THE EFFECT OF UNILATERAL NASAL BLOCKAGE ON THE GROWTH OF THE RAT CRANIOFACIAL COMPLEX - A QUANTIFIED GROSS AND HISTOLOGICAL MICROSCOPIC ASSESSMENT

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The effect of unilateral nasal blockage on the growth of the rat craniofacial complex - a quantified gross and histological microscopic assessment

by

William Nigel Chalk

ABSTRACT

The functional stimulus of nasal airflow on the growth and development of the rat calvarium was investigated. Unilateral surgical closure of the external nares altered the local functional environment. It was hoped to demonstrate the effects, if any, of the functional stimulus of the airflow or, alternatively, to illustrate the effects of surgery, or a combination of the two, on the growth of the craniofacial complex.

A sample of 88 male Long Evans rats of starting weight 90 ± 3 grams was studied, 71 animals having unilateral nasal blockage, 5 having a sham nasal operation unilaterally and 12 non-operated animals as a control. In this mixed longitudinal investigation 18 animals were sacrificed serially as a cross-sectional group. All the rats, except the nine animals in the soft tissue sample, were given intraperitoneal injections of NN'-di-(Carbomethyl) aminomethyl fluorescein (Dcaf), alizarin red S, terramycin, and terramycin and alizarin red S on days 0, 4, 11 and 25, respectively, after attaining the starting weight of 90 ± 3 grams, and were sacrificed on day 46 of the experimental period.

The general and local changes or reactions were investigated using weight gains, bacteriological, pH, temperature and soft tissue data. The cross-sectional data were used to evaluate the morphological changes over the experimental period, using photographic, radiographic and microscopic analyses. It was, therefore, possible to describe statistically the changes due to nasal blockage.

Finally, by a quantified microscopic analysis of the serial bone marking agents, a histological measurement of the effect of nasal blockage was made. A total of 32 paired anatomical sites in four coronal regions in the snout of the rat was investigated. The statistical evaluation of the growth differences bilaterally was achieved using an analysis of variance and, from a Duncan multiple range test, the students t test.

The following conclusions were made from this investigation -

1). The surgical technique was found to be satisfactory in producing unilateral nasal blockage with rapid post-operation recovery.

2). The histological examination showed no abnormal tissue changes except local post-surgical reparative processes.

3). The general health, as measured by the gain in weight, was found to be significantly inhibited by unilateral nasal blockage.

4). The pattern of bone growth of the nasomaxillary region was found to be complex and occurred by external apposition with concomitant internal resorption. Area relocation, differential growth and resorption were also demonstrated.

5). Quantitative coordinate analyses of the photographic and microscopic material, demonstrated as polygons, were effective in showing the growth changes cross-sectionally.

6). No gross skeletal changes were found following the surgical procedures and the minor changes reduced throughout the experimental period.

7). The bone thicknesses and bone increments were not significantly different when statistically compared bilaterally or between the groups.

8). This experimental technique has demonstrated that the effect of nasal blockage on the bone growth of the snout was so small as not to be significant statistically. While this does not rule out the possibility of a dynamic functional stimulus of the airflow, it does suggest that nasal blockage per se has a minimal effect on nasal growth.

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INTRODUCTION

There has been much controversy over the effect of chronic nasal blockage on the overall growth and development of the craniofacial complex. As the majority of children do pass through stages of partial nasal obstruction, this makes the issue important practically and clinically. The obstruction is usually brought about by engorged blood vessels of the mucous membrane linings of the nasal cavity and turbinates or by chronically inflamed or enlarged lymphatic masses such as the adenoids and tubular tonsils. It has been pointed out that, during the development of the permanent dentition, the child's lymphatic tissue is twice that of its mature amount and the airway is still growing.

Many investigators have concluded that nasal obstruction in young children will produce abnormal growth and development of the bony facial form including the shape of the dental arches. Other authors vigorously dispute this hypothesis.

Recent expansions of the functional matrix theory have postulated that such functioning units as the airway and sinuses have an intimate dynamic relationship with the surrounding tissues and play an important role in their growth and development.

Because of the myriad of other environmental factors which may be involved, it is understandable that there have been few human clinical studies in this field. Animal experiments have the advantage that controlled alterations of functional activity can be studied.

The aim of this investigation was to evaluate the effect of artificial, unilateral closure of the external nares in young male Long Evans rats on the normal growth and development of the craniofacial complex. This was a mixed longitudinal study of an altered functional environment on the rat skeletal form. With this experimental technique it was hoped to demonstrate the effects, if any, of the functional stimulus of the airflow or, alternatively, to illustrate the effects of surgery, or a combination of the two, on the growth of the craniofacial complex.

Longitudinal growth changes have been studied in a number of ways but only serial bone marking agents will reveal the sites and the amount of apposition at a histological level. Quantitative normative data have been evaluated for the direction and amounts of bone growth for male Long Evans rats using sequential staining in the midsagittal and coronal planes.

The objectives of this investigation were -

1). To consider concomitant local and general changes or reactions following unilateral nasal blockage, using the weight gains, soft tissue and bacteriological data.

2). To evaluate when the morphological changes occurred, using cross-sectional data.

3). To describe the gross morphological alterations following unilateral nasal blockage, using a quantitative analysis of the standardized photographs and radiographs of the rat crania.

4). To describe internal and external morphological changes in different anatomical regions from microscopic measurements of the hard tissue sections.

5). To give an explanation of the gross changes at the tissue level by a detailed microscopic measurement of the bone increments.

REVIEW OF THE LITERATURE

Human Implications of Nasal Obstruction

Tomes in 1872 suggested that the palatal and dental deformities found in malocclusions may be a direct consequence of nasal obstruction by enlarged tonsils. The anomaly, he felt, was caused by muscle pressures. Kingsley (1879) and Talbot (1894) contested this view and claimed that the facial musculature would be in the opposite direction to the deformity described.

Collier (1898) and Lane (1900) rejected the perioral muscle hypothesis and postulated a pressure hypothesis. They claimed that blocked nasal passages would have a marked negative air pressure during inspiration and that this force would be so great that the turbinates would almost be compressed to the nasal septum. Franke (1893), as cited by Brash et al. (1956), showed experimentally that the normal variations in air pressure within the nasal cavity are comparatively slight and rejected the mechanical pressure hypothesis as being exaggerated.

Spicer (1889) claimed that the lack of function was the cause of deformities following nasal obstruction. The normal functioning airway allowed the full development of the nasal passages and chronic disuse stunted this realization. This view was supported in the works of van der Klaauw (1948) and Moss (1954).

Most early authors felt that the facial pattern of the child would be affected by chronic nasal obstruction, leaving a flat skeletal profile, a narrow maxillary dental arch and a high vault. Angle (1907) claimed that mouth breathing was the most potent, constant and varied factor in the causation of malocclusions. Many of the authors

noted how wider systemic conditions were often associated with nasal blockage. Some of these earlier authors were Brady (1902), Tilley (1908), Hoggan (1914) and Johnson (1936) and they described decrease in resistance to infection, failure to gain weight and many other symptoms of poor physical development.

Duke, in 1930, claimed that nasal allergy produced deformities of the face which were different from the types of deformity due to enlarged tonsils and adenoids, and were due to a lack of aeration of the paranasal sinuses. Todd (1936, 1938) and Cohen (1937) agreed that, in fact, growth was retarded in all three dimensions. Straub (1944) felt from his studies that allergies must be considered as an aetiological factor in the growth of the nasal passages and paranasal sinuses and hence the face and jaws. Rosenberger (1934) divided the nasal obstruction into anterior and posterior types, both affecting the growth of the nasomaxillary complex. He did not feel that heredity was the cause of the anomalies found, as was frequently claimed.

Many clinicians have felt that the maxillary malformations gave rise to respiratory inhibition and thus vital embarrassment, and recommended intra oral expansion appliances. Macary (1954), treating 15 patients in this way, felt a marked improvement was achieved. Isaacson et al. (1964) and Haas (1970) also suggested improvements in the upper respiratory airflow, while Wertz (1968) felt the changes were minimal.

Among the recent investigators, the role of enlarged lymphoid masses obstructing the airway and affecting the growth and development of the dentition and facial skeleton has continued to be controversial.

Subtelny and Sakuda (1966), Ricketts (1958), Yip (1969) and Linder-Aronson (1974) have all noted neuromuscular compensations in the tongue and jaw posture before and after surgical removal of these masses. Yip and Linder-Aronson felt that these compensations before and after surgery were reversible, the latter suggesting a direct influence on the maxillary incisors and the mandibular position, the maxillary width and the depth of the nasopharynx. Undoubtedly, the changes would be expected to be relatively small but possible changes in patterns of deglutition as found by Yip may be sufficient to direct morphological change.

Respiration is the primary function of life which, after birth, is the only natural means of survival. Bosma (1960, 1963) showed how 30 newborn children aerated their lungs by gulping in response to their need. He stated that infants with Pierre Robin Syndrome should not have tracheostomies as the normal neuromuscular patterns would not develop. Removal of the tracheostomy tubes necessitated the opening of the oronasal airway as in the newborn. Turner (1913) reported on children who had worn tracheostomy tubes for long periods and concluded that the lack of nasal breathing had not interfered with nasal development of the dental arches and maxilla.

Congenital anomalies which help the understanding of the growth and development of the nasal cavity are rare. Among these is choanal atresia, first reported in 1829 by Otto. Since then over 500 cases have been reported. While this anomaly may be bilateral or unilateral, it would serve as a non-surgical natural form and function model. Morgenstern (1940) described a case of left sided blockage in which the left side of the face was less well developed than the right.

Radiographs revealed an underdevelopment of the inferior turbinates, left frontal and maxillary sinuses. In other studies McGovern (1950), Cracovander and Goodman (1956), Craig and Simpson (1959), Flake and Ferguson (1964) and Ransome (1964) with over 100 cases did not note or did not find any obvious asymmetry, but stated that these were rare. Most authors, however, did note how these patients had a high infant mortality.

McLaughlin (1949) in reporting a case of absence of a nasal septum assumed it to be acquired as no cases had previously been presented of true congenital absence. He mentioned the high possibility of birth damage and noted poor or retarded nasal development. Moss (1968) reported two cases of congenitally absent nasal septa and suggested that the nasal septum played only a secondary compensatory role in nasal growth.

While much research has been done in the field of human form and function, there is no clear picture of a causal relationship between nasal blockage and orofacial skeletal deformity. Brash et al. (1956) recognized the limitations of the clinical view and believed that nasal blockage might lead to pathological conditions which might interfere with the normal development of the nose or of the body as a whole.

Assessment and Measurement Techniques

A knowledge of the degree of maturity is essential in studies of growing animals. There are no definite criteria for determining the stages of developmental processes leading up to the attainment of maturity. Maturity is taken as meaning the full structural integrity of form with functional development. As body growth and development is an

integrated continuum, different aspects have been used to relate this degree of development. Chronological age is not a maturity indicator, but a time frame for comparing indicators. There is always a positive correlation of these indicators with growth but it can often be very low, to such an extent as to be of little value.

While most investigators using a maturity indicator have used body weight because it is the simplest to measure, it is probably the most susceptible to change due to environmental stress factors. Morphological changes were utilized in many of the earlier studies, e.g. Jackson and Lowry (1912), Sugita (1917), Donaldson (1912, 1924) and Cameron (1925), but their relations were merely guidelines. Jackson and Lowry pointed out that the head of the newborn rat comprised 23% of the body size, reaching a maximum at seven days of 26%. By adulthood, the overall head proportion of body size had dropped to 10% and then to 8% in animals over 300 grams.

As with most animal species, the rat shows a sexual dimorphism, with the male growing to a larger size than the female. The age of sexual maturity and sexual characteristics have been utilized in studies by Engle et al. (1937) and Hughes and Tanner (1970). The fact that there is a basic sexual dimorphism is the reason why mixed sex sample data should perhaps not be pooled as done in 1956 by Baer and Moss, using their data of 1954 and the data of Massler and Schour (1951).

Various techniques have been used to assess the degree of skeletal development. Among these, Bhaskar et al. (1950) used the tibia and Hughes and Tanner did a more comprehensive study of Long Evans rats studying the skull, limbs, pelvis and noserump lengths to evaluate the skeletal norms of development. They calibrated the equivalent hand

wrist radiographical tabulation of ossification centres as had been used for children.

De Groot (1963) and Mosier (1969) used the tail length as the criterion for relative growth of the body under normal and in operational studies of the catch-up phenomena. Hammett et al. (1923) and Dienstein (1956) stated that while the eruption pattern of the teeth in man is a relatively good guide to dental development, its assessment in the rat is practically difficult and there is only one dentition erupting over a short period of growth.

Burr and Mortimer (1938) and Jarabak (1942) described a cephalometric technique on a living rat but did not use it to study growth. Spence (1940) in a longitudinal growth study of 62 Wistar rats pointed out two drawbacks of vital cephalometrics, i.e. the difficulty in accurately positioning the rat skull and the lack of normative data.

Baer (1954), Ford et al. (1959), Asling et al. (1963), Cleall et al. (1968) and Hanada (1969) all used serial longitudinal cephalometric studies of rats, often together with other branches of research. Their overall findings were similar, showing to varying degrees of accuracy the relative two-dimensional growth, but with no good estimate of growth rates because of the difficulty of superimposition and deciding which structures should be used for superimposition.

Although vertex basillar and lateral radiographs have been used in rat craniofacial cephalometrics, the limitations of the small skull, using the loose and fragile tympanic bullae for the positioning of the earrods, will always leave a technical error too large for refined growth evaluation. In any case such radiographical techniques will never reveal the specific regions of growth. Implant markers have been used in

rabbits by Selman and Sarnat (1955) and Erickson and Ogilvie (1958) but the rat appears too small for such a technique without damage to the animal or without movement of the markers.

Other radiographical techniques have been used cross sectionally to evaluate the bone density and the orientation of the trabeculae in rats. Greulich (1959), using a microradiographical examination of foetal, postnatal and adult rat demineralized bone, showed the relative uniformity of bone matrix, regardless of its mineralization. Williams (1964) used a similar technique on undecalcified material in a nutritional study on calcium intake. Cimasoni and Becks (1963) utilized this technique to show differences in alveolar bone density and periodontal membrane thickness following the extraction of molar teeth and hypophysectomies. Das (1965) used the method to show the marked alteration in bone patterns following bilateral condylectomies.

There are many agents now available as research tools to label new forming bone. Any metabolic factor in bone growth theoretically may be used in the uptake of radioisotopes. Jarabak (1953) and Dixon (1961) demonstrated the uptake of radioactive calcium in Wistar rats cross sectionally. Unfortunately, a multiple injection sequence is not suitable with this technique and hot spots and blurred images of beta radiation limit its value.

Other agents have proved more suitable for demonstrating bone apposition. Cleall et al. (1964), Prescott et al. (1968) and Storey (1968) are investigators who have evaluated a range of organic dyes. Jacobson (1970) reviewed the bone marking agents commonly in use today.

Although attempts have been made to evaluate the site of uptake of the bone marking agents, this aspect is still not clear. Wallach (1963) showed how Dcaf could be used specifically in microdetermination for the calcium ions present. Albert (1953) noted the affinity of tetracyclines for metallic ions. Harris (1962) showed how tetracyclines bound at low concentrations to all growing bone sites and also to sites of secondary mineralization, any areas of resorption or remodelling, or sites where the crystal surfaces are not complete.

Urist et al. (1963) felt that tetracycline bound to the crystal surfaces as a complex with calcium but he could not rule out the possibilities of complexes with collagen or polysaccharides in newly mineralized tissues. Milch (1958) and Myers (1968) in comparing tetracyclines with alizarin red S found them to be very similar, being negatively charged and having weak chelating powers requiring a site of apposition either on collagen fibrils or on the crystal surface. Myers supported the view that the carbonyl and hydroxyl groups form a chelating ring with calcium such that alizarin and phosphate ions competed for the same site on the apatite crystal surface.

Several methods of administration have been used for bone marking agents. Belchier (1736) and Brash (1924) used the oral route with madder (from which alizarin red S was obtained). Schour et al. (1941) claimed this portal gave vague marks while, if injected, clear stripes were visible between the multiple injections of alizarin red S in monkeys, rats and hydrocephalic children.

Cameron (1930) showed how subcutaneous, intraperitoneal, intravenous and intramuscular routes could be used for various animals.

The intraperitoneal injection was relatively safe, allowing large volumes to be used with moderate uptake of the dye. The intravenous route involved a more difficult technique with a very rapid uptake and the dangers of toxicity were more evident. The intramuscular route allows only relatively small volumes to be injected with fairly rapid uptake into the bloodstream.

Cameron (1930), Hoyte (1960) and Cleall et al. (1964) showed bone marking agents were present on all bone surfaces following intraperitoneal injections, showing the agent colour within minutes. When the agent is removed from the circulation by excretion, the surfaces where apposition is occurring bind and cover the marked area. The areas undergoing active resorption or no growth remove the weakly bonded agent so that only in appositional areas will the bone marker remain. In serial bone marking techniques, the intervals between stains reveal the apposition in that period of time. Should resorption commence, the stains of former apposition are removed.

Toxicity levels of these drugs have been evaluated by Bevlander et al. (1960), Frost (1960), Cleall et al. (1964) and Suzuki (1966) as well as by many others. They felt that, in the dosages used, the inhibitory effects of these agents are not great and that the animals very quickly recover from any temporary setback. At higher dosages Bevlander (1959, 1960, 1962) showed skeletal inhibition, especially in chick embryos which were deformed. Paff et al. (1950, 1951) showed the inhibition of bone growth in vitro using alizarin red S. Frost felt that tetracyclines were less toxic than alizarin red S.

Schour et al. (1941) and Harris et al. (1964) felt that the

effect of injecting a vital marking agent was to cause erratic bone growth. Alizarin red S caused inhibition of collagen synthesis in vivo and Harris suggested that it also retarded crystal growth but that the toxicity subsided quickly and was without serious significant toxic complications to the animals. Robinson and Sarnat (1955) and Hong et al. (1968) felt that in the dosages commonly used the bone formation was inhibited for several days with alizarin red S. Yen et al. (1972) suggested that the dentine growth was inhibited for up to eight days.

The amount of bone marking agent taken up depends on a number of factors: the age when the animals were injected, which reflects the rate of bone growth at that time, and the method of administration. Higher concentrations and dosages will have a direct effect on the bone mark intensity. Lower concentrations, even though present, may be invisible. If a long period of time follows an injection, new bone may mask the underlying mark or, especially in young animals, remodelling could resorb the marked bone.

Schour (1936) utilized alizarin red S for a quantitative measurement of dentine apposition in the rat using serial vital marking. Massler and Schour (1951), Baer (1954) and others have used these agents for gross assessment of growth areas. Cleall et al. (1968, 1971), Jacobson (1970), Ryll (1972) and Smith (1974) used a sequence of different agents so as to obtain and differentiate the agents which all fluoresce under ultraviolet light, with the exception of trypan blue, thus enabling a longitudinal bone growth assessment.

The Growth and Development of the Rat

The embryological development of the rat follows a similar pattern to that of man, the major differences being the rapidity of development in the rat with birth being approximately 20 days after conception and that the rat is multiparous in comparison to man, having typical litters of six to twelve pups. The neonate rat pup is, however, still less well developed than the human neonate with, for example, the eyelids sealed.

During the first few days after conception the zygote undergoes cleavage and blastula formation after which the gastrula develops. By the 8th or 9th day the primitive streak can be identified to mark the development of the embryonic disc into the trilaminar embryo. These stages have been documented by Witschi (1956) and Snell and Stevens (1966) who continued by stating that the mandibular facial processes fused on the 13th day, followed by the maxillary and frontonasal processes.

As the primary flexure extends, several precartilages become distinctly identifiable around the 15th day. The following days lift the snout off the anterior chest wall. Baume (1967), comparing human and Long Evans rat foetuses, showed that the phylogenetic pattern of the chondocranium was followed, and in the 15 mm rat pup the occipital, sphenoidal, trabecular and septoethmoidal cartilage constituents are bonded together. On the 19th day he noted ossification centres in the basioccipital cartilage, followed rapidly by others in the cranial base.

A number of other authors, among them Hughes et al. (1967), Moriarity (1963) and Coleman (1965), have investigated the prenatal development of the palatal shelves and their

fusion. On the 16th day foetuses' palatal shelves lie on either side of the tongue, elevate and then fuse by the 17th day in utero with concomitant epithelial degeneration.

Mohammed (1957) used normal and ia strain rats, the latter having retarded osteoclastic activity but normal bone apposition, for an embryological and histological description of early maxillary bone formation. His techniques superimposed cross-sectional tracings of so called identical regions to show the growth and development. The nasal capsule development was described and he stated how the lateral nasal cartilage acted as a template for the maxilla. He went on to describe the palatine cartilages and stated how they became templates, lateral to which formed the palatine bones and the accessory nasopalatine, maxillary and palatine cartilages. Only one maxillary ossification centre was observed at 17 days after insemination, a date verified by both Anderson (1967) and Hughes (1967), who stated that the bony processes approximated in the midpalatal region around the 21st day.

Curley and Pelas (1969) showed how the Long Evans rats, while lighter at birth, were more viable than the Sprague Dawley neonates and were faster growers. At 8 days they were even in weight and at 14 days the Long Evans rats were heavier. At the same Symposium (1969) on the Long Evans rat, Stratman and Conejeros demonstrated how this strain had a higher resistance to induced pulmonary and intradermal bacterial infection than the Sprague Dawley and Wistar rats. Kidney damage and the urinary infection tests were least well resisted by the Long Evans sample.

Many strains of rats and other rodents have been inbred to exhibit certain features which make them suitable experimental models

for the biological sciences. The major strains of the Rattus Norwegicus have been used in most growth studies although specific characteristics such as those exhibited by the grey, lethal or ia rat, which have been described above, and the ajax mice which undergo spontaneous cleft palate have been used.

The Long Evans hybrid was developed from the albino and agouti rats in 1915. Collection of data over the years and at the Symposium on the Long Evans rat held in 1969 gave rise to a better understanding of the normal physiological development of these animals. Even so, it is always accepted that within the strain there is a wide biological variation so that data obtained serves as a guide only to the other researchers.

The growth and development of the craniofacial skeleton is complex, being a three-dimensional relationship dependent on time. The growth factors are closely interrelated and are not clearly understood. In this review the major morphological changes will be detailed and then, through elaboration of the hypotheses and experimental data, an understanding of the present state of knowledge of form and function will be discussed.

The postnatal growth of the rat is one of rapid deceleration and the changes in craniofacial morphology to the long snouted adult form are largely of differential growth. As time progresses, the relative generalized rapid growth of the neonatal bones becomes more and more isolated into specific growth sites (Massler and Schour, 1951 and Baer, 1954). The preponderance to anteroposterior growth and elevation of the snout (Baer, Massler and Schour, and Moss) is seen, which Moss termed viscerocranial elevation.

Most experimental work on growth and development of the rat has been done on small samples of animals using two groups, one as a control and the other as an experimental group. The age groups used also fall into two groups, the neonatal and post-weaning. The advantage of the former is larger growth changes while the latter is independent of the maternal care.

Massler and Schour (1951) using 113 Wistar rats from birth to 300 days cross-sectionally injected these animals with alizarin red S, then examined the marked bone. These sites were measured with calipers and while most extrapolations by the authors were probably correct, the accuracy of the technique was not great. However, this description of the rat craniofacial growth served as a good basis for other investigators.

The neonatal head was described as being short, flexed and rounded in proportion to the mature animal, a description with which Baer (1954) concurred. The postnatal growth pattern of the craniofacial complex was in two phases. The first was for up to 60 days when there was generalized bony growth, although this was in differing amounts at different sites. This was a period of rapid increase in the size of the cranial vault with only minor changes in its proportions. In the second phase it was claimed that, after 70 days, the growth was much reduced and primarily localized at certain sites and the cranial vault showed only a slight increase in size.

That the rapid growth of the cranial vault occurred in relation to the brain growth was agreed upon by Moss (1954) and Baer (1954) but they felt that this ceased around 20 days post natum. Baer and Moss (1956), pooling their data with that of Massler and Schour, felt that

this transition or interphase occurred at different ages with the different strains, beginning at 10 to 15 days post natum.

Massler and Schour demonstrated that the cranial vault of the rat showed both ectocranial and endocranial apposition and disagreed with the findings of Brash (1934) who claimed that internal resorption took place with apposition ectocranially. They also explained that the diploe and frontal sinus formed by resorption within these bones. Conclusions drawn from the data suggested that the sutures were important growth sites and that the sutural interdigitation was due to trabecular growth resulting from fibre tension. The growth sites studied were used to explain that the skull grew more rapidly in length than in width and least of all in height.

The total findings of Massler and Schour were that the cranial width was completed by the 20th day, the cranial vault length by the 40th day and that the snout continued to increase beyond 300 days. However, the amounts of growth and the duration of growth of bone at these and other sites were more accurately assessed by serial bone marking techniques.

In a similar experiment Baer (1954) used 77 albino rats of both sexes over an experimental period from birth to the 101st day post natum. A single intraperitoneal injection of alizarin red S was given, then the animals were sacrificed at varying times. A number of anatomical measurements were made as regards the length and width of the bones. A gross assessment was made of the sites of the alizarin red S.

The majority of the findings supported Massler and Schour but did make several important new findings. Baer felt that the changes in cranial form of the vault bones occurred by translation, a hypothesis

hinging at sutures. He also noted how the opposite bone edges of sutures grew at unequal rates, a fact now well appreciated after studies by Sarnat (1968) and Cleall et al. (1968). Baer disagreed with the suggestion of Weinmann and Sicher (1955) that the cranial vault bones flatten by endocranial apposition centrally and resorption near the sutural margins. Sutural areas reveal bone surfaces in depth and macroscopically exaggerate the amount of bone forming in comparison with surface apposition.

Cartilage is the main skeletal support in the neonate as the bones of the craniofacial complex are very thin and fragile. The cranial base at birth is made up of four elements around the foramen magnum known as the occipital complex of the basioccipital, a pair of occipitals and the supraoccipital. These cartilages grow by interstitial growth at their synchondroses and apposition at their borders, allowing craniofacial growth in height and width.

The sphenoid and basisphenoid are primarily responsible for growth in the cranial base in the anteroposterior and lateral dimensions. The cartilages are ossifying from centres within the cartilage such that the active cartilaginous growth reduces with time, but at a slower rate than in man. Cleall et al. (1968) showed the continuation of this growth quantitatively, primarily in the anteroposterior direction up to the 100th day post natum.

Massler and Schour (1951) described the growth from birth to 300 days and noted that the nasal bone growth at the internasal suture ceased early in the postnatal development, around the third day. However, more growth took place in the anteroposterior direction and it was pointed out that, while some growth occurred at the anterior or free margin, far more took place at the frontonasal sutures and continued to the 70th day.

Cleall et al. (1968), in a quantitative manner, gave a more detailed description of the growth of these bones from approximately the 30th to 100th day postnatally, using serial bone marking agents. They verified that the prime bone growth sites were at the frontonasal suture and that a differential growth took place with almost two thirds of the growth occurring on the nasal side of that suture. The growth at the nasal tip was two thirds that of the posterior border and continued throughout the entire experimental period at an ever decelerating rate.

In that study it was shown that the internasal suture did continue growing throughout the experimental period, in conflict with Massler and Schour (1951). The more refined technique of serial bone marking analysis is the most probable reason for this difference. The lateral borders of the nasal bones in proximity to the premaxillary bones were sites of apposition while the most obvious factor, external apposition and internal resorption, was typical of the whole nasal cavity. From an incremental standpoint, flat surface apposition was shown to be less than the sutural growth.

Isotupa et al. (1965) and Isotupa (1974) using alizarin red S as a bone marking agent concurred with these findings after examination of the trabecular patterns and split line techniques.

The premaxillary growth in the postnatal development of the rat is marked, particularly in the anteroposterior direction. Cleall et al. (1971) showed how the interpremaxillary suture showed limited apposition at the approximating bony surfaces of the suture. Bone remodelling of the alveolus around the incisors was noted with medial and inferior apposition and superior and lateral resorption. The alveolar growth at the anterior edge was also significant in an anteroposterior

direction. Apposition superiorly and laterally to the incisors was most rapid while inferiorly oral growth was only moderate. Posteriorly, the inferior premaxillary-maxillary suture was a growth site with twice the growth on the premaxillary surface compared with the maxillary surface, but with less than the frontonasal suture.

Isotupa (1974) showed from the trabecular pattern that this bone grew largely in a distal direction superiorly up against the nasal, frontal and maxillary bones. Like any region of growth, the snout grows in a three-dimensional manner which can be illustrated, he felt, by the trabecular pattern passing through the bones in an internal-external direction, as well as in a curved pattern seen from a lateral macroscopic view. Baer and Isotupa agreed that the bone develops from a single centre of growth.

Baer (1954) suggested that the nasal bones grew medially at the internasal suture, a site of growth which Isotupa (1974) felt was small in extent. The superior surfaces of the nasal bones were areas of apposition. Baer noted that no increments of growth were found on the premaxilla at the nasomaxillary junction, a fact with which Cleall (1971) concurred.

Baer described the maxillary bones as laterally housing the molar dentition. The growth of the maxillary alveolus at the posterior end of the tooth row allows an increase in the maxillary length. Cleall (1974) showed that the alveolar processes and sockets grew inferiorly and laterally.

Ventrally, both Baer and Cleall described the maxillary palate growth at the intermaxillary suture, widening the palate. Isotupa (1974) showed how the orientation of the trabeculae altered with growth in the rat. They all showed rapid growth with nasal resorption and oral deposition.

Massler and Schour stated how the frontomaxillary suture increased the anteroposterior growth vector. Just lateral to this point in the malar process Ryll (1972) and Cleall et al. (1971) found the most rapid growth area with superolateral apposition and internal resorption. The external surface of the lateral aspect of the nasal cavity was an area of uniform apposition with nasal cavity resorption.

Anderson et al. (1967) studied the postnatal growth of the palate and concluded that the midpalatal suture was an epiphyseal-like, endochondral bone forming site up to the 20th postnatal day, by which time growth in width ceased while osteoblastic evidence in palatal thickness remained at 35 days. The hyaline cartilage persisted across the midpalatal suture of the adult rat. They stated that the first molars erupted at 15 days and oriented downward and laterally in eruption.

The palatomaxillary suture was again found to be a large component in an anteroposterior growth by Cleall et al. (1968) with a total almost equal increment of bone growth on both surfaces. Just as in man, the palatine bones are wedged between the cranial base and the facial bones, the connection being through the pterygoid plates and the pre- and basi-sphenoid bones (Greene, 1959).

Weinmann and Sicher (1955) described the simplest bone example, the pair of flat lacrimal bones bordering the inferior orbital foramen. However, they did not mention the point made by Greene that the lacrimal canal passed through the anterior dorsal corner of this bone, connecting the orbit and nasal contents.

Searls and Kinser (1972) illustrated the unpaired vomer bone at the base of the nasal septum above the premaxilla and maxilla in the

midline. While the growth of this bone was not described, the bone requires a great deal of remodelling as it was in the septovomerine area which Searls and Kinser found to be a growth site.

The paired malar or zygomas are thin, strut bones connecting the maxilla with the squamosal bones lateral to the orbital contents, supporting the masseter muscles. Cleall et al. (1968) showed how these bones resorbed medially with apposition laterally.

Dixon (1961), Searls and Kinser (1972) and Nelson (1973) used mixed longitudinal studies of rats labelled with radioisotopes of calcium, tritiated thymidine and proline respectively to study the growth of the nasal septum. The age ranges were from birth to approximately 60 days and suggested that the superior area of the septoethmoidal cartilage and the mid-septonasal junction were involved in the antero-posterior growth.

Growth Mechanisms

The cybernetic chain of reactions and the mechanisms controlling growth and development of the craniofacial complex are largely unknown and the different disciplines are approaching this field in a variety of ways. At the most basic level there is a dichotomy of views as to the effect of the environment on the genetic potential. At one extreme there are those who believe that the environment has almost no effect on the eventual form (Brash, 1924, 1934 and Brodie, 1941) while at the other extreme there are those who feel that the environment is the principal factor in craniofacial morphogenesis (van der Klaauw 1946, 1948 and Moss (1962) and Moss and Salentijn (1969)).

Brodie (1941) after studying the Broadbent human longitudinal cephalometric material concluded that the morphogenetic pattern of growth in the dentofacial region was established early in postnatal life and did not change thereafter. The inference from his articles was that the genetic control could not be regulated by environmental factors.

The functional matrix

Moss, in a series of publications covering twenty years, proposed the functional matrix theory based on the works of van der Klaauw (1946, 1948). This contends that all the functioning tissues are the primary factors in controlling the spatial size and shape of the skeleton. This view supports the strong role of environmental factors in craniofacial growth. He and his co-workers have carried out many experiments and cited many others as illustrations of the effects of the functional matrices, while other interpretations could also be made of their findings. No simple procedure can be devised to evaluate the actions of the proposed matrices.

To elaborate a little further, he proposed that the skeletal components were made up by the functional matrix which performs the function and a skeletal unit which supported it. A skeletal bone could, therefore, be interpreted as being made up of all the microskeletal units with their functions bearing upon it. Functional matrices could be divided into periosteal and capsular, depending on their site and type of activity. Periosteal matrices act primarily for transformative growth, e.g. muscles, glands and blood vessels and the capsular matrices are responsible for translative growth. The capsular matrices of greatest interest in this thesis are the masticatory and respiratory functional spaces.

These airways and sinuses are suggested as dynamically stimulating growth of the adjoining skeletal units.

Moss (1969, 1971) used the term neurotrophism which he had defined as a non-impulse transmitting neural function that involves the axoplasmic transport and interactions to regulate homeostatically the form, composition and functional integrity of the tissues. The nature of these substances remains unknown but by implication it is suggested that embryonic ectodermal tissues continue to regulate mesenchymal tissues. Three subdivisions of the neurotrophic expression are given: neuroepithelial trophism (suggesting that loss of taste and special sensory receptors leads to hypoplasia, a view also held by Bosma (1970)), neuromuscular, and neurovisceral trophism regulating glands and fatty tissues. He suggests that the epithelium lined functional spaces may be regulated trophically.

Cartilage

Connective tissues have been associated as primary growth mechanisms based on a concept of tissue dominance. The role of cartilage in craniofacial growth has been claimed as primary by many authors, the most notable being Scott (1953), Baume (1958, 1961, 1967), Koski (1960). Weinmann and Sicher (1955) and Baume (1958) discussed four types of cartilage: those areas which remain cartilaginous throughout life, like the anterior portion of the nasal septum; the areas of cartilage which form as a temporary measure in situ as is found in the mandibular condyles or coronoid processes; the primary cartilages which play an important role in growth, eventually being replaced by bone, such as the cranial base, a process known as endochondral ossification; and

finally, the fourth role of supporting the facial bones forming around the cartilaginous masses.

The histological appearance of cartilage growth is clearly understood as being both interstitial and appositional. Koski and Ronning (1969, 1970) investigated the growth potentials of the cartilaginous areas of the epiphyseal cartilages, cranial base synchondroses and the condylar cartilages by transplanting them intracerebrally and subcutaneously. Their results showed how the majority of the transplants survived and the growth potentials were limited with most growth in the epiphyseal cartilages and least in the condylar cartilages.

Sutures

The functional significance of sutures as sites of growth has long been discussed. In earlier years Mair (1926) and Troitsky (1932) and, more recently, Weinmann and Sicher (1955) and Prahl (1968) have advocated the hypothesis that sutures form a primary growth site. The loose connective tissue could proliferate, separating the bone ends in an active manner, thus allowing bone growth.

Burdi (1966) stated as a hypothesis that one reason for the long snout in the rat and other animals was that there was non-fusion between the premaxilla and maxilla, while in man synostosis occurs in the embryo. Once a bony bridge forms across a suture, no further active growth will occur between the bone surfaces.

Moss (1954, 1956), Scott (1954) and Pritchard et al. (1956) voiced the opinion that the bones grow at the sutural margins and that the sutures have a rather passive role, as suggested by Brash (1934), of holding the bones together. This second hypothesis is now more generally

accepted and sutures are regarded as readjustment sites (Baer 1954).

Moss (1954) and Weinmann and Sicher (1955) described three layers in sutures, two capsular layers plus a middle zone. Pritchard et al. (1956) further subdivided the suture into five intervening layers and two uniting layers, two cambial or cellular layers in continuity with the bone surface, two capsular fibrous layers covering and uniting the bone ends and a middle zone of loose connective tissue.

Bone

Endochondral and intramembranous ossification is one process of bone formation occurring in two locations with differing templates. Mechanisms of bone formation are dependent on the level of investigation. Brasseur et al. (1946) felt that the architecture of bone was related to its vascular pattern, a view supported by Maximow and Bloom (1957). McLean (1968), Urist (1968) and Gaunt et al. (1971) relate the formation to cell and tissue change.

Glucksmann (1938, 1942) stated that osteogenesis in his in vitro studies of chick osteogenesis was influenced by tensile and compressive forces, the former stimulating osteogenic activity and trabecular orientation. Bassett (1961, 1962, 1968) has shown that both dead and living bone under deformation produces piezoelectric potentials which increase with tension and decrease with pressure. While supportive works have been carried out by Becker (1966), Epker (1966) and others, illustrating bone apposition in areas of electronegativity, no experimental procedure has demonstrated the bone resorption in piezoelectropositive areas.

Bone tissue growth is only by means of apposition, and organ growth depends upon a complex system of differential apposition with simultaneous resorption. Enlow (1963, 1966, 1968) illustrated some of

these complexities, such as area relocation, a phenomenon seen in the snout with internal resorption and external apposition of the facial bones so that the former external points become closer and closer to the internal surface. The 'V' principle allowed elongation and widening of bones and terms such as cortical drift and cortical layering were introduced to explain the differential apposition and resorption.

Hoyte and Enlow (1966) demonstrated that the muscle attachments to the periosteum were sometimes sites of resorption, contrary to the popular belief in the tension pressure theories. Translation or displacement was illustrated by Moss (1954) and Baer (1954) by the brain contents moving the cranial vault bones. Scott (1953) gave as an example the nasal septum separating the facial bones. It had been hoped that the investigation of form and function experiments might have shed more light into the control of growth mechanisms.

Form and function experiments

The whole field of animal form and function experiments has been used to test growth hypotheses. While superficially many techniques have been used, there is in fact a paucity of adequate biometric techniques and a limited number of surgical and non-surgical procedures. Among the non-surgical environmental studies there have been investigations into the effects of seasons (Makino, 1951), exercise (Haitai, 1915), lighting (Holmqvist, 1966) and the effects of drugs - papain (Ronning, 1971) and radioactive sulphate (Young, 1960). Watt and Williams (1951), Horowitz (1954), Hiiemäe(1964), Moore (1965) and Whitely (1966) have all studied the effect of food consistency on the skeletal form. Watt and Williams found that the mandibles of 49 rats fed on a hard diet were

consistently heavier than those of the rats on a soft diet, but the difference was not marked. Moore, using 40 pairs of rats, found that the crania and mandibles were significantly lighter and the masticatory muscles similarly smaller in the soft diet group. Moore also found a reduction in the condylar angle in the soft diet group. Whitely felt that while food consistency could have elicited a change, his research failed to demonstrate it.

Surgical procedures may be regarded as being of two types, local or distant. Moss (1961) and Fenart (1966) studied the craniofacial growth of rats whose fore or hind limbs had been amputated neonatally. This led to changes in head posture and to rotations in the otic capsule. These cranial changes were claimed by Moss to show that the branchial arch rotation relative to the neural cranium was accentuated with forelimb removal and retarded with hindlimb removal.

Soft tissues have been removed from animals to study their actions. Wessely (1920) removed the eyeball of rabbits, dogs and pigs to find the orbital cavity grew less rapidly than the normal side, with a deviation of the snouts to the operated sides. In a further experiment induced glaucoma, increasing the size of the eyeball, also increased the size of the orbital cavity. Sarnat (1965) repeated the earlier experiment of eyeball removal in rabbits and supported the finding of local orbital readjustments, but did not find any deviation of the snout.

The effect of cranial contents on the postnatal growth of Long Evans rats was investigated by Young (1959). By aspirating neural tissue in two-day-old rats, he induced microcephaly and, by injecting a kaolin solution, he produced a hydrocephalic skull. These procedures demonstrated compensatory changes in the rat calvaria. Moss (1954) and

Selman and Sarnat (1957) extirpated sutures in rats and rabbits, respectively. No marked alteration in skull morphology occurred and showed the secondary role of sutures and supported the functional matrix hypothesis.

In attempts to assess the role of muscles in the final form of the craniofacial complex, many of the muscle groups have been sectioned, paralyzed or excised. Nerve sectioning was attempted by Washburn (1946), using 26 neonatal rats, sectioning the facial nerve and swabbing the wound with alcohol. The deviation over the three-month experimental period showed, he suggested, the pull of the facial muscles. On repeating the experiment, in rabbits, he found the opposite deviation of the snout, which he claimed as demonstrating cheek atrophy. Experiments such as these demonstrate the wide variability of the results which may be due to differing techniques or to comparative anatomical differences, and which may be interpreted in different ways.

Because the muscles of mastication are relatively large in the rat, they have been found very suitable for form and function investigations. Temporalis muscle surgery was found by Pratt (1943), Washburn (1947), Horowitz and Shapiro (1951) and Horowitz (1954) to cause failure of growth of the coronoid processes, Horowitz also noting the deviation of the incisors to the operated side. Boyd et al. (1967) using guinea pigs questioned this atrophy as being a result of interfering with the blood supply. Craniofacial asymmetries were found following unilateral masseterectomies by Pratt (1943), Washburn (1946), Horowitz and Shapiro (1955) and Horowitz (1954) with the deviation to the non-operated side. Ryll (1972) agreed but felt that sutural adjustments tended to reduce the asymmetry in a rebound response.

Other muscles have also been studied. Simpson (1966), removing the medial pterygoid, found minimal changes, Cullen (1955) also found minimal changes on removing the anterior belly of the digastric muscles while Horowitz and Shapiro (1955), using a similar procedure, induced mesiocclusions. The only significant demonstrable effect on the form of facial muscle extirpation was when the entire orbicularis oris muscles were removed by Horowitz and Shapiro (1952). Washburn (1946) severed the long neck muscles, noticing the absence of nuchal and mastoid crests.

In a rather unusual experiment Lamorlette (1973) removed the anterior third of the tongue of 39 twenty-day-old rats, sacrificing them at 64 days. The morphological changes due to the orofacial musculature not being balanced by the tongue resulted in a foreshortening of the snout and a general lack of maturity. The palatal vault was shallower, the palatal width was less. The incisor relationship remained normal and the procion vital bone marking agents demonstrated the sites of the maxillofacial growth. While most authors do note the fact that the effects of surgery may have some effect, the great variation in findings suggests that possibly the different results are the result of different surgical techniques and coincident damage, locally and generally.

Hard tissue removal form and function experiments have been used in vivo and in vitro. Baker (1922), Landsberger (1924) and McFee and Kronman (1969) studied the effects of dental extractions in sheep, dogs and rabbits. They found varying degrees of asymmetry, from severe deviation towards the operated side to slight deviation to the untreated side. Baker (1941) removed the formative organs in three-week-old rats leaving the incisors. He noted a deviation to the operated side over

a seven-month period and felt that there may be a potential of growth of the dentition. Young (1960) removed the tooth germs of rats and noted no marked effects on growth. Cimasoni and Becks (1963) removed molars and found no significant changes, while Ryll (1972) found primarily local growth reduction with alveolar increase for repair and re-establishment of functional strength.

The role of the mandibular condyle on mandibular growth has been considered controversial. Collins et al. (1946) and Weinmann and Sicher (1955) described the normal histological appearance and decrease in growth activity with age. Blackwood (1966) supported Koski (1965) in suggesting the condyle was different from epiphyseal cartilage as revealed by autoradiography. Unilateral condylectomies were carried out in 24 rats by Jolly (1961) who noted only a slight asymmetry and a change in the incisor wear facets. The healing of the condylar region was by fibrous tissue. Bilateral condylectomies were carried out by Das (1965) and Gianelly and Moorrees (1965) on Holtzmann albino rats. Das felt growth had not been normal and that muscle hypertrophy was the reason for increased bony apposition as muscle attachments, the mandibular angle and then the coronoid regions re-adapted to the new environment. Gianelly and Moorrees found no change in the overjet or overbite while the mandibles were, on average, shorter.

Fick (1857) suggested that the nasal septum had a mechanical potential which could thrust the palate down and forward. He trephined the nasal bones and removed the anterior nasal septum in several animals and noted that the snouts subsequently grew upwards. Franke (1921) suggested that so much damage may have been done to the nasal bones, the ethmoid sinuses and turbinates that this experiment could not be used to demonstrate the role of the nasal septum. Landsberger (1929) repeated

Fick's experiments on dogs of two weeks of age, sacrificing them at six months, and supported Fick's contention of the role of the nasal septum.

Using non-surgical experiments Baume (1958) suggested that the nasal septum was a growth centre and merely an extension of the cranial base. Young (1960), Dixon (1961) and Nelson (1973) used radioisotopes to illustrate their uptake in the nasal septum. Young (1961), using a radioisotope technique, felt that the nasal septum stimulated growth in 25-day-old rats over the 45-day experimental period. Ronning (1971), using papain which inhibited cartilage growth, demonstrated an inhibition of craniofacial growth and development in a large sample of rats.

A radiographic study was carried out by Moss (1968) who removed the nasal septa from 12-day-old Shermon rats using electrocautery. In his findings he suggested that the nasal septum played a secondary compensatory role even though some dorsal collapse occurred while the overall splanchnocranial growth was normal. Babula (1970) working on Ajax mice found no significant differences between the normal mice and those with spontaneous cleft lip and palates. There was a decrease in the anterior one third of the snout in the latter animals, but he felt that the nasal septum was not a prime growth centre.

A number of surgical experiments have been attempted in various animals to investigate the role of the nasal septum. Sarnat (1968, 1969) removed various amounts of nasal septum in rabbits, finding a lack of growth after the 105-145 day experimental period. There was a shortening of the snout, a relative mandibular prognathism and overeruption of the incisors. When the nasal septum was dislocated, Wexler and Sarnat (1965) found no deformities of the snout, jaws or teeth. Experiments

on the rat model have had similar effects. Ohyama (1969) resected the nasal septum in 34 male Wistar rats using an electric knife. Serial radiographic views revealed an arrest in anteroposterior growth. Ohyama suggested this was due to a lack of facial bone separation and demonstrated changes in the mandible with a mesiocclusion of the molars but a normal incisor relationship.

Ziem (1879), cited by Collier (1889) and Wallace (1927), created unilateral nasal blockage in young sheep, calves and goats using antiseptic wool. Widespread consequences were observed after a long experimental period. The maxilla and frontal bones were retarded in growth and he noted extreme deviation of the nasal septum, along with facial asymmetry. The mucous membranes were exuberantly and chronically swollen. Brash et al. (1956) cited these authors but, after extensive research, could not find traces of Ziem's 1879 article but only the 1883 paper which referred to it and this latter mentioned only dogs and rabbits.

Anderson (1909) used rabbits, guinea pigs and dogs and sutured their nostrils closed. Although he did not record the growth changes, he did note the serious symptoms subsequent to sealing the nostrils. He stated that nasal obstruction led to a lowered resistance to infection, serious impairment of vitality and even death. On studying the blood of the dogs, he found that there was a lack of haemoglobin, oxygenation and dilation of the heart. He also noted that the dogs' coats altered with loss of hair. Emphysema of the lungs could be demonstrated histologically and this condition was reversible, following opening of the occluded nostril.

Emslie (1952) tried to induce mouth breathing in albino rats with no success, stating that the alimentary canal would inflate and the animals all died within a week. His explanation of death was that in its efforts to inhale, the rat suffocated. This experiment reveals how the upper respiratory passages are vital structures and how sensitive they are to surgical intervention.

The only successful experiment in nasal blockage in rats was carried out by Gross (1963). Using post weaning Wistar rats, he used electrocautery to block the nostrils of 25 rats unilaterally, the nostrils of 25 rats bilaterally with another 25 rats as controls, with littermates in each group. In 15 cases the surgery was repeated after incomplete closure of the nostril. In the seventh and eighth week the last six rats which had undergone bilateral nasal blockage died. At these times a control and a unilaterally blocked nostril animal were sacrificed. At the end of the experimental period, the surviving 16 controls and 19 unilaterally blocked nostril animals were sacrificed.

The weights of the animals were taken weekly and significant differences were found between the weights of the control animals and the operated groups and between the unilaterally and bilaterally operated groups. Gross made 20 linear cranial measurements with calipers and two limb measurements and found that the heavier rats were skeletally larger, most values being significant at the 1% or 5% level. As no correction was made for the different weights of the animals, the measurements were of size and not of shape. He concluded that the amount of deviation from the normal growth pattern increased with age.

With the wide range of experimental techniques using animals as experimental models, investigators have had widely differing results. The choice of model, the precision of the method and the care in biometrical analysis may account for many of the differences. A lack of objectivity may also account for different findings, for the results of any form and function experiment cannot necessarily prove a direct cause and effect relationship.

MATERIALS AND METHODS

Sample

The sample used in this study consisted of 88 Long Evans rats. The colony (Animal House, Faculty of Dentistry) was started from four males and six females in 1959 with careful inbreeding and avoiding brother-sister crosses. The prime selection factors for breeding purposes have been fertility and rapidly growing healthy rats. Large litters were reduced in numbers to allow the pups and mother the best opportunities for growth. All these factors have helped to produce a healthy, homogeneous colony. The experimental animals were selected from the 17th to the 20th generation.

Several experimental groups were used and all had a starting weight of 90 ± 3 grams which corresponded to 30 days ± 3 days post natum. Only animals which showed regular weight gains after weaning were chosen and the runts of the litter were avoided. Where possible, littermates were used in different groups to keep the sample well balanced.

All the rats were fed on Purina Laboratory Chow* and water ad libitum. An addition of Megicillin** to the drinking water at a dosage of approximately 6 mg per pound of body weight per day was made, requiring a lower concentration later in the experimental period because of the increase in fluid intake of the larger animals. The rats were housed separately one per cage to reduce the chance of cross-infection.

Any animals not consistent with the growth curve suggested by Cleall et al (1964) were regarded as abnormal and omitted from the data.

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* Ralston Purina Co., St. Louis, Missouri, U.S.A.

** Charles E. Frosst and Co., Kipland, Montreal, Canada.

All animals were weighed regularly throughout the experimental period; the weights on the days of injection, i.e. days 4, 11, 25, were recorded as well as on day 46 at sacrifice. Six animals were omitted from the experiment because they steadily lost weight from the day of operation and did not survive after the injection on day 11. Only those animals which survived were used and the weight gains for each animal of each group were analyzed with an analysis of variance. The significance of the weight gains of the groups was given as an F value, allowing a t value to be calculated using a Duncan test.

Surgical Procedure

Several techniques of nasal blockage were attempted by solid objects such as metal pins, soldered pins, zinc oxide and rubber base pastes, all of which were inconsistent and incomplete in nasal blockage. Nasal closure was achieved by electrocautery and using stainless steel ligature wire after laceration of the nasal aperture. The former, it was felt, was liable to damage the tissues, including possibly the nasal septum, and local bone forming areas, while the latter was traumatic to the soft tissues. Both techniques were capable of producing nasal blockage. Tissue adhesives were not strong enough to seal the nostril. Finally two surgical procedures were adopted in this study.

1. Nasal Blockage Procedure: The technique of nasal blockage finally adopted was a rapid one under ether anaesthesia. The nasal aperture was lacerated or scraped with a fine scalpel so as to expose a raw, bleeding surface. Then a 0000 (40) silk mattress suture was inserted and tied to constrict the nostril and encourage healing by primary intention.

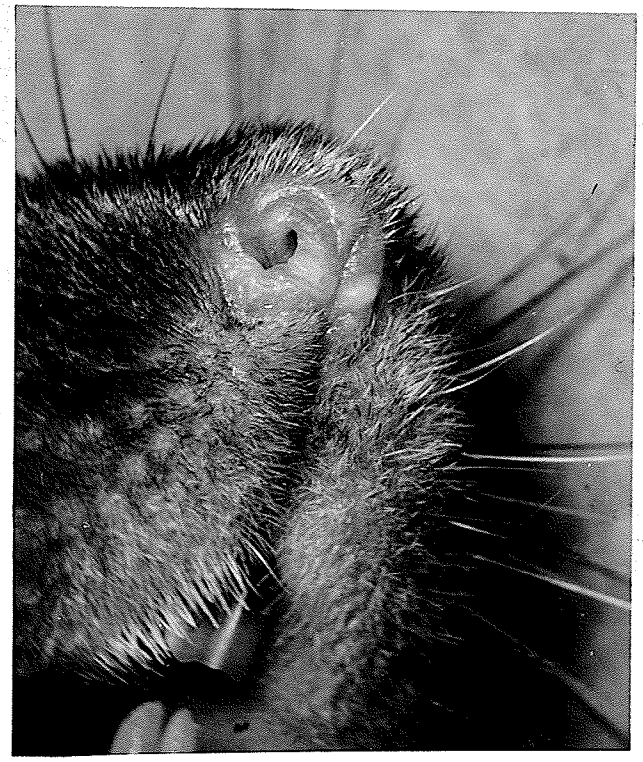

Fig. 1. Photograph of the normal appearance of the rat nostril.

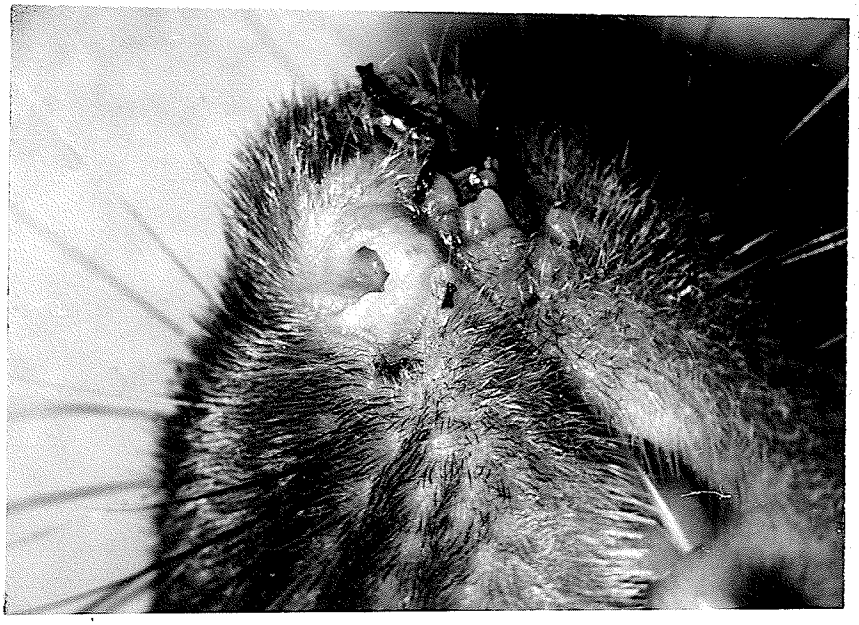

Fig. 2. Photograph of the operated nostril following surgical closure.

Over this surface a tissue cement* was added as illustrated in Fig. 2.

Subjective evaluations were made as to ease of the surgery and the degree of trauma and bleeding. At each weighing the nostril was observed and a drop of water from a pipette was placed over the sealed nostril. If the water was inhaled, then the sealing was not complete and ether anaesthetic was administered and second surgical closure carried out. Eleven of the animals with nasal blockage required this second procedure, most by day 11 of the experimental period. All these animals were given intraperitoneal bone marking agents.

2. Sham Operation Procedure: To help evaluate the effect of surgical trauma, a second surgical procedure identical to that of nasal blockage was performed, except that after the sutures were pulled firmly they were cut and removed. Therefore, these five animals had all the trauma of nasal blockage without the closure of the nasal airway, and were given the bone marking agents as were the operated group.

Control Group

This group underwent no surgery on the nasal aperture and was used for comparison with the operated animals. The normal nostril seen in Fig. 1 appears as an inverted comma shape on the lateral aspect of the snout. These nine animals were all given the intraperitoneal injections of bone marking agents.

Soft Tissue Sample

A group of nine animals was used in the soft tissue sample

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* Permabond (Pearl Chemical Company, Tokyo, Japan)

Figs. 3 & 4. Photographic appearance of the healed, closed nostrils.

as shown in Table I. Of these animals, six had nasal blockage and were sacrificed on days 4, 11, 25 and 46. Three control animals were sacrificed on days 11, 25 and 46.

None of these animals had been injected with any bone marking agents. The animals were sacrificed by decapitation under ether anaesthetic and the heads were immersed in 10% buffered formalin and left in this solution for two weeks to ensure a thorough penetration and fixation of the tissues. These heads were then transferred to 17% formic acid to allow decalcification of the skull which was aided by cutting the heads into blocks.

These blocks were marked and embedded in paraffin wax and coronal sections prepared to correspond with the sites sectioned in the hard tissue sections, as well as sections of the external nares. The sections were made at approximately 7 microns (μ) and were stained with haematoxylin and eosin and mounted for examination.

Bone Marking Agent Injections

Intraperitoneal injections of bone marking agents were given on day 0 using Di-Carbomethyl-aminoethyl fluorescein (Dcaf)* which appears green under ultraviolet light. On day 4 alizarin red S was injected which appears red, then on day 11 terramycin which appears yellow. The final injection given on day 25 was a combination of alizarin red S and terramycin injected separately which stains the bone orange. Table II shows the dosages of these agents.

This is a modification of the serial bone marking technique of Cleall et al (1964) to allow all the serial bone marking agents to

* Hopkin and Williams Ltd., Chadwell Heath, Essex, England.

TABLE I

SOFT TISSUE SAMPLE

DAY OF SACRIFICE*	LEFT SIDE BLOCKAGE	RIGHT SIDE BLOCKAGE	CONTROL
Day 0	Surgery	Surgery	-
Day 4	-	1	-
Day 11	1	-	1
Day 25	1	1	1
Day 46	1	1	1

* At the beginning of the experimental period all animals had a starting weight of 90 ± 3 grams which corresponded to approximately 30 days post natum.

TABLE II

BONE MARKING INJECTION SEQUENCE

STARTING WT 90 ± 3 g	BONE MARKING AGENT	I.P. MARKER VOLUME DOSE	STAIN LINE LETTER	COLOUR UNDER U.V. LIGHT
Day 0	(Dcaf (Surgery	.25 cc 25 mg./kg	A	Green
Day 4	Alizarin Red S	1.30 cc 300 mg./kg	B	Red
Day 11	Terramycin	.25 cc 70 mg./kg	C	Yellow
Day 25	Alizarin Red S + Terramycin	1.55 cc 370 mg./kg	D	Orange
Day 46	Sacrifice		E	Bone Edge

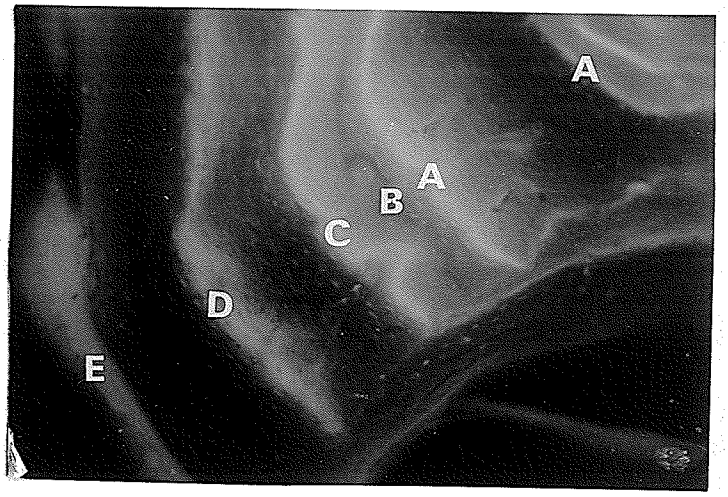

Fig. 5. Photomicrograph of the bone marking agents. A. Dcaf, B. alizarin red S, C. terramycin, D. alizarin red S and terramycin, and E. the bone edge with the periosteum showing green.

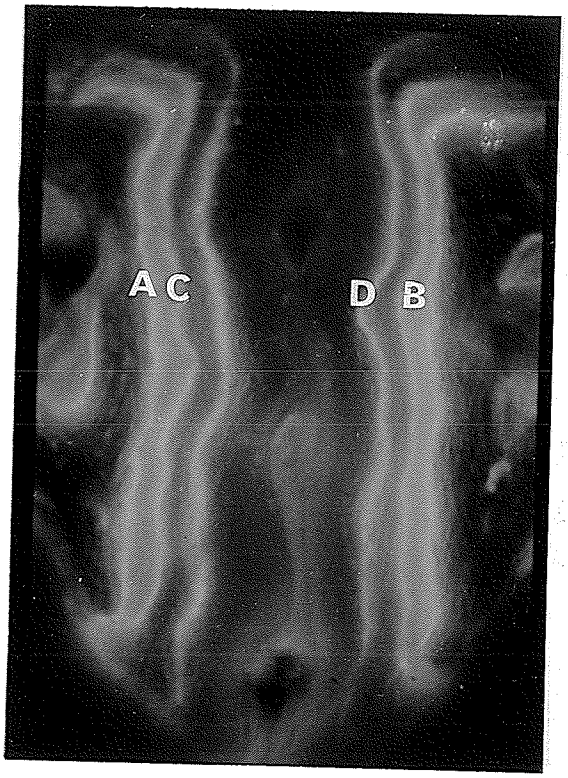

Fig. 6. Photomicrograph of the bone marking agents in the internasal suture. A. Dcaf, B. alizarin red S, C. terramycin, and D. alizarin red S and terramycin.

be visualized under ultraviolet light and to use a less toxic drug, Dcaf. The time periods between the bone marking agent injections were chosen so that fairly even increments of bone were laid down and marked lines were clearly separated by intervals of bone which were suitable for measurement in a longitudinal study of bone growth. The animals were sacrificed on day 46 of the experimental period. Figs. 5 and 6 show the typical bone mark appearance under ultraviolet light.

Each rat was injected while anaesthetized with ether. If the rat was recovering from the anaesthetic, the small volume of Dcaf could be injected without difficulty. Also in a small group of operated animals which were in obvious poor general health on day 11, terramycin was injected without anaesthetic in order to prevent further stress to the respiratory passages. The concentration of these agents used was relatively non-toxic and did not appear to adversely affect bone growth.

Hard Tissue Sample

A longitudinal group of 55 animals was used for hard tissue sectioning and, following the complete bone marking sequence, was sacrificed on day 46. Table III shows the group sizes of these animals.

Another 18 animals were used as a cross-sectional sample for hard tissue section analysis. Table III shows the groups and group sizes of the animals. These represent the stage of development of the longitudinal group at each day of bone marking injections.

Microbiology

Nasal swabs of 35 animals were taken at the time of sacrifice

while the animals were anaesthetized. These swabs were made using orthodontic wire which was gnarled with cotton wrapped around it, and the other end was embedded in a cork. The cork was placed in a glass test tube and the whole sterilized in an autoclave.

The swabs were taken by rotating the cotton tip in the nasal aperture to facilitate entrance into the nasal passage proper and then, by pushing, the swab could be taken through the nasal cavity. The withdrawn swab was replaced in the test tube and identified as to the rat and nostril sampled. The blocked nostril could not be sampled directly except by cutting the entrance open and attempting a nasal swab. Plating on blood agar cultured the bacteria and by examination and sub-plating the gross bacterial flora were identified.

pH

A pH meter* (Fig. 7) was calibrated with buffer solutions of known pH using a thin antimony electrode and a salt bridge to complete the circuit. The pH measurements were made of the external nasal mucosa, internal nasal mucosa, nasopharynx of each side, the oral mucosa and oropharynx. This technique was used on the same group from which the bacteriological swabs were taken, on day 46 of the experimental period.

Temperature Assessment

An attempt was made to evaluate the temperature in the blocked and unblocked nasal cavities. A thermister bead and lead** No. 528, 9604 measured the resistance which was read on a digital ohmeter as seen in Fig. 8

* Bach-Simpson Ltd., London, Ontario, Canada.

** Yellow Spring Instrument Co., Ohio, U.S.A.

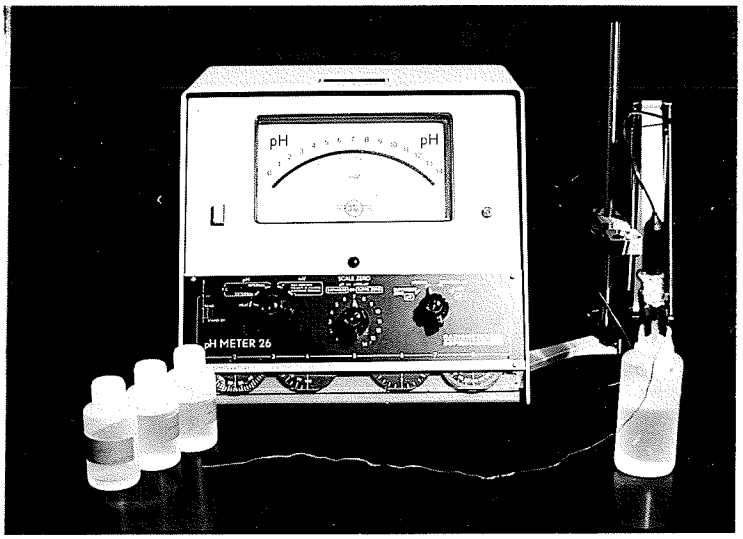

Fig. 7. pH meter.

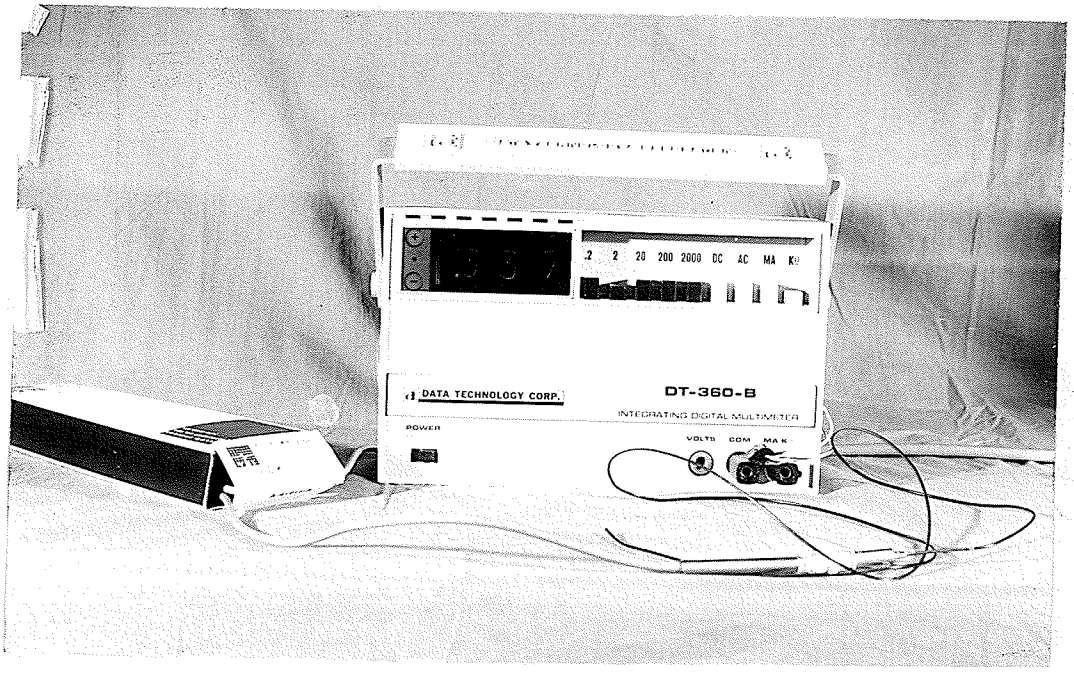

Fig. 8. Ohmeter with thermister bead for temperature assessment.

The conversion resistance tables for the thermister bead allowed the temperature to be read. The thermister bead was very sensitive and measured the temperature at that instant at that point. Unfortunately, the greatest difficulty was in locating the bead in the desired position as touching the mucosa or contamination of nasal secretions would immediately affect the readings. Also the sensitivity of the rat, although anaesthetized, to nasal interruption was great and only a guiding assessment could be made as to the temperature of the nasal passages.

Of the animals which survived to day 46, a sample of 10 was used to take the temperature of the left and right nostrils, the temperature of inspired and expired air and in addition oral, throat and anal temperatures were taken. The closed nostril was surgically opened to allow the thermister to be inserted.

At sacrifice of all the hard tissue sample animals the left kidney, spleen and liver were removed and placed in 10% buffered formalin. These were embedded and soft tissue sections prepared for the analysis of the state of these organs and hence the general state of the body health.

Preparation of the hard tissue material

After decapitation, the heads were wrapped in aluminum foil and stored in a freezer. These crania were cleaned of the soft tissues after cooking at 30 p.s.i. for five minutes and stored in 70% alcohol for at least 24 hours. The crania were allowed to dry for the taking of photographic hard tissue records before being embedded for sectioning.

Photographic Records

Photographic View: Standardized photographic views were taken of all the cleaned rat crania at a distance of one meter. A superior view was taken with the crania held in a rat cephalostat so that the transmeatal axis was at right angles to the plumbline on the camera. A spirit level checked that the film, the base of the tympanic bullae and the incisors were parallel. Fig. 9 shows the photographic appearance and the landmarks measured. The anatomical definition of the landmarks is given in the glossary. In the young rats under day 25 the occipital condyles were not visible in this view so that the most posterior point of the crania was measured, and then in the day 46 rats the most posterior points, the left and right condyles, were measured.

X-ray View: A similar superior view was taken at one meter target film distance with the crania held in the cephalostat on the film so that the base of the tympanic bullae and the incisors were at right angles to the plumbline. Kodak ultraspeed occlusal dental film was used with an exposure of 24/60th second at 90 K.V.P. at 15 milliamperes. Fig. 10 shows the landmarks measured in this view from standardized prints.

Lateral Photographic View: This view was taken at the same distance of one meter with the crania mounted in such a way that the view was at right angles to the vertex superior photograph. The films were all developed and printed to the same magnification so that the photographs were comparable and the radiographs were similar although a slight magnification and distortion factor made them not quite interchangeable. Fig. 11 is an example of the lateral photographic view. Other photographs were taken but were not standardized to the same degree, such as the

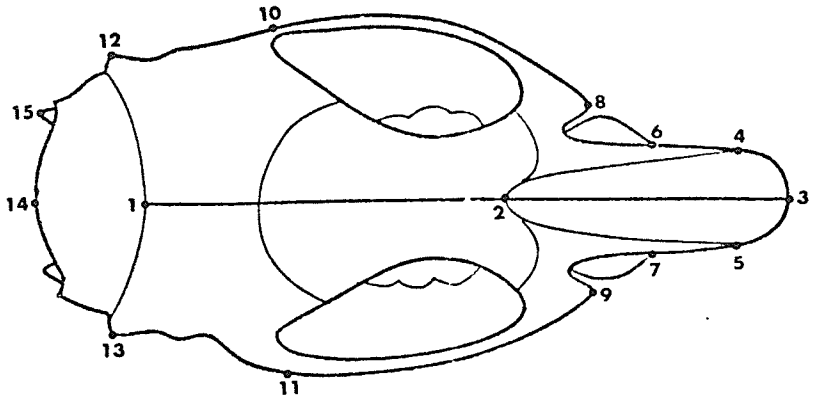

Fig. 9. Landmarks used in the superior photographic assessment.

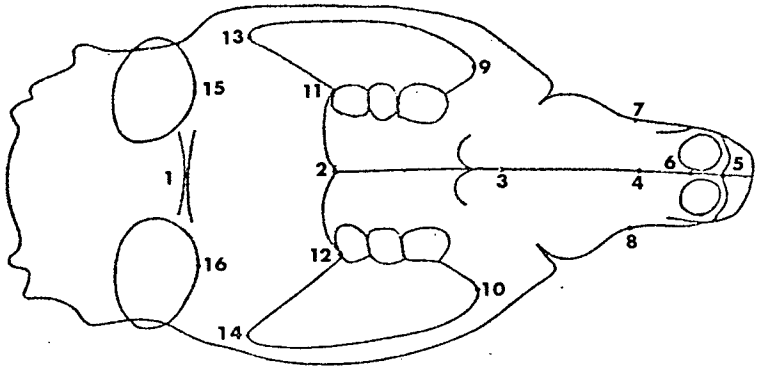

Fig. 10. Landmarks used in the occlusal radiographic assessment.

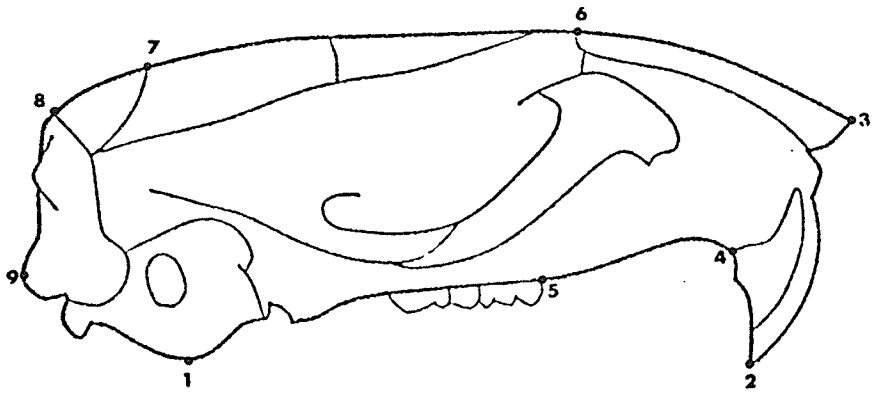

Fig. 11. Landmarks used in the lateral photographic assessment.

anterior view (Fig. 46) and the superior photograph of the crania of the differing age groups (Fig. 12).

A number of anatomical landmarks were measured using each superior photograph, lateral photograph and radiograph to illustrate the overall gross morphological picture of the rat crania. Figs. 9, 10 and 11 show the points measured and their description is in the glossary. The photographs were mounted on an illuminated screen using a rigid acetate template and the X and Y cartesian coordinates could be read using a Ruscom logistic strip chart digitizer* (Fig. 13). Each point was plotted in a preselected order and punched on IBM computer cards.

Use of the computerized method of coordinate analysis described by Cleall and Chebib (1971) allowed a mathematical superimposition of the coordinates on a standardized set of axes, once a common origin and direction had been selected. The mean coordinates and their standard deviations for the operated and non-operated groups were evaluated. From these values, a series of polygon plots was made to give a pictorial representation of the changes between the different groups. A series of linear distances and angular values was evaluated and an analysis of variance was carried out to test the significance statistically of the differences between the groups.

Hard Tissue Section Preparation

Embedding

The dried crania were soaked in Bioplastic** liquid for 24 hours, then placed on a hard base in an aluminum foil container. This container was filled with the monomer-polymer mixture. Along the operated side of the crania a piece of coloured wool was placed, colour

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* Ruscom Logics Ltd., Rexdale, Ontario.

** Ward's, Rochester, N.Y., U.S.A.

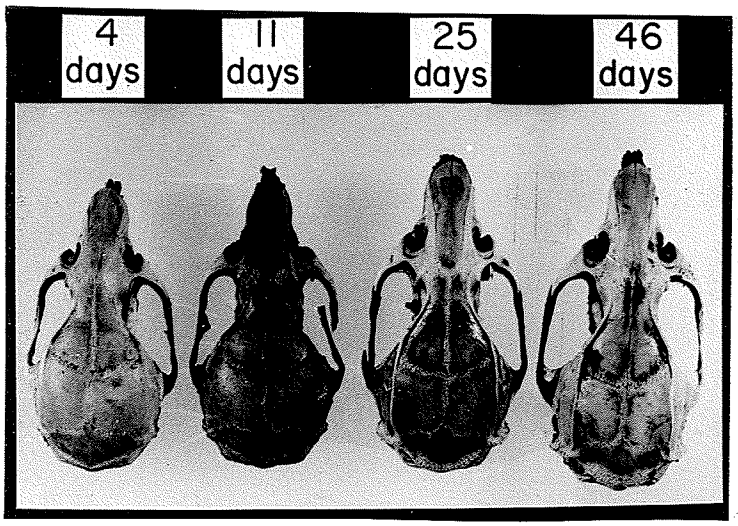

Fig. 12. Photograph of the rat crania, left to right on day 4, 11, 25 and 46, shows the relative increase in size over the experimental period.

Fig. 13. Ruscom logistics strip chart digitizer.

TABLE III

TOTAL HARD TISSUE SAMPLE

DAY OF SACRIFICE	LEFT SIDE BLOCKAGE	RIGHT SIDE BLOCKAGE	CONTROL	SHAM
Day 0	Surgery	Surgery	-	Surgery
Day 4	2	2	-	-
Day 11	4	4	-	-
Day 25	3	3	-	-
Day 46	21	20	9	5

TABLE IV

CORONAL HARD TISSUE SAMPLE

DAY OF SACRIFICE	LEFT SIDE BLOCKAGE	RIGHT SIDE BLOCKAGE	CONTROL	SHAM
Day 0	Surgery	Surgery	-	Surgery
Day 4	1	1	-	-
Day 11	2	2	-	-
Day 25	2	2	-	-
Day 46	12	10	5	3

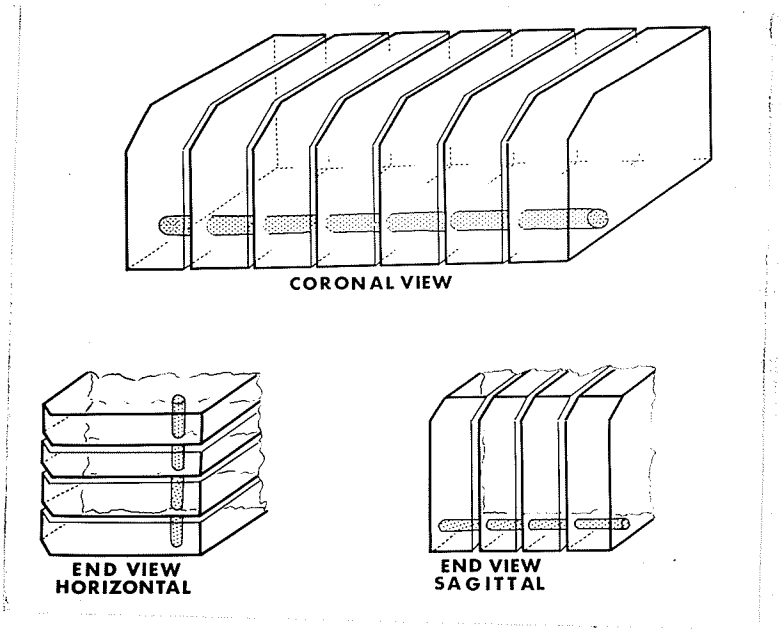

Fig. 14. Diagram showing the regions of section and their orientation on the slides by means of a bevelled corner and coloured wool at right angles to the plane of section.

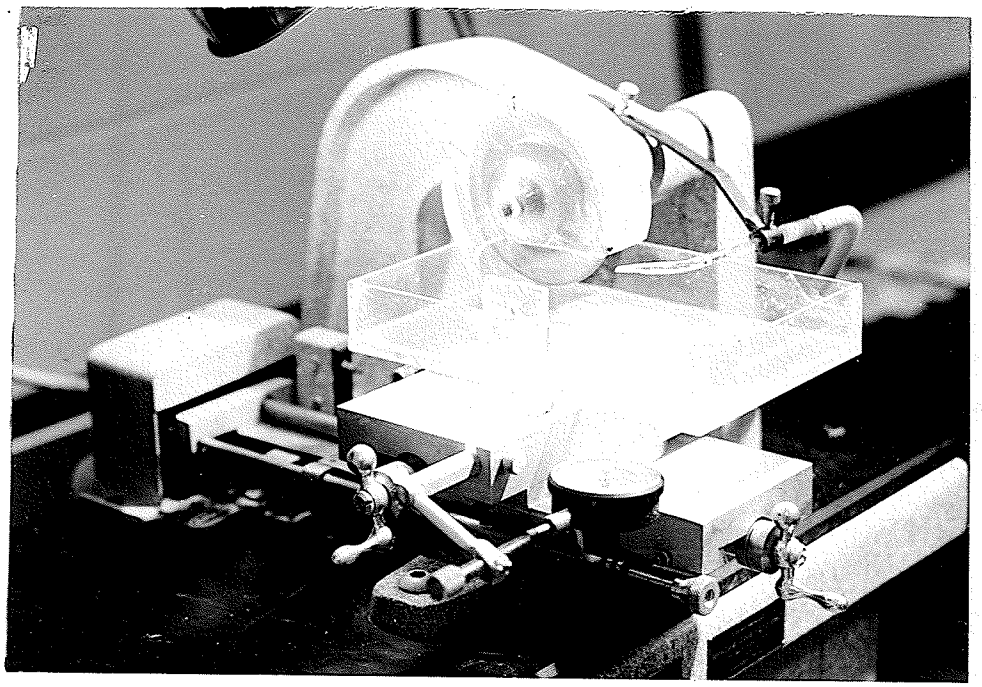

Fig. 15. Photograph of the Gillings Hamco rotary diamond cutting machine, used for cutting the undecalcified blocks.

coded for the experimental groups, and the animal identification number inserted. Fig. 14 shows the orientation of the wool at right angles to the plane of sectioning. After being allowed to set, the blocks were cured for 24 hours at 65°C, squared, marked and mounted for sectioning.

Sectioning

The Gillings-Hamco* thin sectioning machine (Fig. 15) with a diamond wheel was used to cut the undecalcified rat crania in sections of varying thickness, 80 to 120 μ, according to the blade condition and the hardness of the block. These sections were then dehydrated by passing them through 70% and 100% alcohol, then through xylol before mounting them on the glass slide. This is the time when the orientation of the coronal sections was crucial and the wool mark and the trimmed corner, shown in Fig. 14, prevented the section from being reversed. The section was mounted with Permount and a cover slip and allowed to set.

Planes of Section

Three planes of section were used in the hard tissue section material, all using the basal cranial orientation of the incisors and tympanic bullae. The planes of section were the coronal, sagittal and horizontal. The coronal group consisted of seven anatomical regions of each of which three section were taken, as used by Cleall (1968). Figs. 16-18 illustrate the relative anatomical regions in the planes of the sections.

The first region was called the incisor region and given the

* Hamco Machines Inc., Rochester, N.Y., U.S.A.

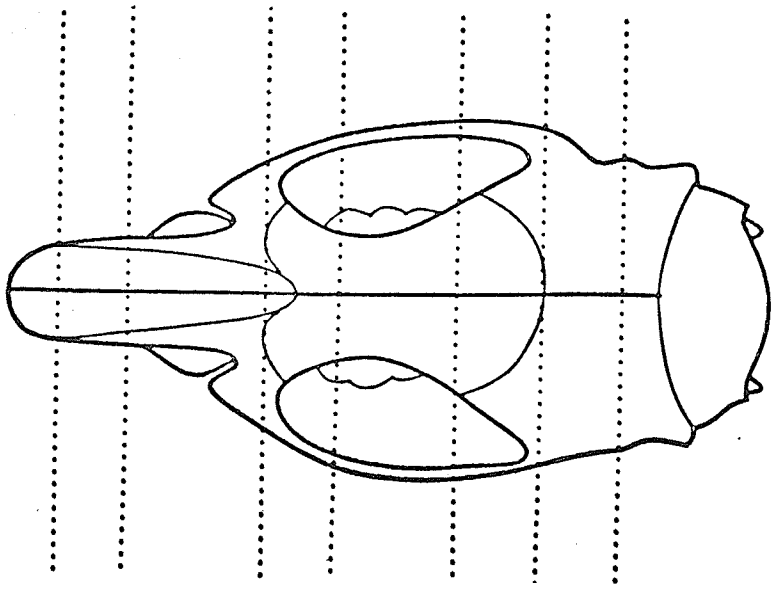

Fig. 16. Coronal planes of region seen from the superior aspect.

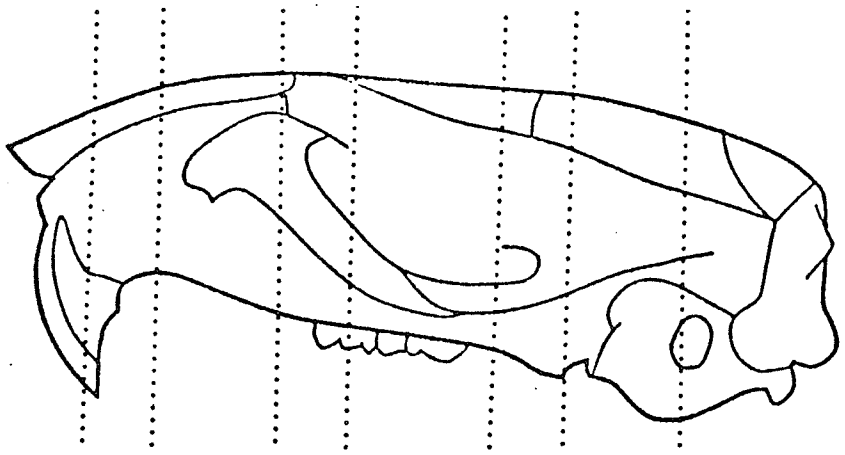

Fig. 17. Planes of the seven coronal regions from the lateral aspect.

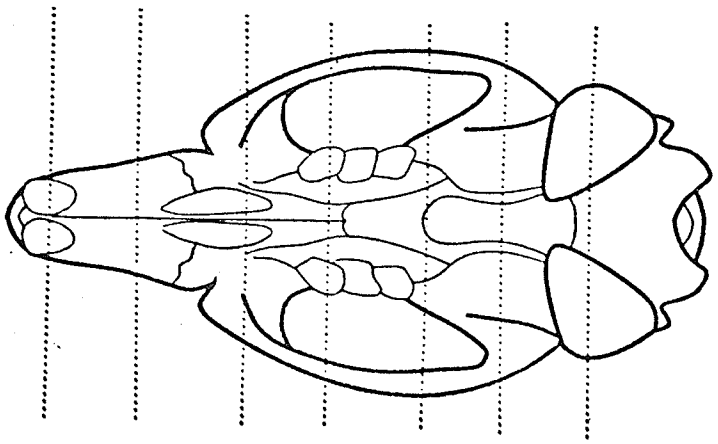

Fig. 18. Location of the coronal regions seen from the inferior aspect.

symbol A and the appearance of the section is shown in Fig. 19; the second was the premaxillary region, given the symbol B, with its morphology illustrated in Fig. 20. The subsequent regions were the malar, given symbol C (Fig. 21), the first molar, symbol D (Fig. 22), the zygomatic, symbol E (Fig. 23), the squamosal, symbol F (Fig. 24), and finally the tympanic bullae, symbol G (Fig. 25). The sample used in this group is shown in Table IV, representing the longitudinal and cross-sectional data and Fig. 26 illustrates the microscope used.

A smaller group was used for preparing the parasagittal hard tissue section and Table V shows the distribution of the sample. The three regions used were the midsagittal and the two incisor regions. Only one section could be taken of the midsagittal plane and two each of the incisor regions. Fig. 27 shows the anatomical regions sectioned in the sagittal plane.

A similar horizontal section group is shown in Table VI with three regions of section illustrated in Fig. 28. The morphology of the sagittal sections is shown in Figs. 29 and 30 and those of the horizontal regions, the molar, malar and zygomatic, are shown in Fig. 31.

Microscopic Examination

Two microscopic series of measurements were made on the hard tissue sections using a Zeiss G.S.L. microscope* equipped with a microscopic stage with two horizontal vernier scales at right angles to one another. The ultraviolet light source was produced from a super pressure 200 watt mercury lamp from a transformed 220 volt alternating current supply. The lens system used was a 12.5 times magnification objective and an occular lens of 6.5 times magnification.

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* Carl Zeiss, Oberkochen, West Germany.

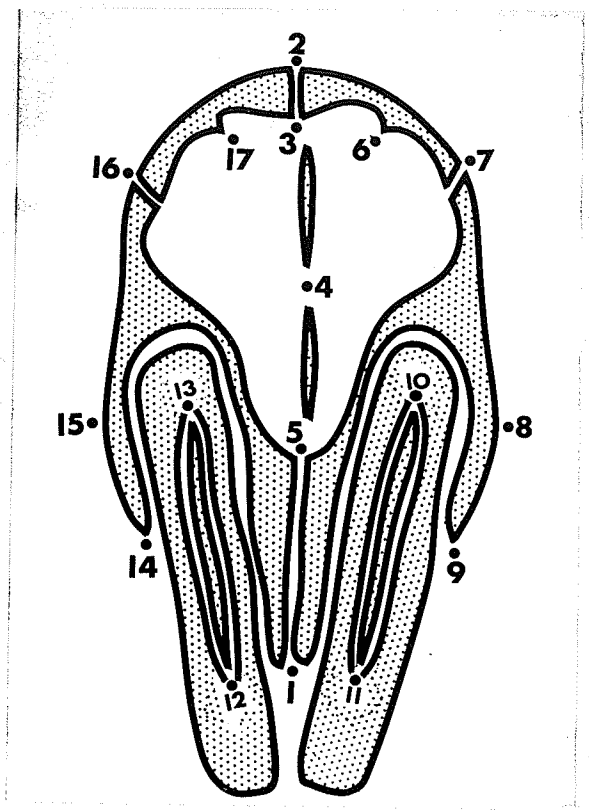


Fig. 19. The morphological appearance of the coronal section in the incisor region and the anatomical landmarks measured in the coordinate analysis.

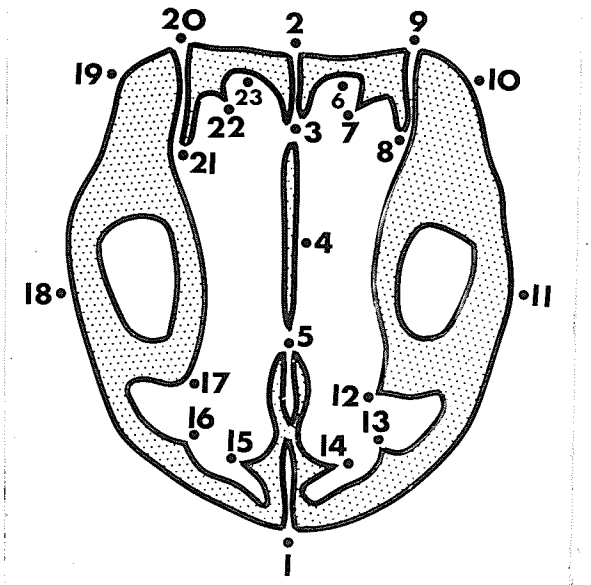


Fig. 20. The morphological appearance of the coronal section in the premaxillary region and the anatomical landmarks measured.

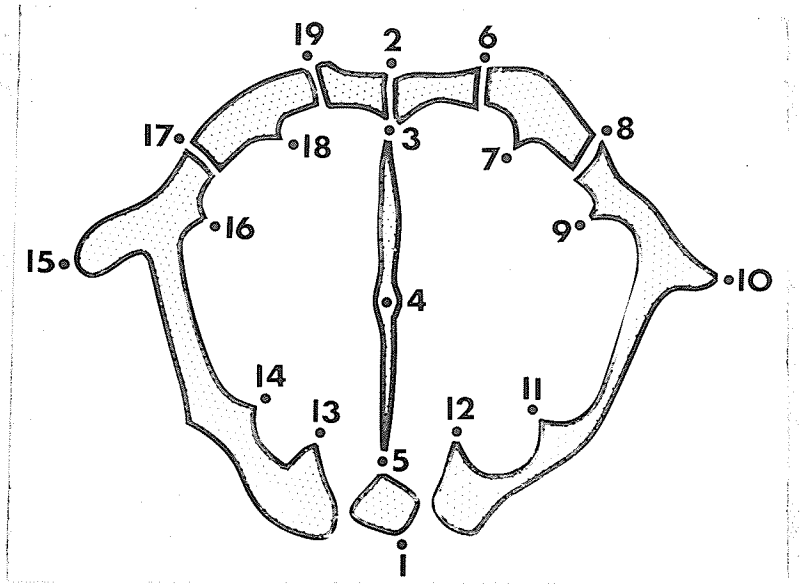


Fig. 21. The morphological appearance of the coronal section in the malar region and the anatomical landmarks measured.

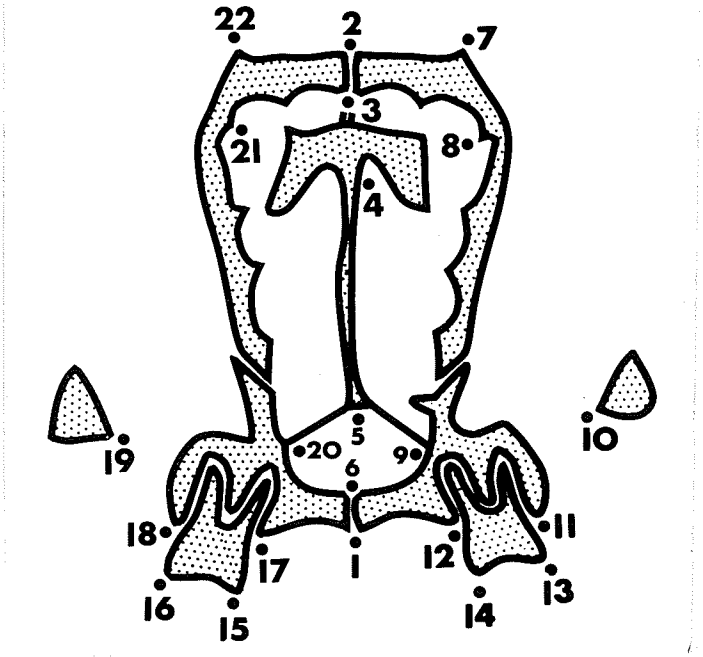


Fig. 22. The morphological appearance of the coronal section in the molar region and the anatomical landmarks measured.

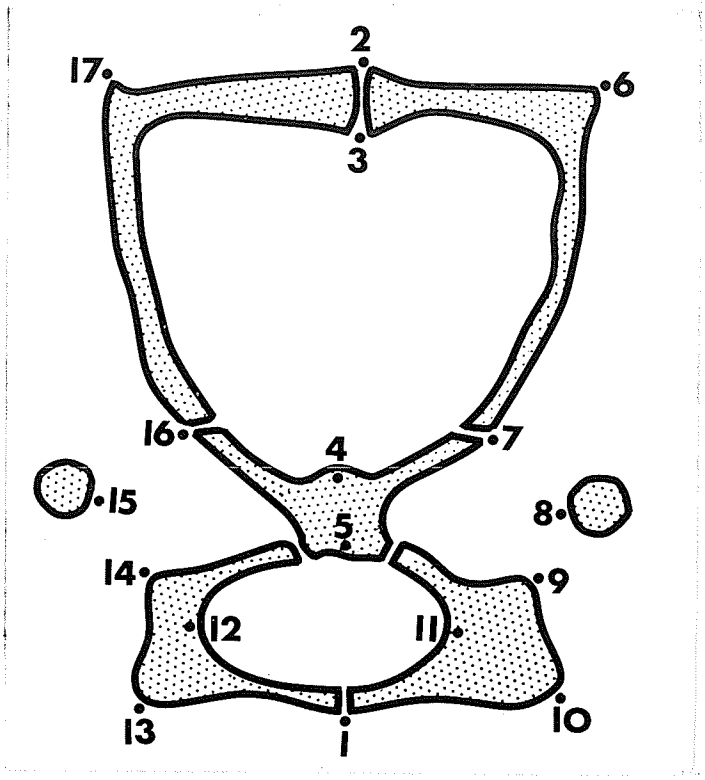


Fig. 23. The morphological appearance of the coronal section in the zygomatic region and the anatomical landmarks measured.

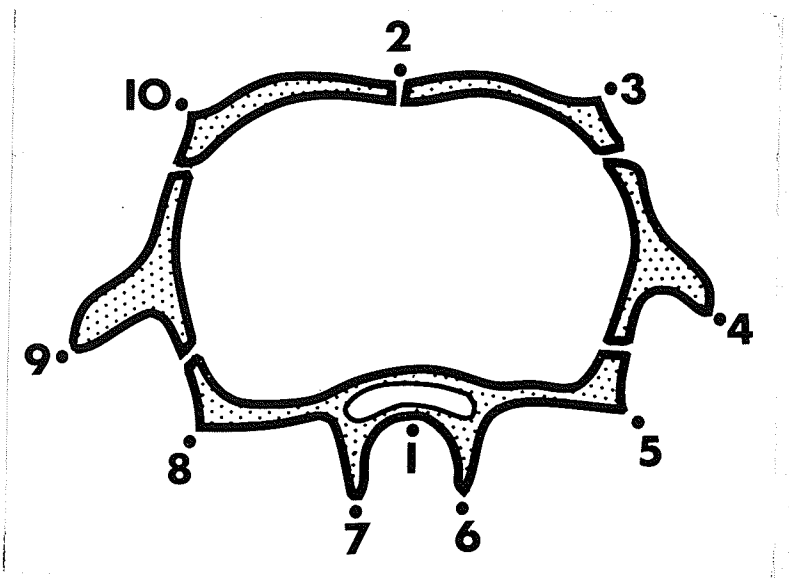


Fig. 24. The morphological appearance of the coronal section in the squamosal region and the anatomical landmarks measured.

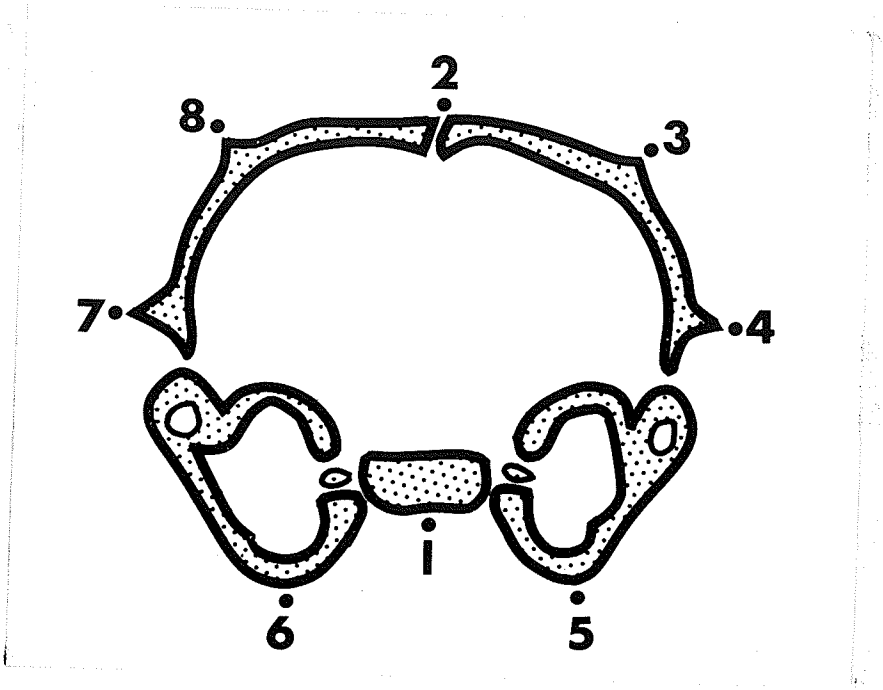


Fig. 25. The morphological appearance of the coronal section in the tympanic bullae region and the anatomical landmarks measured.

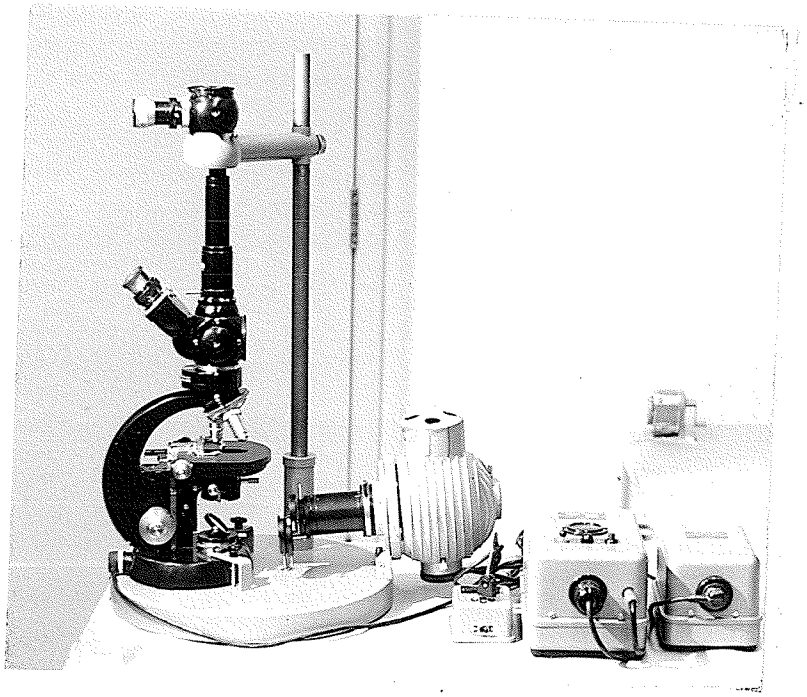


Fig. 26. Carl Zeiss microscope with ultraviolet and tungsten light sources used to study the undecalcified sections.

TABLE V

SAGITTAL HARD TISSUE SAMPLE

DAY OF SACRIFICE	LEFT SIDE BLOCKAGE	RIGHT SIDE BLOCKAGE	CONTROL	SHAM
Day 0	Surgery	Surgery	-	Surgery
Day 4	1	-	-	-
Day 11	1	1	-	-
Day 25	1	-	-	-
Day 46	4	5	2	1

TABLE VI

HORIZONTAL HARD TISSUE SAMPLE

DAY OF SACRIFICE	LEFT SIDE BLOCKAGE	RIGHT SIDE BLOCKAGE	CONTROL	SHAM
Day 0	Surgery	Surgery	-	Surgery
Day 4	-	1	-	-
Day 11	1	1	-	-
Day 25	-	1	-	-
Day 46	5	5	2	1

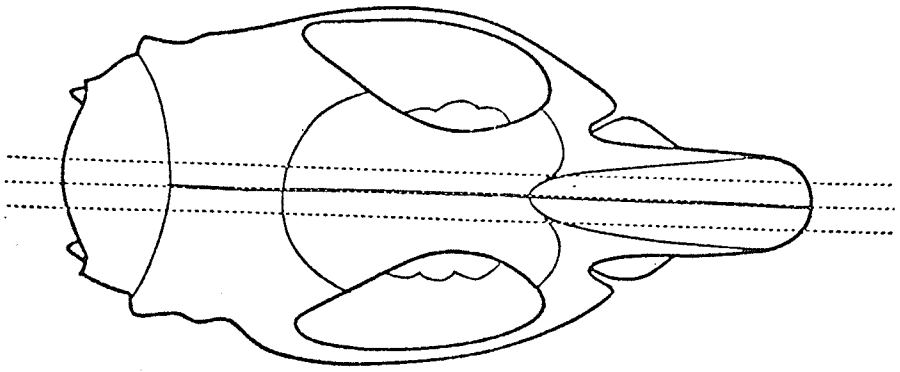


Fig. 27. The location of the three sagittal regions seen from the superior aspect, the incisor and midsaggital regions.

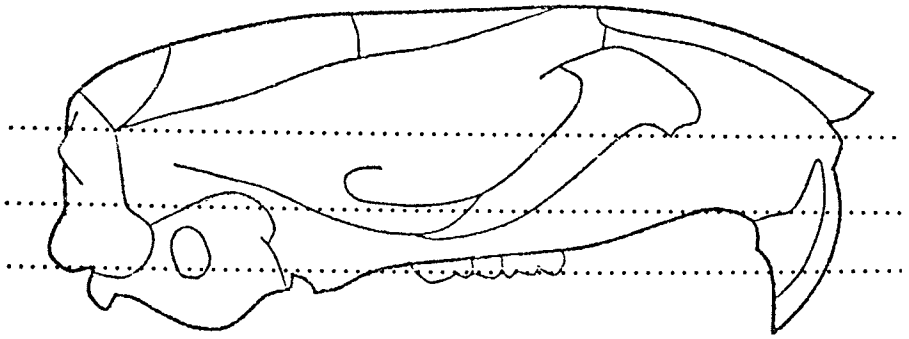


Fig. 28. The location of the three horizontal sections seen from the lateral aspect, the malar, zygomatic and molar regions.

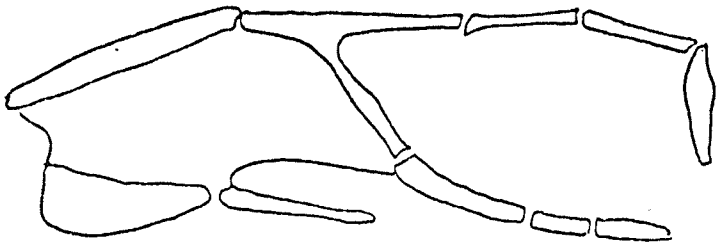


Fig. 29. The morphology of the midsagittal section.

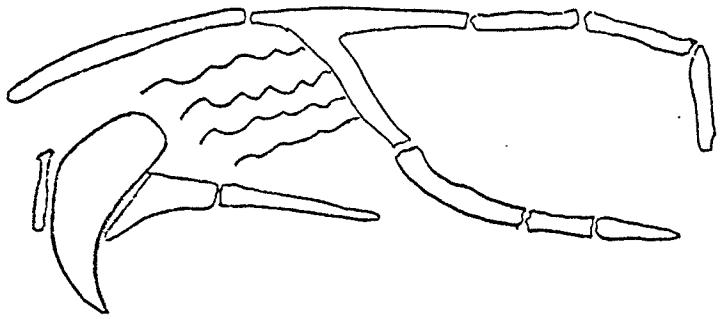


Fig. 30. The morphology of the incisor sagittal section.

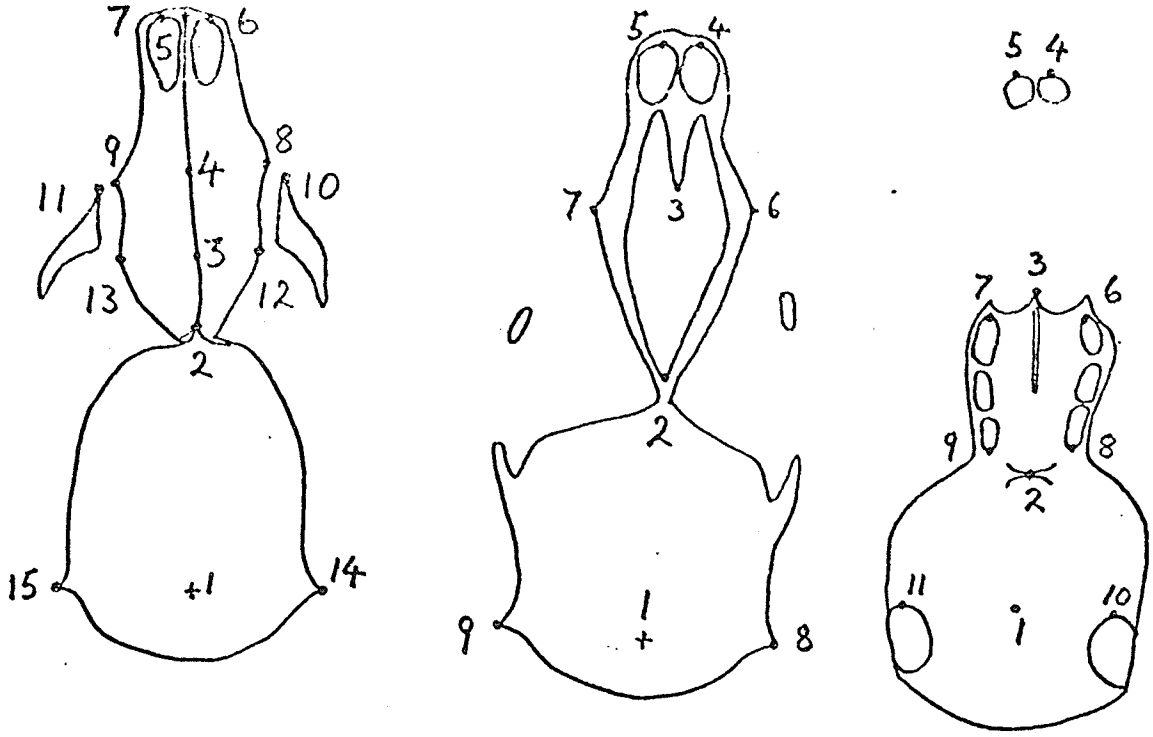


Fig. 31. The morphology of the horizontal regions

Malar Zygomatic Molar

Coordinate Microscopic Analysis

The anatomical landmarks easily distinguished in each of the sections were identified in the centre of the eyepiece which had a fine cross wire in the centre of the field. Each point was measured as X and Y coordinates using the vernier scales to an accuracy of 0.1 mm or equivalent to ± 60 microns on each axis. The data obtained were then dealt with as for the photographic coordinate analysis, being punched on IBM computer cards. However, each region of each animal in the coronal section was evaluated as a mean value of three sections with a mean and standard deviation.

Figs. 19-25 show the number of anatomical landmarks which were measured on each slide and illustrate the morphology of each region. The description for each landmark used is given in the glossary.

Linear Analysis

A series of sites was chosen for the linear analysis in the four coronal regions, Figs. 31-34; these sites were chosen because they demonstrated the bone increments most clearly when illuminated with ultraviolet light, and because they were likely to demonstrate bilateral growth differences in the snout of the rat. The sites numbered show arrowheads which demonstrate the direction of bone apposition.

The measurements were made while the bone marking agents were illuminated with ultraviolet light, using a calibrated ocular lens with a 500 unit scale. The zero was placed on the internal bone edge with the scale at right angles to the bone mark increments. The measurement was made at the centre of each stain line and finally the bone edge.

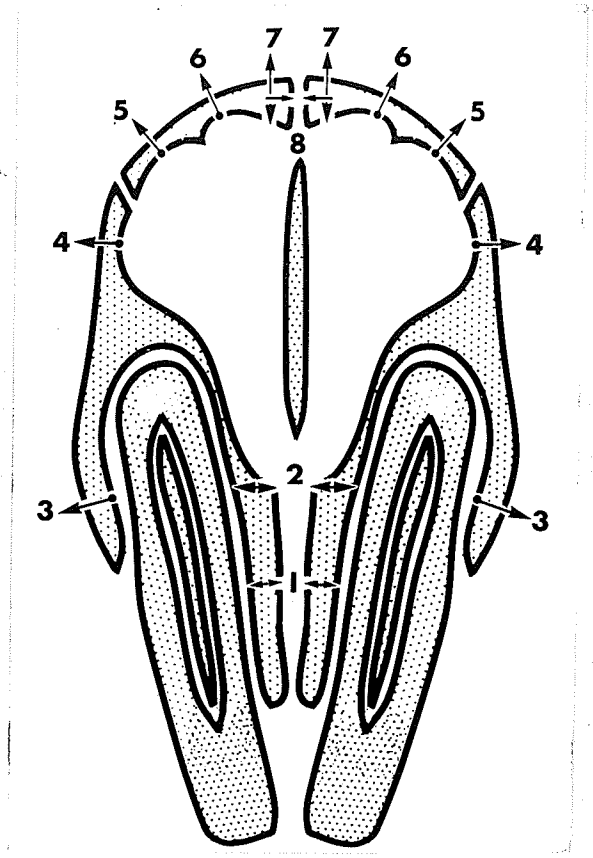


Fig. 32. The sites chosen for the linear microscopic analysis in the incisor region.

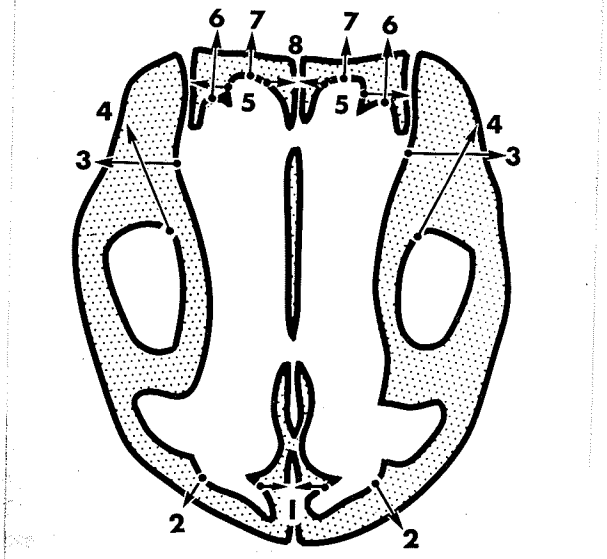


Fig. 33. The sites chosen for the linear microscopic analysis in the premaxillary region.

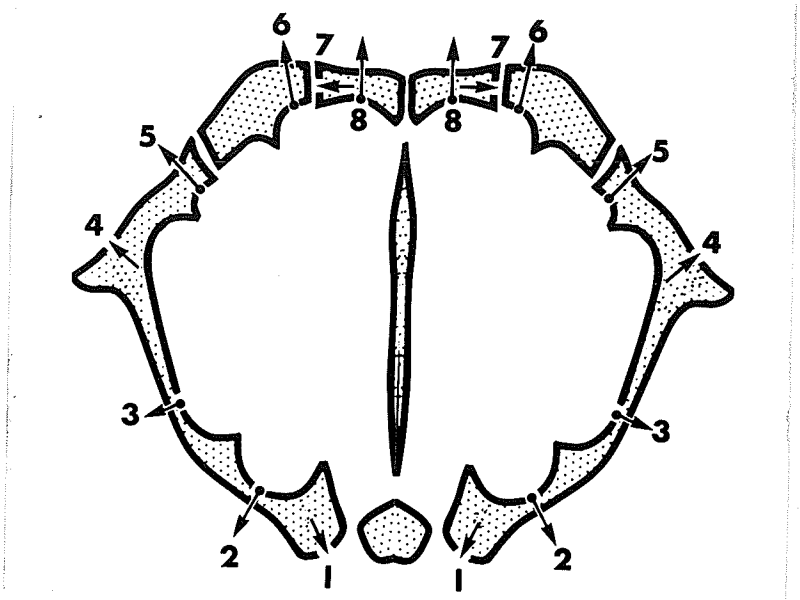


Fig. 34. The sites chosen for the linear microscopic analysis in the malar region.

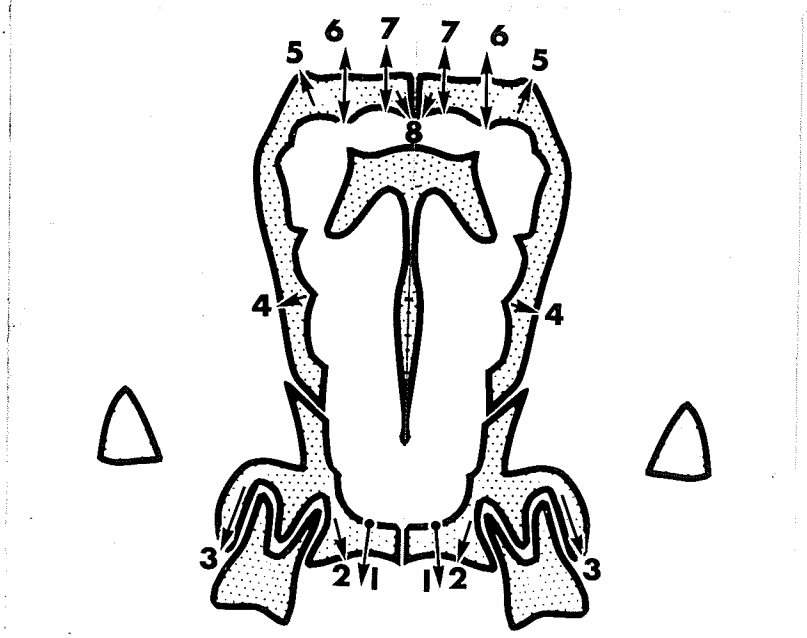


Fig. 35. The sites chosen for the linear microscopic analysis in the molar region.

The values written down in increment lines were then punched onto 80 column IBM computer cards. The computer program yielded a mean, standard deviation and standard error for each increment for each group of animals.

The increment of bone from day 0 to day 4 was the distance between the Dcaf (stain letter A) and alizarin red S (stain letter B) or increment A - B. In an area of apposition the increments become A - B, B - C, C - D and D - E. In some sites there was apposition on both bony surfaces internally and externally when two sets of increments were measured. In other sites internal resorption may have been continuing along with external apposition such that only one or two bone marking agents were visible.

The mean difference of each left side increment was compared with the right side increments for each of the four groups, the left and right nostril blockage groups, the sham operated group and the control group, using a t test. The differences of the four groups were then tested with an analysis of variance. The F values for each increment difference were then evaluated as a t test, using a Duncan test. The same procedure was then followed for the total bone widths from the internal to the external bone surface.

CODES

In order to simplify the illustrations and clarify the text, a number of abbreviations will be used to denote the various study groups.

Symbol	Abbreviation	
	L1	Left nostril blocked, sacrificed at 4 days
	L2	Left nostril blocked, sacrificed at 11 days
	L3	Left nostril blocked, sacrificed at 25 days
	L4	Left nostril blocked, sacrificed at 46 days
	R1	Right nostril blocked, sacrificed at 4 days
	R2	Right nostril blocked, sacrificed at 11 days
	R3	Right nostril blocked, sacrificed at 25 days
	R4	Right nostril blocked, sacrificed at 46 days
	O4	Control group - no surgery - sacrificed at 46 days
	S4	Left nostril sham surgery, sacrificed at 46 days

RESULTS

General health considerations

The most obvious finding of this investigation was the primacy of the airway in the Long Evans rat. Any interference with its nasal passages caused immediate embarrassment and a response to clear them, and the result of unilateral nasal blockage was chronic debilitation of the animals. The debilitation was noticed in a number of ways. First of all, on recovery from surgery, sneezing was almost universal and the paws would attempt to remove the suture inserted in the blocked nostril. This sneezing was a chronic feature day and night throughout the experimental period.

In all, eight animals of this sample died during the experimental period. No animals died as a direct result of surgery from which recovery was rapid, although respiratory arrest occurred in one animal. Weight gains were observed in all animals following surgery by day 11 of the experimental period. Six of these animals died on day 11 following ether anaesthesia and injection of the bone marking agent, terramycin. The other two animals died on day 32 and day 36 of the experimental period, the second animal having otitis media. These results suggested that the debilitation of nasal blockage was not fully evident following surgery and that there was a critical period on day 11, after which the animals were likely to survive.

In a preliminary investigation with animals not included in this study, six animals died. These six animals steadily lost weight from the day of operation and did not survive beyond the injection on day 11 of the experimental period and had death weights of about 70-80 grams. One of

these animals was destroyed on day 10 because of its state of acute respiratory distress. Of the remaining five animals which died on day 11, one animal also had respiratory arrest at the time of the surgical operation but recovered on massaging, and two had otitis media.

It was observed how the operated rats were distressed and, by frequent posturing of the head and sneezing, attempted to clear their nasal passages. The operated, and especially the dying animals, were readily identifiable, being more highly stressed than the control but serially injected animals. The hair or fur condition stood on end in the seriously ill animals.

Several animals in the nasal blockage group were noticed to have an increase in eye secretions which was not observed in the control animals. Their eyelids would become sealed and this could have been due to retrograde infection passing from the lateral aspect of the nasal passages under the malar arch through the lacrimal canal leading to the eye.

Otitis media and ear infections are uncommon but serious illnesses in the rat. There were several cases of possible otitis media, three being fatal, in spite of the addition of megicillin to the drinking water. The route of the infections was most likely a retrograde path through the Eustachian tubes which are very short, opening from the anterior median corner of the tympanic bulla through the nasopharyngeal tube behind the pterygoid plates.

Body weight gains

The body weight gains of the 88 male Long Evans rats used in this study were investigated. Figure 36 shows the relative weight gains graphically and Table VII the means and standard deviations of the four groups.

TABLE VII

LONGITUDINAL WEIGHT GAIN OF SAMPLE GROUPS

DAY OF SACRIFICE	CONTROL + BONE MARKING AGENTS		SHAM + BONE MARKING AGENTS		BLOCKAGE + BONE MARKING AGENTS		BLOCKAGE + NO BONE MARKING AGENTS	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Day 0	89.33	1.58	90.50	2.34	90.69	2.36	91.33	2.40
Day 4	108.44	3.00	102.58	3.87	94.39	5.90	99.91	6.39
Day 11	124.61	12.52	111.60	17.54	111.42	13.63	128.40	10.10
Day 25	229.44	14.00	192.25	21.97	172.76	20.39	184.25	18.44
Day 46	332.33	17.32	292.50	7.32	259.78	34.99	280.50	4.94

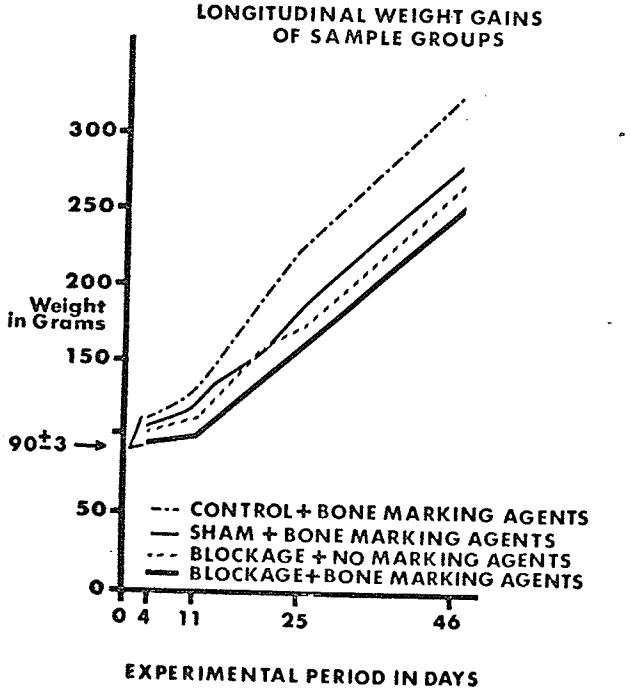


Fig. 36. Graph showing the differences of the sample group weight gains over the experimental period.

The marked lack of weight gain by the operated groups reflects the serious results of unilateral nasal blockage.

The weights of each animal at each injection day, i.e. 0, 4, 11 and 25, were recorded on IBM computer cards as were the weights at the end of the 46-day experimental period. A computer program calculated the weight gains for each increment of time for each animal in each group and an analysis of variance calculated the significance as an F value for the weight gains of the group. From this the students t test was evaluated using a Duncan test.

The most rapid weight gains were recorded by the non-operated or control group of animals which had serial bone marking agents injected. This was significant at the 1% level to all the other groups. The next most rapid weight gains were recorded by the sham operated animals, without nasal blockage but serially injected, which were significantly heavier at the 5% level to the unilateral nasal blockage groups with bone marking agents.

No significant difference was found between the left and right side operated groups, nor between the soft tissue sample which had nasal blockage but no bone marking agents and the operated groups which had been injected with bone marking agents. Although only one soft tissue control animal was used throughout the 46-day experimental period, it was heavier than the controls with bone marking agents.

Microbiological findings

The nasal cavities were not found to be blocked with mucus or nasal secretions as may have been expected. Soft tissue radiographs did not show any noticeable differences and nasal swabs revealed that the open nostrils often had more nasal secretions, possibly indicating a

physiological compensation of the non-functioning nostrils.

The major findings of the bacteriological cultures were that the bacteriological colonies identified in the open nostril were also identified in the closed nostrils and that basically the flora showed no side preference.

While statistical evidence cannot be evaluated, in all nine of the control animals studied, none of the 18 nostrils yielded culturable pathogen colonies. Although a number of the operated animals also failed to produce marked colonies of a pathogenic nature, a number of the bacteria were identified. Eighteen of the 26 animals showed large numbers of colonies of pathogens and non-pathogenic bacteria, the latter being a sign of the debilitation in these animals. The most common pathogen was Pneumococcus while Staphylococcus aureus, Pasteurella haemolytica and Pasteurella pneumotropica were identified as other pathogens. Among the non-pathogens often found different Proteus mirabilis Corynebacterium xerosis were present in such prolific quantities that their presence was regarded as being important. Escherichia coli, identified in one animal, may reflect the habit of the rat of eating its own faeces, or its own growth in debilitated animals. A contamination of the swabs cannot be ruled out completely.

No culturable pathogens were obtained from the blood sample aspirated from the jugular vein, suggesting no pyaemia was present in the unilaterally blocked nostril animal. This does not rule out the possibility that there could be pyaemia in seriously sick animals.

pH

The results of this aspect of the investigation were not statistically significant. While the calibration of the machine from known buffer solutions was simple, the exact location of the antimony electrode could not be known. A wide physiological variation was seen within the animals studied, but essentially no difference was found between the open nostril and the closed nostril. The closed nostril had to be surgically cut to allow the electrode to be inserted, allowing blood to be a contaminant.

The pH at different locations did vary, the nasal aperture being the highest at about 9.5 and the oropharynx the lowest at approximately 7.0, the nasopharynx and nasal passages falling between these values. The values for the operated animals tended to be lower in pH than the control animals but this was not significant. No significant difference was found from open to closed nostril.

Temperature assessment

While the thermister bead is extremely sensitive to thermal change and the evaluation could be extremely accurate, the practical problems of the situation only allowed a generalized assessment.

The rat nostril is extremely narrow and the thermister bead lead almost closed off the nares, causing nasal respiratory passage blockage. This insertion into a normally very sensitive structure was critical for the animals and they required heavy sedation. Any mucus or moisture on the bead would affect the temperature and the constant changes of temperature with inspiration and expiration, along with diurnal rhythms and inflammatory changes, made exact measurements meaningless.

The temperature differential of the atmospheric air and nasal temperature is relatively small - in the order of 20°C - and the relative degree of heat flow conduction of the air is slow and small, so that the local environment temperature variation between the open and the closed nostril would only be expected to be very low.

Soft tissue sample - control

Examination of the decalcified sections stained with haemotoxylin and eosin demonstrated the normal anatomical relations of the soft and hard tissues. The rat snout was surrounded externally by the thick skin and fur and inferiorly below the oral cavity, the submucosal tissue being thick connective tissue overlying the bony nasal bones superiorly and the premaxilla and maxilla laterally and inferiorly. Within these bones a cartilaginous framework supporting the tissues is readily seen as the nasal septum and accessory cartilages.

The nasal septum was seen as being straight in the coronal section and the nasal bones, premaxillae and maxillae, as being bilaterally symmetrical. The sutures were also essentially bilaterally symmetrical as were the nasal conchae structures with the nasal passages similar in shape and size from side to side.

The low power microscopic examination demonstrated the snout dense connective tissues were highly vasculated with the hair follicles intruding deep from the superficial skin. The soft connective tissue was largely comprised of muscle tissue with large numbers of thick nerve bundles, the remaining tissue being dense fibrous connective tissue.

The cartilage of the nasal septum was comprised of a hyaline tissue with a regular outline. The bones around the nasal cavity

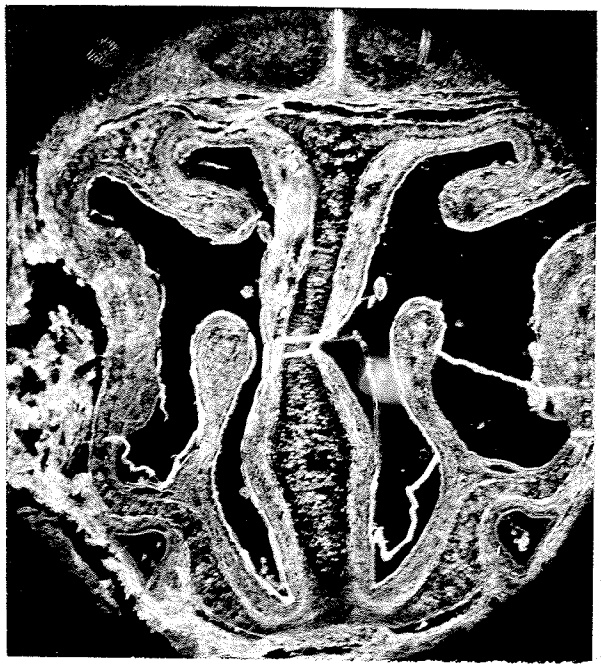


Fig. 37. Coronal soft tissue photomicrograph of the snout showing the nasal bones above and the deviated nasal septum in the animal with the left nostril blocked on day 4. Note the constriction in the lumen and the disturbance of the soft tissues. (x 60)

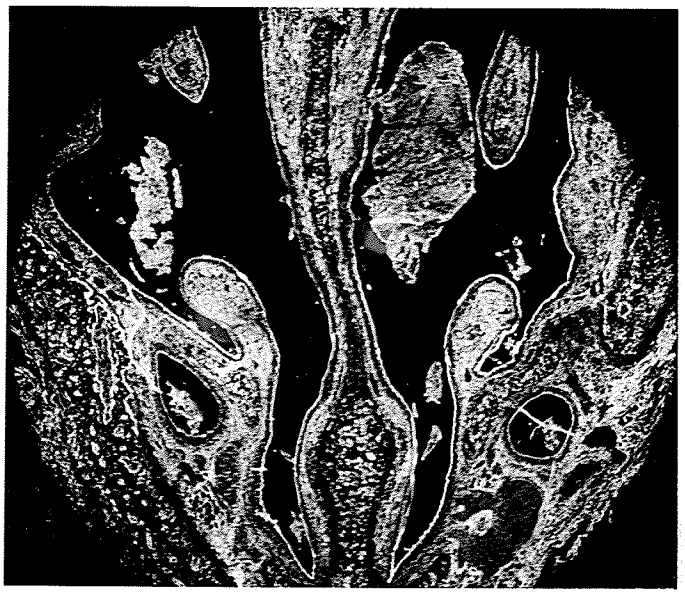


Fig. 38. Photomicrograph of the soft tissue showing the deviation in the nasal septum from its root in the vomer, laterally the premaxillary bone. Day 11, left nostril blocked. (x 60)

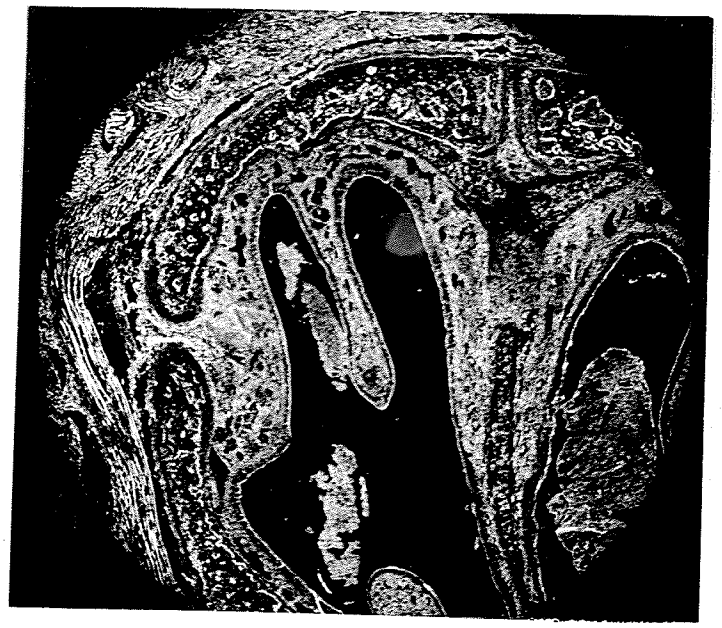


Fig. 39. Photomicrograph of the soft tissue showing the operated side coronal section through the nasal bone and premaxilla on day 11. Note the compressed nasopremaxillary suture (x 60).

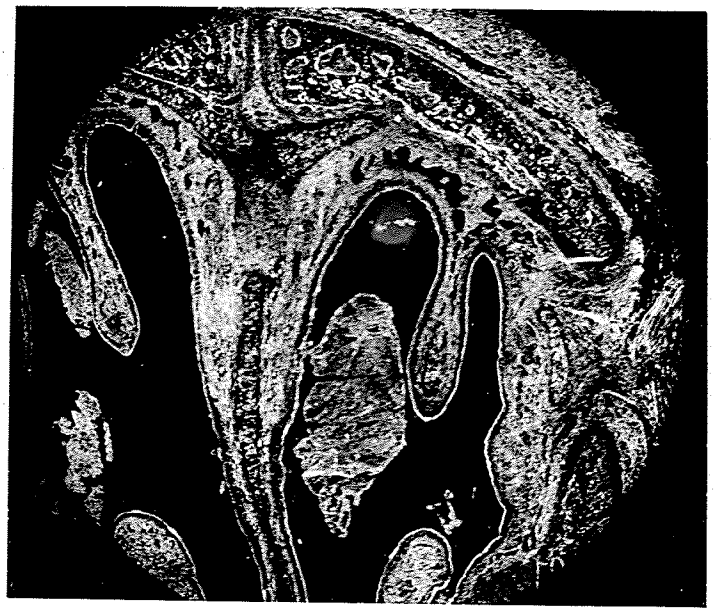


Fig. 40. Photomicrograph of the soft tissue through the adjacent non-closed nostril demonstrating the stretched nasopremaxillary suture. Day 11 (x 60).

could be seen to have a regular external surface and a more irregular internal surface.

At the high power the cellular level of activity could be examined and the interstitial growth activity could be seen in the cartilage. Examination of the bony structures revealed osteoclasts and Howships lacunae on the nasal side of these bones and a more regular osteoblastic activity externally. This is a typical example of bone growth in the nasomaxillary complex.

The fibrous suture structures were regular and gradually reduced in size over the experimental period. The epithelium within the nasal cavities was ciliated respiratory columnar in nature with many goblet cells. The turbinates were covered with olfactory pseudostratified columnar epithelium with specialized bipolar sensory cells. Numerous alveolar glands and short ducts in the lamina propria in the walls and floor of the anterior nasal cavities were found. The oral cavity was a stratified squamous epithelium.

Soft tissue sample - unilateral nasal blockage

Essentially all the gross anatomical structures were similar to those described in the control sample but several differences were noted. On day 4 there was a marked asymmetry which was more noticeable rostrally. The accessory cartilages were locally displaced, being constricted around the closed nostril and deviated to that side. Fig. 37 illustrates the obvious constriction of the lumen on the operated side. The nasal septum showed a flexure near its midheight. By day 11 (Fig. 38) this deviation was less marked and some degree of rebound response or repair was observed with residual deformation less severe than seen previously. This improvement

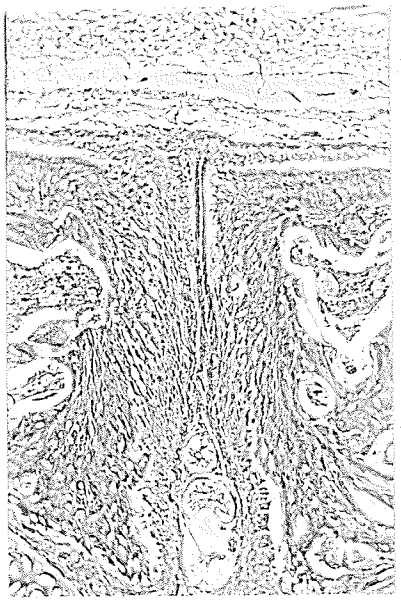


Fig. 41. Photomicrograph of the internasal suture showing a decreased flexure. Day 11 (x 150).

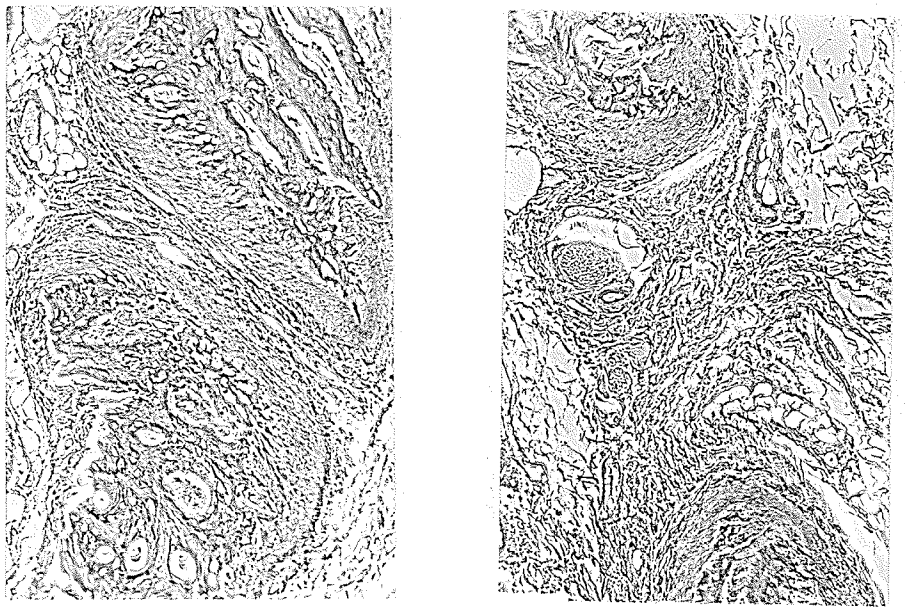


Fig. 42. Photomicrograph of the operated nasopremaxillary suture left and its counterpart, the non-operated suture, right (x 150).

was also seen throughout the experimental period with a continued lessening of the asymmetry.

Fig. 39 illustrates the nasal bone on the operated side in relation to its antemere, the nasal septum and the premaxilla. The internasal suture is little affected while the nasopremaxillary suture is compressed. Conversely, in Fig. 40, the nasopremaxillary suture on the open nostril side is under tension and demonstrates that on day 11 there was still a soft tissue discrepancy at this suture. The biomechanical limits were obviously exceeded following surgery, giving rise to a deviation of the bony units. Over the experimental period of 46 days the degree of asymmetry was reduced.

At a higher magnification the tissue damage could be visualized with disorientation of tissue elements and, in some cases, small haematomas visible in the sutures and submucous connective tissues. These tissue changes had a time scale, being most marked on day 4, still noticeable on day 11, but absent by day 25 of the experimental period. This is related to the resolution of tissue damage. Fig. 41 shows the internasal suture on day 11 and Fig. 42 demonstrates the compressed and the tensed nasopremaxillary sutures at the same age. Besides the reparative process, the growth process showed a gradual reduction in the relative widths of the sutures over the experimental period, a point also noted in the control animals.

The cartilage in the nasal septum, while showing marked deviations on day 4, did not exhibit any observable abnormal cellular activity. The nasal septa of the older operated animals showed normal healthy cartilage (Fig. 43). The vomer bones and groove in which the base of the nasal septum rests were deviated in the operated groups with the loose

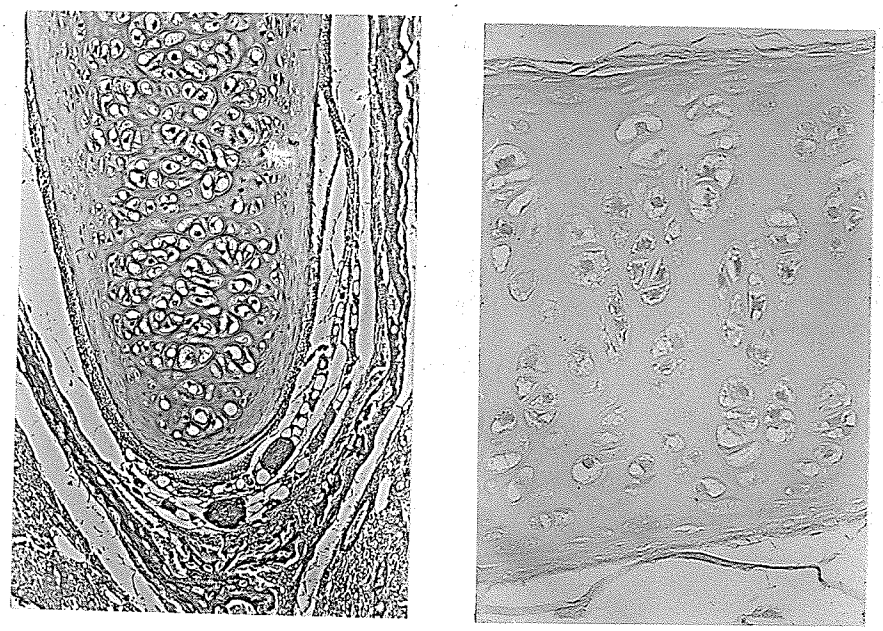


Fig. 43. Photomicrograph of the nasal septal cartilage at its base with its soft tissue sling in the vomer (x 150), and the central portion (x 300).

Fig. 44. Photomicrograph of the ciliated columnar epithelium with goblet cells from the operated nasal cavity, day 25. Submucosal accessory gland can be seen in the stroma (x 400).

alveolar tissues within showing the most damage. While the term dislocation is a relative one, the septum in none of these cases was dislocated from the vomer groove.

The chief area of concern was that of the epithelium. No signs of metaplasia were observed in any of the operated animals, which might have occurred with a marked change in the environment. No signs of ulceration were found in the epithelium lining the nasal cavity, indicating that the nasal contents were no more damaged than normal. The epithelium remained a ciliated pseudostratified columnar epithelium with goblet cells seen in Fig. 44 with submucosal accessory glands.

The turbinates showed very little change although their close proximity on the operated side to the nasal septum, as seen on day 4, suggests that, during surgery, they may have been in contact. Following this first stage, the conchae were never closely related to the septum and appeared to develop in a generalized bilateral symmetry. No aberrant concha growth occurred.

No overt sign of nasal secretion was observed although in the soft tissue preparation these secretions may have been lost. The lumen of the nasal cavities did not exhibit any marked inflammatory response and no engorgement of the submucosa was found. The only inflammatory response was in the areas of localized trauma following surgery.

The bony structure followed a similar pattern of development to that of the control sample. The internasal suture seen in Fig. 45 demonstrates the smooth external apposition of the bone with its periosteum and bone remodelling within. Fig. 46 demonstrates the typical resorptive process within the nasal cavity as exhibited on the internal surface of the nasal bones.

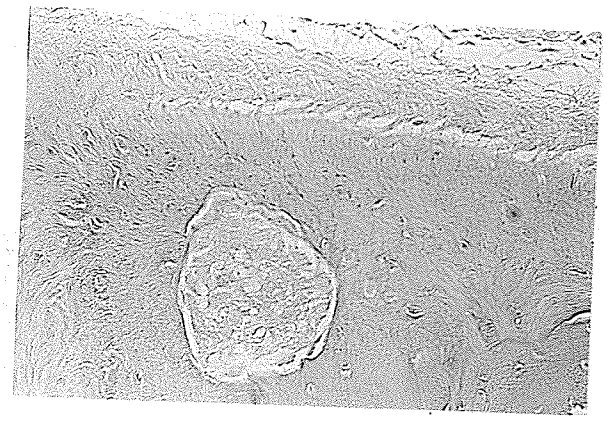


Fig. 45. Photomicrograph of the nasal bone demonstrating the smooth bony outline of the appositional surfaces externally (above) and intersuturally (left) with remodelling within (x 150).

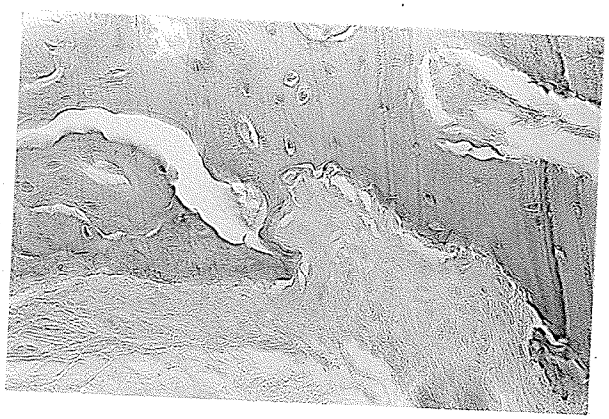


Fig. 46. Photomicrograph of the internal resorptive surface of the nasal bone being more irregular with howships lacunae (x 150).

In conclusion, the soft tissue examination revealed the fact that tissue changes occurred to a marked degree soon after surgery and gradually diminished over the experimental period. No ulceration nor epithelial changes were seen in the operated sample and no gross inflammatory response occurred in the submucosal lining of the nasal cavity.

Photographic assessment - growth changes

The cross-sectional groups on day 4, 11, 25 and 46, allowed polygon plots for each stage of the experimental period. Fig. 47 illustrates the gross morphological growth changes over the experimental period as viewed from the superior aspect. The slower development of the cranial vault can be seen in relation to the growth of the snout. This anteroposterior growth predominates with large bone increments of the nasal bones, premaxilla and maxilla. The width increase is less pronounced especially in the cranial vault. The postero-cranial growth is demonstrated by the enlargement of the nuchal crests and the prominence of the occipital condyles which, while not visible (from the superior aspect) at the earlier age groups, become the most striking posterior landmark.

When the four operational groups were compared by an analysis of variance the effects of nasal blockage could be demonstrated. The overall snout length of the control animals was significantly greater ($P < .01$) than that of the sham operated and nasal blockage groups, reflecting the size differences illustrated by the weight gains. When the nasal deviation data (as measured by the internasal suture points and the posterior midline from the frontonasal suture to the lambdoid suture) were

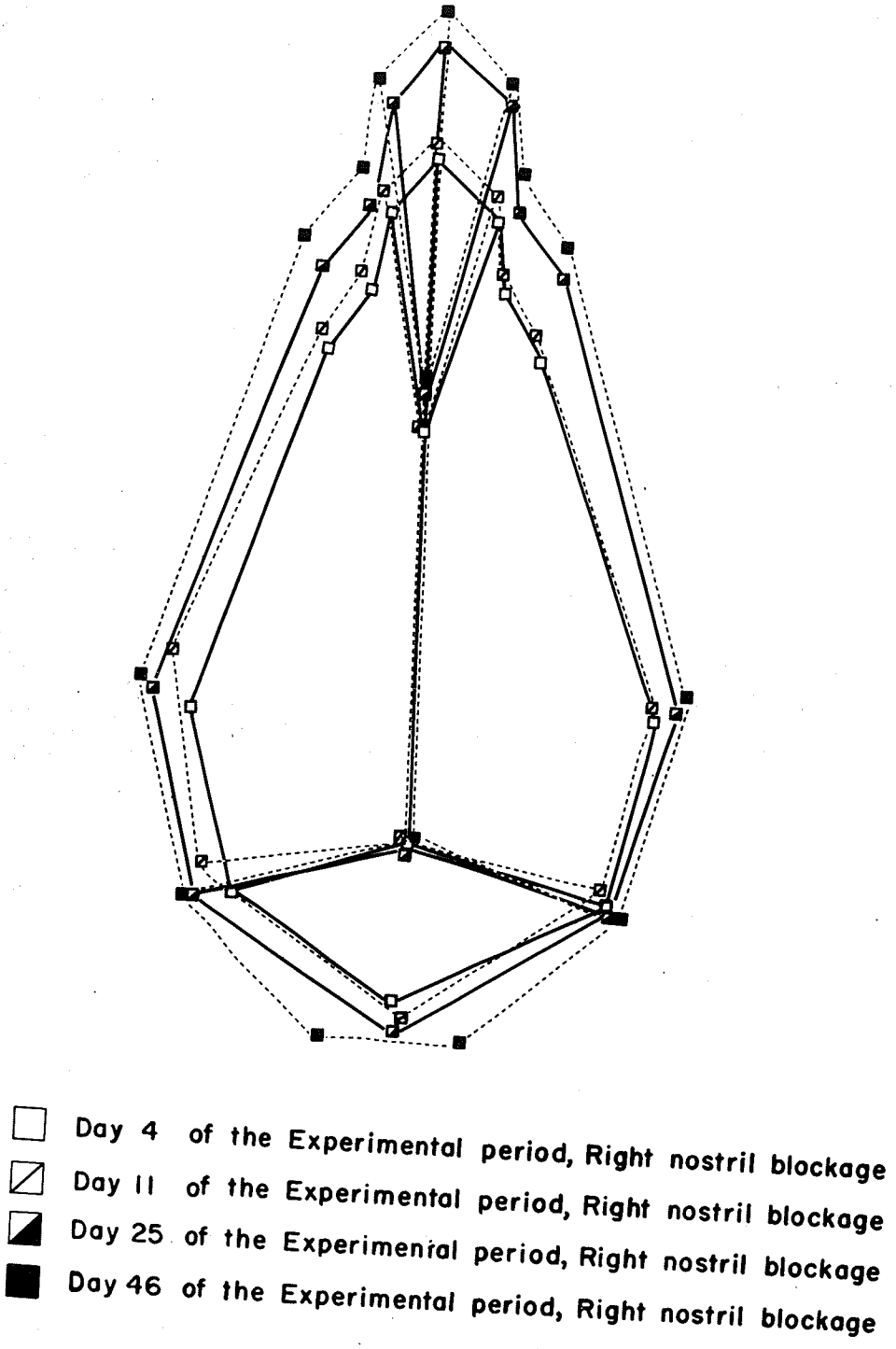


Fig. 47. Polygon plot of the superior photographic coordinate analysis of the right nostril blockage animals, showing the relative growth changes of the four age groups on days 4, 11, 25 and 46.

analyzed, the means for the control, sham, left and right nasal blockage groups were 180.7°, 179.5°, 180.6° and 180.1°, respectively, illustrating the basic lack of midline deviation even though a significant difference was found between the two smaller groups, the control and sham. The fact that there was no statistical difference between the opposite sides on day 46 illustrates the basic symmetry of these animals skulls.

The angular measurement of the most anterior point of the internasal suture to the most anterior premaxillary-maxillary junctions revealed a difference in the shape of the operated animals from that of the controls. The operated animal angles were more acute ($P < .01$) showing that, besides the snout being shorter in the operated animals, they were differentially retarded more laterally with the anterior points of the premaxillary-maxillary sutures more posteriorly.

Lateral photographic assessment - growth changes

The superimposition for the lateral photographic view was the tympanic bullae incisor plane, which was used for cutting the hard tissue sections. When oriented on this plane, there were several striking points as seen in Fig. 48.

The first molar to tympanic bullae plane remained the same throughout this period of growth. The changes in the posteroinferior cranial vault were small and due to the growth of the bony eminences of muscle attachments. Some increase was found in cranial vault height indicating that growth was continuing.

By far the most obvious changes were in the snout region where the viscerocranial elevation was noted. The nasal bones elevated with age and to a lesser extent so did the anterior palate while the incisors elongated. The anteroposterior growth of the snout was impressive.

Fig. 49 illustrates the differences seen in the four groups on day 46. The largest group of animals was the control which was statistically significantly larger ($P < .01$) when comparing linear distances with the three operated groups. While the sham group was larger in size than the two nasal blockage groups, the values were not statistically significant. When the nasal bone elevation to the tympanic bullae-incisor plane was analyzed, again the control group was significantly ($P < .01$) greater than all three operational groups, indicating a skeletal retardation in the normal viscerocranial elevation.

When comparing the incisor angulation (from the anterior point of the nasal bone to the incisal edge) to the tympanic bullae-incisor plane, the control group was larger ($P < .01$) than the two unilateral nasal blockage groups, but not the sham operated group. The sham operated group was significantly larger ($P < .05$) than the two nasal blockage groups revealing a dentoskeletal retardation as the angle steadily decreased in the cross-sectional data. In trying to ascertain the gross morphological changes in the rat crania following nasal blockage, the most striking fact was the lack of any obvious changes. Fig. 50 illustrates the anterior view of three rat crania demonstrating the deviation of the incisors to the operated side. Fig. 51 shows these crania radiographs as a composite photograph, again demonstrating the deviation of the incisors across to the operated side. A possible deviation of the septum to or away from the side of surgery was not significant.

A number of different anatomical structures were measured in the radiographic study (Fig. 52). The increase in the width of the dental arch is shown and the maintenance of the tendency for the incisor

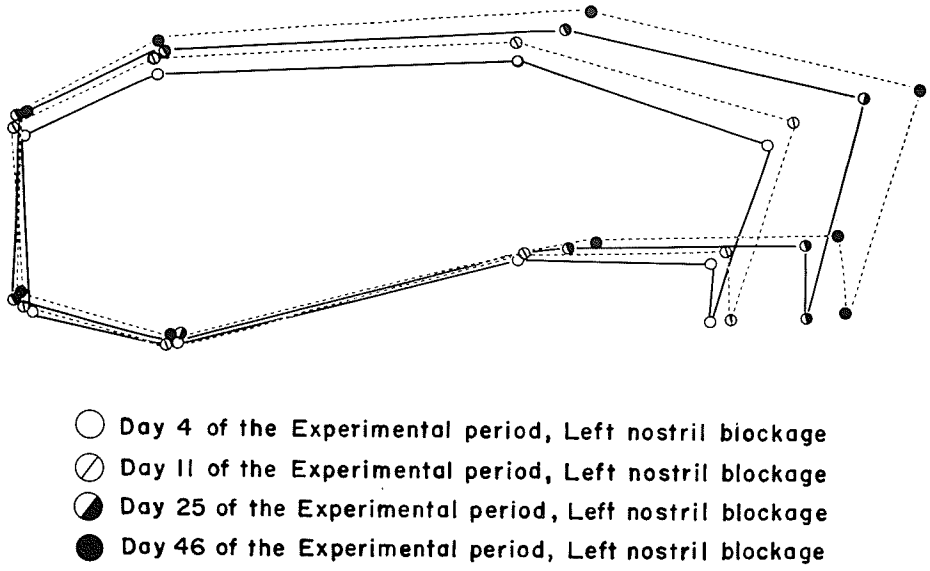


Fig. 48. Polygon plot of the lateral photographic coordinate analysis of the left nostril blockage animals, showing the relative growth changes through the four age groups.

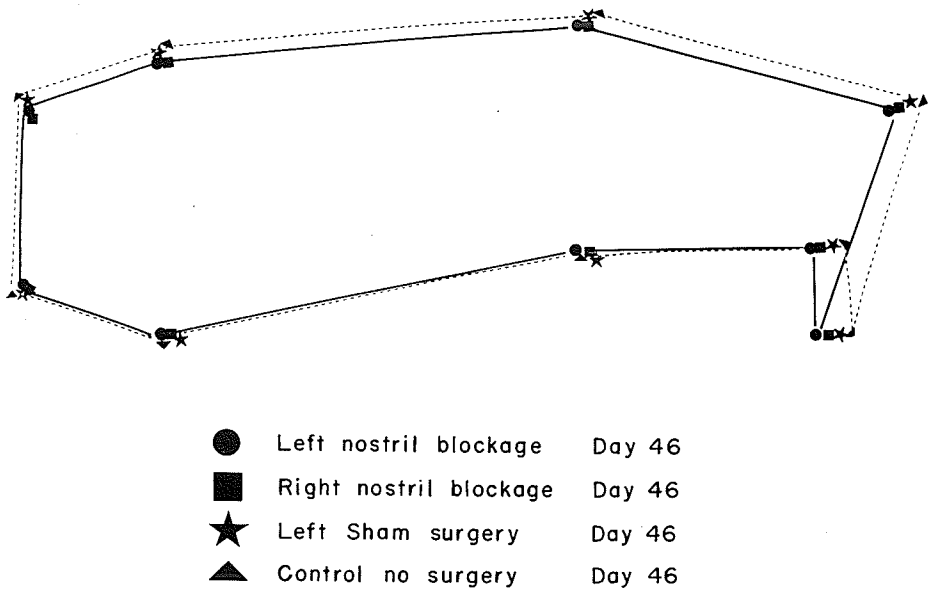


Fig. 49. Polygon plot of the lateral photographic coordinate analysis of the four groups at the end of the 46-day experimental period. The control group is demonstrated as being the largest, then the sham operated group, with the nasal blockage groups, left or right, being the smallest.

TABLE VIII

LATERAL PHOTOGRAPHIC COORDINATE ANALYSIS

		SITES OF MEASUREMENT								
		1†	2	3	4	5	6	7	8	9
LEFT NOSTRIL BLOCKED	Mean X*	0.0Δ	11.16¶	12.50	11.19	7.04	7.18	-0.04	-2.29	-2.44
	Y**	0.0	0.0	3.93	1.54	1.48	5.38	4.74	3.95	0.83
	S.D. X	0.0	0.29	0.34	0.29	0.18	0.24	0.10	0.08	0.10
	Y	0.0	0.0	0.14	0.07	0.11	0.15	0.12	0.14	0.11
RIGHT NOSTRIL BLOCKED	Mean X	0.0	11.07	12.36	11.07	6.97	7.11	-0.09	-2.40	-2.49
	Y	0.0	0.0	3.91	1.52	1.48	5.39	4.72	3.90	0.78
	S.D. X	0.0	0.25	0.28	0.27	0.19	0.28	0.08	0.14	0.10
	Y	0.0	0.0	0.14	0.06	0.13	0.12	0.10	0.18	0.11
CONTROL	Mean X	0.0	11.54	12.76	11.50	7.20	7.25	-0.10	-2.39	-2.61
	Y	0.0	0.0	4.11	1.61	1.54	5.46	4.85	4.03	0.77
	S.D. X	0.0	0.17	0.21	0.18	0.10	0.13	0.08	0.14	0.09
	Y	0.0	0.0	0.11	0.58	0.09	0.07	0.09	0.08	0.09
HAM EFT	Mean X	0.0	11.23	12.45	11.22	7.08	7.14	-0.13	-2.47	-2.65
	Y	0.0	0.0	3.93	1.58	1.41	5.37	4.69	3.97	0.82
	S.D. X	0.0	0.08	0.17	0.10	0.08	0.11	0.03	0.08	0.09
	Y	0.0	0.0	0.11	0.05	0.16	0.14	0.15	0.15	0.05

† Sites measured described in the glossary

* Horizontal coordinate

** Vertical coordinate

Δ Origin of the coordinate analysis

¶ Direction of the coordinate analysis. Measurement of magnified photographic points in centimeters.

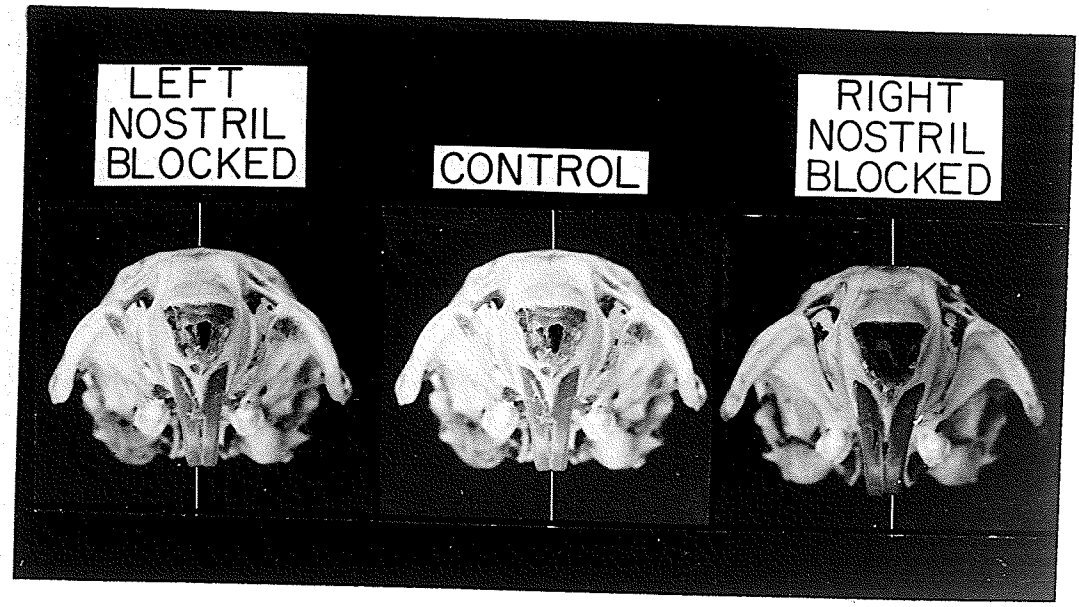


Fig. 50. Anterior view of three rat crania on day 46 showing the deviation of the incisors towards the operated side.

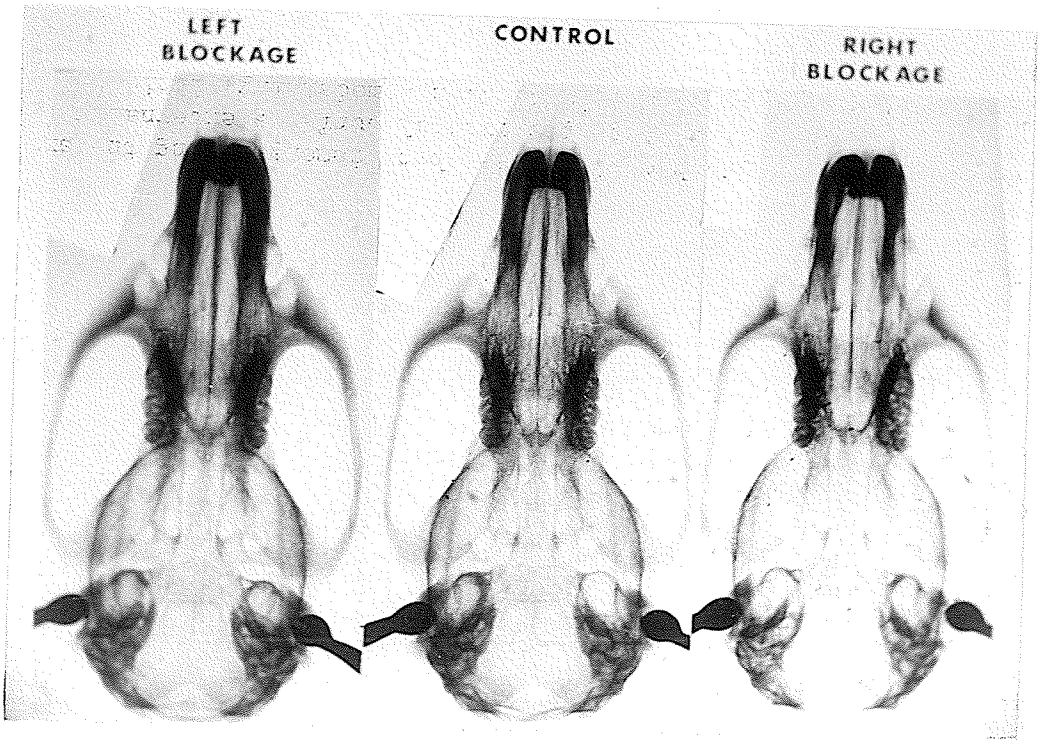


Fig. 51. Superior radiographic view showing a lack of any marked asymmetry while the incisor deviation can be seen towards the side of nasal blockage.

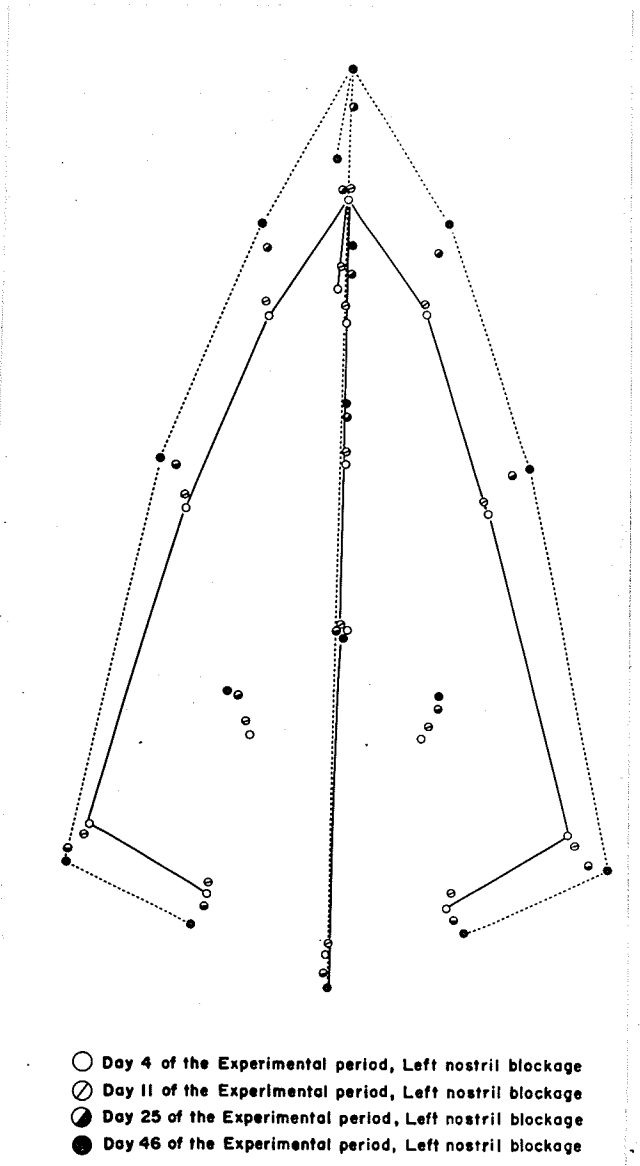


Fig. 52.
Polygon plot of the superior radiographic coordinate analysis showing the relative growth changes of the left nostril blocked animals.

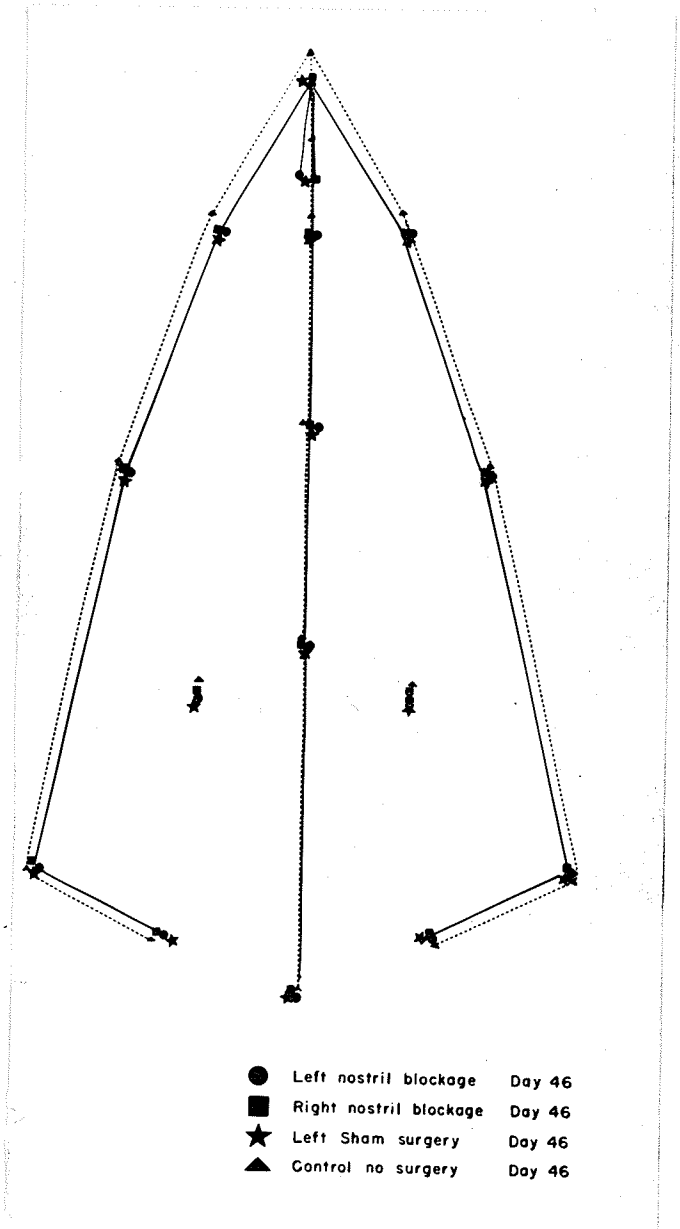


Fig. 53.
Polygon plot of the superior radiographic coordinate analysis showing the differences of the four groups on day 46 with the control group the largest and the operated groups the smallest.

to deviate to the side of surgery. Fig. 53 demonstrates the intergroup differences which were fundamentally as found in the photographic analysis with the control group being significantly larger than the operated groups.

Incisor coronal sections

The coronal section coordinate analysis data were used in the same way as the photographic data, i.e. as polygon plots and a series of analyses of variance. The growth again demonstrated a two-dimensional pattern of changes described in the soft tissue findings with greater increase in height than in width. The nasal bone flexure (Fig. 54) increased throughout the experimental period, the nasal bone distortion following surgery was most asymmetrical on day 4, reducing until on day 46 there was no significant asymmetry.

Fig. 55 shows the differences between the left nasal blockage and the control groups. The tendency of incisor deviation to the operated side was not found to be statistically significant. The nasal bone width was found to be just significantly ($P < .05$) greater in the two nasal blockage groups than in the control group. This would appear to be the opposite of that expected.

Premaxillary coronal sections

In an examination of the cross-sectional appearance changes in the premaxillary region, there were again asymmetries in the base of the nasal septum (Fig. 56) and the nasal bones, but these were reduced over the experimental period. The growth seen in the vertical and horizontal planes was almost equal.

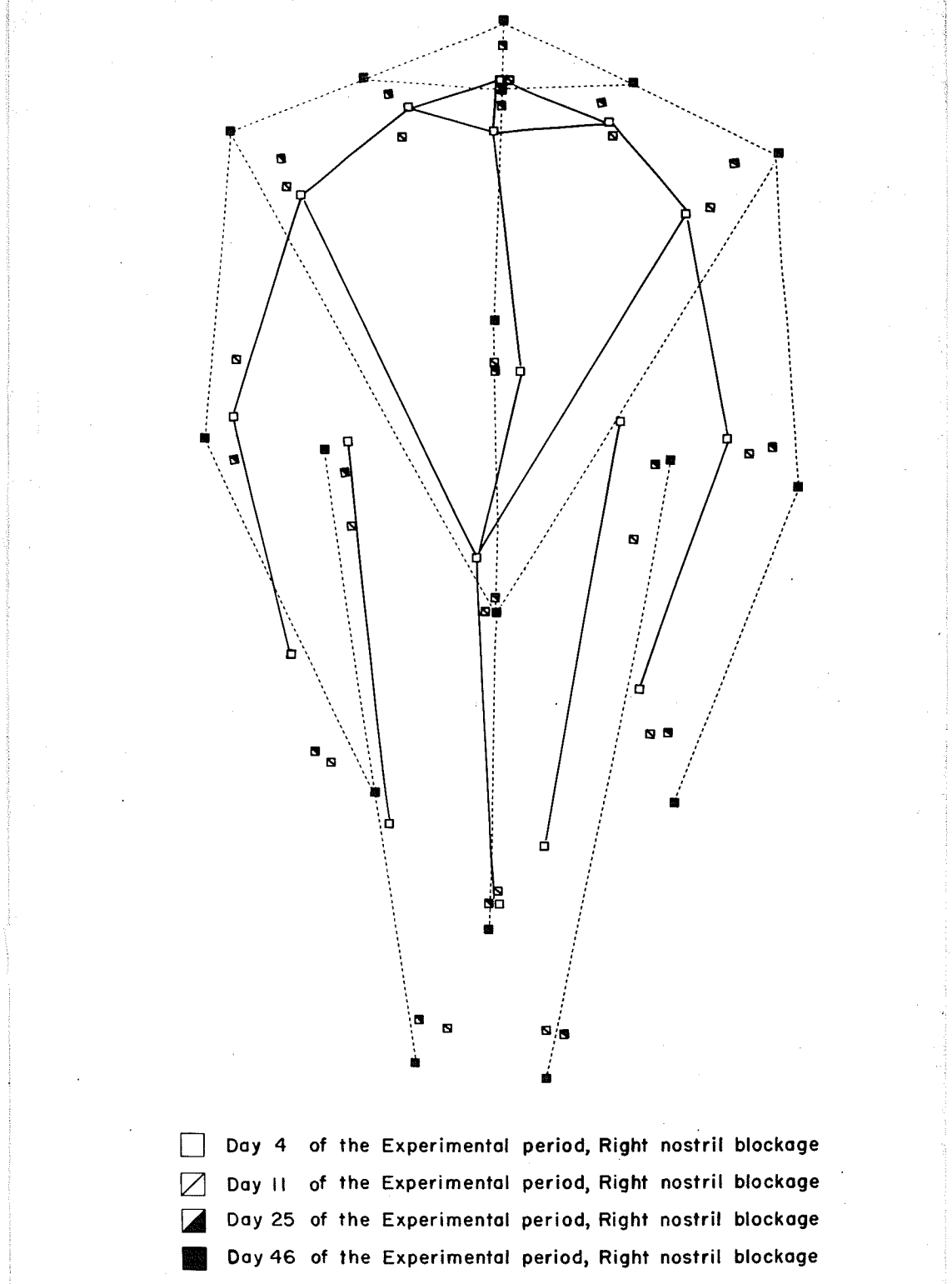


Fig. 54. Polygon plot of the incisor coronal hard tissue region landmarks, showing the growth changes of the right nostril blockage animals for the four age groups.

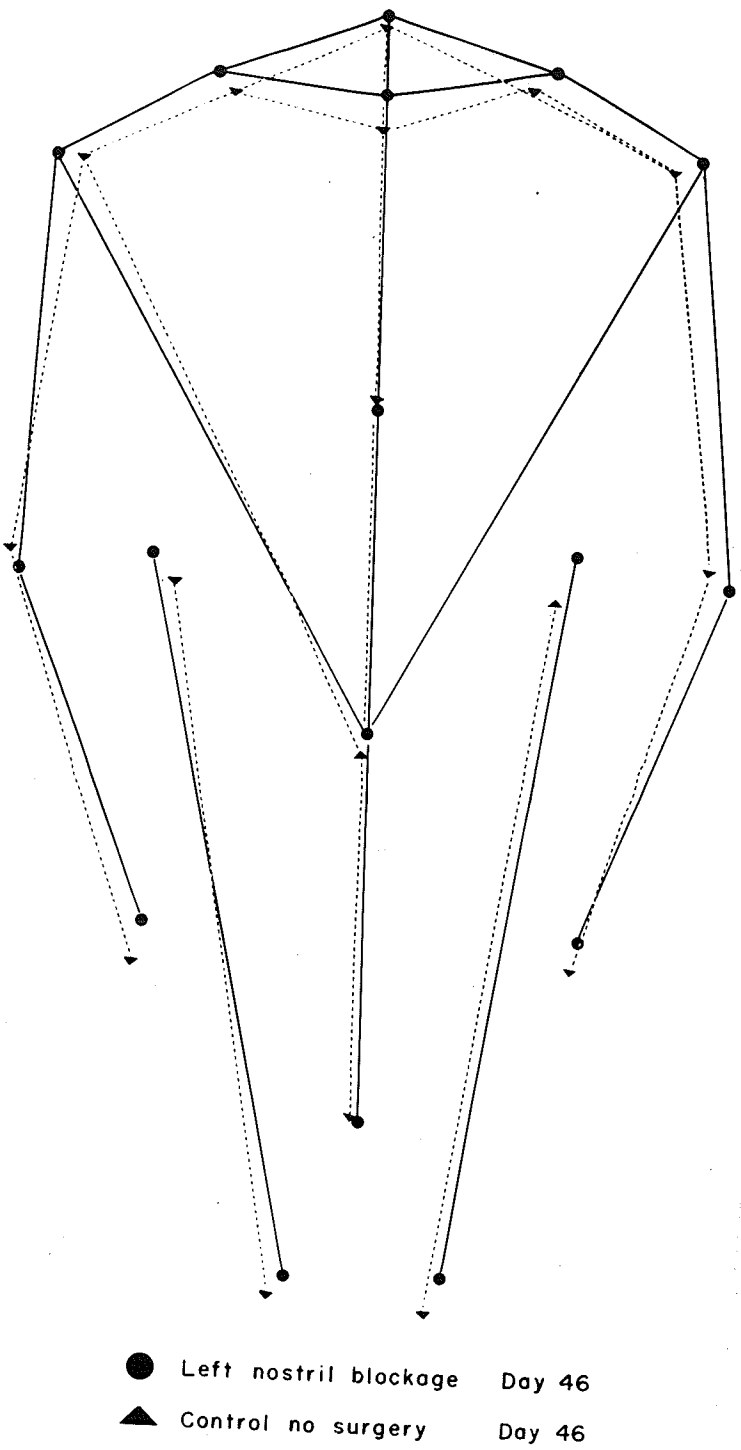


Fig. 55. Polygon plot of the incisor region comparing the control and left nostril blockage animal groups on day 46.

TABLE IX

INCISOR REGION CORONAL COORDINATE ANALYSIS

SITES OF MEASUREMENT

		1†	2	3	4	5	6	7	8	9
	Mean									
LEFT	X*	0.0Δ	9.16¶	8.38	6.08	3.31	8.90	8.18	1.58	4.91
NOSTRIL	Y**	0.0	0.0	0.07	0.04	0.02	-1.46	-2.65	-2.98	-1.79
BLOCKED	S.D.									
	X	0.0	0.59	0.55	0.81	0.36	0.62	0.55	0.41	0.52
	Y	0.0	0.0	0.04	0.15	0.07	0.16	0.20	0.28	0.14
	Mean									
RIGHT	X	0.0	9.03	8.37	6.04	3.15	8.49	7.80	4.39	1.31
NOSTRIL	Y	0.0	0.0	-0.01	0.05	-0.01	-1.55	-2.71	-2.94	-1.79
BLOCKED	S.D.									
	X	0.0	0.40	0.40	0.53	0.29	0.39	0.48	0.35	0.37
	Y	0.0	0.0	0.06	0.11	0.08	0.15	0.08	0.10	0.09
	Mean									
	X	0.0	9.19	8.31	5.97	3.11	8.65	8.03	4.59	1.25
CONTROL	Y	0.0	0.0	0.03	-0.03	0.02	-1.26	-2.43	-2.82	-1.75
	S.D.									
	X	0.0	0.47	0.48	0.33	0.45	0.58	0.50	0.47	0.59
	Y	0.0	0.0	0.04	0.14	0.05	0.09	0.16	0.11	0.11
	Mean									
	X	0.0	9.17	8.33	6.02	3.13	8.45	7.89	4.59	1.27
AM	Y	0.0	0.0	0.04	-0.01	0.03	-0.99	-2.68	-3.11	-1.99
FT	S.D.									
	X	0.0	0.76	0.78	0.40	0.38	0.79	0.47	0.36	0.36
	Y	0.0	0.0	0.01	0.12	0.07	0.11	0.15	0.29	0.01

† Sites measured described in the glossary

* Horizontal coordinate

** Vertical coordinate

Δ Origin of the coordinate analysis

¶ Direction of the coordinate analysis.
Measurement in millimeters.

TABLE X

INCISOR REGION CORONAL COORDINATE ANALYSIS

SITES OF MEASUREMENT

		10†	11	12	13	14	15	16	17
LEFT NOSTRIL BLOCKED	Mean								
	X*	4.91¶	-1.31	-1.27	4.94	1.68	4.80	8.12	8.85
	Y**	-1.69	-0.68	0.61	1.85	1.86	2.97	2.71	1.39
	S.D.								
	X	0.91	0.64	0.63	0.88	0.50	0.46	0.54	0.64
	Y	0.25	0.10	0.11	0.23	0.09	0.09	0.17	0.11
RIGHT NOSTRIL BLOCKED	Mean								
	X	4.75	-1.50	-1.34	4.78	1.30	4.74	7.92	8.46
	Y	-1.71	-0.59	0.67	1.71	1.78	2.88	2.67	1.42
	S.D.								
	X	0.52	0.58	0.57	0.44	0.55	0.50	0.31	0.34
	Y	0.19	0.11	0.13	0.13	0.18	0.12	0.23	0.14
CONTROL	Mean								
	X	4.37	-1.64	-1.49	4.45	1.23	4.61	8.01	8.52
	Y	-1.55	-0.62	0.66	1.60	1.84	2.98	2.47	1.30
	S.D.								
	X	0.37	0.36	0.19	0.47	0.59	0.62	0.50	0.56
	Y	0.14	0.22	0.07	0.11	0.13	0.13	0.22	0.25
LEFT CLAM	Mean								
	X	4.48	-1.88	-1.55	5.02	1.34	4.42	7.98	8.53
	Y	-2.13	-0.89	0.70	1.77	1.97	3.08	2.70	1.40
	S.D.								
	X	0.30	0.56	0.64	0.46	0.33	0.66	0.57	0.76
	Y	0.56	0.77	0.14	0.14	0.03	0.24	0.27	0.04

† Sites measured described in the glossary

* Horizontal coordinate

** Vertical coordinate

¶ Measurement in millimeters.

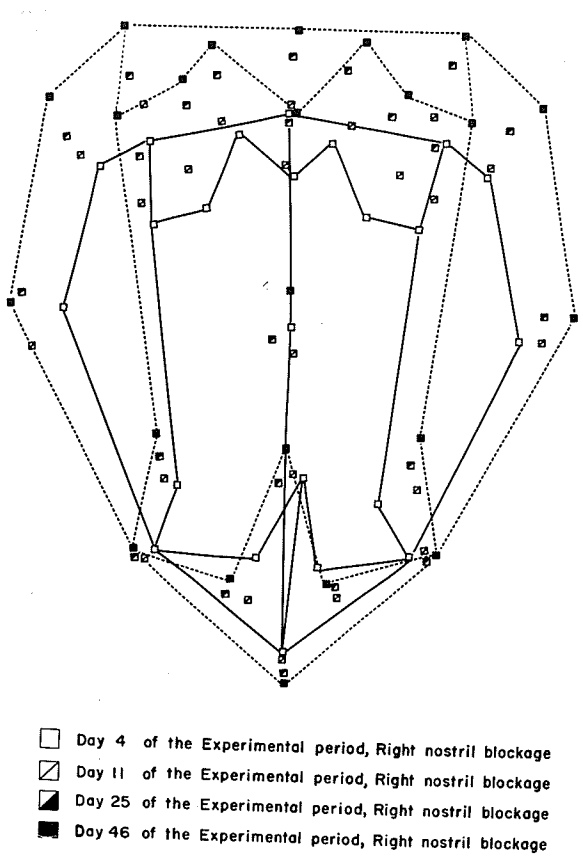


Fig. 56. Polygon plot of the premaxillary region for the four right nostril blockage groups.

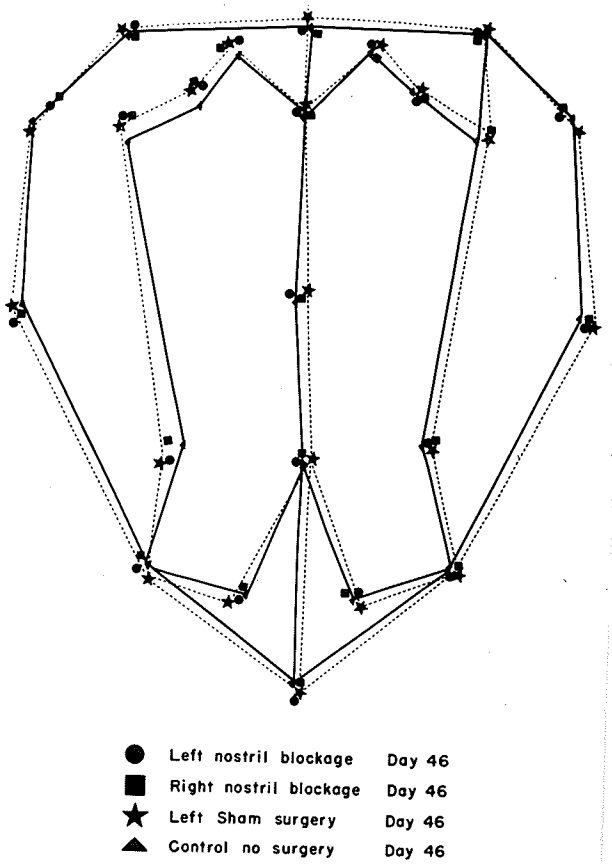


Fig. 57. Polygon plot of the premaxillary region for the four groups on day 46.

Fundamentally, there were no differences in the four groups at the end of the experimental period (Fig. 57). The findings of the sham operated group in the coronal sections was that the morphological changes resembled those of the operated groups. The statistical data suggested that the effect on the sham group was very similar to but a little less severe than on the nasal blockage groups, although most findings were not statistically significant.

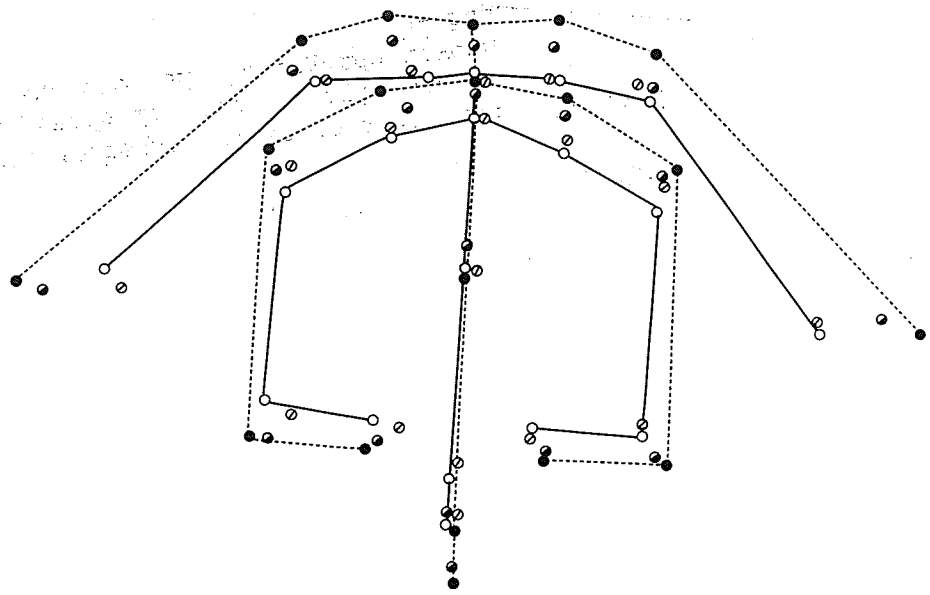
Malar region coronal sections

The malar region polygon plot (Fig. 58) illustrates the deviation of the nasal bones in the nasal blockage groups, although the overall deformation was not as severe as further forward in the snout. The deviation was again reduced with age and by day 46 had essentially disappeared. The lumen size of the nasal cavity appeared smaller on the operated side but this observed difference became smaller as the cavities increased in size and no significant differences were found on day 46.

The four groups on day 46 were basically the same shape but in size there were significant differences. The width and height of the snout were significantly smaller ($P < .05$) in the two nasal blockage groups than in the control group. No statistical correlation was found for the deviation of the nasal septum with the side of the surgical procedure. No changes in the turbinate size or orientation were noted.

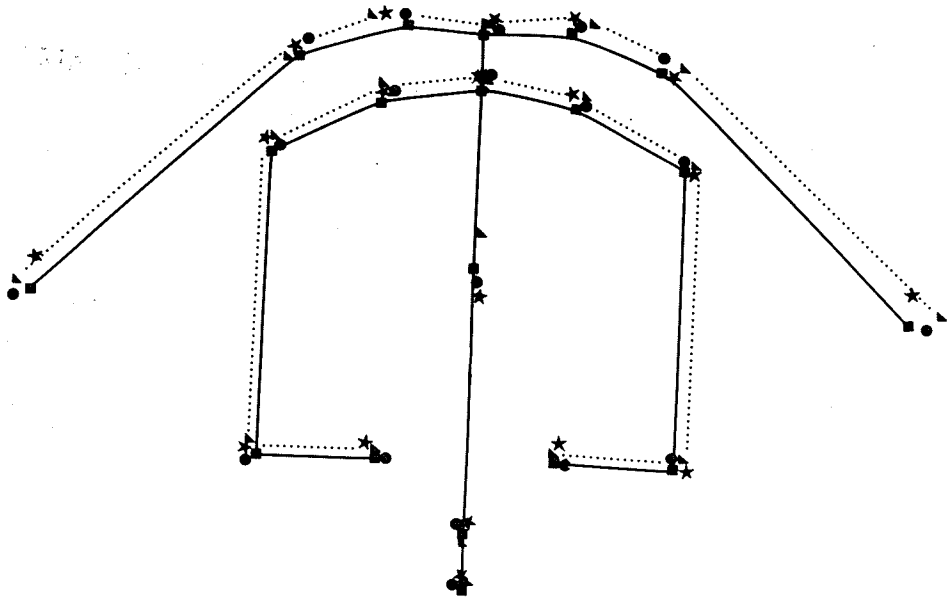
First molar coronal sections

The deviation following surgery could still be identified on day 4 with the palatal plane being tipped. There was a readjustment of the



○ Day 4 of the Experimental period, Left nostril blockage
⊘ Day 11 of the Experimental period, Left nostril blockage
◪ Day 25 of the Experimental period, Left nostril blockage
● Day 46 of the Experimental period, Left nostril blockage

Fig. 58. Polygon plot of the malar region landmarks showing the growth changes of the left nostril blockage animals for the four age groups.



● Left nostril blockage Day 46
■ Right nostril blockage Day 46
★ Left Sham surgery Day 46
▲ Control no surgery Day 46

Fig. 59. Polygon plot of the malar region for the four groups on day 46.

alveolus and palatal floor orientation, probably mediated through the suture system. Fig. 60 shows these changes during the experimental period with more growth in width than in height. The lateral points, the zygomatic arches, grew laterally with the palate and alveolus descending and the ethmoid air sinuses enlarging above the air passage. Because the overall differences between the four groups distal to the malar region were so small, no polygon plot differences on day 46 are necessary. No differences in the zygomatic arch positions were found, nor molar height asymmetry on day 46, following an apparent earlier difference.

Zygomatic coronal sections

The overall changes of this region were those of the growth of the cranial vault above and the facial skeleton with the zygomatic arches laterally and the posterior nasal passage and maxilla below. Greater changes can be seen in the facial skeleton with the zygomatic width increasing more than the height. While no asymmetry could be demonstrated in the cranial vault, the posterior part of the maxilla did appear to be deviated on day 4.

Squamosal coronal sections

No asymmetry was discerned in the cranial vault or the pterygoid plates following surgery in these animals and the amount of growth in this region was limited (Fig. 62). The neurocranial growth was small with the roots of the zygomatic arches elevating slightly.

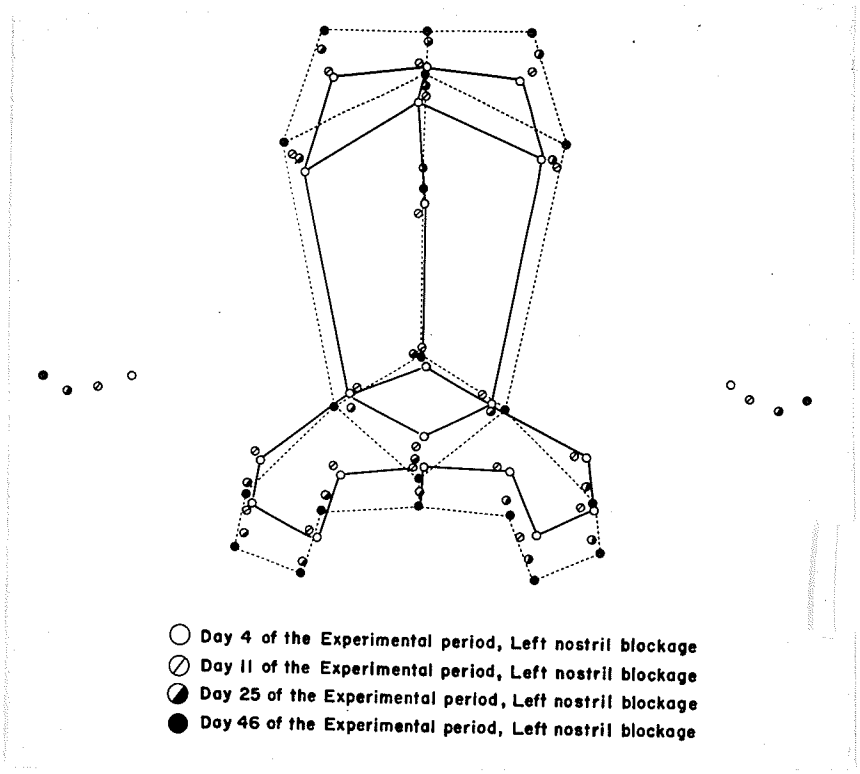


Fig. 60. Polygon plot of the first molar region landmarks showing the growth changes of the left nostril blockage animals for the four age groups.

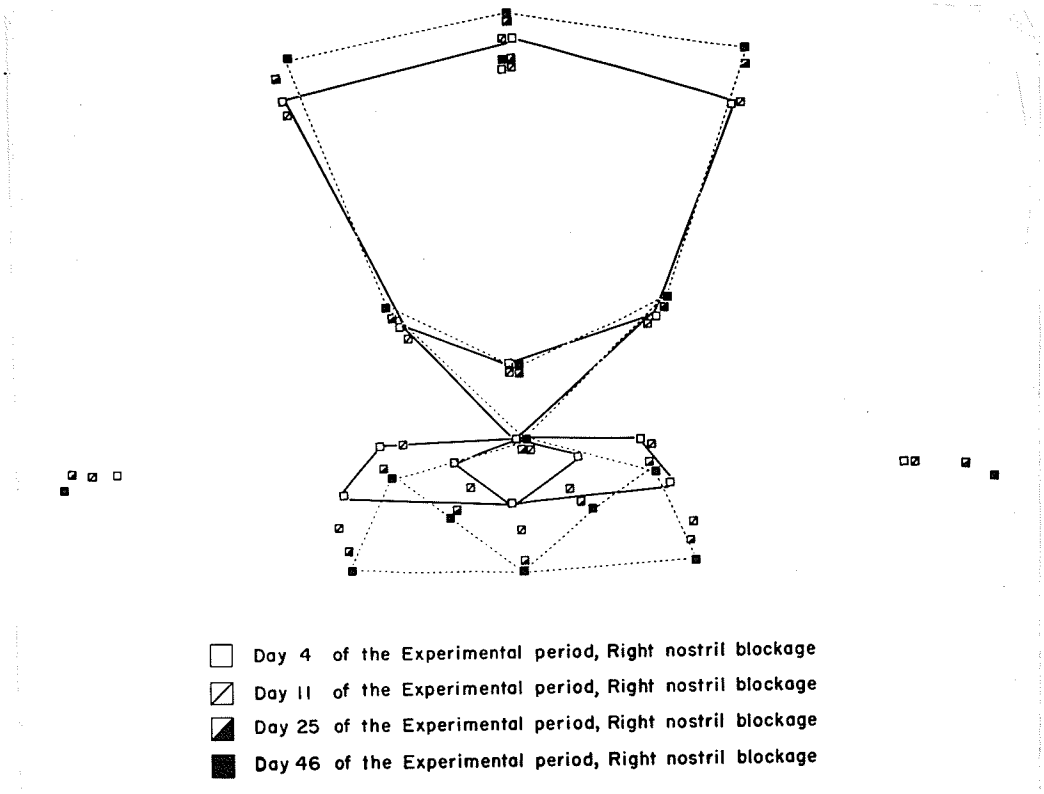


Fig. 61. Polygon plot of the zygomatic region landmarks showing the growth of the right nostril blockage animals for the four age groups.

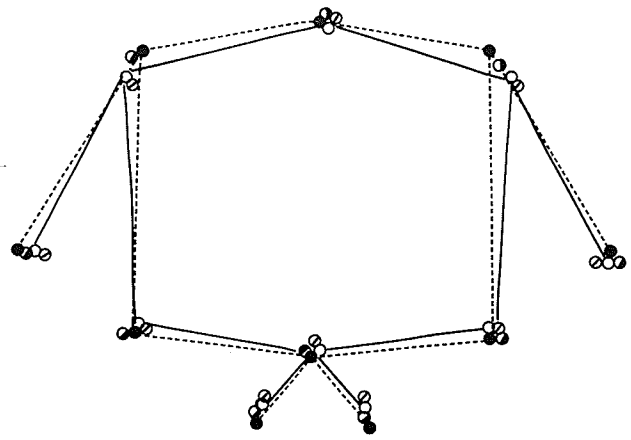
Tympanic bullae coronal sections

There was no observed asymmetry following surgery and there were several growth changes seen (Fig. 63). The tympanic bullae moved laterally and the neurocranial space enlarged with the nuchal crests markedly growing over this period, from being almost absent on day 4 to being prominent on day 46 of the experimental period.

Vital bone marking

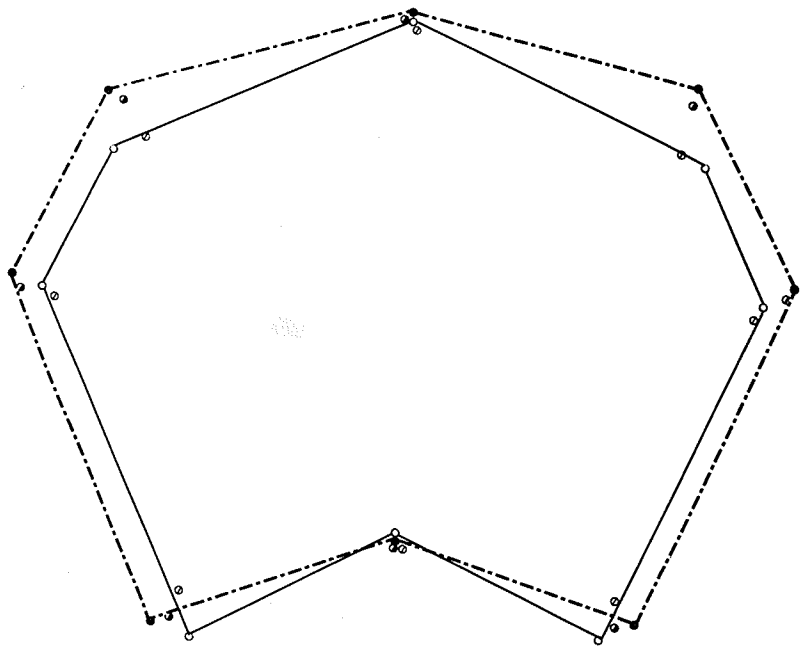
All three agents were clearly visible under ultraviolet light and the four incremental lines were distinguishable as green, red, yellow and orange. On observing the hard tissue sections under ultraviolet light, the bone of the animals sacrificed on day 4 of the experimental period appeared green throughout due to the rapid bone growth by surface apposition and remodelling internally. On day 11 the bone still appeared green throughout with a relatively clear surface bone marked increment of alizarin red S. By day 25 the more mature increment lines were visible with the green of the Dcaf being even more obvious as an increment as were also the red line of alizarin red S and the yellow of the terramycin. The last bone mark was the mixture of alizarin red S and terramcyin which was visible as an orange line, in some cases as two lines superimposed of alizarin red S and terramycin, demonstrating their differences in combining in bone.

Dcaf proved very suitable as a bone marking agent. The volume required was small as was the dosage. Unfortunately, it was markedly alkaline and apolecia and even ulceration were found in the overlying skin of the peritoneum. No signs of damage were seen intraperitoneally in any of these animals at sacrifice. The alizarin red S was markedly acidic and could be seen excreted in the urine after only a matter of minutes



○ Day 4 of the Experimental period, Left nostril blockage
⊘ Day 11 of the Experimental period, Left nostril blockage
◪ Day 25 of the Experimental period, Left nostril blockage
● Day 46 of the Experimental period, Left nostril blockage

Fig. 62. Polygon plot of the squamosal region landmarks showing the growth of the left nostril blockage animals for the four age groups.



○ Day 4 of the Experimental period, Left nostril blockage
⊘ Day 11 of the Experimental period, Left nostril blockage
◪ Day 25 of the Experimental period, Left nostril blockage
● Day 46 of the Experimental period, Left nostril blockage

Fig. 63. Polygon plot of the tympanic bullae region landmarks showing the growth of the left nostril blockage animals for the four age groups.

following injection, and was visible in the urine for up to eight days post injection.

Terramycin was an effective bone marking agent in the same dosage as previous investigations. Unfortunately, its use on day 11 proved toxic in the debilitated rats which, after recovering from the injection, collapsed within minutes with tremors, and several died. This drug was again used with the same dosage in conjunction with alizarin red S on day 25 with no mortality.

Linear analysis

The linear analysis with the calibrated eyepiece was more accurate than the coordinate analysis. The actual measurement technique was modified in this experiment in order to facilitate the recording of the measurements and also the calculation of the bone thickness. Using site 7 (Fig. 64) in the incisor region as an example, the zero of the 500 micron unit scale of the ocular lens was placed on the internal bone edge with the scale crossing the bone marking increments at right angles. Nine positive values were observed and recorded: first the four internal bone mark increments, then the four external bone mark increments and finally the external bone surface.

The bone mark increments were well separated by the apposition of normal bone tissue. The increments allowed a good method of quantifying the bone apposition in the coronal sections. The sagittal sections were less clear as the sections had to be thicker because of the length of the region, and the rapid anteroposterior growth spread out the marked bone making it less distinct. The horizontal sections were obliquely related to the bone surfaces and the increments were least clear in this plane.

The incisor region

Examination of Fig. 64 reveals the general pattern of growth in the coronal plane in the incisor region. The interpremaxillary suture and the medial wall of the alveolar socket (sites 1 and 2) were revealed as areas of apposition only. Fig. 65 shows the relative growth or the cumulative bone apposition at these sites (1 and 2) in microns of growth, both internally or medially and externally. Essentially, through the experimental period, the bone apposition rate reduced slowly and steadily. The values found for the left and right sides for these increments were so similar that no obvious differences could be seen either graphically or in table form.

The values of each increment of bone apposition measured were compared bilaterally and the differences tested for significance by an analysis of variance. All these values were given as an F value (Table XI) and all were not significant. The differences of the mean increments of the bone growth in microns is shown for site 1 medial in Fig. 66. The AB and BC increments of the left nostril blocked animals demonstrated a slightly greater bone apposition on the left side, while later in the CD and DE increments the converse was true.

It was found that frequently the consecutive measurements of the bone increments had a bias in the opposite directions, so that a greater increment on the left side was followed by a smaller increment. If this bias had been found consistently, more importance could have been ascribed to it and the consideration of catch-up phases could have been contemplated. There was no consistent bias of the operated or non-operated sides growing more or less than in the control animals.

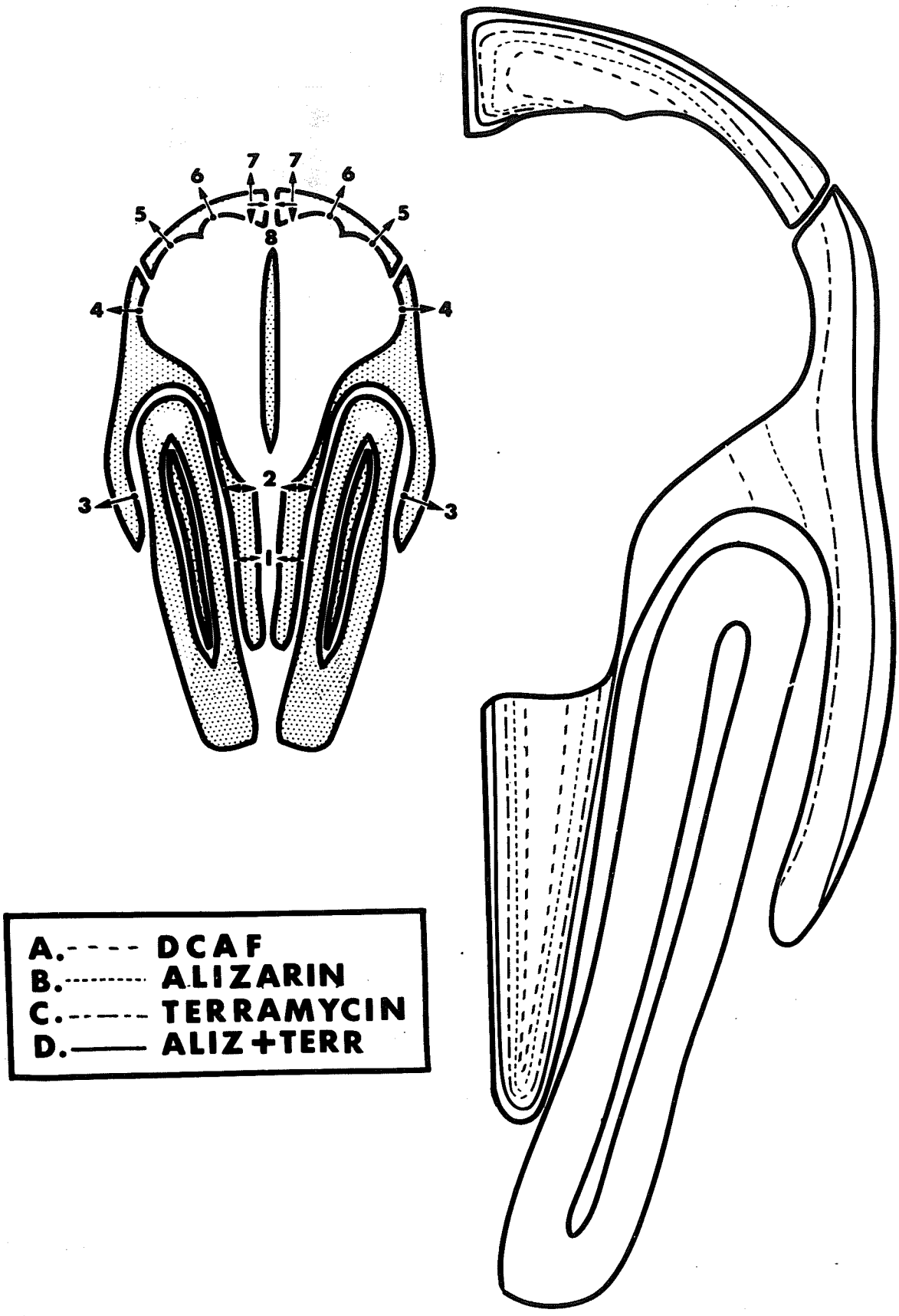


Fig. 64. Diagrammatic representation of the bone mark increments seen in the incisor region.

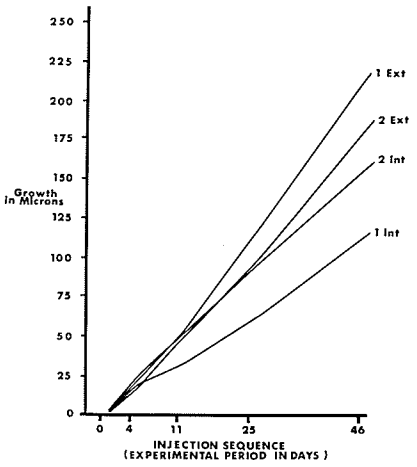


Fig. 65. Cumulative bone apposition in microns in sites 1 and 2, externally and internally in the incisor region.

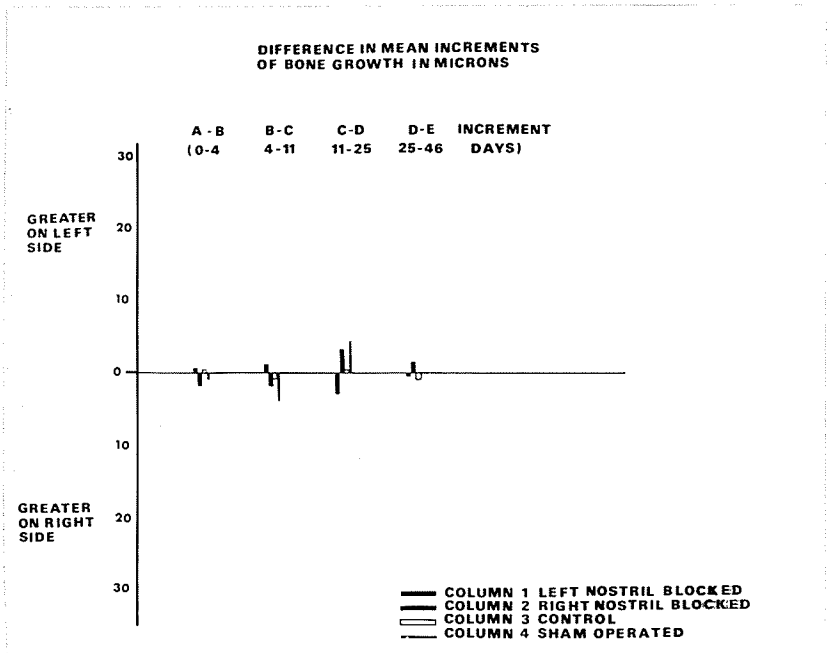


Fig. 66. The mean differences of each of the four increments (from side to side) for the four operational groups on day 46 for the incisor region, site 1 medial.

TABLE XI

INCREMENTS OF BONE APPOSITION IN THE INCISOR REGION, BLOCK A, AS MEANS AND STANDARD ERRORS IN MICRONS

GROUP	SITE	A - B MEAN	A - B SE	B - C MEAN	B - C SE	C - D MEAN	C - D SE	D - E MEAN	D - E SE
INCISOR SITE 1 MEDIAL									
LEFT NOSTRIL BLOCKED	Left	18.3	1.1	16.0	0.9	33.0	2.1	50.1	2.1
	Right	17.3	0.8	14.5	0.9	35.9	2.3	50.5	2.0
	Diff	1.0	1.0	1.5	1.0	-2.9	1.8	-0.5	2.3
RIGHT NOSTRIL BLOCKED	Left	16.7	0.8	14.8	1.0	36.9	2.4	51.2	2.1
	Right	18.0	1.2	16.3	1.0	33.7	2.2	49.8	2.4
	Diff	-1.3	1.0	-1.5	1.1	3.2	1.9	-1.4	2.4
CONTROL	Left	19.4	1.3	16.5	1.6	44.4	4.2	55.6	1.4
	Right	18.7	1.1	17.0	1.5	44.6	4.8	56.5	2.1
	Diff	0.7	1.5	-0.5	1.8	-0.2	4.2	-0.9	2.3
LEFT SHAM	Left	18.7	1.3	19.7	1.1	35.7	9.3	57.3	0.9
	Right	19.0	1.7	23.3	1.3	40.0	8.9	57.3	3.2
	Diff	-0.3	1.6	-3.7	2.2	-4.3	0.4	0.0	2.6
F VALUE		0.55		2.02		1.47		0.11	
INCISOR SITE 1 LATERAL									
LEFT NOSTRIL BLOCKED	Left	22.2	1.9	32.3	3.0	68.9	5.2	98.1	7.1
	Right	23.4	2.8	33.5	3.2	58.9	3.5	103.5	6.3
	Diff	-0.9	2.4	-1.2	3.3	10.0	5.8	-5.4	6.8
RIGHT NOSTRIL BLOCKED	Left	23.0	2.7	32.2	3.3	58.3	3.8	105.5	6.3
	Right	21.7	1.5	30.4	2.9	67.5	5.1	101.3	7.3
	Diff	1.3	2.5	1.7	3.6	09.2	6.0	4.2	7.3
CONTROL	Left	19.7	2.2	36.4	6.4	69.0	11.6	84.0	15.9
	Right	15.5	3.0	35.0	6.0	58.3	12.7	79.6	10.8
	Diff	5.0	1.5	1.4	3.4	10.7	14.8	4.4	11.2
LEFT SHAM	Left	22.5	1.4	21.0	2.3	69.0	6.4	132.5	21.7
	Right	20.0	1.2	17.5	1.4	66.5	19.3	137.5	15.9
	Diff	2.5	2.6	3.5	0.9	2.5	13.0	-5.0	5.8
F VALUE		0.47		0.15		0.87		0.26	

The lateral alveolar socket wall, site 3 in the incisor coronal region, was shown to be an area of greater increments of growth externally but with a coincident lateral incisor socket resorption. Fig. 67 demonstrates the growth pattern similar to that of sites 1 and 2.

The lateral wall of the premaxilla, site 4 measured in the incisor region, was illustrated as having an even greater rate of apposition externally with a rapid rate of intranasal resorption. Fig. 67 shows the steepness of the incremental curve indicative of the rapid rate of apposition, the origin of the line being on day 25 because the AB, BC and CD increments had been removed by resorption.

Site 5 of the incisor region demonstrated the growth of the lateral part of the nasal bone. The internal surface lining the nasal cavity was a site of internal resorption and the external surface a site of apposition. The rate of apposition shown from the cumulative growth in microns was less than in site 3 but greater than in sites 1 and 2.

The anatomical site 6 demonstrated the similar pattern of growth as site 5 with the nasal bone inferior surface resorption and the superior surface apposition. Figs. 69 and 70 again show the cumulative bone apposition and the differences of the mean of the four increments of the four operational groups. Table XII gives the relative values in microns.

The medial edge of the nasal bone increments were seen both internally and externally. Fig. 69 shows the cumulative bone growth curves of these surfaces which are basically the same, although the flexure may indicate a differential rate of growth at the different anatomical sites. The growth increments in microns are shown in Tables XII and XIII for sites 6, 7 and 8 in the incisor region and illustrate a lack of consistent apposition differences with nasal blockage.

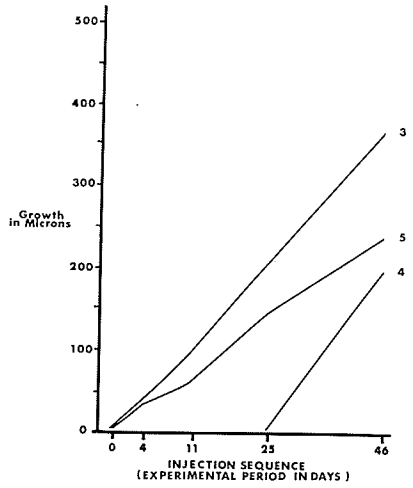


Fig. 67. Cumulative bone apposition in microns in site 3, 4 and 5 in the incisor region.

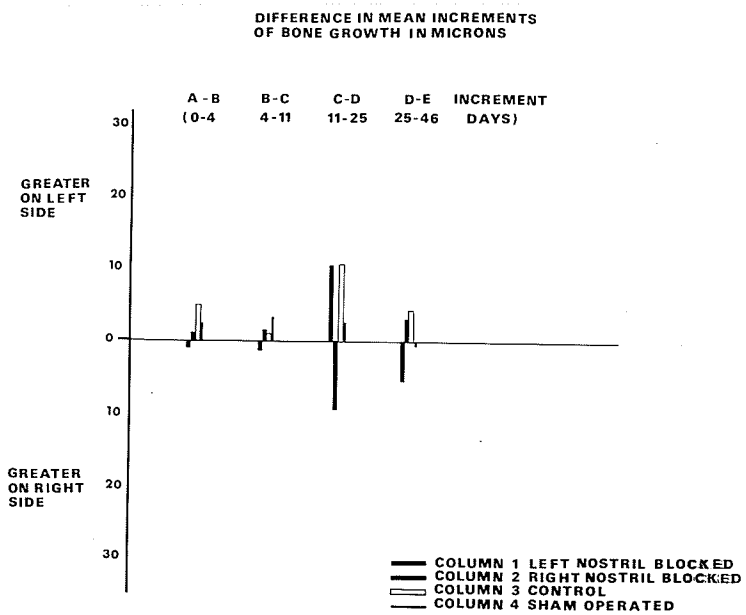


Fig. 68. The mean differences of each of the four increments for the four operational groups on day 46 for the incisor region, site 1 lateral.

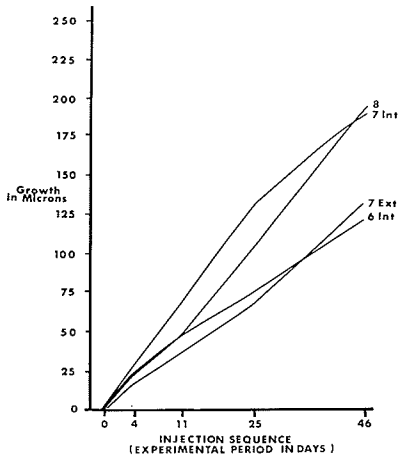


Fig. 69. Cumulative bone apposition in microns for sites 6, 7 internal and external, and 8 of the incisor region.

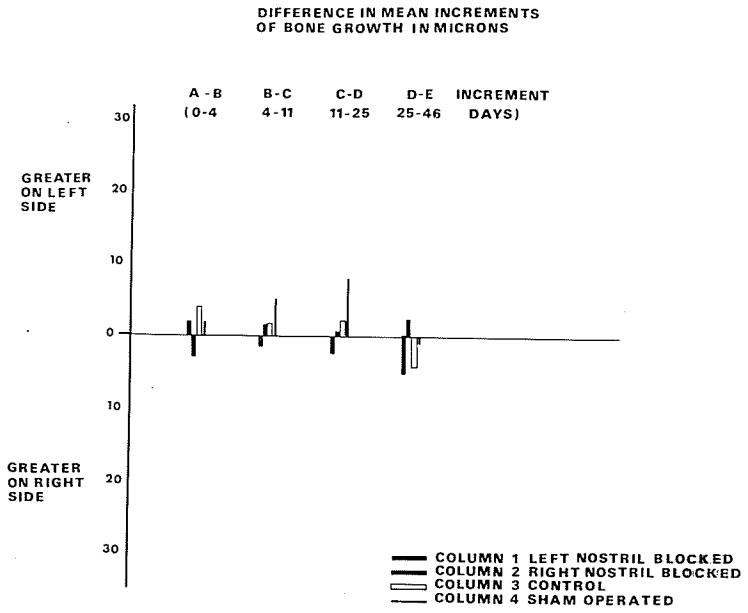


Fig. 70. The mean differences of each of the four increments for the four operational groups on day 46 for the incisor region, site 6.

TABLE XII

INCREMENTS OF BONE APPOSITION IN THE INCISOR REGION, BLOCK A, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				INCISOR SITE 6 EXTERNAL					
LEFT NOSTRIL BLOCKED	Left	21.6	2.6	26.1	2.1	29.3	4.4	46.0	3.6
	Right	19.6	2.2	33.9	7.0	31.3	3.8	49.0	4.4
	Diff	2.1	2.5	-1.4	2.1	-2.1	5.9	-3.1	3.6
RIGHT NOSTRIL BLOCKED	Left	20.2	2.6	34.6	8.5	31.6	4.2	48.4	4.6
	Right	22.8	3.6	25.0	3.0	31.3	5.0	46.1	3.8
	Diff	-2.7	3.1	1.8	3.2	0.6	6.8	2.3	4.0
CONTROL	Left	13.1	1.5	13.5	2.0	30.9	2.4	50.9	8.2
	Right	12.2	1.9	17.2	2.3	28.6	2.8	55.2	7.5
	Diff	0.4	1.7	-1.9	1.4	2.1	2.5	-4.3	7.5
LEFT SHAM	Left	27.0	0.0	22.0	0.0	26.0	0.0	67.0	10.4
	Right	7.0	0.0	17.0	0.0	18.0	0.0	68.5	12.4
	Diff	20.0	0.0	5.0	0.0	8.0	0.0	-1.5	2.0
F VALUE		6.00**		0.69		0.17		0.24	
				INCISOR SITE 7 EXTERNAL					
LEFT NOSTRIL BLOCKED	Left	15.6	0.9	21.1	1.7	32.9	2.6	65.2	7.3
	Right	16.8	1.6	17.1	1.3	37.4	2.1	58.8	5.6
	Diff	-1.1	1.7	4.0	2.0	-4.5	1.9	6.4	6.3
RIGHT NOSTRIL BLOCKED	Left	16.5	1.8	18.5	1.6	37.6	2.3	61.5	6.1
	Right	15.4	1.0	21.0	2.0	33.0	2.8	62.6	7.0
	Diff	1.1	1.8	-2.5	2.3	4.6	1.8	-1.1	5.1
CONTROL	Left	17.7	3.4	18.2	2.8	32.9	4.6	55.8	4.8
	Right	18.6	4.0	16.9	2.6	29.1	3.7	54.6	5.6
	Diff	-0.9	3.5	1.3	2.8	3.8	4.5	1.2	2.2
LEFT SHAM	Left	14.0	0.6	15.0	0.0	17.5	1.4	58.3	2.8
	Right	12.0	0.6	15.5	0.3	20.0	1.1	58.3	2.8
	Diff	2.0	0.0	-0.5	0.3	-2.5	0.3	0.0	0.0
F VALUE		0.31		0.80		2.13		0.23	

** Significant at the 1% level of confidence

TABLE XIII

INCREMENTS OF BONE APPOSITION IN THE INCISOR REGION, BLOCK A, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				INCISOR SITE 7 INTERNAL					
LEFT NOSTRIL BLOCKED	Left	29.0	2.8	42.1	4.0	64.9	4.6	54.9	3.4
	Right	29.9	2.3	41.3	4.1	58.9	4.6	58.7	3.0
	Diff	-1.0	2.6	0.8	3.7	6.0	4.5	-3.8	2.5
RIGHT NOSTRIL BLOCKED	Left	30.3	2.6	40.8	4.6	59.3	5.0	58.6	3.2
	Right	28.3	3.1	42.6	4.4	66.0	4.9	53.4	3.3
	Diff	2.0	3.0	-1.8	4.0	-6.7	4.9	5.1	2.5
CONTROL	Left	28.8	4.2	50.7	5.9	113.4	19.9	100.2	9.0
	Right	30.5	5.4	49.8	6.9	106.5	15.9	107.6	14.0
	Diff	-1.7	4.9	0.9	4.7	6.9	11.6	-7.4	9.2
LEFT SHAM	Left	28.7	3.7	47.3	12.1	43.3	3.8	91.7	5.9
	Right	30.0	2.3	49.3	10.1	55.7	6.9	71.7	12.2
	Diff	-1.3	3.1	-2.0	5.3	-12.3	4.8	20.0	9.7
F VALUE		0.19		0.10		1.73		5.63**	
				INCISOR SITE 8					
LEFT NOSTRIL BLOCKED	Left	22.6	2.6	25.6	3.1	59.3	3.5	88.1	4.6
	Right	18.5	0.8	23.8	2.0	64.8	3.6	89.0	3.4
	Diff	4.1	2.6	1.8	2.6	-5.5	3.9	-0.9	3.4
RIGHT NOSTRIL BLOCKED	Left	18.1	0.9	24.6	2.2	65.0	3.7	89.4	3.6
	Right	22.1	3.1	24.8	3.6	61.4	4.8	88.9	4.7
	Diff	-3.9	3.0	-0.2	3.6	3.6	4.4	0.5	3.6
CONTROL	Left	19.8	1.9	24.8	6.9	55.5	7.6	99.5	5.6
	Right	21.0	2.1	19.0	1.4	65.7	8.1	92.3	5.9
	Diff	-1.2	3.2	5.8	6.8	-10.2	10.5	7.2	6.4
LEFT SHAM	Left	25.0	6.3	35.7	4.4	63.7	13.3	134.7	6.8
	Right	25.0	5.0	51.7	9.6	57.7	10.3	115.0	4.8
	Diff	0.0	2.0	-16.0	5.3	6.0	8.3	19.7	5.0
F VALUE		0.91		0.58		1.32		3.58**	

** Significant at the 1% level of confidence

TABLE XIV

THE SIGNIFICANT DIFFERENCES DETECTED IN THE INCISOR REGION OF THE BILATERAL INCREMENT DIFFERENCES OF EACH INCREMENT BETWEEN THE FOUR OPERATIONAL GROUPS USING THE F TEST

	E-D	D-C	C-B	B-A	E-E†	A-B	B-C	C-D	D-E
Site 1	NS	NS	NS	NS	NS	NS	NS	NS	NS
Site 2	NS	NS	NS	NS	NS	NS	NS	NS	NS
Site 3	-	-	-	-	NS	NS	NS	NS	NS
Site 4	-	-	-	-	NS	-	NS	NS	NS
Site 5	-	-	-	-	NS	-	NS	NS	NS
Site 6	-	-	-	-	NS	**	NS	NS	NS
Site 7	NS	NS	NS	**	*	NS	NS	NS	NS
Site 8	-	-	-	-	-	NS	NS	NS	*

THE SIGNIFICANT DIFFERENCES DETECTED IN THE PREMAXILLARY REGION OF THE BILATERAL INCREMENT DIFFERENCES OF EACH INCREMENT BETWEEN THE FOUR OPERATIONAL GROUPS USING THE F TEST

	E-D	D-C	C-B	B-A	E-E†	A-B	B-C	C-D	D-E
Site 1	-	-	-	-	-	NS	NS	**	*
Site 2	-	-	-	-	NS	NS	NS	NS	NS
Site 3	-	-	-	-	NS	-	-	-	-
Site 4	-	-	-	-	NS	NS	NS	NS	NS
Site 5	-	-	-	-	-	NS	**	NS	**
Site 6	-	-	-	-	**	NS	NS	NS	NS
Site 7	-	-	-	-	NS	**	NS	**	NS
Site 8	-	-	-	-	NS	NS	NS	NS	NS

† Internal to external bone surface, i.e. bone thickness

NS Not significant

* Significant at the 5% level of confidence

** Significant at the 1% level of confidence

Site 8, the internasal suture of the incisor region, demonstrates the normal apposition in this region.

When an overall view of the linear analysis was taken, it was felt that there was no consistent side or increment bias. To test this null hypothesis the F test would confirm or deny that there was no difference between the group variances. The findings of the F tests from the analyses of variance were shown in Table XIV as not significant and show that the null hypothesis stands. Most values were not significant and the few increments which were significant did not show any consistent pattern.

On examination of the three significant increment values of the 48 measured, it was found that they occurred in different sites and were of different amounts. This number of significant values is almost that expected by chance alone. The large number of non-significant variances reflects the small bilateral differences and their relatively large standard errors of the increment measurements because of the biological variation. The variation of the bone thicknesses was only significant at the 5% level in site 7 which may reflect the compensation of the internasal area of the nasal bones.

The premaxillary region

The general plan of bone growth is illustrated in Fig. 71 of the premaxillary region. It reveals the classical features of the root of the incisors within the lateral part of the premaxilla, with the inter-premaxillary suture and the base of the vomer inferomedially and superiorly the nasal bones. The nasal bone increments are found laterally as well as medially in contrast with those findings in the incisor region.

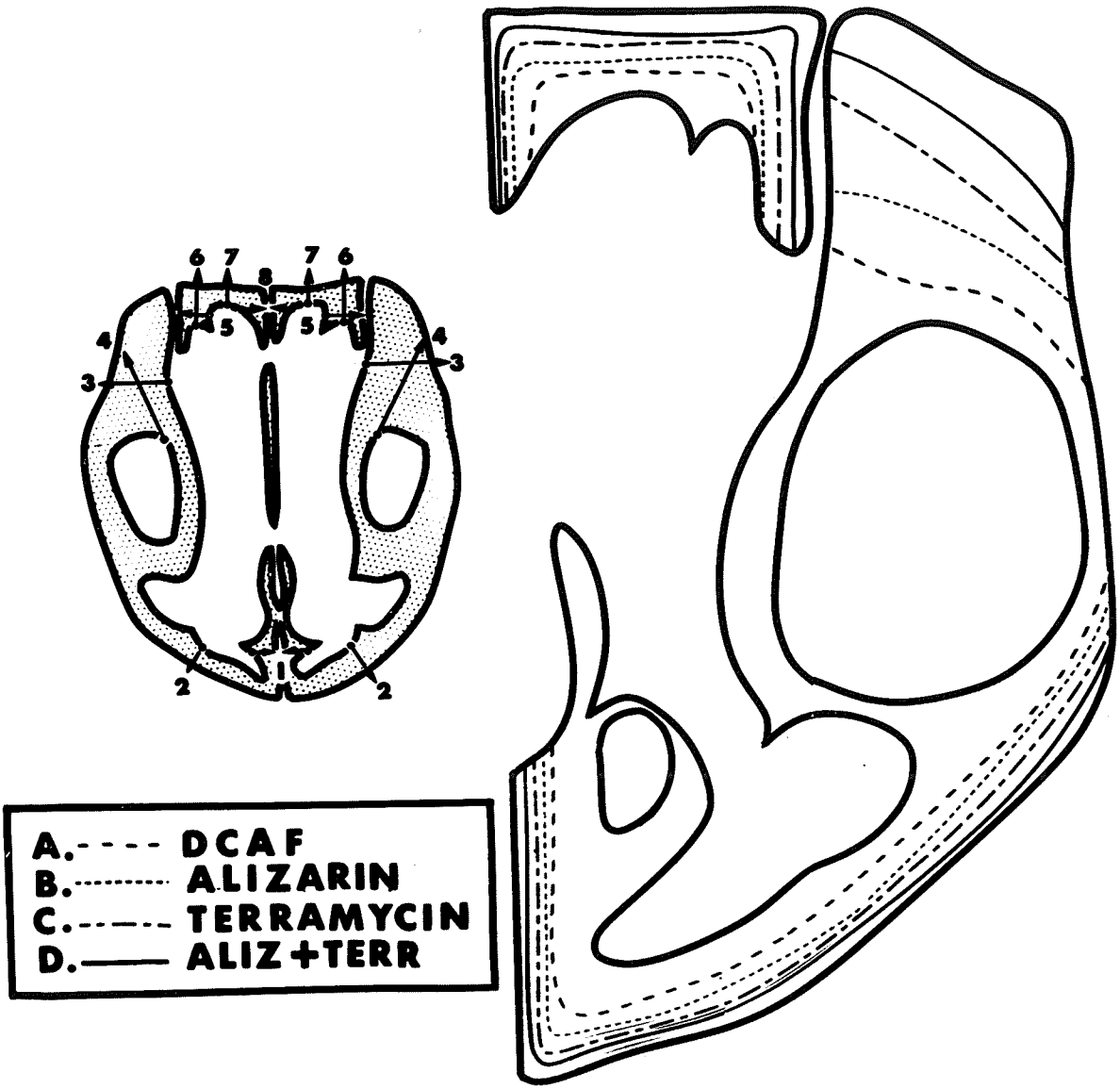


Fig. 71. The general plan of the bone mark increments seen in the premaxillary region.

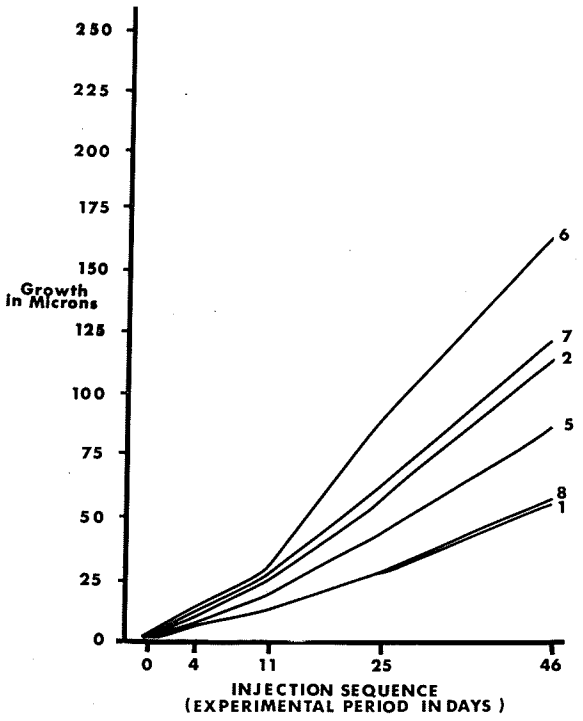


Fig. 72. Cumulative bone apposition in microns for sites 1, 2, 5, 6 and 7 of the premaxillary region.

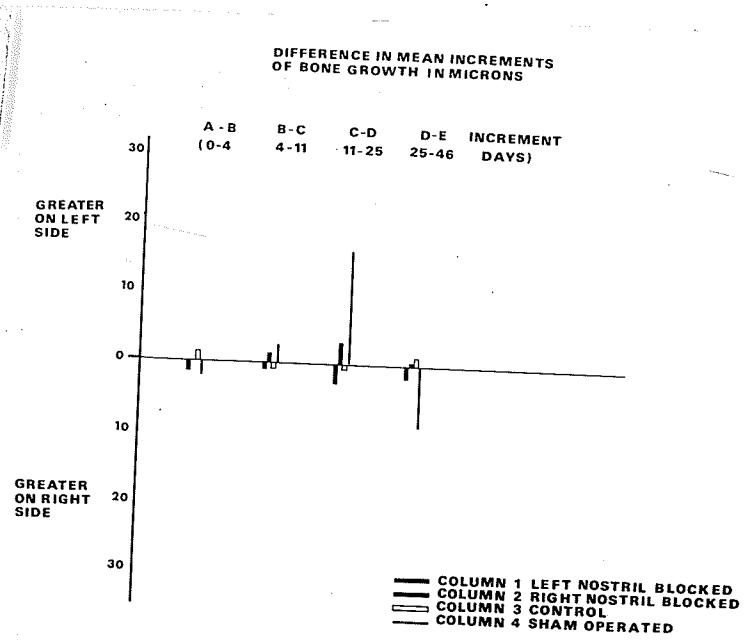


Fig. 73. The mean differences of each of the four increments for the four operational groups on day 46 in the premaxillary region, site 1.

TABLE XV

INCREMENTS OF BONE APPOSITION IN THE PREMAXILLARY REGION, BLOCK B, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				PREMAXILLARY SITE 1					
LEFT NOSTRIL BLOCKED	Left	12.2	0.7	11.4	0.7	32.5	2.1	61.6	3.5
	Right	13.4	1.1	12.3	1.2	35.3	2.4	60.0	3.5
	Diff	-1.2	1.0	-0.9	1.2	-2.8	1.4	1.6	1.4
RIGHT NOSTRIL BLOCKED	Left	12.4	1.2	12.2	1.3	35.0	3.0	56.7	3.3
	Right	12.4	0.9	10.8	0.7	31.9	2.9	57.1	3.2
	Diff	0.0	1.2	1.4	1.2	3.1	1.5	-0.4	1.7
CONTROL	Left	17.9	2.1	19.4	1.5	44.3	3.9	63.4	3.7
	Right	16.4	1.3	20.3	1.6	43.7	3.6	62.3	2.8
	Diff	1.5	1.8	-0.9	2.6	0.6	3.0	1.1	3.7
LEFT SHAM	Left	13.3	1.1	24.7	2.5	45.7	12.4	48.7	16.4
	Right	15.3	1.7	22.0	3.8	31.3	5.0	57.0	19.9
	Diff	-2.0	0.6	2.7	2.6	14.3	7.5	-8.3	3.8
F VALUE		1.01		0.92		7.81**		3.47*	
				PREMAXILLARY SITE 2					
LEFT NOSTRIL BLOCKED	Left	19.7	1.9	24.6	3.0	55.7	4.3	86.2	4.0
	Right	20.1	1.8	21.2	2.7	56.8	3.7	78.1	6.0
	Diff	-0.4	1.9	3.4	2.4	-1.1	4.2	8.2	5.0
RIGHT NOSTRIL BLOCKED	Left	19.2	1.4	22.6	3.0	57.3	4.0	73.6	4.6
	Right	19.1	1.6	24.8	3.7	53.7	4.5	85.9	4.5
	Diff	0.1	1.8	-2.2	2.8	3.6	3.4	-12.3	4.4
CONTROL	Left	24.6	3.7	28.4	3.8	60.9	6.7	90.0	4.8
	Right	23.1	3.8	32.6	4.3	60.1	6.5	89.8	7.3
	Diff	1.5	4.6	-4.2	3.1	0.8	3.2	0.3	7.6
LEFT SHAM	Left	22.3	3.2	35.0	3.8	56.7	2.2	101.0	5.1
	Right	15.0	0.0	33.3	5.6	54.7	1.7	95.3	7.5
	Diff	7.3	3.2	1.7	5.8	2.0	3.8	5.7	3.4
F VALUE		1.45		0.94		0.16		2.08	

* Significant at the 5% level of confidence

** Significant at the 1% level of confidence

Fig. 72 shows the cumulative bone apposition in microns for sites 1, 2, 5, 6, 7 and 8 of the premaxillary region. All of these sites had similar patterns of growth although the amount of growth varied in the direction of measurement. The medial sutural areas, sites 1 and 8, were very similar in rates of apposition. The lateral border of the nasal bones, site 5, was a site of apposition with the apposition on the external surface of the nasal bone, sites 6 and 7, with concomitant inner surface resorption.

On closer examination of site 1 in the premaxillary region the significant differences were found in the CD and DE increments. These are seen in Fig. 73 and it was clear that the sham operated animals were markedly greater in the CD increment on the left side of the snout and conversely greater in the DE increment on the right side, compared with the other operational groups. The sham group had the smallest sample size and these values reflect this.

Table XIV shows the significant differences between the bilateral differences of the four operational groups in the premaxillary region. This again shows an even distribution of the significant values between the increments and the sites.

Fig. 75 illustrates the mean differences of the most rapid growth increments in site 4 of the premaxillary region. The nasopremaxillary suture is therefore shown in Fig. 71 as a sliding suture with non intra-sutural apposition on its lateral surface, the superomedial area of the premaxillary bone. The medial and lateral surface of the lateral wall of the premaxilla was not demonstrated as a definite site of appositional bone growth.

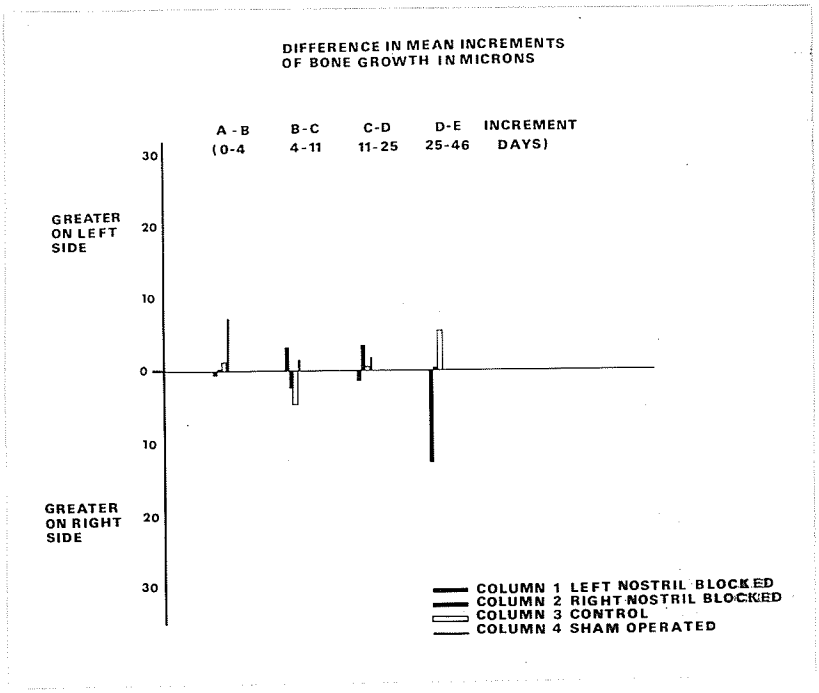


Fig. 74. The mean differences of each of the four increments for the four operational groups on day 46 for the premaxillary region, site 2.

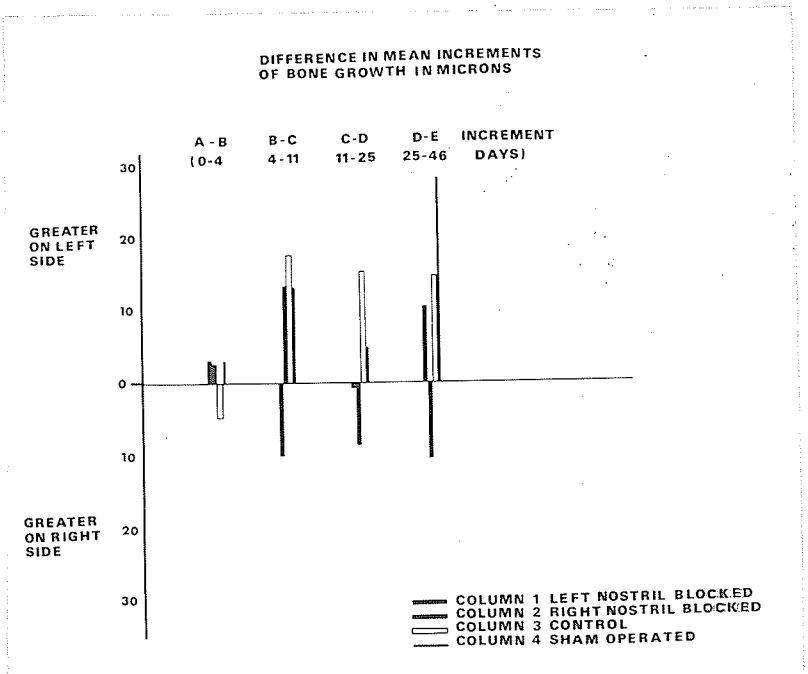


Fig. 75. The mean differences of each of the four increments for the four operational groups on day 46 for the premaxillary region, site 4.

TABLE XVI

INCREMENTS OF BONE APPOSITION IN THE PREMAXILLARY REGION, BLOCK B, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				PREMAXILLARY SITE 4					
LEFT NOSTRIL BLOCKED	Left	173.8	8.4	145.0	8.5	401.2	17.1	345.9	18.6
	Right	170.6	10.7	155.0	7.0	406.2	16.8	335.4	17.1
	Diff	3.2	8.3	-10.0	6.3	-5.0	11.3	10.5	9.3
RIGHT NOSTRIL BLOCKED	Left	170.3	10.8	154.2	7.4	400.0	20.8	334.7	22.4
	Right	167.4	9.2	140.3	9.6	408.2	18.6	345.0	24.2
	Diff	2.9	9.5	13.9	7.1	-8.2	11.9	-10.3	8.9
CONTROL	Left	165.1	21.0	146.2	16.3	430.2	25.1	346.8	15.8
	Right	169.6	20.4	128.6	11.6	415.1	29.8	361.7	16.4
	Diff	-4.5	11.0	17.6	17.4	15.1	21.9	-14.9	12.4
LEFT SHAM	Left	150.0	22.4	186.7	5.9	376.7	15.2	401.7	11.6
	Right	146.7	25.9	173.3	10.5	371.7	27.9	373.3	13.2
	Diff	3.3	5.9	13.3	8.2	5.0	15.6	28.3	13.2
F VALUE		0.11		1.41		0.40		2.57	
				PREMAXILLARY SITE 6					
LEFT NOSTRIL BLOCKED	Left	25.7	5.2	33.6	4.5	122.6	15.0	150.6	11.3
	Right	21.8	4.6	32.3	2.2	147.7	10.1	131.4	12.0
	Diff	-11.5	10.5	8.0	3.1	-16.5	14.0	25.1	11.3
RIGHT NOSTRIL BLOCKED	Left	15.5	1.5	37.6	3.0	152.3	14.5	132.5	14.1
	Right	27.3	7.3	31.7	6.5	137.7	21.1	141.0	13.0
	Diff	0.0	0.0	2.4	7.6	-5.4	13.6	-12.8	11.9
CONTROL	Left	60.3	12.5	29.0	6.5	92.0	16.4	160.0	13.4
	Right	44.5	15.6	31.0	5.8	99.7	16.7	152.9	12.1
	Diff	4.0	24.0	-5.7	12.9	-3.2	16.3	7.1	9.6
LEFT SHAM	Left	0.0	0.0	0.0	0.0	59.0	0.1	192.3	24.2
	Right	0.0	0.0	0.0	0.0	0.0	0.0	164.5	14.7
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	11.0	19.6
F VALUE		0.09		1.00		0.20		1.12	

Table XVI illustrates the increments of bone growth in microns for the premaxillary sites 4 and 6. The measurements for the other sites are found in the appendix. These continue to demonstrate the varied bilateral differences often with the consecutive larger increments varying from side to side in a random manner, suggesting that a wide natural variation occurs bilaterally.

The malar region

The general plan of the bone mark increments seen in the malar region are illustrated in Fig. 76. Superiorly, the nasal bone can be seen with apposition more demonstrable laterally than mesially with resorption inferiorly. The premaxillary extension was seen as being irregular bone growth between the nasal bone mesially and the malar process of the maxilla laterally. This malar process was the most rapid area of apposition in a superolateral direction. Inferiorly, the posterior part of the premaxilla can be seen mesially and laterally to the incisive foramen.

Fig. 77 shows the bone apposition in microns for site 1 and the relative cumulative curve is more irregular than the typical smooth curve. This is either reflecting a differential growth or a consistent increase in the CD increment and a decrease in the DE measurement. Fig. 78 demonstrates the mean differences of the four increments which are enumerated in Table XVII, the greater values being evenly distributed bilaterally.

The external apposition and internal resorption of the snout area was demonstrated in site 3 in the premaxillary bones and Table XVII gives the values of the apposition in microns for the four groups.

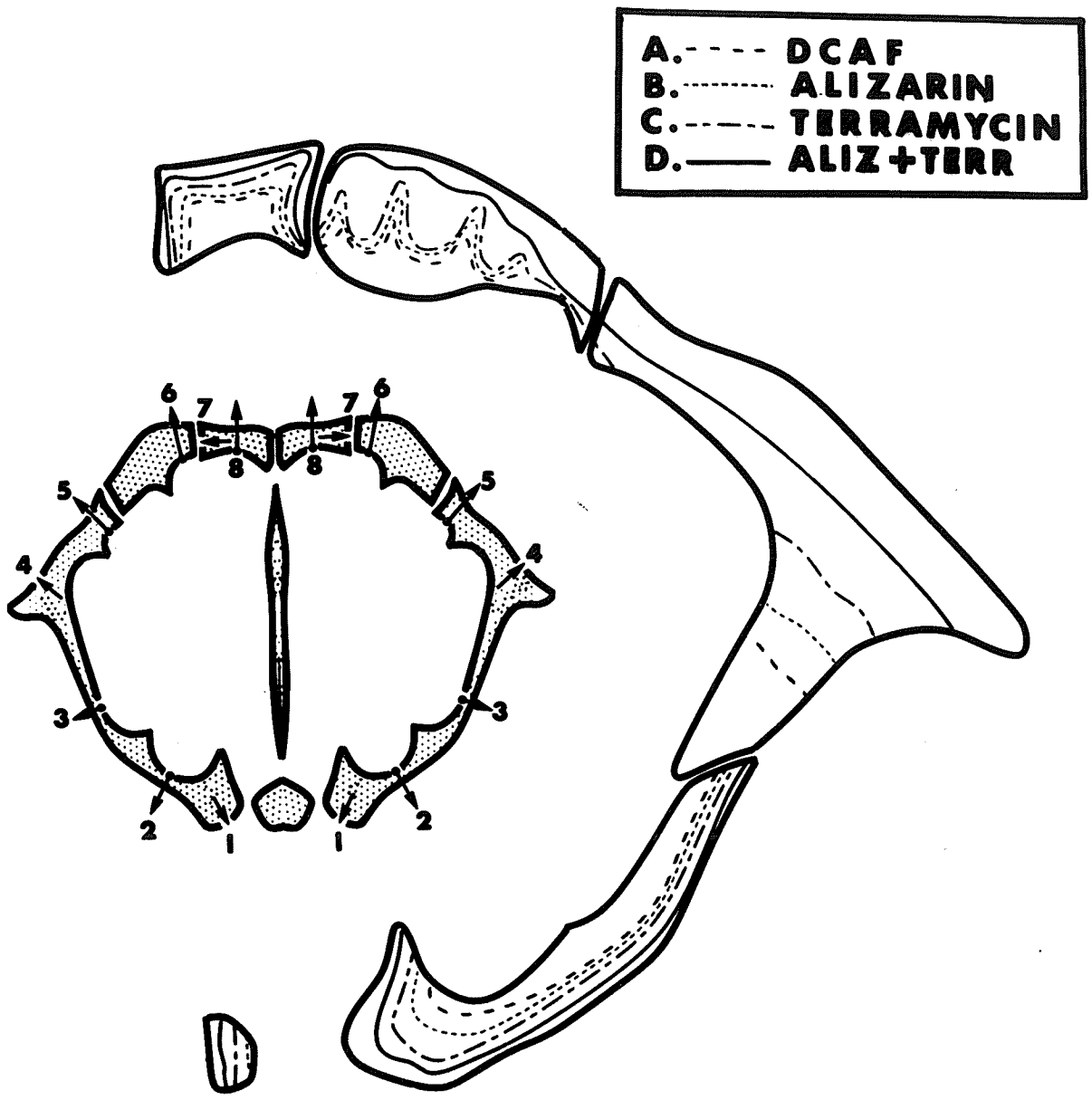


Fig. 76. The general plan of the bone mark increments seen in the malar region.

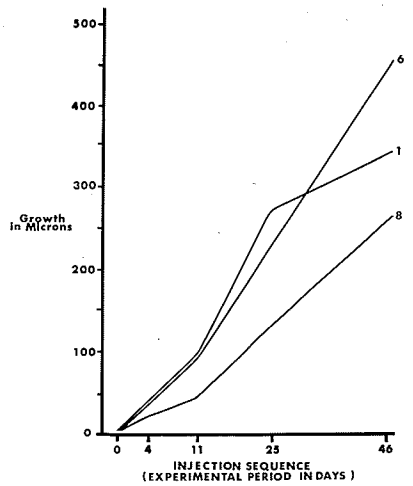


Fig. 77. Cumulative bone apposition in microns for sites 1, 6 and 8 of the malar region.

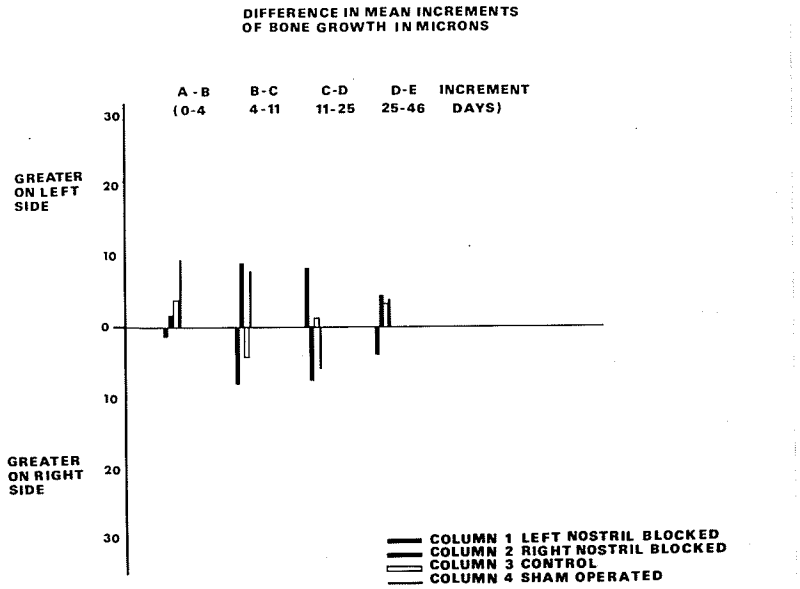


Fig. 78. The mean differences of each of the four increments for the four operational groups on day 46 for the malar region, site 1.

TABLE XVII

INCREMENTS OF BONE APPOSITION IN THE MALAR REGION, BLOCK C, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				MALAR SITE 1					
LEFT NOSTRIL BLOCKED	Left	44.7	4.8	55.9	3.8	174.3	5.2	171.8	9.5
	Right	46.0	6.3	63.8	6.0	165.9	6.1	175.6	9.6
	Diff	-1.3	7.5	-7.9	5.6	8.4	6.5	-3.7	6.1
RIGHT NOSTRIL BLOCKED	Left	47.4	6.5	65.3	6.2	168.8	6.5	179.2	10.1
	Right	47.3	4.8	56.3	4.1	175.9	5.4	174.7	9.9
	Diff	0.2	8.0	9.1	5.9	-7.1	7.0	4.5	6.5
CONTROL	Left	46.7	5.3	80.8	8.7	160.0	7.2	188.8	9.4
	Right	46.3	4.8	84.8	8.1	158.7	7.6	185.5	10.5
	Diff	0.4	3.5	-4.0	4.7	1.3	5.1	3.3	4.5
LEFT SHAM	Left	41.7	5.3	78.3	4.6	136.0	5.5	192.3	5.7
	Right	32.0	1.6	70.3	3.1	141.7	15.5	188.3	10.1
	Diff	9.7	3.7	8.0	7.5	-5.7	10.9	4.0	4.4
F VALUE		0.33		1.52		0.75		0.27	
				MALAR SITE 3					
LEFT NOSTRIL BLOCKED	Left	37.5	5.3	51.6	6.2	46.6	4.7	78.3	5.1
	Right	38.6	4.4	44.2	6.3	56.8	7.2	72.6	4.8
	Diff	-1.1	4.4	7.4	5.2	-10.2	9.3	5.7	7.0
RIGHT NOSTRIL BLOCKED	Left	37.9	4.6	47.2	6.7	49.4	5.4	72.7	5.1
	Right	36.2	5.5	53.0	6.5	50.1	3.9	73.8	4.7
	Diff	1.7	4.6	-5.8	5.5	-0.7	5.0	-1.1	6.6
CONTROL	Left	29.8	3.2	32.7	3.6	68.3	6.7	75.8	6.5
	Right	24.4	4.0	29.8	3.3	65.2	7.2	70.9	5.4
	Diff	5.3	5.8	2.9	3.9	3.1	3.8	4.9	5.7
LEFT SHAM	Left	27.7	5.7	26.7	4.2	41.3	10.2	106.0	9.6
	Right	19.0	5.7	25.0	5.0	34.3	8.6	95.0	29.4
	Diff	8.7	9.0	1.7	1.7	7.0	1.7	11.0	24.7
F VALUE		0.51		0.79		0.73		0.25	

TABLE XVIII

THE SIGNIFICANT DIFFERENCES DETECTED IN THE MALAR REGION OF THE BILATERAL INCREMENT DIFFERENCES OF EACH INCREMENT BETWEEN THE FOUR OPERATIONAL GROUPS USING THE F TEST

	E-D	D-C	C-B	B-A	E-E†	A-B	B-C	C-D	D-E
Site 1	-	-	-	-	-	NS	NS	NS	NS
Site 2	-	-	-	-	NS	*	NS	NS	NS
Site 3	-	-	-	-	NS	NS	NS	NS	NS
Site 4	-	-	-	-	-	**	**	NS	NS
Site 5	-	-	-	-	NS	-	-	NS	*
Site 6	-	-	-	-	NS	NS	NS	**	NS
Site 7	-	-	-	-	-	NS	*	NS	NS
Site 8	-	-	-	-	*	NS	NS	NS	NS

THE SIGNIFICANT DIFFERENCES DETECTED IN THE FIRST MOLAR REGION OF THE BILATERAL INCREMENT DIFFERENCES OF EACH INCREMENT BETWEEN THE FOUR OPERATIONAL GROUPS USING THE F TEST

	E-D	D-C	C-B	B-A	E-E†	A-B	B-C	C-D	D-E
Site 1	-	-	-	-	NS	-	NS	*	NS
Site 2	-	-	-	-	-	NS	NS	**	NS
Site 3	-	-	-	-	-	NS	NS	NS	NS
Site 4	-	-	-	-	-	NS	NS	NS	NS
Site 5	-	-	-	-	-	NS	NS	NS	NS
Site 6	NS	**	NS	NS	NS	**	NS	NS	NS
Site 7	NS	NS	NS	NS	NS	NS	NS	NS	*
Site 8	-	-	-	-	-	NS	NS	NS	**

† Internal to external bone surface, i.e. bone thickness

NS Not significant

* Significant at the 5% level of confidence

** Significant at the 1% level of confidence

Sites 5, 6 and 8 again show this pattern of growth in the superior process of the maxillary bone, the premaxillary bone and the nasal bones, respectively.

The malar process of the maxilla, site 4, was the most rapidly growing site in this region and site 5 demonstrates the rapid rate of internal resorption in this process. The values of all the sites not demonstrated are found in the appendix. On examining the significant F test differences of the malar region in Table XVIII between the four operational groups, the even distribution of the significant values between the increments and sites negates any consistent finding. This supports the null hypothesis of the effects of nasal blockage on bone growth.

First molar region

The overall general plan of the bone mark increments seen in the first molar region is seen in Fig. 79. The palate is seen in sites 1 and 2 as being a site of oral apposition with nasal cavity resorption on the superior surface. The alveolar apposition can be seen measured in site 3 in the maxilla. The lateral wall of the first molar region (site 4) was a site of lateral apposition and internal resorption. The superior surface of the frontal bone was a site of external apposition and the inferior ethmoid sinus surface was also a site of apposition.

Fig. 80 shows the cumulative bone apposition in sites 1, 4, 5, 6 external and 7 external. All these sites reveal a similar growth pattern with site 5 demonstrating the accentuation of the nuchal crest

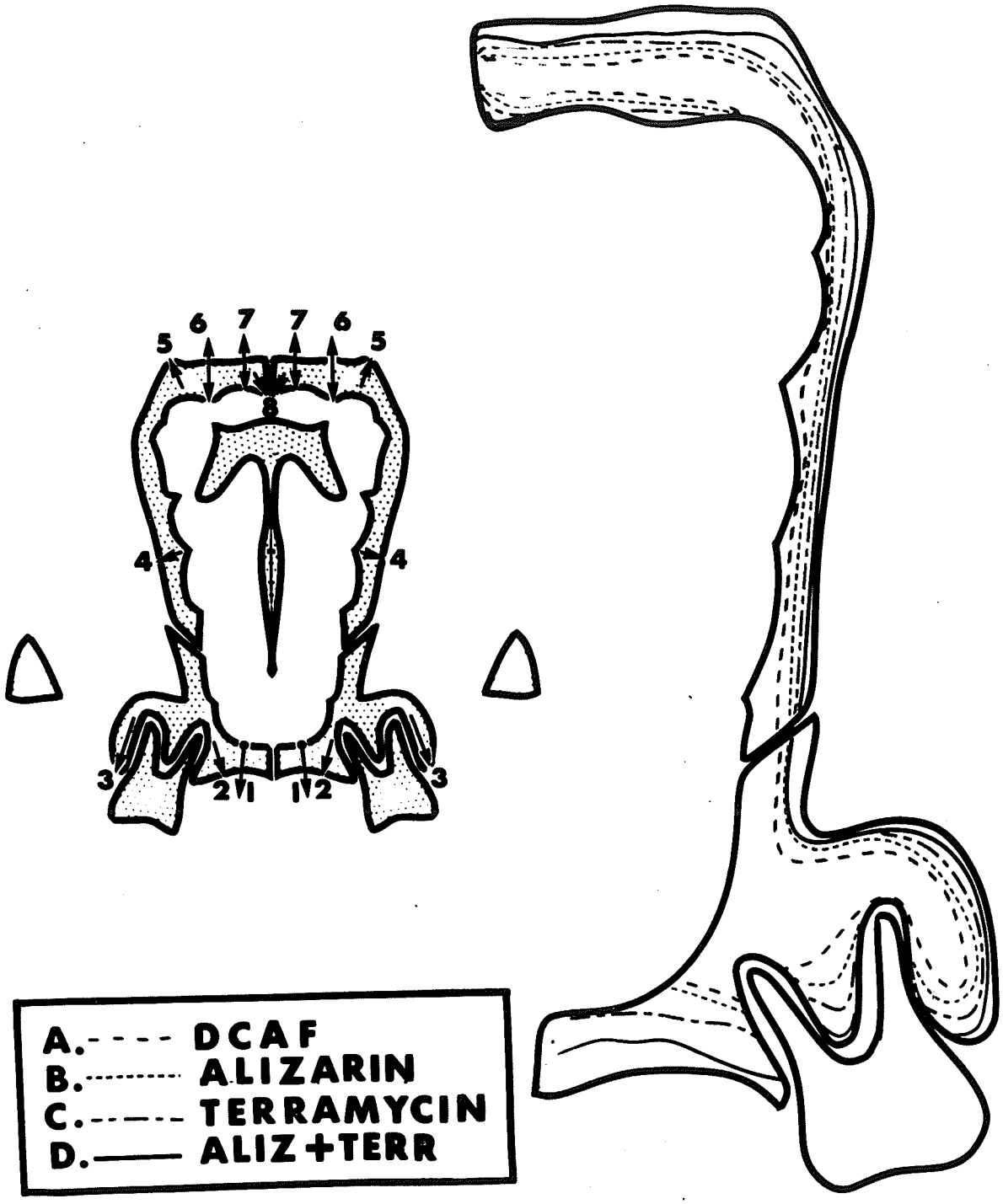


Fig. 79. The general plan of the bone mark increments seen in the first molar region.

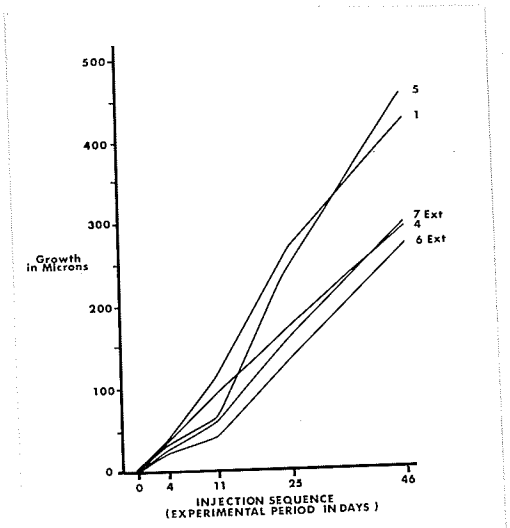


Fig. 80. Cumulative bone apposition in microns for sites 1, 4, 5, 6 external and 7 external of the first molar region.

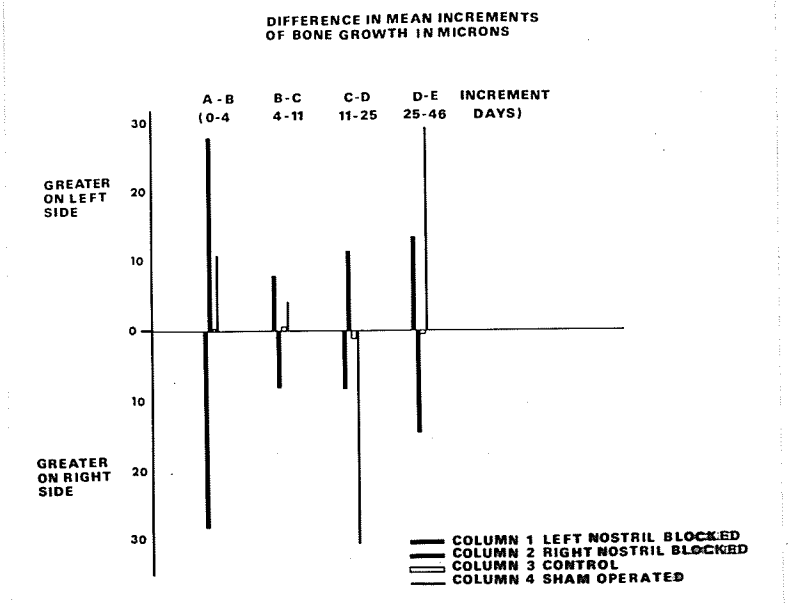


Fig. 81. The mean differences of each of the four increments for the four operational groups on day 46 for the first molar region, site 1.

TABLE XIX

INCREMENTS OF BONE APPOSITION IN THE FIRST MOLAR REGION, BLOCK D, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				FIRST MOLAR SITE 1					
LEFT NOSTRIL BLOCKED	Left	38.2	15.9	77.9	10.8	163.8	15.8	153.2	11.8
	Right	52.7	23.2	60.4	15.7	174.1	13.7	141.4	8.8
	Diff	-28.0	12.0	8.0	20.3	-8.1	10.2	13.2	10.8
RIGHT NOSTRIL BLOCKED	Left	52.7	23.2	60.4	15.7	177.5	14.1	142.4	8.8
	Right	38.2	15.9	83.0	10.7	164.2	17.0	155.2	11.5
	Diff	28.0	12.0	-8.0	20.3	11.6	10.3	-14.4	11.9
CONTROL	Left	22.4	1.9	35.1	2.4	133.9	16.8	113.7	12.8
	Right	22.0	1.9	34.4	2.3	134.0	16.6	113.9	11.8
	Diff	0.4	2.7	0.7	3.5	-0.1	15.3	-0.2	18.2
LEFT SHAM	Left	50.0	0.0	53.0	4.1	100.7	31.4	130.0	31.7
	Right	47.5	4.9	48.7	3.6	140.7	33.0	83.3	24.8
	Diff	11.0	0.0	4.3	4.0	-40.0	10.7	46.7	32.1
F VALUE		3.84**		0.38		3.07*		2.47	
				FIRST MOLAR SITE 2					
LEFT NOSTRIL BLOCKED	Left	54.4	4.3	60.0	4.7	206.9	10.6	205.9	8.6
	Right	55.1	5.0	61.1	3.0	211.4	11.4	207.8	11.4
	Diff	-0.5	3.9	-1.1	4.3	-4.5	6.3	-1.9	8.4
RIGHT NOSTRIL BLOCKED	Left	55.9	5.4	62.8	3.2	217.4	10.8	202.3	9.3
	Right	54.0	4.7	60.3	4.8	210.5	11.9	209.1	8.5
	Diff	1.9	4.4	2.5	4.7	6.8	6.2	-6.8	4.3
CONTROL	Left	38.6	4.1	80.4	6.1	208.6	9.7	169.0	15.1
	Right	38.6	4.0	83.2	8.1	203.8	9.5	171.1	14.8
	Diff	0.0	3.4	-2.8	10.5	4.8	12.4	-2.1	4.3
LEFT SHAM	Left	48.3	9.4	103.3	10.4	151.0	3.1	147.3	17.0
	Right	49.3	3.7	72.3	7.5	178.3	17.0	175.0	9.1
	Diff	01.0	12.4	31.0	8.9	-27.3	14.8	-27.7	8.8
F VALUE		0.05		5.14**		2.74		2.12	

* Significant at the 5% level of confidence

** Significant at the 1% level of confidence

growth later in the experimental period. Fig. 81 and Table XIX reveal the differences in mean increments of the bone growth in microns for site 1. The values for the growth at the other sites are given in the appendix.

Of the over 160 sets of increments measured in the four groups of animals, the left and right nostril blockage groups, the control and sham operated group showed few significant differences of the variances - 21 in all. These significant differences were found three in the incisor region, six in the premaxillary region, six in the malar region and six in the first molar region. Thus, the differences were fairly well distributed between the regions, although with possibly less than expected in the incisor region which is close to the site of surgery.

The distribution of the significant differences were six in the AB increment, two in the BC, seven in the CD and six in the DE. On closer examination of the statistical data it could be seen how these significant values demonstrated the lack of any definite trends. The F test results of this linear analysis did support the lack of any significant difference between the groups, not requiring the use of a t test evaluation.

Even so, the Duncan test was carried out to yield a t test evaluation. Eighty-three increments were found to have a t test value showing significance at the 5% level or more between at least two groups (55 would have been expected by chance alone). The AB increment was significant in 19 cases, the BC in 23 cases, the CD increment had 19 significant values and 22 in the DE increment, allowing the same

distribution as found from the previous data. The distribution of the t test significant values between the regions were 18 in the incisor, 19 in the premaxillary, 22 in the malar and 26 in the first molar. The overall statistical evaluation of the linear analysis failed to demonstrate any definite effect on bone growth in the operational groups, whether the nostrils were blocked, open or surgically disturbed.

DISCUSSION

Surgical procedures

Several techniques of nasal blockage were evaluated, using zinc oxide and rubber base impression pastes as well as using various forms of metal inserts. These techniques were ineffective or traumatic or both. Gross (1963) used a technique of electrocautery and found it to be effective, although he failed to achieve complete nasal blockage at the first attempt in fifteen rats. In this present study blockage was incomplete in eleven animals at the first attempt, with both this study and that of Gross having a similar number of animals. However, based on the findings of a pilot project, this author felt that electrocautery was less successful than suturing the nostrils following laceration of the mucous membranes and that electrocautery could cause serious tissue damage to the nasal septum and adjacent structures.

Ziem (1879), cited by Brash et al. (1956), packed the nostrils of young sheep, calves and goats with antiseptic wool and felt that this incomplete obstruction more resembled the condition in human. It could well be that the physical and chemical irritation of the tissues accounted largely for the widespread changes in the soft and hard tissues during the growth of the crania of these animals. Ziem also scraped the lining of one nostril and united the surfaces in rabbits and dogs but claimed the sutures worked loose due to the powerful alae nasi and facial muscles. The surgical technique finally adopted in this study was that just described by Ziem as being unsuccessful, but used successfully by Anderson (1909). In rats in the present study it was observed how the animals persistently tried to removed the sutures with their paws and by sneezing attempted to clear the airway.

The effect of surgery per se may have contributed to a small amount of local growth inhibition as the snouts of the operated animals were smaller than those of the non-operated or control animals. The hypothesis that local surgery inhibited normal growth was put forward by Kremenak et al. (1970) following five years of research on palatal surgery in beagle pups. Searls and Biggs (1974), with a similar study of the disturbance of growth following surgery of the palate of young rats, supported the findings of Kremenak. While the snouts of the operated rats were overall smaller in size, the finding of this investigation could not demonstrate a local surgical site reduction in growth, as already postulated. The findings suggested that the surgical procedures were followed by rapid compensatory tissue repair.

The rat as an experimental model

The rat was found to be a good model in many respects with its rapid growth in the post weaning period proving eminently suitable for serial bone marking investigations (Cleall et al. 1968, 1971, Jacobson, 1970 and Ryll, 1972). The relatively simple requirements in terms of space, nutrition and husbandry made the experiment more economical than if other animals had been used. The rapid reproductive characteristics and normative data on the Long Evans rat were useful in the expedition of this study. The intact rat cranium was eminently suitable for hard tissue sectioning. The long snouted animal should have shown the greatest changes following unilateral nasal obstruction, especially as this was a period of rapid nasomaxillary growth. The greatest limitation of the rat was its low resistance to infection following nasal blockage,

and its endemic lung disease, described by Stratman and Conejeros (1969) and Lane-Petter (1970).

Several of the major anatomical differences of the rat and man were described in the review citing Greene (1959), Burdi (1966) and Enlow (1968). Enlow (1963) pointed out that the rat did not have haversian or osteone systems, a finding supported in this investigation. Brash et al. (1956) felt that great care must be taken in transferring findings from experimental work in one animal species to another species, as there could never be certainty as to the cause of any changes following a form and function experiment and no direct causal relationship could be justified.

The dog and monkey may be suitable models for nasal blockage, being phylogenetically more closely related to man. The dog, either long or short snouted, should be more suitable for surgery and less prone to lung infections. While the dosage levels of bone marking agents for these animal models have been evaluated there are a number of practical problems, the major ones being associated with husbandry, the lack of normal data and the size of the crania which would require multiple embedding before bilateral coronal sections could be made.

Weight gains

All the animal groups in this investigation gained weight rapidly over the experimental period. The starting weight of 90 ± 3 grams was tripled over the 46-day experimental period, a rate similar to that found by Cleall et al. (1964) using the same strain of rats. Most investigators using other strains of laboratory rats have found a slightly less rapid gain in weight (Schumacher, 1970, Hughes and Tanner, 1970).

Over the 46-day experimental period the differences in weight gain between the non-operated control and the operated groups were still present and suggest a permanent difference due to nasal surgery and not a temporary retardation made up by a catch-up phenomenon following the more severe operation of masseterectomy (Ryll, 1972).

The weight gains of animals with unilateral nasal blockage were significantly reduced in this investigation and concur with the findings of Gross (1963). It was also found that the sham operated animals also gained weight less rapidly than the non-operated control animals, but to a lesser degree than the unilateral blockage animals.

The comparison of the individual animal weight gain increments was a more valuable test statistically than the comparison of mean weights as measured by Gross, although both showed the large effect of systemic stress following nasal blockage. The explanation of this stress was not evaluated but could possibly be due to the embarrassment of the air passages, a relative hypoxia or due to infections. The oxygen and carbon dioxide partial pressures of the blood were not investigated, nor a histological examination made of the lower air passages or lungs. The pathogenic microbiology findings in the operated rats and the relatively negative soft tissue changes do not suggest the physical embarrassment of the upper respiratory passages. The microflora changes may be linked as an effect or cause of the systemic changes.

During acute respiratory distress the rat seemed almost unable to mouth breathe. Emslie et al. (1952) described these difficulties as efforts to exhale and claimed that the gradual inflation of the alimentary canal caused death within a week. In this present investigation small gaseous inflations were noticed in the alimentary canals in both the operated and control animals, suggesting that this may be a normal

phenomenon and unlikely to be the cause of death in Emslie's experiment in nasal blockage of the rat.

Small losses of weight or reduced weight gains were found following intraperitoneal injections although these were temporary in nature and caught up rapidly and support the relative lack of toxicity of these agents as claimed by Cleall et al. (1964) and Suzuki (1966). No significant differences were found between the weight gains in the animals given intraperitoneal bone marking injections and the soft tissue sample animals which did not receive these injections. There was a difference in the results tending to suggest a possible weight gain inhibition or toxicity of the bone marking agents as claimed by Robinson and Sarnat (1955), Hong et al. (1968) and Yen and Shaw (1972). This difference in weight may be accounted for by the stress of the anaesthetics and injections alone, rather than the composition and dosage of the bone marking agents. As all the hard tissue groups had the same dosage of the bone marking agents, the comparison of the groups is still valid even if there were some slight inhibition of bone growth.

Maturity indicators

The animals in this experiment were selected using weight as a maturity indicator. It has been demonstrated how, over the experimental period measured chronologically in days, there was a significant difference in weight gains with reduced weight gains in the operated animals. It may be useful to consider the use of a maturity indicator throughout any surgical technique. In the sequential bone marking technique injections could possibly be given at 90, 120, 170, 240 gm and all animals sacrificed at, say, 330 gm. This would allow the comparisons of morphology of equally mature animals, although the technical problems of the animal

experiment would be more difficult.

As was pointed out in the review, maturity indicators are all positively correlated and the correlation between indicators is often low. The assumption that body weight and skeletal age are completely correlated, while if not true, is more biologically useful than using a chronological period as used in this and most investigations.

Similarly, other maturity indicators could be used, such as the skeletal indicators. Hughes and Tanner (1970) evaluated skeletal norms for the Long Evans rat in the development of the limbs, skull, pelvis and nose rump lengths. De Groot (1963) and Mosier (1969) used the tail lengths. The rats in this experiment have been shown as smaller skeletally after surgery, using linear measurements, and retarded in viscerocranial elevation. These size changes are not as important as shape changes and essentially no shape changes due to nasal blockage have been shown, although shape changes were found following surgery. Gross (1963), using only linear measurements, therefore showed only size differences following nasal blockage and not shape changes.

Bacteriology and general health

While several authors, Brady (1902), Tilly (1908), Hoggan (1914) and Johnson (1936), have suggested that nasal obstruction leads to a decreased resistance to infection in man, this fact has also been shown to be true in experimental animals. Ziem (1899), Anderson (1909), Emslie(1952) and Gross (1963) all described the high mortality of their animals, a view supported by this investigation. The use of separate cages, antibiotics in the drinking water and careful husbandry all aided this study since, in the pilot project housing three animals per cage and without antibiotics, the mortality rate was 50%.

Stratman and Conejeros (1969) showed how the Long Evans rat strain was more resistant to lung infections than other strains of rats. Humphrey and White (1970) demonstrated that once pulmonary exudation occurred there was a high risk of pulmonary infection. Inbred rat colonies are renowned for endemic lung diseases, discussed by Lane-Petter (1970), and for this reason are seldom used in respiratory physiological experiments. The identification of definite pathogens only in the operated animals, which also often had large numbers of non-pathogenic bacteria, were signs of debilitation.

While Anderson (1909) did not study growth per se following nasal blockage, his findings were not at variance with those of this study. He noted a change of condition, loss of hair and skin changes in his operated dogs, a point which Gross (1963) could not support in his study. In this investigation it was found that the hair of seriously ill animals did stand on end, giving an obvious change in their appearance. Anderson found lung emphysema and local emphysema while the only verification of this in this investigation was the spotted appearance on the radiographs of the rat thorax, resembling the appearance of bronchopneumonia.

Anderson found that nasal obstruction led to a dilation of the heart and demonstrated emphysema histologically. He found that there was a disappearance of these symptoms promptly following reopening of the occluded nostril.

Assessment of measurement techniques of the pH and temperature

The accuracy of any technique is limited by its weakest part. The assessment of the animal weights was to the nearest half gram although with live animals in the enclosed pan this error was exceeded. The pH meter was finely calibrated to assess the pH of a small area at the end of

the antimony electrode. Unfortunately, it was found to be practically difficult to know what area was being measured. Similarly, the thermister bead and lead were relatively bulky, being approximately 1 mm in diameter. While the resistance could be measured to a temperature reading of 0.01°C, the proximity of the thermister to the blood vessels or the airflow or contamination with mucus made an accurate assessment impractical in the small nostril of the rat.

The physiology of the air passages has not been studied specifically in the rat, chiefly because of the size problem. Bouhuys (1970) felt that only now were human airway techniques being refined for accurate physiological and biochemical variables to be assessed. He discussed the biomechanical physical dynamics of the human airway and pointed out biological rhythms. Negus (1965) stated that the mucous membrane surface was approximately pH 7.0 and that the ciliary action ceased at 6.4 and 8.5 in man. These values closely approximated the values obtained of the nasal mucosa in this experiment, and the cilia also appeared normal in the histological data.

If ulceration of the epithelium had occurred or if local inflammatory processes had been present, the local pH could have had an effect on the bone forming in the area. As neither overt inflammatory differences were found bilaterally nor ulceration of the epithelium in the nasal cavities, no bony changes could be explained by measuring the pH of the superficial mucosal lining.

Spagnoli et al. (1964) and Bundy et al. (1965) supported the contention that local temperature changes encouraged bone apposition following thermocouple implants in rabbits. Kremenak and his co-workers (1969, 1970) used six beagle pups with wire heated muzzles but found only a small amount of asymmetry. The blocked nasal passage was expected to have an elevated

temperature because of the refrigeration effect of the airflow. The temperature of inhaled and exhaled air is constantly changing and an estimated difference therefore between the open nasal passage and the blocked nasal passage would be small and variable. The subjective evaluation of the nasal cavity temperature difference was felt to be small and to have little bearing on the results of bone apposition in this study.

Soft tissues

The soft tissue examination findings were in accord with the normal histological descriptions by Hummel et al. (1966). The deviation found in the snout following the nasal blockage procedure and its reduction with age would suggest that the major morphological changes were surgical in origin rather than functional. The dislocation of the nasal septa was noticeable in the operated groups and the findings were that no overall deformities of the snout occurred. This supports the findings of Wexler and Sarnat (1965) in their study of growth of rabbits following nasal septa dislocation.

The question as to the primary and secondary growth mechanisms was not closely investigated, but it could be pointed out that cartilage was a dominant tissue in the rat snout, the nasal septum and the nasal capsule, as described by Baume (1958). The appearances of the tissue cartilage and its return to a symmetrical form supports the contention of a primary role of the nasal septum in the craniofacial growth proposed by Scott (1953) in man, and in the rat by Baume (1958), Young (1961), Ohyama (1969) and Ronning (1971).

The sutural areas were obviously disturbed following surgery and gradually returned towards the original symmetrical form. This did not appear to be by proliferation at the separated free margins, which would have produced an asymmetrical form, but by a passive role rebounding after being disturbed. These findings do not support the active role of sutures as a growth site suggested by Weinmann and Sicher (1955) and Prahl (1968) but support the relatively passive role suggested by Brash (1934), Moss (1954), Scott (1954) and Pritchard et al. (1956).

Neither epithelial ulceration nor epithelial metaplasia was found following nasal blockage indicating the lack of any marked environmental changes. The lack of a marked inflammatory response in the submucosa shows that the changes in the bacterial flora of the nasal cavity did not have any gross effect on the deeper tissues. Nasal blockage alone did not appear to have any direct effect on the enlargement of the submucosal tissues and could not support the direct relationship between respiration and compensations before and after nasal obstruction as claimed by Ziem (1899) and Anderson (1909).

Photographic and radiographic assessment

The photographic and radiographic views merely give the two dimensional changes of anatomical landmarks to the accuracy of their definition and the technique. The photometric polygons gave a good impression of the morphological changes during this post-weaning growth period. These changes are the holistic bone organ changes in space rather than growth changes within bone tissues.

Although radiographic studies have been carried out to demonstrate the normal growth pattern of the rat by Baer (1954), Ford et

al. (1959), Asling et al. (1963), Cleall et al. (1968) and Hanada (1969), superimposition of these serial records was difficult (see Figs. 47-53). The skull is growing rapidly as a composite of many parts as demonstrated by the bone apposition so that reservations must be made as to the validity of superimposing on the cranial base (Baer, 1954 and Cleall et al., 1968) or the cranial vault (Baer, 1954) or the nasofrontal contour (Cleall et al., 1968). The superimposition of the lateral photographs on the incisor-tympanic bullae plane (Figs. 48 and 49) proved very useful and simple to reproduce. This plane of superimposition reveals the viscerocranial elevation described by Moss (1954), Baer (1954) and Moss and Baer (1956).

The superior photographic and radiographic views (Figs. 47, 52 and 53) revealed a lack of asymmetry following unilateral nasal blockage and the rapid growth of the snout in the anteroposterior and width dimensions.

Spence (1940) pointed out the fact that the tympanic bullae were fragile and the cranial orientation error was the greatest source of error. Even though the dried crania were held in a cephalostat, using earrods at right angles to the film surface with a spirit level and a plumbline, this orientation error was still regarded as the greatest in this procedure. The errors involved in standardized printing and identification of landmarks were small in comparison with a measurement accuracy with the Ruscom logics strip chart digitizer of about 0.1 mm. This technique was far superior to the use of calipers as employed by Baer (1954) and Gross (1963).

Undecalcified sectioning technique

Most histological studies of bone growth have been qualitative rather than quantitative. Most investigations using alizarin red S bone marking techniques have been essentially subjective in nature, e.g. Isotupa et al. (1965) and Isotupa (1974). Massler and Schour (1951) and Baer (1954) attempted to quantify bone apposition in the rat crania using calipers, a method which was subject to large errors.

In the preparation of the undecalcified hard tissue sections a number of stages were required. These stages included the embedding of the rat crania in Bioplastic, then the trimming of this plastic block after curing. The base of the block was made parallel to the tympanic bullae-incisor plane, with the four sides trimmed at right angles to this plane and to each other. The top surface of the block was made parallel to the base. These blocks were then polished and the surfaces scored in the anatomical regions for sectioning, using a protractor and a set square. For the plane of section in the coronal regions the anatomical midline was used as a common reference line (Fig. 16). For the sagittal regions (Fig. 27) and the horizontal regions, no common reference plane was available. These blocks were then oriented for sectioning with the scored lines parallel to the diamond disc using a set square. While each step involved it own experimental error and these are cumulative in nature, the overall estimate of error was small.

The diamond disc was .012 inches thick and, therefore, every cut destroyed 305 μ of tissue. The histological sections prepared varied from 80 μ to 130 μ in thickness, depending on the condition of the disc, the hardness of the plastic block and the water flow on the disc cutting edge. Consequently, in cutting three sections, the region was

was representative of 850 μ to 1000 μ along the body of the animal. In the incisor, malar and first molar regions cut in the coronal plane it was only possible to prepare three sections with the same morphology, while in the premaxillary region more sections could have been prepared. The bevelling of the edge of the plastic block and the embedding of the colour coded piece of wool (Fig. 14) proved excellent mechanisms for avoiding the possibility of reversal of the sections.

Sectioning of the rat crania was done in three planes of space as shown in Fig. 14, coronally, sagittally and horizontally. The coronal plane of section, as used by Cleall et al. (1971) and Ryll (1972), proved to be the most suitable for this study. The parasagittal sections could not be prepared accurately equidistant from the midline, as required in an asymmetry study. The width of the larger rat crania was greater than the cutting disc height so that the zygomatic arches had to be removed in order to prepare the horizontal sections. Any error in the orientation of the rat crania resulted in all sections being prepared with that small bias. Sections prepared in the parasagittal and horizontal planes of space cut obliquely through the craniofacial bones and, as Jacobson (1970) found in the mandible, were not suitable for the coordinate or linear analysis.

Microscopic coordinate analysis

The coordinate analysis in the coronal plane has been demonstrated as a useful technique to describe the morphology of the animal. The use of this technique employed cross-sectionally has illustrated the combination of changes due to growth, to a change in function and to surgery.

This technique used cross-sectionally would be a valuable adjunct to any form and function experiment and would help to evaluate the effects of the surgical techniques or the function on this form. The coordinate analysis, as used by Ryll (1972) at the end of the experimental period, would only demonstrate the final form.

The coordinate analysis proved to be a useful adjunct to quantitative bone growth assessment in this study, as was also found by Jacobson (1970). The histological coordinate analysis was regarded as being more reliable than the craniometric evaluations of photographic techniques, with the accuracy of the microscopic coordinates being read to the nearest 0.1 mm or 100 microns. The greatest source of error was that of parallax in viewing the vernier scales on the microscopic stage.

In the coronal plane of section the analysis demonstrated the internal and external bony relations in each plane of section. The polygons of the cross-sectional data revealed a spatial reorientation of the incisors and nasal bones following surgery. The lumen of the nasal cavity was reduced on the side of operation and the gradual deviation of all the facial bones could be demonstrated, being greatest at the site of surgery and reducing posteriorly. No deviation was noted in the cranial base or cranial vault. This showed how the changes were largely due to the surgery, as these asymmetry changes became smaller rather than increased over the 46-day experimental period. By the end of this experimental period there were no significant findings between the operated and non-operated nostrils. This was in accordance with the findings of Gross (1963).

In this study there was no evidence to support the extreme deviation of the facial bones found by Ziem (1879) following nasal blockage. There was no support for the hypothesis proposed by Collier (1898) that the airflow produced large forces which would push the nasal septum against the conchae of the blocked nostril. These gross changes were not anticipated in the present investigation.

No clear explanation of the nature of the functional matrices of the functional air spaces were given by Moss (1962, 1968) nor of their exact degree of action. Moss (1969, 1971) stated their roles as the non-impulse transmitting neural functions involving axoplasmic transports and interactions. While most investigators feel that the body tissues and organs are closely interrelated, vague hypotheses such as these are difficult to test. If the physical role of air passing over the epithelium can be regarded as a functional matrix, this has been shown to have no significant effect on the growth of the snout. If there is a functional role of the proximity of air to the epithelium, this was not evaluated.

An examination of the trabecular patterns in the histological sections was best made under tungsten light and the subjective findings tend to support the patterns found by Isotupa (1974). The orientation of these patterns radiated from focal areas in a curved manner, although no verification of the fact that these may be former ossification centres could be made. Because the trabeculae are strands of soft connective tissue extending to the periosteal surface, they may well be related to the surface periosteum and its growth or flow, as claimed by Isotupa following tattooing, split line and bone marking techniques.

Vital bone marking agents

Several modifications to the technique of Cleall et al. (1968), Jacobson (1970) and Ryll (1972) have been made. These included the use of Dcaf as an alternative to trypan blue as a bone marking agent. Dcaf was found to be eminently suitable, as also found by Suzuki (1966) and Sullivan (1972). Apolecia and ulceration were observed over the injection sites, as Suzuki had also noted. At sacrifice no abdominal damage was visible. The green colour of Dcaf was a strong contrast to the other bone marking agents used, when the bone was examined under ultraviolet light.

Alizarin red S was the only bone marking agent used which could be observed excreted in the urine, and it was visible in the urine for over one week following the intraperitoneal injection. This may be explained by its slow uptake from the peritoneum (Isotupa, 1974) or the toxic level of alizarin in the body (Robinson and Sarnat, 1955). Another possible explanation could be that the rapid rate of remodelling of the newly formed bone. The mode of administration may also deserve mention since Isotupa (1974) suggested that an intravenous route would reduce the dosage and possible toxic effect through the use of a buffered solution rather than the markedly acidic alizarin red S. However, it is generally accepted than intravenous injections are technically more demanding and hazardous.

The injection of terramycin on day 11 of the experimental period appeared to be at a critical time. All animals recovered from the anaesthetic immediately following the intraperitoneal injection of the terramycin. However, only the operated animals were observed to collapse approximately 30 seconds later, exhibiting signs of tetany. Of the

operated animals which died on this day, all died at this time. The other animals recovered without further incident. This may be explained by Albert (1953), Milch (1958) and Myers (1968) who pointed out that terramycin had chelating properties and an affinity for metallic ions. The same dosage of terramycin given on day 25 of the experimental period was not found to have any ill effect. The dosage of this drug was similar to that used by other investigators, i.e. Cleall et al. (1968), Jacobson (1970), Ryll (1972), Smith (1974) and Suzuki (1966). The great benefit of Dcaf and terramycin was their small dosage volumes which allowed the intraperitoneal injections without an anaesthetic, while for the larger volumes of alizarin red S anaesthesia was recommended.

Linear analysis

Cleall et al. (1971) and Ryll (1972) illustrated the growth of the coronal regions as diagrammatic representations with the bone marking agents outlined. The overall morphology of these regions was confirmed in this investigation although some minor points of interest did arise. In the incisor region (Fig. 64) areas of interest in this study were those of the bone apposition adjacent to the nasopremaxillary sutures. Unfortunately, these areas are rapidly growing with apposition externally and resorption internally so that the bone marks were frequently broad and indistinct. Although the two ends are connected by the suture, there is no apparent interproximal apposition as seen in the internasal suture and the two bone ends appeared to grow at slightly differing rates rather than specifically in concert. While all the sites of measurement in this investigation were not exactly the same as in Ryll's study, comparative sites yielded very similar bone increments and standard errors, reflecting the reproducibility of these values. The

lateral wall of the premaxilla was demonstrated as an area of apposition with resorption in the incisor region on the lateral wall of the incisor socket.

In the premaxillary region (Fig. 71) no differences were found in the general orientation of the bone mark increments, as found by Cleall et al. and Ryll. It is interesting to note how the lateral borders of the nasal bones in this region were sites of bony apposition, in contrast to the incisal region, while the adjacent medial surface of the premaxillary bone was not a site of interproximal apposition. This indicates the degree of independence of the individual bone growth and the lability of the suture allowing the bony surfaces to slide, as described by Enlow (1968) and Isotupa (1974).

The malar diagrammatic representation (Fig. 76) was again essentially the same as described by the previous authors, with the exception of the posterosuperior portion of the premaxilla. The nasal bone was as described in the premaxillary region with apposition interproximally and externally. The premaxillary bone internal structure was complex with convolutions although the generalized external apposition occurred without interproximal apposition.

The first molar diagrammatic representation (Fig. 79) did differ slightly from those of Cleall et al. (1971), Ryll (1972) and Cleall (1974). The lateral wall of the alveolus was demonstrated by Cleall et al. (1971) and Ryll (1972) to be an area of apposition but as Cleall (1974) showed this was continuous with the apposition in the floor of the molar socket and the palatal roof. No intermaxillary suture apposition was noted as this was an area which did not stain clearly (Fig. 79). This would be explained by Anderson et al. (1967) as being the bridge of hyaline cartilage across

the midpalatal suture. Similarly, there was no obvious bone growth in the midsagittal suture between the bone ends while apposition was noted superiorly and inferiorly at the suture.

The examination of the bone mark increments in the sections in the sagittal plane (Figs. 29, 30) demonstrated an emphasis in the antero-posterior bone growth. The findings of this study were supported by the pattern of growth in the midsagittal region described by Cleall et al. (1968). The horizontal planes of section gave an overall impression of bilateral changes. The horizontal regions and the incisor region of the sagittal planes of section proved to be far less satisfactory than the coronal regions for a linear analysis technique, primarily because the bone marks were cut obliquely and were therefore less clear, but also because of orientation problems.

Jacobson (1970), Ryll (1972) and Smith (1974) have pointed out the accuracy of the linear analysis with which the bone marking increments may be identified. Without doubt the increments may be measured accurately to within ± 2 microns in most cases where the increments are small and clear but the accuracy is reduced where a plane of section cuts the plane of apposition obliquely. When the bone increments are greater than 500 microns, as in the premaxillary site 3 or malar site 4, there is again a greater error in repositioning the microscopic slide to allow the reading from the ocular scale.

At the histological level it was hoped to find significant quantitative differences in either the amount of bone resorption, bone apposition or both, following nasal obstruction. Each increment of bone growth was compared bilaterally in an effort to reveal differential or reparative bony apposition occurring at different times during the

experimental period. In the unilateral nasal blockage group, the non-functional nostril was compared with its hyperfunctional counterpart.

Several hypotheses have been reviewed and discussed to explain why these changes could be expected. The lack of significant differences in bone apposition bilaterally in this experiment (Tables XIV and XVIII) support the null hypothesis that the airflow per se does not have a functioning stimulus to the bony growth of the snout of the rat. Ryll (1972) following masseterectomy and tooth extraction in rats and Smith (1974) in experimental alterations of the coronal sutural area using a similar technique did find significant changes, which may be due to the degree of surgical intervention. It could be argued that the experimental technique may be greater than any small expected change in bony apposition following nasal blockage.

The growth of the craniofacial complex of the rat, as revealed from observations of the sections in the three planes of space - coronal, sagittal and horizontal - were consistent with the findings in the review of the literature. The macroscopic morphological development as described by Massler and Schour (1951), Baer (1954) and Moss (1954) were confirmed. The striking division of the facial skeleton from the cranial vault was especially demonstrated in the zygomatic coronal region (Fig. 61) and the zygomatic horizontal region (Fig. 30). The microscopic qualitative assessment of the bone apposition supports the findings of Cleall et al. (1968, 1971) and Ryll (1972) in their description of the differential bone apposition.

Few methods of bone growth can be evaluated at the histological level quantitatively. The linear analysis, as developed by Cleall et al. (1964),

can be regarded as an excellent means of assessing the amount and deviation of growth in the two planes of space. Metallic implants (as used by Selman and Sarnat, 1955 and Erickson and Ogilvie, 1958) are not as accurate a technique of bone growth assessment in animals, although they have been used to great effect in human clinical studies. Autoradiography has also been investigated in rats by Jarabak (1953) and Dixon (1961), but while the technique relies on systemic uptake of the material, as do vital bone marking agents, its assessment is neither as simple nor as accurate.

This study was in part to test the relative importance of genetic or environmental factors on the growth of the craniofacial complex of the rat. If an environmental change were an independent factor in growth, the alteration of the functional integrity of the skull would modify its final form. The environmental factor examined in this study has been that of the airflow. These data suggest that the cranial morphology was largely predetermined and tended to compensate for the sutural and cartilaginous deformation for surgery.

SUMMARY AND CONCLUSIONS

This study was undertaken to evaluate the function of the airflow on the growth of the rat craniofacial complex. A sample of 88 male Long Evans rats of starting weight 90 ± 3 grams was studied, 71 having unilateral nasal blockage, 5 having a sham nasal operation unilaterally and 12 non-operated animals serving as a control. In this mixed longitudinal investigation 18 animals were sacrificed serially as a cross-sectional group and the remainder were sacrificed on day 46 of the experimental period.

Local and general changes or reactions were investigated using weight gains, bacteriological and soft tissue data. The cross-sectional data were used to evaluate the timing of the morphological changes. Using photographic, radiographic and microscopic analyses it was possible to describe statistically the morphological changes following nasal blockage. Finally, by a quantified microscopic analysis of serial bone marking agents a histological measurement of the effect of nasal blockage was made.

A number of differing opinions have been reviewed and discussed. The findings of this study were -

1). The surgical technique used in this investigation was found to be satisfactory in producing unilateral nasal blockage with a rapid post-operational recovery. The biomechanical limit of the tissues was exceeded with a slight deviation of primarily the nasal bones and the incisors following surgery.

2). No generalized proliferation was found in the submucosa following nasal blockage, although temporary local inflammatory responses were observed in the immediate post-surgical period. No abnormal growth of the nasal septum or nasal conchae was observed. Neither epithelial metaplasia nor epithelial ulceration was found nor was there a significant change in the pH of the mucous membrane in the operated animals.

3). The general health, as measured by the gain in weight, was found to be significantly inhibited by unilateral nasal blockage. The bacteriological data supported the other evaluations of the predisposition to infection by debilitation. Pathogenic bacteria were found only in the unilaterally nostril blocked animals and the deaths only occurred in animals with unilateral nasal blockage.

4). The normal bone growth processes were demonstrated in the rat as being complex. The general pattern of growth of the nasomaxillary region was found to be essentially external apposition with concomitant internal resorption. Area relocation, differential growth and resorption were demonstrated by the bone marking agents.

5). Quantitative analyses of the standardized photographs and radiographs, as well as the microscopic coordinate analysis, proved effective means of measuring changes in the morphology of the rat craniofacial complex. The changes were primarily normal growth changes demonstrating the viscerocranial elevation and marked anteroposterior growth of the snout in relation to the cranial vault in the Long Evans rat. The relative changes of the landmarks were clearly portrayed as polygons while statistically objective measurements could be made from these data.

6). The asymmetry following surgery appeared to be mediated through sutural adjustments and, from the cross-sectional data, it appeared that the deviation became less with age. No gross skeletal changes were found following the surgical procedures, or throughout the experimental period, but the maxillary incisors tended to deviate to the operated side in both the sham and unilaterally blocked groups, while no discernible deviation was seen in the control animals.

7). The bone thicknesses and overall bone increments were not significantly different when comparing the operated and non-operated sites in each animal. No overall consistent statistical differences could be found for each bone increment when compared bilaterally or between the groups.

8). This experimental technique has demonstrated that the effect of nasal blockage on the bone growth of the snout was so small as not to be significant statistically. While this does not rule out the possibility of a functional stimulus resulting from the airflow, it does suggest that nasal blockage per se has a minimal effect on nasal growth.

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APPENDIX

TABLE XX

INCREMENTS OF BONE APPOSITION IN THE INCISOR REGION, BLOCK A, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				INCISOR SITE 2 MEDIAL					
LEFT NOSTRIL BLOCKED	Left	24.5	2.5	28.9	3.8	46.0	2.5	63.0	3.5
	Right	25.3	2.1	28.5	4.3	41.5	3.1	65.7	3.0
	Diff	-0.9	2.5	0.5	3.1	4.5	3.3	-2.7	4.0
RIGHT NOSTRIL BLOCKED	Left	25.4	2.3	28.9	4.6	42.6	3.3	65.0	3.2
	Right	24.4	2.7	28.9	4.1	46.8	2.7	63.9	3.8
	Diff	1.0	2.8	0.0	3.1	-4.2	3.6	1.0	4.2
CONTROL	Left	21.0	2.4	27.1	4.3	71.3	10.4	92.8	7.7
	Right	20.3	2.5	25.9	3.9	70.0	10.1	91.5	7.6
	Diff	0.7	3.3	1.2	3.4	1.3	15.9	1.3	6.0
LEFT SHAM	Left	15.3	1.6	29.7	3.3	41.3	5.8	95.3	9.5
	Right	19.3	1.9	25.7	1.5	40.0	4.8	86.7	9.2
	Diff	-4.0	0.4	4.0	2.9	1.3	1.5	8.7	4.3
F VALUE		0.45		0.20		0.24		0.76	
				INCISOR SITE 2 LATERAL					
LEFT NOSTRIL BLOCKED	Left	18.9	1.3	33.3	4.1	51.2	3.0	84.5	8.0
	Right	23.2	2.1	30.0	2.8	51.8	3.6	82.7	6.7
	Diff	-4.3	1.8	3.3	4.3	-0.6	3.4	1.8	7.0
RIGHT NOSTRIL BLOCKED	Left	21.5	2.0	34.7	6.6	48.1	6.4	83.7	7.3
	Right	18.4	1.4	34.8	4.4	50.8	3.1	85.1	8.8
	Diff	2.7	1.9	-0.1	7.5	-2.7	5.6	-1.5	7.7
CONTROL	Left	16.6	2.5	28.0	4.0	64.3	11.2	57.4	6.1
	Right	16.5	2.3	24.4	4.0	62.6	12.1	51.5	9.9
	Diff	0.1	3.9	3.6	4.4	1.6	11.2	5.9	7.9
LEFT SHAM	Left	12.5	1.4	34.0	2.3	33.5	6.6	97.5	21.7
	Right	15.0	0.0	20.5	1.4	24.5	1.4	122.5	36.1
	Diff	-2.5	1.4	13.5	3.8	9.0	5.2	-25.0	14.4
F VALUE		1.06		0.47		0.45		1.59	

TABLE XXI

INCREMENTS OF BONE APPOSITION IN THE INCISOR REGION, BLOCK A, AS MEANS AND STANDARD ERRORS IN MICRONS

GROUP	SITE	A - B MEAN	A - B SE	B - C MEAN	B - C SE	C - D MEAN	C - D SE	D - E MEAN	D - E SE
				INCISOR SITE 3					
LEFT NOSTRIL BLOCKED	Left	43.5	8.3	52.2	5.6	115.7	10.5	154.9	9.7
	Right	40.4	6.4	48.2	7.3	124.9	10.8	161.4	11.0
	Diff	2.6	11.7	9.0	5.5	-7.2	9.2	-6.5	7.6
RIGHT NOSTRIL BLOCKED	Left	42.1	6.9	49.8	7.8	128.8	10.6	158.8	11.8
	Right	43.6	8.2	51.7	5.8	119.3	10.9	152.9	10.6
	Diff	-2.0	11.7	-8.5	5.6	7.7	9.8	5.9	8.4
CONTROL	Left	24.6	3.1	43.3	7.5	103.8	8.2	152.5	16.6
	Right	23.9	2.9	48.4	8.0	151.1	9.2	148.0	16.4
	Diff	0.6	4.7	-5.1	6.4	-11.3	15.2	4.5	19.9
LEFT SHAM	Left	0.0	0.0	62.0	0.1	140.0	0.0	157.5	24.5
	Right	0.0	0.0	60.0	0.1	140.0	0.0	170.0	23.1
	Diff	0.0	0.0	2.0	0.0	0.0	0.0	-12.5	1.4
F VALUE		0.05		0.95		0.27		0.41	
				INCISOR SITE 4					
LEFT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	76.5	7.5	200.4	12.7
	Right	0.0	0.0	0.0	0.0	0.0	0.0	197.9	11.7
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	-5.7	13.2
RIGHT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	0.0	0.0	189.2	10.4
	Right	0.0	0.0	0.0	0.0	206.5	37.5	198.1	12.1
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	-8.8	12.6
CONTROL	Left	0.0	0.0	0.0	0.0	0.0	0.0	207.3	8.5
	Right	0.0	0.0	0.0	0.0	0.0	0.0	204.0	8.2
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	3.3	5.0
LEFT SHAM	Left	0.0	0.0	0.0	0.0	0.0	0.0	233.3	20.1
	Right	0.0	0.0	0.0	0.0	0.0	0.0	233.3	12.2
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.8
F VALUE		0.0		0.0		0.0		0.13	

TABLE XXII

INCREMENTS OF BONE APPOSITION IN THE INCISOR AND PREMAXILLARY REGIONS AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				INCISOR SITE 5					
LEFT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	89.0	0.0	124.7	10.2
	Right	0.0	0.0	0.0	0.0	119.0	0.0	111.3	6.3
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	14.4	9.8
RIGHT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	119.0	0.0	112.0	7.0
	Right	0.0	0.0	0.0	0.0	89.0	0.0	124.2	10.9
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	-13.2	11.1
CONTROL	Left	0.0	0.0	0.0	0.0	86.0	6.6	94.5	7.6
	Right	0.0	0.0	0.0	0.0	86.1	7.6	101.1	4.4
	Diff	0.0	0.0	0.0	0.0	0.3	10.2	-6.6	9.6
LEFT SHAM	Left	0.0	0.0	0.0	0.0	0.0	0.0	142.5	7.2
	Right	0.0	0.0	0.0	0.0	54.0	0.1	112.5	7.2
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	30.0	0.0
F VALUE		0.0		0.0		0.0		2.60	
				PREMAXILLARY SITE 5					
LEFT NOSTRIL BLOCKED	Left	16.9	1.2	19.5	1.9	49.8	3.3	92.2	6.2
	Right	18.5	1.3	21.0	2.7	47.2	3.0	79.6	5.6
	Diff	-1.5	1.2	-1.6	1.9	2.5	1.8	12.6	4.9
RIGHT NOSTRIL BLOCKED	Left	17.8	1.3	21.9	3.2	45.6	3.6	72.9	5.4
	Right	14.6	1.4	18.9	2.3	48.2	3.7	86.3	6.7
	Diff	3.2	1.3	3.0	2.2	-2.7	2.2	-13.4	5.7
CONTROL	Left	18.4	1.2	22.7	1.4	69.3	3.8	109.4	2.2
	Right	18.1	1.4	19.3	1.1	73.1	5.6	115.0	3.6
	Diff	0.3	2.0	3.4	2.4	-3.8	2.7	-5.6	3.8
LEFT SHAM	Left	40.0	12.6	62.0	22.8	67.5	4.3	114.0	6.4
	Right	60.0	20.6	53.0	5.5	45.0	5.1	83.7	26.2
	Diff	-20.0	8.0	9.0	17.4	14.5	4.3	-11.0	3.5
F VALUE		17.97**		0.83		7.96**		2.51	

** Significant at the 1% level of confidence

TABLE XXIII

INCREMENTS OF BONE APPOSITION IN THE PREMAXILLARY REGION, BLOCK B, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				PREMAXILLARY SITE 7					
LEFT NOSTRIL BLOCKED	Left	25.6	3.0	27.1	1.9	76.8	6.7	112.3	5.0
	Right	25.1	2.7	39.6	5.6	66.3	7.4	115.7	6.1
	Diff	0.3	3.6	-12.6	6.1	12.6	4.5	-3.7	5.4
RIGHT NOSTRIL BLOCKED	Left	21.6	3.8	33.3	5.5	71.0	8.9	109.9	5.8
	Right	21.9	2.6	27.4	2.4	77.4	8.4	107.2	6.1
	Diff	3.1	3.3	6.0	5.5	-8.8	2.9	3.4	5.0
CONTROL	Left	16.6	1.9	26.8	2.3	72.1	6.3	116.9	4.6
	Right	20.1	2.7	22.4	2.0	71.6	6.5	117.9	3.3
	Diff	-2.7	3.1	4.0	3.5	0.5	1.7	-1.0	4.4
LEFT SHAM	Left	16.5	3.2	33.7	4.8	61.7	4.9	138.3	18.0
	Right	30.5	10.1	29.0	1.9	55.0	1.8	139.0	14.6
	Diff	-14.0	6.9	4.7	3.0	6.7	6.0	-0.7	4.7
F VALUE		3.30		2.28		4.29		0.20	
				PREMAXILLARY SITE 8					
LEFT NOSTRIL BLOCKED	Left	12.1	1.0	10.9	0.9	37.6	2.4	56.0	3.6
	Right	12.1	0.8	11.0	0.9	34.9	2.0	54.6	3.7
	Diff	0.0	0.9	-0.1	0.8	2.7	2.0	1.5	1.9
RIGHT NOSTRIL BLOCKED	Left	12.4	0.8	11.1	1.0	34.7	2.5	52.6	3.5
	Right	12.1	1.1	10.8	1.0	36.8	2.5	55.3	3.9
	Diff	0.3	1.0	0.2	0.8	-2.1	2.4	02.7	1.7
CONTROL	Left	13.8	1.7	17.0	2.0	43.4	2.1	66.5	3.5
	Right	13.5	1.3	16.2	1.8	45.1	2.3	65.4	3.4
	Diff	0.3	1.4	0.8	2.1	-1.7	2.6	1.1	1.3
LEFT SHAM	Left	11.0	1.0	19.0	2.4	43.7	6.6	68.3	6.4
	Right	12.3	0.8	15.3	1.8	37.3	6.2	74.0	6.0
	Diff	-1.3	0.2	3.7	3.0	6.3	1.5	-5.7	1.9
F VALUE		0.37		1.53		1.91		2.24	

** Significant at the 1% level of confidence

TABLE XXIV

INCREMENTS OF BONE APPOSITION IN THE MALAR REGION, BLOCK C,
AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				MALAR SITE 2					
LEFT NOSTRIL BLOCKED	Left	29.4	2.4	40.5	4.7	46.2	3.6	82.4	6.3
	Right	32.1	4.0	37.2	4.7	50.8	5.7	75.8	4.8
	Diff	-2.7	4.0	3.4	4.4	-4.7	4.6	6.6	5.2
RIGHT NOSTRIL BLOCKED	Left	32.8	4.2	33.1	3.8	52.0	6.1	78.0	4.8
	Right	31.0	2.7	37.2	4.6	47.8	3.7	82.3	6.8
	Diff	1.8	4.2	-4.1	4.6	4.2	4.8	-4.3	5.5
CONTROL	Left	52.7	33.1	72.8	35.3	108.9	40.3	144.8	75.3
	Right	22.3	3.9	39.8	8.2	62.5	5.6	74.5	9.7
	Diff	30.4	31.9	33.0	34.2	46.4	42.1	70.3	74.4
LEFT SHAM	Left	10.0	0.0	25.0	11.5	42.5	1.4	110.0	4.8
	Right	12.0	4.6	17.0	7.5	38.5	9.5	98.3	17.0
	Diff	0.0	0.0	1.0	0.0	18.0	0.0	11.7	15.5
F VALUE		2.95*		0.60		0.73		1.12	
				MALAR SITE 4					
LEFT NOSTRIL BLOCKED	Left	202.6	14.2	171.8	11.1	630.3	29.9	630.8	25.9
	Right	218.7	13.8	174.7	10.6	624.0	29.1	619.9	30.9
	Diff	-16.1	12.3	-2.9	10.0	6.3	13.9	10.8	15.4
RIGHT NOSTRIL BLOCKED	Left	225.6	10.5	187.5	10.5	618.1	29.1	616.7	32.6
	Right	215.6	18.1	175.8	8.0	623.7	32.0	630.2	27.4
	Diff	10.0	13.6	11.7	9.7	-5.6	15.1	-13.6	16.4
CONTROL	Left	208.0	19.0	261.0	26.4	565.5	29.3	814.5	42.5
	Right	234.5	26.5	224.0	18.4	575.5	35.8	829.5	45.8
	Diff	-26.5	18.7	37.0	15.7	10.0	30.4	-15.0	16.5
LEFT SHAM	Left	279.3	22.5	277.3	28.0	555.0	105.7	738.3	62.9
	Right	360.0	25.3	200.0	21.9	583.3	107.0	726.7	62.9
	Diff	-80.7	26.0	77.3	8.8	-28.3	6.9	11.7	4.6
F VALUE		4.74**		7.39**		0.49		0.60	

* Significant at the 5% level of confidence

** Significant at the 1% level of confidence

TABLE XXV

INCREMENTS OF BONE APPOSITION IN THE MALAR REGION, BLOCK C,
AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				MALAR SITE 5					
LEFT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	0.0	0.0	499.9	38.9
	Right	0.0	0.0	0.0	0.0	0.0	0.0	437.2	21.3
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	70.5	29.5
RIGHT NOSTRIL BLOCKED	Left	0.0	0.0	0.0	0.0	250.0	0.0	430.1	25.0
	Right	0.0	0.0	0.0	0.0	250.0	0.0	447.2	21.3
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	-29.7	11.6
CONTROL	Left	0.0	0.0	0.0	0.0	0.0	0.0	555.7	60.9
	Right	0.0	0.0	0.0	0.0	0.0	0.0	538.6	64.0
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	17.1	17.3
LEFT SHAM	Left	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Right	0.0	0.0	0.0	0.0	0.0	0.0	365.0	0.0
	Diff	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
F VALUE		0.0		0.0		0.0		4.02	
				MALAR SITE 6					
LEFT NOSTRIL BLOCKED	Left	36.1	5.5	56.4	9.1	137.1	13.4	227.5	18.7
	Right	45.4	7.4	54.5	8.5	122.2	11.1	198.9	15.2
	Diff	-9.3	4.5	1.9	11.2	14.8	6.3	28.5	13.7
RIGHT NOSTRIL BLOCKED	Left	42.9	8.1	54.8	9.0	123.8	11.8	209.4	15.7
	Right	34.8	6.4	55.9	9.7	144.1	14.4	232.0	19.3
	Diff	8.1	4.5	-1.2	11.9	-20.2	7.5	-22.6	15.5
CONTROL	Left	0.0	0.0	60.0	15.0	292.5	169.4	192.2	24.6
	Right	0.0	0.0	68.3	9.3	341.2	219.8	195.6	27.8
	Diff	0.0	0.0	-2.5	27.5	-48.8	53.5	-3.4	12.6
LEFT SHAM	Left	16.0	0.0	51.7	12.2	80.7	14.6	155.0	46.6
	Right	20.0	0.0	66.7	10.5	70.0	12.0	150.0	38.5
	Diff	-4.0	0.0	-15.0	1.8	10.7	5.5	5.0	13.8
F VALUE		1.19		0.14		4.36		1.38	

TABLE XXVI

INCREMENTS OF BONE APPOSITION IN THE MALAR REGION, BLOCK C, AS MEANS AND STANDARD ERRORS IN MICRONS

GROUP	SITE	A - B MEAN	A - B SE	B - C MEAN	B - C SE	C - D MEAN	C - D SE	D - E MEAN	D - E SE
MALAR SITE 7									
LEFT NOSTRIL BLOCKED	Left	19.8	1.6	24.8	3.0	74.2	6.4	128.2	10.7
	Right	17.5	1.7	36.2	6.4	79.3	7.5	139.6	11.2
	Diff	2.3	1.9	-11.4	7.2	-5.1	8.0	-11.5	10.6
RIGHT NOSTRIL BLOCKED	Left	17.8	1.7	33.4	3.9	86.0	7.7	134.6	11.9
	Right	19.6	1.8	24.5	3.5	73.6	7.0	123.6	11.2
	Diff	-1.8	2.0	8.8	5.3	12.4	9.1	11.0	11.2
CONTROL	Left	21.7	3.4	49.1	8.0	55.1	10.0	135.7	10.5
	Right	23.2	3.1	44.1	7.9	61.8	15.8	139.8	8.7
	Diff	-1.6	3.1	5.0	12.0	-6.7	10.0	-4.1	6.4
LEFT SHAM	Left	20.3	5.0	35.7	6.1	61.3	15.7	180.3	10.0
	Right	30.0	2.9	37.5	4.3	37.5	7.2	178.3	26.6
	Diff	-3.5	2.0	-11.5	3.8	40.0	11.5	2.0	21.2
F VALUE		0.73		1.25		3.41		0.50	
MALAR SITE 8									
LEFT NOSTRIL BLOCKED	Left	19.9	2.1	24.6	2.6	87.2	15.4	131.6	9.3
	Right	19.6	1.8	22.4	2.2	84.9	13.3	138.1	8.5
	Diff	0.3	2.0	2.2	2.7	2.2	5.1	-6.5	7.8
RIGHT NOSTRIL BLOCKED	Left	20.3	1.8	20.6	1.9	85.2	13.3	136.6	8.5
	Right	20.2	2.2	22.7	2.3	88.8	15.2	136.2	8.9
	Diff	0.1	2.2	-2.2	2.6	-3.7	5.2	0.4	7.1
CONTROL	Left	19.3	1.7	47.9	17.4	82.5	5.9	166.8	26.5
	Right	21.6	5.3	46.9	18.0	79.8	6.1	181.3	13.2
	Diff	-4.5	5.3	1.0	1.9	2.7	3.3	-14.5	21.7
LEFT SHAM	Left	12.7	1.5	27.3	2.0	57.7	9.6	111.7	12.3
	Right	12.7	1.5	38.7	6.9	40.0	2.3	122.7	13.5
	Diff	0.0	0.0	-11.3	6.9	17.7	8.1	-11.0	2.2
F VALUE		0.60		2.72		2.02		0.27	

TABLE XXVII

INCREMENTS OF BONE APPOSITION IN THE FIRST MOLAR REGION, BLOCK D, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				FIRST MOLAR SITE 3					
LEFT NOSTRIL BLOCKED	Left	56.3	4.3	112.2	10.3	255.5	15.5	261.8	12.9
	Right	59.5	5.2	103.8	9.7	257.5	9.8	262.7	14.6
	Diff	-3.1	4.8	8.4	6.5	-2.0	9.1	-0.9	7.2
RIGHT NOSTRIL BLOCKED	Left	61.6	5.5	108.2	10.2	262.5	10.2	268.0	15.4
	Right	57.0	4.4	117.4	10.6	262.2	16.3	267.0	13.6
	Diff	4.6	5.1	-9.2	7.2	0.3	10.0	1.0	7.9
CONTROL	Left	62.1	9.7	125.8	26.7	269.2	27.5	278.7	23.9
	Right	63.6	9.3	92.1	12.5	298.0	18.5	278.1	23.2
	Diff	-1.5	10.5	33.7	31.2	-28.9	33.2	0.6	5.4
LEFT SHAM	Left	66.7	8.6	145.0	24.6	226.7	11.6	188.3	21.8
	Right	72.0	9.8	122.3	15.2	255.0	21.1	161.7	19.7
	Diff	-5.3	3.1	22.7	16.8	-28.3	23.3	26.7	2.1
F VALUE		0.36		1.49		0.79		2.18	
				FIRST MOLAR SITE 4					
LEFT NOSTRIL BLOCKED	Left	37.9	5.1	56.4	6.1	82.5	5.8	119.1	10.2
	Right	38.9	5.1	56.1	5.2	98.0	7.8	113.1	9.6
	Diff	-1.0	3.2	0.3	5.1	-15.4	7.2	6.0	6.3
RIGHT NOSTRIL BLOCKED	Left	40.1	5.5	57.2	5.4	101.4	8.4	106.5	6.5
	Right	39.2	5.5	60.2	6.1	79.5	5.8	114.2	8.3
	Diff	2.6	3.5	-2.9	5.2	21.9	7.0	-7.7	6.4
CONTROL	Left	43.7	8.0	48.9	9.1	121.4	7.4	124.6	6.3
	Right	38.4	5.3	52.2	8.0	127.0	10.1	120.1	7.8
	Diff	5.3	6.9	-3.3	3.1	-5.6	10.2	4.5	6.0
LEFT SHAM	Left	55.0	1.8	76.7	8.4	193.3	20.4	148.3	9.2
	Right	64.3	4.0	70.7	3.4	196.7	27.9	135.0	5.5
	Diff	-9.3	5.1	6.0	5.1	-3.3	11.7	13.3	14.6
F VALUE		1.69		0.50		2.76		1.07	

TABLE XXVIII

INCREMENTS OF BONE APPOSITION IN THE FIRST MOLAR REGION, BLOCK D, AS MEANS AND STANDARD ERRORS IN MICRONS

GROUP	SITE	A - B MEAN	A - B SE	B - C MEAN	B - C SE	C - D MEAN	C - D SE	D - E MEAN	D - E SE
				FIRST MOLAR SITE 5					
LEFT NOSTRIL BLOCKED	Left	25.2	3.1	34.8	3.4	189.9	11.1	206.0	10.7
	Right	29.0	3.6	34.0	4.5	170.2	11.4	210.7	10.0
	Diff	-3.9	3.1	0.9	4.8	19.7	8.4	-4.8	7.9
RIGHT NOSTRIL BLOCKED	Left	29.3	3.9	35.8	5.1	172.7	12.6	212.9	10.6
	Right	27.4	3.5	33.4	3.4	188.2	12.4	206.7	10.7
	Diff	1.8	3.6	2.4	5.3	-15.5	9.5	6.2	8.3
CONTROL	Left	27.6	3.8	66.8	9.7	231.3	15.8	220.0	17.4
	Right	27.7	3.0	65.0	9.7	231.1	18.3	223.5	19.7
	Diff	-0.1	3.5	1.8	3.1	0.2	7.5	-3.5	8.3
LEFT SHAM	Left	24.7	3.3	49.7	9.2	150.3	34.6	235.7	20.0
	Right	31.7	5.9	55.0	3.7	161.7	34.3	223.3	24.1
	Diff	-7.0	6.8	-5.3	5.7	-11.3	0.4	12.3	4.1
F VALUE		0.80		0.34		2.16		0.66	
				FIRST MOLAR SITE 8					
LEFT NOSTRIL BLOCKED	Left	15.9	1.7	24.8	2.6	55.2	5.4	60.1	4.8
	Right	14.8	1.3	30.9	4.6	56.8	5.2	59.0	4.6
	Diff	1.1	1.7	-6.1	3.3	-1.6	5.3	1.0	2.9
RIGHT NOSTRIL BLOCKED	Left	15.3	1.4	30.7	5.1	56.7	5.5	59.2	5.4
	Right	16.5	1.8	25.7	2.9	55.6	5.9	59.4	5.2
	Diff	-1.3	1.9	5.0	3.7	1.2	5.7	-0.2	3.2
CONTROL	Left	13.9	1.0	23.1	5.8	56.3	8.3	53.2	9.4
	Right	14.6	1.4	17.4	2.7	57.4	8.4	53.9	9.3
	Diff	-0.7	1.6	5.7	6.5	-1.1	9.3	-0.7	8.4
LEFT SHAM	Left	12.7	0.9	21.7	2.8	40.0	2.9	47.5	10.1
	Right	17.3	0.9	24.3	3.7	39.5	8.4	18.0	9.8
	Diff	-4.7	0.2	-2.7	4.1	0.5	5.5	29.5	19.9
F VALUE		1.26		1.41		0.02		5.31**	

** Significant at the 1% level of confidence

TABLE XXIX

INCREMENTS OF BONE APPOSITION IN THE FIRST MOLAR REGION, BLOCK D, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				FIRST MOLAR SITE 6 INTERNAL					
LEFT NOSTRIL BLOCKED	Left	13.3	0.9	19.5	4.1	34.1	3.6	39.0	6.1
	Right	13.9	1.2	20.5	3.9	37.5	4.6	38.6	4.4
	Diff	-0.6	1.2	-1.0	1.7	-3.4	5.5	0.4	7.7
RIGHT NOSTRIL BLOCKED	Left	14.3	-.5	24.2	5.0	39.6	4.5	40.4	4.6
	Right	14.1	1.1	20.2	4.3	33.6	3.8	39.4	6.5
	Diff	0.1	1.3	0.9	1.9	5.3	5.6	-0.3	8.2
CONTROL	Left	21.6	3.4	30.9	5.0	40.1	5.1	65.0	4.8
	Right	23.9	2.5	32.9	7.6	42.2	8.0	67.1	4.8
	Diff	-0.7	4.5	-1.0	11.6	-2.1	8.8	-2.1	4.8
LEFT SHAM	Left	65.0	0.0	37.0	0.0	53.0	0.0	30.0	0.0
	Right	65.0	0.0	45.0	0.0	45.0	0.0	30.0	0.0
	Diff	0.0	0.0	-8.0	0.0	8.0	0.0	0.0	0.0
F VALUE		0.02		3.91		0.33		0.01	
				FIRST MOLAR SITE 6 EXTERNAL					
LEFT NOSTRIL BLOCKED	Left	19.0	2.1	23.0	2.1	94.6	10.2	135.3	7.6
	Right	22.7	3.5	29.4	3.2	93.1	11.3	129.2	8.6
	Diff	-3.7	3.6	-6.4	2.7	1.4	5.0	6.1	5.4
RIGHT NOSTRIL BLOCKED	Left	22.8	3.8	28.9	3.5	96.5	12.3	126.9	9.4
	Right	19.1	2.3	22.3	2.3	98.0	10.7	133.3	9.1
	Diff	3.7	4.0	6.7	3.0	-1.5	5.1	-6.4	6.0
CONTROL	Left	21.2	1.7	38.9	5.0	152.0	19.2	165.4	11.5
	Right	20.1	1.9	41.3	5.0	148.7	19.7	162.8	11.4
	Diff	1.1	2.6	-2.4	8.4	3.3	10.3	2.7	11.1
LEFT SHAM	Left	22.7	0.9	39.0	5.1	74.3	20.1	159.0	6.7
	Right	51.0	21.2	33.0	9.0	69.3	20.1	155.0	15.6
	Diff	-28.3	20.5	6.0	4.7	5.0	0.0	4.0	9.6
F VALUE		4.78		1.89		0.16		0.45	

TABLE XXX

INCREMENTS OF BONE APPOSITION IN THE FIRST MOLAR REGION, BLOCK D, AS MEANS AND STANDARD ERRORS IN MICRONS

		A - B		B - C		C - D		D - E	
GROUP	SITE	MEAN	SE	MEAN	SE	MEAN	SE	MEAN	SE
				FIRST MOLAR SITE 7 INTERNAL					
LEFT NOSTRIL BLOCKED	Left	17.7	2.0	31.2	6.5	51.0	6.3	61.2	4.5
	Right	16.6	1.3	26.3	3.6	54.0	5.7	65.3	5.8
	Diff	1.1	2.1	4.9	6.2	-3.0	6.3	-4.1	5.3
RIGHT NOSTRIL BLOCKED	Left	18.5	2.0	24.5	3.7	53.3	5.9	64.1	6.4
	Right	17.2	2.0	31.8	7.2	49.9	6.9	61.1	5.0
	Diff	1.3	2.9	-7.3	6.4	3.3	6.8	3.0	5.7
CONTROL	Left	14.2	1.2	17.5	2.4	52.0	10.4	57.2	11.5
	Right	15.0	2.0	16.7	3.1	51.8	9.3	53.2	9.9
	Diff	-0.8	1.6	0.8	3.8	0.2	10.1	4.0	5.1
LEFT SHAM	Left	12.0	1.2	19.0	5.2	77.5	1.4	65.0	17.3
	Right	12.0	1.2	19.0	2.3	51.5	10.7	68.5	0.9
	Diff	0.0	0.0	0.0	2.9	26.0	12.1	-3.5	16.5
F VALUE		0.08		0.31		1.71		0.25	
				FIRST MOLAR SITE 7 EXTERNAL					
LEFT NOSTRIL BLOCKED	Left	33.3	9.6	30.8	5.0	100.0	9.2	135.0	7.7
	Right	21.0	1.8	39.6	7.5	93.5	7.0	134.6	7.1
	Diff	12.2	9.6	-8.9	7.5	6.5	6.5	0.4	5.2
RIGHT NOSTRIL BLOCKED	Left	21.0	2.1	39.2	8.3	95.3	7.6	135.2	7.9
	Right	24.8	2.5	32.4	5.3	100.9	10.2	134.9	8.4
	Diff	-3.8	2.6	6.8	8.6	-5.6	7.3	0.3	5.7
CONTROL	Left	25.7	2.9	50.6	6.5	165.6	16.6	168.2	6.1
	Right	28.2	2.8	49.0	5.9	166.8	16.2	164.0	8.5
	Diff	-2.5	4.7	1.6	4.9	-1.2	7.9	4.2	7.0
LEFT SHAM	Left	18.0	2.2	47.0	4.2	96.3	17.8	131.0	20.9
	Right	24.7	2.5	48.7	7.0	82.7	14.7	156.3	15.6
	Diff	-6.7	2.6	-1.7	5.3	13.7	3.1	-25.3	6.9
F VALUE		0.93		0.47		0.99		3.69	

GLOSSARY

The landmarks used in this study are listed in the order in which they were measured and are defined below. When two numbers are given, they represent the same structure measured bilaterally with the left side first then the right as seen during the observation.

Vertex Occlusal Photograph

Site 1		Junction of the lambdoid and sagittal sutures
2		Junction of the nasal and frontal sutures
3		Most anterior point of the internasal suture
4	5	Most anterior point of the naso-premaxillary suture
6	7	Most anterior point of the premaxillary-maxillary junction
8	9	Most anterolateral point of the maxilla
10	11	Most lateral point of the zygomatic arch
12	13	Post tympanic hook, the most lateral distal prominence
14		Most posterior point of the cranium (or right condyle when visible)
15		Left condyle most posterior point (when visible)

Vertex Occlusal Radiograph

Site 1	Medial point of the spheno-occipital synchondrosis
2	Most posterior point on the nasal septum
3	Point on the nasal septum between the most anterolateral points of the maxilla
4	Point on the nasal septum between the premaxillary and maxillary junction
5	Prosthion: the most anterior point of the premaxillary suture
6	Interincisal point

Vertex Occlusal Radiograph (cont'd)

7	8	Most anterior point of the premaxillary and maxillary junction
9	10	Most anterior internal point of the zygomatic arch
11	12	Most posterior point of the maxillary third molar crown
13	14	Most posterior internal point of the zygomatic arch
15	16	Most anterior point of the tympanic bulla

Lateral Photograph

Site	1	Base of the tympanic bulla
	2	Incisal edge of the maxillary incisor
	3	Most anterior superior point on the nasal bone
	4	Junction of the incisor and premaxilla on the palate
	5	Most anterior point - junction of the first molar and maxilla
	6	Fronto-nasal suture, most superior point
	7	Most superior point of cranial vault above the tympanic bulla
	8	Nuchal crest - the most distal superior point
	9	Most distal point of the occipital condyle

Incisor Coronal Coordinate Sites

Site	1	Most inferior midpoint of the premaxillary suture
	2	Most superior midpoint of the internasal suture
	3	Most inferior midpoint of the internasal suture
	4	Midpoint on the nasal septum
	5	Most superior point of the premaxillary suture
17	6	Junction of the nasal concha on nasal bone
16	7	Mid-superolateral point of the naso-premaxillary suture

Incisor Coronal Coordinate Sites (cont'd.)

15	8	Most lateral point of the premaxilla
14	9	Most inferolateral point of the premaxilla
13	10	Most superior midpoint of the amelodentinal junction of the incisor
12	11	Most inferior midpoint of the amelodentinal junction of the incisor

Premaxillary Coronal Coordinate Sites

Site	1	Most inferior point of the interpremaxillary suture
	2	Most superior point of the internasal suture
	3	Most inferior point of the internasal suture
	4	Midpoint of the nasal septum
	5	Base of the nasal septum
23	6	Most superior point on the internal surface of the nasal bone
22	7	Junction of the nasal concha on the nasal bone
21	8	Most inferior point of the naso-premaxillary suture
20	9	Most superior point of the naso-premaxillary suture
19	10	Most superolateral point of the premaxilla
18	11	Most lateral point of the premaxilla
17	12	Most medial point of bone on the superior premaxillary concha
16	13	Junction of the inferior premaxillary concha and the premaxilla
15	14	Junction of the vomer and the nasal concha

Malar Coronal Coordinate Sites

Site	1	Most inferior point on the midline of the maxilla
	2	Most superior point of the internasal suture
	3	Most inferior point of the internasal suture
	4	Midpoint of the nasal septum
	5	Most inferior point of the nasal septum
19	6	Most superior point of the naso-premaxillary suture
18	7	Junction of the premaxillary concha and the premaxilla
17	8	Superior point of the premaxillary-maxillary suture
16	9	Junction of the superior maxillary concha on the maxilla
15	10	Most lateral point on the malar process of the maxilla
14	11	Junction of the middle maxillary concha on the maxilla
13	12	Junction of the inferior maxillary concha on the maxilla

First Molar Coronal Coordinate Sites

Site	1	Most inferior point of the intermaxillary suture
	2	Most superior point of the midsagittal suture
	3	Most inferior point of the midsagittal suture
	4	Most inferior point of the ethmoid process
	5	Most inferior point of the nasal septum
	6	Most superior point of the intermaxillary suture
22	7	Most superolateral point on the external surface of the frontal bone
21	8	Junction of superolateral ethmoid concha
20	9	Junction of maxillary concha
19	10	Most medial point of the maxillary bone

First Molar Coronal Coordinate Sites (cont'd.)

18	11	Most inferolateral point of the maxillary alveolar bone
17	12	Most inferolateral point of the maxillary palatal bone
16	13	Most inferolateral occlusal point of the first molar
15	14	Most inferomedial occlusal point of the first molar

Zygomatic Coronal Coordinate Sites

Site	1	Most inferior median suture point
	2	Most superior median suture point
	3	Most inferior point of the midsagittal suture
	4	Most inferior cranial midline point
	5	Most inferior presphenoid midline point
17	6	Most superolateral point of the frontal bone
16	7	Most inferolateral point of the frontal bone
15	8	Most mesial point of the zygomatic arch
14	9	Most superolateral point of the maxilla
13	10	Most inferolateral point of the maxilla
12	11	Most lateral internal point of the maxilla

Squamosal Coronal Coordinate Sites

Site	1	Most inferior midline point
	2	Most superior midpoint of interparietal suture
10	3	Most superolateral point of parietal bone
9	4	Most lateral point on the zygomatic process
8	5	Most inferolateral point of the basisphenoid bone
7	6	Most inferior point of the pterygoid process

Tympanic Bulla Coronal Coordinate Sites

Site	1	Most inferior midline point
	2	Most superior point of lambdoidal suture
8	3	Most superolateral point of parietal bone
7	4	Most lateral point on the zygomatic root
6	5	Most inferior point on the tympanic bullae