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STUDIES OF MANDIBULAR FRACTURE HEALING IN MONKEYS

James R. Hayward
Gerald H. Bonnette
Robert A. Bruce
John M. Gregg

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FOREWORD

The research described in this report was accomplished during the period May 1, 1965, to July 31, 1966.

In conducting the research described in this report, the investigators adhered to the "Principles of Laboratory Animal Care" as established by the National Society for Medical Research.

SUMMARY

Investigations were designed to develop methodology to provide controlled study of jaw fractures in monkeys and evaluate the effect of (1) surgical approach to jaw fractures, (2) duration of the fracture healing period, and (3) mandibular fixation methods on fracture repair.

Twenty-eight young Rhesus monkeys were used and surgical fractures were created in the mid mandibular body. Fixation methods included processed acrylic splints and Risdon wiring incorporating mouth-curing acrylic. Intraoral and extraoral surgical approaches were used and six-, eight-, and ten-week healing periods were evaluated by (1) clinical fracture mobility, (2) radiography, (3) histology, and (4) tetracycline bone labeling.

Parameters for use in the study of jaw fracture healing produced consistent results. Histologic criteria were more precise than clinical rigidity, radiography, or tetracycline labeling. Cartilage production in the lingual surface of the fracture appeared to be related to mobility or ischemia. Lack of immobilization adversely affected fracture repair and caused delayed union. A limited sample revealed no differences in the healing ratio between an extraoral and intraoral approach to the fracture site. Clinical application of the findings are suggested.

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INTRODUCTION

The experimental study of jaw fracture healing poses many challenges. Experimental design, technical procedure, and analysis must plan for the complexities of a unique bacterial environment, the presence of teeth, a complex musculo-osseous anatomy, limitations of animal behavior, and a forced alteration of nutritional habits. Although the literature has described many studies of fracture healing, these investigations frequently have studied fracture healing in the long bones of rodents. In these cases, the application of fixation support has not been necessary due to the natural splinting effects provided by adjacent tibia or fibula. Such fractures heal quickly with minimal care. Unfortunately these studies, although simple in design, do not provide for many of the unique features of jaw fractures. For this reason, our initial studies were concerned with the development of methodology which could develop a model for the controlled study of experimental mandibular fractures in primates.

Preliminary experiments considered fracture healing as it relates to the type of surgical approach (intraoral or extraoral) used to produce the fracture. Based on gross clinical, radiographic, and histologic criteria, the results showed almost an equal distribution of fracture unions and nonunions, with no advantage given to either intraoral or extraoral approaches for the number of observations available in the series. Based on clinical experience with injuries in humans, it was evident that the number of nonunions was exceptionally high. Our study suggested that: (1) healing might be retarded by inadequate immobilization, and (2) a longer duration healing period might be needed for accomplishment of actual fracture union. Because these two important variables, healing time and degree of immobilization, had not been investigated, it seemed necessary and logical to relate these variables to fracture healing on the experimental model developed.

In preface, it was the purpose of continuing studies to evaluate (1) surgical approach to jaw fractures, (2) duration of the fracture healing period, (3) fixation methods for the jaws, and (4) the methodology to provide controlled study of jaw fractures in the monkey. The following information is presented in a manner which will describe the evolutionary development of our present techniques and some conclusions from our accumulated efforts.

MATERIALS AND METHODS

ANIMALS

Our choice of experimental animal has been and remains the young 3-4 kg Macaca Mulatta Rhesus monkey. Because our studies are closely involved with methodology and surgical techniques, it is imperative that the experimental animal morphologically resemble closely the conditions found in man. For example, the animal must be of adequate size for technical considerations. The animal must be able to react physiologically to the surgical procedure (including bacterial contamination), changes in diet, and to the nutritional limitations imposed by mandibular fixation devices. Finally, it is desirable that the metabolism and growth potential of the animal be such that nonunion complications can be produced when there are deterrents to healing. This is important when one considers that so-called nonunion fractures are difficult to produce in many of the smaller subspecies of laboratory animals. We have found the monkey to adequately fulfill all of these requirements and the model fracture to challenge repair processes appropriately.

ANESTHESIA

Our early studies were frustrated in many instances by anesthetic difficulties. We first used intravenous barbiturates titrated through a polyethylene catheter. The technical problems were at times overwhelming. Intravenous catheter placement and maintenance, patent airway without endotracheal tube, anesthesia maintenance, and animal recovery all presented significant barriers to successful experimental surgery. During the past 18 months, we have utilized an experimental drug released by the Parke Davis & Company for animal investigation. This drug has solved most of our anesthesia problems. Phenylcyclidine hydrochloride (Sernylan), described chemically as 1 - (-phenylcyclohexyl) piperidine, hydrochloride, is a potent new drug which produces incapacitation ranging from diminished response to surgical anesthesia. This drug, which acts on the central nervous system, is unlike classical general anesthetic and tranquilizing agents. Even though the animal is incapacitated or completely anesthetized, the following are observed:

1. Simple reflexes, such as the knee jerk, corneal, swallowing, and coughing, are preserved.

2. The eyes may remain open.

3. Muscle tone remains but is not limiting to procedures.

4. Respiration and blood pressure are not depressed, except when near lethal doses are employed.

Utilizing an intramuscular dose of 1 mg/kg of body weight, surgical anesthesia is obtained in 5 to 10 minutes and lasts for about two hours. No endotracheal tube is necessary since the animal can swallow, cough, and clear any obstruction which may occur from blood or debris. It is not necessary to monitor the animal's vital signs. The recovery period, although slightly prolonged (2-3 hours), is uneventful. The drug has been found to work well in primates with effectiveness, but its action diminishes as one progresses down the evolutionary scale. It therefore may have cortical levels as the primary site of action.

SURGICAL PROCEDURE

Our initial experiments which evaluated an extraoral and intraoral surgical approach to the mandible continued, but the design was structured also to evaluate fixation techniques and time of healing.

The surgical procedures were carried out in two stages: First, the mandibular right second primary molar was extracted. One week later the surgical approach was used to (1) remove the permanent tooth bud, (2) create a fracture, and (3) place a transosseous wire at the inferior border of the mandible.

Extraoral Procedure

The anesthetized animal was prepared by shaving the right face and submandibular area and scrubbing the skin with hexachlorophrene soap. The operators scrubbed, gowned, and gloved for the surgical procedure. A 3-centimeter incision was made just below and parallel to the inferior border of the mandible. Facial vessels were tied and divided when encountered. The mandible was then exposed by sharp and blunt dissection. The periosteum was incised and reflected to expose the lateral aspect of the mandible in the area of the deciduous molars and first permanent molar teeth. Sectioning of the mandible was accomplished with a number six round dental bur in the region of the extracted primary second molar. No effort was made to protect or spare the contents of the inferior alveolar canal. Bur holes were then placed approximately 0.5 centimeter above the inferior border of the mandible and the same distance from the fracture margins. A 0.020-inch stainless steel wire was passed through the drill holes and twisted to maintain the fragments of the mandible in a fixed position. The twisted wire was cut and bent over. Soft tissues were closed in layers and the skin was closed with 4-0 black silk sutures. At the completion of the surgery, a postoperative lateral jaw radiograph was obtained.

Intraoral Procedure

A generous buccal mucoperiosteal flap was raised from the right mandible. At the anterior flap margin the incision was extended downward to the buccal sulcus in a vertical oblique direction. The second deciduous molar was extracted and the experimental surgical fracture was completed through the extraction site. Again no attempt was made to spare the inferior alveolar neurovascular structures, and the premolar tooth bud was removed from its crypt. Holes were placed approximately 0.5 centimeter on either side of the fracture lines through the lateral aspect of cortical bone. A 0.020-inch stainless steel wire was passed through the drill holes, and twisted snugly to approximate the fracture fragments. The wire was cut off and bent over to prevent irritation of the soft tissues. The buccal mucoperiosteal flap was then repositioned with 4-0 silk sutures. At the completion of surgery, a postoperative lateral jaw radiograph was obtained.

POSTOPERATIVE MANAGEMENT

In all cases, the animals were placed in their cages in a lateral position with the head hyperextended. Recovery was uneventful following Sernylan anesthesia and the animal was given a commercial animal soft diet postoperatively. All animals maintained or gained weight during the healing period.

METHODS OF IMMOBILIZATION (Figures 1 - 12)

Our earliest animal experiments utilized no intermaxillary fixation. In these experiments a transosseous wire was placed at the inferior border of the mandible, but subsequent results indicated that simple transosseous wiring was an inadequate form of immobilization.

Boyne,* while studying the healing of mandibular vertical ramus osteotomy procedures in the Rhesus monkey, used circummandibular and circumzygomatic wires for intermaxillary fixation. In our laboratory, this technique was applied to three monkeys with less than ideal results. After a short period of time, the wires had become loose, the fracture fragments remained mobile, and, in some cases, the circummandibular wires had cut into the base of the mandible in response to the animals' continuous efforts at opening their mouths.

In a third technique, attempts were made to ligate the teeth with wires for intermaxillary fixation. This method also proved unsatisfactory because of the conical shape and incomplete eruption of the mixed dentition. The technique of intermaxillary fixation had also resulted in significant weight

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*Boyne, P. J. Osseous Healing after Oblique Osteotomy of the Mandibular Ramus. J. Oral Surg., 24:125-33. March 1966.

loss and other technical difficulties.

In yet another method for immobilization, a mandibular splint was constructed. For this procedure the animals were anesthetized with Sernylan, alginate impressions were taken of the mandibles and maxillae, and a labial-lingual acrylic mandibular splint was constructed (Figure 8). The splint was designed to lock into undercuts at the cervical margins of the teeth to facilitate application and retention. In order to augment the retentive properties of the splint, quick-cure acrylic was applied to the processed appliance in two monkeys at the time of fixation. This appliance adequately stabilized the fragments for seven days but then became loose and was expelled by the animals. As a modification of this technique, two circummandibular wires were placed around the posterior aspect of the processed acrylic appliance to gain retention. This technique proved quite successful in eight animals and seemed advantageous in that (1) a significant degree of function was permitted, (2) the animals appeared to tolerate the splint more readily than any previous technique of intermaxillary fixation, and (3) healing appeared favorably influenced.

In our most recent efforts at attaining a more ideal means of immobilization, a combination of Risdon wiring and self-curing acrylic has been utilized. A 0.020-inch stainless steel wire was applied around the teeth in the right mandibular quadrant and acrylic was applied to the buccal aspects of the teeth with a small brush. The acrylic was strengthened by the incorporation of wire ends into the material. This technique has been applied successfully in 6 cases and its advantages include: (1) simplicity: the wiring, application, and polymerization of acrylic requires only a few minutes; (2) strength: the acrylic fortified with wire is extremely strong and rigid; (3) function: when care is taken to avoid the occlusal surfaces of the teeth, considerable movement and occlusion is permitted; (4) healing: bone physiology at the fracture site is enhanced because the osteogenic potential of the fracture is not affected by local shifting movement.

In summary, two techniques of fixation have been found to adequately immobilize the fracture fragments. Therefore, throughout the report to follow, the term "immobilization" will refer to those cases in which either the processed acrylic splint or Risdon wiring and self-curing acrylic splint have been applied.

RADIOGRAPHS

Lateral jaw radiographs were initially taken at weekly intervals under general anesthesia so that the healing process could be studied in series. These radiographs showed many interesting features such as a proliferation of callus at the inferior border, etc. (see Results). However, it was felt that the deleterious systemic effects of weekly general anesthesia required to obtain radiographs far outweighed the value of the information gained.

Our present radiographic routine includes (1) preoperative lateral jaw films at time of tooth extraction, (2) postoperative lateral jaw radiographs, and (3) a final lateral jaw film taken at time of sacrifice. In addition, a horizontal view is obtained of the thin section of fracture area used for tetracycline observations. The lateral jaw films are taken at 65 kv and 0.4 second on Kodak Morlite dental x-ray film and the horizontal view radiographs are taken at 90 kv and 60 seconds on Kodak-type fine grain industrial x-ray film.* This film gives excellent contrast and is ideal for detailed radiographic examination of a fracture site. We have also incorporated an aluminum density key for calcium density comparisons, but the degree of fracture calcification obtained in the initial experiments was so minimal that we were unable in early work to take advantage of this technique. However, this technique is being used for evaluation of our present radiographic results.

TETRACYCLINE LABELING

It is well known that the tetracycline antibiotics are taken up <u>in vivo</u> by any actively mineralizing organic matrix and that the incorporated deposits will yield visible fluorescence when illuminated by ultraviolet light. Our initial experiments attempted to utilize these labeling properties to study fracture site healing. The animals were anesthetized at weekly intervals and 100 mg of tetracycline hydrochloride was injected intravenously. Occasionally, minimal convulsive-like movements were noted; these effects were attributed to anesthetic overdose. However, administration of tetracycline without anesthesia also elicited significant generalized convulsions as well as tachycardia and cardiac arrhythmia. It was then theorized that the tetracycline combination and binding of circulating calcium ions was thereby producing a tetanic condition. On this basis, the dosage was decreased by one-half and administered intramuscularly. This modification eliminated the tetany effects and still afforded adequate bone labeling.

Histologic analysis of the tetracycline labeling results led to some interesting findings. The distinct, concentric rings described by Boyne (see footnote, page 4) and others were observable only in the well formed surrounding lamellar bone. This degree of order was not observed in the reparative callus regions of the early fracture site. Rather, a more diffuse fluorescence pattern was reflective of the fibrous bone which spans the early fracture site. Because of this mode of repair, a time sequence evaluation of repair does not lend itself to tetracycline analysis. We therefore have abandoned weekly tetracycline injections in favor of a single 50 mg intramuscular injection of tetracycline hydrochloride five days before sacrifice. In

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*Our appreciation is extended to Professor A. G. Richards, Chairman of the Department of Radiology, School of Dentistry, The University of Michigan, for his expert assistance in developing the radiographic techniques employed.

this manner, we are able to evaluate the degree of calcification of the organic matrix across the fracture site.

PERIOD OF HEALING

Human mandibular fractures are clinically firm and function satisfactorily in healthy patients after six weeks of immobilization. It seemed logical to assume that healing in monkeys would be comparable to humans, especially since our animals were young and healthy. Therefore, clinical experience had suggested the same six-week period of observation for our experimental animals. In a series of fractures which were allowed to heal the standard six weeks period, the results were disappointing. It appeared both clnically and histologically that six weeks was perhaps too early for evaluation, and future studies were designed to utilize a longer healing period—first eight weeks and presently ten weeks.

In retrospect, we realized that our "surgical fracture" was not a simple fracture of the mandible but a true surgical defect which could be compared to a war wound in which bony substance had been lost. In using a small round bur, a defect was created which would be comparable to a significant bony defect in the adult human mandible. It was decided to use the same type of surgical defect but allow more time for the greater challenge for healing to occur. This accounts for the present program of a ten-week healing interval.

SACRIFICE PROCEDURE

The animals have been sacrificed at various time intervals as described in the "Period of Healing" section. "Veterinary lethal solution" containing pentobarbital in an alcohol vehicle was injected directly into the left ventricle producing instantaneous death. Immediately following death, the splint was carefully removed and the clinical test for union determined. This was evaluated by one investigator (G. H. Bonnette) utilizing palpation and testing in a consistent manner similar to determinations used in human trial for stability. Initial efforts attempted to measure degree of deflection of the mandibular symphysis when loaded with weights. Many variables made this technically impractical and not a true measure of healing. Other investigators have used the deflection test or tensile strength of the fracture site as an objective measurement of healing. However, this results in total disruption of the fracture site so that histologic evaluation is impossible. It also is not a true indication of physiologic bone repair. Although subjective in nature, we feel that clinical firmness is as good an indication of fracture integrity as any presently available.

Following the clinical test, the animal was decapitated and the mandible disarticulated and sectioned between the central incisor teeth, using a small saw. The specimen was placed in 10 percent neutral buffered formalin and stored for histological preparation.

HISTOLOGIC TECHNIQUE

In previous phases of the study, a comparative analysis of radiographic, decalcified, and undecalcified specimens was made difficult because of a lack of uniformity in the planes of tissue sections. Therefore a histologic technique was developed which prepared tissues for all means of analysis by cutting in a standard horizontal plane through the fracture area. This was accomplished with a horizontal section of bone approximately one millimeter in thickness taken at a level five millimeters above the inferior border of the mandible. Both buccal and lingual cortical plates were included in the section, and one of these sections was taken from the bodies of each of the control and operated mandibles.* These cuts were accomplished with a water-cooled rotating silicon carbide blade. The thin undecalcified sections were then radiographed using high resolution industrial film. The same sections were then prepared for ultraviolet microscopic observation of tetracycline fluorescence by processing through progressive alcohol dehydration (70%, 80%, 95%), infiltration and embedding in a clear hard setting plastic (8000 polylite). These sections were then polished to approximately 20 microns and were mounted, unstained, for ultraviolet fluorescence microscopy.

The remaining inferior and superior segments of mandibular segments in the fracture area were decalcified, dehydrated in alcohols, infiltrated, and embedded in paraffin. Serial sections were taken from the horizontal plane adjacent to the ground section level at a thickness of 15 microns, mounted, stained with hematoxylin and eosin, and finally cover-slipped for light microscopic analysis.

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*The mandibular rami, along with a cylindrical section of tibia, were also prepared, undecalcified for possible control use and for future experimental reference. The condyles were decalcified, embedded in paraffin and stored for similar future reference.

A SUMMARY OF ACCUMULATIVE RECORDS

Monkey Number	Surgical Procedure	Period of Healing, weeks	Type of Immobilization
1	extraoral	6	Transosseous wire only[1]
2	extraoral	6	Transosseous wire only[1]
3	extraoral	6	Transosseous wire only[1]
4	extraoral	6	Transosseous wire only[1]
5	extraoral	6	Transosseous wire only[1]
6	extraoral	6	Transosseous wire only[1]
7	intraoral	6	Transosseous wire only[1]
8	intraoral	6	Transosseous wire only[1]
9	intraoral	6	Transosseous wire only[1]
(10)*	intraoral	6	Transosseous wire only[1]
11	intraoral	6	Transosseous wire only[1]
12	intraoral	6	Transosseous wire only[1]
13	extraoral	8	Transosseous wire only[1]
14	intraoral	8	Transosseous wire only[1]
15	extraoral	6	Combined Methods[2]
16	intraoral	6	Combined Methods[2]
(17)	intraoral	8	Combined Methods[2]
18	extraoral	6	Combined Methods[3]
19	extraoral	6	Combined Methods[3]
20	extraoral	6	Combined Methods[4]
(21)	extraoral	6	Combined Methods[4]
22	extraoral	6	Combined Methods[4]
(23)	extraoral	6	Combined Methods[4]
(24)	extraoral	6	Combined Methods[4]
(25)	intraoral	6	Combined Methods[4]
26	intraoral	6	Combined Methods[4]
27	intraoral	6	Combined Methods[4]
(28)	intraoral	6	Combined Methods[4]
29	extraoral	10	Combined Methods[5]
30	extraoral	10	Combined Methods[5]
31	extraoral	10	Combined Methods[5]
32	extraoral	10	Combined Methods[5]
33	extraoral	10	Combined Methods[5]
34	extraoral	10	Combined Methods[5]
35	extraoral	10	Transosseous wire only[1]
36	extraoral	10	Transosseous wire only[1]
37	extraoral	10	Transosseous wire only[1]
38	extraoral	10	Transosseous wire only[1]
39	extraoral	10	Transosseous wire only[1]
40	extraoral	10	Transosseous wire only[1]

*Numbers in parentheses indicate monkey healed completely.

[1]Transosseous wire at the inferior border of the mandible only.

[2]Transosseous wire plus circummandibular and circumzygomatic suspension wires for intermaxillary fixation.

[3]Transosseous wire plus processed acrylic splint plus mouth-curing acrylic.

[4]Transosseous wire plus processed acrylic splint plus circummandibular wires.

[5]Transosseous wire plus Risdon wire plus mouth-curing acrylic splint.

RESULTS

CLINICAL IMPRESSION OF UNION

The firmness of fracture union was ascertained immediately after sacrifice by manual testing and given a value of 1, 2, or 3, indicating (1) rigid, (2) semirigid, (3) nonrigid.

RADIOGRAPHIC EXAMINATION

The amount of bony bridging across the fracture site was evaluated from lateral jaw and occlusal radiographs and given a value of 1, 2, or 3, indicating (1) good bridging, (2) slight bony bridging, (3) no bony bridging.

HISTOLOGIC EXAMINATION

The H & E microscopic sections were evaluated for the amount of new bone formation across the fracture site and given a value of 1, 2, or 3, indicating (1) significant new bone formation, (2) slight new bone formation, (3) no new bone formation.

TETRACYCLINE EXAMINATION

The ground sections were evaluated under ultraviolet light for amount of fluorescence across the fracture site and given a value or 1, 2, or 3, indicating (1) fluorescence, (2) slight amount of fluorescence, (3) no fluorescence.

COMPARISON OF THE FRACTURE EVALUATION METHODS

The methods of evaluation including impression of clinical rigidity, radiography, and histology compared favorably when considering each individual fracture. In general, the fractures having greater mobility exhibited less radiographic and microscopic bone bridging. Of the three methods used to evaluate healing,histologic analysis was adjudged the most objective method and also proved to be the most demanding test of true fracture healing. In all cases, histologic analysis demonstrated less evidence of hard tissue bridging than would have been suspected from clinical and radiographic criteria alone. The tetracycline analysis also showed little or no fluorescence indicating no true calcification across the fracture site in the animals which had been healing for six and eight weeks.

SUMMARY OF DATA

Monkey Number*	Evaluation			
	Rigidity of Segments	Radiography	Histology	Tetracycline
1	1	1	2	2
2	2	2	2	3
3	2	2	2	2
4	2	1	2	3
5	3	3	3	3
6	3	3	3	3
7	3	2	3	3
8	3	3	3	3
9	3	3	3	3
10	1	1	2	2
11	2	1	2	3
12	2	2	2	3
13	3	2	2	3
14	3	3	3	3
15	2	2	3	3
16	2	2	2	3
17	1	1	1	2
18	2	2	2	2
19	2	2	2	2
20	3	2	2	3
21	1	1	1	2
22	3	3	3	3
23	1	2	2	3
24	1	1	2	2
25	1	1	1	2
26	2	2	3	3
27	2	2	3	3
28	1	1	2	3

*29-40 in process.

Note: The group of animals which were allowed to heal ten weeks and which an immediate dental wiring and quick-cure acrylic applicance was used for immobilization await final evaluation and are not included in this report.

DEGREE OF RIGIDITY

The fractures were more rigid in the group of animals in which immobilization was obtained with the processed acrylic splint and circummandibular wiring. Seven of the 9 animals in this group were considered clinical unions. When compared to the group of animals at the 6-week healing period without immobilization, greater fracture stability and a greater number of unions were obtained. (Seven "unions" of 9 animals in the immobilized group as compared to 7 "unions" of 12 animals in the nonimmobilized group.) Clinical evaluation of the surgical approach has shown no real differences between the animals in the extraoral or intraoral group.

RADIOGRAPHIC RESULTS (Figures 13 - 22)

The most useful radiograph was that obtained from a horizontal view of the ground nondecalcified section, used subsequently for tetracycline analysis. In general, the radiographic distance between the fracture segments as measured by venier calipers was less in the immobilized group of animals than in the nonimmobilized group. There were more cases of bony bridging at the fracture site and the amount of submandibular callus was less in the immobilized group. Wider radiolucency in the fracture site correlated with fracture nonunion. Within the six weeks immobilized group in which the two surgical approaches were used, greater proliferation of the submandibular callus in the intraoral group was again noticed in the lateral jaw radiographs. (This same phenomenon was noticed in the nonimmobilized group of animals.) Since there was no apparent difference in the distance between the fracture fragments, it is difficult to explain this phenomenon unless it is related to the position of the transosseous wire and its effect on healing.

The horizontal radiographic views also demonstrated a larger anchoring callus on the buccal aspect of the mandible which could probably be related to surgical trauma and hematoma formation since the approach was from the buccal or lateral surface of the mandible.

HISTOLOGIC RESULTS (Figures 23 - 31)

The decalcified tissue sections showed a greater amount of true bony bridging in the immobilized group of animals when compared to the nonimmobilized group. In sections in which bony bridging had not occurred, there was evidence of osteoblastic activity with osteoid and new bone proliferation in the fracture site. It was the opinion of our Oral Pathology consultant that in most cases, complete bony healing would have occurred in time. This was a guiding influence for establishing a longer healing period.

In fractures which demonstrated little or no bony bridging the fibrous connective tissue proliferated in a perpendicular direction to the fracture site, was greater in amount, and consisted of dense, mature fibrous tissue. It was obvious that pseudoarthrosis was beginning to occur with eburnation of the fracture ends. In many animals, connective tissue, scar formation, and little or no osteoblastic activity between the fragments was noted.

Bone formation in the immobilized group compared favorably to the non-immobilized group except for the amount formed in the same time interval. Intramembranous bone formation was noted in the medullary portion of bone with islands of osteoblasts, osteoid, and young bone forming within the young granulomatous connective tissue. Endochondral bone formation with cartilage production and replacement by bone was noted at the fracture peripheries and most consistently in the region of the lingual cortical plate.

Severe inflammation, and occasionally infection, was noted in areas where the transosseous wire had been placed. Tracts were present, lined with dense mature fibrous connective tissue and heavy lymphocytic infiltration and foreign body giant cells. There was evidence of bony profileration around the wires in which a "walling off" reaction seemed to be occurring.

The developing permanent tooth bud in one instance had not been totally removed but had been curretted and traumatized. Histologically an interesting phenomenon of disorganized osteodentin formation occurred, which possibly could have proliferated to form an odontoma type of lesion if the animal had not been sacrificed. This same phenomena was not seen in any other tissue sections but certainly is worthy of further investigation.

TETRACYCLINE LABELED SECTIONS

The fractures in general demonstrated minimal fluorescence across the fracture site, indicating a lack of mineralization and mature bone formation in a six-week period. The total fluorescence was more prominent in the operated one-half of the mandible as compared to the nonoperated side. Concentric tetracycline labeled rings were noted in the mature lamellar bone at some distance from the fracture site, but no pattern of labeling could be demonstrated within the fracture site, because of the disorganized pattern of woven or fibrous bone formation. No apparent fluorescence differences were noted between the intraoral and extraoral surgical groups. The lack of tetracycline fluorescence across the fracture site prompted us to extend the healing period to ten weeks so that mineralization could be evaluated more objectively.

DISCUSSION

Development of a standardized method of studying fracture healing is a familiar problem for those interested in bone physiology and repair. The problem, however, is compounded when studying the mandible by such additional variables as teeth, oral bacterial contamination, unique form, and nutritional limitations imposed by functional deficiencies. Many of the experimental fracture studies done on dogs, rats, and rabbits are not applicable to mandibular fracture studies. For this reason, many innovations were necessary in the evolution of methodology. Our present methods of animal care, anesthesia, fracture production, splint construction, healing periods and fracture evaluation give consistent results and are worthy of further application.

It is common practice by many men treating facial fractures to utilize techniques such as Kirschner pins and transosseous wires without immobiliztion. Our present studies have indicated that mandibular fracture union is dependent upon immobilization. The actual degree of dependence has not yet been affirmed statistically because of the small sample.

Surgical techniques have been varied between an extraoral and intraoral approach to the mandible throughout our studies. Thus far, we have found no apparent differences between the techniques and their effect on healing. If statistical analysis based on a larger sample size confirms our findings, perhaps current techniques of extraoral open reduction on mandibular fractures should be re-evaluated in favor of the intraoral approach whenever possible. Our limited sample seems to indicate that complications resulting from varying degrees of oral contamination of the fracture site are minimal or nonexistent. It appears that mandibular fractures compounded into the oral cavity could easily and safely be repaired through the area of intraoral trauma whenever possible, thus minimizing the number of necessary operative procedures and approaches.

The production of fractures with dental burs on small animals creates a defect rather than a clinical fracture. Correspondingly, the duration of the healing periods must be increased to allow for rather extensive bone regeneration as well as repair. A six-week healing period has not been adequate to evaluate repair. Rather, a ten-week period seems to be optimum for fracture healing within the specifications of this study.

Histologic study of the fracture is the most rigid and demanding method of evaluation of repair. The method is not dependent upon the strength of the fracture callus, the radiographic technique, or the amount of mineralization present. Osteogenic proliferation and potential can be examined in a more controlled manner.

In our study, cartilage formation has been consistently noted on the medial or lingual aspect of the fracture. In fractures of the long bones, cartilage production has been related to lack of blood supply, degree of mobility or simply as the initial step in the process of bone formation. We have not been able to correlate cartilage formation with the degree of immobilization or surgical technique; and because the cartilage appears in the fracture site in such a random manner, we also find it difficult to relate it to the normal process of fracture repair. Our data suggest that mobility and/or ischemia may play an important role in its production. Further study will be centered on this phenomenon.

SUMMARY AND CONCLUSIONS

1. Experimental studies are currently under way to evaluate the effects of immobilization, healing period, and surgical approach on the repair of mandibular body fractures in young Rhesus monkeys.

2. Parameters for use in the study of jaw fracture healing have been developed.

3. Histologic criteria are the most rigid evaluations. They are more precise than the degree of rigidity, radiography, and tetracycline bone marking.

4. Cartilage was produced on the lingual surface of the repairing fracture and is probably related to mobility or ischemia of the fracture segments. There also may be some specificity of this periosteal surface.

5. Lack of immobilization appears to adversely affect fracture repair and delay union by several criteria.

6. A limited sample reveals no difference in healing ratio between an intraoral approach and an extraoral approach to the fracture site. Clinical application of these findings have been suggested.

ADDENDUM

Animals number 31 through number 36 were sacrificed after ten weeks of healing. Fixation consisted of Risdon wire and mouth curing acrylic splints. Clinical manipulation of the fracture fragments revealed four animals with "unions" and two with "nonunions." Radiographic histologic and tetracycline analysis have not been completed.

These limited results tend to support the hypothesis that once the potential for nonunion has been established, prolongation of the healing period will not influence osteogenesis in the fracture site. Future studies will utilize the six-week healing period.

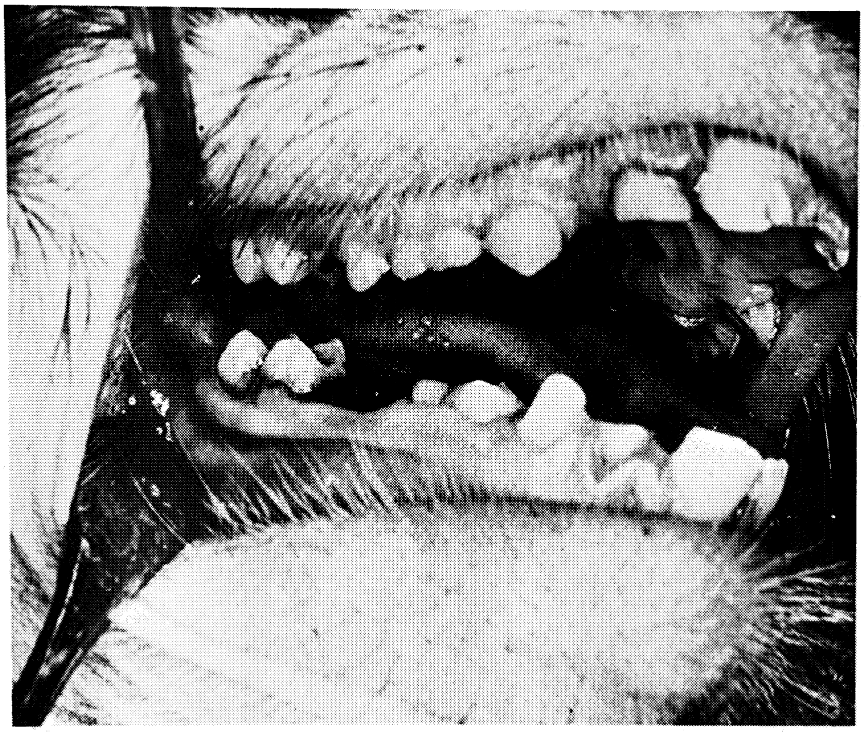


Figure 1. Extraction of second primary molar prior to fracture surgery.

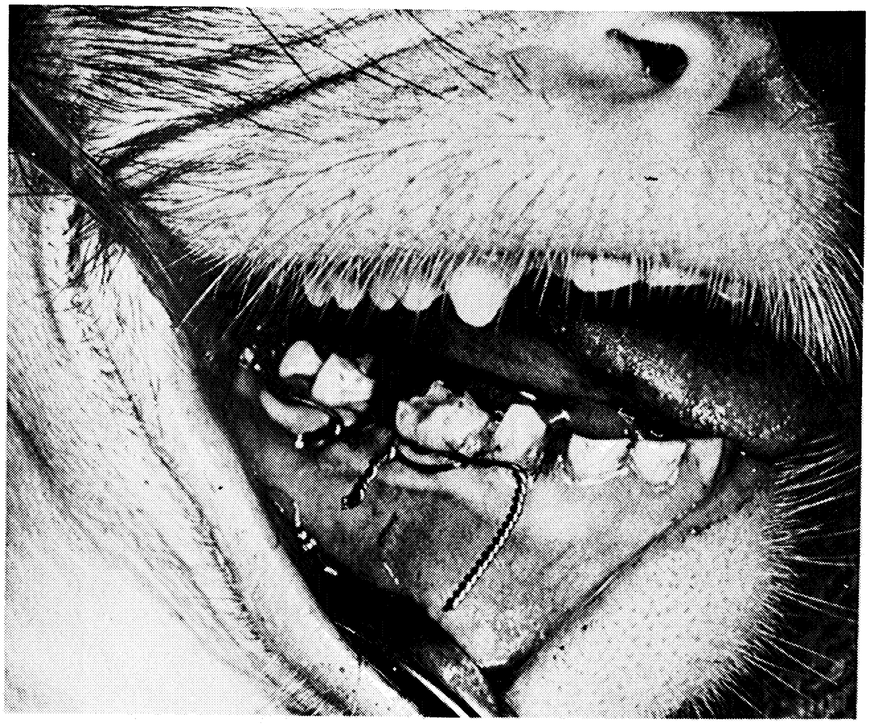


Figure 2. Application of interdental Risdon wires for immobilization of reduced fracture segments.

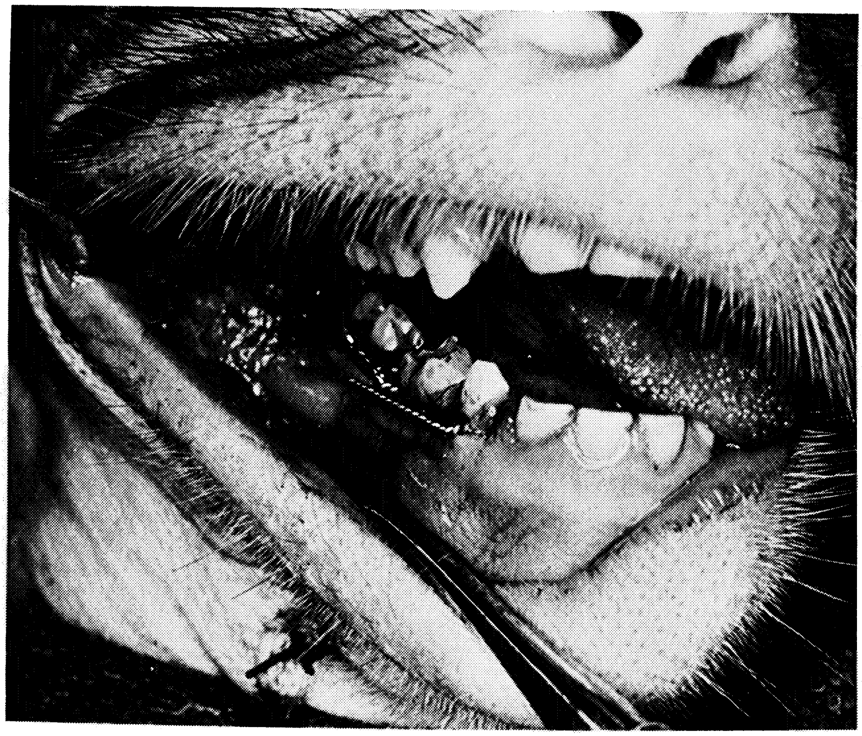


Figure 3. Completion of interdental Risdon wiring.

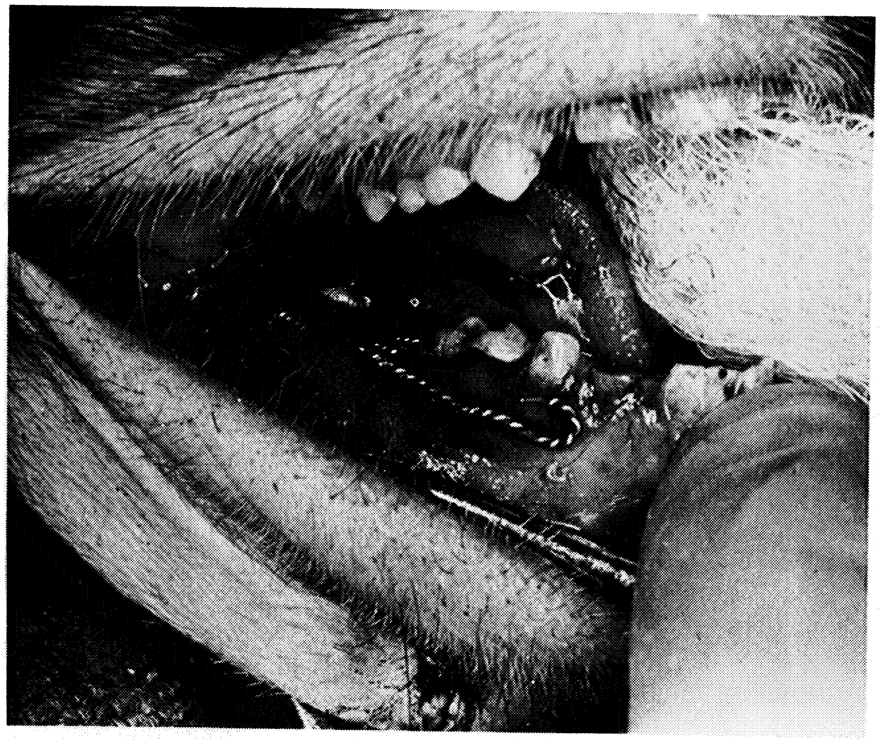


Figure 4. Incorporation of interdental wiring by build-up of quick-cure acrylic.

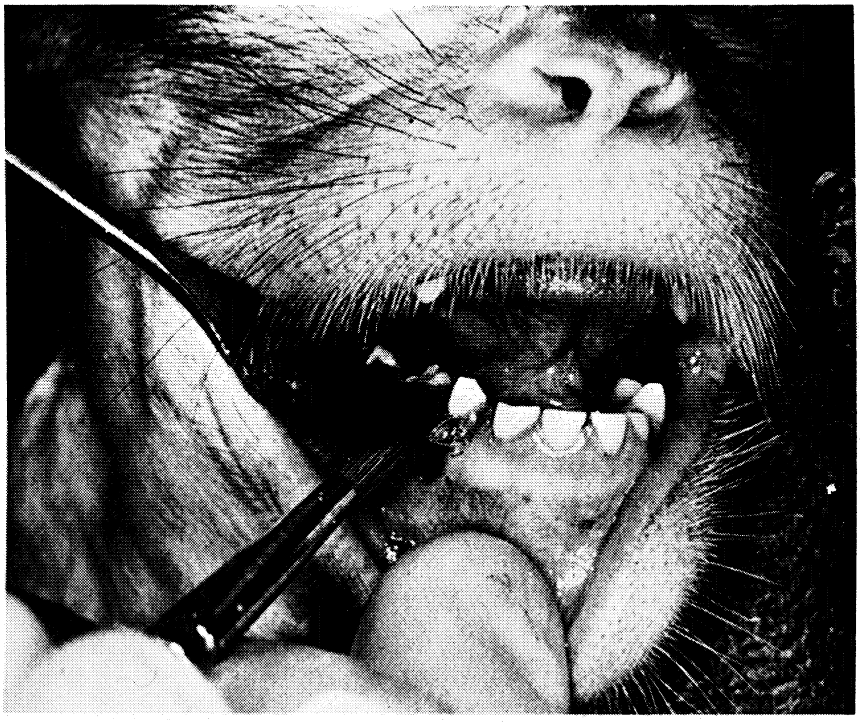


Figure 5. Brush application of quick-cure acrylic to buccal mucosa, around wire and molars.

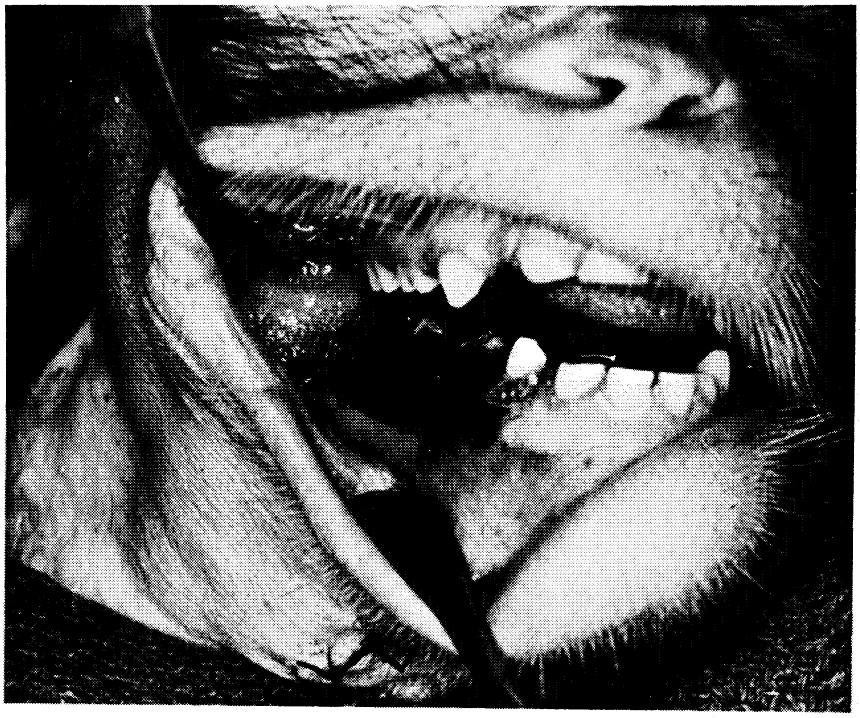


Figure 6. Completed Risdon wiring and quick-cure acrylic splint.

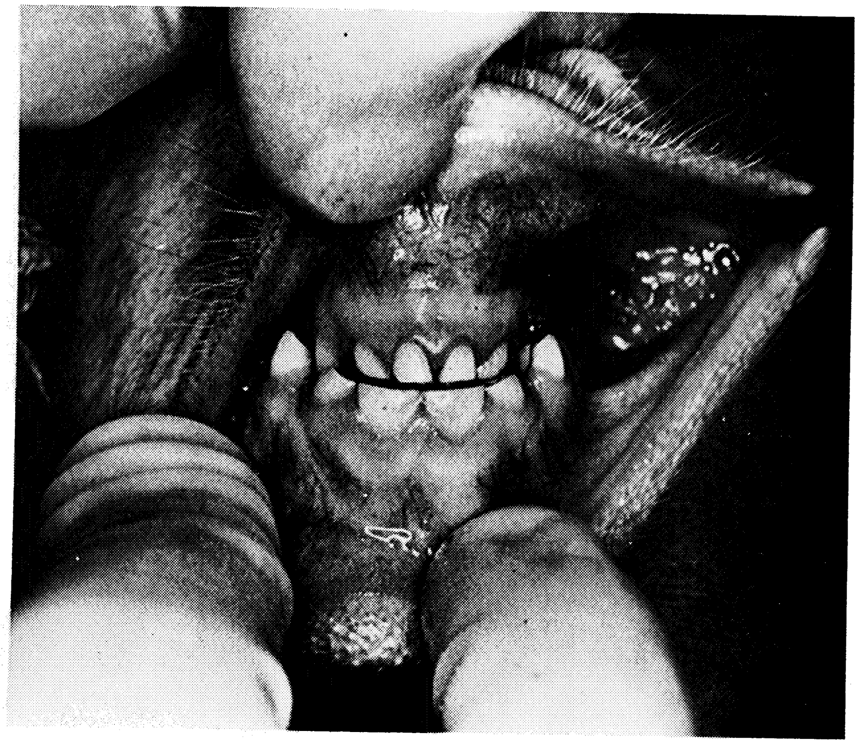


Figure 7. Completed Risdon wiring and quick-cure acrylic splint.

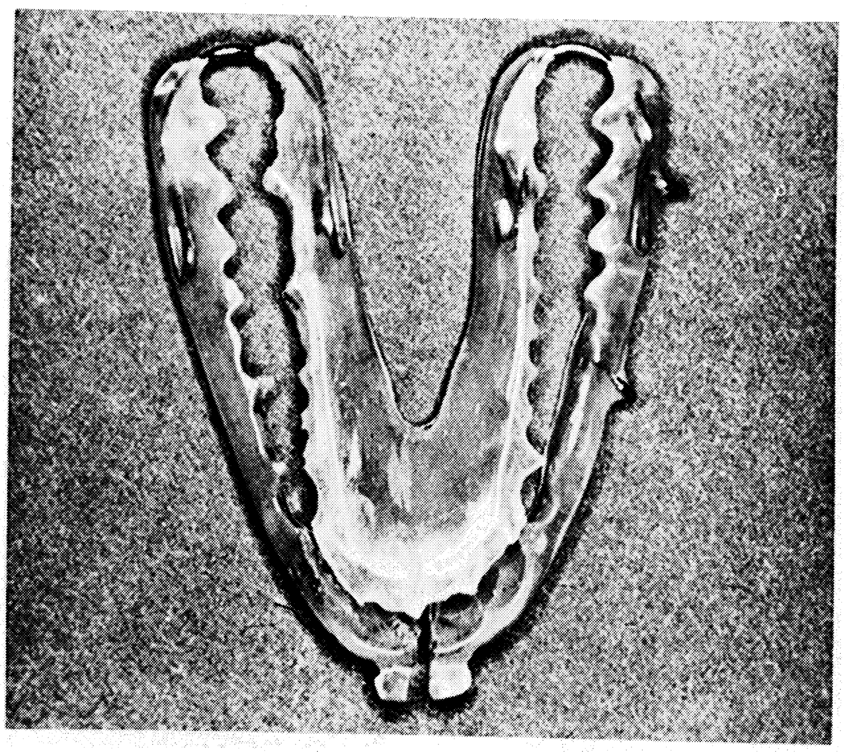


Figure 8. Processed acrylic mandibular splint.

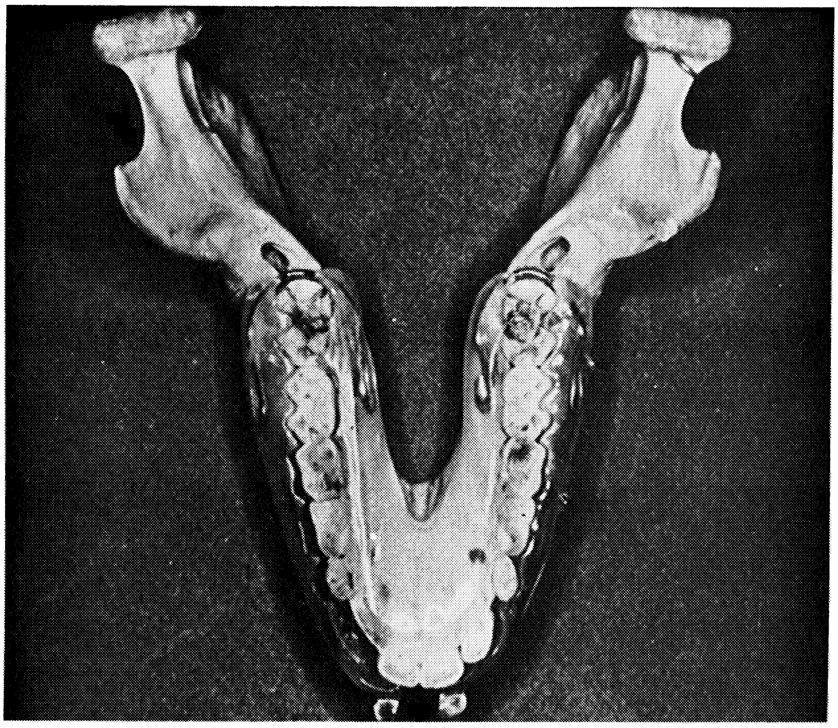


Figure 9. Processed splint positioned on mandible.

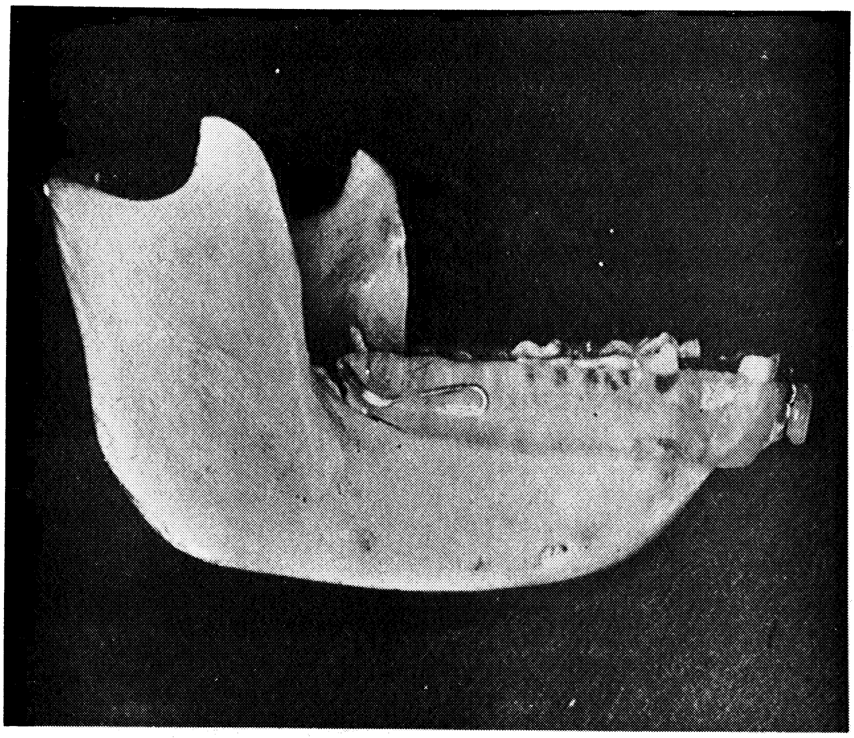


Figure 10. Processed splint positioned on mandible.

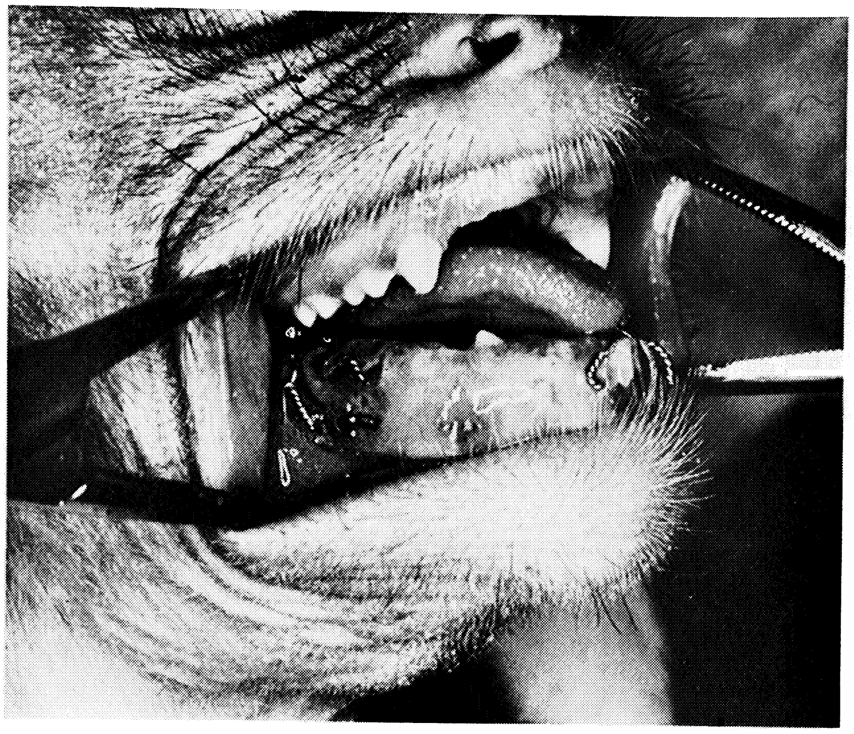


Figure 11. Processed splint secured to reduced mandible.

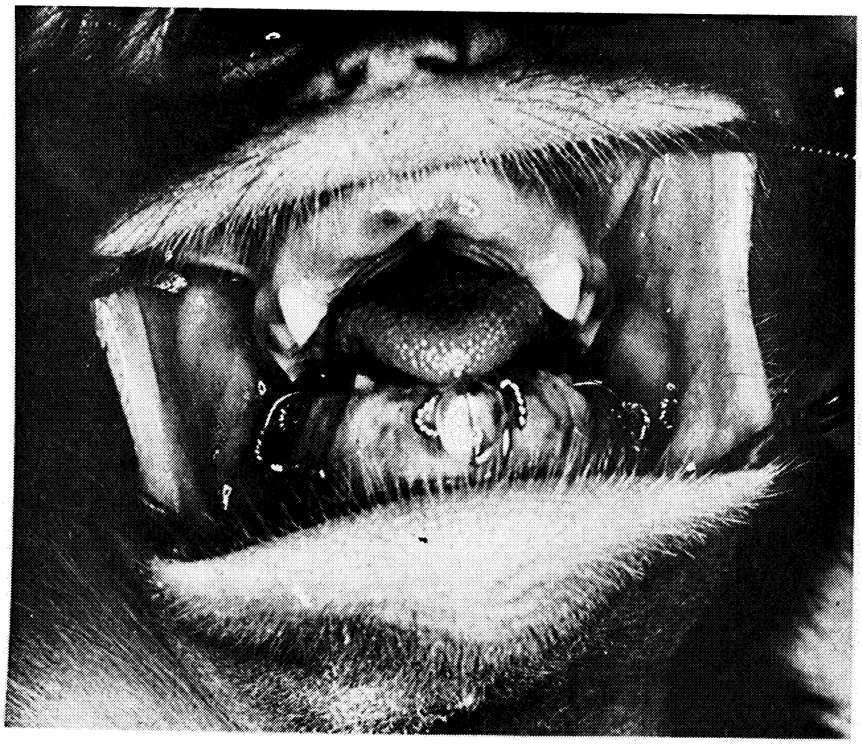


Figure 12. Processed splint secured to reduced mandible.

CLINICAL EVALUATION

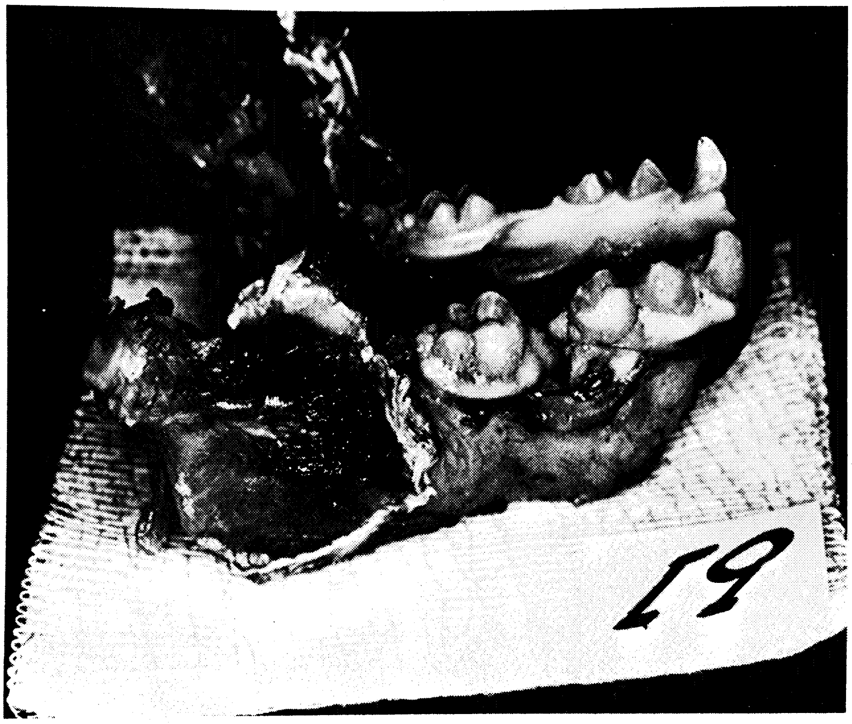


Figure 13. Disarticulated mandible preparatory to clinical evaluation. Observe the excessive buccal callus and mucosal fistula of this clinical non-union.

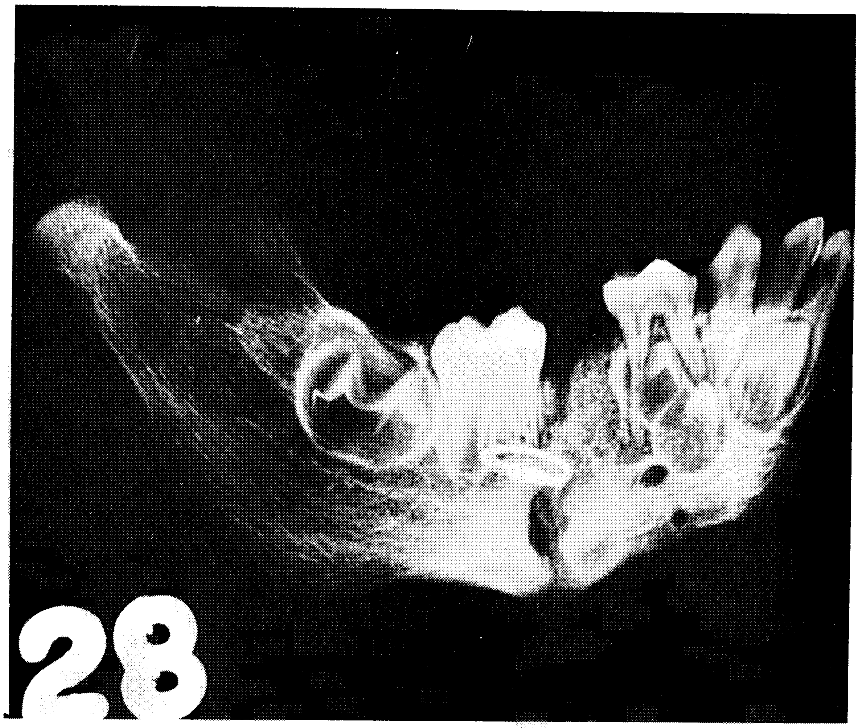


Figure 14. Lateral jaw radiograph of an eight-week clinical union operated intraorally. Note the extreme submandibular callus exostosis.

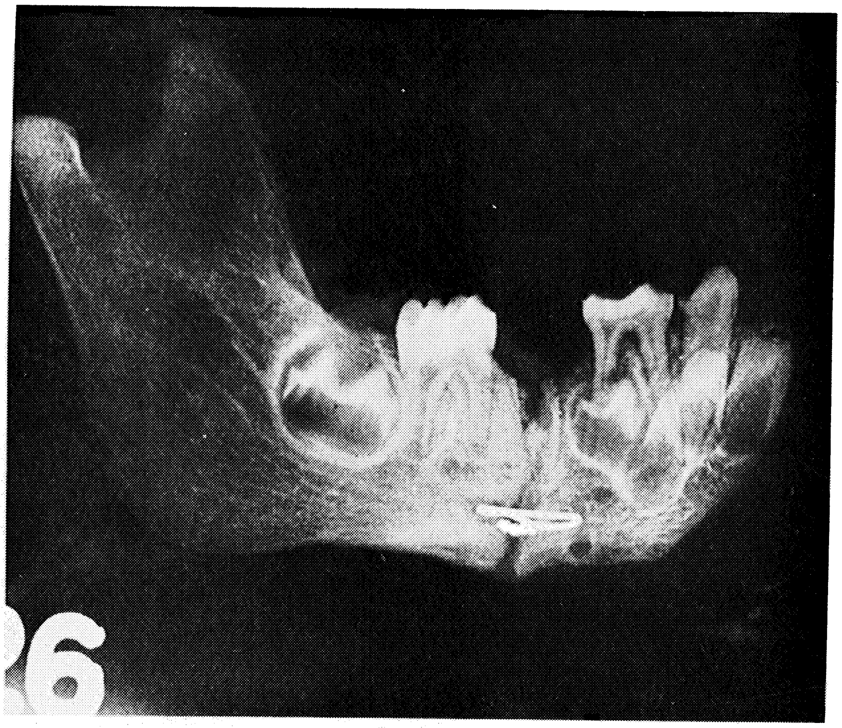


Figure 15. Lateral jaw radiograph of an eight-week clinical union operated extraorally. There is excellent approximation, little submandibular callus and apparent bony bridging of the fracture defect.

RADIOGRAPHIC ANALYSIS

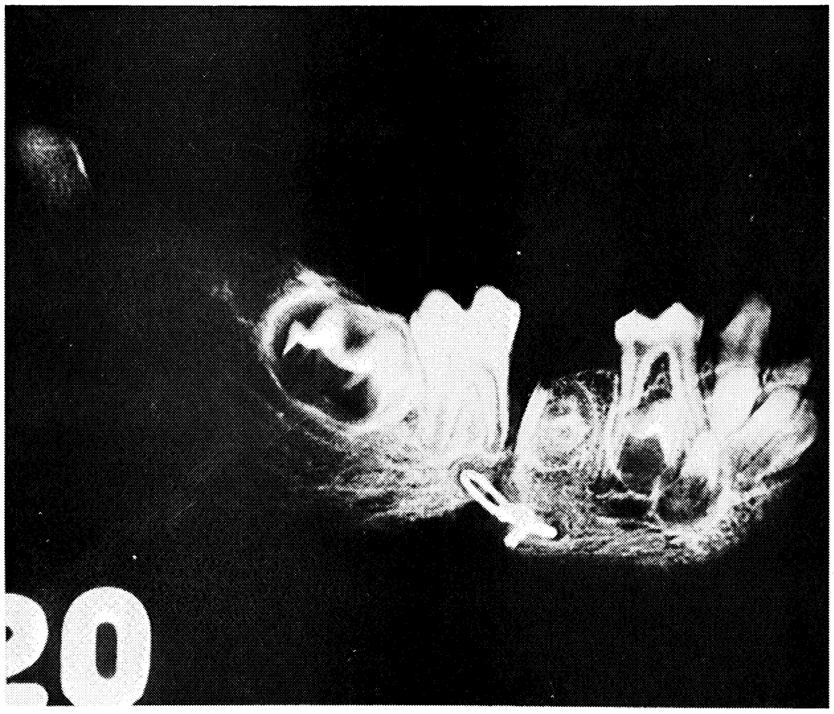


Figure 16. This specimen was operated extraorally and was found to be a clinical nonunion. Bony ebernation and fragmentation is quite evident.

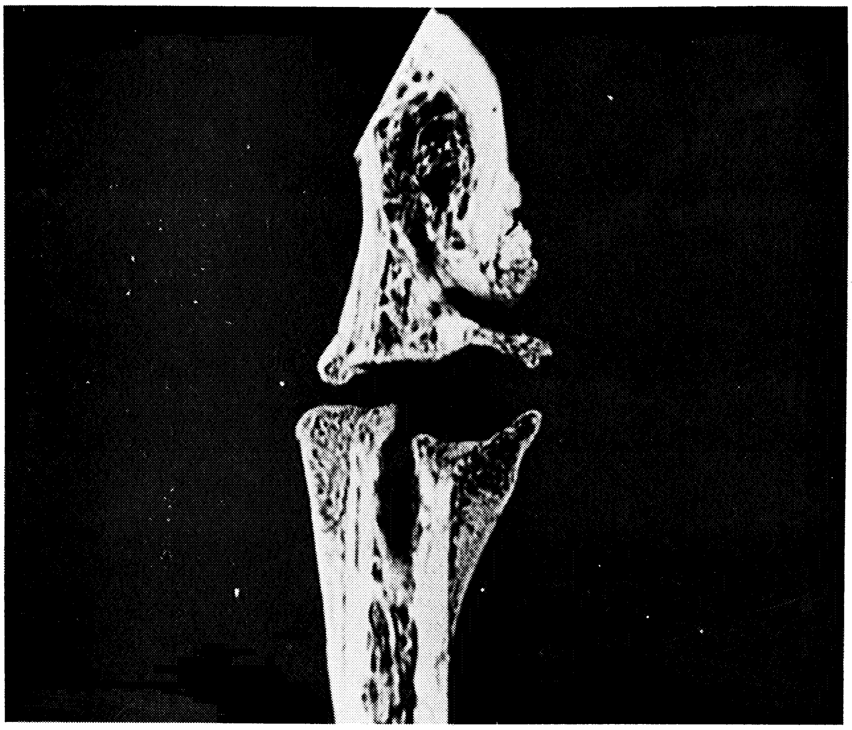


Figure 17. Horizontal radiograph of one millimeter tissue section from clinical nonunion. Observe the extreme buccal and lingual anchoring callus, the bony ebernation, and lack of bridging or uniting calcification.

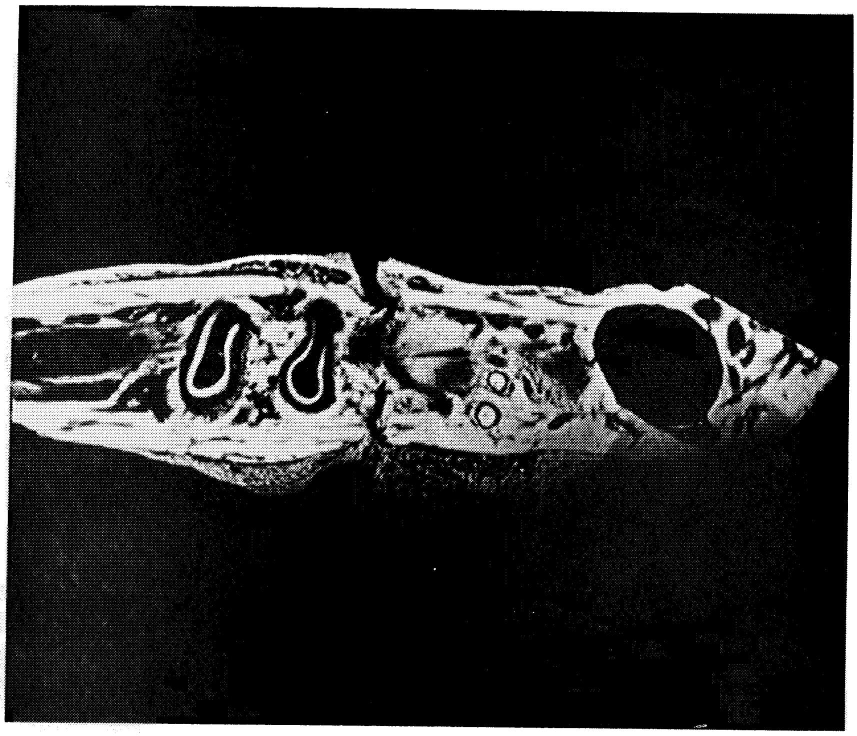


Figure 18. This specimen was taken from a clinically firm union case. Minimal anchoring callus is seen as well as excellent alignment and apparent bony bridging of the fracture defect.

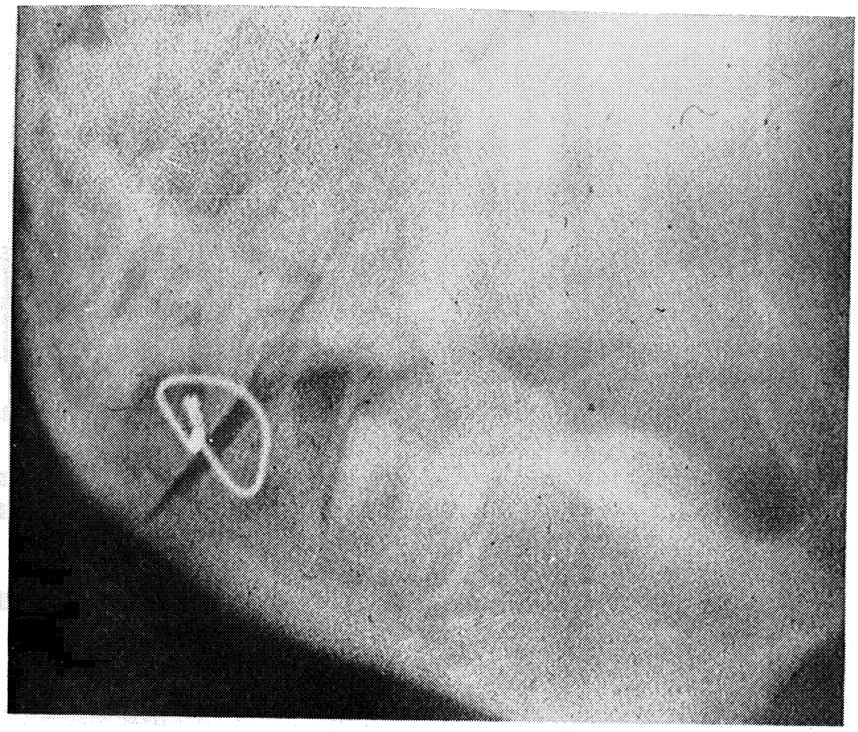


Figure 19. Immediate postoperative projection of intraoral procedure.

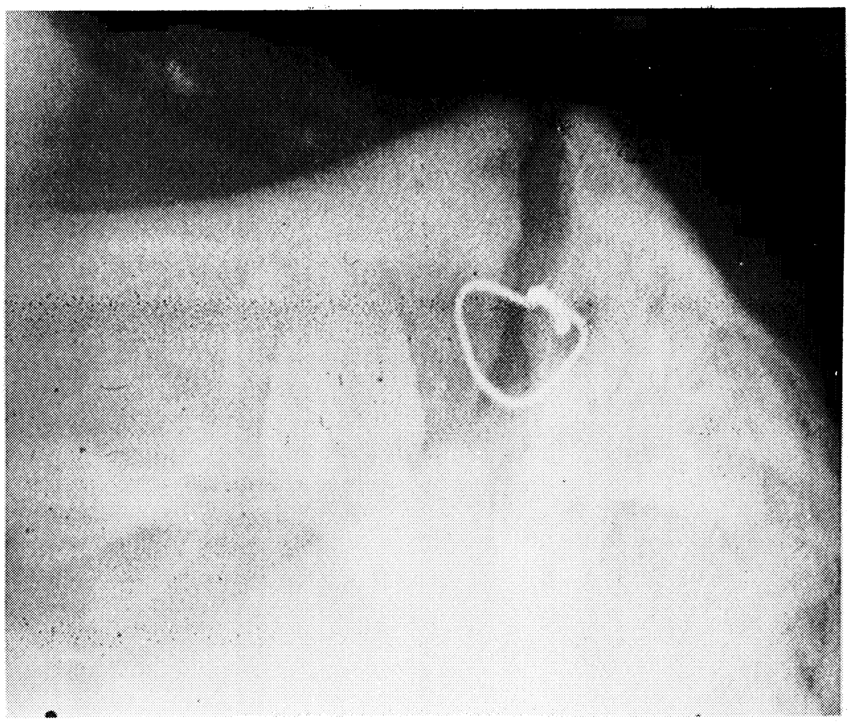


Figure 20. Eight-week lateral jaw radiograph of "Figure 19" specimen. Observe the extreme submandibular callus proliferation. This result was a clinical union.

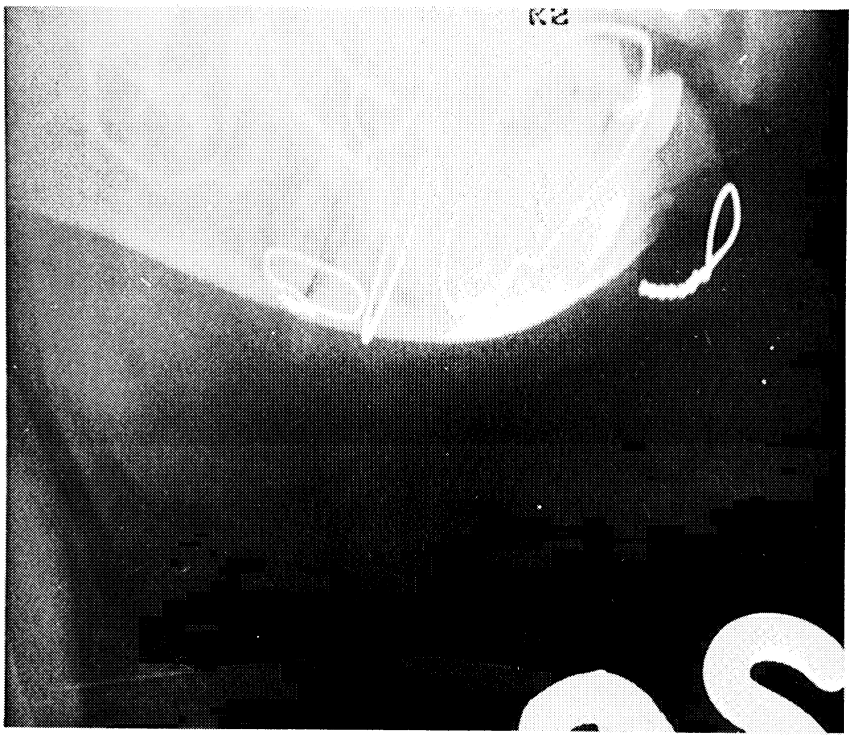


Figure 21. Postoperative projection of extraoral procedure with processed splint secured by circummandibular wires.

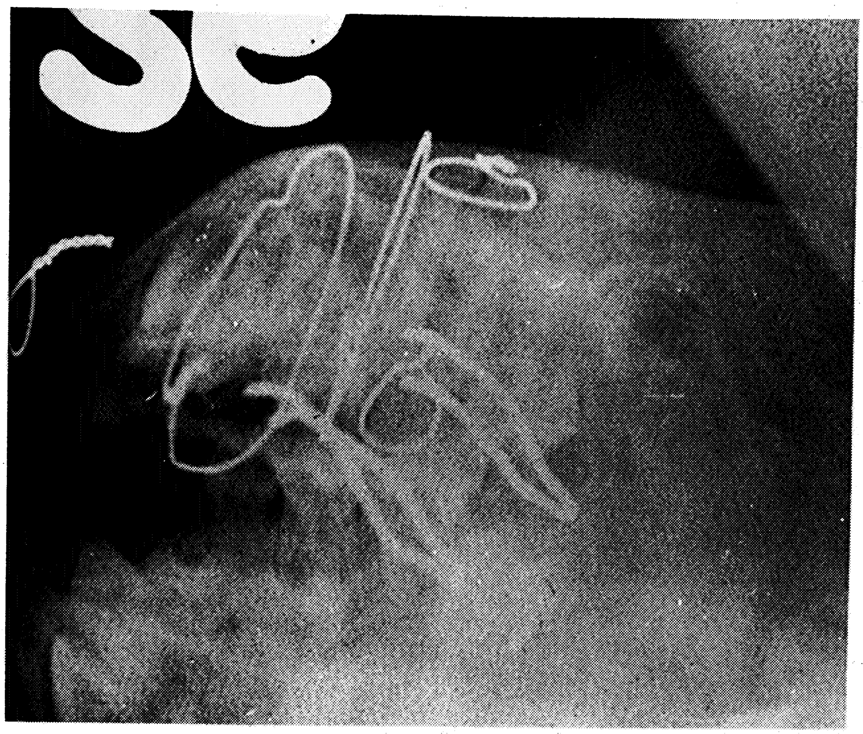
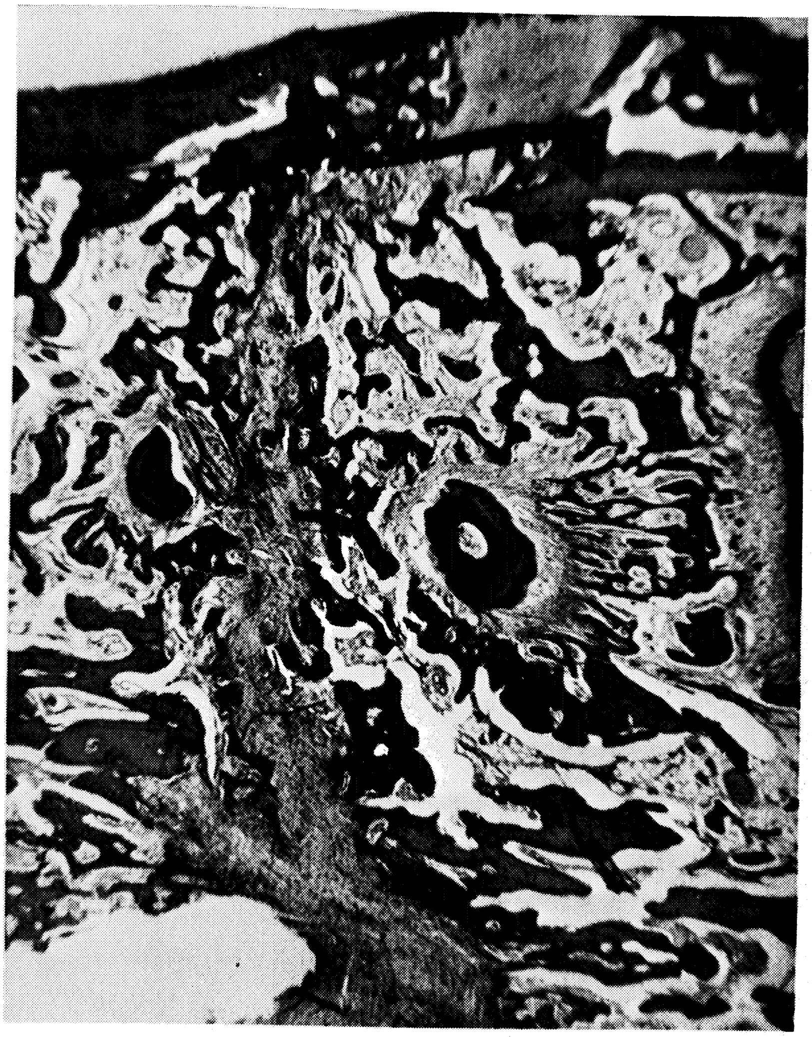


Figure 22. Eight-week lateral jaw radiograph of "Figure 21" specimen. Note the lack of submandibular callus exostosis and contrast this result with the intraoral specimen (Figure 20). This result was a clinical union.

(Magnification 40X)

Figure 23. Microscopic appearance of a clinically united fracture site. The original lingual cortical plate is seen in upper right. Note the great bulk and area of lingual anchoring and bridging callus seen to the left. Immature fibrillar bone and osteoid are seen bridging the defect in a highly irregular pattern. Simultaneously, osteoclastic activity may be seen in other areas.

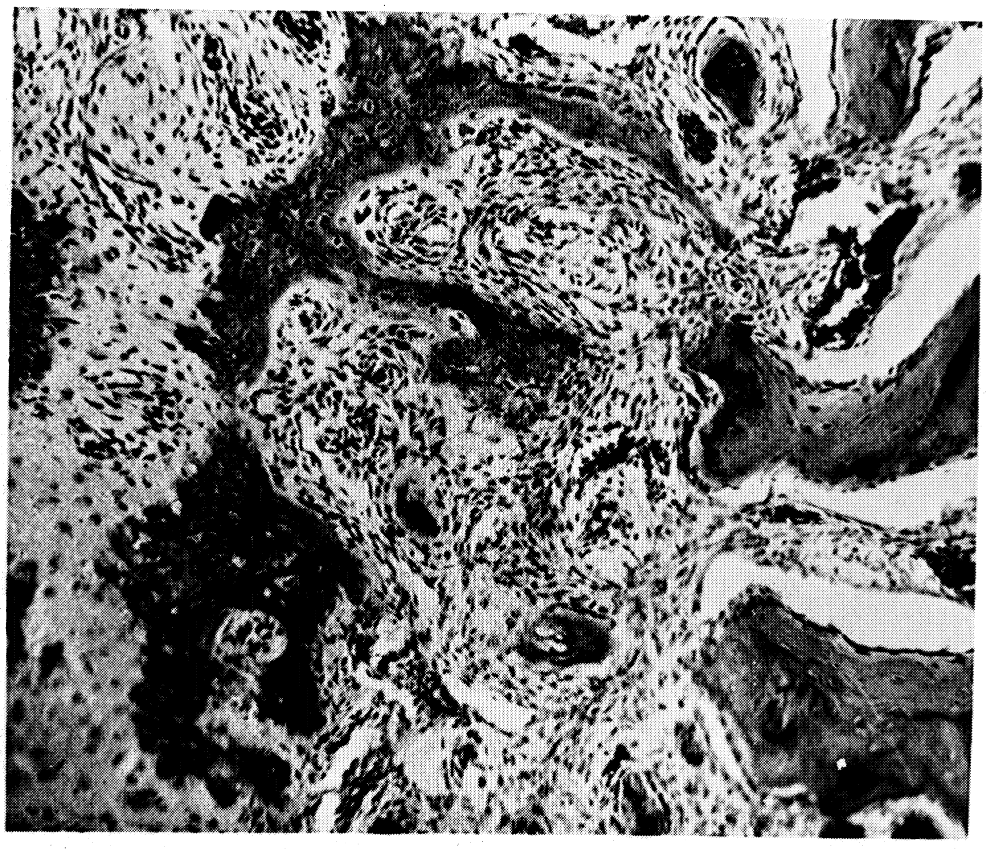


(Magnification 10X)

Figure 24. This section demonstrates the histologic pattern of a clinically firm union. Note the presence of permanent root tips spanning the uniting callus as well as the cartilaginous mass in the bridging callus.

(Magnification 40X)

Figure 25. Dense cartilage occupies the space of bridging callus between anchoring callus on either side. The lingual cortical plates are seen laterally with areas of fibrocartilage interposed. This histologic pattern occurred within a firm clinical union and the presence and position of cartilage was a common finding.



(Magnification 40X)

Figure 26. Intermembranous bone formation within the bridging callus of a firmly united fracture site. Observe the osteofibrous bridges between newly formed intramembranous trabeculae.

(Magnification 100X)

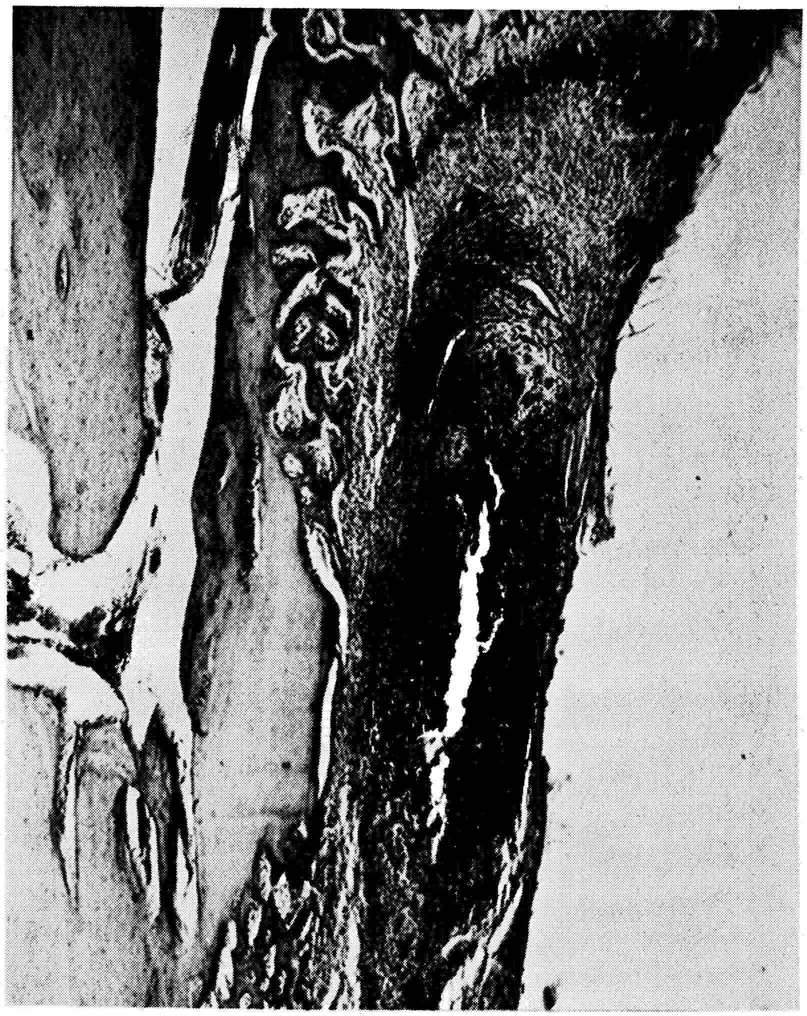
Figure 27. This section characterizes a transition from fibrous callus, through fibrocartilage, hyalinized cartilage, and the final formation of bone. Note the simultaneous osteoblastic formation of bone in lacunar spaces as well as resting lines in the spongy bone itself. This tissue area was located within the uniting callus and the inner layer of periosteum is visible in the lower right adhering to the anchoring callus bone.

(Magnification 10X)

Figure 28. The typical histologic pattern of a gross clinical nonunion is presented. Dense fibrous tissue is predominant in bridging and uniting callus with fiber orientation perpendicular to the bony trabeculae. Anchoring callus is seen to consist of a tremendous bulk of trabecular spongy bone. Note also the variable foci of cartilaginous endochondral bone formation within the fibrous tissue.

(Magnfication 10X)

Figure 29. A fibrous nonunion fracture site is seen at lower right. Note the tremendous overgrowth of fibrillar bone of the buccal and lingual anchoring calluses.



(Magnification 40X)

Figure 30. The effect of transosseous wire presence on callus tissue is seen. Note the intensely cellular inflammatory response and foreign body reaction. The rimming body areas demonstrate variable osteoblastic and osteoclastic activity.

(Magnification 60X)

Figure 31. The presence of proliferating osteodentin within the uniting callus. The osteodentin is seen arising from a permanent tooth left within the fracture site. Its presence may have contributed to the nonunity of the bone fragments.

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13. ABSTRACT

Investigations were designed to develop methodology to provide controlled study of jaw fractures in monkeys and evaluate the effect of (1) surgical approach to jaw fractures, (2) duration of the fracture healing period, and (3) mandibular fixation methods on fracture repair.

Twenty-eight young Rhesus monkeys were used and surgical fractures were created in the mid mandibular body. Fixation methods included processed acrylic splints and Risdon wiring incorporating mouth-curing acrylic. Intraoral and extraoral surgical approaches were used and six-, eight-, and ten-week healing periods were evaluated by (1) clinical fracture mobility, (2) radiography, (3) histology, and (4) tetracycline bone labeling.

Parameters for use in the study of jaw fracture healing produced consistent results. Histologic criteria were more precise than clinical rigidity, radiography, or tetracycline labeling. Cartilage production in the lingual surface of the fracture appeared to be related to mobility or ischemia. Lack of immobilization adversely affected fracture repair and caused delayed union. A limited sample revealed no differences in the healing ratio between an extraoral and intraoral approach to the fracture site. Clinical application of the findings are suggested.

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