elicit no allergic, toxic or oncogenic response from the tissues.

b) Non-biodegradability

The materials should be able to withstand deformation and destruction, nor should they undergo degradation. (This definition was made before resorbable ceramics were in common usage.)

c) Functional performance

The implant should be able to have the function(s) of the tissue(s) it is replacing.
d) Sterilizability

It should be possible to sterilize the implant without affecting its physical or chemical properties.

e) Availability and ease of fabrication

Implants should be reasonably available and their fabrication to the desired end-product should be economical.

Most implants have been used because of their physical properties, yet it would seem that few researchers have given consideration to the tissue-implant surface relationship. Adell et al 1970 condemned the fact that neither atraumatic surgical techniques nor clear guidelines for the evaluation of clinical success were, in general, given the importance they deserved.

Implants have been used in many forms to carry out several roles, yet the evaluation of implant success is vague and often anecdotal (Hall 1971; Johns 1976). Natiella et al (1972) expressed the views of the Council on Dental Materials and Devices of the American Dental Association and its concern about the lack of standardisation of techniques which per force makes the assessment of success difficult especially when, at that time, many reports were anecdotal. This organisation clarified this situation following the Harvard Consensus Development Conference (Schnitman & Shulman 1979). At this meeting, guidelines were proposed on the use of various implant types and opinions recorded as to their progress. This conference also recommended panel review of new clinical trials in conjunction with more rigid criteria for patient selection and case evaluation.

More recently, Adell et al (1981) presented a 15 year review of clinical results of a specific form of endosseous implants which
they labelled osseointegration. The success rate of these implants over 15 years compares favourably to the guidelines and statistics of the Harvard Consensus Development Conference (Schnitman & Shulman 1980). Bergman (1983), as spokesman for the Swedish National Board of Health and Welfare, voiced the opinion that treatment with conventional complete dentures should be attended for at least one year before this form of treatment be attempted. In addition, the Board recommended that this technique should be performed only by teams of specialists.

The success of Brånemark and his co-workers (1983) and the growing importance of ceramics of calcium phosphate (Kent et al 1982; Larsen et al 1985) would appear to give dental implants a degree of success which has, previously been conspicuous by its absence. Given the promise of ceramics, an extensive literature survey was conducted into implant materials in general and ceramic materials in particular. Reports on manufactural and physical properties were also reviewed in animal and human studies.

4. Implant Materials

Many materials have been used for implants. A generalised subdivision of implant materials categorises them as metals, polymers or ceramics.

4.1 Metals

Several metals and their alloys have been used for implantation purposes in animals and man. As a result of their inherent strength, they are the materials of choice where load-bearing functions are required (Cohen 1967).
Metal implants are used to fulfill three functions (Cohen 1967):

(a) as a means of fixation, e.g. in the treatment of fractures,
(b) to apply mechanical forces to bone, and
(c) to replace bone substance.

The following metals are, or have been used as implants:

i) Gold

Gold was used in the 19th Century and early 20th Century but is now not used because it is too expensive.

ii) Silver

Silver, like gold, enjoyed some popularity in the late 19th Century and early 20th Century. Indeed it was used to restore a mandibular defect caused by the removal of a tumour circa 1910, (Robinson 1959). The silver mandibular prosthesis had lost its epithelial cover in places and was in communication with the oral cavity (Robinson 1959).

iii) Lead

Lead was used as an implant material in the late 1890's (Greenfield 1913). Even in these early years of dental implantology, however, the use of lead was questioned as no natural union of implant to host tissue could be proved (Cutright 1983).

Three distinct metal types are now used in dental implants (Williams 1981). These are the stainless steel alloys, the alloys of cobalt chromium and titanium and its alloys.
iv) Stainless steels

Stainless steels were patented in 1913 and were first used as surgical implants. The composition of surgical stainless steels has been specified (B.S.3531:1968) as:-

- Chromium 16.5 - 19.5%
- Nickel 10 - 15%
- Molybdenum 2.25 - 4%
- Silica maximum 1%
- Manganese 0.5 - 2%
- Sulphur 0.03%
- Phosphorous 0.03%
- Carbon 0.03 - 0.07%
- Iron to make up the balance

This composition is known as 316 stainless steel (18:14) and has an austenitic crystal structure. This material is wrought and, as will be discussed later in this chapter, is prone to corrosion. Although still widely used in orthopaedic surgery, these steels, because of their susceptibility to corrosion, have limited use in dental and oral surgery as they would be placed in an environment where they would be even more likely to corrode. In consequence, they are restricted to the treatment of certain fractures where a ready-made wrought means of fixation is desirable.

v) Cobalt-chromium alloys

Cobalt-chromium alloys were first formulated in 1907. They were not used in dentistry, however, until the 4th decade of this Century, when improved casting techniques facilitated their use. Dahl (1943) introduced the concept of subperiosteal implants using
a cast subperiosteal framework of cobalt-chromium alloy. This was later developed by Goldberg & Gershkoff (1949). Early forms of those alloys were known as stellate alloys because of their metallic lustre. In 1936 an improved alloy was introduced with the trade name of Vitallium which had a composition of 30% chromium, 7% tungsten and 0.5% carbon, the remainder being cobalt. Further modifications have resulted in most common surgical cobalt-chromium alloys having a composition according to B.S. 3531:1968:

<table>
<thead>
<tr>
<th>Element</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromium</td>
<td>27 - 30%</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>5 - 7%</td>
</tr>
<tr>
<td>Nickel</td>
<td>2.5% maximum</td>
</tr>
<tr>
<td>Silicon</td>
<td>1% maximum</td>
</tr>
<tr>
<td>Manganese</td>
<td>1% maximum</td>
</tr>
<tr>
<td>Iron</td>
<td>0.75% maximum</td>
</tr>
<tr>
<td>Carbon</td>
<td>0.2% - 0.35%</td>
</tr>
<tr>
<td>Cobalt</td>
<td>to make up the balance</td>
</tr>
</tbody>
</table>

These alloys are intrinsically brittle and hence require to be cast although, nowadays, wrought alloys are feasible. Cobalt-chromium alloys are very strong and their properties are listed in Table 1.1. The biocompatibility of these alloys will be discussed later in this chapter.

vi) Titanium and its alloys

The use of titanium as an implant material has been widely reviewed (Leventhal 1951; Lemons 1975; Bränemark 1983; Williams 1981 and Parr et al 1985): pure titanium tends to be a rather soft material but the addition of trace elements increases its strength. Titanium has a lower density and a lower modulus of elasticity than
<table>
<thead>
<tr>
<th>Materials</th>
<th>Specific Gravity</th>
<th>Tensile Strength</th>
<th>Modulus of Elasticity</th>
<th>Compressive Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Wrought) stainless steel alloys</td>
<td>8</td>
<td>1579 MPa</td>
<td>179 GPa</td>
<td>*</td>
</tr>
<tr>
<td>(Cast) cobalt-chromium alloys</td>
<td>8</td>
<td>870 MPa</td>
<td>223 GPa</td>
<td>*</td>
</tr>
<tr>
<td>Titanium</td>
<td>4.5</td>
<td>1276 MPa</td>
<td>100 GPa</td>
<td>*</td>
</tr>
<tr>
<td>Alumina</td>
<td>3.9</td>
<td>119 MPa</td>
<td>418 GPa</td>
<td>280 MPa</td>
</tr>
<tr>
<td>Dense hydroxyapatite</td>
<td>3.01</td>
<td>25 MPa</td>
<td>*</td>
<td>15-60 MPa</td>
</tr>
<tr>
<td>Compact bone</td>
<td>*</td>
<td>70-100 MPa</td>
<td>15 GPa</td>
<td>&gt;100 MPa</td>
</tr>
</tbody>
</table>

* Not normally recorded
other metals used as implants (Table 1.1). The lower density should, in theory, reduce the patient's awareness of the implant and the low modulus of elasticity means that the titanium implants are more flexible hence they will reduce stresses between implant and bone by flexing with the bone. Titanium oxidises readily and this oxide film, if removed, re-oxides rapidly. There is a lack of information in popular dental material texts concerning titanium and its use as an implant material (Phillips 1982). It has been suggested, however, that the dearth of publications on titanium alloy implants is a sign that they were generally satisfactory (Hille 1966).

Titanium alloys of interest to dentistry exist in three forms: alpha, beta and alpha-beta (Williams 1981¹). These alloys are formed when pure titanium is heated, mixed with aluminium and vanadium in certain proportions and then cooled so as to produce true solid solutions. Aluminium functions as an alpha-phase condition stabiliser and in so doing increases the strength and decreases the weight of the alloy. Vanadium provides beta-phase stabilisation. The alloys most commonly used for dental implants are the alpha-beta; of these the most common alloy contains 6% aluminium and 4% vanadium. Although they are stiffer than bone, their moduli of elasticity are closer to bone than any other popular implant material; the only exception is pure Titanium.

Miscellaneous metals form a fourth category of popular metals of use as implants and these include tantalum, zirconium and ticonium. They are seldom used because they are either expensive or have not been clinically tested in any acceptable way (Williams 1981¹).
4.2 Polymers

These materials have been used in dentistry since the late 19th Century when vulcanite was first used as a denture base material. The polymers in use today are derived as a result of the pioneering work of the 1930's and 40's when many polymers were used as implants (Blais 1983). As the general term polymer encompasses several chemical entities, a review of the polymers in use as dental implants follows:

i) Poly (Methyl) Methacrylate (P.M.M.A.)

This polymer is still a popular material in orthopaedic surgery as a bone cement for joint replacement procedures. The late Sir John Charnley (1964 & 1968) developed its use as a bone cement, although Scales (1968), in an editorial, was more objective as to its uses. As heat-processed P.M.M.A. can be processed to the shape of a tooth root quickly and easily, it had obvious attractions to early researchers such as Flohr (1953) and Waerhaug & Zander (1956). The latter extracted the molar teeth of dogs and duplicated the root forms in heat treated P.M.M.A. They tapped the implants in place to ensure a tight fit. The root forms were either highly polished or had roughened surfaces. Originally the implants extended some distance supragingivally but coronal fracture occurred and it was decided to level off the implants at gingival level. Of 34 implants inserted, 14 were exfoliated within 129 days. In all cases, epithelial downgrowth was observed.

These early "failures" did not deter other researchers. Lam & Poon (1968) and Lam (1972) researched root-shaped implants of P.M.M.A. but not as zealously as Shklar et al (1966) and Hodosh et
al (1967, 1969 & 1971). These workers implanted P.M.M.A. root forms into baboons either as the sole material or as a coating to Vitallium pins. They claimed good adaption to bone, although often the histological evidence was not presented or it was interpreted according to their own opinions. In what may be regarded as a concession that their earlier implants had shortcomings, Hodosh et al (1973) combined the P.M.M.A. with anorganic bone and a foaming agent (dinitrosopentamethylene tetramine) to ensure better implant anchorage. A further avenue of research by the same authors (Hodosh et al 1978) was to combine P.M.M.A. with vitreous carbon micro bubbles. They used baboons as the animal model and again claimed favourable clinical results. Although Healey & Taylor (1973) agreed with Hodosh et al (1978) that these implants were well tolerated in dogs, other workers (Hamner et al 1970) reported otherwise. Hamner et al (1970) implanted 36 P.M.M.A. root forms into baboons; 15 were lost between two and 97 days; five cases exhibited osteomyelitis and the remainder showed signs of inflammation and epithelial downgrowth. Although P.M.M.A. has also been used as a cement for dental implants (Zarb et al 1972; Cardiff et al 1974) this material is currently not used as a dental implant. The concept of using fresh extraction sockets in which to place implants is, however, preferable to other forms of endosseous implants which require that bone surgery be carried out. P.M.M.A. is still used in persistent bony inflammation sites in the form of a therapeutic rosary, antibiotics being incorporated into the P.M.M.A. beads (Law et al 1986).

ii) Silicone Rubbers (polydimethyl siloxane - Silastic™)

This material is widely used in dentistry as either an
impression material, as a resilient lining for dentures, or as an obturator. Silicon rubber may be used as an implant material in plastic surgery, for example, in chin augmentation or malar augmentations (Taicher et al 1984). Boucher (1964) and Gatewood (1968) described its use in ridge augmentation. The disadvantage of this material is that it is either extruded or can cause pressure resorption of the bone it is attempting to augment (Robinson & Shuken 1969). The advantages of using room temperature vulcanising silicone rubbers are outweighed by their lack of strength, working time and short shelf life (Bell et al 1985). The advantages of high temperature vulcanising silicone rubbers in maxillofacial prosthetics include greater colour stability, strength and flexibility and soft lifelike quality. The drawback with these materials, however, is their lengthy fabrication procedure which requires machine milling and the fabrication of metal moulds (Bell et al 1985). For these reasons, these materials, in their present form, have no real future as dental implants.

iii) Polytetrafluoroethylene (P.T.F.E. - Teflon™)

Alone, this material is not used much as a dental implant, although Nalbandian & Hellden (1982) assessed the response of the periodontal tissues of three dogs to P.T.F.E. implants. Compared to control sites, the implant site was found to have bone in apposition to the implant surface. This material may prove useful when tooth reimplantation is a necessary procedure.

P.T.F.E. as a dental implant is combined with carbon fibres which are randomly dispersed within it. This material, known as Proplast I, has a porous structure and a modulus of elasticity
similar to cancellous bone. This material has been used to augment alveolar ridges as it is readily implanted in a block form, Kent et al (1972). A disadvantage of the P.T.F.E. blocks used in ridge augmentation, however, is that when a denture was placed over the augmented ridges, mucosal inflammation results (Kent et al 1975). A further disadvantage of this material is its colour; the carbon imparts a dark colour to the material. In an attempt to overcome the colour problem, Proplast II has been developed and this white material is a composite of P.T.F.E. and aluminium oxide. This latter material is sometimes used as a material for augmentation in maxillofacial and plastic surgery (Williams 1981).

iv) Other polymers

Various other polymers have been used as implants. These include polyvinyl alcohol (Ivalon™), polyhydroxyethyl methacrylate (Hydron™) and poly(ethylene-tetraphthalate) (Dacron™). Of these three materials, only Dacron™ has a dental application.

a) Polyvinyl alcohol (Ivalon™)

This polymer has no apparent significance as a dental implant as the polyvinyl alcohols fail to become structurally and physiologically bound to the surrounding bone (Amler et al 1958).

b) Polyhydroxyethyl methacrylate (Hydron™)

This polymer has been used extensively as a soft contact lens, on account of its biocompatibility and the fact that it forms a gel in the presence of water (Hench & Ethridge 1982).
c) Poly(ethylene-tetraphthalate) (Dacron™)

This polymer has been used in conjunction with a polyether-urethane elastomer to make customised trays for bone grafts, for example in mandibular reconstruction (Leake 1974). This fabricated tray, which can be customised by trimming, has the advantage of being able to be autoclaved (Williams 1981).

d) Polyethylene

Polyethylene has also been used as an implant material, particularly for joint replacement, although it would appear that ultra high molecular weight polyethylene was not a static material but was continually undergoing progressive structural change (Grood et al 1982).

Several workers have investigated the effects of several implanted polymers on host tissues. It was found that when Dacron™, Ivalon™, Nylon™ and Superpolyamide™ were implanted, in the mandible of rats, the last named implant elicited the least foreign body reaction (Boucher & Surwillo (1968).

Although polymers have some applications in plastic and maxillofacial surgery, their relatively poor mechanical properties make them unsuitable for load bearing applications in either the musculoskeletal or oro-masticatory systems.

4.3 Ceramics

Ceramics are structural solids in which one or more metallic elements form a compound or a series of compounds with one or more non-metallic elements (Williams 1981). Chemically, they range from simple carbides such as tungsten carbide to the more complex
silica glasses. The simple ceramics are based on ionic structures; the more complex ceramics have ionic and covalent bonds incorporated in their structure. Ceramics have been utilised for orthopaedic and dental implants principally because they are chemically inert. Although they tend to exhibit reasonable compressive strengths, they tend to be brittle materials (Table 1.1). Hench (1981) has subdivided the broad term of ceramics into three groups. This subdivision is based on what Hench called the relative reactivity of the materials:

a) nearly inert,

b) controlled surface activity, and

c) totally resorbable.

The ceramic materials in common usage include, principally, crystalline materials such as alumina and aluminates, calcium phosphates plus the various forms of carbon. It is accepted that carbon glasses such as the surface-reactive glasses, or bioglasses, are included within this group of materials. The following is an account of ceramic materials which have been and which are used, as dental implants.

i) Calcium Sulphate Dihydrate (Plaster of Paris)

Dressen first reported the use of calcium sulphate dihydrate in 1892 when he implanted it into bony cavities in eight patients (Peltier 1961). Nystrom (1928) implanted a "porridge" of plaster of Paris with a 1:1,000 of aqueous Rivanol (an antibiotic) into bony defects. He reported that the material resorbed and that by 11 weeks, only slight cavities were evident radiographically in bone. Edgberg (1930) also inserted plaster of Paris into bony
cavities and stated that the implant material provided support for only a few weeks as, after that, concentric reduction of the plaster occurred. He noted, however, that bone regeneration was not hindered. Alderman (1969) used plaster of Paris in the treatment of periodontal intra-bony pockets but he was unable to recommend the continued use of this material. When plaster of Paris was coated with n-butyl-2 cyanoacrylate and this implant composite placed on circular defects in the skulls of rabbits, the implants were well tolerated by the tissues but complete bony healing did not result (Frame 1980). Since that time, this material has not featured as a dental implant material because of its obvious limitations in providing support and inability to accelerate bony healing.

ii) Carbon Implants

Carbon has many allotropes. Two which have been used as dental implants are vitreous carbon and pyrolite carbon; these allotropes have a disordered stacking, unlike graphite, and they are termed turbostatic carbons. Vitreous carbon is so called because of its glossy black appearance. It is not, however, a glass but rather a polycrystalline solid with a very small grain size. It is formed by the controlled slow heating of organic polymers. After volatile components are driven off, a 99.9% pure carbon residue remains the resultant material being biocompatible. Benson (1971) and Mooney et al (1971) suggested that vitreous carbon had a potential as an implant material. Grenoble & Kim (1973) evaluated vitreous carbon root replica implants which consisted of hollow stainless steel tubes surrounded by vitreous carbon. This root implant had a
roughened surface, but where it contacted the gingival tissue, a glazed surface was present. When these implants were placed in dogs and in humans, no inflammation was noticed and the implants were considered to be clinically successful. These views were shared by Hucke et al (1973) who gave no clinical or histological evidence to support their findings. Further studies on the effects of vitreous carbon in the alveolar bones of rabbits, dogs and man indicated good preservation of alveolar bone (Mills et al 1974). However, accurate scientific comparison between species is difficult especially as it would appear that alveolar resorption differs between man and dogs (Denissen & de Groot 1979).

Although vitreous carbon implants are well accepted by oral tissues they lack adequate mechanical strength for functional loading (Stanitski & Mooney 1973; Hobkirk 1975). These observations were substantiated by Schnitman & Shulman (1979) who reported the Harvard N.I.D.R. Consensus Conference views that the use of vitreous carbon implants be limited to clinical trials on the basis of insufficient data supporting their efficiency. It was reported, in addition, that splinted teeth were more successful than free-standing teeth. Schnitman & Shulman (1980) were of the opinion that vitreous carbon root implants should no longer be accepted as freestanding implants.

Pyrolytic carbons are essentially solid carbons formed in a fluidised bed by the pyrolysis of a gaseous hydrocarbon at temperatures below 1,500°C. This type of pyrolytic carbon is termed Low Temperature Isotope or L.T.I. pyrolytic carbon. It is possible to incorporate silicone into the L.T.I. pyrolytic carbon and this combination has been considered a suitable composite for
use as a dental implant (Bokros 1977; Kent et al 1977\(^1\)). Williams (1981\(^1\)) whilst agreeing that L.T.I. pyrolytes exhibited the best mechanical properties of all bulk carbons, stated that their absolute mechanical properties were less than ideal and suggested that more favourable results might be obtained if this form of carbon was coated onto a metal.

iii) Aluminas and Aluminates

The pioneer of alumina ceramics was Smith (1963). He developed a material called Cerosium which was a porcelain of 46% porosity produced by combining alumina, silica, calcium carbonate and magnesium carbonate and heating the mixture to 2,000°F. The resulting ceramic, which had a true interconnecting porosity, was impregnated with a hydroxyaliphatic resin. When bars of this new material were inserted into surgically created bony defects in rabbits, they retained the hardness and corrosion resistance of ceramics and, in addition, demonstrated resilience and adequate strength (Smith 1963). A failing of Cerosium was that the resin tended to leach out causing peri-implant inflammation (Rhinelander et al 1971).

From this prototype, however, calcium aluminates were developed. Two such ceramics were prepared by slightly different techniques and slightly differing ratios of calcium oxide to alumina; one was a polyphasic ceramic, the other was a nearly single phase compound. These implants were inserted into the femora of Rhesus monkeys and it was noticed that after 32 weeks, the polyphasic ceramic was completely impregnated with connective tissue whilst undergoing considerable alteration in microstructure.
In contrast, the single phase ceramic was adjudged not to have altered structure significantly (Graves et al 1971). Klawitter (1970) confirmed the potential of calcium aluminates using rods of the material in dog femora and discussed the effect of pore size on tissue ingrowth. Hentrich et al (1971) compared calcium aluminate to alumina and zirconium oxide. These workers placed rods of the test materials into the femora of Rhesus monkeys and assessed the implants histologically after 50 days. Although the periosteum was able to induce bone growth in the implant sites the calcium aluminate implants were considered to stimulate bone growth best (Hentrich et al 1971). Hentrich and his colleagues were perhaps the first to record that the density of the aluminate decreased with time and postulated that one phase of the material was going into solution. Bhaskar et al (1971) investigated implants made of phosphate-bonded alumina. They gave no explanation for their choice of this material but listed the following properties of alumina ceramics:

1. They are similar to bone in terms of specific gravity, coefficient of friction and strength and surface properties.
2. They do not elicit an immune response and do not corrode.
3. They are nonresilient, have low impact strength and fracture resistance and are difficult to mould.

Bhaskar et al (1971) implanted their phosphate-bonded alumina implants into rats, miniature pigs and monkeys but did not state why they used this type of alumina. In the rat, the impact site was the femur, while in the two larger animal models, the implant sites were intra-alveolar. The implants evoked no adverse
reactions and the fact that more bone was stimulated in the rodent rather than the porcine model highlights species difference in such experiments. The significance of species variation has already been reported (Denissen & de Groot 1979).

A feature of early reports on implants is that the precise composition of many implants was not recorded, nor their manufacture. Klawitter (1970) described one method of producing a porous calcium aluminate from alumina and analytical reagent grade calcium hydroxide. The mixture was milled in a glass jar for 1.5 hours using polypropylene rods as the milling agent. Distilled water was added to make a paste. After drying at 110°C and grinding, the material was calcined at 1000°C for 1 hour. This fabricating process which is effectively sintering was a reliable way of producing porosity in the implants (Williams 1981). The polymeric implants used by Hodosh and his co-workers (1969) were condemned by Hamner et al (1970) who assessed calcium aluminate tooth implants as viable alternatives. They inserted whole tooth implants into baboons but noticed the coronal sections discoloured in the oral cavity. In a later study, the problem of discolouration was overcome by glazing the coronal and gingival areas at 1010°C. The teeth were splinted to abutment teeth and the aluminate implant systems were considered to be more successful than the polymeric system (Hamner & Read 1972). These authors, however, offered no histological or radiographic evidence to substantiate their claims.

The physical properties of ceramic tooth implants have promoted research into their ability to bear masticatory loads and, in particular, how they react to concentrated occlusal forces.
In this study he implanted calcium aluminate implants (either as a single or two-part implant) into fresh extraction sockets in dogs. No indication was given of the phasing of the two-stage techniques and the aluminates were considered adequate for use in unloaded sites (Young 1972).

Calcium aluminate has been used to augment alveolar ridges in dogs (Topazian et al 1972). Implantation commenced six weeks after the loss of all mandibular molar and premolar teeth. On one side of the mandible, a bar of calcium aluminate was placed in a subperiosteal tunnel. On the other side, a buccal mucoperiosteal flap was raised to expose the alveolar bone. Calcium aluminate was placed into this defect and the wound closed in layers. The implants were studied clinically, radiographically and histologically. No adverse reactions occurred although Topazian et al (1972) alluded to the fact that denture wearing, in humans, would impart greater pressure and friction on the mucosa and ridges than were experienced in the their canine models.

Hamner et al (1972) and Hamner & Reed (1973) implanted root forms made of calcium aluminate in baboons. No supragingival portions were made, as previous experience had shown that the animal models tended to chew the bars of their cages. Results indicated no sign of inflammatory reaction and there was a lack of mobility on probing. In 1974, Hamner et al reported on six clinical cases where ridge augmentation was effected using calcium aluminate. After two and a half years, four out of the six cases remained satisfactory. Whether the failure rate was attributable to the material, the technique or patient selection, was not clear.

High density alumina ceramic has also been used as an implant
material (Driskel et al. 1973). In this study, in monkeys, temporary stabilisation of the implants was obtained by using acrylic bridges and this precaution may well have accounted for the low failure rate of less than five per cent, although the criteria for success were not detailed. Early aluminates were resorbable and Schnittgrund et al. (1973) used in vitro studies of calcium aluminates to demonstrate that after being held at a constant stress for less than 10 minutes in Ringer's solution, the aluminates lost 40% of their fatigue strength. These same workers found that in vivo ageing in rabbits for 12 weeks was found to decrease the compressive strength of the aluminate by 25% to 30%. Semi-quantitative analyses of the residual ageing medium indicated the leaching of calcium and aluminium ions from the aluminate and an uptake of magnesium from the Ringer's solution.

Several comparative studies using high density aluminas indicate their potential. Dorre & Dawihl (1980) compared high density alumina ceramic prostheses to metal - ultra-high molecular weight (U.H.M.W.) polyethylene joints. The ceramic to ceramic joints were found to improve with time with decreasing friction whereas the metal to U.H.M.W. P.T.F.E. deteriorated with time. Cook et al. (1983) compared L.T.I. carbon-coated aluminium oxide and uncoated aluminium oxide implants. Blade-shaped implants were inserted into monkeys and studied over a two-year period. The object of this experiment was to determine the effect of different moduli of elasticity as well as different surface composition on the performance of the implant. The cortical bone stress levels on the uncoated implant were three times that of the coated implant and it was considered that the alumina implant gave better stress
shielding (Cook et al 1985).

A new development in the field of alumina ceramics was the development in Japan of crystalline alpha aluminium oxide. This produced what was intrinsically a single crystal of alumina, hence the single-crystal sapphire implant. Yamane et al (1979) gave a detailed account of its physical properties, although no details were supplied of the manufacturing process. He only stated that the physical nature of alumina was improved by a special high temperature treatment in single crystal. Animal implantation studies would appear to confirm the biocompatibility of this material as well as its suitability to be used as a dental implant (Kawahara 1979; McKinney & Kohl 1982). Human clinical trials evaluating single sapphire implants are now under way. Twenty nine such implants of a screw-type design were inserted into the mandibles of 18 patients; 23 of these were used as distal abutments for fixed bridges. Clinical success was judged on gingival bleeding, plaque accumulation, crevicular fluid volume and implant mobility. Radiographic assessment and anamnastic reports were included in the evaluation (Koth et al 1983). These workers, after the first year of a projected five year study, estimated a success rate of 91%.

If long term clinical studies confirm these results, then it may be possible to advocate the use of this material as a dental implant.
iv) Glass Ceramics

Hench et al (1971) reported the use of one surface active ceramic, which they termed "Bioglass" although its composition was not disclosed. The constituents of one glass ceramics, 45SS, was reported by Gheysen et al (1983) as being:–

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Percentage by weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicone oxide</td>
<td>45%</td>
</tr>
<tr>
<td>Calcium oxide</td>
<td>24.5%</td>
</tr>
<tr>
<td>Sodium oxide</td>
<td>24.5%</td>
</tr>
<tr>
<td>Phosphate</td>
<td>6%</td>
</tr>
</tbody>
</table>

These materials have several advantages as implant materials principally biocompatibility and controllable surface activity (Hench 1981). It was suggested that the surface active glass ceramics could be coated onto metallic implants hopefully to enable better implant:bone bonding and this technique had three advantages (Hench et al 1971):–

1. Mechanical strength would not be limited as the implant need not be porous.
2. The strength of the implant would not be changed during the implantation as the glass ceramic is only surface active.
3. This material can be coated onto stainless steels, cobalt-chrome alloys or alumina giving a favourable combination of mechanical strength and a biocompatible surface.

When a glass ceramic with a silica content of 45% and a calcium to phosphate ratio of 5:1 was used as an implant, good fixation of implant to bone was established (Greenlee et al 1972). When fluoride was added to the ceramic, it was found that
Implant fixation was not achieved, presumably as the fluoride competed with the calcium in the implant. Bunte et al. (1977) compared the adherence of P.M.M.A., alumina and surface active glass implants to alveolar bone in pigs. After sacrifice, fracture loads of each implant-bone interface were determined by three-point bending tests: those for the glass ceramic were reported to be significantly greater than for the polymer or alumina implants and in the case of the bioglass, the fracture went through bone whereas the other two groups fractured through the implant-bone interface indicating a better bone implant junction with the bioglass.

Bunte & Strunz (1977) augmented human atrophic mandibles with bioglass but with limited success in that clinical stability was achieved only if the implants were fixed in place with sutures. Long term results for this form of treatment have not, as yet, been published (Drummond 1983). An extensive mandibular defect has been restored with surface active glass ceramics and clinical and radiographic observations suggest this material may have a use in reconstruction after treatment for some tumours of the facial skeleton (Bradley 1985).

The main disadvantage of these materials is that, being glasses, they tend to be brittle and exhibit poor fracture strength (Williams 1981). One method of tackling this problem is the coating of a high strength substrate with the bioglass. However, the physical properties of these coatings depend on the reactions and mutual solubilities of the substrate and the coating material in addition to the nature of their thermal expansion rates (Williams 1981). To overcome this, two techniques have been described. These problems may be overcome either through the
application of several layers of the glass-ceramic onto the metal or by coating the metal with an inert ceramic (containing a powder of the bioactive glass) before adding the layer of glass (Heimke et al 1980).

A second means of compensating for the lack of strength of the bioactive glasses was the development of a composite of a bioactive glass and stainless steel fibres (Gheysen et al 1983). These workers dipped a porous compact of 316L Stainless Steel fibres (of predetermined shape and density) into a bath of molten glass. The metallic fibres had diameters of either 50µ or 100µ and a length of 4 mm. The metal:glass ratio was respectively 40:60 volume percent or 60:40 volume percent. Before the fibres were dipped into the bath they were oxidised to ensure good adherence between the fibres and the glass. Plugs of the composite were implanted in the hind legs of dogs for eight weeks. Implants of bioglass and stainless steel served as two-way controls. Shear strengths of the implants was determined using a push-out test and while the stainless steel controls were easily dislodged, the bioglass control and the test implant were of similar strength, both being significantly greater than the stainless steel control. Mechanical testing of the two types of composite involved four point bending tests and the fact that the 50µ composite fractured completely whereas the 100µ composites only bent heavily with local cracks at the surface led to the following conclusions (Gheysen et al 1983):

1. Bioglass composites could be deformed.
2. The strength of bioglass composites was improved by a factor of x 7 or x 8.
3. Bioglass composites could be subjected to tensile stresses.

4. Bioglass composites were machineable.

5. Bioglass composites could withstand impact loading.

6. The low modulus of elasticity of bioglass composites was similar to the surrounding bone.

The fact that the surface activity of these materials is controllable indicates that this implant material may be of use biomedically, subject to long term human clinical trials.

v) Ceramics of Calcium Phosphate

Calcium phosphate, mainly in the crystalline form of hydroxyapatite is the main mineral constituent of bone and tooth (Matthews 1980) and the use of these bioceramics of calcium phosphate as implant materials has obvious advantages. Two thermodynamically stable members of the calcium-phosphate "family" are tricalcium phosphate (especially the \( \beta \) form) and hydroxyapatite (Driessens 1983). As the determining factor in the production of these materials is the ratio of calcium to phosphate in the preparation (Driessens 1983) their manufacture will be presented jointly although their materials per se are discussed separately.

a) Manufacture of calcium-phosphate ceramics

These two bioceramics are synthesised at relatively high temperatures. At temperatures in excess of 800°C, a salt with a calcium to phosphate ratio of 3:2 will undergo a phase transition from apatite to \( \beta \) Whitlockite. If, however, the ratio is 10:6 then the apatite lattice is retained. Temperatures in excess of 1300°C
result in the decomposition of both materials.

There are three sintering techniques used in the manufacture of tricalcium phosphate and hydroxyapatite (de Groot 1983) and these are:

Uniaxial compaction
In this process a block of the ceramic is made by shaping an aqueous slurry and drying it to produce what is called a green body. This is sintered between 1100°C to 1300°C to create a fusion of the ceramic particles. Sintering times are from one to six hours. The longer the sintering process, the less the void volume is between the particles. These voids are termed microporosities (de Groot 1983). If the slurry is made of an aqueous solution of hydrogen peroxide, then heating in excess of 80°C will result in the production of oxygen bubbles; these bubbles escape from the drying slurry leaving bubble-sized pores, which correspond to the bubbles. These pores are usually around 100μ. This process will result in macroporosities (de Groot 1983).

Isostatic compaction
This involves the application of pressure to a powder-filled mould. This pressure, up to 5000 kg/cm², is from all directions by way of an hydraulic press. The resultant green body and final ceramic tend to be denser and stronger than in the uniaxial technique. If organic materials such as naphthalene are incorporated into the powder before sintering, then macroporosity can be achieved.
Continuous hot pressuring
In this process the powder is pressed and heated simultaneously to give the final ceramic body in one process. The finished product is a dense form of hydroxyapatite (Denissen et al. 1980) although for laboratory investigation alone, the high cost of equipment for this technique may prohibit its use (de Groot 1983).

b) Tri-calcium Phosphate \( \text{Ca}_3(\text{PO}_4)_2 \)
Tricalcium phosphate, in a slurry form, has been used to fill bony defects (Albee 1920; Getter et al. 1972). One disadvantage of this material was that it did not have mechanical strength. Bhaskar et al. (1971) implanted plugs of tricalcium phosphate into the tibiae of 66 rats. After one week, bone had formed at the margins of the implants and by two weeks it was found to be difficult to remove the implant from the surrounding bone. A true evaluation of the potential of the tricalcium phosphate from this report is not possible however, as no mention was made of control sites or control materials.

The effects of biodegradable tricalcium phosphate in alveolar bony defects, of five dogs over 22 weeks, were assessed in a controlled study (Levin et al. 1974). In order that these defects could be likened to periodontal lesions, the induced bony defects were filled with cold-cure P.M.M.A. Between six and 10 weeks later, the polymer was removed, the defects curetted and the ceramic inserted. The ceramic resorbed between 19 and 22 weeks. In the early stages this resorption took place at the margins of the ceramic and later on islands of connective tissue were found to have
36.

penetrated the implant. Similar results in monkeys (Levin et al 1974) showed the effectiveness and biocompatibility of tricalcium phosphate although it is worthy of note that in both animal models the ceramic-filled defects healed more slowly than the control sites. A comparative study using iliac marrow and tricalcium phosphate in periodontal defects in beagle dogs showed the ceramic implant healed slower than the bone marrow graft site (Levin et al 1975). Similar findings were reported by other workers, Nery et al (1975), Mors & Kaminski (1975), Jacobs et al (1982) and Hoexter (1983). Streckbeim et al (1981) induced sinus defects in sheep and reported that, after 12 weeks, the ceramic was fully integrated into the bony wall of the sinus without an interceding layer of connective tissue.

Nery et al (1980) investigated the use of tricalcium phosphate in ridge augmentation. They augmented the alveolar ridges of two mongrel dogs and, over this ridge, placed a removable cobalt-chrome denture; after six months, clinically acceptable results were reported. Mathai & Sripathi (1984) implanted tricalcium phosphate to correct labial undercuts in one edentulous patient. They offered photographic and radiographic evidence to illustrate what was, in their opinion, a clinical success. As only a few patients were studied over a relatively short time, and the fact that the quality of reproduced radiographs was poor, credence of this case report was reduced.

The over-riding criticism of tricalcium phosphate is that it is unreliable when subjected to impact loading and should be used only where mechanical forces are primarily compressive (Lavelle et al 1981; Bajpai 1983).
37.

c) Hydroxyapatite \( \text{Ca}_{10} \left( \text{PO}_4 \right)_6 \left( \text{OH} \right)_2 \)

The intrinsic lack of strengths of ceramics prompted Jarcho et al. (1976) to investigate dense hydroxyapatite as an implant material. They did not describe how their material was made but stated that it was well tolerated by the tissues. They chose dense hydroxyapatite as it had no or little macroporosity and it resorbed only very slowly.

Other workers prepared dense hydroxyapatite by a hot continuous pressure technique; the moulds were heated to 900°C while an upper press applied a pressure of 500 kgm/cm² at a pressuring rate of 25 mm/h. It was estimated that the finished product had a density of 97% and this finished product was much stronger than other hydroxyapatites as a result of its low grain sizes (Denissen et al. 1980¹, ²). These workers followed up previous studies on dense hydroxyapatite by implanting rods of the material in the tibias of rats and also into tooth extraction sockets of dogs. Three-point bending tests were used on the rodent model to indicate the strength of the implant:bone interface. As the implant plus tibial bone fractured as one, the bond between the implant and bone was considered to be as strong as the bone itself. Only clinical and histological evaluations were made on the canine model. The conclusion derived from the tests was that the dense hydroxyapatite appeared to be suitable for use in human alveolar bone to act as a substitute for lost bony tissue.

Flatley et al. (1983) implanted hydroxyapatite into defects in the spines of rabbits and reported excellent bony union of the hydroxyapatite. Feenstra and de Groot (1983) mention other medical uses of dense hydroxyapatite and these include middle-ear
prostheses and coatings for metallic implants.

The dental uses of dense hydroxyapatite implants are concerned with the repair of periodontal infrabony pockets and alveolar ridge augmentation. Rabalais et al (1981), Froum et al (1982) and Moskow & Lumbarr (1983) all inserted dense hydroxyapatite granules into infrabony pockets in human patients. In some of these patients the teeth would otherwise have been extracted. In all three articles, the implants were stated to have been well tolerated although the short duration of the study period (six months) in the case of Rabalais et al (1981) would indicate that it would be difficult to prove that new bone did in fact form.

Denissen & de Groot (1979) implanted 50 dense hydroxyapatite root implants into the fresh extraction sockets of dogs and measured their progress radiographically. That these implants were closely surrounded by alveolar bone, both interproximally and superiorly would suggest that this material had a viable dental application. The value of the hydroxyapatite was enhanced by the fact that there was no loss of implants and attempts to remove the implants indicated that ankylosis had taken place. One hundred dense hydroxyapatite root-forms were implanted into 20 patients and the patients were observed weekly for two months and thereafter radiographs were taken every six months for a period of 18 months (Denissen & de Groot 1979). Once again no implants were lost and these researchers were impressed with the implants as they claimed they had created a biostable component of the alveolar ridge. Although no mention was made of a control group the reports of stable, unchanged implants after 18 months are impressive.
Salsbury et al. (1981) repeated this clinical exercise using a proprietary dense hydroxyapatite implant and reported similar results to Denissen and de Groot (1979). De Putter et al. (1983) extended this implant form and evaluated implants which penetrated through the mucosa into the oral cavity. The implants were placed in either fresh extraction sockets or surgically created sockets in healed edentulous ridges of 12 dogs. In all, 58 implants were inserted in such a way that they were load-bearing. Five implants fractured and one implant was removed—all six belonging to the sample where they were inserted into created sockets. After 30 weeks, 50% of all implants had fractured and this had risen to 70% after one year. Although the intraosseous portions were satisfactory, it was concluded that these implants were unable to withstand masticatory forces. A logical extension to the work of Denissen and de Groot (1979) and de Groot (19832) would be the evaluation of this material in ridge augmentation and clinical evidence in support of hydroxyapatite as a suitable material for use in ridge augmentation is considerable (Larsen et al. 1983; Chang et al. 1983 and Gumaer et al. 1985). Detailed accounts of the subperiosteal tunnel technique for alveolar ridge augmentation have been described by Kent et al. (1982) and McCord (1986).

An interesting approach to the manufacture of ceramic materials uses a technique called the replamineform process. In this process the microstructure of a carbonate skeletal form of an echinoderm (Porites) can be altered by replacing the carbonate molecules with phosphate molecules. The process is both complicated and lengthy (White et al. 1972). The coral, plus weighed quantities of reactant and water, was sealed in a gold tube
and heated at specified temperatures and pressures for periods of time ranging from 12 hours to one week to effect this hydrothermal exchange (Roy & Linnehan 1974). Holmes et al (1980) implanted replamineform hydroxyapatite into the radii of dogs and, in another experiment, into the spinous processes of canine lumbar vertebrae. In both cases, iliac autograft was selected as a control material. Histological evaluation and mechanical testing revealed that total incorporation into reagent bone developed but that the porous implants were mechanically weak. This opinion was shared by Chiroff et al (1975). In what can only be described as short-term findings, Kenney et al (1985) reported clinical success with this type of implant, in 24 patients over six months.

Piecuch et al (1983) placed blocks of this material in ridge augmentation procedures in dogs. Where a tunnel procedure was used, they reported 70% retention of implants whereas in the alternative design, only 49% remained.

It would appear that that the hydroxyapatite material of choice for alveolar ridge augmentation is the particulate dense variety produced by continuous hot pressuring and not a block form. In addition, the subperiosteal tunnel technique would appear to be superior to the open wound method of insertion. Another application for dense hydroxyapatite is the immediate implantation of this material in fresh extraction sockets; further studies are indicated to determine whether solid or granular forms of hydroxyapatite are preferable.
5. Implant:Tissue Interface

5.1 General Review

Most people have, at some time in their lives, accidentally "implanted" a splinter of wood into a finger or forearm. This usually results in an acute inflammatory reaction and in some cases this resolves with, apparently, little cellular response. Another sequel, infection, may occur and this infective process may be acute, chronic or sub-acute. In addition hypersensitivity may complicate the process of healing.

When an implant material is placed in the body, the sequelae to implantation may include some, or all, of the complications mentioned in the preceding paragraph, depending on the stability of the implant material in the body, the effects of the implant and its constituents on body tissues and the surface texture of the material. Some of these reactions are described by Meachin & Pedley (1981). This section's purpose is to review the reactions of tissues to implant materials.

In any research review pertinent to the reactions of tissues to metal, plastic and ceramic implants it is insufficient to consider solely the "biologic adaptability" of the tissues to the implant. In addition the mechanical adaptability of the bone around the implant should also be assessed and the assessment of tissue tolerance to the implants indicates the biocompatibility of the implant material or, at the other end of the tissue tolerance scale, records the degree of toxicity of the material (Bienstock 1955). Williams (1981) has defined biocompatibility as "the term used to describe the state of affairs when a biomaterial exists within a physiological environment, without either the material
adversely and significantly affecting the body or the environment of the body adversely and significantly affecting the material". This definition is in no way absolute; biocompatibility is a complex, multifactorial concept, often showing varying degrees of implant:tissue interactions. Henceforth it will be used in accordance with Williams' definition.

One technique of evaluating biocompatibility is the use of tissue culture tests. Kawahara et al (1968) have discussed the use of tissue culture in dentistry. Tissue culture tests enable visual assessment of either toxicity (indicated by clear colourless zones around the samples) or no toxicity (indicated by the integrity of the monolayer of cells) (Powell et al 1970). In 1973, Homsy et al discussed the biological and functional criteria for oral implantation and concluded that tissue culture testing and animal testing were valid techniques.

Autian (1970) reviewed animal implantation methods and tissue cellular tests which were used to evaluate potential implant materials. He described a screening technique whereby the test materials were implanted in the paravertebral muscles of rabbits. Macroscopic examination of the implant site enabled screening, ranging from 0 (no response is regular control) to 3+ (a marked response). In 1974, Autian elaborated on his previous article and advocated three levels of toxicity testing:

1. A screening test incorporating both in vitro and animal tests involving the finished material and its constituents,

2. Testing the material in sites similar to its intended use in humans, and

3. Long-term clinical trials on humans.
Calnan (1970) supported the views of Autian and stressed the importance of knowing the environment of the implants - he likened it to the "milieu interior" of Claude Bernard. Homsy (1970) was of the opinion that the width of the tissue reaction adjacent to the implant could be taken as a gross indication of the degree of implant compatibility. He classified the cellular response into five groups (0-4) varying from normal cell morphology (score 0) to total inhibition of growth (score 5). Moreover, he considered it intolerable that, at that time, there was an absence of systematic preclinical procedures.

Coleman et al (1974) detailed an experimental protocol to test the reaction of tissues of the rabbit to implanted materials. They attempted to standardise variations in both the animal and the implanted materials by using reproducible techniques although this protocol was designed to give quantitative rather than qualitative results.

Garcia et al (1981) described a similar method to evaluate dental materials. Tissue culture tests have been described by Sisca et al (1967) and Helgeland & Leirskar (1972) but Baier (1982) introduced a note of caution when he mentioned that false results were possible because of gas inclusions in the tissue monolayer. Nevertheless, he acknowledged the value of this technique.

The reactions of host tissues to implants will be discussed in the same order as that used to describe the implant materials, namely metals, polymers and ceramics. The concluding section of this chapter is concerned with oncogenic properties of implants.
5.2 Tissue:Metal Implant Interface.

An early account of adverse tissue reactions was given by two French physicians who used a metal wire (the exact composition was not revealed) to unite a fractured humerus; the wire was not sterile and a fatal infection occurred (Hench & Ethridge 1982\(^3\)). One of the earliest reviews of tissue:metal reactions was by Venable et al (1937) who reviewed the literature on the effects of metal on the repair of bone and noted that findings were often contradictory. When they examined tissue taken from around bone screws plus some renal and hepatic tissue they considered that macroscopic, microscopic and radiographic findings were too variable. In their opinion, moreover, the tissue reactions were a result of electrolytic potentials between different metals. Pure metals gave no such actions and these workers like, Jones & Lieberman (1936), considered Vitallium (an alloy of cobalt, chromium and tungsten) to be the least reactive alloy. Other researchers considered that tissue reactions were in response to physical and chemical properties of the metals (Bothe & Davenport 1942).

Bienstock (1955) stated that the unfavourable reaction of alloys was due to the presence of toxic metals which were soluble in body fluids. Ferguson et al (1960) placed metal implants in rabbits and found that metallic elements appeared around the implants in concentrations significantly greater than normal, irrespective of how non-corrosive these implants proved to be in other environments. Ferguson et al (1962\(^1\)) determined metal concentrations in the liver, kidney, spleen and lung of rabbits and compared these findings to the equivalent values in humans. The same authors in 1962\(^2\), implanted specimens of stainless steel and
cobalt-chromium alloys into rabbits and determined the trace ions released from the implants: nickel was the principal ion released from the steels and cobalt the principal ion released from the cobalt-chromium alloys. It was found that in addition to the peri-implant tissues, ions were stored mainly in the spleen and lungs but also in the liver and kidneys.

Cohen (1967) emphasised the importance of careful techniques and discouraged the use of plated metals which he found could be scratched easily. The importance of standardising techniques, materials and material management was emphasised by Martz (1969).

The earlier work of Ferguson and co-workers (1962\textsuperscript{1,2}) was updated by Laing (1966) and Laing et al (1967). In these experiments, metal implants were placed in either the paravertebral muscles or in the femora of 400 rabbits. Nine corrosion-resistant metals were used including stainless steel, cobalt chromium alloys and alloys of titanium, zirconium, aluminium and nickel. Histological and spectrochemical analyses were used to study the thickening of the fibrous envelope surrounding the implants in an attempt to evaluate the tissue response. The fibrous capsule varied from 0.02 mm to several millimetres thick. In some cases, the muscle tissue was replaced by fibrous and adipose tissue, whilst in the more extreme cases, chronic inflammatory cells were found between the implant and the fibrous tissue. Titanium and its alloys were found to be the least reactive metal. In general, tissue reaction tended to be proportional to the concentration of metal ions but high concentrations of titanium and aluminium produced minimal tissue reaction. Cobalt-chromium alloys tend to elicit more fibrosis than alloys of titanium (Laing 1973).
The biocompatibility of metals is, then, governed by the rate of corrosion and the toxicity of the metal ions. Corrosion of metal alloys is a complicated process and was reviewed comprehensively by Williams (1981): two types of corrosion, pitting corrosion and intergranular corrosion, have been observed in stainless steel orthopaedic implants of which more than 55% had corroded (Hughes & Jordan 1972; Williams & Meachim 1974). Scales (1956) said that there was no knowledge of corrosion of cobalt-chromium alloys although Coleman et al (1973) observed that concentrations of cobalt and chromium increased in the hair, blood and urine in patients who had total hip replacement prostheses. This finding they attributed to wear of the joint components.

Meachim & Williams (1973) used neutron-activated analyses to study the periosteum adjacent to 19 titanium implants which were removed for varying reasons. Nine out of 19 had titanium levels more than 100 parts per million by weight, while three out of 19 had more than 2,000 parts per million. However there was no correlation between the concentrations of ions and the duration of the implants. Two types of intracellular particles were noted. The first, or Type A particle, gave a positive response to Perl's free iron test indicative of the presence of haemosiderin and may be a consequence of haemorrhage. Type B gave a negative result to Perl's Test. The negative result was considered to be caused either by titanium or a by-product of titanium.

Histological studies on metallic dental implants are concerned principally with the alloys of cobalt-chromium and titanium. A range of tissue responses have been reported, the range arising from varying techniques, different objectives and different
interpretation. The fact that fibrous tissue is produced when metals are implanted in soft tissue is utilised where subperiosteal implants are concerned as this helps implant retention. Several investigators have expressed varying opinions on this form of implant system. Obwegeser (1959) clearly was not an advocate of subperiosteal implants. He listed some complications resulting from subperiosteal implants and reported on 35 clinical cases, one third of which had complications after 18 months. Only 13 cases were free from inflammation but of those only nine were free from pain or paraesthesia. He declared that success was a product of subjectivity on the part of the operator and not of objective testing.

Bodine & Mohammed (1969) had the unique opportunity to examine, macroscopically and histologically, the mandible of a patient who had worn a subperiosteal Vitallium implant for 12 years; the patient had died from an unrelated cause. This implant was apparently well tolerated by the tissues and the oral epithelium had migrated down the permucosal strut by only two or three millimetres. This finding was in contrast to Mack (1960) who implanted Vitallium subperiosteal implants in two monkeys. Histological examination, between 251 days and one year, revealed that the framework was mechanically retained in position by fibrous tissue. Moreover in most histological sections there was a lining of epithelial cells between the metal and the fibrous tissue. Some round cell infiltration was also seen.

Bodine (1974) presented a 16 year assessment of 17 subperiosteal implants. He reported a 96% success rate after five years and 52% after 16 years. His criteria for success, however,
were not made clear. Young et al (1983) questioned the parameters of success of subperiosteal implants. They alluded to the 1978 Harvard Conference on Dental Implants (Schnitman & Shulman 1979) and, in particular, to its criteria for success. Young and his colleagues (1983) presented clinical reports on 11 patients although the formulation of their group was unclear. It has to be admitted that the tenuous nature of clinical criteria of success have to be tempered by anamnastic reports. This fact was reinforced by Hobkirk (1985) who has conducted the only scientific long-term (16 years) report of subperiosteal and endosteal implants in Great Britain.

Endosseous implants are not intended to rely on the fibrous reaction from the periosteum and understandably have been the subject of more investigation than subperiosteal implants (Hulbert & Bennett 1975). Seidenberg & Lord (1963) placed Vitallium intraosseous implants in dogs and reported on the healing socket. Little histology was shown but the authors were encouraged by this material. Babbush (1972) inserted blade vent implants in dogs and reported that after one year no signs of rejection were apparent. Cranin et al (1973) placed 260 blade anchor implants in dogs over a period of three years. They presented radiographs to give evidence of bone to metal contact. Unfortunately no histological photographs were presented and of the 260 implants, 19 were reported as failures. Harris (1969) studied the effects of cobalt-chromium tooth forms and in 1973 Harris & Lossin compared Vitallium implants of the blade vent form to titanium implants of several forms. They considered that titanium was a more tissue-acceptable material than Vitallium. In addition, they stated
that only a limited amount of epithelial downgrowth had taken place.

In contrast to this Manderson (1971) implanted titanium blade vent implants in miniature pigs. He showed evidence of considerable epithelial downgrowth after only 21 days. Linkow (1970) presented a seven year progress report on transosseous implants and, understandably, endorsed his design of blade-vent implant. Armitage et al (1971) evaluated early bone changes following the insertion of 20 endosseous implants into the jaws of 10 monkeys. Although they commented on the fact that a list of detailed criteria was almost impossible to formulate, they reported no unfavourable response to the implants. If implants are to be successful, then it is important that primary stability, reduced stress on the implants and lack of damage caused by temperature changes to the surrounding bone should be the goals of the surgeon (Tetsch & Peppmeier 1973).

Brånemark et al (1969) gave a detailed account of experimental evaluation of intra-oral anchorage while Lundskog (1972) and Ericsson & Albrektsson (1983) have investigated the effect of temperature on bone viability. The critical temperature for bone is 47°C; above that, osteonecrosis can result and unless temperature controlled atraumatic surgical techniques are used, then fibrosis can result (Ericsson & Albrektsson 1983).

A careful surgical procedure is the basis of an exciting new technique described by Adell et al (1981) and Albrektsson (1983). This procedure uses a meticulous surgical technique and a deliberate two-stage, hollow, cylindrical titanium implant concept to produce what is termed osseointegration. Details of this
technique were given by Bränemark (1983). Possible reasons for the nature of the osseo-integration have been offered by Gould et al (1981), Hansson et al (1983), and Skalak (1983) although the exact explanation for osseointegration is still not clear it may be a paradigm of the careful, atraumatic surgery, the metallic oxide properties of the implant and the planned avoidance of implant loading (McCord 1986).

Haraldson & Carlsson (1982) have indicated that masticatory function of this form of implant is usually superior not only to complete denture function but, by inference, to the performance of patients with other implants as measured by de Hernandez & Bodine (1969).

The technique of osseointegration would appear to be the most promising of all of the metal implants as this is the only technique not to result in fibrosis. It substantiates the hypothesis of Southam & Selwyn (1971) who attributed the failure of metal implants largely to the effects of careless treatment of bone.

5.3 Tissue: Polymer Implant Interface

A fundamental concept of relevance to the differences between the reactions of the tissues to metals and to polymers is that the metals used as implants can be found in the tissues as trace elements whereas the synthetic polymers must be considered intrinsically foreign materials (Autian 1981). In addition, many polymers have additives such as dyes, plasticisers or unused monomers which are all capable of inducing tissue reactions in their own right. Calnan (1963) pioneered the systematic
histological investigation of polymers used in soft tissue. He noticed that solid sheets of the polymeric implants were encapsulated by solid sheets of fibrous tissue while meshed materials were penetrated by fibrous tissue without encapsulation of the implant as a whole. P.M.M.A. was found to elicit the least response of the tested materials. The lower the molecular weight of the polymer, the more toxic it tended to be. The same was said of any residual monomer (Kapsimalis 1960; Williams 1981).

Boucher (1964) and Boucher & Surwillo (1968) investigated the effects of Dacron, Ivalon, Nylon and superpolyamides in the alveolar tissues of the rabbit. The superpolyamide produced minimal reaction whereas the others elicited considerable inflammatory response. Polyvinyl sponge was used in restoring bony defects in dogs and it was considered that, in small defects, the sponge appeared to impair bone regeneration whilst in larger defects it provided unsuitable scaffolding (Freidenberg & Lawrence 1959).

Charnley & Crawford (1968) examined the histology of bone in contact with self-curing acrylic cement in a review of 13 post-mortem examinations of patients with hip-prostheses. A general finding was that the cement was surrounded by fibrous tissue and that in some regions lacunae were devoid of osteocytes indicating cell death. A similar conclusion was reached by Laing (1973) when he implanted plugs of P.M.M.A. into the paravertebral muscles of rabbits. Histological sections revealed that, three days after implantation, the cells adjacent to the implants were necrotic. Between one and two weeks after implantation, the cells were being removed by macrophages and a fibrous capsule was being
formed around the implant. The cells adjacent to the implant reacted less strongly and the only change seen, with time, was a thickening of the fibrous membrane. This thickening was attributed to the release of residual monomer.

Turner et al (1973), however, did not find a facile direct relationship between the thickness of the fibrous capsule and the extent of tissue reaction to polymers. These workers stated that when inert controls were implanted the inflammatory infiltrate was dominated by neutrophil leucocytes and some necrotic tissue was removed by macrophages and giant cells after about four days. After one week, fibrous tissue encapsulation was seen around the specimens evoking the least inflammatory reaction. Moreover as the degree of inflammation increased, fibrosis tended to be delayed and, on occasion, samples with marked necrosis often failed to form a fibrous capsule. After the acute inflammation had subsided, the number of lymphocytes and, to a lesser extent, the number of plasma cells increased until the process of repair and regeneration dominated (Nedelman 1968; Turner et al 1973).

Polymers used as dental implants include P.M.M.A., dimethyl siloxane and polytetrafluoroethylene. Tooth implants of P.M.M.A. were reported by some workers to produce gingivitis (Shklar et al 1966). Hodosh et al (1967) stated that the result of implanting P.M.M.A. replacement teeth was a peri-implant fibrous capsule which they likened to the periodontal membrane and they described this a pseudo-periodontal membrane. When a porous polymer-composite was tested as an implant material, consisting of a composite of P.M.M.A., an organic bone and a foaming agent (Dinitrosopentamethylene tetramine) a good penetration of tissue into the implants
was observed (Hodosh et al. 1969). A year previously these workers implanted P.M.M.A.-coated vitallium pins in baboons for a six month period (Hodosh et al. 1968). They claimed good adaption to bone on the basis of radiographic findings and argued that as they were unable to section the metal, soft tissue would have been lost or destroyed if the Vitallium pins were pulled out during the histological processing.

Hamner et al. (1970) were unable to concur with Hodosh. They implanted 36 P.M.M.A. implants into baboons. Fifteen were lost within 97 days and no histological evidence of attachment was found although apical downgrowth of epithelium between the fibrous tissue and the implants occurred. In addition, inflammatory cells were also present. Indeed, in five cases osteomyelitis was diagnosed. Similar findings were reported by Zarb et al. (1972) although Nathanson et al. (1978) claimed that ossified tissue was found within the large pores of his implants of P.M.M.A.

Comparative Studies of Polymers

Fitzpatrick (19681,2) compared tissue reaction of the rat to dimethyl siloxane, P.T.F.E., P.M.M.A. and vitallium and found that the last two had the thinnest fibrous capsules, that adjacent to the metal being thinner than the polymer. Harris & Lossin (1973) and Harris (1974) compared the reactions of canine tissue to silicone rubber, P.T.F.E. (in block and felt form) and bovine cartilage. Histological sections showed clearly that the silicone was separated from bone by a layer of dense fibrous tissue which was covered by several layers of flattened epithelial cells; no inflammation was reported. The felt form of P.T.F.E. was seen to
be surrounded by copious fibrous tissue, in which multinucleated giant cells were present. In contrast, no inflammatory reaction was reported in the block form and some bone had apparently grown into perforations in the block. These findings were also observed by Calnan (1970). Lam (1972) compared four implant alternatives in humans. These were root implants made of P.M.M.A., roots of extracted teeth which were re-implanted, plaster of Paris implants and cartilage from costo-chondral junctions. Out of a total of 12 subjects only six were usable and the time span was four years. No histology was presented and assessment was by clinical and radiographic evaluation. None of the implants caused the socket to be restored with new bone and Lam considered that the P.M.M.A. functioned as an acceptable scaffold.

In another comparative test, Matlaga et al (1976) compared six polymers, each of three basic shapes, circular, pentagonal and triangular. These samples were inserted into the gluteal muscle of the rat for two weeks and the tissues assessed microspectrophotometrically for cellular and phosphatase activity. The triangular forms elicited greatest enzyme activity and the circular ones least. It was concluded that the sharper, more acute angles created greater friction hence resulting in greater tissue response.

Perhaps Autian (1981) summed up the problem of tissue reaction to polymers best of all when he postulated that many metals are found naturally as trace elements in the tissues. This is plainly not so in the case of these synthetic polymers which, per force, must be considered as "foreign" by the tissues. In addition, most polymers contain other chemicals which are added to improve physical and/or chemical properties of the polymers. These additives may be
used as stabilisers, plasticisers, fillers or dyes and they are all capable of leaching. In consequence, acute or chronic inflammation may result after the implantation of polymers containing additives.

5.4 Tissue:Ceramic Interface

Introduction

Ceramics would appear to have two attractive features as implant materials: (a) they are reasonably inert materials and (b) they tend to have high compressive strengths. Smith (1963) outlined these desirable qualities of ceramics but also remarked on their brittle nature. He attempted to overcome this by manufacturing Cerosium - a composite of alumina, silica, calcium and magnesium carbonates embedded in a close-bonding hydroxyaliphatic epoxy resin. He claimed favourable age and abrasion resistance following their implantation in rabbits. Cook et al (1965) and Petersen et al (1969) claimed fusion between bone and these implants of Cerosium. Satisfactory tissue tolerance was also reported. Rhinelander et al (1971), however, placed implant discs of Cerosium in the tibiae of dogs and investigated the tissue responses to Cerosium and some metals, histologically, at between six weeks and six months. They reported greater vascularity around the discs of Cerosium than around discs of titanium. All metals became invested with a bony wall which was lined with fibrous tissue. They noted there was less tendency to form a bony wall with Cerosium and inflammation around the Cerosium discs was attributed to the resin leaking out of the surface to a depth of 50μ-70μ. Implants of Cerosium failed over a period of time because of biodegradation of the polymer and the polymer-ceramic bond
Hench (1981) has classified ceramics according to their biological actions and he suggested three groups, (i) nearly inert, (ii) those with controlled surface activity and (iii) totally resorbable.

i) Nearly inert ceramics

Although several materials in this group have been investigated, only alumina ($\text{Al}_2\text{O}_3$) and its calcium salt has been studied intensively and used in clinical situations. Klawitter (1970) and Hulbert et al (1970) have demonstrated that implants of calcium aluminate when placed in canine femora gave strong histological evidence of being compatible with bone.

In a classic experiment, Klawitter (1970) demonstrated that bone did not resorb away from the alumina implant. This led him to consider bony ingrowth into porous implants. Using a resin-embedding technique, he matched microradiographic techniques with routine histological techniques to relate the type of tissue ingrowth into increasing pore sizes. His findings can be summarised as follows:-

1. No fibrous tissue entered the pores when their diameters were less than 15µ.
2. Fibrous tissue only was found in pores in the range 15µ-45µ.
3. Osteoid tissue was found when the pores had a diameter of 45µ-95µ but no mineralised tissue was present.
4. Mineralised tissue was only found in pores greater than 100µ.

Porous calcium aluminate tooth shaped implants were implanted
into tooth sockets. Although radicular portions remained stable, coronal fracture of the implant resulted and an extension to this study using root implants only resulted in stable, non-mobile implants although no histological or radiograph evidence was supplied (Young 1972). Hamner et al (1972) and Hamner & Reed (1972, 1973) placed calcium aluminate root forms into extraction sockets of baboons. These porous implants had pore sizes ranging from 50μ to 200μ. Evaluation revealed no mobility of the implants and histological results showed an absence of the marked chronic inflammatory tissue associated with implants of P.M.M.A. Their findings with regard to porosity were in accordance with Klawitter (1970). Bhaskar et al (1971) recorded similar results when they inserted phosphate-bonded alumina implants into rats, miniature pigs and monkeys.

Calcium aluminate has been recommended for ridge augmentation and Topazian et al (1971, 1972) and Hamner et al (1973, 1974) have reported the success of this material in dogs and humans. In an attempt to increase the strength of porous alumina Driskell et al (1973) coated tantalum substrates with a glass enamel onto which was laid a porous phosphate-bonded alumina. Driskell et al (1973) and Dorre & Dawihl (1980) have reported favourably on the use of high density alumina as did Hobkirk (1975, 1977). Hobkirk (1975) summed up the finding of these workers when he stated that bone was deposited near to the implants not in intimate contact to it. Atkinson et al (1984) thought that bone formations often occurred in the fibrous tissue between implant and bone.

Busing et al (1983), however, investigated some high density alumina "explants" (four implants which had to be extracted as a
result of accidents. Light and electron microscopic studies gave strong indications of direct contact between the alumina implants and the adjacent bone. Hentrich et al (1971) implanted specimens of alumina, zirconium oxide and calcium aluminate into the femora of monkeys. They were removed after 50 days and the animals sacrificed when it was found that in the defect filled with calcium aluminate, substantially more ingrowth of bone had taken place than in the other two. In addition, the density of the aluminate decreased with time, indicating that it was dissolving in vivo. Schnittgrund et al (1973) used a resin-embedding technique to study tissue reactions to dense alumina implants in baboons. They used high magnification and reported extremely dense and well organised bone on the portion of the implant. At one point the picture was almost one of ankylosis although they did not claim a chemical bonding. This would appear to confirm the views of Williams (1981^) that the dense alumina implant had a very thin hydration layer (less than 50 Angstroms thick) of aluminium hydroxide formed on exposure to tissue fluids and this he considered negligible. The findings of Kawahara et al (1980) and Akagawa et al (1985) with regard to single crystal alumina implants would appear to confirm this.

The isotopes of carbon may also be considered as nearly inert. When non-functional vitreous carbon tooth root forms were implanted into canine mandibles for over 180 days, there was a complete lack of inflammation in the peri-implant tissues and a layer of fibrous tissue enveloped the implant, although bone tended to adapt to the shape of the implant (Grenoble & Kim 1973). Stallard et al (1974) reported similar findings on Rhesus monkeys. Hobkirk (1975^) gave the most comprehensive histological account of the tissue: vitreous
carbon reactions. He implanted vitreous carbon rods into the iliac crest and mandible of rabbits and reported on their histological effects. After three weeks there was no evidence of any acute or chronic inflammatory processes. Lamellar bone was said to have been deposited in close proximity to the vitreous carbon and a thin fibrous layer was reported, in places, between implant and bone. Some giant cells were observed in the sections but it was suggested that these were involved in post-surgical repair. More peri-implant fibrous tissue was laid down in the mandible than in the iliac crest. Hobkirk's research (1975\textsuperscript{1,2}) involved comparing vitreous carbon rods to rods of high density alumina and more fibrous tissue resulted adjacent to the alumina implant than to vitreous carbon, demonstrating the biocompatibility of carbon. Hobkirk (1975\textsuperscript{1,2}) also presented histological evidence of, in places, direct bone to carbon contact. Kent & Bokros (1980) reported similar findings when blade-type implants were inserted into baboons, although they did mention they found some areas of mild gingival inflammation but this inflammation was present also in natural teeth.

An additional finding of these authors was that implant design was important; implants which were flared in the region of the alveolar crest resulted in less alveolar bone loss than more rounded forms. Less alveolar bone was lost when the bone:implant angle was acute (Hobkirk 1981). Hobkirk (1982) evaluated bony response of rabbits to 96 carbon fibre reinforced carbon implants over a period of 18 months and reported, again, that new bone was deposited with lamellae parallel to the implant with some direct apposition of bone on the implant. Schnitman & Schulman (1980)
however presented a five year report of vitreous carbon implants in baboons and their findings indicated that despite their early promise, clinical results with fibrous carbon tooth roots were not ideal. They could not be recommended as free-standing implants and high morbidity ruled out their use in anterior locations.

ii) Surface-active ceramics

Beckham *et al* (1971) implanted a non-porous glass ceramic into rat femora. In their opinion the ceramic:bone interface could not be evaluated under light microscopy as the implant had to be removed before sectioning; no adverse cellular responses were seen in the peri-implant tissues. When some sections were examined under the scanning electron microscope, however, there was no appreciable gap noticeable at the junction of bone and glass ceramics. Similar results were recorded by Hench *et al* (1971) although Davis *et al* (1972) highlighted the disadvantages of certain glasses which could corrode especially those containing lead and borosilica. Greenlee *et al* (1972) determined that implants with a calcium:phosphate ratio of 5:1 and which contained 45% silica gave good bone-implant fixation although the addition of fluoride to the glasses was found to decrease the surface reactivity of the glass ceramics hence preventing bone-implant fixation.

One theoretical basis for implant materials selection was that an ideal implant should have a dynamic surface chemistry that would induce histological changes at the implant interface which would normally occur if the implant were not present (*Clark et al* 1976). Their theory was put into practice and explained histologically by Hench *et al* (1977) who studied bioglass fixation of hip prosthesis
in rats. When implants had a phosphate composition of 2-6%, it was noticed that osteoblasts differentiated near the implant surface. These osteoblasts produced collagen fibres and mucopolysaccharides which became incorporated in the inorganic gel layer on the surface of the implant. This bonding zone on the implant surface was formed after 10 days and by 30 days 100% of the implants were stated to have been bonded. In this gel layer, mineralisation occurred between three and six weeks; concurrently the mineralisation took place in the newer bone in the vicinity of the implant. These two mineralising zones united giving an ankylosis between bone and implant.

When bioglass implants were inserted in subperiostele tunnels, to assess ridge augmentation in pigs, true bony fixation only occurred if the implants were fixed in place (Sunte et al 1977). Gross et al (1981) examined, in detail, the ultrastructure of the bone-glass ceramic interface. They implanted a glass ceramic of low alkali content into rat femora. The early surface activity or degradation of the glasses resulted in the presence of macrophages and multi-nucleated cells at the soft-tissue interface. The authors were unable to give any reasons for the cessation of the surface activity or corrosion but they observed that bone bonded to the glass via an organic matrix composed of collagen and mucopolysaccharide.

Unlike Hench et al (1977), Gross et al (1981) were unable to detect the fusion of the organic matrix to the gel-like surface of the implant. What they observed was the deposition of a granular material inside and outside macrophages which they hypothesised might form a barrier to the products of dissolution of the implant.
Another finding was the deposition of a material of unknown composition which may have been mineralised. The mechanisms of mineralisation were unclear to Gross and his colleagues although light microscopic examinations revealed bone connections to the implant on up to 95% of its surface. Unfortunately no time scales were supplied to indicate the chronology of these events (Gross et al 1981). Although the histo-biochemical mechanisms of the surface active glass ceramics are still unclear, histological evidence of integration with host bone tissue was clearly demonstrated by Hench et al (1977) and Gross et al (1981). An extension to the surface active concept has been the coating of, for example, metallic implants with either alumina, carbon or a glass-ceramic. Another alternative is the use of porous implants. Berman (1955) coated vitallium subperiosteal implants with polyvinyl alcohol sponge before implanting them in the jaws of monkeys and reported no irritation or inflammation present in the monkeys but supplied no histological evidence to substantiate his findings. He reported, however, that two out of four patients had a foreign body reaction to the polyvinyl alcohol. Lueck et al (1969) extended the porous sponge concept to an open pore titanium implant system. The titanium was sintered in a furnace at 2,000°F for two hours and fashioned into cylindrical forms of 45%, 50% and 65% porosity which were implanted in rabbits. After three weeks, bone had grown into the pores, the greatest penetration found in these of 45% porosity. When porous titanium implants were placed in femora of sheep, high bond strength, indicative of bone binding, resulted (Hahn & Palich 1970). Porous titanium with a pore size of more than 200μ also produced excellent bony ingrowth (Hirshorn et al 1971).
Klawitter (1970) determined the effect of pore size on tissue ingrowth. Porous aluminate cylinders with pore sizes less than 50\(\mu\) implanted in the femora of dogs contained only fibrous tissue whereas pores between 50\(\mu\) and 100\(\mu\) would contain unmineralised osteoid-like material and mineralisation occurred only in those pores with more than 100\(\mu\). This was confirmed by Karagianes et al. (1976).

When excessive load bearing is not a primary requirement, porous ceramics can provide a mechanism for attachment of a material to the system (Klawitter & Hulbert 1972). Hulbert and his co-workers (1972) reasoned that the principal advantage of a porous alumina implant was this inert ceramic would be unlikely to cause later toxicological problems. A second advantage of porous ceramics was that the mechanical strength of the convoluted interface increased when bone grew into the pores of the ceramic. The advantages of porous implants are:

- a) The pores result in a much greater area of mechanical integration between implant and tissue over which more positive and uniform interlocking may occur.
- b) The presence of pores suggests, in theory, improved tissue vascularity within the porous implant.
- c) More rapid fixation of the implant should be obtained through the mechanisations of (a) and (b).

(Homsy et al. 1972; Clemow et al. 1981; Bobyn et al. 1982).

In a study determining the kinetics of canine femur bone ingrowth into cylindrical channels of dense alumina and titanium, Predecki et al. (1972) varied pore sizes from 95\(\mu\) to 1,000\(\mu\). They found that in the larger pore sizes (500\(\mu\) - 1000\(\mu\)) rapid ingrowth
to depths of over 2,000μ occurred by four weeks although the larger channels had a smaller cross-sectional fraction of the defect filled with bone than the pores in the 200μ-500μ range. Klawitter et al (1976), however, reported conflicting results, namely that larger sizes do not result in a faster bone growth rate. It has to be noted, however, that in this experiment, porous polyethylene was the material under test.

A basic fact worthy of mention is that the prime requirement for bone ingrowth into porous materials is the development of an adequate vascular system. This can take several weeks because of the time required for the establishment of viable haversian systems which take about six weeks in man (Simmons 1980). The average cross section of an haversian system is of the order of 300μ hence an indication of the optimal size of the porous system is possible.

A theoretical disadvantage of a porous system is that, having potential dead spaces, this form of implant is vulnerable to bacteriological or microbiological infection (Elek & Conen 1957; Merritt et al 1979). Merritt et al (1979) compared porous implants to dense implants. In a controlled study they infected 40 test animals with coagulase positive Staphylococcus aureas on the implant site, under acute and chronic conditions. The acute animal models were infected at the time of insertion of implant and the chronic animal models were infected one month post-insertion. Where bacteria were present at the time of implantation, the incidence of infection was found to be greater in porous implants than in the dense forms. If, however, host tissue had grown into the porous structure before the bacteria were introduced, then the incidence of infection of the porous implants was less than the dense
implant. The clinical implications of this are clear, no implant should be placed on an area of infection.

iii) Totally resorbable ceramics - tissue reactions

The ceramics of calcium phosphate combine both surface reactivity and porous surfaces to varying degrees. The porous, also resorbable, tricalcium phosphates and hydroxyapatite could be said to exhibit both surface reactivity around or inside their pores whereas dense, almost non-resorbable, hydroxyapatite exhibits surface activity only. Jarcho (1981) reviewed bio-resorbability and agreed with de Groot (19831), Klein et al (19831,2) and Misiek et al (1984) that chemistry and material structure play key roles in the resorbability of the implant.

de Groot (19831) suggested that micropores (frequently found at the grain boundaries of individual crystals) determined the rate of bio-resorbability and that macroporosity determined the vascularity and hence ingrowth of new bone, although the process may be multifactoral (Ducheyne & de Groot 1981). Jarcho (1981) stated that the attractiveness of calcium phosphate implants was that they elicited no local or systemic toxicity. He also stated that there was an absence of inflammatory or foreign body response and that no fibrous tissue was noticed between bone and calcium phosphate implants.

Tricalcium Phosphate

Bhaskar et al (19712) implanted pellets of porous tricalcium phosphate into the tibias of 66 rats and reported on the tissue
responses under light and electron microscopy. After three days the outline of the ceramic was observed clearly. Connective tissue had extended into some of the pores of the ceramic and it was reported that no inflammatory cells were present. One week after insertion the implants were adjudged to be smaller and new bone formation was described as extensive. In some areas this new bone was deposited directly onto the ceramic whereas in others a thin cellular layer intervened between the new bone and the ceramic. It was postulated that the replacement of tricalcium phosphate by new bone was by ingrowth and arching back of trabeculae. After two weeks, connective tissue ingrowth had reached the centre of the implants. Indeed, some central pores contained bone. Some peripheral areas of the ceramic were reported to have been replaced by bone. This bony replacement peaked at four weeks where bone and connective tissue had replaced most of the implant. Six and eight week sections showed essentially similar histology except that the bony trabeculae became lamellated and thicker.

Electron microscopic examination showed that, after two weeks, the principal activity was resorption of the ceramic and some electron dense material (presumed to be ceramic) was observed in some cells. After five weeks the ceramic pores were observed to be completely occluded by new bone which was seen deep within the implant and was directly apposed to ceramic.

When tricalcium phosphate chips were implanted into surgically created periodontal defects in six monkeys, osteoid material formed after three weeks at which time osteoblastic activity was also seen (Levin et al 1974). Inflammation was resolved by four weeks and also at this time, woven bone had filled the central areas of
control sites but this finding was not seen in the experimental sites until nine weeks. Flatley et al. (1983) implanted porous blocks of tricalcium phosphate and hydroxyapatite in the vertebral columns of 21 rabbits and analysed histological sections taken three, six, eight, 12 and 24 weeks later and the findings compared to microradiographs of the implant sites. After three weeks, no inflammatory reactions were seen and osteoblastic differentiation of cells was the dominant cellular activity. Microradiographs revealed minimal calcification at the periphery of the implant. This calcification had increased greatly by six weeks although the bone within the pores was predominantly woven bone. Between eight and 12 weeks the formation of osteoid was noticed. By 24 weeks, the implant space was bridged by radiographically dense bony tissue which completely incorporated the ceramic block. Controls failed to show evidence of bony fusion.

Hydroxyapatite

Hydroxyapatite, in the porous form, behaves in a similar manner to tricalcium phosphate although the dense hydroxyapatite with no macroporosities and few microporosities is barely resorbable (de Groot 1981). Jarcho et al. (1976) described the definitive histological report on the tissue interface of dense hydroxyapatite. Three holes were created in the femora of each of six dogs. The holes were filled at random by two types of hydroxyapatite (particulate and solid) and one site was left unfilled to serve as a control. The particulate implant consisted of granules, whilst the plug form was a sintered cylinder. Evaluation at one month and six months was by light, scanning
electron and transmission electron microscopy in addition to electron probe analysis.

After one month, all sites exhibited increased endosteal bone growth especially the implant sites. By six months the plug and particulate implants were completely invested by bone. In some areas haaversian canals were seen directly on the ceramic surface. The histological and microprobe results indicated excellent tolerance of the implants by the bone. This is in contrast to the 50μ seam reported around calcium aluminates by Klawitter & Hulbert (1972). The particulate form of hydroxyapatite caused a more rapid proliferation of osteoblasts than the plug form and this resulted in a greater rate of healing of the particulate form and would suggest that the particulate form would give more rapid stabilisation.

The fact that strong bonding of the bone to hydroxyapatite results from non-porous implants which, therefore, permit no ingrowth would suggest a chemical surface-reactive nature of the interaction (Denissen et al 1980). These authors implanted dense hydroxyapatite into the tibiae of three rats. The implants, at time of insertion, had no close contact with the walls of the cavity. In this report, no mention was made of controls. Six months after implantation, the surface of implants was almost completely covered by bone and it was estimated that 10-20% of the implants were still in contact with a thin capsule. Similar studies of blocks of implants in canine mandibles indicated that, radiographically, no radiolucencies were present between hydroxyapatite implants and bone.
Froum et al (1982) were unable to demonstrate osteogenesis in hydroxyapatite periodontal implants in four medically compromised patients. These patients who had severe periodontal osseous lesions had routine periodontal treatment prior to implantation. Between eight and 18 months after implantation the teeth concerned were removed in block sections. Although the results were described as being acceptable, clinically, no new bone was formed. These findings were not endorsed by Rabalais et al (1981) whose clinical radiographs indicated that osseous repair had taken place. Although tooth mobility was not mentioned in their report, it is perhaps the mobility of the teeth in the patients studied by Froum (1982) which contributed to the lack of new bone growth.

Drobeck et al (1984) and Misiek et al (1984) have observed soft tissue responses to varying shapes of hydroxyapatite and although all forms were well tolerated, inflammation resolved quicker when round implant shapes were used. Piecuch (1982) implanted coralline hydroxyapatite subcutaneously in dogs and reported favourable tissue reaction. Bone was not found, indicating that the hydroxyapatite is not osteo-inductive. Jarcho (1981) considered that hydroxyapatite should be described as either osteoconductive or osteophilic. Ducheyne et al (1980) reported on bone binding to implants of stainless steel coated with hydroxyapatite and Denissen et al (1983) considered that metal implants should not be coated with resorbable materials as, after resorption of the coating, a fibrous interface would be the final outcome. Non-resorbable hydroxyapatite appeared to be an attractive alternative to load bearing skeletal implants where the mechanical advantages of the metal are coupled to the excellent
bone-bonding property of the hydroxyapatite. The metal-hydroxyapatite junction is, however, the main weakness of this implant type.

The type of bond which exists between an implant and bone is decisive for the long-term biofunction of the implant (Osborn & Newesely 1979). These authors also stated that metals and plastics became incorporated in bone in accordance with contact osteogenesis while the bioactive implants were incorporated according to the pattern of bond osteogenesis.

6. Tumour Induction by Implant Materials

The purpose of this section is to review the results on studies on oncogenic effects of implants in animals and humans.

6.1 Animal studies

Autian (1981) and Pedley et al (1981) have reviewed this important features of adverse tissue reaction to implants. Turner (1941) was the first researcher to attribute tumour formation to an implanted material. He implanted bakelite discs subcutaneously in female rats and found that after 20 months almost 50% of the experimental animals had developed sarcomas adjacent to the discs. Oppenheimer et al (1955) implanted films of polymers subcutaneously in rats. They reported that 35% of the animals which had cellulose film implants developed sarcomas adjacent to the polymer films after a latent period of one to two years. It was the opinion of those workers that the tumour developed in the fibrous capsule adjacent to the implant. Hueper (1960) in a similar experiment observed that polyurethane foam induced carcinomatous changes after
12-18 months. Shulman et al (1963) compared implants of polyethylene in films and mesh forms. They implanted discs of each form into the abdomen and concluded that the films of polyethylene were more likely to produce tumours than the solid forms. They agreed with the theory proposed by Oppenheimer et al (1955) that the films of material blocked the interchange of body fluids and brought about sarcomatous changes adjacent to the implants as a consequence of altered ionic exchange. Brand et al (1967) extended this research and implanted tissues (initially adherent to the films of polymer) into mice. Nine months later, sarcomas had developed suggesting that tumour induction originated not from the fibrous capsule but from altered cells themselves.

Heath et al (1971) injected wear particles of cobalt-chromium molybdenum alloys into spinal muscles of 80 female rats. They reported that in 22 of the 80 rats sarcomas had been induced adjacent to the implants after a latent period of between 16 and 72 weeks. This finding was not endorsed by Meachim et al (1982) who placed dry particles of chromium alloys into the paraspinal muscles of two strains of rats and one strain of guinea pigs. In all, 210 animals were studied, including 50 used as controls. No malignant neoplasms developed. They stated that Heath et al (1971) had particle sizes ranging from 0.1µ-5µ whereas they had used particles ranging in size from 0.5µ-50µ. Although no neoplasia was induced in their experiments, Meachim et al (1982) accepted that some particulate metals, for example nickel, could produce tumours in rats. Not all of these tumours are reproducible; some tumours are specific to implant site, in specific species. Smaller animals such as some strains of mice and
rats may show adverse reactions to implants not recorded in larger animals although in the smaller animals, the latent period for tissue transformation is shorter (Autian 1981; Pedley et al 1981).

6.2 Tumour formation in human implant sites

In contrast to tumour formation associated with animal implant studies, actual clinical evidence that a human implant has led to a tumour has not been substantiated. Autian (1981) and Pedley et al (1981) listed only five clinical cases where a neoplasm was noted adjacent to an implant. Thompson & Entin (1969) and Burns et al (1972) reported sarcomas arising adjacent to two implanted polymers while McDougall (1956), Delgado (1958) and Dube & Fisher (1972) reported neoplastic changes adjacent to metallic bone plates and screws in single cases. As the implants have not been proven to be direct causal agents of the neoplasia and given the many thousands of human implants used, it would appear that screening methods for implants have been satisfactory. One must, however, consider latent periods of tumour induction in man and further long term studies would appear to be indicated.

No neoplasms have been reported adjacent to dental implants.

7. Summary

The review of literature in the preceding pages adequately demonstrates the plethora of implant materials and forms which have been utilised in dentistry. Included in the literature review are accounts, often confusing and anecdotal, of animal and human evaluations of the suitability of implant materials or implant forms.
Metals, because of their intrinsic strength, have the physical properties required of supportive implants. Strength alone, however, is insufficient to guarantee the success of an implant and a general finding is that the reaction of tissues to metals results in an intervening fibrous layer between the metallic implant and bone. Although this fibrous layer is used to retain subperiosteal implants, it serves no useful physiological or mechanical purpose where endosseous implants are concerned, as infection is often a consequence. The long-term clinical success of osseointegration, using titanium endosseous implants, clearly shows, for the first time, that metallic endosseous implants can be used to perform oral function with reliability: a drawback, however, is that this system is extremely expensive.

In contrast to metals, polymeric materials lack physical strength. A second disadvantage of polymeric implants is that they, too, elicit a fibrous response from host tissues. Because of these disadvantages, polymeric materials are no longer used as functioning dental implants.

Ceramic materials, like polymeric materials, do not have the desirable physical strength properties of metals. However, their excellent biocompatibility makes them the materials of choice for implants not subjected to flexural forces. Two bioceramics of calcium phosphate, tricalcium phosphate and hydroxyapatite, are both present in normal bone and both of these materials have been used successfully in ridge augmentation and in the treatment of periodontal bony lesions.

The purpose of this study was to investigate the effects of tricalcium phosphate and hydroxyapatite on the strength of bone.
Cryosurgery may be defined as the deliberate destruction of diseased tissue by cold in a controlled manner (Shepherd & Dawber 1982). The purpose of this chapter is to outline a brief review of the mechanisms of cryosurgery, to review its applications to surgery, particularly its dental applications, and to justify the use of cryosurgery in this research.

The effects of freezing on the body tissues have been known since the 18th century. Three separate stages were observed; first of all there was vascular stasis, then tissue necrosis occurred and, finally, excellent healing ensued (Hunter 1772). Bracco (1980) and Shepherd & Dawber (1982) have reviewed the historical and scientific development of cryosurgery from the time of Hunter to the present day. According to Whittaker (1984) modern cryosurgical instruments are of three basic types. The first type achieves freezing via evaporation of a liquid. The second type uses a thermoelectric effect and the third utilises adiabatic expansion of a compressed gas; this third type is most commonly used in dentistry at present.

The applications of extreme cold to tissues results in a cryolesion. In the creation of a cryolesion it would appear that tissue is destroyed as a result of the formation of ice crystals which may form intracellularly and/or extracellularly. These ice crystals would appear to damage the cells by either direct
physical damage of the cell and its constituents, or by disturbing the biochemistry of the cell (Fraser & Gill 1967; Le Pivert 1980; Whittaker 1984). Microvascular arrest resulting in tissue ischaemia is also considered a contributory factor to the tissue destruction (Kuylenschierna et al 1980). According to Fraser & Gill (1967), cell death in in vitro experiments could be attributed to direct physical or biochemical trauma. In animal studies, however, Fraser & Gill (1967) were of the opinion that vascular stasis was ultimately responsible for all destruction in the cryolesion. Another finding was that the cell-lethal temperature was -12°C. Whittaker (1984) was in general agreement with these findings but considered that the effects of ischaemia took effect an hour or so after the initial cell damage.

Cryosurgery has been used in Oral Medicine and Oral Surgery in the treatment of soft tissue lesions for over a decade and Barnard et al (1978) and Barnard (1980) described its use in the treatment of facial pain not of herpetic origin. Cryosurgery, in addition, might provide a means of treating lesions in sites which would be otherwise difficult to treat, such as haemangiomas of lip and bone (Barnard 1980). Al-Drouby (1983) discussed the effectiveness of cryosurgery in the treatment of potentially pre-malignant conditions of the floor of the mouth such as leucoplakia.

Cryosurgery of bone has lately received much attention, especially in the treatment of malignant tumours. Gage et al (1966) carried out an in vivo experiment in adult dogs to determine how bone was destroyed and ultimately repaired in the femora of dogs. They coiled latex rubber tubing around the femoral shaft and periosteum in dogs and liquid nitrogen (boiling point -196°C) was
passed through the tube after the soft tissues had been raised. The femora were removed at varying intervals after freezing. For some animals, the freezing level was varied. It was observed that the length of freezing made no significant histological difference. Gage and his co-workers noted that bony cell death was produced at any temperature below 0°C.

Emmings et al (1966) carried out similar experiments in canine mandibles and concluded that if cryosurgery was used in the treatment of bony tumours, all living cells would be destroyed yet the bony matrix would be retained to act as a scaffolding in the rebuilding of the mandible, hence avoiding unsightly disfiguration resulting from conventional surgery. Emmings et al (1971) described a case report on the effect of curettage and cryosurgery on a recurrent mandibular ameloblastoma. They mentioned that, as a result of thinness of the mandible, fracture occurred in the third post-operative month.

There are three distinct histological phases following cryosurgery of bone (Bradley 1986):-

1. Necrotic Phase: This is observed histologically after a few days. In mandibular bone subjected to a cell-lethal temperature (which Bradley considered to be -15°C), the following changes were reported:-

   a) Loss of osteocytes, the empty lacunae often being enlarged.

   b) Necrosis of endosteal tissue, periodontal membrane and dental soft tissue.

   c) Within the neurovascular bundle, thrombosis of the artery and loss of cellular elements.
2. Osteogenic Phase: This took place several weeks after the cryolesion was produced. New subperiosteal woven bone was formed at the margins of the lesion.

3. Remodelling Phase: This takes place over several months.

Kuylenstierna et al (1980², 1981) have described the healing of rabbit mandibles following cryosurgery and confirm Bradley’s findings. Kuylenstierna & Lundquist (1984) were also in accordance with Bradley (1986) when they stated that for complete destruction of bone, a temperature of -50°C is required.

Although it is outwith the scope of this thesis to review the treatment, by cryosurgery, for bony tumours, it is interesting to note the findings of Emmings et al (1971) that mandibular fracture occurred after cryosurgery. Gage et al (1966) also reported bony fracture after cryosurgery and Marcove et al (1978) reported fractures in 28% of patients who had cryosurgery for giant cell lesions. In order to examine the effect of cryosurgery on bone strength, Fisher et al (1977) induced a cryolesion in the left mandibles of rats. Following sacrifice (at two, four, eight, 16 and 26 weeks) the left and right mandibles were dissected from the skull and subjected to a three-point bending test to determine bone fracture strength. It was found that between the second and third post-treatment months, the strength of the left mandible was 70% that of the right (control) mandible, which is in accordance with the findings of Gage et al (1966) and Emmings et al (1971).

Cryosurgery can thus be used to provide a reliable means of weakening bone. The purpose of the research reported in the following chapters is to evaluate the effects of two biomaterials
on the fracture strength of bone. With the knowledge that a bone may be weakened following cryosurgery, it was decided to evaluate the test materials on weakened and unweakened bone, to determine if these materials would be of value to standard oral surgery procedures.
CHAPTER 3

AIMS OF THE PRESENT STUDY

The review of the literature indicates that although bioceramics of calcium phosphate are biocompatible, certain properties have not been elucidated.

The objectives of this investigation are therefore to:-

1. Develop an experimental model to assess the effects of both dense hydroxyapatite and tricalcium phosphate on bone strength.
2. Compare the relative effects of the two implant materials on the strength of weakened and unweakened bone.
3. Compare, histologically, the effects of both implant materials on weakened and unweakened bone.
4. Relate these findings to clinical aspects of dentistry.
CHAPTER 4

MATERIALS AND METHODS

Introduction

The object of this research project was to assess the effect of two bioceramics on weakened and unweakened bone. The materials used were dense, sintered hydroxyapatite\(^1\) and sintered 3\textsuperscript{rd} tricalcium phosphate\(^2\). The former is non-porous and is essentially non-resorbable while the latter is porous and resorbable (de Groot 1983\(^1\)). Although studies by Levin et al. (1975), Jarcho et al. (1976) and Jarcho (1981) showed that both materials bind well to bone, the effects of these materials on bone strength have, so far, not been reported. Both materials have been used to augment alveolar ridges but it would appear that no comparability studies have been carried out to demonstrate any similarity or contrast of these materials on bone or in the strength of bone after the implantation of these materials. Thorough screening tests on implants, before their use in humans should include tissue culture tests (to assess biocompatibility and toxicity of materials) and, if these tests prove favourable, experimental testing in an animal model (Autian 1971; Coleman et al. 1974). Tissue culture has been shown to be a reliable means of evaluating the biocompatibility of dental materials (Kawahara et al. 1968).

The bioceramics of calcium phosphate have been studied extensively by Jarcho et al. (1976), Jarcho (1981) and de Groot (1983\(^2\)) and yet the authors did not mention the use of tissue

---

1 Calcitec Inc., 4125-B Sorrento Valley Boulevard, San Diego, California 9212, U.S.A.

2 Johnson and Johnson, East Windsor, New Jersey, 08520, U.S.A.
culture tests on these materials. It was decided that this comparative study would combine in vivo experiments on an animal model, with in vitro experiments using tissue culture tests to confirm the biocompatibility and lack of toxicity of these bioceramics of calcium phosphate.

Cytotoxicity testing of biomaterials, is based on the addition of a test material to a healthy cell culture. If cells adjacent to the test material die, then the material may be considered toxic. If, however, the cells are seen to survive and to remain healthy in contact with the material, then that material can be considered biocompatible (Freshney 1983).

The use of experimental animals in biological and medical research has led to many of the medical and surgical advances of the last half century. Many animals have been used, ranging from small rodents such as mice to primates. Some advantages of the rodent are its availability and cost whilst disadvantages are its size and resultant operational difficulties (Murphy 1971). In a comprehensive appraisal, Frame (1984) analysed the range of animal models and suggested possible uses for each model; rats, rabbits, dogs, pigs and primates could all be used to evaluate implants in bone and soft tissue. Kavanagh et al (1985) described, in detail, their evaluation of titanium implants in rats and suggested that as rats, relative to larger animals, were inexpensive to purchase and maintain, it was possible to use a greater number of animals for study.

Bone may be weakened as a result of fracture or through pathological processes. Another means of weakening bone is by means of cryosurgery (Fisher et al 1977) and it was decided, in
this investigation to evaluate this one-step means of incorporating bone weakening with implant evaluation. Fisher et al (1977) investigated the bone weakening effect of cryosurgery on the mandibles of rats. They did this by inducing a cryolesion in the left mandibular ramus after it had been exposed. Measurement of the percentage differences in the fracture strength between the right mandibular ramus and the contralateral (experimental) side, enabled these researchers to quantify strength changes when these ratios were charted against time. Fracture strength of mandibular bone, following cryosurgery was seen to have decreased by 35% by eight weeks post-operatively.

The rat model was thus selected to investigate the comparative effects of β-tricalcium phosphate and hydroxyapatite on unweakened bone and on bone weakened after cryosurgery. This chapter describes cytotoxicity and biocompatibility testing, surgical procedures, strength measuring procedures and histological techniques.

1. Cytotoxicity and Biocompatibility Testing

In this investigation, pieces of gingivae, excised during the extraction of human wisdom teeth in the Department of Oral Surgery, Edinburgh Dental Hospital, were grown in explant culture. The tissue was chopped into fragments less than 1 mm³ in size and the explants were anchored in place in petri dishes with a sterile coverslip. The tissue was bathed in 10% Eagle's Minimum Essential

1 Lux, Labetch, 3220, Illinois, U.S.A.
Medium \(^1\) (x 10) supplemented with L-glutamine, 200 m Mol L\(^{-1}\), 1\% non-essential amino acids (x 100), sodium pyruvate 1 m Mol L\(^{-1}\), sodium bicarbonate 20 m Mol L\(^{-1}\), Hepes buffer 50 m Mol L\(^{-1}\), the antibiotics penicillin 100 U ml\(^{-1}\) streptomycin 100 \(\mu\)g ml\(^{-1}\) and fungizone 0.25 \(\mu\)g ml\(^{-1}\) plus human serum. The final serum concentration was 10\%. Cultures were incubated at 37°C in a humidified atmosphere of 95\% O\(_2\) and 5\% CO\(_2\).

Outgrowth of fibroblasts was monitored under a phase-contrast microscope (Leitz dialux\(^2\)). As the cells reached confluence, the cells were trypsinised (Bernstine et al. 1973) and, after thorough washing in phosphate-buffered saline, the cells were seeded onto Lux Permanox culture dishes, 60 mm x 15 mm, at a density of 2.5 \(\times 10^6\) cells/ml.

A few granules of either dense hydroxyapatite or \(\beta\) tricalcium phosphate were placed into each culture dish and the dishes incubated at 37°C in the same humidified atmospheric conditions mentioned previously. After a period of 10 days, the dishes were examined microscopically to assess toxicity and biocompatibility and the results photographed.

2. Animal Experimental Method

In this investigation, rat mandibular bone was selected as the donor site for the implants, as it is subjected to similar stresses and strains to human mandibular bone, for example flexion, compression and tension through mastication. The dense

---

\(^1\) Flow Labs Ltd., P.O. Box 17, Second Avenue, Irvine.

\(^2\) E. Leitz (Instruments) Ltd., 48 Park Street, Luton LU1 3HP.
hydroxyapatite and β tricalcium phosphate functioned as implants as, following placement on the exposed mandible, (technique to be described) they were immediately covered with periosteum which held the implants in position.

2.1 Experimental Design

Two hundred and thirty one male Sprague-Dawley rats were used in this experiment; each animal was obtained from the breeding stock, Faculty of Medicine Animal Area, University of Edinburgh. The weights of the animals ranged from 350 gm to 750 gm with a mean weight of 456.5 gm. The animals were assigned into seven distinct surgical groups (Table 4.1). In Group 1, which constituted the surgical control, the left mandibular ramus was exposed by elevation of the left masseter muscle and its periosteum. The wound was closed in layers and the animals sacrificed after two, eight and 26 weeks; seven animals were sacrificed in each group for each time interval.

Groups 2 and 3 differed from Group 1 only in that, in Group 2, 100 mg of dense granular hydroxyapatite were placed on the exposed ramus before wound closure whilst in Group 3, 100 mg of granular sintered β tricalcium phosphate were similarly inserted before wound closure. The sacrifice times for Groups 2 and 3 was the same as for Group 1. These three groups formed the study of both implant materials on unweakened bone.

The animals in Group 4 served as cryosurgical controls. A cryolesion was induced on each exposed left mandibular ramus, before wound closure. In order that a comprehensive evaluation of the effects of cryosurgery on mandibular bone could be achieved, the
**TABLE 4.1**

Plan of experimental procedure including a summary of surgical procedure(s) and sacrifice periods

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Summary of Experiment</th>
<th>Sacrifice Periods (in weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surgery only</td>
<td>2, 8, 26</td>
</tr>
<tr>
<td>2</td>
<td>Surgery + Hydroxyapatite (granules)</td>
<td>2, 8, 26</td>
</tr>
<tr>
<td>3</td>
<td>Surgery + Tricalcium Phosphate (granules)</td>
<td>2, 8, 26</td>
</tr>
<tr>
<td>4</td>
<td>Surgery + Cryolesion</td>
<td>2, 4, 6, 8, 10, 12, 26</td>
</tr>
<tr>
<td>5</td>
<td>Surgery + Cryolesion + Hydroxyapatite (granules)</td>
<td>2, 4, 6, 8, 10, 12, 26</td>
</tr>
<tr>
<td>6</td>
<td>Surgery + Cryolesion + Tricalcium Phosphate (granules)</td>
<td>2, 4, 6, 8, 10, 12, 26</td>
</tr>
<tr>
<td>7</td>
<td>Surgery + Cryolesion + Hydroxyapatite (block)</td>
<td>2, 8, 26</td>
</tr>
</tbody>
</table>
animals were sacrificed fortnightly between two and 12 weeks and also at 26 weeks after cryosurgery. In Group 5, 100 mg of dense, granular hydroxyapatite were placed over the cryolesion before wound closure. In Group 6, 100 mg of sintered \( \beta \) tricalcium phosphate were placed over the cryolesion before wound closure. The animals in Groups 5 and 6 were sacrificed in parallel to the time intervals selected for Group 4.

The animals in Group 7 had 100 mg of a sintered block of hydroxyapatite placed over the cryolesion before wound closure. In this group, animals were sacrificed after two, eight and 26 weeks and this group was used in conjunction with Group 5 to evaluate the effects of implant form on the fracture strength of mandibular bone. Groups 4, 5, 6 and 7 formed the study of both implant materials on mandibular bone.

All animal experimentations were performed in the Faculty of Medicine Animal Area, University of Edinburgh. In addition, all experiments were performed under Home Office Licence (No. E.D. 6034) in accordance with the Cruelty to Animals Act, 1976.

2.2 Surgical Technique

After weighing, the animals were anaesthetised with etorphine hydrochloride (Small Animal Immobilon\(^1\)). This anaesthetic is dangerous to man and its antedote diprenorphine hydrochloride (Revivon \(^1\)) should be drawn and prepared before using the Immobilon. The anaesthetic was administered intramuscularly into the left biceps femoris muscle. The dosage used was 0.10 ml

---

\(^1\) C-Vet Ltd., Minster House, Western Way, Bury St. Edmunds.
per 100 gm body weight of a 50/50 solution of Small Animal Immobilon and sterile water. Induction time of the anaesthetic took, on average, seven minutes, at which time the animal was shaved of fur over the left mandibular and submandibular regions. The operating site was sterilised with 70% isopropyl alcohol swabs (Sterek\textsuperscript{1}). A semilunar submandibular incision was used to expose the lateral surface of the left mandible and masseter muscle. All the investing fascia was removed by blunt dissection. In order that the masseter could be reflected clear of the mandible, it was necessary to ligate the left facial vessels at the anterior aspect of the masseter muscle. This was done using an atraumatic needle and a resorbable suture material, polyglycolic acid (Dexon 3.20\textsuperscript{2}). Two sutures were placed, superiorly and inferiorly, to the vessels and a horizontal incision made along the lower border of the mandible (Figure 1). The left masseter and its periosteum were then reflected to expose the entire mandibular ramus (Figure 2) in order to permit the placement, where required, of the implant materials described later. The wound was closed in layers using cutting needles and sterile catgut sutures (W520\textsuperscript{3}) and the animal injected, also in the left pectoris femoris muscle with an equivalent dose of 50% Revivon to 50% sterile water by volume. Recovery from the anaesthetic took, on average 5-7 minutes.

1 Schering-Prebbles Ltd., Liverpool L20 8NT.
2 Davis & Geck, Cyanamid of Great Britain Ltd., Gosport.
3 Ethicon Ltd., Bankhead Avenue, Sighthill Industrial Estate, Edinburgh.
Post-operatively, the animals were caged in groups of three and fed on water and standard mouse and rat diet. This technique was used alone in Group 1 (Table 4.1), which served as a surgical control group.

Animals in Group 2 and Group 3 had identical surgical procedures except that in Group 2, 100 mg of dense hydroxyapatite granules were placed in the lateral surface of the mandible (Figure 3) before wound closure in such a manner that the implant was sandwiched between the mandibular ramus and its periosteum. In Group 3 the implant material was sintered β tricalcium phosphate granules.

Group 4 comprised the group which served as a control for cryosurgery. When the lateral surface of the mandibular ramus had been fully exposed, a cryolesion was produced using a liquid nitrogen apparatus (Figure 4). The cryolesion was produced by placing a tonsillar probe (Figure 4) on the lateral surface of the mandible and an external thermocouple placed on the medial surface of the mandible in approximate juxtaposition to the tonsillar probe. The temperature of the external thermocouple was set for \(-16^\circ\text{C}\). The cell-lethal temperature for bone is \(-15^\circ\text{C}\) (Bradley 1986). The time from the iceball formation to the external thermocouple reaching \(-16^\circ\text{C}\) was, on average 1 min 45 secs. When the preset temperature was reached, the lesion was allowed to defrost naturally. Only one freeze was used. In 5% of cases the desired temperature was not reached.

1 Special Diet Services Ltd., 1 Stepfield, Witham, Essex.
2 Spembly Ltd., Newsbury Road, Andover, Hants.
Figure 3: Rectangular block of dense hydroxyapatite placed on implant 'site'.
Figure 4: Spembly DFS 30 cryosurgery apparatus. The tonsillar probe and external thermocouple are indicated.
In these animals the temperature only reached between -10°C and -16°C and a time limit of 2 min 30 secs was imposed on the cryosurgery, to prevent the possibility of respiratory distress to the animals. After defrosting, the thermocouple and probe were removed and the wound closed in layers.

Group 5 and Group 6 were identical to Group 4 except that 100 mg of dense hydroxyapatite granules were placed over the cryolesion prior to wound closure in Group 5 and 100 mg of sintered β tricalcium phosphate were added to the cryolesion of Group 6 animals.

Group 7 was identical to Groups 4, 5 and 6 except 100 mg blocks of dense hydroxyapatite were placed onto the cryolesion before wound closure.

The animals in Groups 1, 2 and 3 were sacrificed, two, eight and 26 weeks post-operatively, to assess the overall effect of the surgical technique on the strength of unweakened bone. Groups 4, 5 and 6 were sacrificed at fortnightly intervals up to 12 weeks and thereafter at 26 weeks. Group 7 animals were sacrificed after two, eight and 26 weeks as only a broad assessment of the effects of blocks of implants was required. In each group of seven animals, five were killed by desiccation (CO₂/O₂ mixture) and were used for fracture strength testing while two were sacrificed by the perfusion of 2.5% buffered gluteraldehyde (in 0.2 M phosphate buffer) into the left ventricle to ensure adequate histological sections.

Subsequent to this investigation, a preliminary appraisal of results indicated that an implant control group was justified in order that a complete appraisal of the effects of the two test
biomaterials on bone could be made. It was decided to include such a control group as a supplementary study. The implant control material was a sterilised, carbonised wood (Ethicon Ltd.). This material is biocompatible and chemically inert and therefore satisfies the criteria of a bland control material. A rectangular block of carbonised wood, weighing 100 mg was placed on the exposed left mandibular ramus of four adult male Sprague-Dawley rats. In two animals the control material was placed on bone previously subjected to cryosurgery; in the other two the implant control was placed on unweakened bone, again prior to wound closure. As this study was prompted for philosophical reasons, to be discussed in Chapter 8, it was decided to evaluate this study from a strictly histological perspective and the animals were sacrificed at two and eight weeks post-operatively.

3. Fracture Strength Measuring

Immediately after sacrifice, the mandibles of the animals killed by desiccation were removed from the skull, stripped of all soft tissue and separated into left and right mandibles. The mandibles were placed in Ringer's solution and were transferred immediately to be tested for their respective fracture strengths. The fracture strengths of left and right mandibles were compared within each group as well as between groups. The fracture strengths of each mandible were measured on an Howden servo-hydraulic universal testing machine¹ (Type U-M 5/2) (Figure 5a,b) adapted to use a three-point bending test. The universal testing machine used

¹ R D P Howden Ltd., Leamington Spa, Warks.
Figure 5a: Load cell of RDP Howden servo-hydraulic universal testing machine (Type U-M 5/2). The ascending ramp and the fixed point are indicated.
Figure 5b: Recording unit of R D P Howden universal testing machine. Electronic connections to other components are labelled.
consists of a load cell (Figure 5a), and a recording unit (Figure 5b). The recording unit was connected, electronically, to a linear graphic chart recorder\(^1\), Watanabe Servo-Corder, (Figure 6). In this test, an ascending ramp with two raised rods (to hold the test specimen) was raised at a cross head speed of 3 mm per minute towards the fixed rod. The fixed rod was positioned in such a way that it lay between the two raised points when the ramp was in the fully raised position.

Preliminary three-point bending tests resulted in inconsistent fracture sites and it was found that reliable fracture sites were obtained if a 2 mm piece of polyurethane foam supported the mandible on the rods of the ascending ramp (Fisher et al 1977). The fracture strengths of each mandible were fed through a computer which supplied a linear tracing for each test (Figure 7).

Before the 33 groups of rats were tested (Table 4.1), pilot studies on the fracture strengths of right and left mandibles from five male and five female rats were carried out. These animals, which were not operated on, served as non-surgical controls.

4. Preparation of Tissues for Histological Investigation

The animals selected for histological investigation were injected intramuscularly with etorphine hydrochloride (Small Animal Immobilon). Following induction of anaesthesia, the left ventricle was exposed and the animals perfused with 2.5% gluteraldehyde in 0.2 M phosphate buffer, pH 7.2.

\(^1\) Watanabe Instruments Ltd., Tokyo, Japan.
Figure 6: Watanabe linear graphic servo-corder, Type S.R. 657.
Figure 7: Photograph of a typical graphic recording of a fracture test. The value recorded is one half of the actual fracture load, expressed in kiloNewtons.
The mandibles were then removed and placed in 10% formal-saline solution to be fixed. The mandibles were allowed to fix for no less than four days before being prepared for decalcified sections.

4.1 Decalcified Section Technique
a) Laboratory preparation of tissues

The mandibles in fixative were rinsed in a phosphate-buffer bath for 30 minutes to remove any formalin. After this the mandibles were immersed in decalcifying fluid (10% formic acid in 7% tri-sodium citrate).

Decalcification took between five and 10 days and this was assessed radiographically. When decalcification had been achieved, the mandibles were rinsed for 30 minutes in water to remove the decalcifying fluid, prior to dehydration of the tissues.

Dehydration was achieved through gradual concentration of alcohols (20%; 30%; 50%; 70% and absolute). The tissues were immersed in two changes of absolute alcohol before "clearing" in chloroform. This clearing was performed in two changes of chloroform, each of one hour and the tissues left overnight in fresh chloroform.

The tissues were then taken through three changes of paraffin wax, under vacuum, for two hours each and were then blocked out.

b) Preparation of decalcified sections

Serial sections were cut between lines A - A1 and B - B1 (Figure 8). Sections of 6μ were cut with a Spencer 1 820

1 American Optical Co., Instrument Division, Buffalo, New York, 14215, U.S.A.
Figure 8: Photograph of lateral aspect of a left mandible of a rat (x 2). Histological sections were taken at 6μ intervals from the area between the lines A-A₁ and B-B₁.
A/O microtome using Reichert\(^1\) disposable blades. The sections were "floated" in 1 per cent albumin in water, mounted on a glass slide and dried overnight at 60°C.

Staining was carried out after deparaffinisation in two changes of Xylene. The sections were hydrated by rinsing in decreasing alcohol levels in coping jars at five minutes per rinse until the tissues were washed in running water. The hydrated sections were stained for 20 minutes with Weigharts iron haematoxylin (which was differentiated in 1% acid alcohol based in water) and blued in Scott's tap water solution.

The stained sections were washed for five minutes in running water, then for 5 minutes in 1% alcohol Eisers and dehydrated rapidly in absolute alcohol (two changes) and rinsed in absolute alcohol. Clearing was achieved (two changes) with Xylene and the sections mounted under glass using DPX and glass cover slips.

\(^1\) Reichert-Jung Ltd., 820 Yeovil Road, Slough SL1 4JB.
CHAPTER 5

INVESTIGATION OF ERRORS

The ultimate responsibility of any researcher should be the presentation of results which are both scientifically acceptable and reproducible. To achieve this, the researcher should design the project such that the techniques employed, including technical equipment, should be standardised in order that potential sources of error may be reduced or even eliminated.

These potential sources of error may be present in recording instruments and their values are, commonly, stated in operating manuals. Errors may also arise during operative procedures where measurements are recorded and may be affected by the methods of analyses used to interpret data.

One method used to reduce errors is the standardisation of techniques, while others must be investigated, in order that any effects these errors may have on results may be ascertained, and/or quantified.

In this study, the sources of error may be considered to arise from measurements or out of random.

1. Measurement Errors

1.1 Errors in Weighing

The quantity of implanted materials inserted into each animal was adjudged to weigh 100 milligrams. Measurement of each 100 milligrams was carried out on a standard laboratory balance. To

---

1 Stanton Instruments Ltd., Copper Mill Lane, London (now as W. & T. Avery Ltd., Smethwick, Warley).
determine the accuracy of the weighing process, 100 milligrams of hydroxyapatite was weighed on the balance: the balance was zeroed and the material reweighed. A total of 10 readings was taken to determine intra-observer errors. Two other observers then carried out similar readings to determine the extent of inter-observer errors. The error from the readings was calculated from the formula:

\[
\text{Mean Deviation} = \frac{(x-x)\,^2}{n}
\]

where \(x\) is one observation, \(\bar{x}\) the mean of the observations and \(n\) is the number of recordings (Bailey 1974). The results of these calculations are shown in Table 5.1. It can be seen that, for each 100 mg measured, the errors were small, ranging between 0.49 and 1.10 mg: added to this of course must be any error inherent in the balance. This has been designated by the manufacturer to be 0.01 mg. Addition of both factors results in weighing error of less than 2%.

1.2 Errors in Strength-testing

The strength-testing was done by three-point bending tests on a Howden universal testing machine which comprises of a load cell (Figure 5a) and a recording unit (Figure 5b). There is an electronic connection from the recording unit to a graphic recorder (Figure 6).

In theory, errors may arise from either the testing machine or the graphic recorder. The accuracy of the testing machine may be tested in accordance with British Standards Specification 1610-1964.
### TABLE 5.1

Lists of intra and inter observer readings used to determine error of measurement

<table>
<thead>
<tr>
<th></th>
<th>Observer 1</th>
<th>Observer 2</th>
<th>Observer 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Readings</td>
<td>100</td>
<td>101</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>101</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>102</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>101</td>
<td>101</td>
<td>99</td>
</tr>
<tr>
<td></td>
<td>102</td>
<td>102</td>
<td>100</td>
</tr>
<tr>
<td>Mean Reading</td>
<td>100.7</td>
<td>101.1</td>
<td>99.7</td>
</tr>
<tr>
<td>Maximum Errors</td>
<td>+1.3 - 1.7</td>
<td>+2 - 0.0</td>
<td>+2.0 - 2.0</td>
</tr>
<tr>
<td>Mean Deviation</td>
<td>1.1</td>
<td>0.49</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Using these guidelines, a steel proving ring was used. The proving ring was certified by the National Physical Laboratory (117-3-615.1572) to be correct from 2 kiloNewtons to 20 kiloNewtons. Test compressions on the proving ring, by two operators, resulted in both operators recording Grade-A results, indicating that the testing machine was operating with negligible error (0.1%).

The graphic chart recorder was calibrated with a standard current and voltage source. This voltage source was designated to have an error range of ± 0.1%.

These findings suggest that any errors arising from strength-testing are within acceptable limits.

2. Random Errors

2.1 Operator-induced Errors

The importance of standardised techniques has been alluded to earlier in this chapter and this standardisation must be employed in surgical techniques as well as to laboratory tests. In this study, the surgical techniques described in Chapter 4 were standard for every animal, this reporter being the sole operator throughout the study. The creation of a cryolesion, however, necessitated the assistance of two other workers: one reflected all soft tissues clear of the mandibular ramus, while the other placed the external thermocouple on the medial surface of the ramus. This researcher was thus able to apply the cryoprobe in such a way as to create a cryolesion in bone and not in soft tissue.

1 Time Electronics Ltd., Botany Industrial Estate, Tonbridge, Kent.
Although errors can never be wholly eliminated in any surgical procedure, the fact that a standardised operating technique was rigidly adhered to meant that any random errors were reduced to the level coincident with normal biological variation.

2.2 Apparatus-induced Random Errors

A possible source of error in the creation of the cryolesion was the accuracy of the external thermocouple. A standard laboratory thermometer\(^1\), THM -440-050S, was used to determine the accuracy of the thermocouple. This thermometer is designed to measure temperature ranges of -20°C to +20°C with 0.1° divisions. The British Standard Specification for this type of thermometer (B.S. 593: Designation A20c/100) demands a maximum error of 0.3 of a degree. Ten experimental freezings of (dead) porcine tongue were performed to determine the accuracy of the thermocouple. Both the thermocouple and the thermometer were placed on the tongue (a fresh site each time) and a cryolesion produced near them. The maximum difference between the readings was 0.8°C. As the pre-set temperature was 1°C below the cell-lethal temperature for bone, apparatus related error was not large enough to affect the standardised weakening of bone.

3. Conclusions

A thorough appraisal of the possible sources of errors indicated that the effects of these errors, if any, on the results of this study were not significant.

\(^{1}\) Gallenkamp Ltd., Technical House, Christopher Street, London.
1. Introduction

Statistics is the science which deals with the collection, processing and analysing of raw data for the purpose of drawing inferences and making predictions (Barks 1972).

In this thesis, statistical analyses were used to compare results among similar groups. These analyses determined if any differences or similarities, between the groups, were of statistical significance and not the result of a chance occurrence which might recur under similar experimental conditions. The result of these analyses is an accurate and meaningful interpretation of results obtained in any experiment or survey.

2. Symbols

The following symbols have been employed in the statistical analyses used:-

\( n \) - The number of observations in a sample.
\( x \) - A single observation.
\( \bar{x} \) - The mean of the observations.
\( \Sigma \) - The summation of data.
S.D. - The standard deviation or root mean-square deviation.
\( S^2 \) - The variance or mean of the squares of the deviations from the mean.
\( t \) - A normally distributed deviate, expressed in units of standard deviation.
\( 'P' \) - Probability.
3. **Statistical Methods**

The statistical methods used in this study were standard ones contained in most statistical textbooks. Particular reference was paid to Croxton et al (1968), Barks (1972) and Bailey (1974).

4. **Analyses of Data**

Analyses of the data were performed to determine mean values, standard deviations, variance and significant differences among the groups.

Standard deviation was determined from the following formula:

\[
S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}
\]

where the sum of the squares of the deviations is divided by \( n-1 \).

This is because the standard deviation is an estimation of the variability in a population and division by \( n-1 \) instead of \( n \) results in a more conservative estimate when dealing with small numbered samples (Bailey 1974).

Comparisons of the means and standard deviations of different groups were performed using two-sample t-tests. These tests established variance \( (S^2) \) using the formula:

\[
S^2 = \frac{(n_1-1) \text{ S.D.}_1^2 + (n_2-1) \text{ S.D.}_2^2}{n_1 + n_2 - 2}
\]

The t value was then obtained using the following formula:

\[
t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S^2 (\frac{1}{n_1} + \frac{1}{n_2})}{n_1 n_2}}}
\]
The t-test assumes that there is a normal distribution in each group and also that there is equal variance in each group.

From the values of t, a value for the probability ('P') can be obtained from standard tables of t, using the equation $n_1 + n_2 - 2$ to calculate the degrees of freedom.

From the 'P' value, the significance was determined as follows:-

If 'P' equals or is less than .05, the results may be considered to be probably significant.

If 'P' equals or is less than .01, the results may be termed significant.

If 'P' equals or is less than .005, the results may be termed highly significant.

5. Calculations

All statistical calculations were performed on a desk-top computer-calculator (Hewlett-Packard H.P. 33C)\(^1\). This apparatus was programmed to evaluate means, standard deviations, variance and values for t.

Ratification of each result was performed by using a computer package supplied by Edinburgh Regional Computing Centre. This package, Peritz one-Way ANOVA test, amassed data and analysed the variance of the results of each group.

\(^1\) Hewlett-Packard Ltd., Winnersh, Wokingham
CHAPTER 7
RESULTS

Introduction

The purpose of this study was to evaluate the effects of sintered dense hydroxyapatite and sintered β tricalcium phosphate on mandibular bone. If any evaluation of implant materials is to be thorough, then in vitro and in vivo experiments should be carried out before clinical testing with patients is performed (Autian 1981).

In this chapter, results are presented of in vitro testing, where tissue culture tests were used and also of in vivo testing on rats. Two types of results from the animal model are presented. First of all, bone fracture strength assessed mechanically is recorded in graphic and tabular form. Second, histological photographs are presented to illustrate the changes, with time, of the effect of the surgical method on mandibular bone. The interpretation of these results is discussed in Chapter 8.

1. In Vitro Tests

The in vitro investigation selected involved tissue culture, using human fibroblasts obtained via explant-culture of gingivae excised during the extraction of wisdom teeth in the Department of Oral Surgery, Edinburgh Dental Hospital. Ten days after the addition of samples of dense hydroxyapatite and β sintered tricalcium phosphate, to petri dishes containing fibroblasts in cultures, photographic assessments were made to determine levels of toxicity or biocompatibility. Figures 9 and 10 demonstrate the
Figure 9: Granules of dense hydroxyapatite in tissue culture. Intimate contact between fibroblasts and granule demonstrated with no evidence of toxicity. Mag. x 300.
Figure 10: Granules of $\beta$ tricalcium phosphate in tissue culture. Obvious biocompatibility is demonstrated by close contact of cells and implant material. Mag. $\times$ 300.
biocompatibility of both implant materials. The absence of clear zones between the fibroblasts and both implant materials indicates non-toxicity; biocompatibility is implied by the growth of fibroblasts around the margins of both implant materials.

2. **In Vivo Experiments**

   *In vivo* evaluation of the effects of dense hydroxyapatite and sintered β-tricalcium phosphate was performed on 231 adult, male Sprague-Dawley rats. A comprehensive evaluation of the effects of the two implant materials on rat mandibular bone was achieved by conducting mechanical testing studies in parallel to histological assessment of the bone.

   In order to ensure that no ambiguity occurs in the interpretation of the results presented in this chapter, the experimental groups studied in this investigation (Table 4.1) have been summarised:

   **Group 1**, which served as a surgical control, had no implant material placed on the left mandible exposed by elevation of the left masseter muscle and its periosteum. Following wound closure, the animals were sacrificed two, eight and 26 weeks post-operatively. At every sacrifice time, seven animals were sacrificed; two animals were perfused for histological examination and five for fracture strength testing.

   **Group 2** differed from **Group 1** in that 100 mgm of granular dense hydroxyapatite were placed on the exposed mandibular ramus of each animal before wound closure.

   **Group 3**. In this group, the only addition to the operation for **Group 1** was that 100mgm of granular β-tricalcium phosphate were
placed in the exposed left mandible of each animal before wound closure.

Groups 1, 2 and 3 thus comprised that part of the study evaluating the effects of both dense hydroxyapatite and sintered β tricalcium phosphate on unweakened mandibular bone.

Group 4 served as the control group for the effects of cryosurgery on bone. For each animal, the exposed left mandible was subjected to a cryolesion by a cryoprobe, set to -16°C prior to wound closure. Animals were sacrificed after two, four, six, eight, 10, 12 and 26 weeks post-operatively. As in the other control group, Group 1, no implant materials were added. The number of animals in each time group was the same as the groups in the study on unweakened bone.

Group 5. In this group, 100 mgm of granular, dense hydroxyapatite were placed over the cryolesion, before wound closure.

Group 6. The modification for this group was that 100 mgm of granular sintered β tricalcium phosphate were placed over the cryolesion, before wound closure.

Group 7 differed from Groups 4, in that a 100 mgm sintered block of dense hydroxyapatite was placed over the cryolesion, before wound closure.

Groups 4, 5, 6 and 7 comprised the study on bone which was previously weakened.

2.1 Mechanical (Fracture Strength) Test Results

Assessment of fracture strengths of rat mandibular bone was achieved by three-point bending tests on a Howden servo-hydraulic universal testing machine (Figures 5a and 5b).
In a pilot study, the fracture strengths of left and right mandibles of five adult male Sprague-Dawley rats were compared to those of five adult female Sprague-Dawley rats. The results, listed in Table 7.1, show that for the male rats, the mean fracture strength of the right mandibles was 9.22 with a standard deviation of $\pm$ 0.526. The mean fracture strength of the left male mandibles was 9.225 with a standard deviation of $\pm$ 0.475. The mean ratio of left:right male mandibles (L:R ratio) was 1.001 $\pm$ 0.0521. The mean fracture strength of the right female mandibles was 7.48, with a standard deviation of $\pm$ 0.757 while the mean value of the female left mandibular fracture strength was 7.49 with a standard deviation of $\pm$ 0.720; the mean ratio of left:right fracture strengths of the female mandibles was 1.0028 $\pm$ 0.1033. The mean ratio of left:right fracture strength was selected to indicate any change between the test side and the (right) control side.

The results of the study on unweakened bone are presented in Table 7.2.

In the surgical control group, the mean reading for the right mandibular fracture of the two week group was 4.31. (As has been explained in Figure 7, this value represents a graphic record representing one half of the value in kiloNewtons; and this was common in every result in Groups 1 to 7). The equivalent values of the left fracture results range from readings of 4.70 maximum to 4.00 minimum. The mean ratio of left:right fracture strengths is 1.016 with a standard deviation of $\pm$ 0.072.

The mean value of right mandibular fracture test for the Group 1 animals sacrificed eight weeks after surgery was recorded as
TABLE 7.1

Recorded right and left fracture strengths of five male and five female rats (non surgical controls)

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Results</th>
<th>Mean Right</th>
<th>Left Results</th>
<th>Mean L:R Ratio</th>
<th>L:R Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Controls</td>
<td>8.80</td>
<td>8.90</td>
<td>8.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.90</td>
<td>8.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9.00</td>
<td>9.22</td>
<td>9.10</td>
<td>1.00108</td>
<td>0.0521</td>
</tr>
<tr>
<td></td>
<td>9.30</td>
<td>9.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10.10</td>
<td>9.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female Controls</td>
<td>6.30</td>
<td>6.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>7.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.85</td>
<td>7.48</td>
<td>8.00</td>
<td>1.0028</td>
<td>0.1033</td>
</tr>
<tr>
<td></td>
<td>8.35</td>
<td>8.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.40</td>
<td>7.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Right Results</td>
<td>Mean Right</td>
<td>Left Results</td>
<td>Mean L:R Ratio</td>
<td>L:R Standard Deviation</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>------------</td>
<td>--------------</td>
<td>----------------</td>
<td>------------------------</td>
</tr>
<tr>
<td>Surgery</td>
<td>4.20</td>
<td>4.70</td>
<td>4.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.75</td>
<td>4.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>4.20</td>
<td>4.31</td>
<td>4.50</td>
<td>1.016</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td>4.00</td>
<td>4.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.40</td>
<td>4.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.60</td>
<td></td>
<td>5.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.50</td>
<td></td>
<td>5.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>4.50</td>
<td>4.85</td>
<td>5.20</td>
<td>1.08</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td></td>
<td>5.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.90</td>
<td></td>
<td>5.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5.00</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.10</td>
<td></td>
<td>4.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>5.00</td>
<td>5.18</td>
<td>5.50</td>
<td>1.002</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td></td>
<td>5.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.30</td>
<td></td>
<td>5.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.50</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>3.90</td>
<td></td>
<td>5.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.30</td>
<td>4.24</td>
<td>5.50</td>
<td>1.309</td>
<td>0.087</td>
</tr>
<tr>
<td>2 weeks</td>
<td>4.10</td>
<td></td>
<td>5.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.40</td>
<td></td>
<td>6.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>5.50</td>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>6.00</td>
<td></td>
<td>7.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>6.50</td>
<td>5.70</td>
<td>8.10</td>
<td>1.389</td>
<td>0.180</td>
</tr>
<tr>
<td>8 weeks</td>
<td>5.00</td>
<td></td>
<td>8.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td></td>
<td>9.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.70</td>
<td></td>
<td>6.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.55</td>
<td></td>
<td>6.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>5.80</td>
<td>5.01</td>
<td>8.60</td>
<td>1.377</td>
<td>0.192</td>
</tr>
<tr>
<td>26 weeks</td>
<td>4.50</td>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.00</td>
<td></td>
<td>3.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.30</td>
<td></td>
<td>4.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium</td>
<td>3.75</td>
<td>4.06</td>
<td>4.25</td>
<td>1.025</td>
<td>0.089</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.50</td>
<td></td>
<td>4.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>3.75</td>
<td></td>
<td>3.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.65</td>
<td></td>
<td>6.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.05</td>
<td></td>
<td>8.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium</td>
<td>4.05</td>
<td>4.19</td>
<td>6.60</td>
<td>1.79</td>
<td>0.244</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.00</td>
<td></td>
<td>8.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>4.25</td>
<td></td>
<td>7.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgery</td>
<td>4.00</td>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.50</td>
<td></td>
<td>6.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium</td>
<td>4.85</td>
<td>4.31</td>
<td>8.50</td>
<td>1.605</td>
<td>0.177</td>
</tr>
<tr>
<td>Phosphate</td>
<td>4.80</td>
<td></td>
<td>7.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>4.40</td>
<td></td>
<td>7.50</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4.85. The maximum fracture strength reading for the corresponding left mandible for this subgroup was 5.70 while the minimum value recorded was 5.00. The mean ratio of left:right fracture strengths was 1.08 with a standard deviation of ± 0.056.

For the animals in Group 1 sacrificed 26 weeks after surgery, the mean right fracture reading was 5.18. The maximum left fracture reading was 5.60 and the minimum value was 4.75; the mean ratio of left:right fracture strengths was 1.002 with a standard deviation of ± 0.068.

The effect of surgery, alone, on the fracture strength of unweakened bone is illustrated graphically in Figure 11, which indicates that negligible change occurred over the three periods of study.

The fracture strength results after the addition of 100 mgm of granular dense hydroxyapatite to exposed mandibular bone (Group 2) are also listed in Table 7.2. Two weeks after implantation of hydroxyapatite, the mean fracture readings of the right mandibles was 4.24; the maximum left fracture reading was 6.00 while the minimum value was 5.00. The mean ratio of left:right fracture strengths was 1.309 with a standard deviation of ± 0.087.

The animals in Group 2, which were sacrificed eight weeks after the implantation of dense hydroxyapatite had a mean right fracture reading of 5.70. The maximum left fracture reading was 9.00 while the minimum value was 6.50; the mean ratio of left:right fracture strengths was 1.389 with a standard deviation of ± 0.180.

The mean right fracture reading for Group 2 animals, sacrificed 26 weeks post-implantation of 100 mgm of dense hydroxyapatite was 5.01. The maximum recorded reading for the left
Figure 11: Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of surgery alone, surgery plus granules of dense hydroxyapatite (HA) and surgery plus β tricalcium phosphate (TCP).
fracture tests was 8.60 with the minimum value 6.25; the mean ratio of left to right fracture strengths was 1.377 with a standard deviation of \(\pm 0.192\).

Graphic illustration of the effects of adding granular hydroxyapatite to unweakened bone are presented in Figure 11.

The results of the third group studied are also seen in Table 7.2; this third group represents the addition of 100 mgm of sintered \(\beta\) tricalcium phosphate to bone subjected only to surgical exposure of the left mandibular ramus. In the animals sacrificed two weeks after the addition of \(\beta\) tricalcium phosphate to unweakened bone, the mean right fracture reading was 4.06. The maximum left fracture reading was 4.75 while the minimum left fracture value was 3.90; the mean ratio of left:right fracture strengths was 1.025 with a standard deviation of \(\pm 0.089\).

The mean right fracture reading for Group 3 animals, sacrificed eight weeks post-implantation, was 4.19. The maximum left reading was 8.50, while the minimum value was 6.25; the mean ratio of left:right fracture strengths was 1.79 with a standard deviation of \(\pm 0.244\).

Twenty six weeks after the addition of \(\beta\) tricalcium phosphate to the exposed left mandible, the mean right fracture reading was 4.51. The maximum left reading was 8.50 while the minimum value was 6.50; the mean ratio of left:right fracture strengths was 1.605 with a standard deviation of \(\pm 0.177\).

Figure 11 illustrates, graphically, the effects of adding sintered \(\beta\) tricalcium phosphate to mandibular bone. This figure, by comparing mean ratios of left:right fracture strengths against time, in weeks, of Groups 1, 2 and 3 demonstrates the effect of the
three modalities on bone. These modalities are surgical exposure alone, surgical exposure plus dense hydroxyapatite and surgical exposure plus sintered β tricalcium phosphate.

The mean values of the pilot study on male rats were compared to those of the two week surgical control and the readings subjected to analyses of variance. No significant difference, statistically, was found. 'P' = >.1 [Table 7.3(a)].

The results of Groups 2 and 3 were subjected to analysis of variance with Group 1 and the results listed in Table 7.3(b). It can be seen that two weeks post-implantation, the effects of the addition of dense hydroxyapatite to weakened bone are highly significant ('P' = <.001), whereas the 'P' value for the comparative Group 3 animals was >.1, a figure taken to be not significant. Eight weeks after implantation, however, the 'P' values for Group 2 (hydroxyapatite) were probably significant ('P' = <.02) whereas those for Group 3 (β tricalcium phosphate) were highly significant ('P' = <.001). Both Groups 2 and 3 had levels of significance considered highly significant 26 weeks after implantation ('P' = <.001).

The fracture readings for Group 4 animals only are shown in Table 7.4. The group was selected to be the cryosurgical control group. Two weeks post-cryosurgery, the mean right fracture figure was 5.44 while the maximum left result was 6.00 and the minimum 5.35. The mean ratio of left:right fracture strengths was 1.031 with a standard deviation of ± 0.051.

In the animals sacrificed four and six weeks after cryosurgery the fracture readings were similar; at four weeks the mean right fracture reading was 4.35 while the reading on the six week group
TABLE 7.3(a)

'P' value of differences in Left:Right ratios of mean values between non-surgical and surgical controls (2 weeks)

<table>
<thead>
<tr>
<th>Experimental Control</th>
<th>Mean L:R Ratio</th>
<th>Standard Deviation</th>
<th>'P' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/ Non-surgical</td>
<td>1.00108</td>
<td>0.0521</td>
<td>&gt;.1</td>
</tr>
<tr>
<td>Surgical</td>
<td>1.016</td>
<td>0.072</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 7.3(b)

''P' values of differences in Left:Right mean values between surgical controls and groups where implants were placed on unweakened bone

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>2 weeks</th>
<th>8 weeks</th>
<th>26 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Groups 2) Surgery + Hydroxyapatite</td>
<td>&lt;.001</td>
<td>&lt;.02</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>(Group 3) Surgery + Tricalcium Phosphate</td>
<td>&gt;.1</td>
<td>&lt;.001</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
TABLE 7.4
Results of the effects of cryosurgery on the fracture strength of mandibular bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Results</th>
<th>Mean Right</th>
<th>Left Results</th>
<th>Mean L:R Ratio</th>
<th>L:R Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>6.00</td>
<td>5.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.80</td>
<td>5.44</td>
<td>5.35</td>
<td>1.031</td>
<td>0.051</td>
</tr>
<tr>
<td>2 weeks</td>
<td>5.15</td>
<td>6.00</td>
<td>5.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td>5.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>4.20</td>
<td>3.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.10</td>
<td>3.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>4.75</td>
<td>4.35</td>
<td>4.30</td>
<td>0.862</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td>4.15</td>
<td>4.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.55</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>5.25</td>
<td>4.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.00</td>
<td>3.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>4.35</td>
<td>4.75</td>
<td>4.20</td>
<td>0.851</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>4.65</td>
<td>4.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>4.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>5.45</td>
<td>3.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.60</td>
<td>4.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>4.70</td>
<td>5.35</td>
<td>4.25</td>
<td>0.697</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>3.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>4.00</td>
<td>3.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.00</td>
<td>3.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 weeks</td>
<td>6.00</td>
<td>4.66</td>
<td>4.25</td>
<td>0.828</td>
<td>0.065</td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>4.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>3.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>4.50</td>
<td>4.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5.50</td>
<td>5.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
<td>0.96</td>
<td>0.068</td>
</tr>
<tr>
<td></td>
<td>5.25</td>
<td>4.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>5.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>7.50</td>
<td>8.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.80</td>
<td>8.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>5.60</td>
<td>6.80</td>
<td>7.30</td>
<td>1.162</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>5.60</td>
<td>7.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.50</td>
<td>8.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
was 4.75. The maximum and minimum figures in the four week group were 4.30 and 3.25 respectively while those in the six week group were 4.20 and 3.85 respectively. The mean ratio of left:right fracture strengths after four weeks was 0.862 (standard deviation ± 0.095); the mean ratio for six weeks was 0.851 (standard deviation ± 0.027).

More noticeable changes occurred in the eight week cryosurgery group. The mean right fracture reading was 5.35 while the maximum left reading was 4.25 and the minimum value 3.20. The mean ratio of left:right fracture strengths had fallen to 0.697 (standard deviation ± 0.081), a reduction of over 30%.

The results of the animals sacrificed 10 weeks after cryosurgery are similar to those in the four and six week time groups; the mean right fracture reading was 4.66, the maximum left fracture reading was 4.25 whilst the minimum value was 3.50. The mean ratio of left:right fracture strengths was 0.828, standard deviation ± 0.065.

For these animals sacrificed 12 weeks after cryosurgery the mean right fracture reading was 5.00; the maximum left reading was 5.10 while the minimum value was 4.30. The mean ratio of left:right fracture strengths had risen to 0.96 while the standard deviation was ± 0.068.

Greater values were recorded in these animals sacrificed 26 weeks after cryosurgery. The mean right fracture reading was 6.80 while the maximum left reading was 8.20 and the minimum 7.30. The mean ratio of left:right fracture strengths had risen to 1.162 with a standard deviation of ± 0.052.
The gradual reduction in fracture strength to eight weeks post-cryosurgery is shown, graphically, in Figure 12. In this figure, mean ratios of left:right fracture strengths are graphed against time in weeks, and a reduction of 30% in the ratio is seen at eight weeks before the ratio rises to just above the level of unity by 26 weeks. The significance of the tabular and graphic results of Group 4 are shown in Table 7.5. Displayed in this table are 'P' values determined by analysis of variance between Groups 1 and 4 at two, eight and 26 weeks. After two weeks, there is no significant difference between the results of the surgical control group and the cryosurgery control group, 'P' = .946. After eight weeks, the bone in the cryosurgery group is significantly weaker than the surgery control group ('P' = <.001). After 26 weeks, the difference between both groups is significant ('P' = <.01), the cryosurgery group being stronger than the surgery control group.

Figure 12, represents in part, the effects of the addition of granular dense hydroxyapatite to bone previously subjected to cryosurgery and indicates a considerable difference from the effects of cryosurgery alone. The results of Group 5 are listed in Table 7.6. The results seen in animals sacrificed two and four weeks after the addition of 100 mgm of dense hydroxyapatite granules to bone which had been subjected to cryosurgery, are similar. The mean right fracture reading of the two week animals was 5.44 while that for the four week group was 4.50. The maximum left fracture strength reading was 6.30 and the minimum value 4.80 in the two week group while, in the four week group, the maximum reading was 6.30 and the minimum value 4.80. In the two week group the mean ratio of left:right fracture strengths was 1.20 with a
Figure 12: Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of cryosurgery alone, cryosurgery plus granules of dense hydroxyapatite (HA) and cryosurgery plus β tricalcium phosphate (TCP).
TABLE 7.5

'P' values of differences in left:right mean values between surgery controls and groups where implants were placed on bone subjected to cryosurgery

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>'P' Value</th>
<th>2 weeks</th>
<th>8 weeks</th>
<th>26 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery + Cryolesion</td>
<td>.946</td>
<td>&lt;.001</td>
<td>.01</td>
<td></td>
</tr>
</tbody>
</table>
TABLE 7.6

Results of the effects of cryosurgery plus dense hydroxyapatite (granules) on the fracture strength of bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Results</th>
<th>Mean Right</th>
<th>Left Results</th>
<th>Mean L:R Ratio</th>
<th>L:R Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryosurgery + granular</td>
<td>5.50</td>
<td>6.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.50</td>
<td>4.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>5.50</td>
<td>5.440</td>
<td>6.30</td>
<td>1.20</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.00</td>
<td>5.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>4.35</td>
<td>6.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.30</td>
<td>4.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 weeks</td>
<td>4.85</td>
<td>4.50</td>
<td>6.30</td>
<td>1.26</td>
<td>0.129</td>
</tr>
<tr>
<td></td>
<td>4.25</td>
<td>5.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.75</td>
<td>5.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>4.30</td>
<td>5.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.00</td>
<td>4.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 weeks</td>
<td>4.00</td>
<td>4.10</td>
<td>4.40</td>
<td>1.195</td>
<td>0.081</td>
</tr>
<tr>
<td></td>
<td>4.30</td>
<td>5.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.90</td>
<td>4.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>4.30</td>
<td>8.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.45</td>
<td>7.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>5.40</td>
<td>5.00</td>
<td>6.85</td>
<td>1.441</td>
<td>0.165</td>
</tr>
<tr>
<td></td>
<td>5.60</td>
<td>6.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.55</td>
<td>6.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>4.00</td>
<td>5.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>5.00</td>
<td>6.050</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 weeks</td>
<td>6.00</td>
<td>5.00</td>
<td>8.00</td>
<td>1.32</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>7.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td>6.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>5.00</td>
<td>8.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>5.20</td>
<td>8.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 weeks</td>
<td>4.30</td>
<td>4.82</td>
<td>6.60</td>
<td>1.587</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>5.10</td>
<td>8.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.50</td>
<td>7.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery + granular</td>
<td>4.40</td>
<td>8.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.00</td>
<td>10.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>4.50</td>
<td>4.98</td>
<td>10.00</td>
<td>1.789</td>
<td>0.213</td>
</tr>
<tr>
<td></td>
<td>6.50</td>
<td>7.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td>8.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
standard deviation of ± 0.129 while the corresponding values for the four week group were 1.26 and ± 0.129.

Similar results are also seen in the animals sacrificed after six weeks; the mean right fracture reading was 4.10. The maximum left fracture strength reading was 5.20 while the minimum was 4.40. The mean ratio of left:right fracture strengths was 1.195 with a standard deviation of ± 0.081.

More marked effects were seen in these animals sacrificed after eight weeks. The mean right fracture reading was 5.00; the maximum left fracture reading was 8.40 while the minimum value was 6.50. This resulted in a mean ratio of left:right fracture strengths of 1.441, with a standard deviation of ± 0.165.

Ten weeks after implantation the mean right fracture reading was 5.00. The maximum left reading was 8.00 while the minimum value was 5.30; the resultant mean ratio of left:right sides was 1.32 with a standard deviation of ± 0.199.

The readings for the animals in Group 5 sacrificed 12 weeks after implantation can be seen to have risen. The mean right fracture reading was similar to the previous time group, viz. 4.82. The maximum left fracture reading was 8.20 while the minimum value recorded was 6.60; this gave a mean ratio of left:right fracture strengths of 1.587 with a standard deviation of ± 0.146.

Although in the 26 week group the mean right fracture figure (4.98) remained reasonably similar to the corresponding figures in other time groups, other readings rose. The maximum left fracture reading was 10.00 while the minimum value was 7.60; the resultant mean ratio of left:right fracture strengths was 1.789 with a standard deviation of ± 0.213. The collective results of the left to
right ratios are graphed against time in Figure 12.

Differences, in mean ratios of left:right fracture strengths, between results in Groups 4 and 5, when analysed statistically, are listed in Table 7.7. It can be seen that the differences between Group 4 and Group 5 are probably significant after two weeks, 'P' = <.09. Thereafter, for all time figures, the increase in strength of bone, into which dense granular hydroxyapatite was added, was significantly greater than the effect of cryosurgery alone ('P' = <.001).

The results of Group 6 are shown in Table 7.8. In the two week sacrifice group, the mean right fracture reading was 4.07. The maximum left fracture reading recorded was 5.30, and the minimum was 3.90; the mean ratio of left:right fracture strengths was 1.101 with a standard deviation of ± 0.128.

The results of the fracture tests of the animals sacrificed four and six weeks were similar. The mean right fracture reading of the four week group was 4.56, that of the six week group was 4.45. The maximum and minimum left values for the four week group were 6.30 and 6.00 respectively while those for the six week group were 6.60 and 5.25 respectively. The mean ratio of left:right fracture strengths for the four week group was 1.351 ± 0.029 while that of the six week group was 1.337 ± 0.110.

The results of the fracture tests, for Group 6, after eight, 10 and 12 weeks post-implantation, can also be seen to be similar.

The mean right fracture reading for the eight, 10 and 12 week groups were 4.97, 4.787 and 4.55 respectively. In the eight week group the maximum and minimum fracture values were 8.00 and 6.35 respectively, giving a mean ratio of left:right fracture strengths.
TABLE 7.7

'P' values of differences in left:right mean values between cryosurgery controls and groups where implants were placed on bone subjected to cryosurgery

<table>
<thead>
<tr>
<th>Experimental Model</th>
<th>'P' Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 weeks</td>
</tr>
<tr>
<td>Surgery + Cryolesion + Hydroxyapatite</td>
<td>&lt;.09</td>
</tr>
<tr>
<td>(granules)</td>
<td></td>
</tr>
<tr>
<td>Surgery + Cryolesion + Tricalcium</td>
<td>&lt;.07</td>
</tr>
<tr>
<td>Phosphate (granules)</td>
<td></td>
</tr>
<tr>
<td>Surgery + Cryolesion + Hydroxyapatite</td>
<td>&lt;.9</td>
</tr>
<tr>
<td>(block)</td>
<td></td>
</tr>
</tbody>
</table>
Results of the effects of cryosurgery plus β tricalcium phosphate on the fracture strength of mandibular bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Results</th>
<th>Mean Right</th>
<th>Left Results</th>
<th>Mean L:R Ratio</th>
<th>L:R Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryosurgery plus</td>
<td>3.75</td>
<td>3.90</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 2 weeks</td>
<td>4.20</td>
<td>4.07</td>
<td>4.45</td>
<td>1.101</td>
<td>0.128</td>
</tr>
<tr>
<td>Phosphate 2 weeks</td>
<td>4.25</td>
<td></td>
<td>5.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.15</td>
<td></td>
<td>4.55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>4.90</td>
<td>6.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 4 weeks</td>
<td>4.70</td>
<td>4.56</td>
<td>6.10</td>
<td>1.351</td>
<td>0.029</td>
</tr>
<tr>
<td>Phosphate 4 weeks</td>
<td>4.80</td>
<td></td>
<td>6.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.30</td>
<td></td>
<td>6.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>4.85</td>
<td>5.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 6 weeks</td>
<td>4.00</td>
<td>4.45</td>
<td>6.00</td>
<td>1.337</td>
<td>0.110</td>
</tr>
<tr>
<td>Phosphate 6 weeks</td>
<td>4.20</td>
<td></td>
<td>6.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.70</td>
<td></td>
<td>5.80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>4.45</td>
<td>8.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 8 weeks</td>
<td>4.65</td>
<td>4.97</td>
<td>7.25</td>
<td>1.421</td>
<td>0.133</td>
</tr>
<tr>
<td>Phosphate 8 weeks</td>
<td>4.95</td>
<td></td>
<td>6.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.50</td>
<td></td>
<td>6.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>5.00</td>
<td>6.70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 10 weeks</td>
<td>4.90</td>
<td>4.787</td>
<td>6.85</td>
<td>1.462</td>
<td>0.057</td>
</tr>
<tr>
<td>Phosphate 10 weeks</td>
<td>4.25</td>
<td></td>
<td>7.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>4.90</td>
<td>7.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 12 weeks</td>
<td>4.75</td>
<td>4.55</td>
<td>6.40</td>
<td>1.451</td>
<td>0.092</td>
</tr>
<tr>
<td>Phosphate 12 weeks</td>
<td>4.40</td>
<td></td>
<td>6.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.70</td>
<td></td>
<td>7.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery plus</td>
<td>4.00</td>
<td>6.65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tricalcium 26 weeks</td>
<td>5.00</td>
<td>4.375</td>
<td>7.15</td>
<td>1.569</td>
<td>0.101</td>
</tr>
<tr>
<td>Phosphate 26 weeks</td>
<td>4.50</td>
<td></td>
<td>7.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00</td>
<td></td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
of 1.421 and a standard deviation of \( \pm 0.133 \). Similar values were found in the 10 week group; maximum and minimum left fracture values were 7.30 and 6.70 respectively while the mean ratio of left:right fracture strengths was 1.462 \( \pm 0.057 \). In the animals sacrificed 12 weeks after implantation, the maximum and minimum left fracture figures recorded were 7.10 and 6.20. These gave a mean ratio of left:right fracture strengths of 1.451 with a standard deviation of \( \pm 0.092 \).

In those animals sacrificed after 26 weeks, the mean right fracture reading was 4.375. The maximum left fracture reading was 7.30 while the minimum value recorded was 6.35. As in the 10 week group the zero figures are not included as they represent animals which died shortly before sacrifice. The mean ratio of left:right fracture strengths of the 26 weeks group was seen to have risen to 1.569 with a standard deviation of \( \pm 0.101 \).

A graphic illustration, for Group 6, of mean ratios of left:right fracture strengths is seen in Figure 12.

Table 7.7 lists the 'P' values obtained when the results of Group 4 and Group 6 are subjected to analyses of variance (Peritz one-way Anova Test). It can be seen that, after two weeks, the effect of sintered tricalcium phosphate on bone subjected to cryosurgery may be considered probably significant when compared to the effects of cryosurgery alone, ('P' = <.07) but not when compared to the effects of dense hydroxyapatite on bone subjected to cryosurgery. At all other times, however, Group 6 is seen to give increased fracture strength results compared to Group 4; these increases are highly significant ('P' = <.001). Figure 12 and Table 7.7 indicate, however, that any differences in bone strength
between Group 5 and Group 6 are not significant.

The results of the fracture tests of the animals in Group 7 are listed in Table 7.9.

The mean right fracture reading for the animals sacrificed after two weeks was 5.19. The maximum left fracture reading was 5.40 while the minimum value recorded was 4.75; the resultant mean ratio of left:right fracture strengths was 0.979 ± 0.068.

In those animals sacrificed after eight weeks, the mean right fracture reading was 4.10. The maximum left fracture reading was 6.20 while the minimum value was 5.50. The mean ratio of left:right fracture strengths was 1.420 with a standard deviation of ± 0.080.

The mean right fracture reading of the 26 weeks group of animals, was 4.11. The maximum left reading was 7.40 while the minimum figure was 6.00; the mean ratio of left:right fracture strengths was 1.657 with a standard deviation of ± 0.151.

Figure 13 in addition to illustrating the graph of the L:R ratio of Group 7 against time in weeks, also compares the effects of the two forms of hydroxyapatite, granular and sintered blocks, on bone strength. This figure indicates that, other than at the two week group, there is no significant difference between the effects of on bone fracture strength. This observation is ratified in Table 7.7 where the 'P' values of Group 7 against Group 4 are shown. At two weeks, no significant difference is found between Group 7 and Group 4 ('P' = <.9). Thereafter, Group 7 gave fracture strength results which were highly significantly greater than Group 4 ('P' = <.001). Similarly, when Group 7 was compared to Groups 5 and 6 only at two weeks was Group 7 seen to be weaker than Groups 5
TABLE 7.9

Results of the effects of cryosurgery plus hydroxyapatite (blocks) on the fracture strength of mandibular bone

<table>
<thead>
<tr>
<th>Group</th>
<th>Right Results</th>
<th>Mean Right</th>
<th>Left Results</th>
<th>Mean L:R Ratio</th>
<th>L:R Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryosurgery</td>
<td>4.90</td>
<td>4.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>5.60</td>
<td>5.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>5.25</td>
<td>5.19</td>
<td>5.00</td>
<td>0.979</td>
<td>0.068</td>
</tr>
<tr>
<td>Blocks</td>
<td>4.70</td>
<td>4.75</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 weeks</td>
<td>5.50</td>
<td>5.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>4.00</td>
<td>6.10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.30</td>
<td>6.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>4.10</td>
<td>4.10</td>
<td>5.50</td>
<td>1.420</td>
<td>0.080</td>
</tr>
<tr>
<td>Blocks</td>
<td>4.00</td>
<td>5.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 weeks</td>
<td>4.10</td>
<td>5.80</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryosurgery</td>
<td>3.80</td>
<td>6.30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>plus</td>
<td>4.10</td>
<td>7.15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite</td>
<td>3.90</td>
<td>4.11</td>
<td>6.00</td>
<td>1.657</td>
<td>0.151</td>
</tr>
<tr>
<td>Blocks</td>
<td>4.55</td>
<td>7.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26 weeks</td>
<td>4.20</td>
<td>7.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 13: Graph plotting the (mean) ratio of L:R fracture strengths against time (in weeks) of the effects on bone of cryosurgery plus dense hydroxyapatite (HA) granules and cryosurgery plus dense hydroxyapatite (HA) blocks.
and $6 - 'P' = .9$ compared to $'P' = .09$ and $'P' = .07$ respectively.

### 2.2 Histological Findings

The experimental groups comprising the studies on weakened bone are listed in Table 4.1. Seven animals were ascribed to each time interval for each group; five animals were sacrificed for fracture strength testing and two for histological investigation. Adequate fixation was achieved by perfusion, via the left ventricle, with 2.5% gluteraldehyde in 0.2 M phosphate buffer, pH 7.2. The perfused mandibles were immersed in 10% formal saline to complete fixation. Decalcified sections were prepared throughout and achieved by immersion of the mandibles in 10% formic acid in 7% trisodium citrate. Decalcification was assessed radiographically; when decalcified, the specimens were routinely processed for light microscopy. Each specimen (6μ thick) was stained with haematoxylin and eosin (H & E).

Photographs of histological sections are presented for animals sacrificed two, eight and 26 weeks post-operatively. As with the fracture strength results, histological findings of each group are described in toto. Each photograph is of a coronal section of the left mandible, sectioned at the level of the lingula (Figure 8).
Group 1: Surgery only

Two weeks post-operation (Figure 14):

Section shows a thin layer of new bone over mature vital bone which contains plentiful osteoblasts. The periosteum is hyperplastic at this stage at it adjusts to being repositioned. Where reposition was not precise, some new bone has been deposited. There are no signs of acute inflammation present in this section. (H & E x 100)

Eight weeks post-operation (Figure 15):

Section shows a similar field to that of the previous group, but more re-organisation has taken place, with more prominent osteoblasts present on mature vital bone. (H & E x 100)

Twenty six weeks post-operation (Figure 16):

Section shows complete re-attachment of periosteum. Beneath the periosteum, normal, vital bone is seen. In addition, when compared to Figures 14 and 15 this section shows no significant morphological difference attributable to age changes. (H & E x 100)

Group 2: Surgery plus granules of dense hydroxyapatite

Two weeks post-implantation (Figure 17):

Section shows, on the left, a focus of dense hydroxyapatite granules is included within fibrous, hyperplastic periosteum. Where the granules were placed subperiosteally, a layer of new bone, characterised by plentiful osteoblasts and osteocytes, is present on the cortical surface of the ramus. A cellular inflammatory exudate is not identified. (H & E x 40)
Figure 14: Coronal section of the left mandibular ramus of a rat at the level of the lingula: two weeks after surgery only. (H & E x 100).
Figure 15: Coronal section of the left mandibular ramus of a rat at the level of the lingula; eight weeks after surgery only. (H & E x 100).
Figure 16: Coronal section of the left mandibular ramus of a rat at the level of the lingula: 26 weeks after surgery only. (H & E x 100).
Figure 17: Coronal section through the left mandibular ramus of a rat at the level of lingula. Granules of dense hydroxyapatite were added to unweakened bone: two weeks post-implantation. (H & E x 100).
Figure 18: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite were added to unweakened bone: eight weeks post-implantation. (H & E x 40).
Figure 19: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite were added to unweakened bone: 26 weeks post-implantation. (H & E x 40).
Eight weeks post-implantation (Figure 18):

Section shows several, irregular trabeculae of new, woven bone. These trabeculae are superficial to normal, vital, mature bone and marrow. (H & E x 40)

Twenty six weeks post-implantation (Figure 19):

Section shows substantial, mature, vital bone merged with the underlying bone. Some granules of dense hydroxyapatite are seen within the new bone. (H & E x 40)

Group 3: Surgery plus granules of β tricalcium phosphate

Two weeks post-implantation (Figure 20):

Section shows multiple foci of granules of β tricalcium phosphate are contained within a highly cellular hyperplastic periosteum. Several multinucleated giant cells can be seen at the peripheries of the granule-containing foci. A thin layer of woven bone is identified on the surface of the cortical bone and in some fields this is arranged in prominent trabeculae. No inflammatory exudate is evident in this section. (H & E x 40)

Eight weeks post-implantation (Figure 21):

Section shows thick and irregular new bone overlying vital mature bone. Within this new bone, multiple foci of granules of β tricalcium phosphate are seen. (H & E x 40)

Twenty six weeks post-implantation (Figure 22):

Section shows the copious mature bone which has resulted from the implantation of β tricalcium phosphate on mandibular bone. Remodelling has taken place within the new bone. (H & E 40)
Figure 20: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of \( \beta \) tricalcium phosphate were added to unweakened bone: two weeks post-implantation. (H & E x 40).
Figure 21: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of β tricalcium phosphate were added to unweakened bone: eight weeks post-implantation. (H & E x 40).
Figure 22: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of β tricalcium phosphate were added to unweakened bone: 26 weeks post-implantation. (H & E x 40).
Group 4: Cryosurgery only

Two weeks post-cryosurgery (Figure 23):

Section shows the osteocytes within the existing bone are either not present or are undergoing necrotic changes, suggesting the presence of non-vital bone. A small focus of new bone is seen where the periosteum is not in intimate contact with the underlying bone. No signs of acute inflammatory exudate are identified in this field. (H & E x 40)

Eight weeks post-cryosurgery (Figure 24):

Section shows the presence of non-vital bone, as is evidenced by lacunae, void of osteocytes. (H & E x 100)

Twenty six weeks post-cryosurgery (Figure 25):

Section shows that although some non-vital bone is present in the deeper layers of the mandible, vital, new surface bone is seen overlying the non-vital bone. In addition, excellent endosteal regeneration is seen in deeper layers. (H & E x 100)

Group 5: Cryosurgery plus granules of dense hydroxyapatite

Two weeks post-implantation (Figure 26):

Section shows non-vital cortical bone on the surface of which are trabeculae of vital, woven bone. Signs of acute inflammatory exudate are not present in this section. (H & E x 40)

Eight weeks post-implantation (Figure 27):

Section shows sheets of vital new bone on the surface of the non-vital mandibular bone. Within the new bone, osteocytes and osteoblasts with large, plump nuclei are seen. The surface of the new bone is irregular. (H & E x 40)
Figure 23: Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: two weeks post-cryosurgery. (H & E × 100).
Figure 24: Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: eight weeks post-cryosurgery. (H & E x 100).
Figure 25: Coronal section through the left mandibular ramus of a rat at the level of the lingula. The ramus was subjected to cryosurgery: 26 weeks post-cryosurgery. (H & E x 100).
Figure 26: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: two weeks post-implantation. (H & E x 100).
Figure 27: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: eight weeks post-implantation. (H & E x 100).
Figure 28: Coronal section through the left mandibular ramus of a rat at the level of the lingula. Granules of dense hydroxyapatite added to a cryolesion: 26 weeks post-implantation. (H & E x 40).
Twenty six weeks post-implantation (Figure 28):
Section shows that within the mandibular bone some non-vital bone can be identified. Overlying this, however, regular thick, remodelled bone, indicative of considerable new bone formation, is seen. (H & E x 40)

Group 6: Cryosurgery plus granules of $\beta$ tricalcium phosphate
Two weeks post-implantation (Figure 29):
Section shows, non-vital mandibular bone with morphological evidence of endosteal regeneration and some subperiosteal new bone formation. There is no cellular inflammatory exudate suggestive of acute inflammation present. (H & E x 40)

Eight weeks post-implantation (Figure 30):
Section shows new, vital endosteal bone can been adjacent to the marrow. Although the mandibular bone is non-vital, a layer of new bone is present on the cortical surface. (H & E x 40)

Twenty six weeks post-implantation (Figure 31):
Section shows thick new cortical bone which has undergone remodelling. Some non-vital bone is present within the body of the ramus. (H & E x 40)

Group 7: Cryosurgery plus sintered blocks of dense hydroxyapatite
Two weeks post-implantation (Figure 32):
Section shows non-vital bone overlying which a small amount of bone and fibrous tissue are seen. (H & E x 40)

Eight weeks post-implantation (Figure 33):
Section shows some trabeculae of new bone overlying non-vital bone. (H & E x 40)
Figure 29: Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: two weeks post-implantation. (H & E x 100).
Figure 30: Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: eight weeks post-implantation. (H & E x 40).
Figure 31: Coronal section through the left mandibular ramus of a rat, at the level of the lingula. Granules of β tricalcium phosphate added to a cryolesion: 26 weeks post-implantation. (H & E x 40).
Figure 32: Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: two weeks post-implantation. (H & E x 100).
Figure 33: Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: eight weeks post-implantation. (H & E x 40).
Figure 34: Coronal section through left mandibular ramus of a rat at the level of the lingula. A rectangular block of dense hydroxyapatite has been added to a cryolesion: 26 weeks post-implantation. (H & E x 40).
Twenty six weeks post-implantation (Figure 34):
Section shows thick, mature, remodelled bone overlying non-vital bone. (H & E x 40)

In order that the effects of both implant materials could be evaluated more fully, an implant control group, using carbonised wood, was added to the study. The implant control was implanted on unweakened bone and also on bone subjected to cryosurgery. Histological specimens were prepared two and eight weeks post-implantation. The results of the histological sections prepared eight weeks after cryosurgery and implantation are presented in this section.

Implant Controls (Carbonised Wood)

Surgery plus wood (Figure 35):
Section shows a thin layer of new bone at the margins of the implant, although no new bone is present within the implant. No cellular inflammatory exudate is evident. (H & E x 40)

Cryosurgery plus wood (Figure 36):
Section shows non-vital bone, evidenced by empty lacunae, throughout the mandibular bone. A thin layer of new bone is present at the margin of the implant where the periosteum has lifted. This section shows no signs of cellular inflammatory exudate. (H & E x 100)
Figure 35: Section through left mandibular ramus of a rat at the level of the lingula. Carbonised wood implant control added to unweakened bone: eight weeks post-implantation. (H & E x 40). IS = Implant Space.
Figure 36: Section through left mandibular ramus of a rat at the level of the lingula. Implant control added to a cryolesion: eight weeks post-implantation. (H & E x 40). IS = Implant Space.
"A patient with a false eye cannot see, a patient with a false leg cannot run, but many patients expect to look and function with dentures as well as, or better than, they did with their natural dentition."

This aphorism by Appelbaum (1984) concisely illustrates the problems faced by the prosthodontist. These problems are aggravated where excessive alveolar resorption has taken place, especially in the atrophic mandible; in these cases, the provision of, and wearing of, satisfactory complete dentures is a taxing problem. It is for these patients, principally, that dental implants have been developed.

This thesis contains a comprehensive review of dental implants, including their classification and ideal properties. The mechanical and physical properties of most implant materials are described, in addition to a detailed account of a wide range of host-tissue: implant interfacial reactions; these interfacial reactions range from intimate bone:implant contact, through varying degrees of fibrous tissue enveloping the implant, to tumour induction.

Scientific evaluation of dental implant materials and techniques have not all been either ideal or objective (Adell et al 1970; Natiella et al 1972). Recent research, however, suggests that two implant techniques have combined clinical success with long term scientific evaluation. The first of these is called osseointegration, where, after a careful surgical technique and a two-stage surgical procedure, titanium implants are effectively
ankylosed into the alveolar bone (Adell et al. 1981). Although clinical success over 15 years is described, this technique is unlikely to be widely practised in Great Britain for two reasons; firstly as 54% of the population of Great Britain aged 55-64 years are edentulous (Todd et al. 1982) this means the numbers of patients involved are considerable. Secondly, as the titanium implants, titanium instruments and accessory equipment are extremely expensive, the financial cost of treating even a proportion of these patients would be inordinately high.

The second technique of promise is the use of the bioceramics of calcium phosphate to augment resorbed ridges. Two such materials used are hydroxyapatite and tricalcium phosphate. As both materials are normal constituents of bone (Matthews 1980), then their use as dental implants is logical; how they are best used remains to be determined; although case reports indicate that both materials satisfactorily augment resorbed ridges, their effects on bone strengths, until now, have not been investigated.

Thus the aims of this investigation were to:-

1. Develop an experimental model to assess the effects of both dense hydroxyapatite and ß tricalcium phosphate on bone strength.

2. Compare the relative effects of the two implant materials on the strength of weakened and unweakened bone.

3. Compare, histologically, the effects of both implant materials on weakened and unweakened bone.
4. Relate these findings to clinical aspects of dentistry.

Although both hydroxyapatite and tricalcium phosphate have been used in humans, a survey of the literature failed to record any details of previous in vitro assessment of these materials. Initial investigation of any biomaterial should combine in vitro and in vivo trials before the biomaterial is used on humans (Autian 1981). It was therefore decided to include in vitro testing of both hydroxyapatite and tricalcium phosphate in this study. This was performed by the addition of samples of each material to culture petri dishes in which confluent explant cultures of human fibroblasts had been incubated. After 10 days, the test cultures were photographed in their dishes. That both test materials were clearly biocompatible to human fibroblasts is demonstrated in Figures 9 and 10.

The in vitro study indicated the biocompatibility of both hydroxyapatite and tricalcium phosphate and justified their use in a controlled in vivo study. Before in vivo clinical trials are performed it is customary, if not advisable, to assess the implant material on an experimental animal model (Autian 1981). Animal experiments may be justified on the need to know basis before clinical trials are performed in order that any technique or material may be evaluated scientifically. In addition, if an animal experiment is to be meaningful, and justifiable, then it should be standardised with sufficient controls to ensure reproducibility of results. A major disadvantage of animal experimental procedures is that no direct or facile comparison can be made to any human situation; furthermore, species difference
often prohibits the use of more than one animal type within any experiment. These factors, taken collectively, indicate that a considerable number of experimental animals may be required in any experiment. Moreover, when large numbers of animals are required, the choice of animal is often restricted to rats or mice purely on the grounds of cost (Murphy 1971; Frame 1984; Kavanagh et al 1985). A further consideration is that established experimental techniques should be used, whenever possible, to reduce unnecessary use of animals. For these reasons, it was decided to use the rat as the animal model in this investigation and to modify the experimental technique used by Fisher et al (1977). In this technique, the left masseter and its periosteum were elevated off the left mandibular ramus and a cryolesion induced on the exposed ramus before wound closure; animals were sacrificed at two, four, eight, 16 and 26 weeks. Left and right mandibles were dissected free of all soft tissue and the fracture strength of each mandible was determined by a three-point bending test; percentage differences between test and control sides were used to evaluate the statistical significance of any difference. Histological sections were made from the fractured mandibles.

The necessity for controlled experiments in any scientific investigation has been alluded to previously in this chapter. For this reason, 231 adult male Sprague-Dawley rats were placed into seven groups in order that controlled studies of the effects of adding dense hydroxyapatite and β-tricalcium phosphate to exposed rat mandibular bone, could be evaluated. Table 4.1 itemises these groups. The animals in Group I served as the surgical control; wound closure followed exposure of the left mandibular ramus by
elevation of the left masseter muscle and its periosteum. The animals in this group were sacrificed after two, eight and 26 weeks. The animals in Group 2 underwent an identical surgical procedure to those in Group 1, except that 100 mg of dense hydroxyapatite were added to the exposed mandible prior to wound closure. The animals in Group 3 also underwent an identical surgical procedure to those in Group 1, except that 100 mg of β-tricalcium phosphate granules were added to the exposed mandible prior to wound closure. The animals in Groups 2 and 3 as per those in Group 1 were sacrificed two, eight and 26 weeks post-operatively. Groups 1, 2 and 3 comprised the study on unweakened bone.

The animals in Group 4, after exposure of the left mandibular ramus, were subjected to a cryolesion at -16°C prior to wound closure. The animals in Group 4 were sacrificed after two, four, six, eight, 10, 12 and 26 weeks post-operatively. Previous studies by Fisher et al. (1977) demonstrated that bone subjected to cryosurgery is significantly weaker especially eight weeks after the induction of a cryolesion; this group served as a control group for weakened bone. The animals in Group 5 received identical treatment to those in Group 4 except that, prior to wound closure, 100 mg of dense hydroxyapatite granules were added to the cryolesion. The animals in Group 6 also received identical treatment to the animals in Group 4 except that, prior to wound closure, 100 mg of β-tricalcium phosphate granules were added to the cryolesion. The animals in Groups 5 and 6 as per those in Group 4 were sacrificed two, four, six, eight, 10, 12 and 26 weeks post-operatively. The animals in Group 7 received identical
treatment to those in Group 4 except that, prior to wound closure, 100 mg blocks of dense hydroxyapatite, one per animal, were added to the cryolesion. The animals in Groups 4, 5, 6 and 7 comprised the study on bone weakened by cryosurgery. In addition it was possible to evaluate the effects of implant forms on bone strength by comparing the granular form of dense hydroxyapatite (Group 5) to a sintered block of dense hydroxyapatite which was of equal weight (Group 7).

Prior to the investigation proper, it was decided to run a pilot study on the fracture strengths of five adult male and five adult female Sprague-Dawley rats. The purposes of this pilot study were twofold: first it afforded an opportunity to standardise the three-point bending tests on a Howden servo-hydraulic universal testing machine. Second it served as a non-surgical control.

In this study five male and five female animals were sacrificed without being subjected to any previous surgery. Sacrifice was by desiccation. It was determined (Table 7.1) that for the male animals the mean for the right mandibles was 9.22 kiloNewtons (standard deviation ± 0.526) while the mean value for the left mandible was 9.225 (standard deviation ± 0.475). The mean ratio of left:right mandibular fracture strengths for the male group was 1.001 ± 0.0521. The corresponding values for the female group were 7.48 ± 0.720 for the right mandibles and 7.49 ± 0.720 for the left mandibles. The mean left:right ratio for the female fracture strengths was 1.0028 ± 0.1033. These results indicate that left and right fracture strengths in this control group were virtually identical. For this reason it was decided to use the
ratio of left mandibular fracture strength to right mandibular fracture strength (L:R) to evaluate the effects of dense hydroxyapatite and β tricalcium phosphate on mandibular bone. As the right mandibles serve as an 'animal' control, the left:right ratio thus enables a clearer, more comparative, assessment of the investigative technique than the percentage difference chosen by Fisher et al (1977). Further, each left fracture strength was divided by the mean of the right fracture strengths and five ratios determined. From these five ratios, a more conservative left:right ratio was evolved.

The amount of implant material used was determined during a preliminary study. In this study, a 100 mg block of dense hydroxyapatite adequately covered the area of cryolesion induced after cryosurgery of the mandible. Similarly 100 mg of granular hydroxyapatite adequately covered the lesion and this weight of hydroxyapatite was therefore used in the study. One hundred milligram weights of β tricalcium phosphate were used to standardise the comparative study. Dense hydroxyapatite is denser than β tricalcium phosphate as is evidenced by granules of β tricalcium phosphate occupying a larger volume than those of hydroxyapatite. Granules of β tricalcium phosphate are porous and irregular in shape whereas granules of dense hydroxyapatite are rounded and non-porous and volumetric methods of measurement of equal amounts of both materials were discounted in favour of equal weights of material being tested.

Both male and female results demonstrate that for each sex, little difference occurred in the fracture strengths of left and right mandibles. The female group, however, gave lower mean
fracture strengths and higher standard deviations than the male group; this may be attributable to an altered calcium:phosphate metabolism in the breeding female rat brought about by the fact that the female rat goes into oestrus shortly after the birth of a litter. The gestation period of only three weeks means that up to six litters a year are possible. For these reasons it was decided to use adult male rats in the investigation proper. A further value of the pilot study was to create a non-surgical control group. Any surgical operation or careless handling may result in a rat being highly stressed (Kreezer 1949). One possible effect of stress is an increased production of cortisol which may result in dissolution of bone (Ganong 1983). Comparison of the fracture strengths of the two week surgery control and those of the male non-surgical controls using a paired t-test resulted in a 'P' value >.1 indicating no significant difference. The effects of stress could thus be discounted in this experiment.

The experimental procedure itself proved to be very successful; it is not uncommon to experience an appreciable number of deaths under anaesthesia if, for example, barbiturates are used as the anaesthetic agent. This has been attributed to a higher fat content in the adult rat, increasing the body weight of the animal and hence increasing the likelihood of overdosage. In this experiment the anaesthetic agent used was etorphine hydrochloride (Small Animal Immobilon). The dosage used was 0.10 per 100 gm body weight of a 50/50 solution of Small Animal Immobilon and sterile water. Although this chemical is potentially dangerous to man, only five animal deaths occurred out of 231, amounting to a 2.1% mortality rate. No animal deaths occurred after an equal dose of
the antidote diprenorphine hydrochloride was administered. In all cases, the animals were able to eat standard rat and mouse diet within two days after operation. In those animals sacrificed two weeks after operation, it was found that the weight of the animals in each group was ± 20 gm of their pre-operative weight, in each group, indicating that the experimental procedure produced no lasting adverse effects. Of the 231 animals in the study, only two died before sacrifice: both animals were in that group investigating the effects of β tricalcium phosphate on both previously subjected to cryosurgery. Both animals died one week before their allocated sacrifice time, viz. 10 and 26 weeks. Post-mortem studies revealed they had middle ear disease; abscess cavities were present in their auditory canals. Both animals were excluded from the study and in each group only four animals per sub-group have been included in the table of fracture results.

The measurement of fracture strengths by three-point bending tests involves a combination of compression, tensile and flexural strengths of bone. The rate of flexure may affect fracture values and, in this experiment, was standardised by pre-setting the cross-head speed of the ascending ramp to 3 mm per minute; this rate had previously been shown to give consistent results (Fisher et al 1977).

When control rat mandibles were three-point bend tested, fracture occurred at irregular sites; the site of fracture was made consistent, however, by supporting the mandible, under test, on a rectangular block of polyurethane foam. Although the presence of the foam precludes measurement of the elastic modulus of bone, it has no effect on the measurement of fracture strength.
The effect of surgery, alone, on the fracture strength of bone is illustrated, graphically, in Figure 11. It can be seen that two weeks after operation, the mean ratio of left:right fracture strengths was 1.016 with a standard deviation of ± 0.072. As has been discussed, these results were not significantly different from non-surgical controls, when analysed statistically, 'P' = .1. Eight weeks post-operatively, the mean ratio of left:right fracture strengths was 1.08 with a standard deviation of ± 0.056. Similarly the mean ratio of left:right fracture strengths 26 weeks after surgery was 1.002 with a standard deviation of ± 0.068. As all three ratios are banded closely together, barely above unity, it can be concluded that the operative technique does not weaken bone. This fact was confirmed histologically by the presence of apparently healthy osteoblasts on and osteoclasts within bone in Figures 15 and 16 indicating that the mature bone present was healthy. No, or negligible amounts of new bone were formed when the periosteum was repositioned in close proximity to the mandible; where spacing existed between bone and periosteum, some new bone was deposited (Figure 14).

These findings confirm that the first aim of the investigation was achieved, namely that an experimental model be developed to assess the effects of both dense hydroxyapatite and tricalcium phosphate on bone strength. The mean ratio of left:right fracture strength tests performed two weeks after the addition of dense hydroxyapatite granules to exposed mandibular bone are seen in Table 7.2 to be 1.309 with a standard deviation of ± 0.087. Where these values were compared to the equivalent surgical controls, by analysis of variance, a 'P' value of <.001
was obtained (Table 7.3b). The increase in fracture strength two weeks after the addition of 100 mg of dense hydroxyapatite is, therefore, highly significant. Histological sections taken at this time (Figure 17) show considerable amounts of new woven bone being deposited in a trabecular pattern. Eight weeks after the addition of dense hydroxyapatite granules, the mean ratio of left:right fracture strengths had risen to 1.389 with a standard deviation of ± 0.180. This represents a mean increase in strength of the left mandibles of almost 40%. This result is also significant when compared to the corresponding surgical control. Similarly the mean ratio of left:right fracture strengths obtained after 26 weeks, 1.377 ± 0.192 is also highly significant when compared to its corresponding surgical control. Parallel histological sections at eight and 26 weeks (Figures 18 and 19) indicate that the new woven bone produced after two weeks (Figure 17) has matured with time to produce mature lamellar bone with haevsian systems.

In comparison, the mean ratio of left:right fracture strengths, obtained two weeks after the addition of β tricalcium phosphate granules to exposed, unweakened mandibular bone, of 1.025 ± 0.089 is not significantly different from the two week surgical control group 'P' > .1. This is in accordance with the histological examination of the two week β tricalcium phosphate implant group where no appreciable new bone was formed (Figure 20). This may be due to the fact that the porous nature of the β tricalcium phosphate granules elicited a different response from the dense hydroxyapatite granules. In marked contrast, the mean ratio of left:right fracture strengths eight weeks after the addition of tricalcium phosphate to exposed mandibular bone had risen to 1.79,
with a standard deviation of ± 0.244. When these figures were compared to the corresponding values of the surgical control group, the 'P' value of <.001 (Table 7.3b) was highly significant. Peritz one-way Anova tests of all pairs of categories determined that the values of the eight week β tricalcium phosphate group, when compared to the eight week dense hydroxyapatite group are also highly significant, 'P' = .00621. Histological sections of the eight week β tricalcium phosphate group (Figure 21) indicate appreciable amounts of new bone which appeared to be more mature in nature than that seen in the dense hydroxyapatite group, also indicating a different response of the bone to these two implant materials. Twenty six weeks after the addition of β tricalcium phosphate granules, the mean ratio of left:right fracture strengths had fallen slightly to 1.605 ± 0.177. These values, however, were still highly significant, 'P' = <.001 (Table 7.3b) when compared to the surgical control group. Compared to the 26 week dense hydroxyapatite group, by Peritz one-way Anova test of all pairs of categories, however, the statistical significance 'P' was .08687. These findings are shown graphically in Figure 11 and indicate that granules of β tricalcium phosphate impart greater fracture strength to unweakened bone than granules of dense hydroxyapatite except after two weeks. The dense hydroxyapatite granules, however, significantly increase the fracture strength of mandibular bone, especially after two weeks.

If these results on an animal model could be related to a human clinical situation, then both materials might be used to increase the strength of mandibular bone. Possible clinical examples of this could be the augmentation of atrophic mandibles or
the infilling of large cavities within the mandible resulting from the enucleation of large dentigerous cysts. The latter surgical operations, potentially render mandibles prone to the possibility of post surgical fracture.

Both dense hydroxyapatite and β tricalcium phosphate have the potential to be of use clinically to increase bone strengths. The investigation on unweakened bone has, in part, fulfilled some of the aims of the investigation by comparing both implant materials and indicating possible clinical roles of both materials.

The animals in Group 4 (Table 4.1) served as cryosurgery controls. In order that a thorough analysis of the effects of cryosurgery on the fracture strength of bone be performed, fracture tests were performed two, four, six, eight, 10, 12 and 26 weeks post-cryosurgery. The results are listed in full in Table 7.4. Two weeks after cryosurgery, the mean ratio of the fracture strengths of left and right mandibles was 1.031 with a standard deviation of $\pm$ 0.051. When analysed against the corresponding surgical control, no significant difference occurred, 'P' = .946. Four weeks after cryosurgery, the mean ratio of left:right fracture strengths was reduced to 0.862 $\pm$ 0.095. A further fall had occurred by six weeks where the ratio was 0.851, standard deviation $\pm$ 0.027. More dramatic was the reduction in the ratio seen at eight weeks post-cryosurgery; the value of the ratio at eight weeks was 0.697 $\pm$ 0.081, a reduction in fracture strength of 30% compared to bone not subjected to cryosurgery. This point represents the nadir of the graph illustrating the effects of cryosurgery on bone strength (Figure12). When the values of the eight week cryosurgery group were analysed statistically against the corresponding surgery
controls, the 'P' value obtained was <.001 which was highly significant (Table 7.5). Ten weeks after surgery, the mean ratio of left:right fracture strengths was 0.828 ± 0.065 and after 12 weeks the ratio was 0.96 ± 0.068. Twenty six weeks after cryosurgery the mean ratio of left:right fracture strengths was 1.162 ± 0.052. When these results were analysed against the corresponding surgical controls, the resulting 'P' value of .01 was significant indicating increased bone strengths some six months after cryosurgery. The physiological aspects of these changes are discussed later in this chapter.

Histological sections of the effects of cryosurgery were studied in parallel to the fracture testing. For uniformity, photographs of histological sections are presented for the two, eight and 26 week sub-groups. Two weeks after cryosurgery some signs of early changes in bone are seen (Figure 23) but not as dramatic as the necrotic bone evidenced by empty lacunae seen in the section eight weeks after cryosurgery (Figure 24). Twenty six weeks after cryosurgery healthy new bone is seen overlying some necrotic bone (Figure 25).

Earlier studies by Fisher et al (1977) suggested that cryosurgery decreased the fracture strength of bone, especially after eight weeks. This investigation, through the use of increased temporal assessment, more powerful statistical analysis and superior histological fixation and analysis has confirmed that bone subjected to cryosurgery suffers a reduction in fracture strength of more than 30% eight weeks post-operatively.

In addition, the results obtained from the cryosurgery control group confirm that this experimental animal model could be
used to investigate the effects of both dense hydroxyapatite and β sintered tricalcium phosphate on bone previously subjected to cryosurgery, i.e. weakened bone.

The results of adding granules of dense hydroxyapatite to mandibular bone previously subjected to cryosurgery are listed in Table 7.6. Two weeks after the addition of granules of dense hydroxyapatite, the mean ratio of left:right fracture strengths was $1.20 \pm 0.129$. Statistical analysis comparing these results to equivalent cryosurgery controls gave a 'P' value of $<.09$ (Table 7.7), a value which is probably biologically significant. Four weeks after adding dense hydroxyapatite to the cryolesions, the mean ratio of left:right fracture strengths was $1.26 \pm 0.129$ and the value after six weeks was $1.195 \pm 0.081$ compared to the corresponding cryosurgery controls, the results after four and six weeks are highly significant, 'P' $= <.001$. Eight weeks after the addition of dense hydroxyapatite granules to the cryolesion, the mean ratio of left:right fracture strengths was $1.441 \pm 0.165$. When compared to the cryosurgery control for the same time group these results were found to be statistically significant 'P' $= <.001$. Peritz one-way Anova testing of all pairs of categories results in a 'P' value of $2.2 \times 10^{-9}$ indicating a highly significant increase in bone strength. This finding is illustrated graphically in Figure 12. The graphs for the effects of cryosurgery on bone are featured in the same graph illustrating the effects of adding 100 mg of dense hydroxyapatite granules to bone previously subjected to cryosurgery.

Ten weeks after the addition of dense hydroxyapatite granules to the cryolesion, the mean ratio of left:right fracture strengths
was $1.32 \pm 0.199$ whereas the value obtained after 12 weeks was $1.587 \pm 0.146$. Twenty six weeks after the addition of dense hydroxyapatite to a cryolesion, the mean ratio of left:right fracture strengths was $1.789 \pm 0.213$. The statistical significance of the results obtained at 10, 12 and 26 weeks was $P' = <.001$ which is highly significant. Peritz one-way Anova test for all pairs of categories gives a 'P' value, for the 26 week time group of $2.8 \times 10^{-5}$ indicating that a highly significant increase in fracture strength has occurred although not as high as that which occurred after eight weeks ($2.2 \times 10^{-9}$). Figure 12 shows that the graph plotting fracture strength ratio against time is not a straight line. Depressions are seen after six weeks and after ten weeks in the graph indicating that bone is not being actively deposited at these points in line; such observations suggest short remodelling cycles of about a week; these findings support the finding of Vignery & Baron (1980) who estimated that the remodelling cycle of rat mandibular bone was of the order of six days compared to 60-120 days in adult human trabecular bone.

Histological examination of animals given in Group 5 (100 mg of dense hydroxyapatite granules added onto bone subjected to cryosurgery and before wound closure) was performed. Two weeks after cryosurgery, fibroblasts with plump nuclei are enclosed in trabeculae of woven bone indicating a rapid rate of bone deposition. This immature bone is seen (Figure 26) overlying bone in which osteocytes, although present, do not look to be healthy.

Eight weeks after operation, the animals in Group 5 are seen (Figure 27) to have considerable amounts of new bone, more mature than that seen in the two week group. This new bone is found
superficial to non-vital bone; the non-vital bone is a consequence of cryosurgery. As nuclei are seen in the new bone, although not in the lesion, the possibility of artefacts causing the lack of nuclei in the mature bone is discounted.

The results following the addition of β tricalcium phosphate to bone previously subjected to cryosurgery are listed in full in Table 7.8. The mean ratio of left:right fracture strengths of the two weeks group was 1.101 with a standard deviation of 0.128. When these results are compared to the corresponding results of the cryosurgery control group (1.031 ± 0.051) and the two pairs analysed for statistical significance, a 'P' value of <.07 (Table 7.7) indicates that the increase in fracture strength is probably significant two weeks after the addition of tricalcium phosphate to bone subjected to cryosurgery. The four week and six week mean ratios of left to right fracture strengths are calculated to be 1.351 ± 0.029 and 1.337 ± 0.110 respectively. These values, when compared statistically to corresponding time groups of cryosurgery controls, were found to be highly significant, 'P' = <.001.

Eight weeks after the addition of β tricalcium phosphate granules to a cryolesion, the mean ratio of left:right fracture strengths was determined to be 1.421 ± 0.133. Peritz one-way Anova test of all pairs of categories determined the level of significance to be 'P' = 5.8 x 10^-9. Although signified as 'P' = <.001 in Table 7.7 this value like the corresponding time group in Group 5, is highly significant and indicated that both β tricalcium phosphate and dense hydroxyapatite double the fracture strength of bone weakened by cryosurgery: eight weeks after cryosurgery the mean ratio of left to right fracture strengths of the cryosurgery
control group was $0.697 \pm 0.081$; the equivalent mean ratios of the eight week tests in Groups 5 and 6 were $1.441 \pm 0.165$ and $1.421 \pm 0.133$ respectively. Ten and 12 weeks post-operatively, the mean ratios of fracture strengths of animals which received $\beta$ tricalcium phosphate granules over cryolesions, were slightly higher than the eight week groups. The values of the 10 and 12 week ratios were $1.462 \pm 0.057$ and $1.451 \pm 0.092$ respectively; both of these values when compared statistically to equivalent time groups of cryosurgery contents were both highly significant, '$P$' = <.001. Although the mean ratio of left:right fracture strengths of the 26 week time group of animals, in Group 6, was $1.569 \pm 0.101$, statistical analysis against equivalent cryosurgery control values was found to have reduced '$P$' = .003. This is listed in Table 7.7 as '$P$' = >.001 but <.005 and may still be considered highly significant.

Histological photographs of sections reported two weeks after $\beta$ tricalcium phosphate granules were added to a cryolesion (Figure 29) indicate the presence of new bone on the lateral surface of the the mandible. This bone appears to be more mature than the trabecular bone seen in the corresponding time sub-group of Group 5. More new bone has been deposited by eight weeks (Figure 30) and this bone is seen overlaying non-vital bone. In the 26 week group the histological appearance (Figure 31) was similar to that of Group 5 indicating that any osteogenic effects of both materials had ceased to exert any influence and that normal mature bone with haversian systems and reversal lines was present.

The final group of this investigation, Group 7, was similar in design to Group 5; the only exception was that 100 mg of dense
hydroxyapatite, in the form of a sintered block, were placed over the cryolesion before wound closure. Fracture strength testing and histological investigations were carried out after two, eight and 26 weeks post-operatively. The results of mandibular fracture strength testing of animals which had blocks of dense hydroxyapatite placed over a cryolesion are detailed in Table 7.9. Two weeks after implantation of blocks of dense hydroxyapatite, the mean ratio for fracture strengths was 0.979±0.068. These values when compared to the corresponding cryosurgery control group 1.031±.051 are not significant, 'P' = <.9 (Table 7.7) indicating no statistically significant increase in fracture strength; indeed there is a reduction in the mean fracture ratios which indicate a reduction in fracture strength although this is not statistically significant. In the eight week group, however, the mean ratios of fracture strength, for Group 7, have risen to 1.420±0.080 a value near to Group 5 at eight weeks (1.441±0.165). When the eight week readings of fracture strength for Group 7 animals are compared statistically to those in the equivalent cryosurgery controls the results are highly significant, 'P' = <.001. More detailed analysis using Peritz one-way Anova test for all pairs of categories, the value for 'P' obtained was 5.9 x 10^-8. This is highly significant and compares favourably with the corresponding 'P' values of Group 5 fracture test results ('P' = 2.2 x 10^-9) and Group 6 fracture test results ('P' = 5.8 x 10^-9). Even higher mean ratios of fracture strength tests, 26 weeks post-operatively (1.657±0.151) result in 'P' values of <.001. Histological photographs of sections prepared two weeks after operation show a small amount of new bone on the mandible although
some fibrous tissue is present (Figure 34). Figure 35, however, is representative of the eight week group and here trabeculae of woven bone can be seen overlying non-vital bone. The photograph taken after 26 weeks shows mature bone with haversian systems (Figure 36) similar in appearance to the bone seen in the corresponding Group 5 and Group 6 sub-groups.

Group 7, by virtue of the block form of the dense hydroxyapatite, enabled a comparison of the strength effects of forms of hydroxyapatite. The granular form produces greater fracture strength than the block form after two weeks; this increase in strength is highly significant; 'P' = <.005, >.001. Thereafter no significant difference between Group 7 and Group 5 were found either after eight weeks ('P' = .99509) or after 26 weeks 'P' = .70051). An explanation for these findings may be that the granular form of dense hydroxyapatite has a greater surface area than the block form and thus fibrous tissue, before progressing to calcified tissue via osteoid, is able to envelop the implant more comprehensively. Another possible explanation for the poor early strength of the block form of dense hydroxyapatite is that any rectangular block has eight surface angles which would cause irritation before they are enveloped by fibrous tissue prior to mineralisation.

The investigation into the effects of dense hydroxyapatite and β tricalcium phosphate, on bone subjected to cryosurgery, has confirmed that both materials increase the strength of bone subjected to cryosurgery. The increase in strength is probably significant at two weeks after the addition of granules of dense hydroxyaptite and granules of β tricalcium phosphate, but not
significant when dense hydroxyapatite is in the form of a block. At all other times, the increase in fracture strength of all forms of dense hydroxyapatite and of $\beta$ tricalcium phosphate is highly significant.

It may be that all of these findings are species dependant; to determine if these findings are species dependant or not, further studies are indicated in other animals. If these studies indicate that the increases in fracture strength are not species dependant, then human clinical valuation is possible. Cryosurgery has been used in the treatment of oral cysts and oral tumours, often with a potential for post-surgical fractures of the mandible to occur (Gage et al 1966; Marcove et al 1978). Where the possibility exists for post-surgical or post-cryosurgical fracture of the mandible, then dense hydroxyapatite or $\beta$ tricalcium phosphate might be used to augment and strengthen residual mandibular bone.

In the study of both dense hydroxyapatite and $\beta$ tricalcium phosphate on unweakened bone, the latter proved to elicit the greater increase in fracture strength; in the study on bone subjected to cryosurgery, dense hydroxyapatite elicited the greater increase in mean ratios of fracture strength. When the bone under investigation was unweakened bone, the optimum mean ratio of fracture strengths was $1.790 \pm 0.244$; this value was recorded eight weeks after $\beta$ tricalcium phosphate granules were added to exposed but unweakened mandibular bone. In comparison, in the same study, dense hydroxyapatite produced its highest effect 26 weeks after it was applied to exposed, unweakened bone where the mean ratio of fracture strengths was $1.377 \pm 0.192$. 
In the study on unweakened bone, the poor early strength achieved by β tricalcium phosphate is probably a result of its porous nature, early bone forming within the pores of the implant material before it forms on the mandible; in contrast, dense hydroxyapatite has no pores and would encourage new bone formation on the surface of the mandibular bone. The nature of these osteogenic properties will be discussed in a following paragraph.

An interesting observation is that dense hydroxyapatite elicits greater fracture strength increases on weakened bone and that this is achieved at 26 weeks, with a mean ratio of $1.789 \pm 0.212$. This value is almost identical to the optimum value attained by β tricalcium phosphate in the study on unweakened bone ($1.790 \pm 0.244$) indicating that there is, perhaps an optimal value for bone fracture strength. A second interesting observation concerning granular dense hydroxyapatite is that its values for mean ratios of fracture strengths at eight weeks, on the studies of both weakened and unweakened bone, are similar, $1.441 \pm 0.165$ in the study of weakened bone and $1.389 \pm 0.180$ in the study on weakened bone.

The reasons for the changes in fracture strengths of both materials may be explained by changes in blood supply. Kuylenstierna et al (1980$^1$) stated that cryosurgery on bone achieves its effect through microvascular arrest. As blood vessels within bone are destroyed, then bone, which is a highly dynamic and sensitive organ, is rendered effectively necrotic, hence by eight weeks the non-vital bone found in the study of weakened bone. If blood flow has ceased temporarily, and therefore as oxygen gradient is decreased, granulation tissue (which organises into osteoid and,
eventually bone) cannot grow into pores of $\beta$ tricalcium phosphate to form new bone. As $\beta$ tricalcium phosphate is resorbable perhaps resorption of this biomaterial is taking place concurrently with the delayed formation of bone within it, hence producing reduced fracture levels compared to the study on unweakened bone.

The loss or reduction in blood flow hypothesis may also explain the reduction in the fracture strength ratio of dense hydroxyapatite seen after two weeks. In the study on unweakened bone, the mean ratio of fracture strength was $1.309 \pm 0.087$; in the study on weakened bone, the mean fracture ratio was $1.20 \pm 0.129$. As dense hydroxyapatite is non-resorbable and non-porous, it is less dependant on blood flow than the porous $\beta$ tricalcium phosphate, and this factor may well explain the differences between the two materials in the study on weakened bone.

Although histological findings permit a comparative study of the effects of dense hydroxyapatite, they also reveal some similarities. These histological findings support and confirm the opinions of Jarcho (1981) and Piecuch (1982) who stated that neither of the materials was osteoconductive; this means that when either or both materials are placed in soft tissue, bone will not form. This is clearly demonstrated in Figure 20, where some particles of $\beta$ tricalcium phosphate have been "implanted" in soft tissue. A fibrous reaction has taken place, with no bone formation evident. It would therefore appear that both materials are osteoconductive and that they achieve their effects by acting as a scaffold to raise the periosteum. To verify this hypothesis, a small study was initiated whereby biocompatible carbonised wood was placed on exposed weakened and unweakened bone as per dense hydroxyapatite and
β tricalcium phosphate. Histological assessments were performed after two and eight weeks. It was seen (Figures 35 and 36) that the scaffolding effect of carbonised wood produced bone in both weakened and unweakened bone but the new bone formed around this implant and not on the mandibular bone as was seen on β tricalcium phosphate and dense hydroxyapatite. One can therefore conclude that β tricalcium phosphate and dense hydroxyapatite achieve their effects through a combination of scaffolding and also through some chemical or physio-chemical means.

In light of the above discussion, the following conclusions may be drawn from the results of the studies reported in this thesis.

The non-toxicity and biocompatibility of both dense hydroxyapatite and β tricalcium phosphate have been confirmed through in vivo testing testing with explant culture of human fibroblasts and the results illustrated.

The rat model used in the investigation reported in this thesis has been demonstrated to be suitable for the study of implant materials on mandibular bone. In addition, the model may be extended to allow study of the effects of new implants on bone that is either weakened or unweakened. Moreover, this surgical technique has been demonstrated through the use of non-surgical controls to result in no appreciable increase in stress. Further confirmation of the efficacy of the experimental method was that, out of 231 rats, five animal deaths occurred, a mortality rate of 2.1%. This low mortality rate was undoubtedly attributable to the suitability of etorphine hydrochloride as the anaesthetic agent.

The results of a pilot study indicated that regardless of the sex of the rat, the fracture strengths on left and right side
are essentially equal. The female rats, however, exhibited lower fracture strengths than the male with much higher standard deviations of these fracture strengths. A possible explanation for this is an altered calcium:phosphate metabolism in the breeding female rat.

When fracture strengths between groups were compared, the method adopted was to compare mean ratios of left:right fracture strengths. This simple calculation resulted in values which permitted visual comparison between groups and was much easier to follow than the use of percentage difference referred to in a previous study (Fisher et al 1977). In addition, the decision to divide each left fracture strength by the mean right fracture strength resulted in a mean ratio of left:right fracture strengths which enabled conservative evaluation of any changes in the ratio, with time, or as a result of experimental method.

When the left masseter and its periosteum were elevated to expose the entire lateral border of the mandible and the wound closed without any implant being inserted, then the effect of surgery alone, on bone strength, was not significant. Histological investigations of this technique indicated that when periosteum was laid down in close proximity to bone, no new bone formed; where a space existed, however, some new bone developed.

When granules of dense hydroxyapatite were implanted between the left mandibular ramus and its periosteum, the fracture strength of mandibular bone was found to have increased significantly; whereas mean ratios of left:right fracture values of control animals would be expected to be unity, the values found in this group at two, eight and 26 weeks were $1.309 \pm 0.087; 1.389 \pm 0.180$ and $1.377$
respectively. Histological investigations used in parallel to fracture strength tests indicated that significant quantities of new bone were developed.

The effects of granules of β tricalcium phosphate on unweakened bone were not as marked as those of dense hydroxyapatite after two weeks. After eight weeks, however, the mean ratios of left to right fracture strengths had risen to 1.79 ± 0.244. Although this value fell to 1.605 ± 0.177 after 26 weeks it indicates, nevertheless, highly significant increases in bone strength.

β tricalcium phosphate has been demonstrated to produce higher fracture strength than dense hydroxyapatite, on unweakened bone. It must be stated, however, that greater early strength is achieved with dense hydroxyapatite than with β tricalcium phosphate.

The parallel use of fracture strength testing and histological testing allows a comprehensive assessment of the effects of biomaterials on bone. The planned use of seven animals in each group enabled a complete temporal sub-group to be operated on in one session, helping to reduce operator, machine induced and random errors.

This study has confirmed that when bone is subjected to cryosurgery then, after a period of eight weeks the fracture strength of that bone has decreased by over 30%. This reduction in bone strength is considerable and, as will be discussed later, has obvious clinical relevance. A further finding within this thesis, however, was that bone subjected to cryosurgery had increased fracture strength after a period of six months; the mean ratio of left to right strengths rose to 1.162 ± 0.052. This tardy increase in fracture strength is attributable to the presence of
the non-vital bone itself, which forms a perfect physio-chemical scaffold for new bone to form under the repositioned periosteum. The planned use of a cryosurgery control model, followed closely at fortnightly intervals from two to 12 weeks, confirmed earlier findings of Fisher et al (1977) that cryosurgery weakens bone. The rat, with a bone remodelling cycle of six days (Vignery & Baron 1980) is an ideal animal to evaluate bony changes; thus fortnightly intervals of the study of weakened bone enabled accurate assessment of bony changes.

When granules of dense hydroxyapatite were implanted onto weakened bone, highly significant increases in the fracture strength of the weakened bone occurred, especially from after the fourth implantation week. The increases in bone strength achieved after the implantation of dense hydroxyapatite increased up to 26 weeks where a mean ratio of left:right fracture strengths of 1.789 ± 0.213 occurred. Rectangular blocks of dense hydroxyapatite produce no biologically significant increases in bone strength until the eighth post-implantation week. The presence of the block of dense hydroxyapatite was found to have decreased the fracture strength of bone slightly at the second post-operative week, as is evidenced in Figure 13. This has been attributed to reduced surface area and to potentially irritant effects of eight face angles in the rectangular block compared to granular forms of dense hydroxyapatite.

The effects of the addition of granules of β tricalcium phosphate to previously weakened mandibular bone were also found to be significant although the increases in bone fracture strength were less than those observed for granular dense hydroxyapatite.
Moreover, the optimal mean ratio of left:right fracture strengths on weakened bone was lower than the corresponding value after \( \beta \) tricalcium phosphate was added to unweakened bone. This latter fact has, it is hypothesised, been attributed to the microvascular arrest of blood vessels in the mandibular bone caused as a result of cryosurgery (Kuylenstierna et al. 1980). An additional finding of these studies was confirmation of the resorbability of \( \beta \) tricalcium phosphate; Figure 20 illustrates resorption of this material by multinuclear giant cells, presumably osteoclasts.

Results from the study on weakened bone allow favourable comparison between implants of dense hydroxyapatite and \( \beta \) tricalcium phosphate. Moreover, although similar comparisons were possible in the studies on unweakened bone, different results occurred. Where bone has been weakened after cryosurgery, higher fracture strengths of bone are achieved from implants of dense hydroxyapatite than from \( \beta \) tricalcium phosphate.

When an inert biocompatible implant control was evaluated, it was observed that new bone formed between the inert implant and the overlying periosteum. This supports the observation made earlier that if the periosteum is not replaced in intimate contact with bone, then some new bone will form. Jarcho (1981) and Piecuch (1982) stated that neither \( \beta \) tricalcium phosphate nor dense hydroxyapatite were osteoconductive, a fact confirmed in Figures 17 and 20, which illustrate that if these biomaterials are implanted in connective tissue, fibrous tissue and not bone is formed. These two factors therefore suggest that when \( \beta \) tricalcium phosphate and dense hydroxyapatite are implanted, they promote new bone growth by means of a scaffolding effect, in addition to some chemical or
physiochemical means.

Review of the above conclusions allows certain clinical protocols to be formulated for the use of these materials, under the assumption that these conclusions are tenable for human clinical conditions.

1. Granules of both \( \beta \) tricalcium phosphate and dense hydroxyapatite may be used to increase the strength of unweakened bone. If no immediate gain on fracture strength is desired, then tricalcium phosphate may be used, for example after periodontal surgery. If, however, a clinical condition predisposes mandibular bone to considerable stresses and early increases in fracture strength are desired, then granular dense hydroxyapatite is preferable; such a clinical condition could be the enucleation of a large dentigerous cyst near the angle of the mandible or in a very resorbed mandible. Furthermore, as dense hydroxyapatite is non-resorbable, in contrast to tricalcium phosphate, then the former material is more ideal for ridge augmentation case where complete dentures are to be worn subsequently as pressure from the dentures themselves and/or from forces of mastication would be likely to accelerate resorption of \( \beta \) tricalcium phosphate.

2. Cryosurgery is used routinely in oral conditions and oral surgeons should be aware of the potential consequences of freezing mandibular bone. Where extensive mandibular cryosurgery is proposed, additional fracture strength could be achieved by the incorporation of granules of dense hydroxyapatite immediately after the cryosurgery.

3. Granular dense hydroxyapatite is the preferred implant material for use on weakened bone, especially where cryosurgery has been used
to treat multilocular lesions of the angle of the mandible. Although rectangular blocks of dense hydroxyapatite give good increased fracture strength after eight weeks, they may be of value in the maxilla, but not in mandibular defects where the possibility of pathological fracture necessitates early increases in bone strength.

The studies in this thesis have been an attempt to evaluate the effects of two implant materials on rat mandibular bone. It is hoped that the data presented may be useful in the understanding of the effects of dental implants on bone.
BIBLIOGRAPHY


DAHL, G.S.A. (1943) Om möjligheten för implantation i kaken av metallskellest som bas eller retention for fasta eller avtag bava protesor. Odontologisk Tidskrift, 51, 440-446.


Calcium phosphate resorbable ceramics: a potential alternative to bone grafting. Journal of Dental Research, 52, (Special Issue) 123, Abstract No. 259.


GREENFIELD, E.J. (1913) Implantation of artificial crown and bridge abutments. The Dental Cosmos, 28, 364-369.


SMITH, D.C. (1975) Biomaterials in dentistry. Journal of Dental Research, 54, (Special Issue B), 146-152.


TURNER, F.C. (1941) Sarcomas at sites of subcutaneously implanted bakelite discs in rats. Journal of the National Cancer Institute, 2, 81-83.


WILLIAMS, D.F. (1981)\textsuperscript{1} Implants in dental and maxillofacial surgery. Biomaterials, 2, 133-146.


Figure 1: Left masseter muscle exposed after submandibular incision. The associated anatomy and two tying-off ligatures (A&B) are labelled on the overlay.
Figure 1: Left masseter muscle exposed after submandibular incision. The associated anatomy and two tying-off ligatures (A&B) are labelled on the overlay.
Figure 2: Left mandibular ramus exposed by reflection of left masseter muscle and its periosteum. An outline of the mandible is drawn on the overlay to facilitate orientation.
Figure 2: Left mandibular ramus exposed by reflection of left masseter muscle and its periosteum. An outline of the mandible is drawn on the overlay to facilitate orientation.