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Experimental alteration of the rat skull base and its effect upon the position of the larynx and hyoid bone

Reidenberg, Joy Spring, Ph.D.

City University of New York, 1988

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EXPERIMENTAL ALTERATION OF THE RAT SKULL BASE AND ITS EFFECT UPON THE POSITION OF THE LARYNX AND HYOID BONE.

by

JOY SPRING REIDENBERG

A dissertation submitted to the Graduate Faculty in Biomedical Sciences in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1988

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Abstract

EXPERIMENTAL ALTERATION OF THE RAT SKULL BASE AND ITS EFFECT UPON THE POSITION OF THE LARYNX AND HYOID BONE.

by

JOY SPRING REIDENBERG

Advisor: Jeffrey T. Laitman, Ph.D.

Laryngeal position is important for regulating a mammal's breathing, swallowing, and vocalizing abilities. A larynx located high in the neck allows for simultaneous respiration and deglutition, while a larynx positioned low in the neck increases the area available as a resonating chamber for laryngeal sounds.

This study investigates the relationship between basicranial shape and the position of the larynx and hyoid bone. Comparative anatomical dissections reveal that a flat skull base and high larynx is the typical mammalian pattern, found in both terrestrial and aquatic species. The flexed skull base and low larynx is characteristic of adult humans. The relationship between the skull base and the upper respiratory tract is explored in an experimental study utilizing rats. Basicranial flexion was artificially induced and any resulting effects on laryngeal and hyoidal position recorded.

The spheno-occipital synchondrosis was surgically ablated with a dental drill in 129 infant rats at 13 days of age. Sham operations were performed on 15 rat pups at 13 days old. Eighteen normal control rats were used for comparison. Radiopaque dye was placed on the soft palate, epiglottis, and valleculae to visualize the larynx, and lateral radiographs were taken at 40, 60, 80, 100, and 120 days. Angular measurements of basicranial contour and the position of the larynx and the hyoid bone were recorded. Changes in overall skull shape were also monitored. Rats were sacrificed at 130 days and the skull base examined histologically.

Results indicate that removal of the spheno-occipital synchondrosis induces flexion (kyphosis) and shortening of the basicranium, arching of the cranial vault, and an endocranial elevation resembling the human dorsum sellae and clivus. The induced basicranial flexion caused the larynx and hyoid bone to be inferiorly displaced. Statistical analysis shows significant differences between experimental and control groups for basicranial, hyoidal, and laryngeal angles, and an inverse correlation for basicranial vs. hyoidal angles, and basicranial vs. laryngeal angles. Histological examination reveals a premature formation of a synostosis between the basisphenoid and basioccipital bones.

These results reveal that larynx and hyoid positions are affected by changes in the shape of the basicranium, thus indicating a mechanical relationship between the skull and upper respiratory tract structures.

THIS DISSERTATION IS DEDICATED TO:
MY PARENTS, IRA AND MARLENE GAYLINN,
AND MY HUSBAND, BRUCE REIDENBERG.
THEIR LOVE, PATIENCE, AND ENCOURAGEMENT
ENABLED ME TO COMPLETE THIS WORK.

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PART I

COMPARATIVE ANATOMICAL STUDY

CHAPTER 1: INTRODUCTION

The purpose of this investigation is to study the anatomical relationship between the skull base (cranial base or basicranium) and the larynx in mammals. The anatomy of the upper respiratory region will be described through original postmortem dissections, and the positional relationships between the skull base and respiratory structures will be compared among a large sample of mammals. The mechanical relationship between basicranial shape and the position of the larynx and hyoid bone will be examined experimentally in laboratory rats.

The upper respiratory tract region consists of both soft and hard tissue structures. The soft (visceral) anatomy includes the oral and nasal cavities, pharynx, larynx, trachea, and all associated musculature. The hard (bony) anatomy includes the skeletal structures, such as the hyoid bone and areas of the skull base. These structures interact as a coordinated unit enabling mammals to breathe, swallow, and vocalize efficiently.

The anatomical and functional relationship between the basicranium and respiratory tract may not appear obvious, at first. The skull is, however, anatomically associated with the upper respiratory tract.

Anteriorly, it houses the nasal cavity, while posteriorly, its inferior or external surface constitutes the supporting frame for the attachments of the constrictor muscles of the pharynx and the muscles and ligaments that suspend the larynx and hyoid bone. The contour of the external surface of the skull base is thus anatomically contiguous with the superior aspect of the upper respiratory tract.

The basicranium is an important structure due to its critical location. Besides supporting the brain, protecting the pituitary, and providing the foramina through which the cranial nerves exit the skull and the carotid artery enters the skull, the basicranium also provides the framework to which many muscles and ligaments attach. Since the basicranium houses so many important structures, any modifications of its anatomy must be slight so as not to impinge upon any of these vital structures. Changes in the anatomy of this region may occur as the result of both normal (e.g., developmental or evolutionary) and abnormal (e.g., pathological or traumatic) events. Drastic changes in this region are often incompatible with life. As a result, the basicranium tends to be an evolutionarily conservative structure, showing limited variation in shape compared with the more "plastic" facial or vault bones.

The anatomical configuration of the skull base and upper respiratory tract is of prime importance, since slight changes in the anatomy of this region can severely alter an individual's respiration, deglutition, and phonation. Disturbances of the skull base may affect the contiguous anatomy of the upper respiratory tract. Any change in the position of the larynx is of particular importance, since it lies at the intersection of the respiratory/vocal and digestive pathways.

The anatomical positioning of the larynx may be partially, if not wholly, dependent upon the anatomy of the basicranium, from which it is suspended. Shape changes in the skull which flex the basicranium may depress the larynx below its usual position. Likewise, any change which results in an elevation of the skull base may similarly elevate the larynx above its normal position. These relationships may be the result of mechanical interactions between the larynx and the basicranium. This study investigates whether these relationships do indeed exist, and to what extent, in both a natural and artificial setting.

This study is divided into two parts. Part I (chapters 1, 2 and 3) describes the comparative anatomy of the upper respiratory tract and skull base; Part II (chapters 4, 5, 6, and 7) describes an experimental study of the relationship between the basicranium and upper respiratory tract in rats.

Part I, chapter 1, is the introduction to the thesis. In chapter 2, the anatomical relationships between the skull base and the position of the larynx will be examined through comparative anatomical dissections across a wide range of mammals. The functional significance of the basic anatomical pattern which appears in these groups is discussed. Chapter 3 focuses on two groups which show extreme specializations away from the general anatomical pattern in this region: cetaceans and adult humans. Functional explanations for the benefit of these divergences away from the basic anatomical pattern are discussed.

In Part II, the mechanical relationships between the skull and respiratory structures are investigated experimentally in laboratory rats. Chapter 4 describes surgical and radiological methods developed specifically for these experiments. Results are presented in chapter 5: 1) data describing whether the skull base was successfully altered, 2) histological results depicting how the surgery affected skull base growth, and 3) data documenting any resulting changes in the position of the hyoid bone and larynx in rats with an altered basicranial shape, during growth and development.

Chapter 6 is a discussion of the results. Explanations are given for any observed relationship between basicranial shape and laryngeal and hyoidal position. Chapter 7 concludes with a discussion of the significance of understanding skull-respiratory system relationships.

This study draws its conclusions from results in two modalities: original comparative anatomical dissections, and an experimental investigation in rats. While the former provides information regarding the anatomical relationships between the skull and upper respiratory tract, the latter is necessary to better understand the functional interactions between these two regions in living mammals.

Basicranial growth, development, and pathology, are subjects of ongoing importance in fields ranging from developmental biology to paleoanthropology. Understanding how the position of the larynx affects its function during respiration, deglutition, and phonation is of concern to the clinician as well as the comparative anatomist. This study investigates the relationship between the skull base and the upper respiratory tract through postmortem dissections of typical mammals (i.e., mammals exhibiting the basic anatomical pattern), and "natural experiments" (i.e., mammals which deviate from this basic pattern). The interactions of these structures in living mammals, however, are currently not well understood. This study thus examines the functional interactions between basicranial shape and the position of the larynx and hyoid bone in an experimental study using live rats.

CHAPTER 2

BACKGROUND: Comparative Anatomy

This chapter introduces the reader to the region of the upper respiratory tract in mammals. The first section gives a brief historical background to the subject. The functional importance of the region is discussed. The second section is an overview of comparative anatomy of the upper respiratory tract. The third section presents the results of my own comparative anatomical dissections, focusing on the position of the larynx.

Background studies

The upper respiratory tract region includes the oral and nasal cavities, pharynx, larynx, trachea, any associated musculature, and bony structures (such as the hyoid bone and the skull base). Slight changes in the anatomy of this area can drastically affect respiratory and olfactory function, swallowing efficiency, and vocalization potentials.

The skeletal supports for the upper respiratory tract are the skull and hyoid bone. The skull base, hyoid bone, and indirectly the mandible, comprise the bony framework to which many muscles attach, including those muscles and ligaments which suspend the larynx and comprise the borders of the pharynx. Due to its anatomical relationship with the nasal cavity, pharynx, and larynx, the basicranium has been described as the "roof" of the upper respiratory tract (Laitman, 1985). The skull base is located at the interface of an anatomically complex region. Inferiorly, it supports the muscles and ligaments of the respiratory and digestive tracts. Superiorly, it supports the brain and the vascular cerebroarterial circle (circle of Willis), and protects the pituitary. In addition, it provides the foramina through which the spinal cord and the carotid and vertebral arteries enter the skull, and cranial nerves and jugular vein exit the skull. Since the basicranium houses many critical structures, developmental or evolutionary changes in its anatomy are relatively limited, compared to the more variable configurations of the face or skull vault. Extensive changes in the basicranium (e.g., from trauma or pathology) are often incompatible with life, as they may adversely affect these vital structures (Hamberger and Wersall, 1969; Bosma, 1976; Samii and Brihaye, 1983). As a result, this area tends to be embryologically stable (Kean and Houghton, 1987), and evolutionarily conservative, showing limited intraspecific variation, and often comparatively little interspecific variation (Olson, 1981; 1985; Laitman, 1983).

The comparative functional anatomy of the mammalian upper respiratory tract has been described in great detail, most notably by Bowles (1889), Negus (1929a, 1949), Pressman and Kelemen (1955), and, more recently, by Laitman and his co-workers (Laitman, 1977; Laitman et al., 1978; Laitman and Crelin, 1980a; Laitman and Heimbuch, 1984; Magriples and Laitman, 1987a). Bowles (1889) described the presence of the lateral food channels (piriform sinuses) and the apposition of the soft palate and the epiglottis, but did not draw any functional conclusions from these observations. Although most of Negus' work concentrates upon the structure of the larynx itself, he was one of the first to note the importance of the positioning of the larynx relative to other structures. He describes the height of the larynx (and its ability to contact the soft palate) as important criteria in determining whether an animal is an obligate nose breather, and thus its ability to simultaneously eat or drink, breathe, and smell (Negus, 1929a; 1949). A larynx positioned high in the neck allows for efficient separation of the respiratory/olfactory and digestive pathways. A larynx low in the neck, on the other hand, results in a crossing of the two tracts in the pharynx. As a result, the pharynx, which is now shared by both pathways, is restricted to only one function at a time. Although this condition makes adult humans particularly vulnerable to aspirating food, research by Crelin (1973, 1976), Laitman and Crelin (1976, 1980a, 1980b), Laitman (1977, 1983), Lieberman (1984), and Laitman and Reidenberg (1988) has shown that the benefit of such an alteration is the presence of a larger resonating chamber above the glottis, thus giving adult humans the ability to produce articulate speech.

The relationship of the upper respiratory tract to the basicranium has been elucidated only recently in studies by Gibbons (1974), George (1978), Grosmangin (1979), and Laitman and his co-workers (Laitman, 1977; 1983; 1986a; Laitman and Crelin, 1976; 1980a; 1980b; Laitman et al., 1978; 1979; and Laitman and Heimbuch, 1982; 1984). The upper respiratory tract spans from nose to trachea and involves both visceral and skeletal structures, including the basicranium. The inferior surface of the basicranium is contiguous with the posterior choanae. The constrictor muscles of the pharynx, as well as the muscles and ligaments which suspend the hyoid bone and larynx, all attach to the external surface of the basicranium. These studies describe a relationship between laryngeal positioning and the degree of basicranial flexion. The basicranial shape (described by Laitman's "basicranial line") appears relatively flat in mammals with a larynx placed high in the neck. In adult humans, however, the basicranium is highly flexed (kyphosed) and the larynx is found very low in the neck. These studies have shown that there appears to be a relationship between basicranial shape and laryngeal height. They have not, however, investigated any of the mechanisms governing this relationship. As a result, our understanding of the mechanisms involved in the relationship between different laryngeal positions and the degree of basicranial bending remains unclear.

Comparative anatomy of the respiratory tract

The anatomical arrangement of structures in the upper respiratory tract of terrestrial mammals has been shown to be remarkably similar (see Negus, 1929a; 1949; Wind, 1970; Laitman, 1977; Reidenberg and Laitman, 1986a). The respiratory pathway is uninterrupted from nose to trachea. It serves to channel air from outside the body to the lungs, where oxygenation of blood occurs. In all mammals, the upper respiratory system consists of the nasal cavity (the initial segment of the respiratory tract), the pharynx (a passageway derived from embryonic foregut), the larynx (an organ used for regulating air flow through the respiratory system), and the trachea (a passageway channeling air into the lungs) up to the carina or tracheal bifurcation. The upper respiratory tract also includes the skeletal structures which provide support for the soft tissues. These skeletal structures include the basicranium and the hyoid bone or hyoid apparatus.

Nasal Cavity

Inspired air first passes from the external nares into the nasal cavity. The nasal cavity opens externally at the nostrils and extends to the choanae. It is divided medially into left and right sides by a nasal septum composed of septal cartilage and two bones: the vomer and perpendicular plate of the ethmoid (Walike, 1977). In the rat (*Rattus norvegicus*), this septum is open along part of its ventral border, allowing communication between the left and right nasal cavities (Kelemen, 1947). Along the lateral wall of each chamber are at least three conchae or turbinate bones (dorsal or superior, middle, and ventral or inferior), and often several more (e.g., the ethmoturbinates, of which the middle concha is only one) (Graeger, 1958). Inspired air is passed over the complex infoldings of these conchae, where inhaled foreign particles are trapped and filtered out and soluble gases absorbed through the mucous membrane (Schreider and Raabe, 1981). This highly vascularized region also serves to humidify and warm the inspired air. Olfactory nerves richly supply the more caudally placed ethmoturbinates and the superio-caudal part of the dorsal turbinate (Read, 1908). In addition, the nasal cavity functions as a resonating chamber for laryngeally produced sounds.

Pharynx

Inspired air passes from the posterior choanae of the nasal cavity into the pharynx. The pharynx is an anatomical space lined with mucous membrane and enveloped by muscles. It is derived from the embryonic foregut (for review of the development of the pharynx, see Edgeworth, 1916; Bosma and Fletcher, 1961; Crelin, 1973; Johnston, 1975). Since the pharynx is intersected by the upper respiratory tract in adult humans, it has become a shared passageway for both the alimentary canal and the respiratory tract. The pharynx is usually divided topographically into three parts: the nasopharynx (epipharynx), the oropharynx (mesopharynx), and the laryngopharynx (hypopharynx), (Skolnik, 1953). The nasopharynx is the space bounded posteriorly by the superior constrictor muscle, laterally by the salpingo-pharyngeal folds, and anteriorly by the soft palate. It is continuous via the choanae with the nasal cavity, and extends from the choanae to the level of the free edge of the soft palate (Walike, 1977). The oropharynx lies immediately inferior to the nasopharynx, extending from the level of the soft palate to the tip of the epiglottis of the larynx in humans. The space of the oropharynx communicates directly with the oral cavity. It is bounded anteriorly by the palatoglossal arch and the base of the tongue, posteriorly by the epiglottis, and laterally by the palatopharyngeal arch. The laryngopharynx begins at the tip of the epiglottis and the palatopharyngeal arch, and extends around and posterior to the larynx, communicating directly with the cavity of the larynx. The lateral recesses are called piriform sinuses and the posterior boundary of the laryngopharynx is composed of the middle and inferior constrictor muscles. The pharynx terminates inferiorly at the laryngeal aditus and the esophageal opening.

The size and shape of different pharyngeal regions can vary between different species. In most terrestrial mammals, for example, the nasopharynx and laryngopharynx are reduced and the oropharynx is almost nonexistent, since the larynx is located high in the neck with the epiglottis locking directly into the nasopharynx. As a result, inspired air passes directly from the nasal cavity to the laryngeal vestibule without going through a true oropharynx as in humans (see chapter 3 for discussion of human anatomy).

Larynx

The larynx is thought to have evolved primarily as a protective organ. In less derived forms, such as fish, it exists as a mere sphincter designed to separate and close off the piscine gas bladder (destined to evolve into the lung in more derived forms) from the digestive tract (Negus, 1932; Wind, 1970). The demands of terrestrial life, however, necessitated a change in the respiratory organs. The upper respiratory tract was modified and strengthened by the presence of the cartilaginous larynx. This may have helped to keep the respiratory opening from collapsing, while preventing liquids and solids from blocking the laryngeal aperture (for further information on the evolution of the larynx, see Wind, 1970; Bowles, 1889; and many works by Negus: 1928, 1929b, 1937, 1949, 1955.)

The larynx is composed of three unpaired cartilages (thyroid, cricoid, and epiglottis) and three paired cartilages (arytenoids, cuneiforms, and corniculates). The arytenoid, thyroid, and cricoid cartilages are composed of hyaline cartilage, while the epiglottic, corniculate, and cuneiform cartilages are composed of elastic cartilage. The thyroid cartilage is derived from the fourth branchial arch, while the cricoid and arytenoid cartilages are derived from the fused fifth and sixth branchial arches (Frazer, 1910; Crelin, 1976; Smith and Catlin, 1984). The epiglottis is a uniquely mammalian trait, and thus is not derived from any branchial arch (Crelin, 1976; Magriples and Laitman, 1987a). (For more detailed information and review of literature on larynx development, see Frazer, 1910; Hast, 1972; Tucker and O'Rahilly, 1972; O'Rahilly and Tucker, 1973; Muller et al., 1985; Zaw-Tun and Burdi, 1985; Magriples and Laitman, 1987a; 1987b; Wolfson and Laitman, 1988.)

There are interspecies variations in the shapes and sizes, and sometimes presence of, these cartilages, but most mammals possess all of the unpaired cartilages and most of the paired ones. For example, in the sheep (*Ovis aries*), the epiglottis is more pointed, the arytenoid cartilage is proportionally larger, and the arytenoid apex is posteriorly inclined, compared with the human epiglottis and arytenoid (Zrunek et al., 1988). The epiglottis of the rat (*Rattus norvegicus*) is flat and triangular, and there is an additional small U-shaped cartilage around the "ventral depression," located on either side of the base of the epiglottis (Smith, 1977).

Many ligaments, membranes, and muscles connect the cartilaginous laryngeal skeleton. Some only provide support, while others, such as the quadrangular membrane or the vocal ligament, play an important role in deglutition and phonation. The quadrangular membrane spans the gap between the arytenoid and corniculate cartilages and the epiglottis. It is covered by a mucous membrane which, together, produce the aryepiglottic fold. The cuneiform cartilages also attach to this membrane and help to keep it spread open like a funnel. This membrane is important in deglutition during which food, especially liquids, must bypass the laryngo-tracheal opening en route to the esophagus. The quadrangular membrane forms a wall which prevents flooding of the trachea with liquids. Thus, fluids are channeled through the piriform recesses on either side of the larynx.

The extrinsic muscles of the larynx are the thyrohyoid, hyoepiglottis (in humans this is reduced merely to a hyoepiglottic ligament), the sternothyrohyoid (divisible in humans as the sternothyroid and sternohyoid), and the inferior pharyngeal constrictor. These muscles attach the larynx to other nearby structures, namely the hyoid bone and sternum. These extrinsic muscles are responsible for moving the larynx relative to its resting position, for example during a swallow. The thyrohyoid elevates the larynx rostrally or superiorly, while the hyoepiglottis approximates the epiglottis to the hyoid bone. These two muscles are innervated by the descendens hypoglossi (nerve fibers from the first cervical spinal nerve which are carried in the hypoglossal nerve). The sternothyrohyoid (sternothyroid and sternohyoid) retracts the hyoid bone and larynx caudally or inferiorly. It is innervated by the ansa cervicalis (ventral branches of the first three cervical spinal nerves). The pharyngeal constrictor stabilizes the larynx against the posterior pharyngeal wall (Landman, 1970).

The intrinsic muscles of the larynx are responsible for movements within the larynx. The cricothyroids tense the vocal ligaments and folds by approximating thyroid and cricoid cartilages ventrally. They are innervated by the external branch of the superior laryngeal nerve (generally known as the cranial laryngeal nerve in animals other than primates). The dorsal cricoarytenoid (posterior cricoarytenoid in humans) and the transverse arytenoid, abduct the vocal processes of the arytenoids, thereby opening the glottis. In the dog (*Canis familiaris*), however, the transverse arytenoid has the opposite action (Evans and Christensen, 1979). Perhaps this discrepancy is due to variations in the attachments of this muscle (for information on variations of attachments of internal laryngeal muscles, see Mossallam et al., 1987.) The lateral cricoarytenoid and the thyroarytenoid adduct the vocal processes of the arytenoids, thereby narrowing the glottis. The thyroarytenoid is divided into the vocalis and vestibularis (e.g., dog, horse - *Equus caballus*, or donkey - *Equus asinus*, Lindsay and Clayton, 1986).

With the single exception of the cricothyroid, all other intrinsic laryngeal muscles are usually innervated by the recurrent laryngeal nerve (also known as the caudal or inferior laryngeal nerve). The tensor muscle of the vocal fold (cricothyroid) is supplied by the vagus nerve via the superior laryngeal nerve, while the abductor muscle (posterior cricoarytenoid) and adductor muscles (thyroarytenoid and lateral cricoarytenoid) are supplied by vagus and accessory nerves. The fibers from the cranial portion of the accessory nerve emerge with the cranial root of the accessory nerve, but are quickly divided off to join the vagus nerve. Thus the adductor muscles are supplied by the accessory nerve via the inferior (recurrent) laryngeal nerve of the vagus. In the rabbit (Oryctolagus cuniculus), there is experimental evidence that the cell bodies for the accessory root may not arise from the nucleus ambiguous, but rather, arise from a region overlapping with the vagal nucleus (Kitamura et al., 1987). These observations are histologically supported in human embryos (Muller et al., 1985). The internal branch of the superior laryngeal nerve provides sensory innervation to the larynx above the glottis, while the recurrent laryngeal nerve provides sensory innervation below the glottis. The cricothyroid probably derives from the fourth branchial arch, while the remaining intrinsic laryngeal muscles probably derive from the sixth branchial arch (Hast, 1978).

The vascular supply for the larynx arises from the laryngeal (also called pharyngeal, in the dog - Evans and Christensen, 1979) branch of the superior (cranial) and inferior (caudal) thyroid arteries, and the cricothyroid branch of the superior (cranial) thyroid artery. These arteries are variable in their origin. In some animals, they originate from branches off the common carotid artery, while in others, they are branches of the thyrocervical trunk.

The vocal folds (plica vocalis or true vocal cords) are an opposing pair of projections from the wall of the larynx towards the interior. The mucous membrane of the vocal fold covers the vocal ligament, which forms the free border of the crico-thyroid ligament. Together, the cricothyroid and its free border form the conus elasticus. The free borders of the vocal folds, supported by the vocal ligaments, are capable of abduction and adduction. When held apart (abduction), their medial edges define the lateral border an aperture called the rima glottidis. Air is allowed to enter or leave the trachea through this opening. When they are tightly apposed (adduction), however, the rima glottidis is closed and air is prevented from either exiting or entering the trachea.

The vocal folds, rima glottidis, and a narrow portion of the larynx at the level of the vocal folds comprise what is generally referred to as the "glottis" (see discussions of the different definitions of the glottis in Moore, 1985; Fink et al., 1975; Williams and Warwick, 1980) According to Hirano et al. (1986), the glottis may be divided into an anterior glottis (intermembranous portion, important in phonation) and a posterior glottis (intercartilaginous portion, important in respiration). The vocal folds close at an angle that is of mechanical advantage to their function, acting as one way valves (Sasaki, 1977). The free borders of the folds meet at the midline and are wedge shaped. Their tight closure produces an apex at the midline which is directed nasally. This allows unimpeded egress of air, but resistance to the ingress of air (Brunton, 1883). Upon forceful inspiration, the folds cannot buckle under the positive pressure superior to the glottis (Sasaki, 1977). (For information on pressure flow relationships within the larynx, see Scherer and Titze, 1983.)

The presence of vocal folds is a consistent mammalian feature, but their orientation within the larynx varies from vertical, to oblique, to horizontal, with respect to the long axis of the larynx (see chapter 3, discussion of vocal folds in odontocetes). The length and angle of the vocal folds is largely dependent upon the shape of the arytenoid cartilages, i.e. how far inferiorly their vocal processes project into the lumen of the larynx.

The vestibular folds (plica vestibularis or false vocal cords), which lie superior to the vocal folds, are also thought to adduct the folds. There is some controversy over the actual function, if any, of the vestibular folds (see review by Fink et al., 1975). They may, for example, play a role as a one way valve responsible for preventing air from leaving the lungs during forced expiration against a closed glottis (Brunton, 1883; Negus, 1924; Pressman and Kelemen, 1955; Myerson, 1964; Sasaki, 1977; Bateman, 1977; Dickson and Maue-Dickson, 1982). When adducted, the vestibular folds are wedged against each other in the closed position so that the apex is directed caudally. This is also at a mechanical advantage in that forceful expiration which produces positive pressure inferior to the folds (as in "bearing down", or Valsalva's maneuver) merely helps to wedge them even further shut (Pressman and Kelemen, 1955; Sasaki, 1977).

Located between the vestibular and vocal folds is a slit-like invagination called the laryngeal ventricle or, when enlarged in size, a laryngeal acccule or laryngeal air sac. Differences in the depth of these pouches may be found among mammals. In some mammals, most notably the highly vocal primates, these ventricles can be greatly enlarged (Negus, 1929; 1949; Laitman, 1977). In the donkey, large laryngeal saccules are thought to function during braying (Lindsay and Clayton, 1986). The laryngeal saccules provide an additional volume of air to be used for intensifying vocalizations (Kelemen, 1963). The laryngeal ventricles are derived from the fifth pharyngeal pouches (Zaw-Tun and Burdi, 1985).

The vocal folds (and possibly the vestibular folds as well), are used to seal off the lower respiratory system from the upper respiratory system. Apposition of the vocal folds prevents inspiration, while apposition of the vestibular folds may prevent expiration under extreme exertion. Regulation of inspiration and expiration is important in maintaining intra-thoracic pressure. For example, by contracting thoracic musculature in an attempt to expire forcefully against a closed glottis, intra-thoracic pressure will rise, thus maintaining the diaphragm as a rigid wall. When inspiratory air flow is blocked (by adducting the vocal folds) negative intra-thoracic pressure builds up and can be maintained against contraction of the diaphragm (Brunton, 1883; Negus, 1924; Sasaki, 1977). This causes the ribs to be stabilized, thus creating a rigid structure against which the pectoral muscles can pull. Contraction of the pectoral muscles will result in adduction of the upper (anterior) extremity while straining, rather than elevation of the chest as in respiration. This ability is especially apparent in lifting or carrying heavy objects (Negus, 1924; 1955; Kaplan, 1971).

Similarly, contraction of the abdominal rectus, external oblique, internal oblique, and transversus muscles against the rigid thoracic cavity will then allow pressure to rise within the abdominal cavity. This increased abdominal pressure allows an animal to exert a squeezing force on the viscera, thereby aiding in processes such as defecation, micturition, flatulation, emesis, and parturition.

If a piece of food or a bolus of mucus has managed to evade the protective features of the larynx and has come to lie at the glottis, high intra-thoracic pressure from forced expiration against a closed glottis can be effectively used in the cough. The tightly apposed vestibular folds are thought to keep the glottis closed as the pressure head builds (Sasaki, 1977). When it is suddenly opened, air is forced out at a tremendously high speed and with enough force to dislodge the obstructing material (Heimlich and Uhley, 1979).

Trachea

Inspired air travels through the larynx and passes into the trachea. The trachea is a tube supported by incomplete ("C"-shaped) cartilaginous rings (except in cetaceans, which possess complete "O"-shaped rings, see discussion in chapter 3). The intervals between the cartilaginous segments are connected by fibrous membrane. Dorsally (or posteriorly in humans), where the cartilaginous rings do not connect, the trachea is composed of fibrous and elastic tissue, and nonstriated muscular fibers.

Tracheal length is variable, depending upon the length of the animal's neck. The trachea is a flexible structure which can be extended or compressed along its long axis, or curved ventrally (anteriorly in humans) and dorsally (posteriorly in humans).

Air is channeled through the trachea to the carina (tracheal bifurcation), into the primary and then secondary bronchi, numerous lobar bronchi, and then the bronchioles. From the terminal bronchioles, air finally passes into the alveoli of the lungs, where gas exchange occurs.

Basicranium

The basicranium is not usually associated with the respiratory tract, since most descriptions of the respiratory passageway focus only on the soft tissue structures. The basicranium and the respiratory organs are generally considered as independent units. In this study, the basicranium is recognized as an integral part of the upper respiratory tract, as it provides the supporting skeletal framework for the muscular, osseous, and ligamentous attachments which suspend the larynx and comprise the pharyngeal walls.

The basicranium has been studied in great detail. Much has been written about its growth and development, as well as its comparative anatomy (see reviews by

Scott, 1958; Bosma, 1976; Koski, 1985). Some have described the growth and development of the basicranium as a unit (Bjork, 1955a; Moss and Greenberg, 1955; Ford, 1958; Stramrud, 1959; Koski, 1960; Vilmann, 1969; 1971; Kvinnsland, 1971; Melsen, 1971; 1974; Latham, 1972; Laitman and Crelin, 1976; Dean and Wood, 1984). Others have studied the results of artificial deformation and normal biomechanical stresses on basicranial bones (Oetteking, 1924; McNeill and Newton, 1965; Demes, 1985). Still others have examined longitudinal growth, shape changes, and angular measurements (or basicranial "axis") of the components of the cranial base (Zuckerman, 1955; Angst, 1967; Michejda and Lamey, 1971; Laitman et al., 1978; Laitman and Heimbuch, 1984; Trenouth, 1984; also see review by Schulter, 1976). The growth of the skull is regulated by genetic, epigenetic, and environmental factors, each of which exerts differing amounts of influence on different regions of the skull (Limborgh, 1970; 1972; Devor, 1987). Although many studies only consider the growth of isolated basicranial bones (e.g., Hoyte, 1965; Koski and Ronning, 1970; Harkness, 1974; 1976; Olivier, 1975; Shapiro and Robinson, 1976; Harkness and Trotter, 1980), it is apparent that the skull and the surrounding musculature and organs must interact dynamically, each affecting the growth of its neighbor, to produce their final form (Kean and Houghton, 1987). (For more detailed information on regulation and mechanisms of basicranial growth, see Hoyte, 1971; Moss, 1972).

Particular attention has been paid to the growth of the basisphenoid and basioccipital bones. These bones are joined to each other by a cartilaginous joint, the spheno-occipital synchondrosis. This synchondrosis is the major growth center of the skull base and thus growth at this synchondrosis contributes to the shape and elongation of the cranial base. The spheno-occipital synchondrosis has received considerable attention in studies utilizing the mouse (Servoss, 1973), rat (Dorenbos, 1973; Kvinnsland and Kvinnsland, 1975; Roberts, 1975; Moss, 1976; Roberts and Blackwood, 1983; 1984), horse (Ackerman et al., 1974), monkey such as *Macaca* (Adams and Harkness, 1972; Michejda, 1972; Giles et al., 1981), and human (Powell and Brodie, 1963; Melsen, 1969; 1972; Ingervall and Thilander, 1972; Thilander and Ingervall, 1973; Moss-Salentijn, 1975).

The anterior midline of the basicranium is composed of the incisive (premaxilla), maxilla, and horizontal portion of the palatine bone (hard palate), and the vomer. Posteriorly, the basisphenoid and basioccipital bones comprise the midline of the basicranium. Laterally, the posterior skull base consists of the petrous portion of the temporal bone. In most terrestrial mammals, the basicranium has a relatively flat shape (Laitman, 1977; Laitman and Heimbuch, 1984; Laitman et al., 1978). When midsagittally bisected, the basicranial contour is usually linear and oriented in a horizontal plane. The contour of the basisphenoid and basioccipital bones are of particular interest, since it is from this posterior region of the skull base that most of the muscles, ligaments, and connective tissue attachments for the suspension of the larynx and hyoid bone are found.

Hyoid bone or hyoid apparatus

In apes and humans, the hyoid is a U-shaped bone comprised of a body, two lesser cornua, and two greater cornua. This bone does not articulate with any other bone. The hyoid is suspended from the styloid process of the temporal bone of the skull base by the stylohyoid ligament. Hyoid position is maintained by the muscles which connect it to the rest of the skeleton (geniohyoid, middle pharyngeal constrictor, stylohyoid, omohyoid, mylohyoid, sternohyoid and, via a fibrous connective tissue loop, from the digastric in the human), the tongue (hyoglossus, genioglossus, chondroglossus), or the larynx (thyrohyoid membrane, hyoepiglottic ligament in primates, hyoepiglottic muscle in other mammals). The lesser cornu is derived from the second branchial arch, the greater cornu from the third arch, and the body from the fused ventral portions of both the second and third arches (Crelin, 1976).

Many mammals, such as the dog, horse, cattle, sheep, or goat, do not have a hyoid bone of the type found in apes and humans. Instead, they have a basihyoid, which is linked to the skull by a direct stylohyoid skeleton (Bosma and Fletcher, 1961). This entire structure is collectively called the "hyoid apparatus." The bony hyoid apparatus is composed of a single basihyoid, and paired thyrohyoids, ceratohyoids, epihyoids, and stylohyoids. The bones are united by cartilaginous synchondroses.

The basihyoid is the homologue of the body of the hyoid bone, and often contains a lingual process. It is attached, posteriorly, to the thyrohyoid, which fuses with it in older animals. The thyrohyoid is the homologue of the greater cornu of the hyoid bone. In some mammals, the thyrohyoid attaches via a synchondrosis to the superior cornu of the thyroid cartilage. Superiorly, the basihyoid attaches to the ceratohyoid. The ceratohyoid is the homologue of the superior or lesser cornu of the hyoid bone. The basihyoid, thyrohyoid, and ceratohyoid, which comprise the inferior portion of the hyoid apparatus, are homologous to the hyoid bone. The ceratohyoid is derived from the second branchial arch, the thyrohyoid from the third arch, and the basihyoid from the fused ventral portions of both the second and third arches.

The superior portion of the hyoid apparatus is composed of the epihyoid and the stylohyoid. The epihyoid is attached to the ceratohyoid below and the stylohyoid above. The epihyoid and stylohyoid unite in adults. The stylohyoid, in turn, attaches to the basicranium at the styloid process of the petrous part of the temporal bone via the tympanohyoid cartilage. These structures, homologous to the stylohyoid ligament, are largely cartilaginous in infants, but ossify in adults. This superior portion of the hyoid apparatus, sometimes called the stylohyoid chain, is derived from the second branchial arch (see Cambas, 1983 for further discussion of hyoid arch).

Muscles which suspend the hyoid bone from the basicranium

Many of the muscles, ligaments, and connective tissues which attach to the basicranium have an important function in the suspension of upper respiratory structures. The larynx is attached to the hyoid bone by the thyrohyoid muscle, thyrohyoid ligament, and hyoepiglottic muscle (in some mammals) or hyoepiglottic ligament (in primates). Thus, any muscle which suspends the hyoid bone also indirectly suspends the larynx.

The digastric muscle, which spans between the mandible and the skull base, suspends the hyoid bone from a fascial loop in humans. This fibrous loop connects the posterior horn and body of the hyoid bone to the round intermediate tendon of the digastric muscle. This rounded tendon is located at the junction between the anterior and posterior digastric bellies, and sometimes perforates the tendon of insertion of the stylohyoideus. The posterior belly of the digastric muscle has a variable origin in primates, attaching to a lateral area of the basioccipital bone in the orangutan (Pongo pygmaeus), the mastoid process or the occipitomastoid suture in the chimpanzee (Pan troglodytes), and the temporal bone in the gorilla (Gorilla gorilla), (Dean, 1984). In some quadrupeds, such as the dog, horse, cattle, sheep, or goat, it attaches just behind the auditory bulla at the jugular process of the occipital bone.

A styloid process (or muscular process of the temporal bone), which projects from the skull base in a ventral, anterior, and medial direction, is found in mammals with a hyoid bone. It is the attachment point for three muscles and one ligament: stylopharyngeus, styloglossus, and stylohyoid muscles, and the stylohyoid ligament. Of these four structures, at least three play a role in suspension of the larynx. The stylohyoid muscle attaches from the base of the styloid process to the body of the hyoid bone at its junction with the greater cornu, thus suspending the hyoid bone from the skull base. In animals posessing a stylohyoid ligament, it runs from the tip of the styloid process to the lesser cornu of the hyoid bone.

In mammals with a hyoid apparatus, the stylohyoid muscle attaches from the muscular process of the dorsal extremity of the stylohyoid bone to the rostral part of the thyrohyoid bone (e.g., horse, cattle, sheep, or goat) or the basihyal bone (e.g., dog). There are also a number of other small muscles which connect the hyoid apparatus to the skull or larynx. The occipitohyoid (or jugulohyoid) muscle arises from the jugular process of the occipital bone and inserts on the stylohyoid bone (e.g., dog, cattle, sheep, or goat) or the thyrohyoid bone (e.g., horse). The ceratohyoid muscle connects the ceratohyoid and thyrohyoid bones. The thyrohyoid muscle connects the thyrohyoid portion of the hyoid bone to the thyroid cartilage.

Muscles suspending the larynx from the hyoid bone and basicranium

Many of the muscles, ligaments, and connective tissues which attach to the basicranium have an important function in the suspension of upper respiratory structures. Since the larynx is attached to the hyoid bone by the thyrohyoid muscle and thyrohyoid ligament, muscles suspending the hyoid bone also indirectly suspend the larynx. In addition, there are muscles which insert on the larynx itself, thus directly suspending it from the basicranium.

The muscles which suspend the larynx are the same muscles which comprise the muscular walls of the pharynx. These muscles include three pairs of pharyngeal constrictors (rostral or superior, middle, and caudal or inferior) and three pairs of longitudinal elevator muscles (stylopharyngeus, salpingopharyngeus, and palatopharyngeus).

The stylopharyngeus arises from the base of the styloid process in primates (Dean, 1984) or the stylohyoid bone in mammals possessing a hyoid apparatus (Dyce, 1957), and usually inserts between the rostral/superior constrictor and the middle constrictor, with some fibers merging with those of the middle constrictor. The salpingopharyngeus, which is variably present in humans (Hollinshead, 1978), arises from the pharyngeal opening of the auditory (eustachian) tube and merges with the palatopharyngeus. Fibers from the palatopharyngeus and the stylopharyngeus directly insert on the posterior border of the thyroid cartilage of the larynx.

The pharyngeal constrictors also play a role in suspension of the larynx from the skull base. The pharyngeal constrictors are actually three groups of muscles (rostral/superior, middle, and caudal/inferior) which overlap each other to form a continuous muscular sheath. These muscles form the posterior and lateral walls of the pharynx, while anteriorly the pharynx opens into the nasal, oral, and laryngeal cavities. Posteriorly, the constrictors insert into each other, forming the median pharyngeal raphe. This raphe spans the entire posterior midline from the esophagus to its insertion via the pharyngeal aponeurosis to the pharyngeal tubercle of the occipital bone in the human (Skolnik, 1953). Dissections by Tagaki et al. (1962), however, show that the pharyngeal aponeurosis attaches to a "pharyngeal crest" located on the basilar portion of the basisphenoid bone in the human, cat (_Felis domestica_), guinea pig (_Cavia cobaya_), rabbit, and cattle, and attaches to the presphenoid bone in the opossum (_Didelphis marsupialis_).

In humans, the superior pharyngeal constrictor may be divided into four muscles according to their origins: pterygopharyngeus, buccopharyngeus, mylopharyngeus, and glossopharyngeus (Skolnik, 1953). In other mammals, such as the dog, horse, cattle, sheep, or goat, the rostral pharyngeal constrictor usually comprises two muscles: the pterygopharyngeus and the palatopharyngeus (Dyce, 1957; Kunzel et al., 1966). Cranially (superiorly in humans), the rostral/superior constrictor inserts onto the base of the skull at the pterygoid hamulus and the pterygoid plate, and laterally it is separated from the buccinator by the pterygomandibular raphe. In the orangutan (Pongo pygmaeus), the chimpanzee (Pan troglodytes), and the gorilla (Gorilla gorilla), the superior constrictor may attach directly to the anterior region of the basioccipital bone, the undersurface of the apex of the petrous temporal bone, and the lower portion of the medial pterygoid plate (Dean, 1985).

The middle pharyngeal constrictor (also called the hyopharyngeus), is divisible into two parts: ceratopharyngeus and chondropharyngeus. Anteriorly and inferiorly, the middle pharyngeal constrictor attaches to the stylohyoid ligament and lesser cornu of the hyoid bone (chondropharyngeal part), and the greater cornu of the hyoid bone (ceratopharyngeal part) in primates. In animals possessing a hyoid apparatus, the ceratopharyngeus inserts on the stylohyoid and ceratohyoid bones, while the chondropharyngeus attaches anteriorly to the thyrohyoid bone and the thyroid cartilage.

The caudal, or inferior, pharyngeal constrictor is comprised of the thyropharyngeus and the cricopharyngeus. These muscles insert anteriorly on the thyroid and cricoid cartilages of the larynx, respectively. The thyropharyngeus attaches to the posterior and lateral surface of the thyroid cartilage, including the superior and inferior cornua. The cricopharyngeus inserts on the lateral surface of the cricoid cartilage, just posterior to the cricothyroid muscle. This muscle is important for regulating the opening to the esophagus. By using this muscle to control the release of swallowed air, laryngectomized humans are able to use an "esophageal voice" to produce alaryngeal speech. (For review of muscular actions of the pharynx in humans during respiration, deglutition, and phonation, see Bosma et al., 1962.)

COMPARATIVE ANATOMY:
POSITION OF THE LARYNX

Methods

This section briefly discusses the positional relationships of the larynx in terrestrial mammals. These dissections were performed for two purposes: 1) to learn, through original observations, the normal anatomy of the upper respiratory tract, and 2) to establish as a baseline reference the typical mammalian pattern of laryngeal positioning against which some of the more unusual anatomical arrangements may be compared (see chapter 3). The observations presented are the result of original dissections performed on a comparative sample of over 200 mammalian specimens representing 29 genera. Species, sample size, age, and sex for specimens are given in table 1. Specimens were frozen to prevent spoilage and to facilitate cutting with a Biro electric band saw. Specimens were examined through whole head bisection. They were cut in the midsagittal plane to expose the midline anatomy of the upper respiratory and digestive tracts and basicranial shape. The bisected heads also allowed easy visualization of the skeletal level of the larynx. Specimens were then preserved by immersion fixation in

TABLE 1

Common name	Genus species	N	Ages	Sexes
bat	*Myotis thysanodes*	4	A	?
mouse	*Mus musculus*	10	A	M,F
spiny mouse	*Liomys* sp.	4	A	M
Norway rat	*Rattus norvegicus*	>150	A	M,F
chipmunk	*Tamias striatus*	1	A	F
gray squirrel	*Sciurus carolinensis*	2	A	M
guinea pig	*Cavia porcellus*	2	A	F
hamster	*Mesocricetus auratus*	4	A	F
muskrat	*Ondatra zibethica*	1	A	M
woodchuck	*Marmota monax*	6	A	M,F
rabbit	*Oryctolagus cuniculus*	12	A	M,F
cottontail rabbit	*Sylvilagus floridanus*	2	J	F
opossum	*Didelphis virginiana*	2	A, J	F
raccoon	*Procyon lotor*	5	A, J	M,F
cat	*Felis domestica*	2	A	F
river otter	*Lutra canadensis*	1	A	F
harbor seal	*Phoca vitulina*	5	A, I	M,F
fur seal	*Callorhinus ursinus*	1	A	F
California sea lion	*Zalophus californianus*	1	A	M
common red fox	*Vulpes vulpes*	2	A	M
dog	*Canis familiaris*	17	A,F	M,F
pig	*Sus scrofa*	8	A,J	M,F
goat	*Capra hircus*	1	A	M
sheep	*Ovis aries*	5	A	F
white-tailed deer	*Odocoileus virginianus*	18	A	M,F
pronghorn antelope	*Antilocapra americana*	2	A	F
horse	*Equus caballas*	1	A	?
cynomolgus macaque	*Macaca dascicularis*	5	A	M,F
olive baboon	*Papio anubis*	1	A	M

phosphate buffered 10% formalin.

The position of the larynx was measured relative to vertebral levels and portions of the skull base. The level was determined by extending a line perpendicular to the long (vertical) axis of the larynx and trachea and noting which skeletal structures were intersected. The lines were drawn at the most rostral (superior) and the most caudal (inferior) portions of the larynx. The most rostral extension of the larynx is the superior tip of the epiglottis, and the most caudal part is the inferior border of the body of the cricoid cartilage.

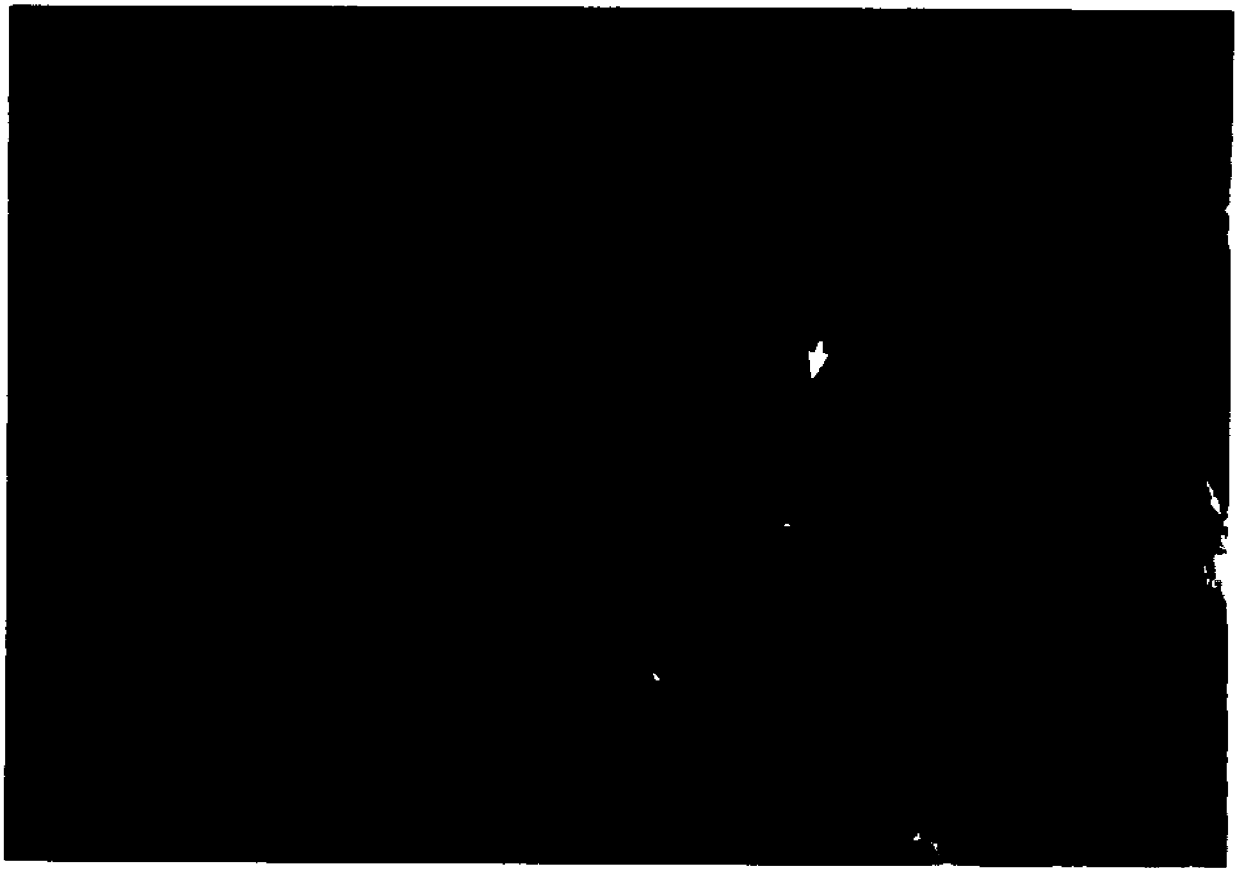
The specimens discussed in this chapter were donated post mortem by several departments or laboratories at The Mount Sinai Medical Center/Mount Sinai School of Medicine. Additional specimens were donated by The New York State College of Veterinary Medicine at Cornell University, The Westport Nature Center, New England Aquarium, and Mystic Marinelife Aquarium. Other specimens were donated by Mr. J. DeBone (taxidermist), Ms. S. Katz, and Mr. and Mrs. I. Gaylinn.

Results

In all nonhuman mammals dissected, the larynx is located high in the neck. This high position allows the epiglottis to overlap behind the soft palate. The curved anterior surface of the epiglottis is in contact with the curved posterior surface of the soft palate for almost their entire lengths, thus displaying a large overlap between these two structures.

Figure 1 displays a midsagittal view of the pig, (Sus scrofa). This omnivorous mammal shows the typical anatomical arrangement of a high larynx. The epiglottis is a relatively elongated structure. Its overlap with the soft palate is extensive. Note that the tongue lies completely within the oral cavity, with no portion of it forming the anterior wall of the pharynx, as in adult humans (see chapter 3). The nasopharynx is limited to the space visible above the epiglottis, leading into the nasal cavity, while the laryngopharynx is visible only as the space below the level of the tip of the epiglottis and communicating

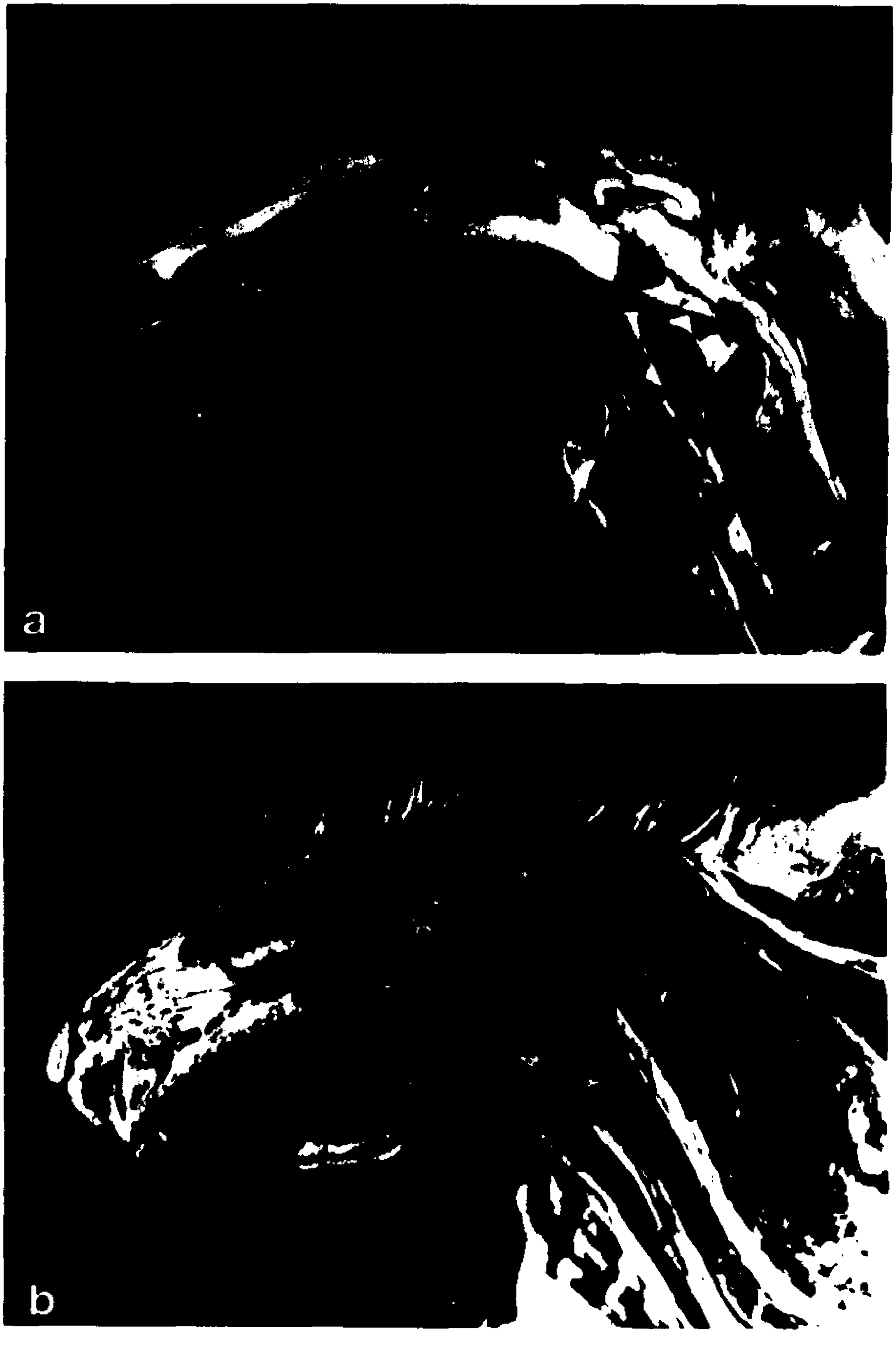
Figure 1: Midsagittal section through the head of a pig, (Sus scrofa). Note the high position of the larynx, with the epiglottis overlapping the soft palate. HP = hard palate, T = tongue, H = hyoid bone, S = soft palate, E = epiglottis, L = larynx, white arrow indocates spheno-occipital synchondrosis of the skull base.



directly with the laryngeal vestibule. There is almost no oropharynx visible in the pig. The skull base is relatively flat and oriented in a nearly horizontal plane, parallel to the hard palate.

Herbivores also display the typical arrangement of a larynx positioned high in the neck with the epiglottis overlapping with the soft palate. Figure 2a shows a midsagittal view of the sheep, (*Ovis aries*). Note the extensive overlap of the epiglottis and soft palate. The epiglottis was also found to be elongated in other large herbivores, including the goat (*Capra hircus*), pronghorn antelope (*Antilopcapra americana*), white-tailed deer (*Odocoileus virginianus*), and horse (*Equus caballas*). In the horse, the palatopharyngeal arch is rotated into a horizontal plane, and its posterior portion projects from the pharyngeal wall. This structure thus resembles a ring, into which the larynx is inserted. The epiglottis overlaps behind the anterior portion, as in other herbivores. The posterior portion, however, is also overlapped by the enlarged arytenoids, which curve backwards to facilitate this interlocking. This interlocking, while extensive in herbivores, is even more exaggerated in the odontocetes (see chapter 3). The skull base is aligned along an essentially horizontal plane, roughly parallel to the hard palate.

Figure 2: a) Midsagittal section through the head of a sheep, (*Ovis aries*). b) midsagittal section through the head of a rabbit, (*Oryctolagus cuniculus*). Note the extensive overlap of the epiglottis and soft palate.


a
b

Small herbivores and omnivores, such as the lagomorphs and rodents, display a similar pattern. The high epiglottis overlaps behind the soft palate, although the degree of overlap is not as extensive. Figure 2b is a midsagittal view of the domestic rabbit, (*Oryctolagus cuniculus*). This pattern is also evident in the cottontail rabbit (*Sylvilagus floridanus*), as well as several rodents such as the woodchuck (also known as marmot or groundhog, *Marmota monax*), muskrat (*Ondatra zibethica*), guinea pig (*Cavia porcellus*), eastern gray squirrel (*Sciurus carolinensis*), Norway rat (*Rattus norvegicus*, see figures in chapters 4 and 5), hamster (*Mesocricetus auratus*), chipmunk (*Tamias striatus*), mouse (*Mus musculus*), and spiny mouse (*Liomys* sp.). The bat (*Myotis thysanodes*) also display a similar arrangement. The skull base of these mammals is unflexed and usually aligned in a horizontal plane. The rabbit has a very curved hard palate, and thus the skull base does not always parallel the palatal line.

The marsupial opossum (*Didelphis virginiana*) is shown in figure 3a, and the raccoon (*Procyon lotor*) is depicted in figure 3b. These small omnivores also have a larynx positioned high in the neck. Although the epiglottis is not elongated, as in the herbivores, it fully overlaps the soft palate. The oropharynx is very

Figure 3: a) Midsagittal section of an opossum head, (Didelphis virginiana). b) Midsagittal section of the head of a raccoon, (Procyon lotor). Note the overlap between the soft palate and the epiglottis.


a
b

small and not visible in these sections, and the tongue is located entirely within the oral cavity. Portions of the nasopharynx and laryngopharynx are visible above and posterior to the epiglottis and soft palate. The basicranium is flat and horizontally oriented.

Two representative carnivores are shown in figure 4. The dog (*Canis familiaris*) is shown in figure 4a, while the harbor seal (*Phoca vitulina*) is depicted in figure 4b. These carnivores show the same arrangement of a larynx positioned high in the neck with the epiglottis overlapping the soft palate. The overlap in these specimens indicates an ability to maintain a patent airway while swallowing. This pattern is also seen in the cat (*Felis domestica*), common red fox (*Vulpes vulpes*), river otter (*Lutra canadensis*), fur seal (*Callorhinus ursinus*), and California sea lion (*Zalophus californianus*). In most terrestrial carnivores, the skull base is remarkably flat. In aquatic mammals (otter, seal, fur seal, and sea lion), the skull base is slightly lordosed (i.e., convex inferiorly).

Nonhuman primates also display this same arrangement of a larynx positioned high in the neck. The cynomolgus monkey or crab-eating macaque (*Macaca fascicularis*) is shown in figure 5a, and the olive baboon (*Papio anubis*) is seen in figure 5b. As in the

Figure 4: a) Midsagittal section through the head of a dog, (*Canis familiaris*). b) Midsagittal section through the head of a seal, (*Phoca vitulina*). Note the interlocking of the epiglottis and soft palate.

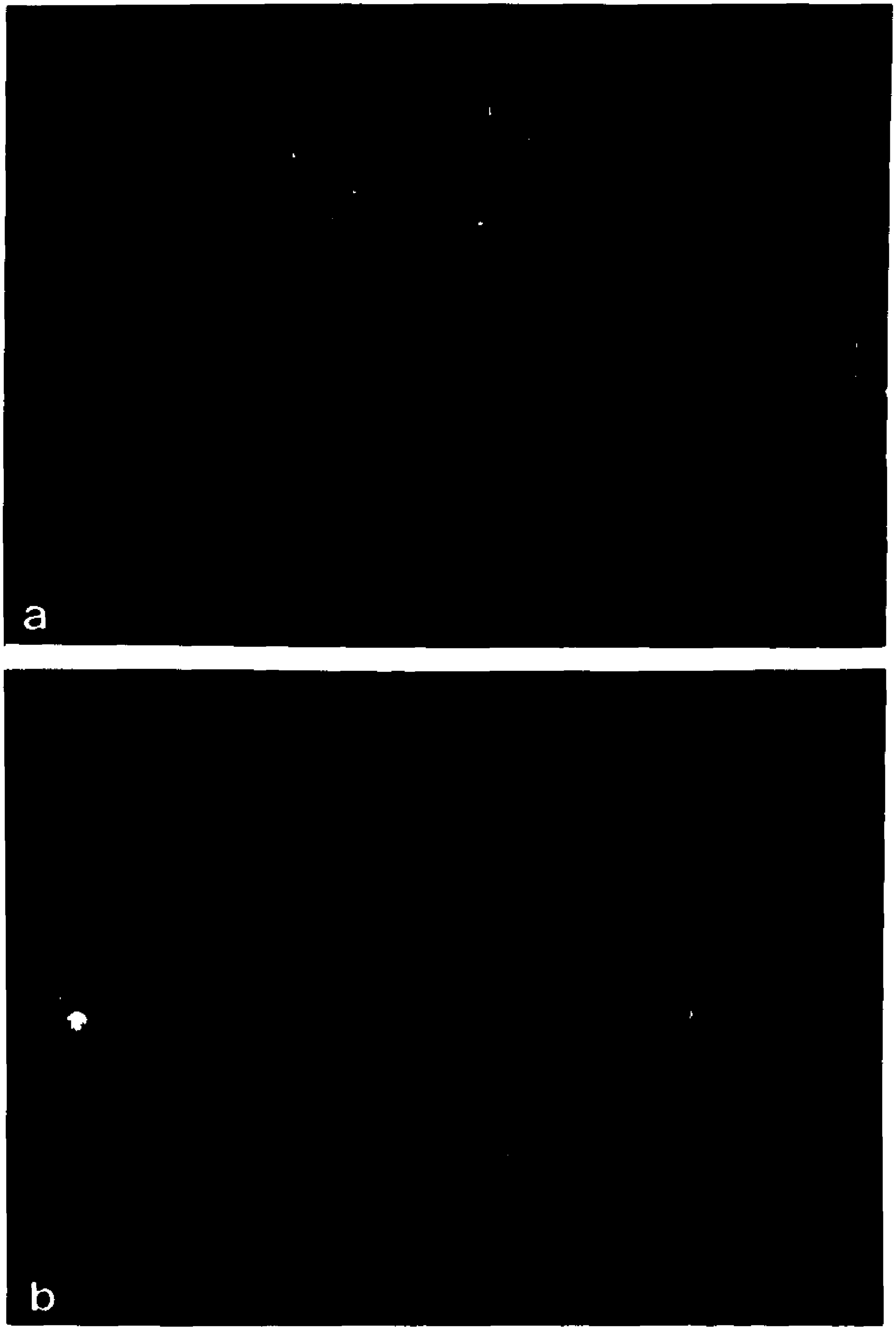

a


b

other terrestrial nonhuman mammals examined, these primates have an epiglottis which contacts the soft palate, overlapping behind it. The nasopharynx and laryngopharynx are visible above the larynx and soft palate, opening into the nasal cavity. Unlike adult humans, these primates do not have an enlarged oropharynx, and the posterior third of the tongue does not form the anterior wall of the pharynx. Rather, it is contained completely within the oral cavity (see chapter 3). The skull base is relatively flat and aligned in a roughly horizontal plane.

The position of the larynx is found to be high in the neck in all mammals listed in table 1. This high laryngeal position would allow for efficient separation of the respiratory and digestive pathways. The epiglottis overlaps the soft palate thus providing a continuous pathway for air from nose to lungs, and a separate pathway for food from mouth to stomach (Reidenberg and Laitman, 1986a). The larynx, from the tip of the epiglottis to the bottom of the cricoid cartilage, is usually positioned from the level of the basiocciput or first cervical vertebra to the level of the second, third, and occasionally fourth cervical vertebrae. The basicranium is found to be relatively flat in the mammals studied. Using the hard palate as a reference plane, the basisphenoid and basioccipital

Figure 5: a) Midsagittal section through the head of a cynomolgus monkey (crab-eating macaque), (*Macaca fascicularis*). b) Midsagittal section through the head of an olive baboon, (*Papio anubis*). Note the contact between the epiglottis and soft palate.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bones were found to be aligned in a largely horizontal plane roughly parallel to the plane of the hard palate. (For detailed discussion of the anatomy in cetaceans and humans, see chapter 3.)

Discussion

Digestive tract and larynx: the two pathway system

The digestive pathway in the animals studied runs uninterrupted from the mouth to the stomach. Food can be collected and manipulated by the lips, teeth, and tongue. Next, food may be propelled from the oral cavity into the oropharynx via the isthmus faucium. Normally, this opening appears to be closed by the base of the tongue, and may open only during swallowing. If the food is liquid or soft in consistency, it may be divided by the exposed portion of the erect epiglottis to pass through the piriform fossae to either side of the larynx in the channels of the piriform sinuses. During the swallow, the larynx is elevated, the arytenoids tilt towards the epiglottis, and the vocal folds close to seal the larynx from accidental inspiration of food. The usual path of food is from the mouth, through the lateral food channels (piriform sinuses) of the laryngopharynx, into the esophagus, and then the stomach (Laitman, 1977; Laitman et al., 1977; Laitman and Crelin, 1980b).

Carnivores devour large pieces of meat which may often be too large to bypass the larynx via the narrow and shallow lateral food channels. Instead, large chunks may be forced to pass over the larynx. In order to prevent choking, the laryngo-tracheal opening must be closed or protected, and thus respiration may momentarily cease as the bolus passes over the larynx. This movement may be aided by the folding over of the epiglottis, which may also act a secondary protective mechanism shielding the glottis from food.

Unlike carnivores, herbivores must consume much more food and thus a major portion of the day is spent feeding. It would be disadvantageous if an herbivore was required to cease breathing with every swallow, as this would interrupt not only its respiration, but also its olfaction and thereby decrease its awareness of approaching predators (Negus, 1927; 1942). As a result, the mechanism described below probably developed to allow deglutition and respiration to occur simultaneously.

In herbivores, the diet consists of well masticated food, which, when swallowed, is in a semi-liquid form. Portions of the anterior wall of the erect epiglottis, combined with the anterior surface of the soft palate, may function as a splashguard (like the bow of a boat). Soft foods reaching the interlocked epiglottis and soft palate may be separated by this structure and diverted to either side of the larynx, via the piriform recesses, en route to the esophagus (Laitman et al., 1977). Additionally, the lateral food channels are widened to accommodate the constant stream of liquid or semi-liquid food. The medial wall of the piriform recess, the aryepiglottic fold, prevents inundation of the larynx by food (Negus, 1955). The larynx is found high in the pharynx with the epiglottis protruding beyond the nasopharynx into the nasal cavity. The tip of its curved anterior surface interlocks with the curved posterior surface of the soft palate (Bosma and Fletcher, 1961). Thus, although the structures are anatomically separable, they act physiologically as one, forming a protective wall for the respiratory tract. The anterior epiglottic surface shunts fluids laterally to the piriform recesses, while the posterior surface funnels air directly into the larynx, and then into the trachea and lungs.

Carnivores may also utilize this arrangement for maintaining a patent airway while swallowing liquid, semi-liquid food, smaller pieces of meat, or while the mouth is occupied (e.g., carrying or grooming its young; carrying, dragging, killing or dismembering prey; carrying or toying with other objects during play or while constructing a nest or shelter).

Although the epiglottis may protect the larynx from the food, it is thought to function primarily in the process of respiration and olfaction. The epiglottis guides the larynx cranially and dorsally to fit just behind the soft palate, thereby locking into the nasopharynx and establishing a separate airway (Laitman et al., 1977).

It has been argued that the intranarial epiglottis is also important in the sense of olfaction (Negus, 1927). Since feeding animals are subject to predation, their vulnerability can be reduced by increasing their awareness of their environment. A very important sense for detecting potential predators is olfaction. Since the epiglottis is intranarial, the two pathway system of respiration and deglutition is utilized (Laitman and Crelin, 1976; Laitman, 1977). Without a two pathway system, important scent-bearing air would be inspired more readily via the wider oral opening rather than through the narrow nasal openings, and thus the olfactory sense would be dulled. In order to sharpen the sense of smell, the mouth would have to be closed and feeding would have to cease. With an intranarial epiglottis, however, air from the mouth is less likely to enter the lungs, thus leaving the mouth free for eating and drinking, while the nose is simultaneously freed for breathing and smelling. In this way the important scent-bearing air is forced to pass exclusively over the olfactory mucosa, allowing the animal to spend more time eating while retaining a continually utilized keen sense of smell (Negus, 1957). The intranarial epiglottis would also be of great importance in this fashion in carnivores which posess a keen sense of smell.

Although the normal position of the mammalian larynx is intranarial, with the epiglottis contacting the soft palate, there are occasional conditions when this connection can be broken. Unlocking the epiglottis from behind the soft palate occurs during oral ventilation (such as panting) or oral vocalization (such as barking or squealing) (Bowles, 1889; Laitman, 1977; Laitman and Crelin, 1980). While an antevelar position of the epiglottis allows for brief oral expirations, oral inspiration does not appear to be possible. This is because both negative pressure and the inspiratory air current might suck the epiglottis against the soft palate, thus blocking the oral route of inspiration. In addition, this arrangement is not desirable, since it would initiate a vagal response from the tactile stimulation of the soft palate against the posterior surface of the epiglottis.

Summary

Several muscles, one ligament (stylohyoid), a cartilaginous or bony chain (stylohyoid chain or hyoid apparatus), and one connective tissue (aponeurosis of the median pharyngeal raphe) were found to attach to the ventral surface of the skull base. These structures were also found to be responsible for suspending the hyoid bone and the larynx from the basicranium. A flat basicranium was noted to be the general pattern among the terrestrial mammals. The larynx was found to be positioned high in the neck with the epiglottis inserted intranarially.

CHAPTER 3

UPPER RESPIRATORY TRACT ANATOMY OF CETACEANS AND HUMANS:

How these two groups differ from the typical mammalian pattern

The Basic Mammalian Pattern

As described in the previous chapter, the position of the larynx in the neck largely determines a mammal's breathing, swallowing, and vocalizing abilities. Studies of terrestrial mammals have shown that a larynx located high in the neck allows the epiglottis to contact the soft palate and overlap behind it (see chapter 2). This places the laryngeal vestibule in close proximity to the internal nares, in what has been called the intranarial position of the epiglottis (Howes, 1889; Bowles, 1889; Wood Jones, 1940; Negus, 1949; Laitman and Crelin, 1976). In most terrestrial mammals, the larynx is positioned opposite the basiocciput or the first cervical vertebra, and extends as low as the second or third cervical vertebrae (Laitman and Reidenberg, 1988). The intranarial epiglottis allows for efficient separation of the respiratory/olfactory and the digestive pathways. It can be unlocked from behind the soft palate to permit oral ventilation (as in panting) or oral vocalizations, or occasionally, to swallow a very large bolus of food (Bowles, 1889; DuBrul, 1976; Laitman and Crelin, 1980; Laitman et al., 1977).

While we are beginning to understand the functional anatomy of the upper respiratory tract in mammals, our knowledge is largely limited to the general anatomical pattern of this region in typical terrestrial mammals. In order to further understand the factors influencing the position of the larynx, specimens whose anatomy differs from this general pattern were studied. In such specimens, anatomical structures which may be important for determining laryngeal position, such as the basicranium, may differ from the general pattern seen in typical terrestrial mammals. Any changes in laryngeal position may then be related to these basicranial changes.

For this study, specimens whose anatomy naturally deviated from the typical mammalian pattern were examined. In this case, subjects of this "natural experiment" have had their skeletal and respiratory anatomy altered through the natural effects of evolutionary change. Two mammalian groups were chosen: cetaceans (whales, including dolphins and porpoises) and humans. These mammals are adapted for respiratory activity under conditions very different from that of most terrestrial mammals. Cetaceans are most appropriate for this study, as the anatomy of their respiratory tract is considerably modified from the terrestrial pattern to accomodate their aquatic existence. Humans, on the other hand, show a pattern radically different from that of most terrestrial mammals. The anatomy of the human upper respiratory tract is highly modified, allowing for the unique use of oral tidal respiration, as well as the production of complex speech sounds.

CETACEAN ANATOMY

Odontocetes (toothed whales, including dolphins and porpoises) exist in a fully aquatic environment. Alterations in their external anatomy reflect these adaptations. In this study, the anatomy of their upper respiratory tract is examined to determine whether the intrinsic anatomy and position of the larynx is also modified for an aquatic existence. First, this study investigates the anatomy of the odontocete upper respiratory tract, focusing on the intrinsic anatomy of the larynx, such as the appearance of the vocal folds. Second, the position of the larynx relative to other upper respiratory tract structures, particularly the basicranium, is examined. The function of the larynx during feeding, breathing, and sound production for communication and echolocation is also discussed.

Materials and Methods

Twenty-four odontocetes representing ten genera (*Delphinus*, *Stenella*, *Lagenorhynchus*, *Tursiops*, *Grampus*, *Delphinapterus*, *Globicephala*, *Kogia*, *Mesoplodon*, and *Phocoena*) were examined post mortem. Specimens were obtained from the Marine Mammal Stranding Center (Brigantine, NJ), the New England Aquarium (Boston, MA), the Mystic Marinelife Aquarium (Mystic, CT), the Okeanos Ocean Research Foundation (Hampton Bays, NY), the New York State College of Veterinary Medicine at Cornell University (Ithaca, NY), and the New York Zoological Society (New York, NY).

Species, sex, age, length, condition of death, and method of dissection for each specimen are given in table 2. The age of each specimen was determined by the following criteria: fetus - umbilical cord still attached; infant - fringed tongue, teeth not yet erupted, pre-weaned (milk diet), small size; juvenile - dentition erupting or erupted with relatively little wear, immature skin color or scarring patterns (e.g.

Delphinapterus or Grampus), weaned (fish or squid diet), sexually immature (determined by postmortem examination of genitalia at autopsy); adult - full dentition erupted with moderate or extensive wear or tooth loss, mature skin color or pattern, sexually mature. For consistency in identifying our specimens with those described by others, the taxonomic classification of Honacki et al. (1982) has been used. As the common names for many of these species vary regionally, only the genus and species names will be used here. The sample size for this study is large, considering the restricted availability of these protected, and often rare, species. Of the 24 specimens studied, 20 were recovered dead from beach strandings along the northeast Atlantic coastline of the United States, two Tursiops died in captivity, Stenella accidentally drowned in a fishing net, and Delphinapterus was found shot dead of bullet wounds. Specimens were frozen to prevent spoilage and to facilitate cutting with a Biro electric band saw. They were then preserved by immersion fixation in phosphate buffered 10% formalin.

Specimens were examined through either whole head bisection or by opening the larynx from a dorsal approach. In nine specimens, the head was midsagittally bisected and the larynx examined in situ.

The bisected heads allowed easy visualization of the skeletal level of the larynx, as well as the lateral attachments of any internal folds of the larynx. Twelve specimens could not be bisected for reasons of excessive size or condition of preservation. For these specimens, the position of the larynx was noted in situ before the larynx was removed. The larynx was excised whole from a ventral approach, noting the relationship of the larynx and other respiratory and digestive structures to each other and to the skeletal anatomy. Isolated larynges were then opened from a dorsal approach to reveal internal anatomy. The cricoid cartilage was cut in the dorsal midline and reflected laterally. Opening the larynx from this approach afforded another perspective of any internal folds. In three additional specimens, the larynx was examined in situ from a dorsal approach before the head was bisected. In this procedure the hyoid bone was detached from the skull on one side and reflected inferiorly. The larynx was disengaged from the palatopharyngeal sphincter, retracted laterally, and then incised along its dorsal midline. After the folds were examined and photographed, the larynx was replaced in anatomical position and the head subsequently bisected along the midsagittal plane.

The position of the larynx was measured relative to vertebral levels and portions of the skull base. The level was determined by extending a line perpendicular to the long (horizontal) axis of the larynx and trachea and noting which skeletal structures were intersected. The lines were drawn at the most rostral (anterior) and the most caudal (posterior) portions of the larynx. The most rostral extension of the odontocete larynx is the epiglottis, and the most caudal part is the caudal border of the body of the cricoid cartilage.

The internal anatomy of the larynx was also examined histologically. Tissues from folds found inside the larynx were taken from Delphinapterus, Kogia, and Mesoplodon. They were fixed in phosphate buffered 10% formalin, embedded in paraffin, cut at 6 microns in thickness, and stained with hematoxylin and eosin. Additional sections from each specimen were also stained with Masson's trichrome to better visualize and differentiate muscle cells and connective tissue. In describing laryngeal anatomy, the term "fold" will refer to both its surface layer and the underlying internal components contained within.

Dissections

Anatomy of the odontocete skull

The odontocete skull shows many unusual modifications which are probably adaptations for existence in an aquatic environment. The piriform aperture (anterior nasal aperture) is located on the dorsum of the skull near the nuchal crest, and is usually offset to the left side (Yurick and Gaskin, 1988). The openings are sloped so that the rostral border is lower than the caudal border. In the intact specimen, these two openings are asymmetrical in size (Arvy, 1977), and lead to a fleshy tube, which terminates as single opening called the blowhole (external nares).

Anteriorly, the rostrum is composed of the premaxilla and maxilla, and contains the vomer, palatine bone, and a large cartilaginous "septum." This cartilaginous structure does not actually divide the nasal cavity into right and left sides, as in other mammals, since the odontocete nasal cavity is not located in the rostrum. There do not appear to be any conchae in either the rostrum or the bony nasal cavity. The rostrum supports several conical teeth (e.g., in dolphins and larger toothed whales, such as the sperm whale) or spade-shaped teeth (e.g., in porpoises).

Odontocetes are homodonts, posessing a variable number of teeth depending upon species, sex, and age (see Gaskin, 1982 for discussion of odontocete tooth morphology in relation to diet). Posteriorly and inferiorly (ventrally), the rostrum joins the rest of the cranium at the greatly enlarged pterygoid plates.

The nasal bones are greatly reduced. Only very small portions of the frontal and parietal bones are visible on the external (exocranial) surface, since they are largely covered by the maxilla and occipital bone. The parietal and frontal bones are visible on the internal (endocranial) surface. This overlapping of the skull bones contributes to the unusual thickness of the skull. The ethmoid bone is also visible on the endocranial surface, but it is located anteriorly. Although Kernan (1918) reports the presence of a crista galli in Cuvier's beaked whale (Ziphius cavirostris), there was no such structure observed in the odontocetes dissected in this study. There were, however, depressions or pits in the cribriform plate despite the absence of olfactory bulbs (Rankin, 1956; Swerdtfeger et al., 1984). No perpendicular plate of the ethmoid was observed.

The lateral surface (figure 6b), displays a relatively small temporal bone, no sagittal crest, and a very thin zygomatic bone. This is probably related to a reduction in the surface area for attachment of the temporalis and masseter muscles. Odontocetes do not chew their food, but rather, swallow it whole (and thus their homodont dentition resembles that of reptiles). As a result, the masseter and temporalis muscles are much reduced (see Lawrence and Schevill, 1965 for muscular anatomy of delphinid head and neck). Below the mastoid is a loose tympano-periotic complex, composed of two fused bones which are acoustically isolated from the skull (Oeschlager, 1986).

The odontocete basicranium, as seen in the midsagittally bisected heads (figures 7, 8, and 9), is aligned in the horizontal plane. The midline of the basioccipital and basisphenoid bones comprises a flat line, which is continuous with the line of the midline of the hard palate. The foramen magnum is aligned in the vertical plane. This is consistent with the horizontal alignment of the skull with the cervical vertebrae. It is interesting to note that the posterior portion of the basioccipital bone is curved dorsally, just at the inferior edge of foramen magnum (basion). This gives the otherwise flat odontocete basicranium a slight posterior lordosis. When compared with typical terrestrial mammals, the odontocete skull base thus appears to be an exaggeration of the "standard plan" of a flat basicranial contour. In fact, it is flatter than most mammals in the middle and anterior portions, and is even slightly lordosed in the posterior region.

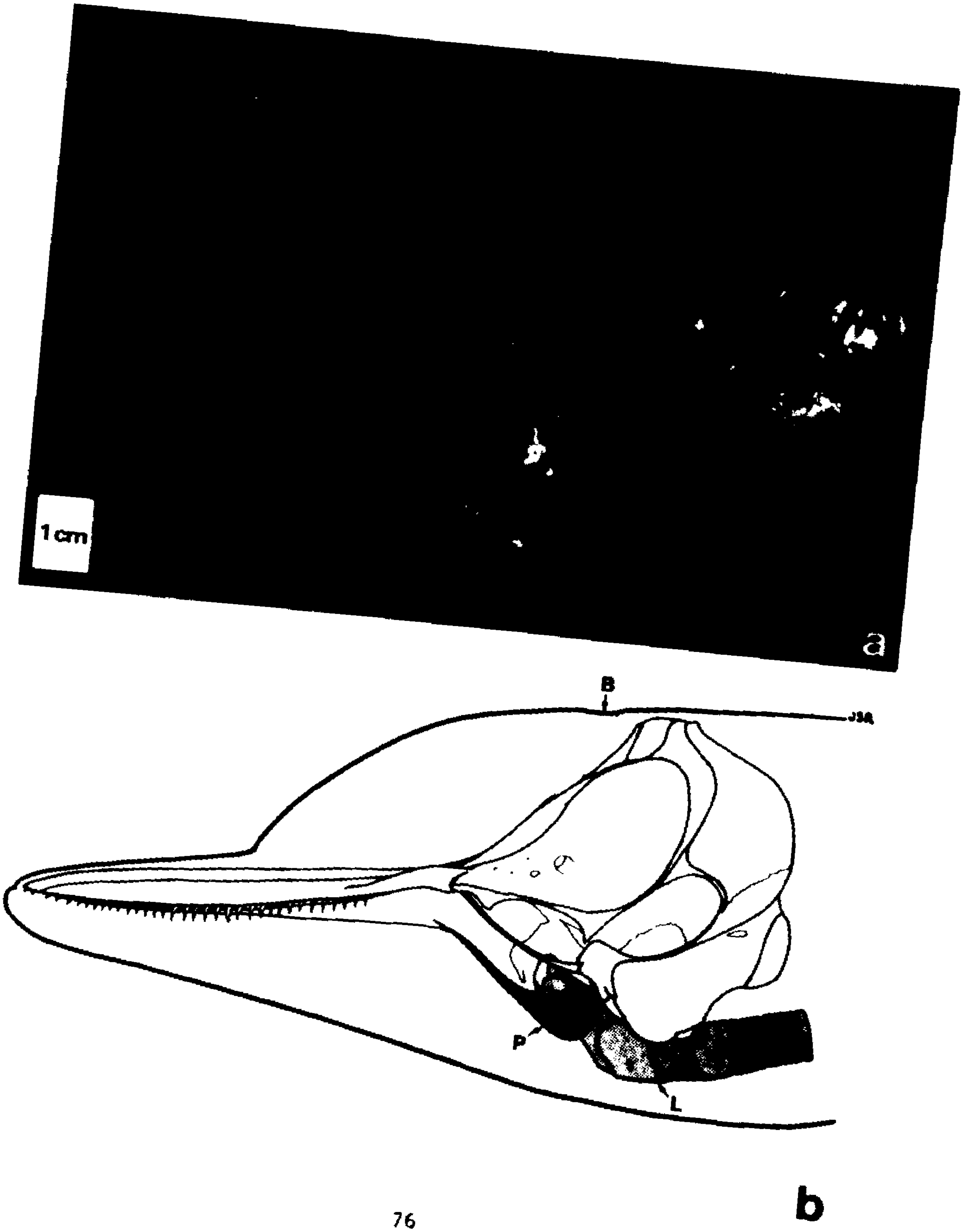
Nasal anatomy

Cetaceans, perhaps more so than most terrestrial mammals, are obligate nose breathers. All breathing occurs through the blowhole (a single nostril in odontocetes, and a pair of nostrils in mysticetes) which is located on top of the head (Lawrence and Schevill, 1956; Mead, 1959; Schenkkan, 1973). The blowhole is often asymmetrically placed to the left of the midline. The opening is sealed by the nasal plug, a muscular tongue-like structure. There are a number of expandable sacs attached to the nasal tract external to the skull. These sacs can inflate with air (and perhaps water in some species, such as the sperm whale [Clarke, 1978]). Expansion of the tubular sac, which is located just under the blowhole, may aid the nasal plug by pneumatically sealing the blowhole (Lawrence and Schevill, 1956). Although the premaxillary and vestibular sacs have been observed to move (inflate, deflate, vibrate) during sound production, it is not clear what role these sacs play in producing or modifying vocalizations for communication or echolocation. The bony nares consist of two (often assymetrically sized) curved tubes passing through the skull. There are no conchae or olfactory epithelium in the odontocete nose, and the absence of olfactory bulbs in the brain indicates an anosmotic condition (Evans and Maderson, 1973; Swerdtfeger et al., 1984).

Intrinsic Anatomy of the Odontocete Larynx

The odontocete larynx is composed of the same homologous cartilages as are found in terrestrial mammals: epiglottic, corniculate, arytenoid, cuneiform, cricoid, and thyroid cartilages (Reidenberg and Laitman, 1987a; 1987b; 1987c). An example of an odontocete larynx is pictured in figure 6a. The epiglottis forms the most rostral extension of the larynx. It is elongated and trough shaped, with its tip curled forward to create a rounded lip at the laryngeal aditus. It is opposed posteriorly by a pair of elongated cartilages which fit into the trough-shaped cradle of the epiglottis. These elongated cartilages articulate with the superior-rostral surface of the paired arytenoids, and thus appear to be the corniculates. Although the corniculate cartilages are unusually large compared with those of other mammals, their location and site of articulation appear homologous with that of the corniculates of terrestrial mammals. Together, the

Figure 6: a) Left lateral view of a dissected larynx of Stenella coeruleoalba (striped dolphin). b) Schematic drawing of a typical dolphin head showing placement of the larynx under the skull base, with the laryngeal spout encircled by the palatopharyngeal sphincter. B = blowhole, C = corniculate , E = epiglottis, T = thyroid cartilage, A = arytenoid, Cr = cricoid, Tr = trachea, P = palatopharyngeal sphincter, L = larynx, White arrow indicates laryngeal aditus. Figure from Reidenberg and Laitman, (1987a).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a
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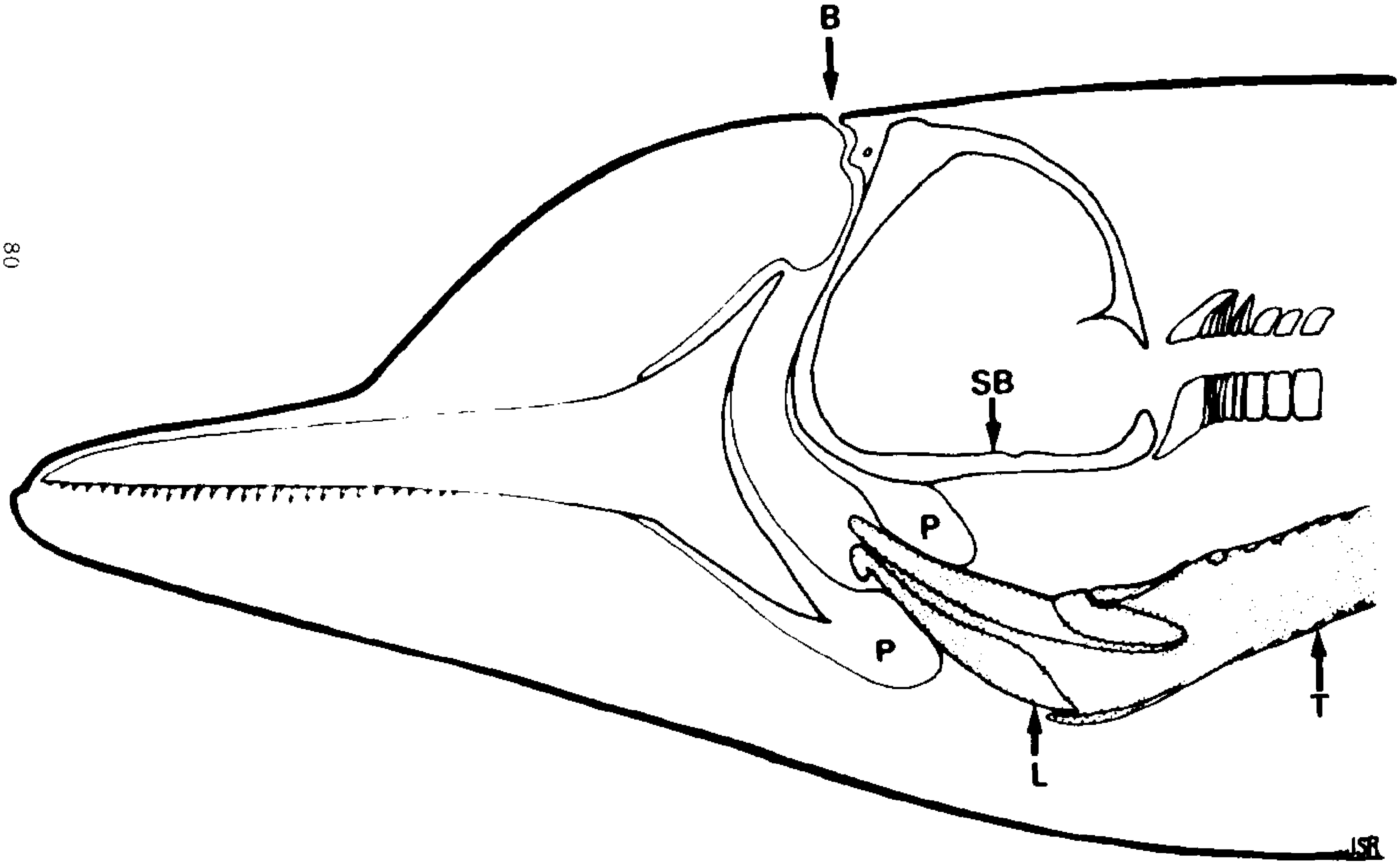
corniculates and the epiglottis compose the laryngeal spout (also called the laryngeal tube, epiglottic spout/tube, cuneo-epiglottic spout/tube, or aryepiglottic spout/tube).

The arytenoid cartilages articulate with the rostral-dorsal surface of the cricoid. Our observations confirm previous findings (Hosokawa, 1950; Blevins and Parkins, 1973; Green et al., 1980) of a large, synovial cricoarytenoid joint, whereas the other laryngeal joints are fibrous. A small pair of cartilages, which may be remnants of the cuneiforms, is found caudal to the epiglottis and ventral to the articulation of the corniculates and arytenoids. The cricoid cartilage is unusual in that it is not a complete ring as in other mammals, but instead opens ventrally. This ventral opening is largely covered by the thyroid cartilage, which opens dorsally. The trachea is short and composed of complete cartilaginous rings. It trifurcates into two right bronchi and one left bronchus.

It is of note that considerable confusion exists concerning the naming of the paired laryngeal cartilages. In some studies on cetaceans, for example, the cartilages described here as the corniculates (cartilages of Santorini), have been varyingly identified as the cuneiforms or cartilages of Wrisberg, arytenoids, supra-arytenoids, or the corniculate processes of the arytenoids. For a more detailed discussion of the intrinsic anatomy of the odontocete larynx, see Murie (1870), Howes (1880), Thompson (1890), Rawitz (1900), Blevins and Parkins (1973), Green et al. (1980), Purves and Pilleri (1983), and Reidenberg and Laitman (1987b); and for the mysticetes (baleen whales), see Hosokawa (1950), Sukhovskaya and Yablokov (1979), and Henry et al. (1983).

The internal, ventral surface of the odontocete larynx shows grossly observable midline folds which appear to be a continuation of the internal laryngeal membrane (cricothyroid ligament or cricovocal membrane of terrestrial mammals). They span from the arytenoid cartilage to the thyroid cartilage at the epiglottic base. The morphology of the midline folds is variable. They appear as: 1) true bifurcated structures reminiscent of the condition in terrestrial mammals; 2) folds exhibiting a trifurcated appearance; or 3) a single, large midline fold (Reidenberg and Laitman, 1988a). Histological examination of Delphinapterus, Kogia, and Mesoplodon, shows that the folds contain a large component of collagen and elastic tissue. Although the epithelial lining is totally decomposed, muscle fibers and mucous glands appear to be present in some sections. Given these similarities of gross

Figure 7: Schematic drawing of dolphin showing interlocking of larynx into nasopharynx. B = blowhole, SB = skull base, P = palatopharyngeal sphincter, L = larynx, T = trachea.


B
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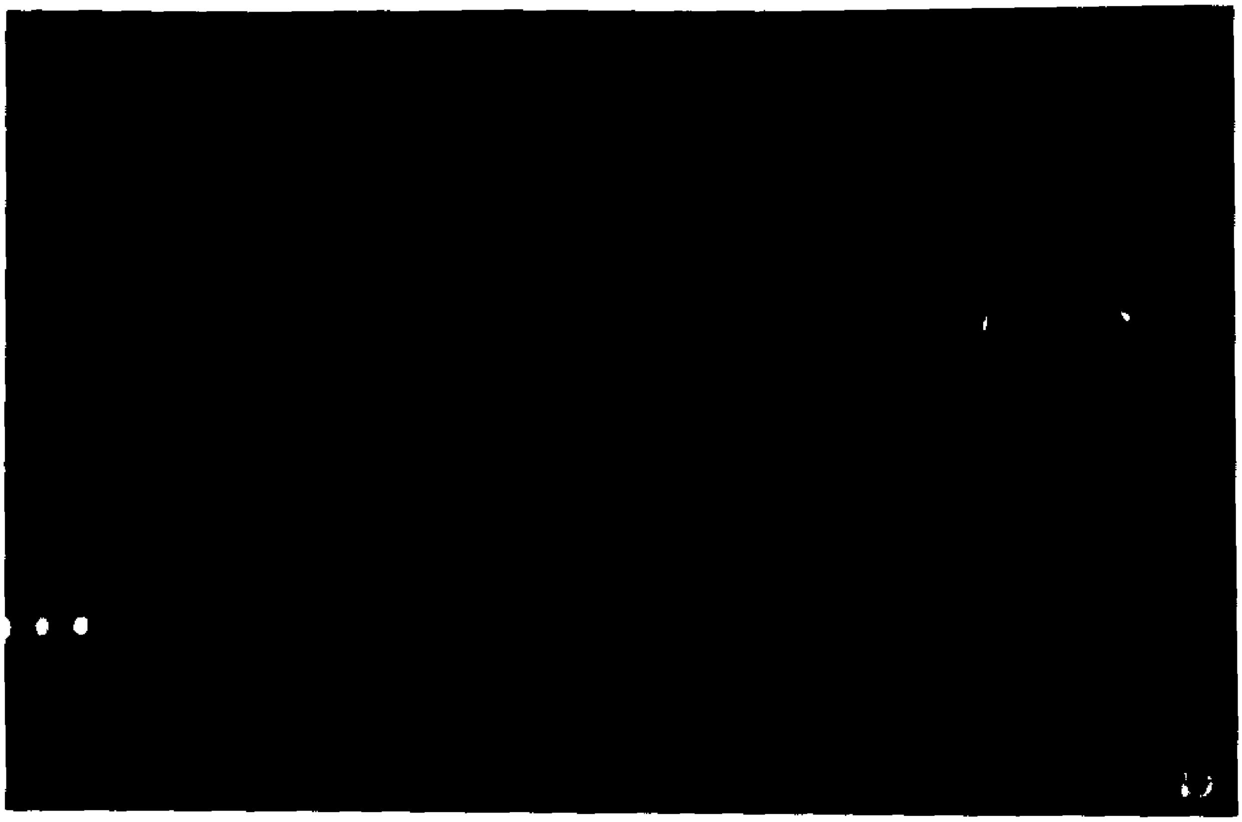
morphology, attachment, and histological composition, the midline and lateral folds appear structurally homologous to the vocal and vestibular folds of terrestrial mammals. These findings are particularly interesting, given the long-standing belief that cetaceans lack vocal folds. For a more complete discussion on odontocete vocal folds, and the historical arguments surrounding their existence, see Reidenberg and Laitman (1988b).

Positional Relationships of the Odontocete Larynx

The odontocete larynx is located under the skull base, rather than adjacent to the cervical vertebrae as in terrestrial mammals (Figs. 7, 8 and 9). As noted in chapter 2, the position of the larynx ranges from the levels of the basiocciput or first cervical vertebra to the second, third, or occasionally fourth cervical vertebrae in most terrestrial mammals. In all the odontocete specimens studied, however, the larynx is positioned directly under the skull base such that the entire structure, from the tip of the laryngeal spout to the caudal border of the cricoid cartilage, lies rostral to the level of the first cervical vertebra (Reidenberg and Laitman, 1986b). The epiglottic tip is usually found at the level of the presphenoidal synchondrosis (also referred to as the

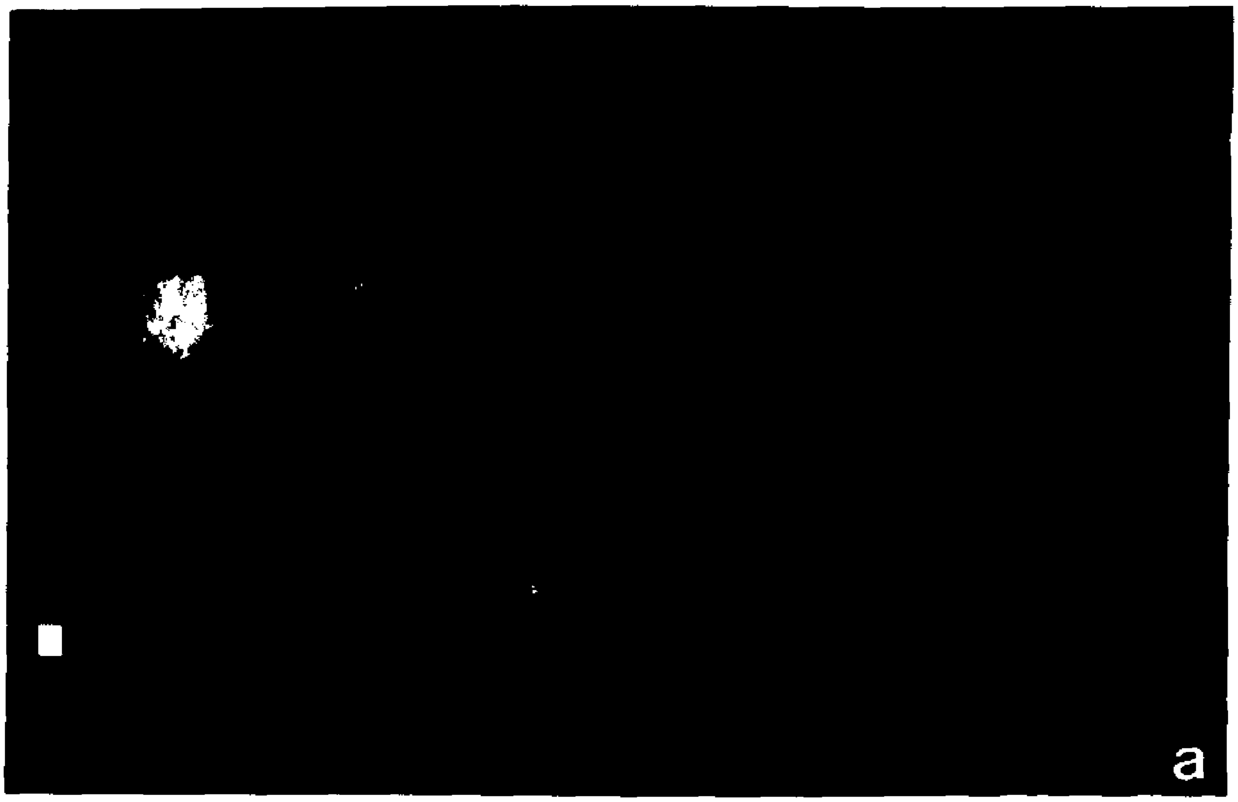
Figure 8: a) Midsagittal view of _Delphinus delphis_ (common dolphin). b) Midsagittal view of _Grampus griseus_, (Risso's dolphin). B = blowhole, PS = presphenoidal synchondrosis, C = corniculate, P = palatopharyngeal sphincter, E = epiglottis, T = thyroid cartilage, Cr = cricoid cartilage. Figure from Reidenberg and Laitman (1987a).



prespheno-basisphenoidal, intersphenoidal, or midsphenoidal synchondrosis) of the skull base, directly posterior to the most caudal portion of the vomer. The most caudal portion of the cricoid cartilage is usually found at the level of the foramen magnum. The levels of the larynx in the specimens studied are given in table 3. Gross midsagittal sections of the head and neck of four representative odontocetes are shown in figures 8 and 9.

The position of the odontocete larynx appears to be the result of three anatomical modifications of the cetacean body (Reidenberg and Laitman, 1987b). First, the structure of the larynx has been modified so that the epiglottis and the corniculate cartilages form an elongated laryngeal spout which traverses the digestive tract into the nasal cavity, where it remains in the erect position. The elongated epiglottis allows for an extensive degree of overlap of the epiglottis and the soft palate in the specimens studied. This arrangement extends the most rostral portion of the larynx into the nasopharyx, to the level of the presphenoidal synchondrosis of the skull base. In all of the specimens, the laryngeal aditus lies completely within the separate nasopharyngeal chamber, opening directly into the posterior choanae. The larynx appears to be permanently intranarial. It could only be retracted

Figure 9: a) Midsagittal view of *Globicephala melaena* (pilot whale). b) Midsagittal view of *Phocoena phocoena*, (harbor porpoise). B = blowhole, PS = presphenoidal synchondrosis, C = corniculate, P = palatopharyngeal sphincter, E = epiglottis, T = thyroid cartilage, Cr = cricoid cartilage. Figure from Reidenberg and Laitman (1987a).


a


b

from this position with great difficulty when dissected from a ventral approach. The nasopharynx is sealed ventrally by the sphincteric action of the palatopharyngeal muscle (also called the arcus palato-pharyngeus or the laryngo-pharyngeal sphincter). The laryngeal spout is completely surrounded by the lateral food channels (piriform fossae) which open anteriorly into the oral cavity and posteriorly into the esophagus. There is no true laryngopharynx, as in humans, since the opening into the laryngeal vestibule lies within the separate chamber of the nasopharynx. Since there is no separation of the larynx and the soft palate, there is no space lying simultaneously superior to the larynx and inferior to the soft palate, as in adult humans. As a result, an enlarged supralaryngeal oropharynx cannot exist. Since both the epiglottis and the corniculate cartilages are intranarially located, odontocetes may be the only mammals which have a truly intranarial laryngeal aditus.

The second modification of the cetacean body is the elimination of a functional neck region. The cervical vertebrae are highly compressed and some were fused in the specimens studied. Most pelagic odontocetes have lost the flexibility of their necks as a result of the compression and sometimes fusion of the cervical vertebrae (Evans, 1973; Pilleri et al.,

TABLE 2 Specimens studied

Genus species	Sex	Age	Length (cm)[1]	Condition of death	Method of opening larynx
Delphinus delphis (common dolphin)	F	Adult	226.0	Beach-stranded	Bisected head[2]
	F	Adult	231.1	Beach-stranded	Bisected head
	F	Juvenile	—	Beach-stranded	Bisected head
Stenella coeruleoalba (striped dolphin)	F	Juvenile	131.0	Drowned in net	Opened dorsally[3]
Lagenorhynchus acutus (whitesided dolphin)	M	Adult	247.0	Beach-stranded	Opened dorsally then bisected[4]
	F	Adult	239.0	Beach-stranded	Opened dorsally
Lagenorhynchus albirostris (whitebeaked dolphin)	F	Adult	250.0	Beach-stranded	Opened dorsally
Tursiops truncatus (bottlenosed dolphin)	F	Adult	270.0	Died in captivity	Opened dorsally
	F	Adult	262.5	Beach-stranded	Opened dorsally, then bisected
	M	Adult	237.5	Beach-stranded	Opened dorsally
	M	Adult	225.0	Beach-stranded	Opened dorsally
	M	Fetus	114.0	Died in captivity, stillborn	Opened dorsally, then bisected
Grampus griseus (Risso's dolphin)	M	Adult	289.6	Beach-stranded	Bisected head
Delphinapterus leucas (beluga whale)	F	Juvenile	313.0	Found dead of bullet wounds	Opened dorsally
Globicephala melaena (pilot whale)	F	Juvenile	289.6	Beach-stranded	Opened dorsally
	F	Fetus	127.0	Removed from beach-stranded mother	Bisected head
	F	Fetus	121.0	Removed from beach-stranded mother	Bisected head
Kogia breviceps (pygmy sperm whale)	M	Adult	290.0	Beach-stranded	Opened dorsally
Mesoplodon mirus (True's beaked whale)	F	Adult	464.8	Beach-stranded	Opened dorsally
Phocoena phocoena (harbor porpoise)	—	Adult	—	Beach-stranded	Opened dorsally
	M	Adult	137.2	Beach-stranded	Opened dorsally
	F	Juvenile	111.8	Beach-stranded	Bisected head
	M	Infant	85.5	Beach-stranded	Bisected head
	F	Infant	84.3	Beach-stranded	Bisected head

[1] Lengths measured in centimeters from the tip of the rostrum to the midline tail notch.

[2] Whole head was bisected in the midsagittal plane and the larynx examined in situ.

[3] Larynx was removed whole from head and opened along the dorsal midline, leaving ventral region intact for examination.

[4] Larynx was retracted laterally, opened along its dorsal midline, examined, replaced in anatomical position, and then whole head bisected along the midsagittal plane

—Indicates information not available

Table from: Reidenberg and Laitman, (1987a).

TABLE 3 Laryngeal position in odontocetes studied

Genus species	Common name	Sex	Age	Skeletal level of the larynx
Delphinus delphis	Common dolphin	F	A	Eth to OC
		F	A	PS to OC
		F	J	PS to FM
Grampus griseus	Risso's dolphin	M	A	Eth to FM
Globicephala melaena	Pilot whale	M	Fe	PS to FM
		F	Fe	BS to OC
Kogia breviceps	Pygmy sperm whale	M	A	PS to FM[1]
Mesoplodon mirus	True's beaked whale	F	A	PS to FM[1]
Phocoena phocoena	Harbor porpoise	M	A	PS to FM[1]
		F	J	BS to FM
		F	I	PS to FM
		M	I	PS to FM

Abbreviations: A, adult; BS, basisphenoid bone; Eth, ethmoid bone; F, female; Fe, fetus; FM, foramen magnum; I, infant; J, juvenile; M, male; PS, presphenoidal synchondrosis; OC, occipital condyles.

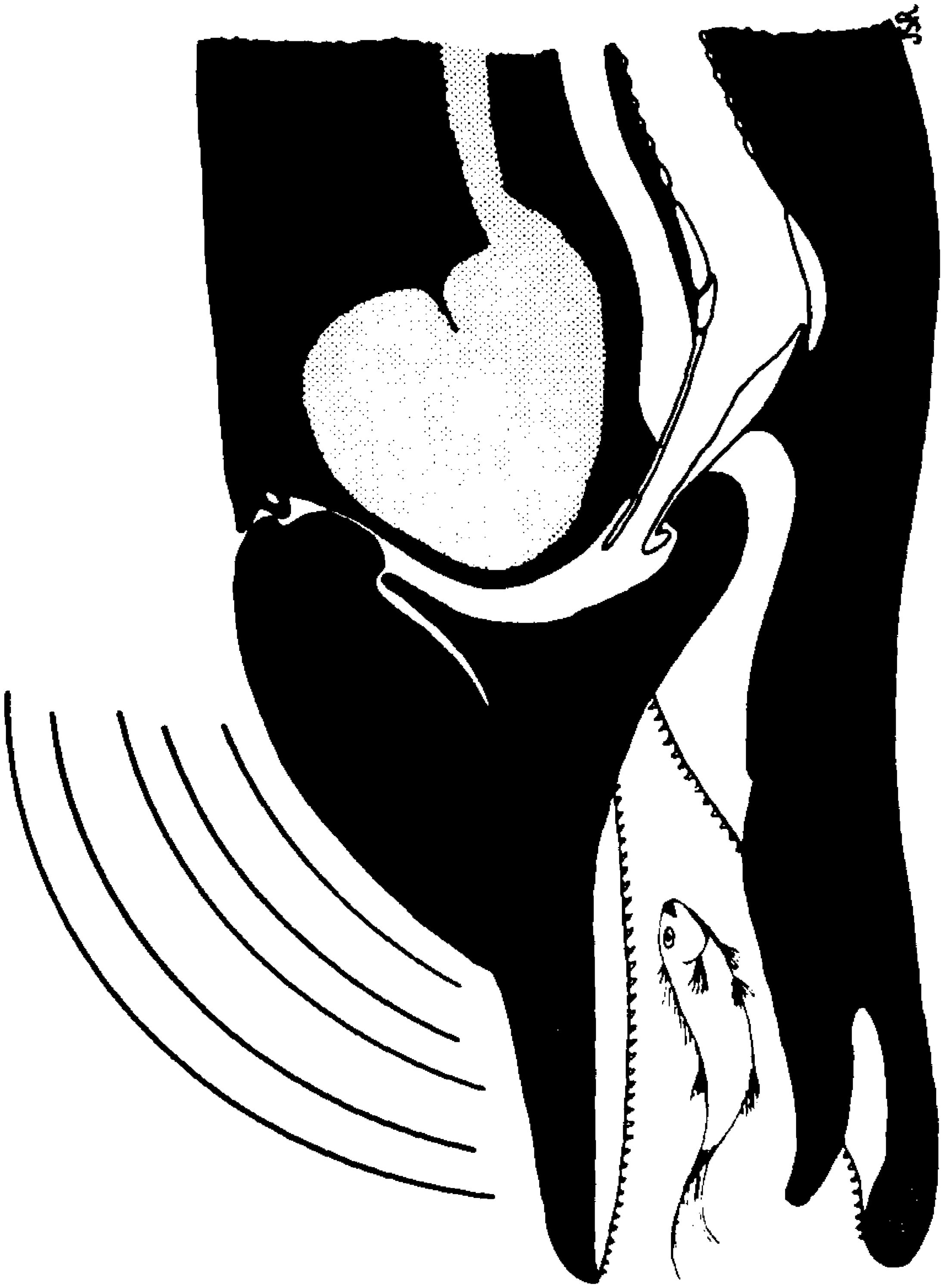
[1] Specimens used for measurements of skeletal levels were bisected in the midsagittal plane, with the exception of *Kogia breviceps*, *Mesoplodon mirus*, and the adult *Phocoena phocoena*, which were examined in situ.

Table from: Reidenberg and Laitman, (1987a).

1976a). This shortening of the cervical region appears to result in an elimination of an anatomical "neck" in many species, and a loss of mobility in this area. This restricts any significant flexion to the atlanto-occipital joint, and limits flexion in the cervical region. Through shortening of the neck, the cetacean head is followed almost immediately by the thorax, thus forcing the larynx out of the practically nonexistent cervical region and into a cranial position. The most caudal portion of the larynx (the caudal border of the cricoid cartilage) never reaches beyond the level of the occipital condyles. Restricted movement of the head may limit mobility of the larynx. The trachea is also correspondingly shortened, thus placing the carina very close to the cricoid cartilage.

The third rearrangement is the horizontal head position. Unlike terrestrial mammals, the head does not form an angle with the neck and body. Instead, the fusiform body of the odontocetes has resulted in a realignment of the skull base with the horizontal axis of the body. Both the skulls and the bodies of the specimens studied are aligned in the horizontal plane, while the foramen magnum is aligned in the vertical plane. As a result, the contour of the midline of the skull base lies either on the same plane or on a plane parallel with the axis of the cervical vertebrae. This

Figure 10: Schematic drawing of a typical dolphin breathing, swallowing, and echolocating simultaneously. The intranarial position of the laryngeal aditus allows complete separation of the respiratory and digestive pathways. This arrangement may permit the dolphin to swallow a fish while simultaneously emitting sounds for echolocation (represented by curved lines), prey stunning, or communication. The respiratory tract may also be kept separate from the digestive tract while breathing and swallowing simultaneously.



streamlined alignment of the skull and cervical vertebrae may be related to the lack of a functional neck. In terrestrial mammals, angulation of the head and neck allows the larynx to be situated simultaneously under the skull base and adjacent to the cervical vertebrae. In odontocetes, this is not possible as the position of the larynx under the skull base places it rostral to the cervical vertebrae. Since there is restricted movement in the heads of most pelagic odontocetes (Evans, 1973), their larynges do not appear to be significantly pulled or pushed out of position by slight flexions at the atlanto-occipital joint.

As a result of these unique modifications in the shape and positioning of the cartilages, the larynx seems to have lost some of its mobility. Unlike the larynx of terrestrial mammals, the odontocete larynx appears rigidly locked into the nasopharynx. The apposing, elongated corniculate cartilages seem to prevent the epiglottis from folding over caudally and unlocking from the nasopharynx to allow food to pass over it. These three cartilages are encircled by the palatopharyngeal sphincter. This sphincter contracts just under the curled lip of the epiglottis to form a seal around the laryngeal spout. This arrangement locks the laryngeal aditus into an intranarial position, providing a direct conduit for inspired air from the blowhole and nasal cavity to the larynx, trachea, and lungs. Contracting the palatopharyngeal sphincter around the laryngeal spout during deglutition prevents aspiration of water or food into the larynx, nasopharynx, or nasal cavity (Hosokawa, 1950). Thus, odontocetes are able to swallow a solid bolus of food lateral to the intranarially placed larynx (Rawitz, 1900; Green et al., 1980). These observations are further supported by the anatomical work of Lawrence and Schevill (1965) on the throat musculature of a number of odontocetes. They describe the powerful muscles which elevate and hold the larynx in its intranarial position, as well as the sealing action of the palatopharyngeal sphincter during deglutition. They also point out the lack of opposing musculature for retracting the larynx from behind the bony nares.

Odontocetes have developed wide lateral food channels for swallowing their prey whole without mastication. Since the epiglottis cannot fold over to allow bulky foods to pass over the larynx, food must pass via these piriform recesses around the larynx and into the esophagus during deglutition (Rawitz, 1900; Green et al., 1980). These large lateral food channels are completely isolated from the respiratory tract. The pharyngeal space in odontocetes has thus been greatly modified. There is no true laryngopharynx or enlarged oropharynx, as in adult humans, due to the intranarial placement of the laryngeal aditus. The nasopharynx is sealed ventrally from the digestive pathway by the palatopharyngeal sphincter. The odontocete larynx is ideally positioned to allow the animal to swallow solid food without aspirating either food or water into the respiratory tract. Its structure and intranarial location effectively separates the respiratory tract from the digestive tract to a greater extent than that found in any other mammal.

Functional anatomy of the upper respiratory tract

The intranarial position of the larynx in most terrestrial mammals restricts the supralaryngeal respiratory pathway to the nasal cavity and nasopharynx, rather than the oral cavity or oropharynx. This, in turn, enhances their sense of olfaction, since all inspired air passes over the olfactory epithelium. As a result, terrestrial mammals can simultaneously breathe, feed, and sample the air for the scent of predators or prey. Cetaceans, perhaps more so than most terrestrial mammals, are obligate nose breathers. Although odontocetes appear to be anosmotic (Evans and Maderson, 1973; Swerdtfeger et al., 1984), they are still able to detect predators and prey in their environment. They accomplish this by using echolocation instead of olfaction (or instead of vision in some species) to investigate their surroundings for predators, prey, and obstacles (Kellogg, 1958, 1959; Norris et al., 1961; Evans, 1973). The intranarial position of the larynx may play an important role in phonation, as well as during respiration and deglutition. The rostral placement of the larynx under the skull allows for a more complete interlocking of the laryngeal aditus and the nasopharynx than that found in terrestrial mammals. Echolocation sounds may well be generated while feeding due to this efficient separation of the air and food pathways (see figure 10).

Odontocetes vocalize for both echolocation (Kellogg, 1958; Norris et al., 1961) and communication (Lilly and Miller, 1961). They are able to produce a wide variety of sounds ranging from whistles to clicks (Evans and Prescott, 1962; Mackay and Liaw, 1981). The methods by which they produce these sounds are still unclear. While many investigators believe that movements of structures in the nasal region are responsible for producing sound, others attribute sound generation to vibrations of laryngeal cartilages (see reviews by Dormer, 1979; Evans, 1973; Evans and

Maderson, 1973). Our own recent observations on the existence of odontocete vocal folds suggest their participation in sound production as well (Reidenberg and Laitman, 1987c; 1988b; 1988c).

Combinations of whistles and clicks may be produced simultaneously by odontocetes (Lilly, 1961; Lilly and Miller, 1961; Evans and Prescott, 1962; Purves, 1966; Mackay and Liaw, 1981), thus pointing to the existence of more than one sound source (Lilly, 1978). These sound sources may be located within one structure, such as the larynx. Alternatively, these sources may exist simultaneously in two different areas, e.g., in both the laryngeal and nasal regions. The intranarial position of the odontocete larynx enables air to pass through the larynx and into the nasal sacs (Reidenberg and Laitman, 1987b), and perhaps back again to the larynx (Dormer, 1979). This arrangement would allow both the larynx and the nasal sacs to participate simultaneously in sound generation and air recycling.

Although the role of the larynx as the generator of the fundamental frequencies is still unclear, the position of the larynx is clearly important for directing air through this region. The permanently intranarial location of the odontocete larynx is essential for channeling air from the lungs or laryngeal air sacs directly into the nasal cavity. Sound could thus be generated by pneumatic vibrations of the vocal folds or other structures of the nasolaryngeal tract. As a result of this high position, the laryngeal aditus does not appear to be unlocked from behind the soft palate to allow for oral vocalizations, as in terrestrial mammals. An intranarial laryngeal aditus would restrict any airborne sounds to being emitted nasally, rather than orally. The sound waves produced could then be modified by the resonating chambers of the nasal sacs, and then transmitted by vibrations along the bony or cartilaginous rostrum or the fatty melon to the water as echolocation beams. As noted, the permanently intranarial larynx may thus be an adaptation enabling odontocetes to scan their environment via echolocation while feeding.

Summary

This "natural experiment" has yielded some interesting results. Odontocetes possess a highly modified respiratory apparatus suited to an aquatic existence. Although the shape of the larynx may differ from most mammals, its position is consistent with the basic mammalian pattern. In most mammals, the larynx is located "high" in the neck, i.e., usually between the basiocciput or first cervical vertebrae and the second, third, third or even fourth cervical vertebrae. In the odontocetes, the level of the larynx is so "high" that it cannot be measured against the cervical vertebrae, but rather, is usually located between the presphenoidal (intersphenoidal or midsphenoidal) synchondrosis and the foramen magnum. In addition, most mammals possess an epiglottis which overlaps the soft palate, thus providing two pathways for respiration/vocalization and deglutition. In the odontocetes, however, the separation of these two pathways is made <u>complete</u> by the intranarial laryngeal aditus, sealed by the palatopharyngeal sphincter.

In the basic mammalian pattern, the high position of the larynx corresponds to a relatively flat basicranial contour, with extremely slight or no kyphosis. In this "natural experiment", the skull base of odontocetes was found to be among the flattest of the mammals. In the posterior region, it is even slightly lordosed. Thus, it is evident that the odontocete arrangement is an extreme variation of the basic mammalian condition. Based upon these relationships, a pattern is beginning to emerge: the "flatter" (or more lordosed) the basicranium, the "higher" (or more cranial) the position of the larynx.

HUMAN ANATOMY

The second mammal chosen for this "natural experiment" was the human. Human infants are practically identical to other terrestrial mammals in their laryngeal positioning and skull base contour. The larynx is located high in the neck, with the epiglottis overlapping the soft palate. The skull base is relatively unflexed, showing a pattern very similar to other terrestrial mammals. Human adults, on the other hand, are known to exhibit a markedly flexed (kyphosed) basicranium (Laitman, 1977). As a natural experiment, it is interesting to see whether the relationships observed for terrestrial mammals and odontocetes applies to adult humans. In this case, the anatomy of the adult human was examined to investigate whether or not a flexed basicranium corresponds to a larynx positioned low in the neck.

Materials and Methods

For this study, 125 adult human cadaver heads were studied, using specimens from the human gross anatomy course at The Mount Sinai School of Medicine. Three human fetuses obtained from the Department of Anatomy at the Mount Sinai School of Medicine, were also studied. The adult cadavers (including males and females) ranged in age from approximately 30 to well over 70 years of age. All specimens were preserved in formalin and phenol, and were then sectioned in the midsagittal plane. The shape of the skull base was noted, and the position of the larynx was measured relative to vertebral levels.

Dissections

Basicranial anatomy

The anterior midline of the human skull base is composed of the palatine process of the maxilla and horizontal plate of the palatine bone (comprising the hard palate), and the vomer. Posteriorly, the midline basicranium consists of the basisphenoid and basioccipital bones. Laterally, the posterior skull base receives a contribution from the petrous portion of the temporal bone. The external contour of the basisphenoid and basioccipital bones are of particular interest, since it is from this posterior region of the skull base that most of the muscles, ligaments, and connective tissue attachments for the suspension of the larynx and hyoid bone are found.

In human infants below the age of 1 1/2 years, the skull base is relatively flat. This shape is consistent with the pattern seen in most terrestrial mammals. In human adults, however, the pattern has reversed. The skull base is highly flexed or kyphosed. The basisphenoid and basioccipital bones have moved from a horizontal orientation to almost a vertical orientation. (For further discussion of basicranial shape changes with age in the human adult and infant, see discussions in: Zuckerman, 1955; Laitman, 1977; Laitman et al., 1978; George, 1978; Lestrel and Roche, 1986. For general anatomy of the human skull, see: Muller and O'Rahilly, 1980 [embryonic]; Diewert, 1985 [late embryonic and early fetal]; Ford, 1956 [fetal]; Swischuk, 1974 [newborn]; 1972 [infant]; Binet, 1974 [adult].)

In order to understand this change in the shape of the skull base, one must first understand the nature of the normal flexion process between the basisphenoid and basioccipital bones. These bones are joined to each other by a synchondrosis. Synchondrosal growth is responsible for elongation of the basicranium (Bjork, 1955; Knott, 1969; 1971; Lewis and Roche, 1972; 1974; Roche and Lewis, 1974). The major growth center of the skull base is believed to be the spheno-occipital synchondrosis (Koski, 1960; Michejda and Lamey, 1971; Roberts, 1975; Giles et al., 1981). Much work has been done on the growth of the spheno-occipital synchondrosis (e.g., Stamrud, 1959; Michejda, 1972; Moss, 1976; Roberts and Blackwood, 1983; 1984). Growth at this synchondrosis also contributes to the extent of flexion (kyphosis) of the basicranium (Melsen, 1971; Michejda and Lamey, 1971; Laitman et al., 1978; Giles et al., 1981; Laitman and Heimbuch, 1984). As the bones elongate, the spheno-occipital synchondrosis becomes a pivoting point around which they bend. It is thought that the bending, or flexion, which occurs between the two bones at this joint is due to differential growth within the synchondrosis itself (Moss, 1972; 1976; Hoyte, 1973).

Upper respiratory tract anatomy

The human larynx is composed of the same nine cartilages found in most terrestrial mammals: three unpaired cartilages (epiglottic, thyroid, and cricoid), and three paired cartilages (arytenoids, corniculates, and cuneiforms). Sometimes there is an additional pair of cartilages known as the triticeal cartilages, which are found embedded in the thyro-hyoid membrane stretching from the superior horn of the thyroid cartilage to the greater cornu of the hyoid bone.

Many of the muscles, ligaments, and connective tissues which attach to the skull base have an important function in the suspension of upper respiratory structures. Since the larynx is attached to the hyoid bone by the thyrohyoid ligament, any muscle suspending the hyoid bone will indirectly suspend the larynx.

The digastric muscle, which spans between the mandible and the skull base, suspends the hyoid bone from a fascial loop. This fibrous loop connects the greater cornu and corpus of the hyoid bone to the round intermediate tendon of the digastric muscle. This rounded tendon (located at the junction between the anterior and posterior digastric bellies), perforates the tendon of insertion of the stylohyoideus. The posterior belly of the digastric muscle attaches to the lateral skull base at the mastoid notch of the temporal bone.

Humans possess a large styloid process, which projects from the skull base in a ventral, anterior, and medial direction. It is the attachment point for three muscles and one ligament: stylopharyngeus, styloglossus, and stylohyoid muscles, and the stylohyoid ligament. Of these four structures, at least three function in suspension of the larynx. The stylohyoid muscle attaches from the base of the styloid process to the body of the hyoid bone at its junction with the greater cornu, thus suspending the hyoid bone from the skull base. The stylohyoid ligament runs from the tip of the styloid process to the lesser cornu of the hyoid bone. The stylopharyngeus arises from the base of the styloid process and inserts between the superior and middle constrictors. Its fibers then merge with the fibers of the superior, middle, and inferior pharyngeal constrictors. Some fibers directly insert on the posterior border of the thyroid cartilage of the larynx.

The pharyngeal constrictors also play a role in suspension of the larynx from the skull base. The pharyngeal constrictors are actually three muscles (superior, middle, and inferior) which overlap with each other to form a continuous muscular sheath. They form the posterior and lateral walls of the pharynx. Posteriorly, they all insert into the median pharyngeal raphe. This raphe spans the entire posterior midline from the esophagus inferiorly to the pharyngeal aponeurosis superiorly. The pharyngeal aponeurosis inserts on the pharyngeal tubercle, located in the midline of the basioccipital bone of the skull base. Anteriorly, the superior constrictor inserts on the pterygoid hamulus, the pterygomandibular raphe, and even the mandible and tongue. The middle constrictor attaches anteriorly to the lesser cornu of the hyoid bone, the stylohyoid ligament, the upper border of the body of the hyoid bone, and the greater cornu of the hyoid bone. The inferior constrictor is divided into two parts: the thyropharyngeus and the cricopharyngeus. These two divisions insert anteriorly on the thyroid and cricoid cartilages of the larynx. The thyropharyngeus attaches to the posterior and lateral surface of the thyroid cartilage, including the superior and inferior cornuae. The cricopharyngeus inserts on the lateral surface of the cricoid cartilage, just posterior to the cricothyroid muscle.

Several muscles, one ligament (stylohyoid), and one connective tissue (aponeurosis of the median pharyngeal raphe) attach to the ventral surface of the skull base. These structures are responsible for suspending the hyoid bone and the larynx from the basicranium. A mechanical relationship thus appears to exist between basicranial shape and the position of the hyoid bone and the larynx. If the skull base is deformed, malformed, or changed through normal growth and development, then the attachments of these muscles, ligament, and connective tissue may be affected. If a mechanical relationship does exist, then the positions of the hyoid bone and the larynx may be affected as well.

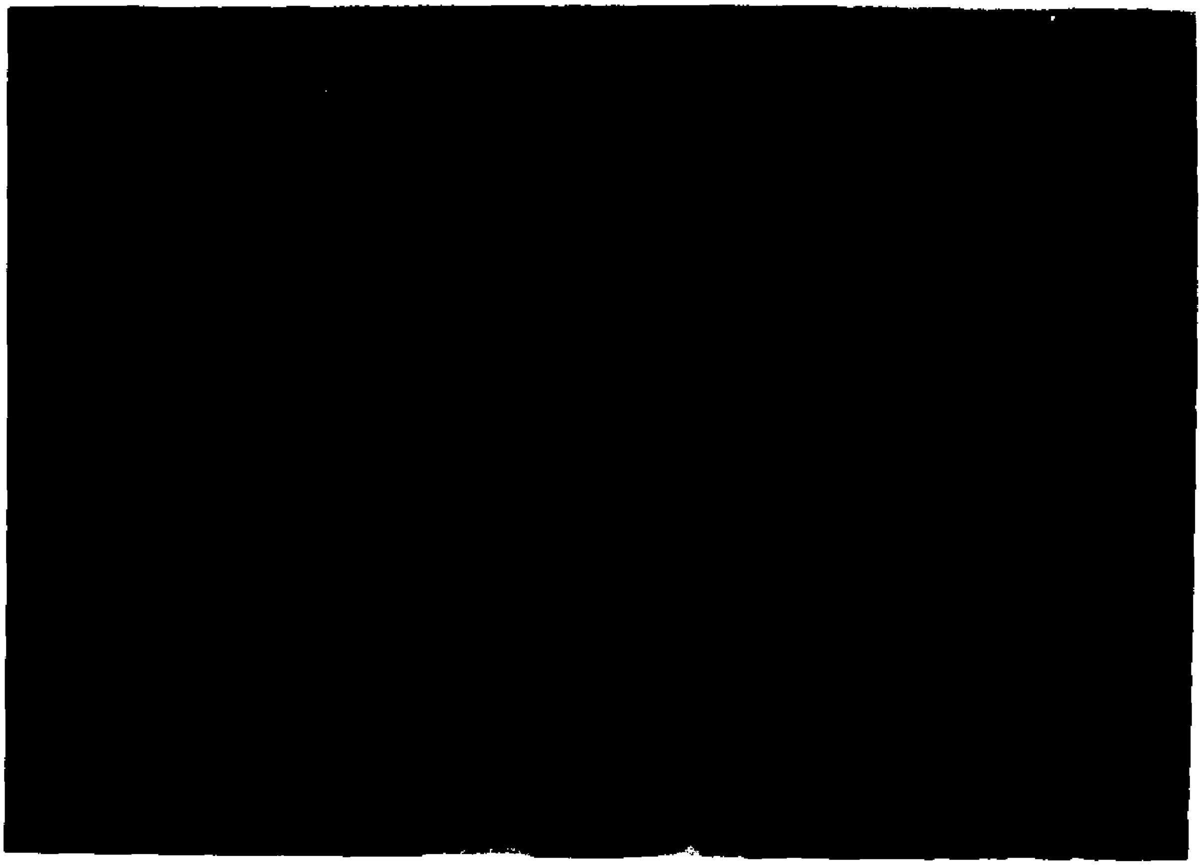
Positional Relationships

The upper respiratory anatomy of human infants is very similar to the structural pattern seen in most terrestrial mammals. (For the anatomy and positional relationships of the human fetal and infant larynx, see Symington, 1885; Noback, 1923; Roche and Barkla, 1965; Fried et al., 1982; Pracy, 1983; Magriples and Laitman, 1987a; 1987b; Wolfson and Laitman, 1988; Laitman, 1988). The larynx not only functions to regulate the processes of respiration and protection during deglutition, but also functions as a sound source in phonation (For review of upper respiratory anatomy and function in human infants, see Bosma and Showacre, 1975). The infant larynx lies very high in the pharynx, with the epiglottis in contact with the soft palate (Bowles, 1889; Laitman, 1977; Laitman and Crelin, 1976; 1980). As in herbivores, this arrangement allows simultaneous deglutition and respiration to occur uninterrupted (Crelin, 1976; Laitman and Crelin, 1980). Only when a heavy bolus is swallowed must respiration cease as the bolus is passed over the closed larynx (Laitman et al., 1977). Human infants, as most mammals, are habitual nose breathers (Bosma and Fletcher, 1962; Laitman et al., 1977; Sasaki et al., 1977). The epiglottis thus functions to keep the airway patent by channeling nasally inspired air directly into the trachea, while preventing orally ingested material from entering the larynx (Laitman, 1977; Laitman et al, 1977). The diet of a human infant consists largely of liquids (mostly milk) which easily flow around the larynx via the piriform recesses to the esophagus.

The skull base is relatively flat in human infants under the age of 1 1/2 years (Laitman, 1977; Laitman et al., 1978). This contour is consistent with the pattern seen in most terrestrial mammals. It is not surprising, then, that the upper respiratory pattern exhibited is also very similar to that of other terrestrial mammals. The larynx is positioned relatively high in the neck, corresponding to cervical levels C1 through C3 (Laitman and Crelin, 1980). The epiglottis is found in apposition with the soft palate, thus allowing a two pathway system for respiration and deglutition to exist. Thus, in young humans and in most other terrestrial mammals, there appears to be a common pattern of a flat skull base and a larynx positioned high in the neck (see figure 11).

As the infant matures, the larynx begins a gradual descent into the neck (Symington, 1885; Roche and Barkla, 1965; Noback, 1923; Bosma, 1975; Laitman and Crelin, 1976; 1980; Laitman et al, 1977; 1979; Sasaki

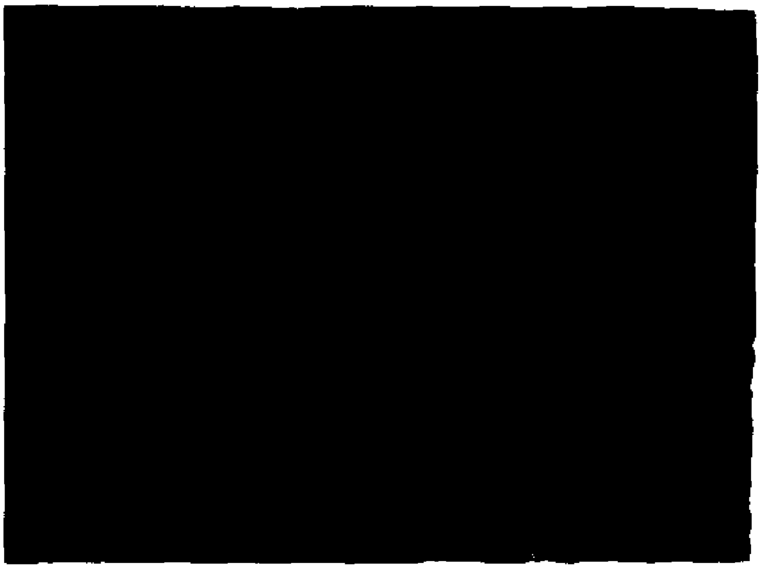
Figure 11: a) Midsagittal section through the head of a newborn human. b) Midsagittal section through the head of a 7 month old human infant. Note the high position of the larynx, with the epiglottic tip contacting the soft palate. Figure from Laitman (1977).

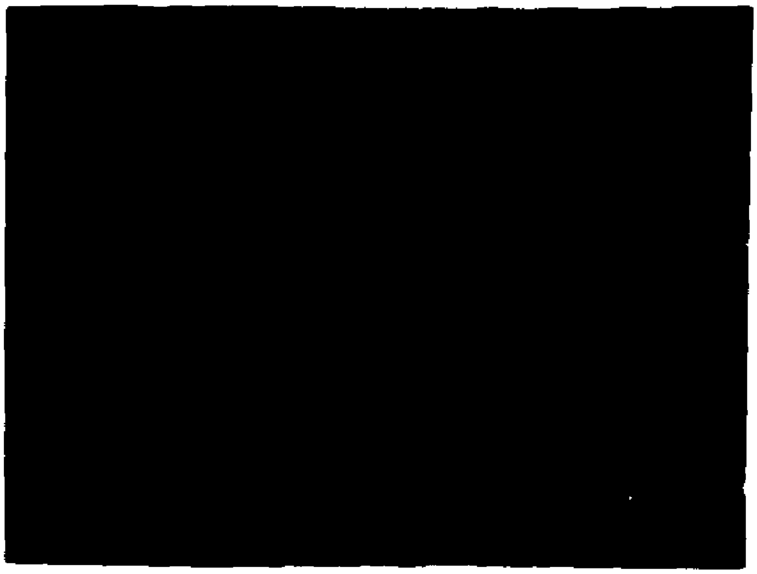


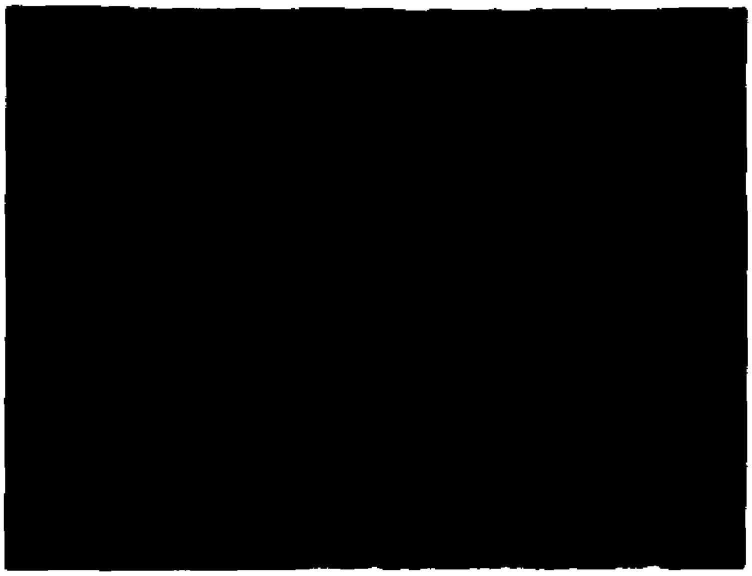
et al., 1977). By adulthood, this descent is complete. The skull base is highly flexed or kyphosed as noted throughout the sample studied (see also Laitman et al., 1978 for craniometric analysis of skull flexion). The basisphenoid and basioccipital bones have moved from a horizontal alignment to an almost right angle orientation to each other. Likewise, the larynx is located very low in the neck, corresponding to cervical vertebral levels C4 through C7. This pattern was found to be largely consistent throughout the sample studied. In this arrangement in adult humans, the epiglottis can no longer contact the soft palate to create two separate pathways for respiration and deglutition (see figure 12). In none of the adult cadavers studied was the epiglottis ever found to appose the soft palate. This low-lying position of the larynx is unique among mammals. One major consequence of such a separation of the epiglottis from the nasopharynx is a dangerous crossing of the alimentary canal over the respiratory tract. This leaves the mature human particularly prone to choking by aspirating food into the larynx (Crelin, 1976; Laitman, 1977; Heimlich and Uhley, 1979; Laitman and Crelin, 1980a).

To compensate for the deleterious effects of a larynx low in the neck, a complicated swallowing process has developed. Unlike the infant's, the

Figure 12: a) Midsagittal section through the head of an adult female human. b) Midsagittal section through the head of an adult male human. c) Midsagittal section through the head of another adult male human. Note the low position of the larynx. The epiglottis no longer contacts the soft palate. Also, note the highly flexed skull base.







adult's diet no longer consists of just liquids. Since bulky pieces of food are too large to pass easily around the larynx through the lateral food channels, the swallow must be initiated to allow the bolus to pass over the larynx and into the esophagus. First, the larynx is elevated cranially, thus raising the aryepiglottic folds, widening the lateral food channels and narrowing the laryngeal aperture (Landman, 1970; Logemann, 1983). Then, as the larynx is raised even higher, the epiglottis (which has been holding the bolus from passing into the laryngeal vestibule) is folded over caudally as the bolus passes safely over the larynx (Curtis and Sepulveda, 1983; Curtis and Cruess, 1984). The liquid portions of the bolus may be prevented from entering the laryngeal vestibule by the epiglottis, which often carries liquid residues back on its rostral surface, as it rises back to the erect position (Ardran and Kemp, 1952). During this process, both the vocal and vestibular folds are adducted, and the arytenoids tilt forward towards the epiglottic base. This protective closure prevents any liquids which may have entered the larynx from continuing into the trachea and lungs. Obviously, respiration must cease during the swallow. Fortunately, swallows are of a very brief duration in the human adult (compared, for example, with the slow swallow of a snake as it engulfs its prey), and thus temporary suspension of breathing is not detrimental.

Given the low position of the larynx in the adult human, the epiglottis can no longer interlock with the nasopharynx to maintain a separate airway (Laitman et al., 1977). It is not surprising, then, that humans are not very keen scented. Unlike herbivores, we must close the mouth and inhale deeply through the nose in order to determine the character of a detected odor. Some believe that, due to laryngeal descent, the epiglottis has become a vestigial structure (Negus, 1927; 1937; Hast, 1978). Others, however, have maintained the classic belief that its function is to protect the larynx from inundation by food during swallowing (Ardran and Kemp, 1952; 1967). Evidence to the contrary, however, suggests that the epiglottis is not necessary for swallowing. For example, people in whom the epiglottis has been removed (epiglottectomy or partial epiglottectomy) are able to swallow without choking (Negus, 1927; 1943; Pressman and Kelemen, 1955; Olson and Sullivan, 1985; Hirano et al., 1987). Protection of the laryngeal airway is obtained, rather, via closure of the vocal folds, anterior tilting of the arytenoids, and elevation of the larynx itself (Negus, 1943, 1948; Laitman, 1977).

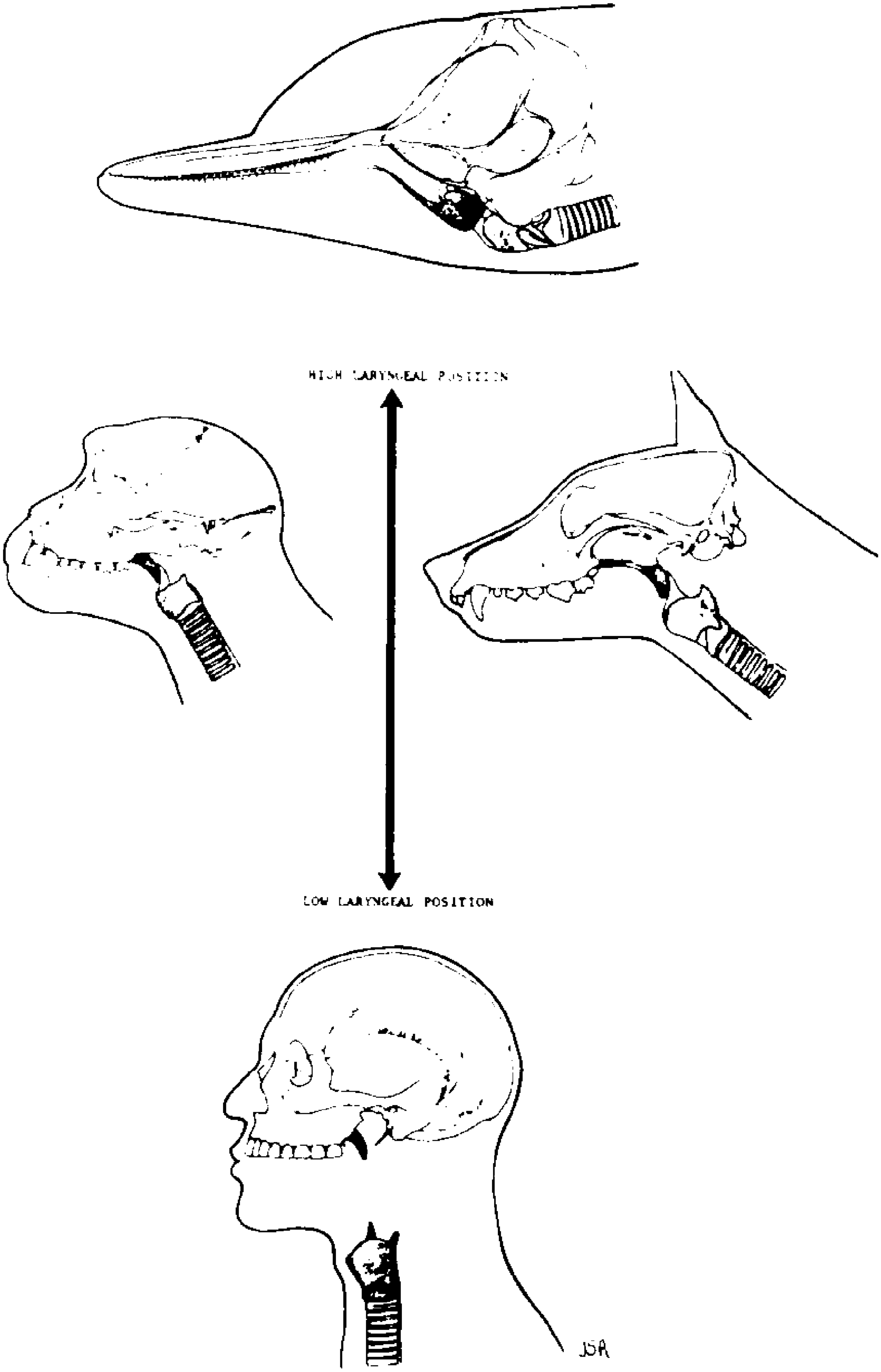
Unlike odontocetes, which have exaggerated the intranarial position of the larynx, the adult human larynx has migrated in the exact opposite direction - towards the lungs. Indeed, no other mammal has a larynx placed so low in the neck (Laitman, 1983; 1984; Reidenberg and Laitman, 1986). Although the respiratory system has been jeapordized by its intersection with the upper digestive system, the benefit of the enlarged pharyngeal space above the larynx is an increased ability to modify sound. Regulation of the expanded supralaryngeal pharynx allows adult humans to modify the sounds generated at the vocal folds (Laitman et al, 1978; Lieberman, 1984; Laitman, 1986). Humans appear to be the only mammals that have perfected the larynx as an organ of sound production, resulting in fully articulate speech.

Vibration of the vocal folds causes the creation of a fundamental frequency (Pressman and Kelemen, 1955; Lieberman, 1984). The length, tautness, closeness of approximation to each other, sharpness or roundedness of the edges, and degree of vibration of the vocal folds, all contribute to the quality of the sound produced (Myerson, 1964). Volume is controlled by the pressure of the air expired. Further modifications of the sound occur above the larynx in the pharyngeal, oral, and nasal cavities. The length of the pharynx is a critical factor. A longer pharynx gives rise to lower pitches, while a shorter pharynx allows the production of higher pitches. The tube-like structure can be likened to a pipe organ, trombone, or any other musical instrument in which the resonating tubes are of variable lengths. The longer pipes produce lower notes than the shorter pipes. Pharyngeal length can be controlled by elevation of the larynx. The nasal cavity and its various extensions into the paranasal sinuses serve as resonating chambers for sound produced at the larynx. Resonating chambers help to amplify the fundamental frequency and the overtones (Bateman, 1977). The oral cavity is the location of much sound modification. Structures which have movement, such as the tongue, soft palate, lips, and even movement of the larynx itself are capable of reshaping the volume of the supralaryngeal cavity, thus resulting in a modification of the sound waves produced and further regulation of the volume of air released (Bateman, 1977). Through movements of the tongue, pharynx, velum, lips, and jaw, the various vowels and consonants characteristic of human speech are created. Changing the size of the resonating chamber picks out and exaggerates certain harmonics or overtones, giving rise to vowel sounds, while partial or complete interruptions in the outflow of air along the respiratory tract produce the consonants (Hollender, 1953; Lieberman, 1984).

Summary

Adult humans and odontocetes appear on opposite ends of a spectrum concerning the positioning of the larynx (with reference to the pharyngeal cavity and skeletal structures). In an attempt to maintain nasal breathing while swallowing, terrestrial mammals have adopted the two channel system of respiration and deglutition by approximation of the epiglottis to the soft palate. The odontocetes, in response to the demands of aquatic life, were forced to improve upon this system in order to prevent drowning during deglutition. As a result, the rostral extensions of the larynx have become permanently intranarial and thus a watertight respiratory passageway was developed. It is interesting to note, however, that the odontocete larynx, although structurally reshaped, still remains high in the pharynx, and thus follows the same pattern established by its terrestrial mammalian ancestors. Only the degree of insertion into the nasopharynx is exaggerated from the terrestrial condition, thereby resulting in two separate tubes for respiration/vocalization and deglutition. Adult

Figure 13: Schematic representation of "laryngeal positions". The adult human possesses the lowest laryngeal position, while odontocetes (represented by the dolphin) have the highest laryngeal position. All other terrestrial mammals studied (represented here by the macaque and dog) fall in an intermediate range, but closer to the condition of the dolphin.


HIGH LARYNGEAL POSITION
LOW LARYNGEAL POSITION
JSR

humans, on the other hand, have altered the positioning of the larynx but not its basic intrinsic structure. As the human matures, the larynx descends into the neck. This radical change allows the two pathway system of respiration and deglutition to degenerate. Although physiologically detrimental to the integrity of the respiratory tract, it is balanced by the attainment of a larger pharynx. This increase in pharyngeal length gives adult humans a large resonating chamber and thus the ability to modify speech sounds to a greater extent than any other mammal.

This "natural experiment" has revealed two patterns of respiratory and basicranial anatomy. The typical mammalian pattern is exhibited by terrestrial mammals, infant humans, and odontocetes. In this arrangement, the skull base is relatively flat, and the larynx is positioned very high in the neck, with the epiglottis contacting the soft palate. In the odontocetes, an exaggeration of this pattern is seen in which the skull base is extremely flat (with slight lordosis in the posterior region), and the larynx is located above (i.e., rostral to) the compressed cervical region. The second pattern is exhibited solely by adult humans. In this arrangement, the basicranium is no longer flat, but instead is flexed into a kyphosis. In addition, the larynx is located very low in the neck. This flexed basicranial contour and low larynx differs greatly from the arrangement in typical terrestrial mammals, and is opposite to the pattern seen in odontocetes (see figure 13).

A basic relationship has thus emerged in the study of these "natural experiments": a flat basicranium appears to correspond to a larynx placed high in the neck, while a flexed (kyphosed) basicranium appears to correspond to a larynx placed low in the neck. This pattern, evident through comparative anatomy, indicates that a structural relationship indeed exists between basicranial shape and laryngeal position. These natural experiments have thus shown much about the interrelationships between soft tissue and bony anatomy.

PART II

EXPERIMENTAL STUDY IN RATS

CHAPTER 4

MATERIALS AND METHODS

Introduction

Studies of the comparative anatomy of terrestrial mammals have established that a basic pattern of upper respiratory tract anatomy exists. A relatively flat basicranial shape was found to correspond to a larynx positioned high in the neck, with the epiglottis overlapping the soft palate. In the previous chapter, a study of "natural experiments" has shown that mammals, such as cetaceans can exaggerate the basic anatomical arrangement while still maintaining this critical relationship between basicranial shape and laryngeal position. Adult humans, on the other hand, were found to deviate from the typical mammalian arrangement, while maintaining this important relationship between laryngeal position and basicranial shape. The adult human basicranium is markedly flexed (kyphosed) and the larynx is found positioned low in the neck.

Although these "natural experiments" have shown that the position of the larynx appears to be related to the shape of the basicranium, the central question of whether a direct functional interaction exists between these structures remains unanswered. To address this question, an experiment was designed to test whether or not a relationship exists between basicranial shape and laryngeal position. In this experiment, the skull base of rats was artifically flexed (via surgically induced basicranial kyphosis) to determine whether this change affects laryngeal or hyoidal positioning.

Background and rationale

Rats were chosen as experimental animals. As rats have been important animals used widely in biomedical research, many studies on bone growth have used them as models. Much has been written about the anatomy of the rat skeleton (Donaldson, 1919; 1932; Strong, 1926; Zucker and Zucker, 1946; Dunn et al., 1947; Enlow, 1962; Hughes and Tanner, 1970; Hansson et al., 1972; Feik and Storey, 1983). Many studies have focused on the anatomy of the rat skull (Hatai, 1908; Gentile, 1952; Young, 1959; Fenart and Destombes, 1971; Hoyte, 1971; Lavelle, 1976; Hughes et al., 1978; Baer et al., 1983; Smit-Vis, 1984). Similarly, a number of investigations have used the rat as a model to study the basicranium (Vilmann, 1969; 1971; Dorenbos, 1973;

Geelen, 1973; Schramm et al., 1974; Roberts, 1975; Moss, 1976; Roberts and Blackwood, 1983; 1984). The rat is also widely used in craniofacial growth and development studies (Pritchard et al., 1956; Cleall et al., 1968; 1971; Riesenfeld, 1974; Pucciarelli, 1978; Engstrom et al., 1982; Lozanoff and Diewert, 1986; Fanghanel et al., 1987; Mierzwa et al., 1987; Pucciarelli and Oyhenart, 1987; Schumacher et al., 1987). The rat has also been used in studies of the upper respiratory tract (Kelemen, 1947; Smith, 1977; Schreider and Raabe, 1981). Since there is a large collection of literature on the anatomy, growth, and development of the rat, our methodology and results can be reviewed and compared to those of others.

The surgical approach described below was developed for this project after review of the literature describing the conditions during which kyphosis was induced or observed. The majority of these investigations were designed to study regulation of growth of cranial components by inducing or observing skull growth disorders. Many of these studies involved pharmacologically induced malformations or deformations of the skull and brain. The chemicals used include: methylazoxymethanol (MAM) to produce microcephaly (Spatz and Laqueur, 1968; Haddad et al., 1969; Rabe and Haddad, 1972; Moss, 1975b; 1976; Melsen and Moss, 1977), diazo-oxo-norleucine (DON) to induce cranial stunting (Lozanoff and Diewert, 1986), papain (Ronning, 1968; Ronning, 1971; Kvinnsland, 1974; Schramm et al., 1974), kaolin to produce hydrocephaly (Young, 1959; Fenart and Destombes, 1971), and ingestion of the plant Veratrum californicum to cause cyclopia (Evans et al., 1966). Other induced cranial changes were accomplished by hypervitaminosis (Baume et al., 1972; Geelen, 1973), hypovitaminosis (Engstrom et al., 1982), undernourishment (Smit-Vis, 1984; Pucciarelli and Oyhenart, 1987), or hormonal manipulations (Riesenfeld, 1974). Some studies focused on naturally occurring genetic mutants exhibiting pathological malformations of the skull, such as congenital hydrocephalus (Grunberg and Wickramaratne, 1974), microcephaly or anencephaly (Moss, 1975b), achondroplasia (Julian et al., 1957; Cohen et al., 1985; Wang et al., 1987), cranial synostosis (Ousterhout and Melsen, 1982; Burdi et al., 1985; Farkas et al., 1985; Lavelle, 1985; Sax and Flannery, 1986; Richtsmeier, 1987), or hemifacial microsomia (Figueroa and Friede, 1985). (See Cohen, 1985 for review of craniofacial dysmorphic growth and development.)

Unfortunately, many of these disorders are not restricted to the skull base alone, but rather, affect the subject systemically (Cohen, 1985). Often, the pathologies (both naturally occurring and induced) affect systemic bone and cartilage growth, including malformations of laryngeal cartilages (Grunberg and Wickramaratne, 1974; Hochwald, 1985). In addition, there are other associated effects which could alter laryngeal positioning, such as systemic neuromuscular disorders affecting breathing, swallowing, and vocalizing (Gabriel and McComb, 1985).

To avoid complications arising from pathological conditions, a surgical procedure in which the effects can be limited to a defined region of the skull base was deemed the most suitable choice. A mechanically induced flexion, localized to the posterior basicranium, should not affect the skeleton systemically. Likewise, this procedure should not affect cartilaginous growth of the larynx. Any observed changes in laryngeal position could thus be attributed to the induced flexion of the skull base.

The purpose of the surgical procedure used in this study was to cause basicranial kyphosis. This method involves eliminating the spheno-occipital synchondrosis, one of the major cartilaginous growth centers of the skull base (see figures 14 and 15). Removal of this important growth center should cause early ossification of the basicranium by premature fusion of the basisphenoid and basioccipital bones. This premature basicranial synostosis should thus result in a shortened basicranium (also reported recently by Brons, 1988). Stunting the growth of the basicranium should not inhibit growth of the cranial vault. Thus, as the skull vault continues to enlarge, it should overgrow the skull base, resulting in basicranial kyphosis.

Elimination of the spheno-occipital synchondrosis could be accomplished via either a dorsal or a ventral approach. A dorsal approach to the synchondrosis would involve destruction of the brain, particularly the pons and the pituitary (for description of structures overlying the spheno-occipital synchondrosis in the rat, see de Groot, 1959). Destruction of the pituitary would result in changes in growth unrelated to the elimination of the spheno-occipital synchondrosis. Damage to the pons could have disastrous affects upon the cardiovascular and respiratory control centers of the nervous system. The pons controls the pneumotaxic center of the brain which is responsible for terminating apnea (Lambertsen, 1980), and the vasopressor region of the brain which maintains arterial pressure (Milnor, 1980). A lesion in this region could result in either respiratory or cardiac arrest. In addition, the basilar artery lies directly over the spheno-occipital synchondrosis. If it is damaged, hemorrhage into the brain is possible (creating a hemorrhagic stroke), as well as anoxia of the pons due to lack of sufficient blood flow through the pontine arteries. Thus, in order to avoid damage to the pons, pituitary, and basilar artery, a ventral approach to the spheno-occipital synchondrosis was used.

Materials

The experimental subjects used in this study were Long-Evans hooded rats (Rattus norvegicus). Rats were chosen for several reasons: 1) The rat shows the typical mammalian upper respiratory tract configuration: a larynx placed high in the neck with the epiglottis overlapping the soft palate. 2) The rat skull has a very flat basicranium (i.e. the skull base is neither convex nor concave). Deviations from this outline are thus easily observed and measured. 3) The spheno-occipital synchondrosis is an easily visualised, rather large cartilaginous strip in the young rat. It is accessible via a ventral surgical approach with minimal destruction to the surrounding musculature. 4) Infant rats are large, compared with infant mice, and thus are easier to operate on (average weights for a 1 day old litter of mice were 2.05g for females and 2.11g for males, while average weights for a 1 day old litter of rats were 6.17g for females and 6.40g for males). 5) Rats are remarkably tolerant of surgical procedures and rarely show infection at wound sites. 6) Rats have a very short gestation (21 days) and a short maturation (weaned at 21-25 days; sexually mature at 40-80 days, depending upon sex of animal). 7) Rat strains show very little genetic variability, thus artifically induced deviations from the normal pattern are immediately apparent as such. 8) Rats are relatively inexpensive, can be obtained in large numbers, and are easily housed. 9) Much work has already been done on the growth of the rat skull, (see review by Baer et al., 1983) and thus these results could be reviewed in light of this work.

Surgical operations removing the spheno-occipital synchondrosis were performed on 129 infant rats. Forty of these rats were pilot study subjects, and the remaining 89 rats were experimental subjects for this study. Sham operations were performed on 15 rats. Five of these rats were used as sham operated controls for the pilot study, and the remaining ten rats were used as sham operated controls for this study. Eighteen rats were used as normal controls. Twelve of these rats were used as normal controls in the pilot study, and the remaining six rats were used as normal controls in this study.

Surgical Methods

The surgical approach to the spheno-occipital synchondrosis was based upon the works of Smith (1930), DuBrul and Laskin (1961), and Ingle and Griffith (1963). It is similar to the procedure used for hypophysectomy. This procedure was modified to prevent the traumatic effects of a midline approach on the larynx and trachea. Retracting the larynx, trachea, and esophagus, or cutting and intubating the trachea, would stress the respiratory system of the anesthetized rat. In addition, tracheal transection has been shown to result in hyoid elevation (Polansky et al., 1984). The method developed for this study, however, avoids this trauma by utilizing a ventral-oblique approach to the spheno-occipital synchondrosis.

Pilot Study

A pilot study was conducted to determine which was the best age for performing this operation. In this study, 40 rats were operated on in groups of 10 at 12, 13, 14, and 15 days old. Survival past weaning was 50%, 70%, 80%, and 90% respectively. Since the spheno-occipital synchondrosis is a major growth center for the skull base, the timing of its destruction will significantly affect its growth. Therefore, it is desirable to operate on rats at the youngest age possible while avoiding excessive mortality of the sample. This pilot study indicated a greater mortality rate at 12 days old, as compared to the other ages (as seen by gaps of 10% from 15 to 14 days old and 14 to 13 days old, but a gap of 20% from 13 to 12 days old). Accordingly, it was decided that operations on pups at 13 days old offered the optimal combination of earliest age with relatively low mortality rate.

Experimental Study

For this study, 89 experimental rats, 12 sham operated controls, and 6 normal controls were used. Litters were weighed and culled to six pups on the first day after birth, keeping the three largest males and the three largest females. At 13 days old, the infant rat was anesthetized for surgery with intramuscular injections of ketamine hydrochloride (Ketalar) 50 mg/ml and xylazene (Rompun) 20 mg/ml. Ketamine is a dissociative anesthetic drug with profound analgesic properties, while Rompun is a long acting sedative analgesic drug with centrally-acting muscle relaxant properties. Ketamine was injected intramuscularly at 30 mg/kg (or 0.0006 ml/gm of a 50 mg/ml concentration solution) body weight initially, and Rompun was injected intramuscularly at 12 mg/kg (or 0.0006 ml/gm of a 20 mg/ml concentration solution) initially. Doses were measured and delivered with sterile 25 gauge 5/8, 1cc tuberculin syringes. Supplemental doses of ketamine were administered intramuscularly at half the initial dosage, if needed. Rompun and ketamine were used for all sham rats and the first 49 experimental rats. Of these 49 experimental rats, 30 rats (59%) died from respiratory arrest which appeared to result from anesthetic complications. This elevated mortality rate may be due to increased stress on the cardio-pulmonary system during the experimental operation (drilling). This, in turn, may contribute to an increased sensitivity to the anesthesia, thus resulting in more frequent or more severe episodes of apnea (often leading to irreversible respiratory arrest) in the experimentals relative to the shams (see discussion in chapter 6). Since 59% is an unacceptable mortality rate, an alternative anesthetic was sought. Cooling the rat pups with ice was not effective for anesthetizing them since they are too old and already have a coat of fur. Diethyl ether was chosen because the level of anesthesia could be varied during the surgery. As a result, apneic episodes were often reversible. The remaining 40 experimental rats were anesthetized with diethyl ether. Only 12 of these rats died of respiratory arrest (30%). Since ether is highly explosive, it was used in a very well ventilated room. To confirm that the level of anesthesia was satisfactory, the rat was checked for any motor response by pinching its toes.

The rat was placed on its back (supine) on a sterilely draped styrofoam board with the head facing away from the operator. To avoid movement of the head, a soft cradle made of gauze was used to position the head (see figure 16a). The head was further anchored (and the neck was maximally extended) by a string which

Figure 14: Ventral surface of the skull of a normal adult rat. Note the spheno-occipital synchondrosis (arrow), located between the basisphenoid bone (rostrally) and basioccipital bone (caudally). This synchondrosis traverses the skull base between the left and right bullae. It is a major growth center of the skull base.

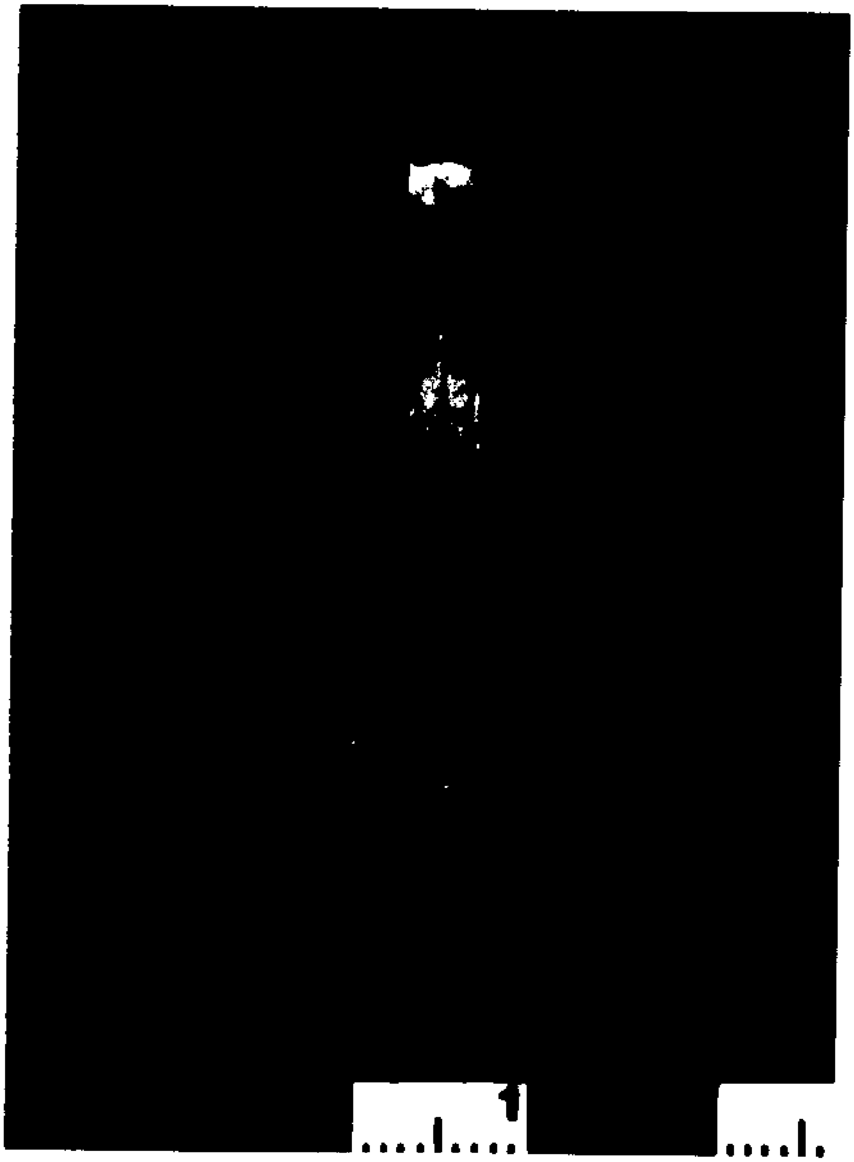

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Figure 15: Lateral view of a midsagittally bisected head of a normal adult rat. Note high position of the larynx and the flat basicranium. The spheno-occipital synchondrosis is visible as a whitish stripe running vertically between the basisphenoid and basioccipital bones. There is no elevated ridge resembling a dorsum sellae above the synchondrosis.

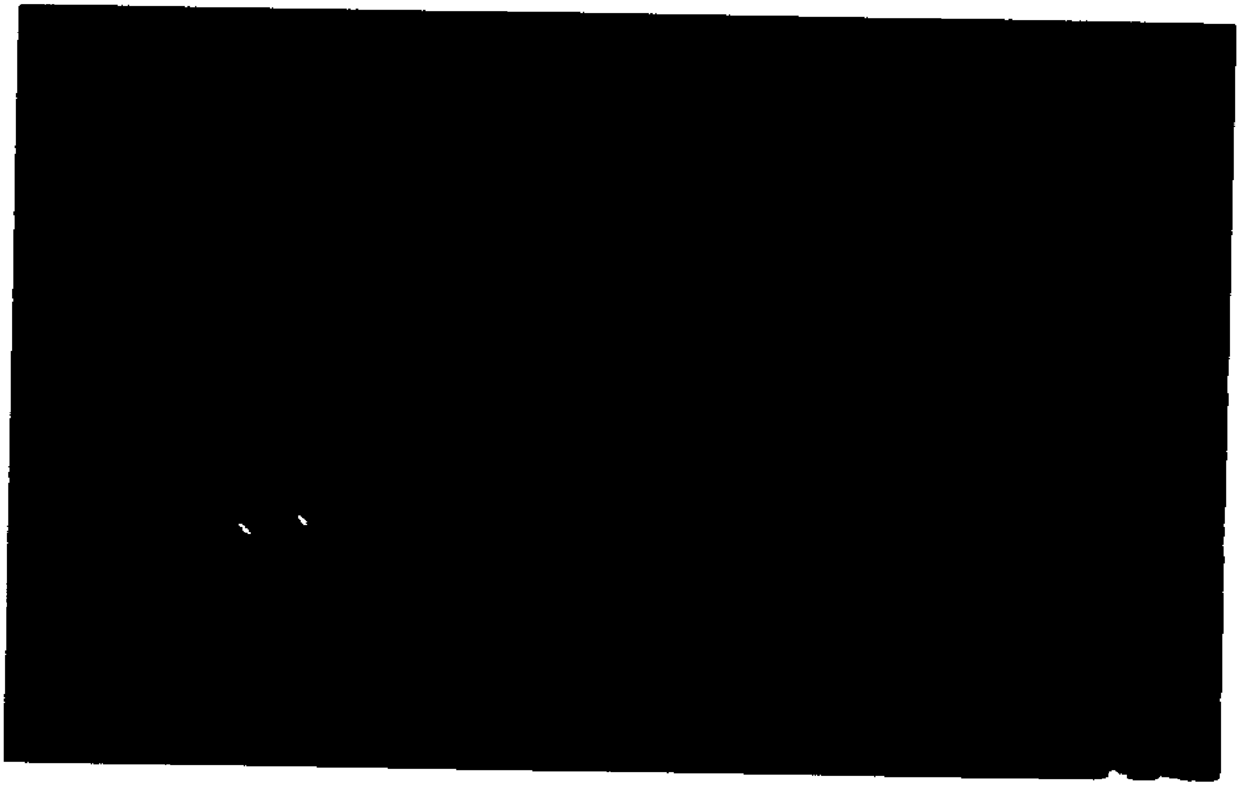
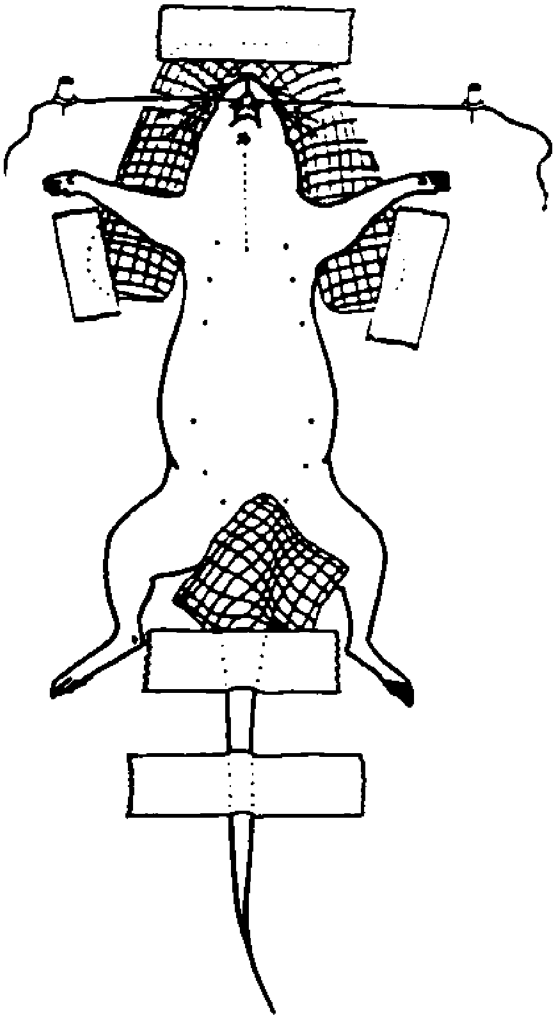


Figure 16: Operator's view of the rat as it is positioned for surgery (a). Note the exposed ventral surface of neck and the incision line (vertical dotted line), string under incisors, gauze head cushion, gauze to absorb urine, and tape restraint on tail. View of the operative field (b) with anatomical structures labelled. Large arrows indicate the angle of approach for the drill.

a



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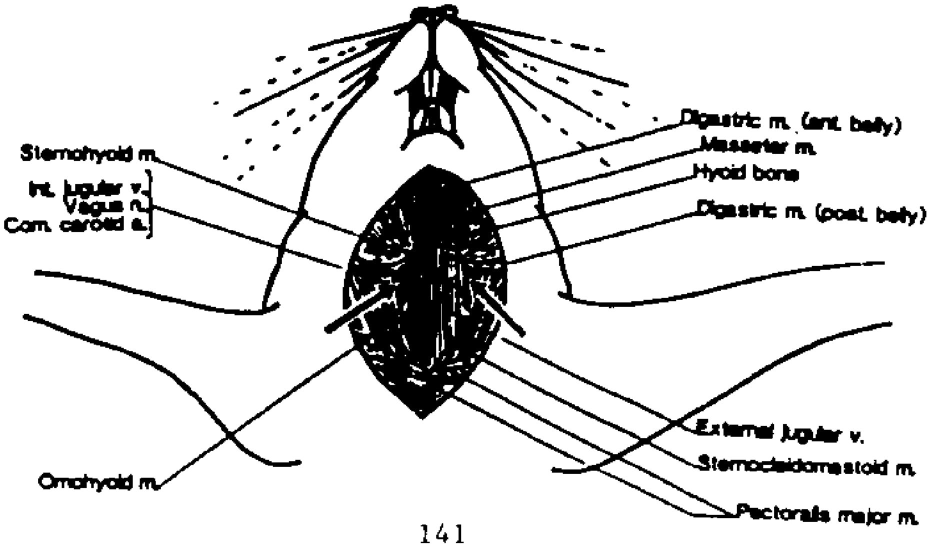

Sternohyoid m.
Int. jugular v.
Vagus n.
Com. carotid a.
Omohyoid m.
Digastric m. (ant. belly)
Masseter m.
Hyoid bone
Digastric m. (post. belly)
External jugular v.
Sternocleidomastoid m.
Pectoralis major m.

ran under the upper incisors, and which was tacked to the styrofoam board on either side of the animal's head. Tape was placed over the tail to keep the body extended. Another piece of tape held some gauze over the urethral opening to absorb any urine, should it be released during the procedure. No other restraints were used. The respiratory movements of the chest and abdomen were monitored to ascertain depth of anesthesia. If respirations were too shallow, the ether cone was removed. In the event of respiratory arrest, artificial respiration was performed by gently blowing small puffs of air through plastic tubing placed over the mouth and nose of the rat.

Betadine (iodine antiseptic solution) was then placed over the throat. The operative field was visualized through a Zeiss operating microscope (OPMI 1) with magnifications of 4x, 6x, 10x, 16x, and 25x. Using a pair of 14mm microdissecting scissors, a midline incision was made extending from the genion to the suprasternal notch. The incision cut through the platysma muscle, but did not penetrate deeply. The skin was pulled laterally, using two 0.6mm delicate rat-toothed forceps, exposing the deep fascia, salivary glands (submandibular and sublingual), and lymph nodes. The glands were gently separated in the midline and retracted laterally. No vessels were cut in this procedure. The skin and glands were spread and held apart for the duration of the surgery by a self-retaining Tico ratchet retractor.

The superficial muscles of the mandible and neck were then visible, including: sternocleidomastoid, omohyoid, sternohyoid (sternothyroid was slightly hidden under the sternohyoid), anterior and posterior bellies of the digastric, mylohyoid, and masseter. There was a semicircular ridge formed from the attachments of these muscles (except for the masseter and the sternothyroid muscles). This ridge indicated the position of the hyoid bone. Just inferior (caudal) to this bone was the thyroid cartilage of the larynx, whose prominence was barely visible. In order not to disturb the trachea and larynx, a midline approach, dividing the infrahyoid muscles and retracting the trachea, was not used. Instead, an oblique approach was used, entering laterally from either side of the trachea (see figure 16b).

A triangular space was visible bounded laterally by the sternocleidomastoid muscle, medially by the omohyoid muscle, and anteriorly (rostrally) by the posterior belly of the digastric muscle. The fascia in this space was gently parted by blunt dissection, with blunt-tipped microdissecting forceps, and Halsted mosquito forceps. The carotid artery (and branches of the external carotid such as the superior thyroid) and the vagus nerve (and two of its branches, the internal laryngeal and external laryngeal) were seen lateral to this area. The recurrent laryngeal nerve ran posterio-laterally alongside the trachea with its branches coursing medially. As this space was parted, the common carotid artery and vagus nerve were carefully isolated so that they would not be damaged. They were retracted superior-laterally with a mastoid probe, which was held in position by a cable lock device.

The micro-dissection was continued with two pairs of very delicate, blunt-tipped micro-dissecting forceps. Connective tissue immediately adjacent to and medial to the carotid artery and vagus nerve was parted so as not to disturb these retracted structures or the bordering muscles. This triangular space was gently widened as the forceps moved deeper until the longus capitus muscle was visualized. The dissection did not go any deeper, but at this point, proceeded anteriorly (rostrally). The forceps were used to widen the opening and dissect rostrally towards the skull base. The cartilaginous synchondrosis was visualized at the rostral border of the insertion of the longus capitus muscles on the skull base. The synchondrosis between the basisphenoid and basioccipital bones lay on a transverse ridge. Grooves for the insertion of the longus capitus muscles were felt caudal to this ridge. The spheno-occipital synchondrosis was located deep to the hyoid bone, and was found between the openings into the auditory bullae. It appeared as a blue-gray colored strip embedded in the white bone of the skull base, was relatively wide in the young rat, and easily visualized.

Delicate curved microdissecting forceps were now used to keep this opening patent. The triangular hole was spread by these forceps medially and caudally, and by the mastoid probe rostrally. If any longus capitus muscle fibers inserted over the synchondrosis, they were gently scraped back from the rostral edge of their insertion until the synchondrosis was clearly and completely visualized. A drill with a small bone cutting bur (2.3mm diameter, round head) was used to destroy the spheno-occipital synchondrosis. The tip of the drill bur was inserted into this hole and placed on the synchondrosis. A side-to-side (from bulla to bulla) motion was used while cutting to ensure complete elimination of the synchondrosal strip. Drilling proceeded only as deep as the dura so as not to injure the dura, basilar artery, or brain. Although accidental dural puncture could result in cerebrospinal fluid leakage, any resultant changes in pressure and volume are corrected by internal buffering mechanisms which preserve volume and pressure at a steady-state equilibrium (Shapiro et al., 1985). The drill was held steadily so as not to catch any of the muscles, arteries, or nerves surrounding the drilling site or the hole through which the shaft of the drill was inserted. The rat was watched carefully for any signs of respiratory distress. Bleeding was not usually encountered during this procedure, since no vessels are cut. However, sometimes slight bleeding did occur as blood oozed from the marrow spaces. It was stopped and absorbed by applying microcrystalline collagen (gelfoam) or by pressure with a cotton swab. Blood was not suctioned from the field since vacuum suction was too strong and caused more bleeding to occur.

The opposite side was opened in the same manner as the first side and the procedure repeated to ensure complete removal of the synchondrosis on both the left and right sides as well as in the midline.

Once the drilling was completed, the forceps and mastoid probe were removed, the salivary glands repositioned, and the skin sutured. Sterile chromic gut surgical sutures (6-0 chromic gut sutures with curved cutting needle) were used since they resorb, and thus a second procedure would not be necessary to remove the sutures. Next, 0.1cc saline was injected intraperitoneally to reduce any dehydration which may have occurred, since the rats were not nursing during this operation. The rat pups were then ear marked (using an ear punch) and toe marked. To help reduce the chances of maternal cannibalism of her seemingly injured pups, any blood stains from ear or toe clipping or from the incision site were washed off with sterile saline. The pups were left to recuperate on a warm surface (a plastic bag filled with warm tap water), and were gently warmed by the incandescent light bulb of a small lamp positioned over the cage. The warmth helped to lessen shock. The pups were also scented with the mother's smell (to lower the risk of cannibalism by the mother) by sprinkling shavings from the mother's cage over the pups. They were placed back with the mother only after fully recovering from the anesthesia, as evidenced by their ability to ambulate.

There were two sets of controls for this study. The first set was a group of normal, unoperated rats. They were not littermates of the experimental rats, since healthy littermates will outcompete the operated animals which are weaker initially. The second set of control animals was a group of rat pups which have undergone a sham operation at 13 days old. The procedure for this was identical to that described above for the experimental animals, except that no drilling was done. As with the normal controls, these animals were not littermates of the experimental rats, since they may be less weakened than the experimental animals initially, and would outcompete them for nursing time. Studies have shown that craniofacial growth is affected by litter size in rats, with large litters resulting in undernourished pups (Park, 1968; Smit-Vis, 1984). Malnourished preweaned rats display stunted growth, smaller skulls, and altered skull shape (Warren and Bedi, 1985; Pucciarelli and Oyhenart, 1987). Since a large litter size leads to runting, all litters (whether destined to be experimental, sham, or normal) were culled to six pups on the first day after birth.

After the pups were weaned (25 days old), the rats were weighed daily and their weights compared to identify malnourished or sick individuals, or those whose growth may have been severely retarded as a result of the surgery. As a restricted diet can affect the growth of weaned rats (Dunn et al., 1947), all rats were given unrestricted access to standard laboratory rat chow and water. Rats were housed in standard plastic rat cages, with two or three rats of the same sex and age per cage. Rats were maintained under artificial lighting on a 24-hour light/dark schedule of 12 hours light and 12 hours dark.

Radiological Methods

At 40, 60, 80, 100, and 120 days of age, the rats were laterally radiographed (X-rayed) to document the position of the larynx and hyoid bone, and any changes in the shape of the skull base. For this procedure, they were initially anesthetized with ether, weighed, and then given intramuscular injections of ketamine hydrochloride (Ketalar) 50 mg/ml and xylazene (Rompun) 20 mg/ml. Ketamine was injected intramuscularly at 30 mg/kg body weight (or 0.0006 ml/gm of a 50 mg/ml concentration solution) initially, and Rompun was injected intramuscularly at 12 mg/kg (or 0.0006 ml/gm of a 20 mg/ml concentration solution) initially. Doses were measured and delivered with sterile 25 gauge 5/8, 1cc tuberculin syringes. Supplemental doses of ketamine were administered intramuscularly at half the initial dosage, if needed. The rat was placed supine in a modified stereotaxic device. The ear bars were used to position the head. A loop of string was used to hold the upper incisors in position, and a tissue elevator was used as a tongue depressor to keep the mandible open and keep the tongue from slipping into the operative field. Radiopaque contrast medium (a solution of tantalum dust suspended in barium paste) was applied to surfaces of the soft palate, epiglottic tip, and valleculae with a 25 gauge tuberculin syringe via a transoral approach. This contrast material helped to visualize the location of the larynx on the radiogram. The rats were placed with their right side down against the x-ray cassette to obtain a lateral image on the film. The head was positioned to avoid parallax. The mandible was relaxed, but closed so that the molars are touching. Curix Ortho-G 35x45cm chest X-ray film was used. In addition to these lateral radiograms, a series of lateral tomograms were obtained on two specimens (one experimental and one normal) at 80 days old.

Analytical Methods

A developmental series of lateral radiograms were taken of the growing rats to determine the success of the surgery and the effects of the operation. These radiographic images, taken of living rats at 40, 60, 80, 100, and 120 days old, were used as negatives to make 5X enlarged positive prints. The Kontron image analyzer (with stylus and tabulator/keypad) and Zeiss MOP Videoplan software was used for measuring angles directly from these prints. As our concern was with shape, rather than size differences, angular measurements reflective of this were chosen. It should be noted that these measurements were obtained from radiograms taken while the rats were alive. As a result, distortions which could affect laryngeal and hyoidal position because of postmortem artifacts, such as rigor mortis or shrinkage, were avoided.

Statistical tests used

All data were statistically analyzed on an IBM 4080 mainframe computer using a standard scientific stastical package (SAS, Cary, N.C., copyright 1986, release 5.16). The data were analyzed by using the General Linear Models (GLM) of Analysis of Variance (ANOVA) for repeated measures, followed by the Tukey multiple comparison test. All computer time was donated by the City University of New York Computer Center.

ANOVA is used to determine whether there were significant differences between the data groups. ANOVA is an ultimately robust test in that it is relatively insensitive to deviations from normality of distribution and uneven sample sizes. Additionally, ANOVA can be performed under conditions that result in a low probability of committing a Type I error (i.e. rejecting a true null hypothesis and thus falsely concluding significant differences between groups), yet remaining sufficiently powerful to determine significance at a minimum detectable level (i.e. low probability of committing a Type II error of accepting a false null hypothesis). Use of ANOVA is appropriate for multiple comparisons, whereas individual Student-T tests for each pair of means results in an increased alpha level with each increase in the number of means compared, thereby resulting in an increased probability of committing a Type I error. ANOVA preserves the experimentwise alpha = 0.05 significance level despite multiple comparisons, and, through the use of harmonic means, adjusts for unequal sample sizes. It is appropriate to use a parametric test here since these data are not ranked ordinal scale data, and ANOVA is more powerful than the Kruskal-Wallis test, an essentially non-parametric ANOVA which is at best only 95% as powerful as ANOVA (Zar, 1984).

There were three factors tested (age, sex, and operation), and thus a three-way ANOVA was performed resulting in statistical tests for each factor separately and for all combinations (interactions) of these factors. Age and sex were tested to determine whether any observed differences could be due to conditions other than the effects of the surgical operation. For example, differences due to weight, size, shape, hormonal levels, or development between males and females or young and old rats may affect results. Operation was further divisible into three factor levels (normal, sham, and experimental, with experimental further subdivided into group T and group P). As these factors were compared to three variables (angles S, H, and L), three different three-way ANOVAs were conducted.

As ANOVA merely indicates the presence or absence of significant differences, a test subsequent to ANOVA such as the Tukey test (Tukey, 1953) is needed to determine where these differences lay. The Tukey test is recommended by Zar (1984) as it is more conservative than any of the other tests subsequent to ANOVA (e.g. Student-Newman-Keuls or Duncan tests). By controlling for the experimentwise error rate (probability of committing a Type I error), the Tukey test is more likely to accept the null hypothesis and conclude no significant differences between groups. In addition, the Tukey test can be performed on data with unequal sample sizes without a decrease in power.

Establishing a reference line

A stable (non-moving) region of the rat skeleton, which was not affected by the surgery, was sought in order to establish a reference line against which the angular measurements could be taken. Any bony structure which could move (such as the mandible or cervical vertebrae) with respect to the area being measured, could not be used. Therefore, the only available region on which points could be located had to be on the body of the cranium. Points on the cranium itself, however, had to be unaffected by the surgery. A region which appears to meet the dual criteria of being both stable and unaffected by surgery was the hard palate, as it is both a part of the cranium and is removed from the immediate region of the surgery.

In order to test the validity of using the hard palate as a reference line the following was done. Enlarged positive prints from the radiograms of the 45 pilot study rats were examined. A linear measurement of hard palate length was taken for each rat. Measurements from pilot study experimentals were compared to measurements from pilot study normals and pilot study shams (see table 4) using ANOVA (see table 5) and Tukey test (see table 6), at alpha = 0.05. There were no significant differences between the means for pilot study experimentals (2.4cm, std. dev.= 0.08), shams (2.3cm, std. dev.= 0.05), and normals (2.3cm, std. dev.= 0.08). Hard palate length thus appeared to be a stable cranial measurement which was not affected by the surgery, and thus was chosen as a reference line for angular measurements of skull base contour and laryngeal and hyoidal positions.

Cranial base measurements

Skull base flexion was documented by measuring the angle S (see figure 17). Angle S was formed at the intersection between two lines connecting three points visible in the lateral radiograms: 1) the midline point between the upper incisors at the front of the hard palate (the approximate equivalent of craniometric point orale in humans), 2) the posterior edge of the hard palate (an approximation of craniometric point staphylion in humans), and 3) the front edge of foramen magnum (the approximate equivalent of craniometric point basion in humans). The vertex for this basicranial angle was located at the edge of the hard palate (staphylion). (For descriptions of human craniometric points see Wilder, 1920; Martin, 1928; Stewart, 1952.) The degree of kyphosis present in the experimentals relative to sham operated and normal controls was then statistically assessed using ANOVA and Tukey's test.

Figure 17: Positive print from radiogram of a normal rat to show the angular measurements of rat skull: angle S indicates skull base angle, angle L indicates larynx angle, angle H indicates hyoid angle.


L
H

Hyoidal position measurements

The position of the hyoid bone was recorded by measuring the angle H (see figure 17). Angle H was formed by the intersection between two lines connecting three points visible in the lateral radiograms: staphylion, orale, and the hyoid bone (at its anterior-inferior edge in the midline) with the vertex located at orale. Differences in position of the hyoid bone of experimentals, shams, and normal controls was then statistically assessed using ANOVA and Tukey's test.

Laryngeal position measurements

The position of the larynx was recorded by measuring the angle L (see figure 17). Angle L was formed at the intersection between two lines connecting three points visible in the lateral radiograms: staphylion, orale, and the larynx (at the base of the epiglottis, where the contrast dye outlined the laryngeal valleculae), with the vertex located at orale. Differences in position of the larynx of experimentals, shams, and normal controls was then statistically assessed using ANOVA and Tukey's test.

Post mortem histological examination

All rats were allowed to mature to 130 days old before being sacrified by a lethal dose of diethyl ether and transcardiac perfusion with phosphate buffered formalin (10% formaldehyde). The heads were then removed and decalcified with versene (EDTA). Decalcification of each skull was confirmed radiographically. The heads were then dehydrated, double embedded in 3% celloidin and then paraffin, and mounted in paraffin blocks. Sections were cut at 10 microns and stained with hematoxylin and eosin. The region of the spheno-occipital synchondrosis was examined for the presence of a prematurely formed synostosis. The skull base was also examined to confirm that: 1) the drilling was performed in the correct location on both the left and right sides, 2) the cartilage of the synchondrosis was completely destroyed or removed, 3) the drilling did not puncture the dura and damage the brain, particularly the pituitary or the pons.

Postmortem gross anatomy

Post mortem examination was done on the 45 pilot study rats killed at 80 and 87 days old. Fifteen pilot study experimental rats, 6 pilot study normal rats, and 2 pilot study sham rats were sectioned in the midsagittal plane and the anatomy of this region was visually inspected and compared. Specimens were then photographed to record their anatomy. Fourteen pilot study experimentals, 6 pilot study normals, and 2 pilot study shams were dissected from a ventral approach to inspect for any muscular damage and gross changes on the ventral surface of the skull base. These specimens were then defleshed and cleaned to reveal gross changes in the morphology of the whole skull.

CHAPTER 5

EXPERIMENTAL RESULTS

Sample size

Surgical operations removing the spheno-occipital synchondrosis were performed on 89 experimental rats aged 13 days old. Ten sham rats underwent the surgery, minus the drilling, at 13 days old. Of the 89 experimental rats that underwent surgery, 42 died of respiratory arrest during the surgery. Of the remaining 47 experimental rats, 17 died before being weaned at 25 days old (including 4 that were cannibalized by their mothers within 6 days after surgery). Thirty experimental rats, 10 sham rats, and 6 normal control rats survived past weaning. Measurements were taken from 27 experimental rats (as the other 3 were sickly), ten sham operated rats, and six normal control rats. All rats were sacrificed at 130 days old.

Weights

After weaning all rats were weighed daily. Their weights (in grams) were then compared to each other to help identify any differences in growth based upon the surgery. Weight charts for the experimentals, shams, normals, and all three groups combined are presented in figures 18-22. The weights of the females were found to be less than those of the males. Weight curves for experimentals, shams, and normals were found to overlap from ages 25 to 130 days, thus indicating no significant differences between these three groups at any age based upon surgery. Mean weights are presented in table 7.

Figure 18: Weight chart of normal rats. Weights were recorded daily for each rat. Top four lines represent males, bottom two lines represent females.

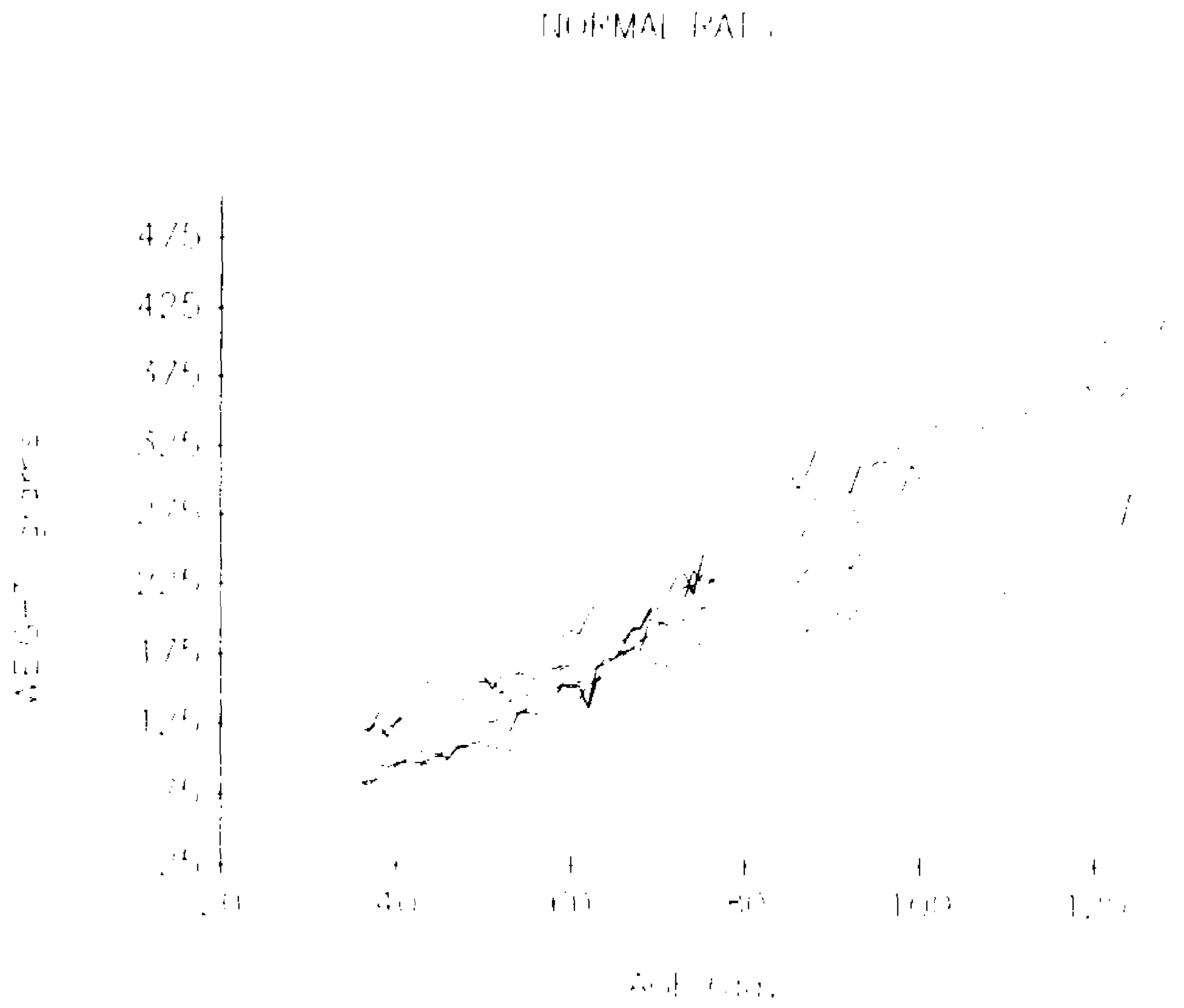


Figure 18

Figure 19: Weight chart of Sham operated rats. Weights were recorded daily for each rat. Top five lines represent males, bottom five lines represent females.

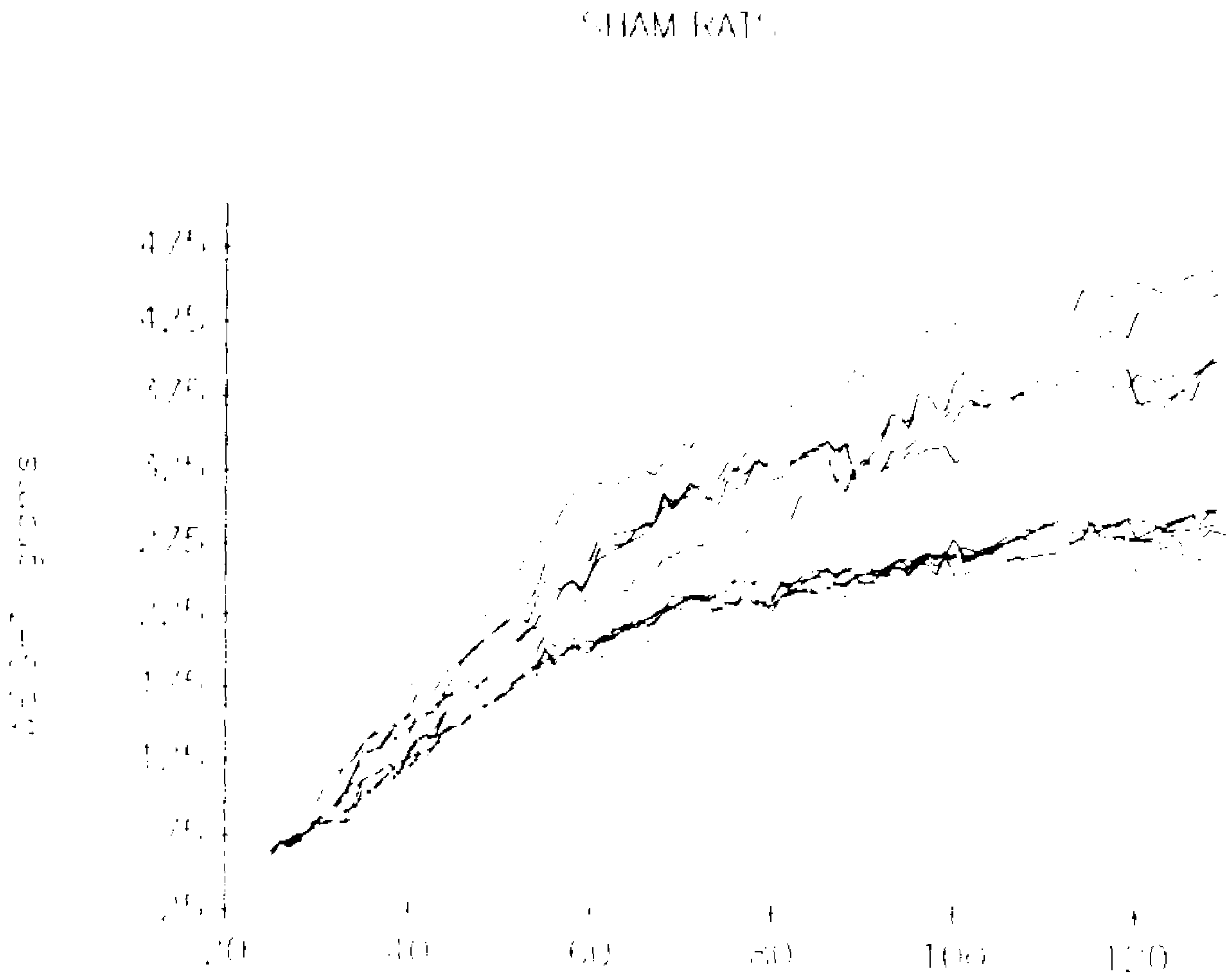

SHAM RATS
4.75
4.25
3.75
3.25
2.75
2.25
1.75
1.25
.75
.25
20
40
60
80
100
120
AGE

Figure 19

Figure 20: Weight chart of Experimental rats. Weights were recorded daily for each rat. Top fifteen lines represent males, bottom twelve lines represent females.

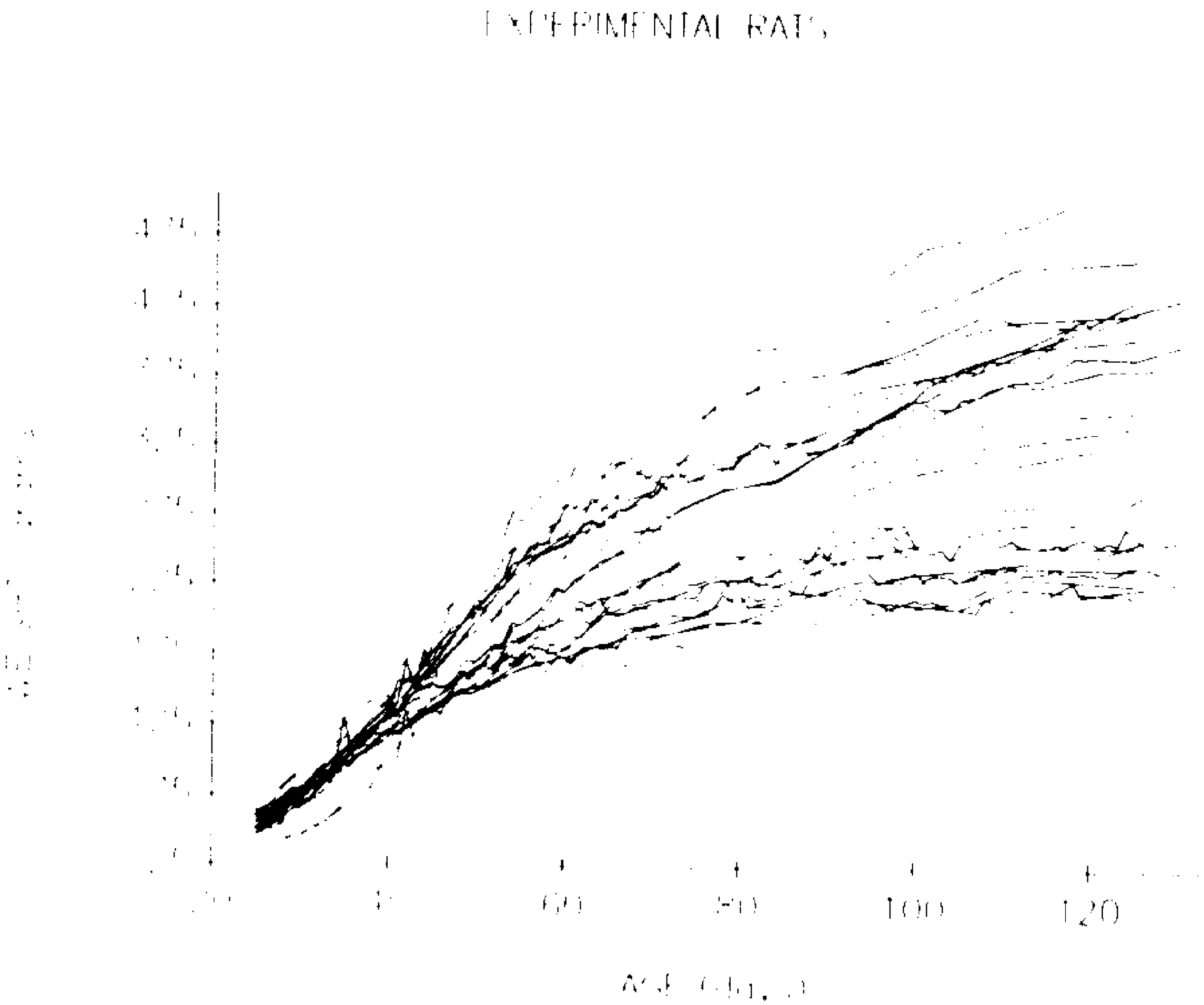


Figure 20

Figure 21: Weight chart of Normal, Sham operated, and Experimental rats. Weights were recorded daily for each rat. Top twenty-four lines represent males, bottom nineteen lines represent females.

NORMAL, SHAM, AND EXPERIMENTAL RATS

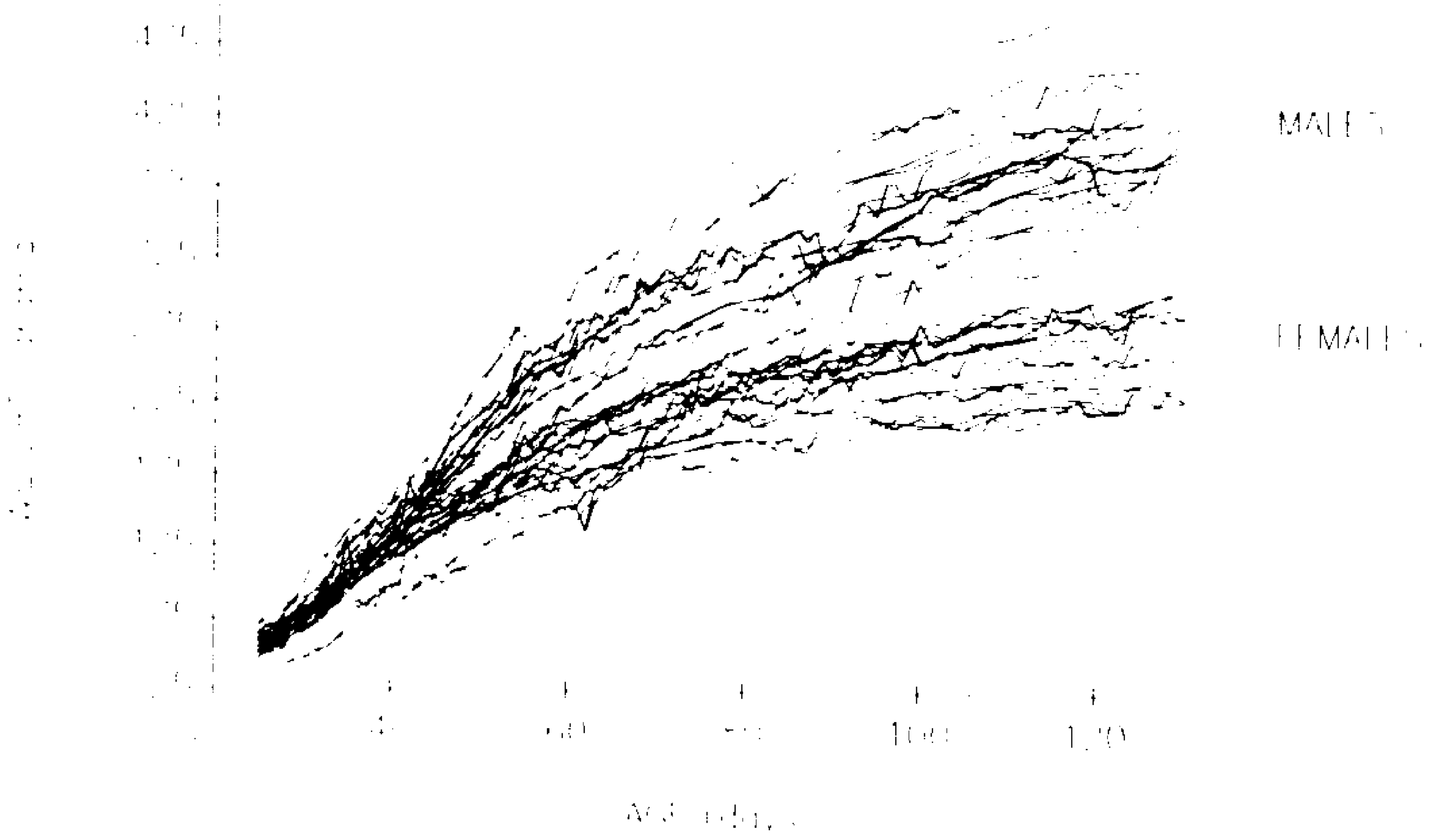


Figure 21

Figure 22: Weight chart of Normal, Sham operated, and Experimental rats. Mean weights and standard error bars are shown for males and females of each group (normal, sham, experimental) at 40, 60, 80, 100, 120, and 130 days old.

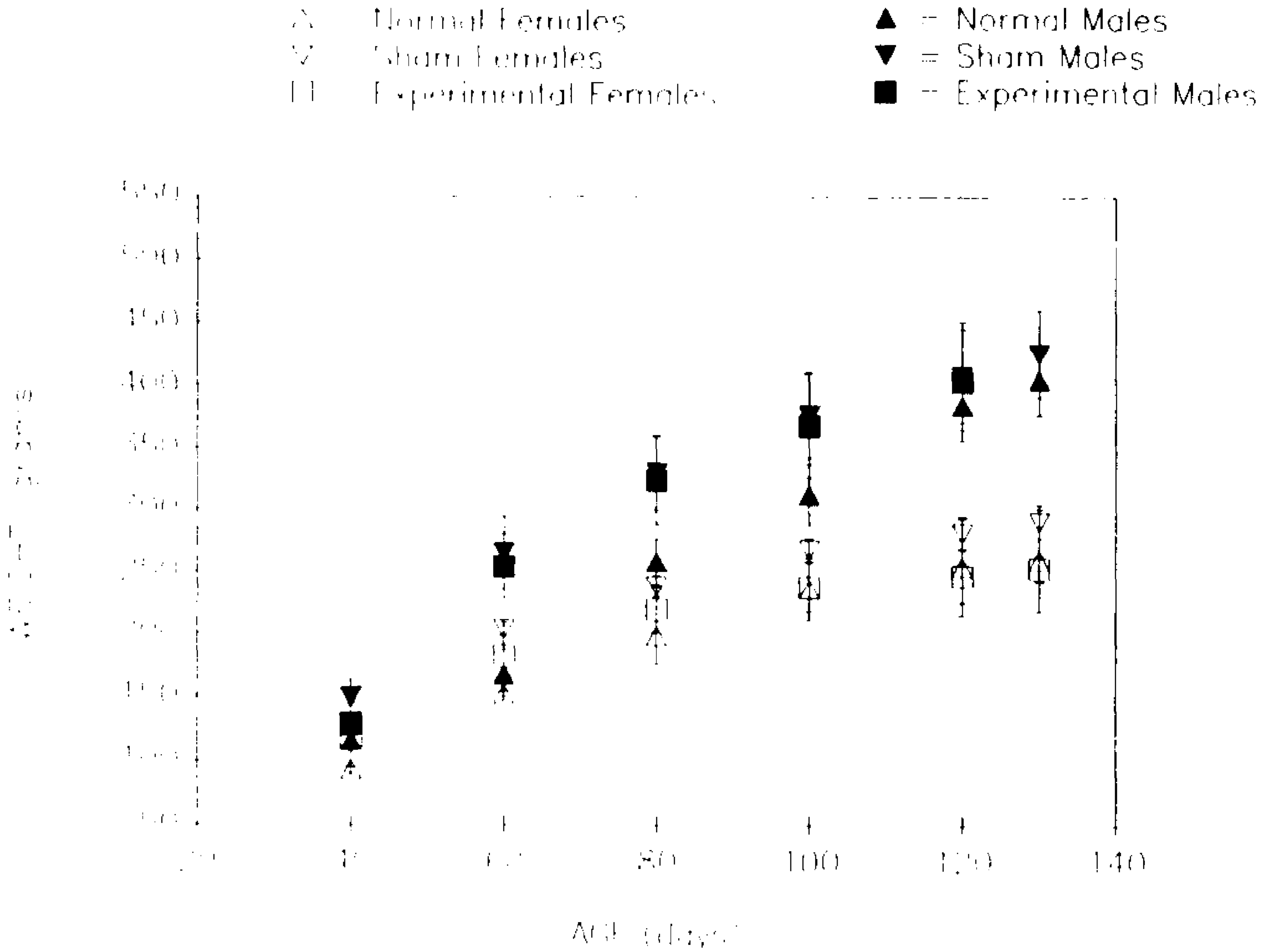

△ = Normal Females
▽ = Sham Females
□ = Experimental Females
▲ = Normal Males
▼ = Sham Males
■ = Experimental Males
550
500
450
400
350
300
250
200
150
100
50
20
40
60
80
100
120
140
AGE (days)

Figure 22

In Vivo observations

Visual inspection of radiograms revealed observable differences in skull shape and size between the experimentals and the controls (both sham operated and normal). Examination of the serial radiograms by age revealed the expected enlargement in overall skull size with progressive age in all groups. Normal changes in skull shape were observed in the sham operated and normal control groups. These changes consisted of lengthening of the skull base accompanied by a parallel lengthening of the skull vault, and elongation of the facial region. Younger rats (age 40 days) had rounded skull vaults, while older rats (age 120 days) had flatter skull vaults. The overall calvarial shape changed from a relatively oval appearance in the young rats, to a rectangular shape in the older rats (see figures 23-28).

In the experimental rats, the skull vault was more rounded than that of the sham operated or normal controls. This rounded shape was especially apparent in the older rats (80 to 120 days). In addition, the basicranium appeared more kyphosed and shorter in the experimentals than in sham operated or normal control rats. The overall skull size also appeared to be slightly smaller in the experimentals than in the controls (both normal and sham operated). The foramen magnum opened more ventrally in experimentals than in normal and sham controls. The internal surface of the skull base in experimentals often displayed a peculiar "peaking" above the region where the spheno-occipital synchondrosis was drilled, slightly posterior to the location of the pituitary. This triangular elevation was not present in the sham operated or normal controls. This difference was especially apparent in the lateral tomograms, which were focused to emphasize midline structures (see figures 29 and 30).

Figure 23: Lateral radiogram of a normal rat. S = soft palate, H = hyoid, V = valleculae, E = epiglottis, black arrows indicate spheno-occipital synchondrosis. The white area above the vallecula and anterior to the epiglottis is a radiopaque marker (tantalum dust suspended in barium paste) placed in the laryngeal valleculae. It indicates laryngeal position and also faintly outlines the ventral surface and the posterior portion of the dorsal surface of the soft palate.

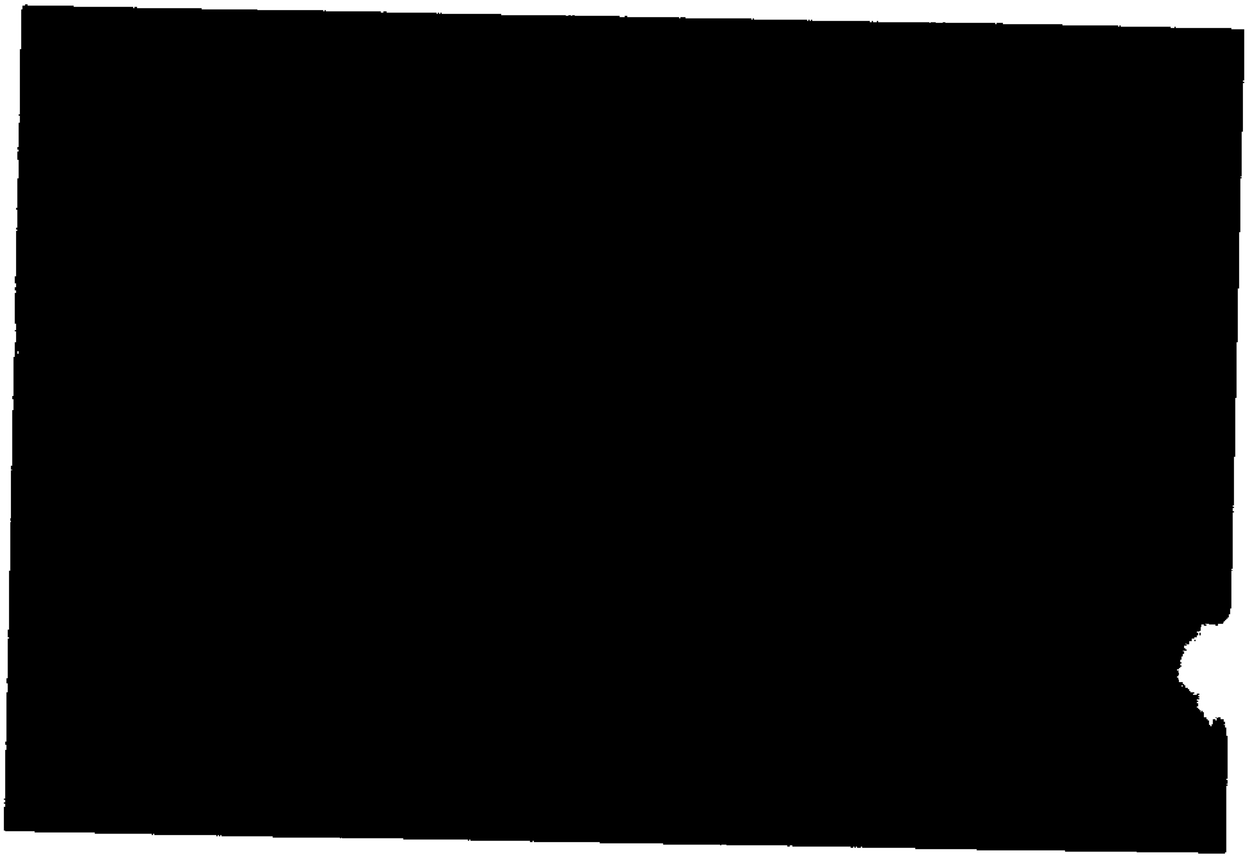


Figure 24: Lateral radiograms of typical normal (N), sham (S), and experimental (E) rats at age 40 days. Note flexed skull base and low position of the hyoid and larynx in the experimental rat.

40 DAYS

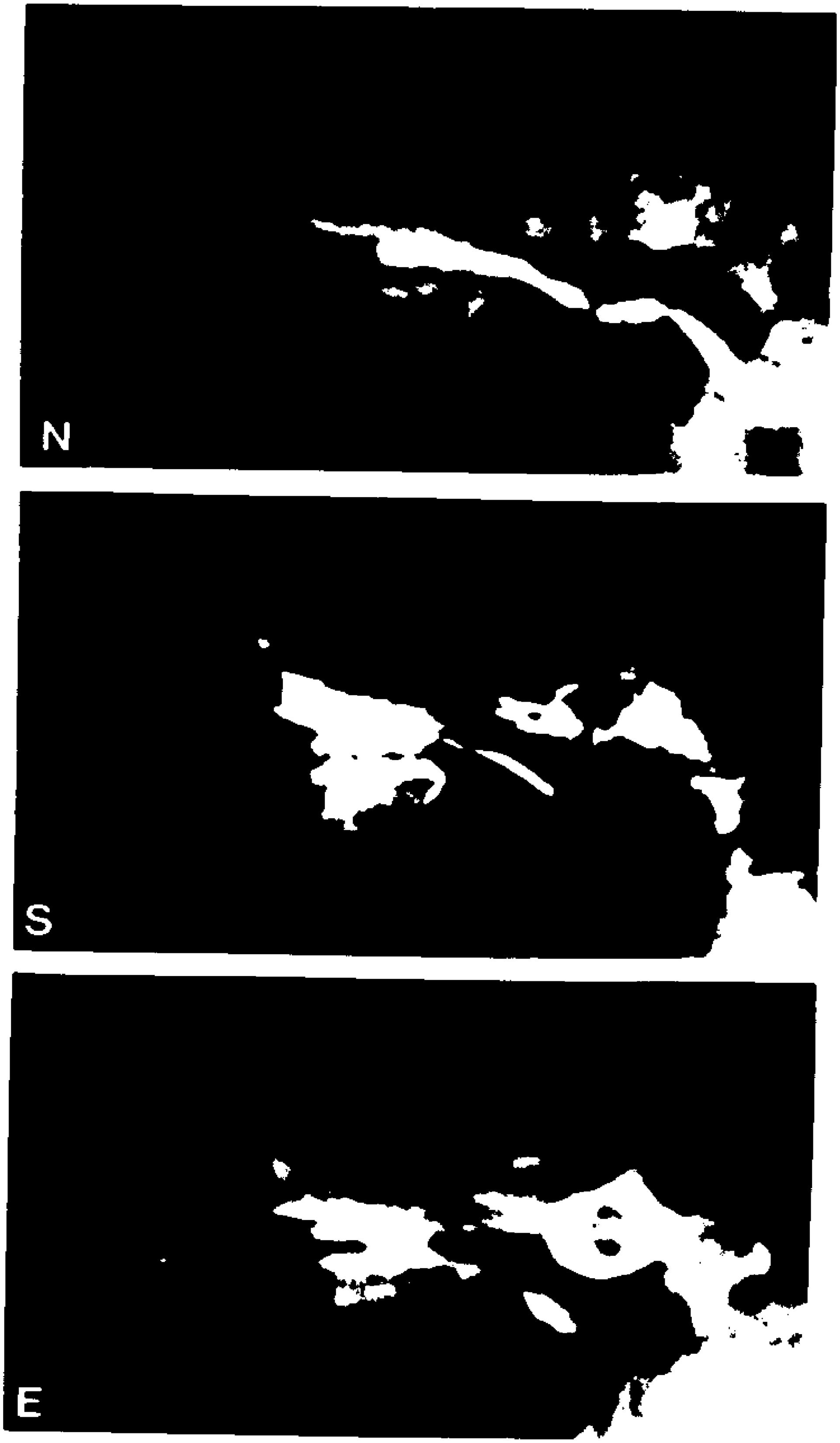


Figure 25: Lateral radiograms of typical normal (N), sham (S), and experimental (E) rats at age 60 days. Note flexed skull base and low position of the hyoid and larynx in the experimental rat.

60 DAYS

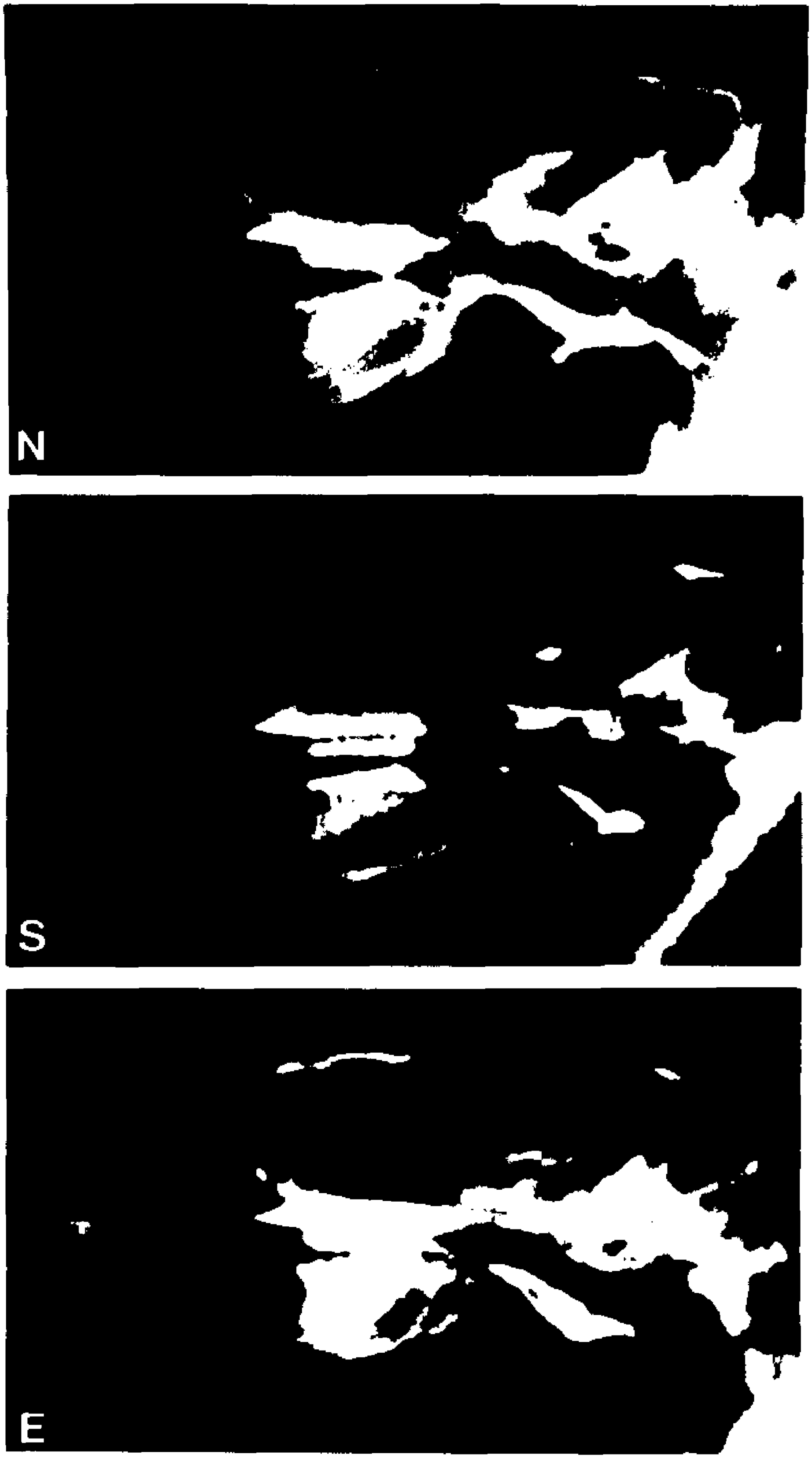


Figure 26: Lateral radiograms of typical normal (N), sham (S), and experimental (E) rats at age 80 days. Note flexed skull base and low position of the hyoid and larynx in the experimental rat.

80 DAYS

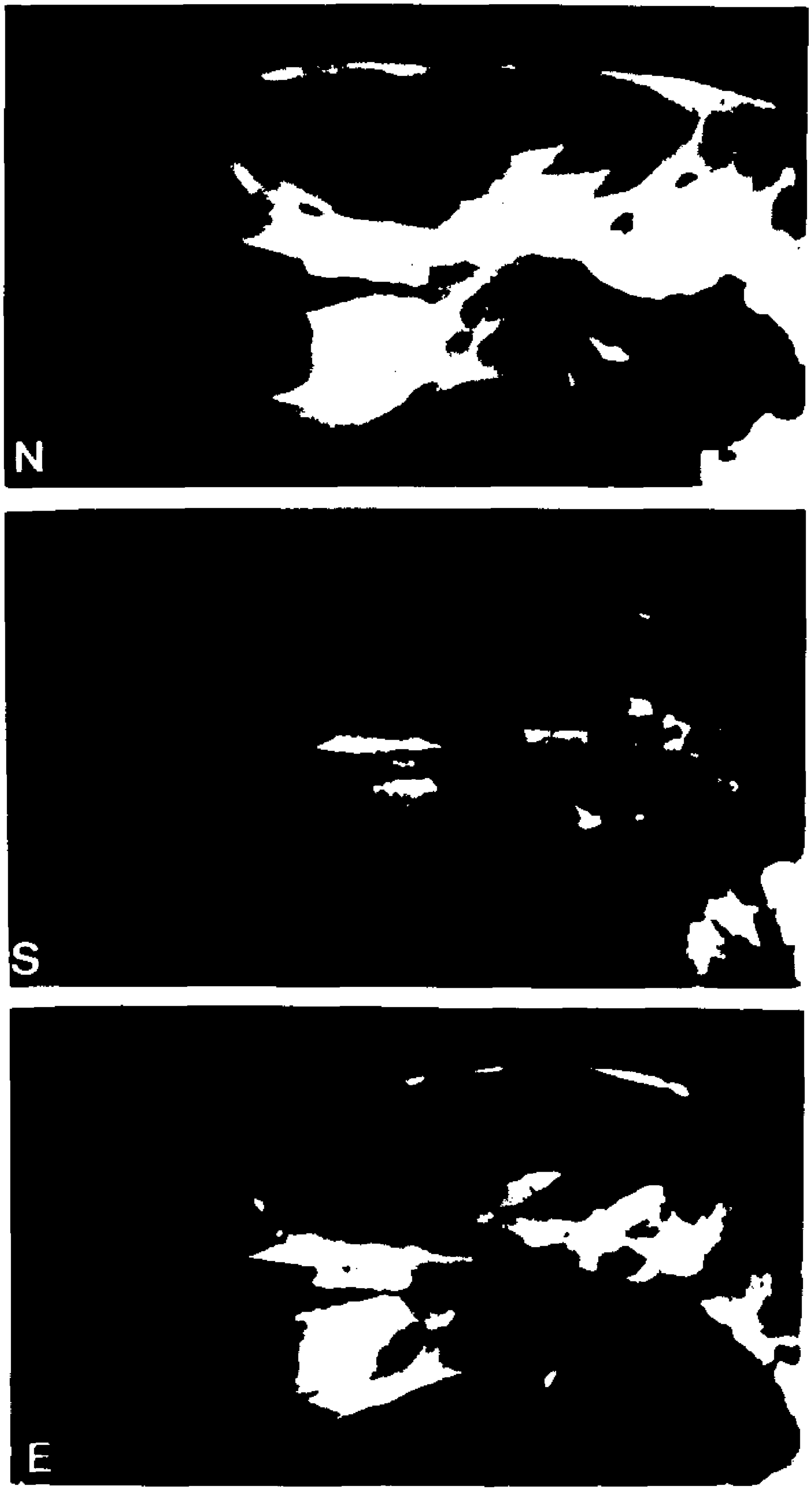


Figure 27: Lateral radiograms of typical normal (N), sham (S), and experimental (E) rats at age 100 days. Note flexed skull base and low position of the hyoid and larynx in the experimental rat.

100 DAYS

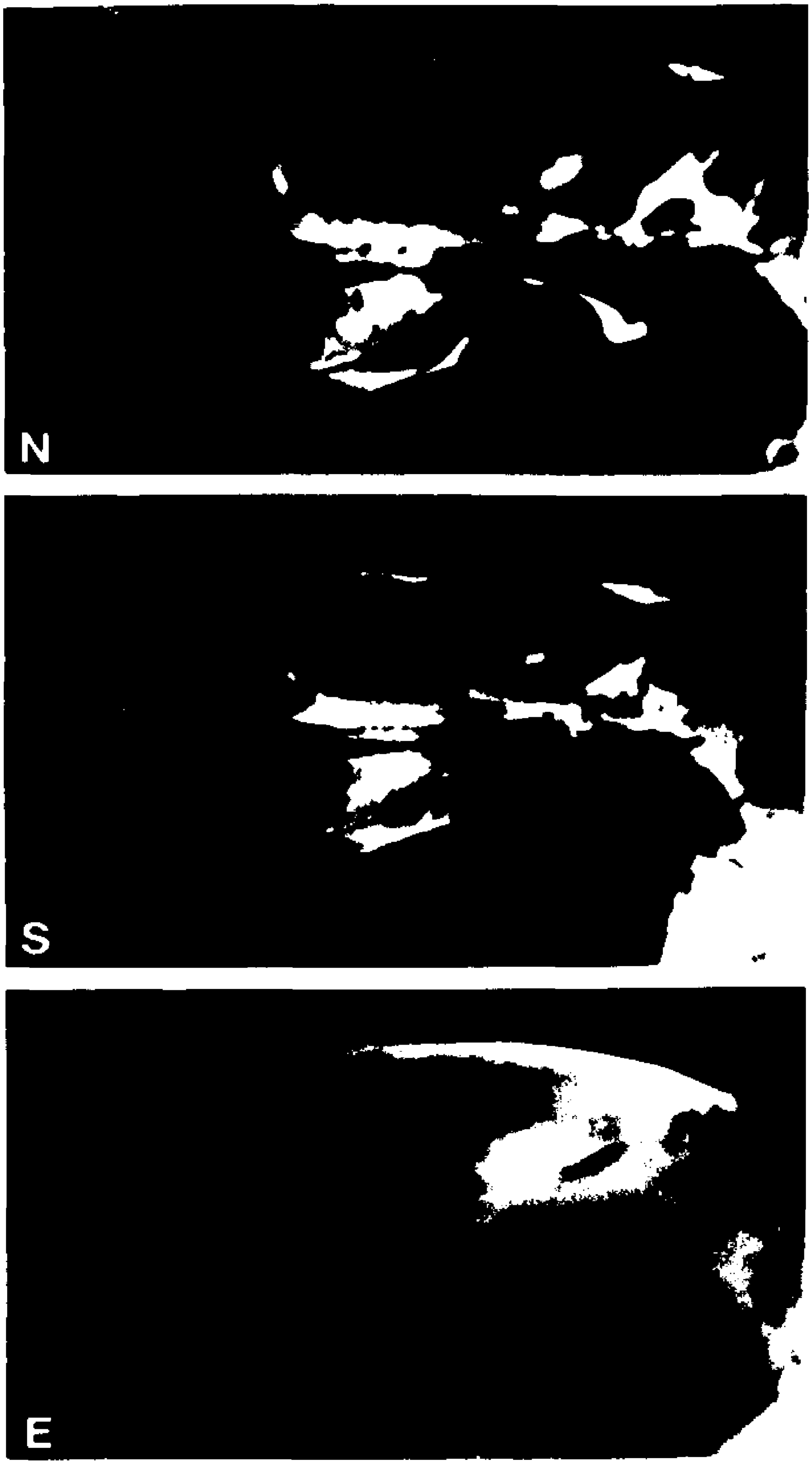


Figure 28: Lateral radiograms of typical normal (N), sham (S), and experimental (E) rats at age 120 days. Note flexed skull base and low position of the hyoid and larynx in the experimental rat.

120 DAYS

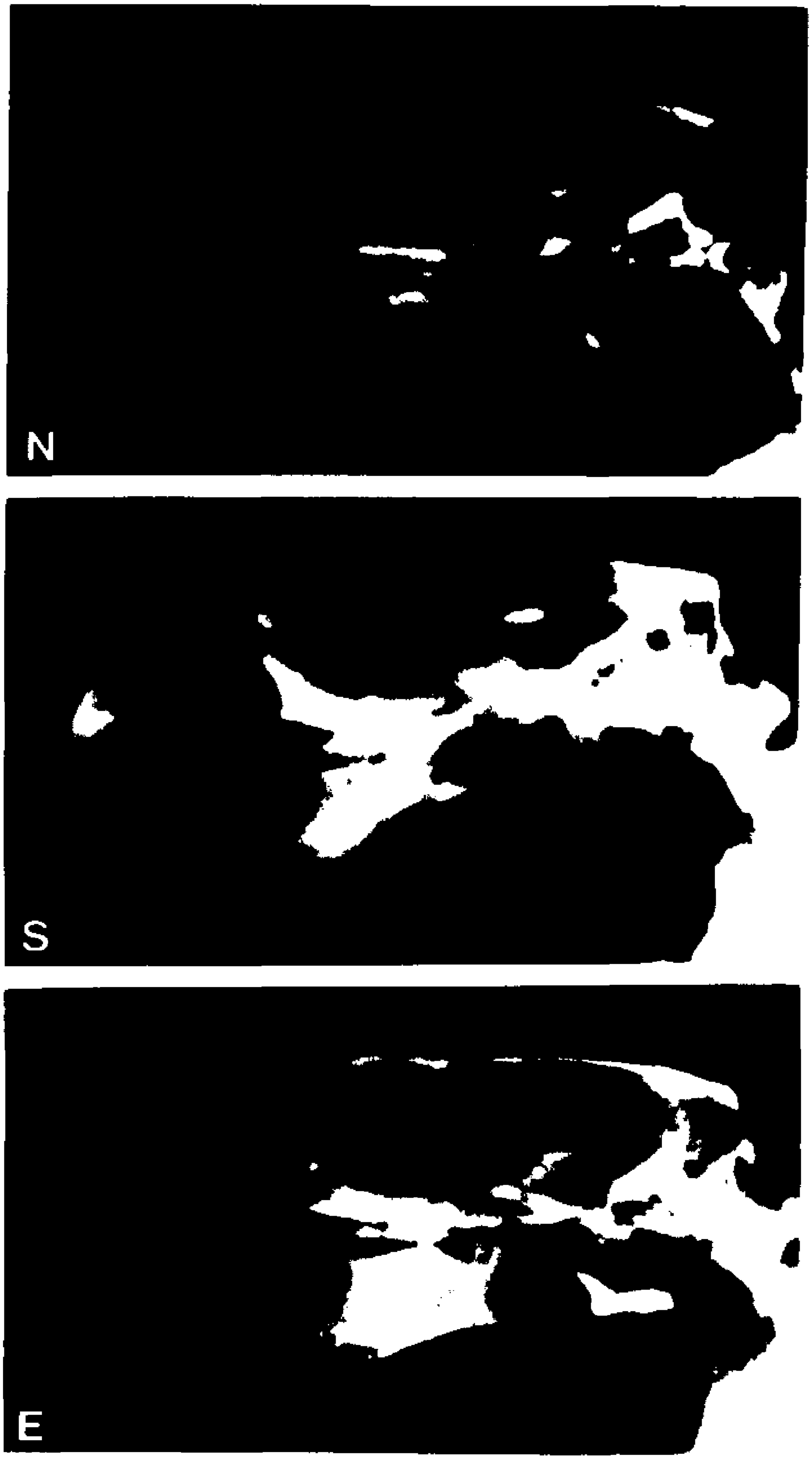
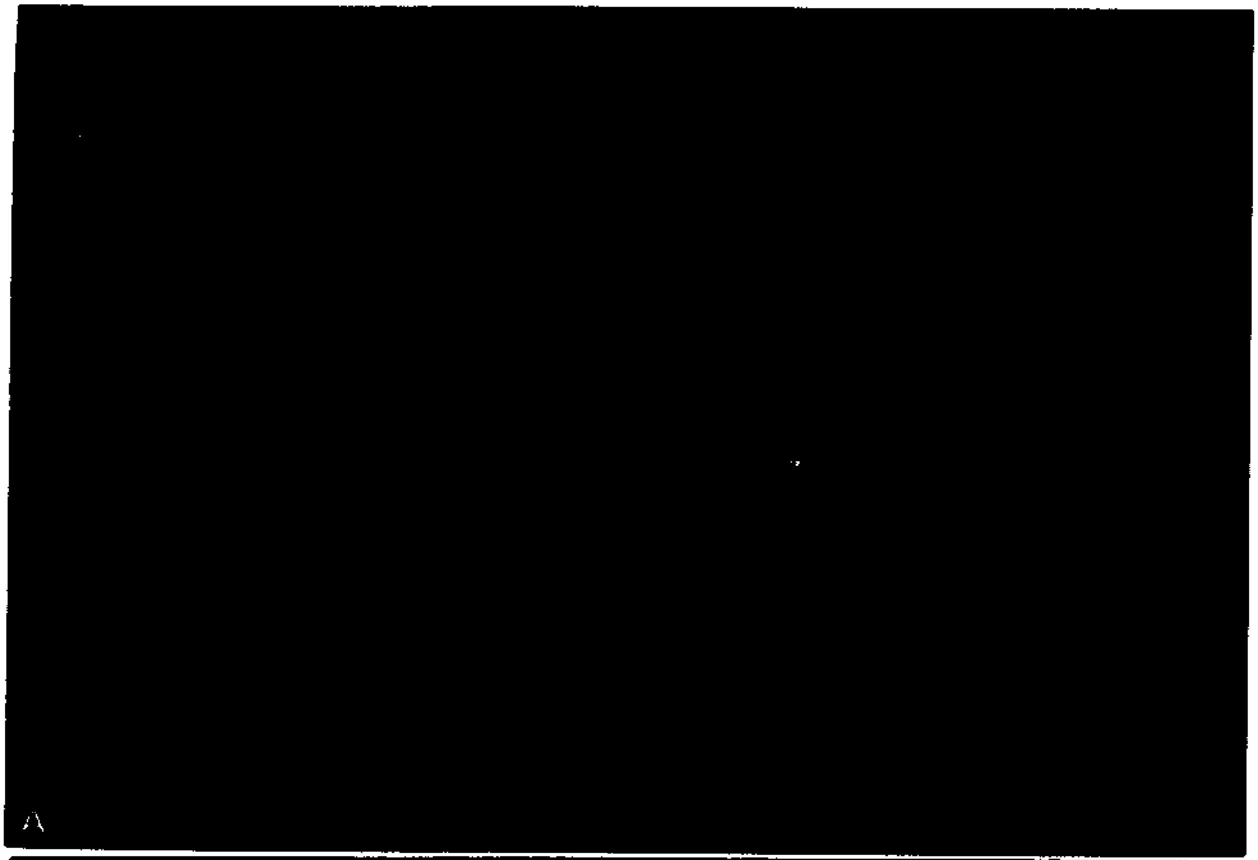


Figure 29: Tomograms of normal rat (A) and experimental rat (B). Note the high hyoid, flat skull base, and squared cranial vault in the normal rat. The experimental rat shows a low hyoid, curved skull base, and domed cranial vault. Also note the elevated ridge, resembling the dorsum sellae of the human skull, on the endocranial surface of the basicranium of the experimental rat.


A

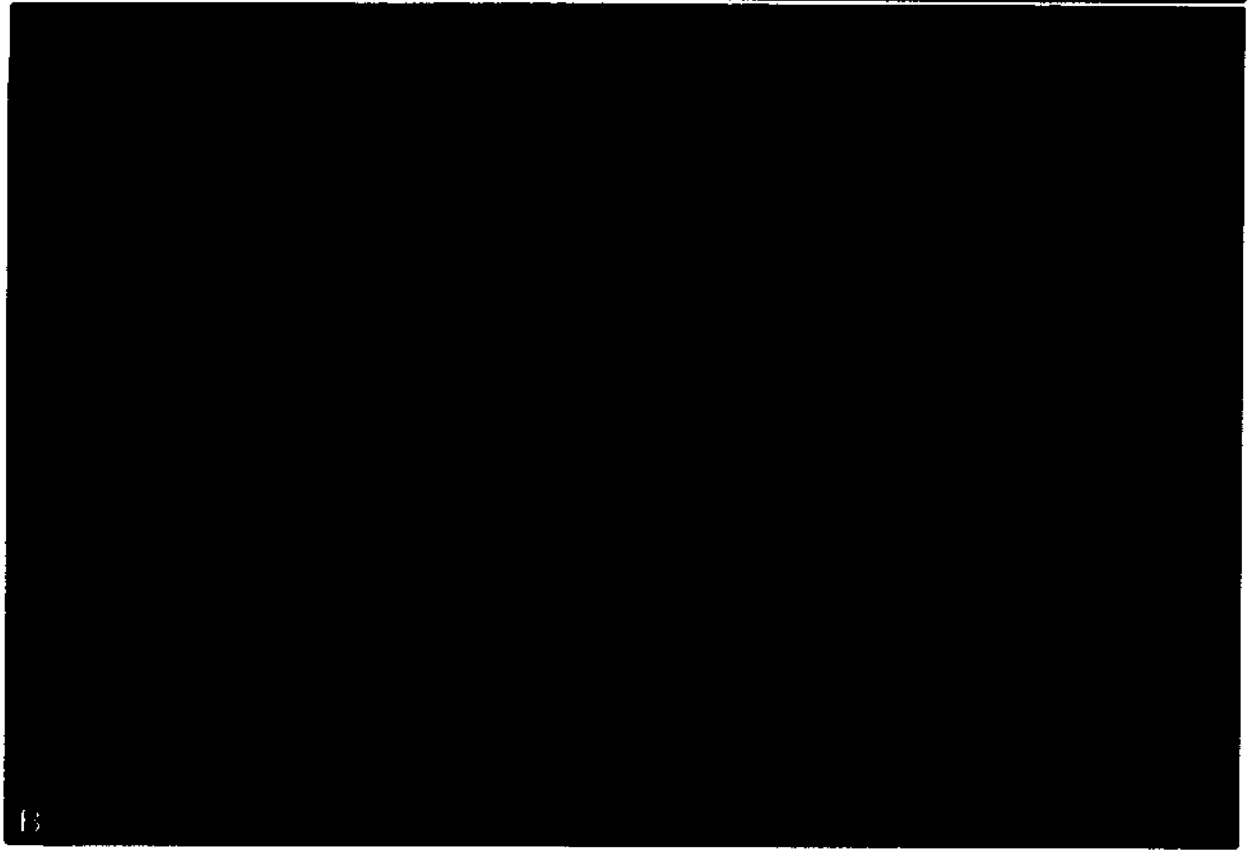
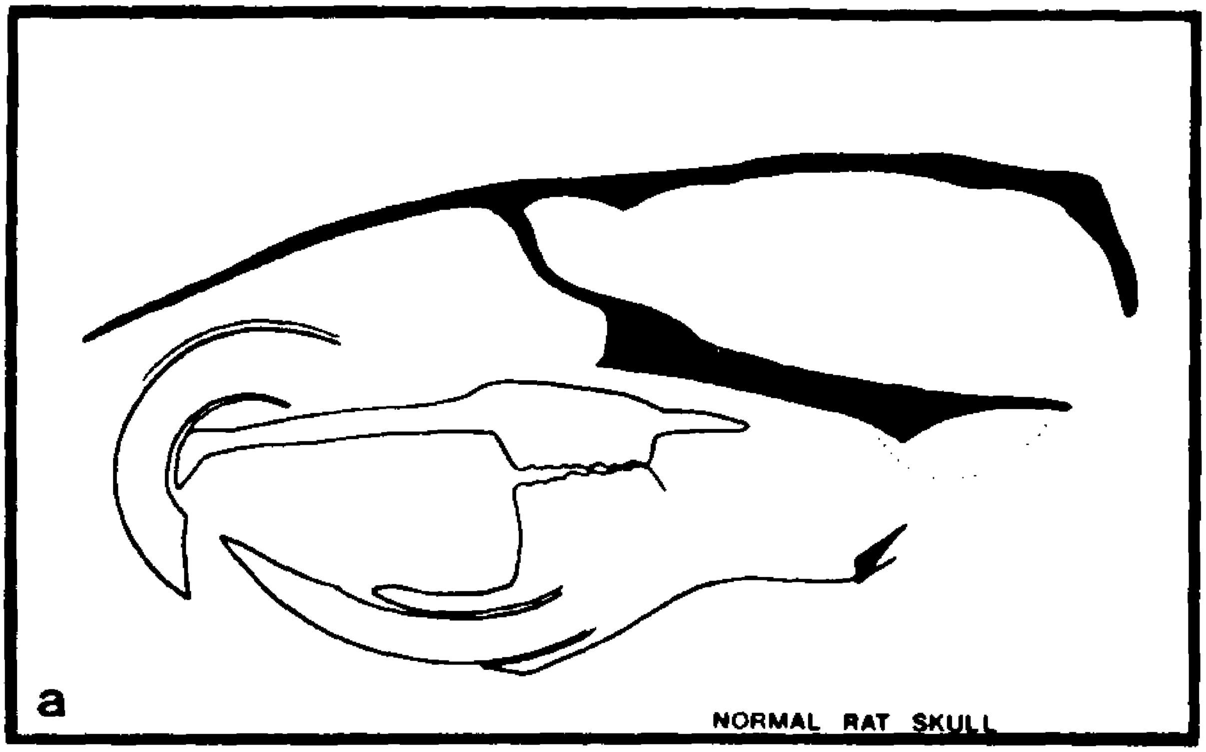
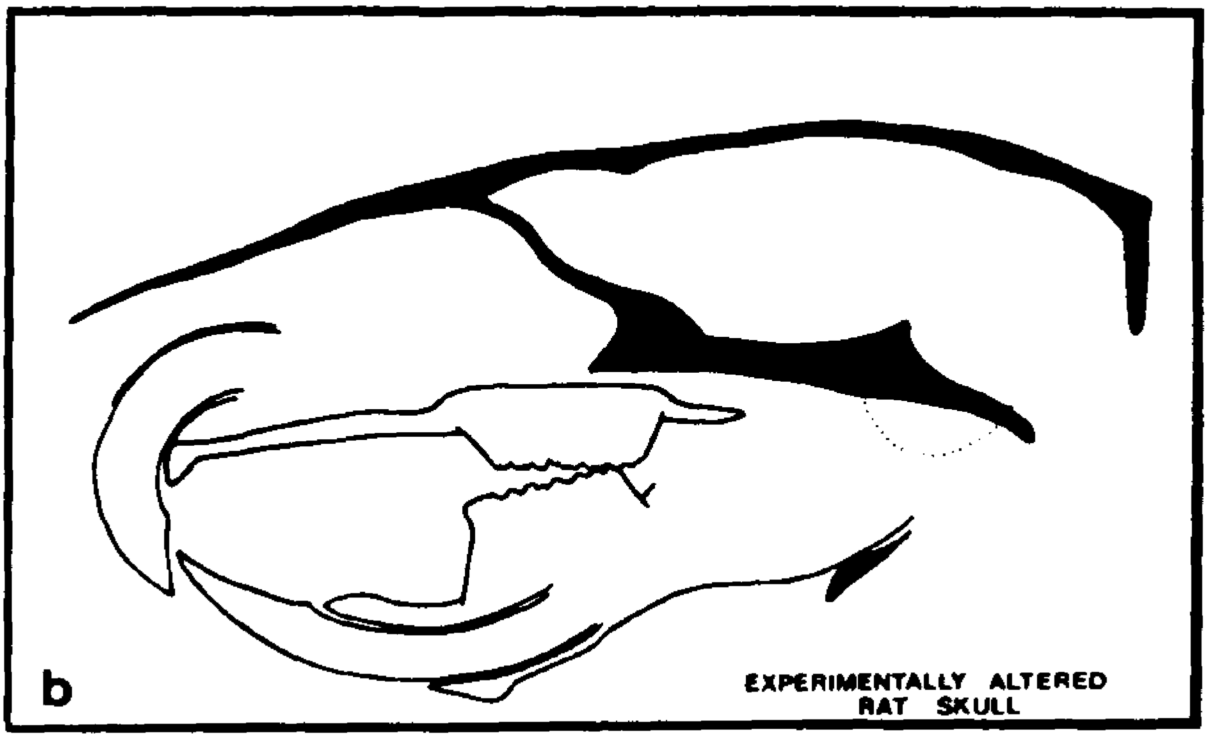

B

Figure 30: Direct tracings from the tomograms shown in figure 29. These outlines show the higher position of the hyoid bone and the flat skull base and vault in the normal rat (a), compared with the lower position of the hyoid bone and the curved skull base and vault in the experimental rat (b). An elevated ridge resembling the dorsum sellae of the human skull is also evident on the endocranial surface of the skull base of the experimental rat.


a
NORMAL RAT SKULL


b
EXPERIMENTALLY ALTERED
RAT SKULL

Statistical Analysis of Radiological Data

Angular measurements were taken from radiograms of experimental, sham, and normal control rats at 40, 60, 80, 100, and 120 days old for skull base angles, hyoid angles, and laryngeal angles. These raw data are given for each specimen at each age in table 8. Analysis of variance (ANOVA) results are presented in table 9. Tukey test results are given in table 10. Means for all angles are given for each operative group at each age and sex, and for all combinations of these groups in tables 11-16. Pearson correlation coefficients are listed in table 17.

Three-way analysis of variance, or ANOVA, (alpha = 0.05) were performed separately on the data from each group for angle S (skull base angle), angle H (hyoid angle), and angle L (laryngeal angle), versus indices of operation, sex, and age. The ANOVA results show that there were significant differences ($P > 0.0001$) between the groups based upon surgical operation (experimental=E, sham=S, normal=N) for angle S, angle H, and angle L. There were also significant differences ($P > 0.0006$) between the groups based upon sex for angle S, but not for angles H or L. There were slightly significant differences ($P > 0.0095$) between the groups based on age for angle S, but not for angles

H or L. There were no significant differences due to any interactions of operation and age, operation and sex, or operation and age and sex.

The Tukey multiple comparison test was performed subsequent to ANOVA to determine which groups were significantly different. The Tukey test results indicate that there were significant differences (alpha = 0.05) between the experimental (E), sham (S), and normal control (N) groups for angle S, angle H, and angle L based upon operation. These results indicate that the experimental, sham, and normal control groups were significantly different from each other for all three angles. The experimental rats had the smallest mean for angle S (i.e., the skull base was flexed), the normal rats had the largest mean for angle S (i.e., the skull base was the flattest), and the shams had an intermediate mean for angle S. The experimental rats had the largest means for angles H and L (i.e., the hyoid and larynx were lower), the normal rats had the smallest means for angles H and L (i.e., the hyoid and larynx were higher), and the shams had intermediate means for angles H and L.

The Tukey test also detected significant differences between groups based upon sex. For angle S, males were significantly larger than females. This sexual difference was not apparent in angles H or L.

The Tukey test was also used to determine differences between the groups based upon age. There were significant differences between the 40 day old rats and the 120 day old rats for angle S, but not amongst the 60, 80, and 100 day old rats. There were no significant differences between the means of any age for angles H and L.

Correlation coefficients were calculated to determine whether there was any correlation between the skull base contour (angle S) and the position of the hyoid (angle H) and the larynx (angle L). The Pearson correlation coefficient (r) for experimentals, shams, and normals at 40, 60, 80, 100, and 120 days old was -0.743 ($P < 0.0001$) for angle S vs. angle H, and -0.799 ($P < 0.0001$) for angle S vs. angle L. Correlation coefficients were also calculated separately by groups. In normals, $r = -0.439$ ($P < 0.0006$) for angles S vs. H, and $r = -0.440$ ($P < 0.0005$) for angles S vs. L. In shams, $r = -0.676$ ($P < 0.0001$) for angles S vs. H, and $r = -0.725$ ($P < 0.0001$) for angles S vs. L. In experimentals, $r = -0.691$ ($P < 0.0001$) for angles S vs. H, and $r = -0.665$ ($P < 0.0001$) for angles S vs. L. These values indicate significant negative correlations (i.e., as the skull base flattens, the hyoid and larynx are positioned higher, while as the skull base flexes, the hyoid and larynx are positioned lower).

TABLE 4
MEANS FOR HARD PALATE MEASUREMENTS

Operation	Age	Sex	N	Length (cm)
N	-	F	6	2.2 ±0.05
N	-	M	6	2.3 ±0.08
S	12	F	3	2.3 ±0.06
S	12	M	1	2.3 ±0.00
E	12	F	4	2.3 ±0.05
E	12	M	1	2.4 ±0.00
E	13	F	2	2.3 ±0.00
E	13	M	5	2.4 ±0.09
E	14	F	2	2.3 ±0.07
E	14	M	6	2.4 ±0.06
E	15	F	4	2.3 ±0.05
E	15	M	5	2.4 ±0.09

Age = age at time of surgery.
Standard deviations are given for each mean length.

TABLE 5
ANALYSIS OF VARIANCE (ANOVA)
Dependent variable = hard palate length

Groups	F value	PR > F
operation	5.59	0.0081
age	0.80	0.5008
sex	17.56	0.0002
operation*age	0.0	0.0
operation*sex	1.36	0.2696
operation*age*sex	0.48	0.6981

Age = age at time of surgery.

TABLE 6
TUKEY ANALYSIS FOR MULTIPLE COMPARISONS
Variable = hard palate length

Grouping	Mean	N	Operation
A	2.4	29	E
A	2.3	4	S
A	2.3	12	N

TABLE 7

MEAN WEIGHTS FOR NORMALS, SHAMS, AND EXPERIMENTALS

Age	Sex	Operation	Mean Weight ± SD (g)
40	F	Normal	93.1 ± 1.8
40	F	Sham	125.9 ± 10.5
40	F	Experimental	118.4 ± 11.5
40	M	Normal	115.7 ± 26.5
40	M	Sham	149.1 ± 14.1
40	M	Experimental	128.3 ± 16.1
60	F	Normal	152.0 ± 1.9
60	F	Sham	203.6 ± 5.3
60	F	Experimental	181.6 ± 16.3
60	M	Normal	166.7 ± 20.1
60	M	Sham	265.5 ± 29.4
60	M	Experimental	254.0 ± 26.2
80	F	Normal	198.0 ± 22.6
80	F	Sham	238.4 ± 8.3
80	F	Experimental	219.2 ± 18.5
80	M	Normal	257.9 ± 18.8
80	M	Sham	330.6 ± 30.3
80	M	Experimental	324.0 ± 36.0
100	F	Normal	236.0 ± 25.5
100	F	Sham	268.2 ± 9.4
100	F	Experimental	237.7 ± 19.9
100	M	Normal	312.5 ± 24.4
100	M	Sham	376.8 ± 34.6
100	M	Experimental	368.5 ± 41.8
120	F	Normal	254.0 ± 39.6
120	F	Sham	281.2 ± 13.6
120	F	Experimental	246.5 ± 22.1
120	M	Normal	384.5 ± 19.1
120	M	Sham	408.2 ± 36.9
120	M	Experimental	403.5 ± 47.5
130	F	Normal	259.5 ± 41.7
130	F	Sham	290.8 ± 13.9
130	F	Experimental	252.5 ± 10.6
130	M	Normal	405.0 ± 28.3
130	M	Sham	425.6 ± 34.6

SD = standard deviation.

TABLE 8

Operat.	Age	Sex	Angle S	Angle H	Angle L
T	40	M	168.3	15.1	11.7
T	40	M	167.8	15.2	12.0
P	40	M	170.7	13.8	11.6
P	40	M	172.6	14.4	11.8
P	40	M	171.7	14.4	13.1
P	40	M	170.6	14.0	12.2
T	40	M	168.2	15.6	12.5
P	40	M	171.1	16.0	12.5
P	40	M	170.6	15.2	12.8
T	40	M	168.2	16.4	13.5
P	40	M	172.7	14.9	11.1
T	40	M	168.7	16.1	13.3
T	40	M	169.1	15.7	12.0
T	40	M	171.7	16.9	13.1
T	40	M	165.4	17.7	13.9
P	40	F	169.9	13.4	11.4
P	40	F	170.8	13.7	11.5
T	40	F	166.7	15.7	13.3
P	40	F	172.3	14.2	12.0
T	40	F	164.2	17.6	14.1
P	40	F	170.0	15.2	12.9
P	40	F	169.6	16.4	13.1
P	40	F	168.7	15.9	13.3
T	40	F	165.5	16.6	14.5
T	40	F	165.5	16.5	15.8
T	40	F	167.6	15.7	14.4
P	40	F	172.6	14.1	13.2
T	60	M	161.1	16.3	12.6
T	60	M	171.5	15.4	12.9
P	60	M	171.2	12.8	11.2
P	60	M	174.3	15.1	12.6
P	60	M	172.5	14.8	12.4
P	60	M	174.4	13.7	10.7
T	60	M	169.3	17.3	12.8
P	60	M	171.2	15.1	12.1
P	60	M	173.5	15.1	13.7
P	60	M	178.4	12.9	10.7
T	60	M	170.1	16.8	14.8
T	60	M	170.8	15.8	13.3
T	60	M	171.6	15.3	12.2
T	60	M	168.0	18.1	14.3
P	60	F	171.3	14.2	11.4
P	60	F	170.0	15.0	12.2
T	60	F	169.7	16.4	12.8
T	60	F	165.1	17.8	15.7
P	60	F	171.0	14.8	12.6
P	60	F	173.0	14.8	12.1
P	60	F	170.3	13.8	11.1
T	60	F	166.8	16.8	13.9
T	60	F	169.5	15.9	13.7
T	60	F	168.9	15.7	12.8
P	60	F	169.1	15.8	13.4
T	80	M	168.1	15.2	12.3
T	80	M	170.3	15.2	12.1
P	80	M	171.9	13.5	11.2

Operat.	Age	Sex	Angle S	Angle H	Angle L
P	80	M	176.7	12.6	10.3
P	80	M	173.6	14.7	11.6
P	80	M	172.2	16.0	12.4
T	80	M	173.3	15.8	11.9
P	80	M	172.3	15.8	12.1
P	80	M	174.6	15.2	10.9
T	80	M	168.0	16.3	13.2
T	80	M	165.3	17.7	14.4
T	80	M	164.6	17.6	13.7
T	80	M	162.7	18.9	14.1
P	80	F	169.8	15.4	11.7
P	80	F	169.5	15.6	12.9
T	80	F	170.0	15.9	12.8
T	80	F	164.0	18.2	14.8
P	80	F	171.8	15.0	12.2
P	80	F	169.1	15.1	12.0
P	80	F	172.2	15.4	11.2
T	80	F	169.1	17.1	13.5
T	80	F	168.0	17.2	13.0
T	80	F	166.8	16.9	13.1
P	80	F	169.8	15.3	12.3
T	100	M	169.6	14.9	12.1
T	100	M	172.2	16.4	13.3
P	100	M	170.5	13.9	10.5
P	100	M	174.0	15.0	11.7
P	100	M	177.0	14.9	10.4
P	100	M	172.3	15.7	11.2
T	100	M	170.9	16.5	12.6
P	100	M	174.0	14.6	11.3
P	100	M	170.9	15.9	11.9
T	100	M	165.8	18.3	14.3
T	100	M	171.5	16.4	13.2
T	100	M	164.6	18.4	15.0
T	100	M	162.1	20.2	14.8
P	100	F	168.4	16.0	13.1
P	100	F	169.6	15.6	12.9
T	100	F	169.8	16.6	13.0
T	100	F	165.6	18.2	15.1
P	100	F	171.4	15.9	12.2
P	100	F	173.9	14.7	10.6
P	100	F	171.2	15.0	11.8
T	100	F	165.4	17.8	14.2
T	100	F	167.5	16.7	13.4
T	100	F	168.8	16.1	12.6
P	100	F	170.2	15.4	11.2
T	120	M	164.7	16.6	12.7
T	120	M	171.0	16.3	12.5
P	120	M	173.5	14.0	10.9
P	120	M	174.6	15.0	12.5
P	120	M	178.0	14.5	10.5
P	120	M	174.2	15.7	12.2
T	120	M	172.5	16.3	12.1
P	120	M	174.5	14.3	10.7
P	120	M	175.4	14.9	11.8
T	120	M	170.7	17.4	14.6
T	120	M	171.6	18.0	14.4
T	120	M	167.3	18.3	15.2
T	120	M	169.9	19.5	15.8

Operat.	Age	Sex	Angle S	Angle H	Angle L
P	120	F	170.2	15.1	11.8
T	120	F	171.8	16.4	12.0
T	120	F	166.3	17.8	12.2
P	120	F	172.6	15.8	12.9
P	120	F	170.6	15.1	12.1
P	120	F	170.8	16.1	11.6
T	120	F	165.8	17.3	12.0
T	120	F	166.0	17.6	14.2
T	120	F	167.6	17.2	13.3
P	120	F	172.1	15.1	13.6
S	40	M	174.9	14.0	11.6
S	40	M	172.7	15.3	11.9
S	40	M	171.8	16.1	11.8
S	40	M	177.3	14.6	10.9
S	40	F	170.8	16.1	13.0
S	40	F	172.5	16.2	12.6
S	40	F	175.5	13.1	10.6
S	60	M	173.2	15.5	12.0
S	60	M	176.1	13.9	10.1
S	60	M	172.8	16.3	11.9
S	60	M	175.3	14.9	12.5
S	60	M	174.3	14.9	12.2
S	60	F	172.0	15.8	11.7
S	60	F	176.6	14.5	10.1
S	60	F	175.3	15.5	12.3
S	60	F	174.9	15.6	11.9
S	60	F	171.2	15.4	11.9
S	80	M	175.1	14.9	11.2
S	80	M	173.7	15.9	11.5
S	80	M	174.1	15.7	12.3
S	80	M	178.0	14.9	11.2
S	80	M	174.5	16.7	12.7
S	80	F	176.0	14.5	11.5
S	80	F	175.6	15.0	11.4
S	80	F	174.3	16.7	11.6
S	80	F	173.5	15.6	11.9
S	80	F	172.6	15.3	12.7
S	100	M	177.0	14.3	10.9
S	100	M	177.1	14.7	10.9
S	100	M	173.7	15.4	11.0
S	100	M	177.3	14.9	10.4
S	100	M	175.0	15.9	12.6
S	100	F	178.5	13.8	10.0
S	100	F	175.5	14.3	10.3
S	100	F	176.0	15.5	11.5
S	100	F	173.9	15.6	11.8
S	100	F	172.7	14.7	11.1
S	120	M	175.8	15.0	11.9
S	120	M	177.3	15.1	10.5
S	120	M	180.6	12.0	9.1
S	120	M	178.3	13.7	10.4
S	120	M	173.8	16.4	12.0
S	120	F	177.3	14.3	10.6
S	120	F	177.2	14.4	10.0
S	120	F	175.9	15.2	11.1
S	120	F	173.9	14.6	11.0
S	120	F	173.1	14.7	11.4
N	40	M	174.2	15.8	12.3

Operat.	Age	Sex	Angle S	Angle H	Angle L
N	40	M	177.1	13.6	10.3
N	40	M	176.6	13.3	9.6
N	40	M	176.3	14.0	10.0
N	40	M	178.1	14.3	9.3
N	40	M	176.7	14.6	10.9
N	40	M	173.3	15.7	11.7
N	40	F	170.4	14.8	12.2
N	40	F	178.4	12.2	8.8
N	40	F	176.3	13.6	11.5
N	40	F	178.7	13.0	10.0
N	40	F	175.9	13.7	10.4
N	60	M	177.4	13.1	9.6
N	60	M	175.2	15.5	11.6
N	60	M	173.5	14.8	10.4
N	60	M	175.6	14.7	11.4
N	60	M	180.4	13.2	10.4
N	60	M	178.6	14.3	10.4
N	60	M	178.6	14.2	11.6
N	60	F	173.7	15.4	12.3
N	60	F	175.5	13.9	11.0
N	60	F	178.6	13.2	10.0
N	60	F	176.6	14.6	10.7
N	60	F	177.0	14.0	11.3
N	80	M	177.0	15.4	10.8
N	80	M	177.7	15.1	11.3
N	80	M	175.5	15.2	10.8
N	80	M	178.5	14.5	11.6
N	80	M	179.8	13.9	10.7
N	80	M	179.7	13.8	10.4
N	80	M	181.2	13.8	10.7
N	80	F	175.9	15.1	11.1
N	80	F	176.5	14.8	11.4
N	80	F	177.6	14.3	11.5
N	80	F	178.9	15.8	11.8
N	80	F	175.5	13.6	11.4
N	100	M	181.5	15.3	10.4
N	100	M	179.5	13.8	10.1
N	100	M	178.4	14.7	10.4
N	100	M	178.8	14.4	11.4
N	100	M	180.9	13.1	10.6
N	100	M	178.9	14.2	11.3
N	100	M	178.2	14.5	11.4
N	100	M	177.2	15.4	11.5
N	100	F	178.1	14.4	11.4
N	100	F	177.6	14.0	10.4
N	100	F	179.3	13.7	10.2
N	100	F	179.3	12.3	9.0
N	120	M	180.4	13.5	9.9
N	120	M	178.8	14.8	11.4
N	120	M	176.4	14.3	10.9
N	120	M	178.6	14.6	10.8
N	120	M	178.1	13.4	10.2
N	120	M	178.7	12.2	10.3
N	120	F	175.5	14.6	10.6
N	120	F	177.5	13.9	10.0
N	120	F	180.4	13.4	9.7
N	120	F	178.2	12.7	8.5

TABLE 9

ANALYSIS OF VARIANCE (ANOVA) STATISTICAL RESULTS

Dependent variable = angle S

groups	F value	PR > F
operation	166.04	0.0001
age	3.44	0.0095
sex	12.08	0.0006
operation*age	0.82	0.5901
operation*sex	0.58	0.5610
operation*age*sex	0.19	0.9986

Dependent variable = angle H

groups	F value	PR > F
operation	37.55	0.0001
age	1.46	0.2157
sex	0.02	0.8971
operation*age	1.51	0.1544
operation*sex	0.79	0.4535
operation*age*sex	0.25	0.9951

Dependent variable = angle L

groups	F value	PR > F
operation	70.27	0.0001
age	1.10	0.3570
sex	1.00	0.3191
operation*age	1.03	0.4178
operation*sex	0.86	0.4247
operation*age*sex	0.57	0.8617

TABLE 10

TUKEY TEST FOR MULTIPLE COMPARISONS

Variable = angle S

Grouping	Mean	N	Operation
A	177.5	58	N
B	174.9	47	S
C	170.0	123	E

Variable = angle H

Grouping	Mean	N	Operation
A	15.8	123	E
B	15.0	47	S
C	14.2	58	N

Variable = angle L

Grouping	Mean	N	Operation
A	12.7	123	E
B	11.4	47	S
C	10.7	58	N

TABLE 11

MEANS FOR ANGLES S, H, AND L
GROUPS: OPERATION, AGE, AND SEX

Operat.	Age	Sex	N	Angle S	Angle H	Angle L
N	40	F	5	175.9 ±3.3	13.5 ±0.9	10.6 ±1.3
N	40	M	7	176.0 ±1.7	14.5 ±1.0	10.6 ±1.1
N	60	F	5	176.3 ±1.8	14.2 ±0.8	11.1 ±0.8
N	60	M	7	177.0 ±2.4	14.3 ±0.9	10.8 ±0.8
N	80	F	5	176.9 ±1.4	14.7 ±0.8	11.4 ±0.3
N	80	M	7	178.5 ±1.9	14.5 ±0.7	10.9 ±0.4
N	100	F	4	178.6 ±0.9	13.6 ±0.9	10.2 ±1.0
N	100	M	8	179.2 ±1.4	14.4 ±0.8	11.0 ±0.6
N	120	F	4	177.9 ±2.0	13.6 ±0.8	9.7 ±0.9
N	120	M	6	178.5 ±1.3	13.8 ±1.0	10.6 ±0.5
S	40	F	3	172.9 ±2.3	15.1 ±1.7	12.1 ±1.3
S	40	M	4	174.2 ±2.5	15.0 ±0.9	11.5 ±0.5
S	60	F	5	174.0 ±2.3	15.3 ±0.5	11.6 ±0.9
S	60	M	5	174.4 ±1.4	15.1 ±0.9	11.7 ±0.9
S	80	F	5	174.4 ±1.4	15.4 ±0.8	11.8 ±0.5
S	80	M	5	175.1 ±1.7	15.6 ±0.8	11.8 ±0.7
S	100	F	5	175.3 ±2.2	14.8 ±0.8	10.9 ±0.8
S	100	M	5	176.0 ±1.6	15.1 ±0.6	11.2 ±0.8
S	120	F	5	175.5 ±1.9	14.6 ±0.4	10.8 ±0.5
S	120	M	5	177.2 ±2.6	14.4 ±1.7	10.8 ±1.2
P	40	F	7	170.6 ±1.4	14.7 ±1.1	12.5 ±0.8
P	40	M	7	171.4 ±0.9	14.7 ±0.8	12.2 ±0.7
P	60	F	6	170.8 ±1.3	14.7 ±0.7	12.1 ±0.8
P	60	M	7	173.6 ±2.5	14.2 ±1.1	11.9 ±1.1
P	80	F	6	170.3 ±1.3	15.3 ±0.3	12.0 ±0.6
P	80	M	6	173.6 ±1.9	14.6 ±1.3	11.4 ±0.8
P	100	F	6	170.8 ±1.9	15.4 ±0.5	12.0 ±1.0
P	100	M	6	173.1 ±2.4	15.0 ±0.7	11.2 ±0.6
P	120	F	5	171.3 ±1.0	15.4 ±0.5	12.4 ±0.8
P	120	M	6	175.0 ±1.6	14.7 ±0.6	11.4 ±0.8
T	40	F	5	165.9 ±1.3	16.4 ±0.7	14.4 ±0.9
T	40	M	8	168.4 ±1.7	16.1 ±0.9	12.8 ±0.8
T	60	F	5	168.0 ±1.9	16.5 ±0.8	13.8 ±1.2
T	60	M	7	168.9 ±3.7	16.4 ±1.0	13.3 ±0.9
T	80	F	5	167.6 ±2.3	17.0 ±0.8	13.4 ±0.8
T	80	M	7	167.5 ±3.6	16.7 ±1.4	13.1 ±1.0
T	100	F	5	167.4 ±1.9	17.1 ±0.9	13.7 ±1.0
T	100	M	7	168.1 ±3.9	17.3 ±1.7	13.6 ±1.1
T	120	F	5	167.5 ±2.5	17.3 ±0.5	12.7 ±1.0
T	120	M	7	169.7 ±2.7	17.5 ±1.2	13.9 ±1.5

Standard deviations given after each angle's mean.

TABLE 12

MEANS FOR ANGLES S, H, AND L
GROUPS: OPERATION AND AGE

Operat.	Age	N	Angle S	Angle H	Angle L
N	40	12	176.0 ±2.4	14.1 ±1.1	10.6 ±1.1
N	60	12	176.7 ±2.1	14.3 ±0.8	10.9 ±0.8
N	80	12	177.8 ±1.8	14.6 ±0.7	11.1 ±0.4
N	100	12	179.0 ±1.3	14.1 ±0.9	10.7 ±0.8
N	120	10	178.3 ±1.5	13.7 ±0.9	10.2 ±0.8
S	40	7	173.6 ±2.3	15.0 ±1.2	11.8 ±0.9
S	60	10	174.2 ±1.8	15.2 ±0.7	11.7 ±0.8
S	80	10	174.7 ±1.5	15.5 ±0.8	11.8 ±0.6
S	100	10	175.7 ±1.9	14.9 ±0.7	11.0 ±0.8
S	120	10	176.3 ±2.3	14.5 ±1.1	10.8 ±0.9
P	40	14	171.0 ±1.2	14.7 ±0.9	12.3 ±0.8
P	60	13	172.3 ±2.5	14.5 ±0.9	12.0 ±1.0
P	80	12	172.0 ±2.3	14.9 ±1.0	11.7 ±0.7
P	100	12	172.0 ±2.4	15.2 ±0.6	11.6 ±0.9
P	120	11	173.3 ±2.4	15.0 ±0.6	11.9 ±0.9
T	40	13	167.5 ±2.0	16.2 ±0.8	13.4 ±1.2
T	60	12	168.5 ±3.0	16.5 ±0.9	13.5 ±1.0
T	80	12	167.5 ±3.0	16.8 ±1.2	13.2 ±0.9
T	100	12	167.8 ±3.1	17.2 ±1.4	13.6 ±1.0
T	120	12	168.8 ±2.8	17.4 ±0.9	13.4 ±1.4

Standard deviations given after each angle's mean.

TABLE 13

MEANS FOR ANGLES S, H, AND L
GROUPS: OPERATION AND SEX

Operat.	Sex	N	Angle S	Angle H	Angle L
N	F	23	177.0 ±2.1	14.0 ±0.1	10.7 ±1.0
N	M	35	177.9 ±2.1	14.3 ±0.8	10.8 ±0.7
S	F	23	174.6 ±2.0	15.1 ±0.8	11.4 ±0.9
S	M	24	175.4 ±2.1	15.0 ±1.0	11.4 ±0.9
P	F	30	170.7 ±1.4	15.1 ±0.7	12.2 ±0.8
P	M	32	173.3 ±2.2	14.6 ±0.9	11.6 ±0.9
T	F	25	167.3 ±2.0	16.9 ±0.8	13.6 ±1.1
T	M	36	168.5 ±3.1	16.8 ±1.3	13.3 ±1.1

Standard deviations given after each angle's mean.

TABLE 14

MEANS FOR ANGLES S, H, AND L
GROUPS: AGE

Age	N	Angle S	Angle H	Angle L
40	46	171.7 ±3.8	15.0 ±1.3	12.1 ±1.4
60	47	172.9 ±3.8	15.1 ±1.2	12.0 ±1.3
80	46	172.9 ±4.5	15.5 ±1.3	12.0 ±1.0
100	46	173.5 ±4.8	15.4 ±1.5	11.8 ±1.4
120	43	173.9 ±4.3	15.3 ±1.7	11.7 ±1.6

Standard deviations given after each angle's mean.

TABLE 15

MEANS FOR ANGLES S, H, AND L
GROUPS: SEX

Sex	N	Angle S	Angle H	Angle L
F	101	172.2 ±4.0	15.3 ±1.3	12.0 ±1.4
M	127	173.6 ±4.3	15.2 ±1.4	11.8 ±1.3

Standard deviations given after each angle's mean.

TABLE 16

MEANS FOR ANGLES S, H, AND L
GROUPS: OPERATION

Operation	N	Angle S	Angle H	Angle L
N	58	177.5 ±2.1	14.2 ±0.9	10.7 ±0.8
S	47	175.0 ±2.1	15.1 ±0.9	11.4 ±0.9
P	62	172.1 ±2.2	14.9 ±0.9	11.9 ±0.9
T	61	168.0 ±2.8	16.8 ±1.1	13.4 ±1.1

Standard deviations given after each angle's mean.

TABLE 17

PEARSON CORRELATION COEFFICIENTS (r)

Normal, Sham, and Experimental combined

angle S vs. angle H, $r = -0.743$ ($P < 0.0001$)
angle S vs. angle L, $r = -0.799$ ($P < 0.0001$)

Normals

angle S vs. angle H, $r = -0.439$ ($P < 0.0006$)
angle S vs. angle L, $r = -0.440$ ($P < 0.0005$)

Shams

angle S vs. angle H, $r = -0.676$ ($P < 0.0001$)
angle S vs. angle L, $r = -0.725$ ($P < 0.0001$)

Experimentals, Groups P and T combined

angle S vs. angle H, $r = -0.691$ ($P < 0.0001$)
angle S vs. angle L, $r = -0.665$ ($P < 0.0001$)

Experimental Group P

angle S vs. angle H, $r = -0.363$ ($P < 0.0037$)
angle S vs. angle L, $r = -0.500$ ($P < 0.0001$)

Experimental Group T

angle S vs. angle H, $r = -0.503$ ($P < 0.0001$)
angle S vs. angle L, $r = -0.422$ ($P < 0.0007$)

TABLE 18

TUKEY TEST FOR MULTIPLE COMPARISONS, SECOND ANALYSIS

Variable = angle S

Grouping	Mean	N	Operation
A	177.5	58	N
B	175.0	47	S
C	172.1	62	P
D	168.0	61	T

Variable = angle H

Grouping	Mean	N	Operation
A	16.8	61	T
B	15.0	47	S
B	14.9	62	P
C	14.2	58	N

Variable = angle L

Grouping	Mean	N	Operation
A	13.4	61	T
B	11.9	62	P
C	11.4	47	S
D	10.7	58	N

Postmortem examination

Postmortem examination was performed via both gross dissection and histological sectioning of whole rats heads.

Dissection

Fifteen pilot study experimental rats, 2 sham pilot study rats, and 6 pilot study normals were sectioned in the midsagittal plane and their anatomy compared to each other. In addition, the ventral surfaces of the intact basicrania of 14 pilot study experimental rats and 2 pilot study sham rats were compared to those of 6 pilot study normal rats. Gross examination of all 45 pilot study rats revealed no evidence of muscle destruction in the region of the insertion of the longus capitus muscle. Although initially scraped off from its insertion during surgery in experimentals and shams, the longus capitus was found firmly reattached in its original position. Scraping off the longus capitus revealed bony overgrowth in the region where drilling was performed in experimentals. The ventral surface of the basicranium was often thickened and rough in texture at the drilling site. The spheno-occipital synchondrosis appeared to be replaced by a synostosis in the experimentals, but remained a synchondrosis in shams and normals. There was no apparent damage to the dura mater, pons, or pituitary.

Necropsy

Necropsies were performed on the 23 experimental rats which survived the surgery, but died before they were scheduled to be sacrificed at 130 days. Seventeen of the 23 rats died before weaning, and the remaining six rats died after weaning but before the age of 40 days. As these rats did not survive long enough to be radiographed, no angular measurements were obtained. These necropsies were performed to help ascertain the causes of premature death.

Of the 17 preweaned rats, four were cannibalized by the mother, and the remaining 13 were found to have abscesses in the lungs and occasionally in the liver. Histological sections of the lung, liver, and salivary gland were prepared and standard bacterial cultures of the lungs were taken. (The salivary gland was examined to determine whether the infection entered via the wound site.) Tissue sections revealed necrotic areas with gram positive cocci in the liver and lungs, and pus and blood in the alveolar spaces. Bacterial cultures revealed *Staphylococcus sp*. and beta-hemolytic *Streptococcus sp*. It is suspected that these rats died of pneumonia, perhaps as a consequence of the effects of increased skull base flexion on the position and function of the larynx during swallowing. It is not clear whether the pneumonia was caused primarily by infection or by aspiration with secondary infection. Hepatic involvement may have occurred during an episode of bacteremia secondary to pneumonia.

Of the six rats which died postweaning, one was partially cannibalized by the rat sharing its cage (either they fought and it was killed or it was sickly and died before being cannibalized), one died from complications arising from an eye infection, two died of respiratory arrest from the anesthesia used during radiographic procedures, and two died from a cryptogenic illness affecting the gastrointestinal tract.

Histological Data

Histological analysis was performed to confirm whether the spheno-occipital synchondroses were successfully obliterated in the experimentals. Sagittal and parasagittal histological sections of the heads of all 27 experimental rats were compared to sagittal and parasagittal histological sections prepared from the 10 sham operated and 6 normal control rat heads.

Examination of histological sections revealed no evidence of destruction of the dura mater. There was no indication of any damage to the pituitary and pons, which directly overlay the region of the spheno-occipital synchondrosis, or to any other portion of the brain (see figures 31-37).

An unusual triangular elevation was found on the endocranial surface in experimental rats in which the spheno-occipital synchondrosis was completely obliterated. This bony ridge ran laterally from left to right sides, and was continuous ventrally with the rest of the basicranium. It was positioned just posterior to the pituitary, and was seen cradling the pituitary on its rostral sloping surface (see figure 37). The endocranial surface of the basicranium was flat in sham operated and normal control rats.

The exocranial (ventral) surface of the basicranium exhibited a thickened mantle of bone at the drilling site in some experimentals. There was no unusual thickening of bone in the sham operated or normal control rats. The longus capitus muscle was seen attaching along the ventral surface of the spheno-occipital synchondrosis and the basioccipital bone in experimentals, shams, and normals. There was no evidence of damage to the longus capitus mucle fibers, or disruption along its insertion on the basicranium in experimental and sham rats.

An intact spheno-occipital synchondrosis was present in all of the sham operated and normal control rats. Thirteen of the experimental rats displayed complete obliteration of the spheno-occipital synchondrosis. In these rats, a synostosis was present between the basisphenoid and basioccipital bones. In the remaining 14 experimentals, the spheno-occipital synchondrosis was partially destroyed. Experimental rats with only a portion of the synchondrosis present were categorized as "partially destroyed," while those lacking any trace of a spheno-occipital synchondrosis were categorized as "totally destroyed."

Figure 31:

A) Histological section through the midline of a normal rat head. Note the high position of the larynx (L), with the epiglottis overlapping the soft palate. The normal rat head shows a flat basicranium, a sphenoid sinus (S) located rostral to the pituitary (P), and a large spheno-occipital synchondrosis (SOS). An enlargement of the region indicated by box is shown below.

B) Closeup of a normal skull base showing the large cartilaginous strip of the spheno-occipital synchondrosis (S) under the pituitary (P). This section was taken through the midline, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis).

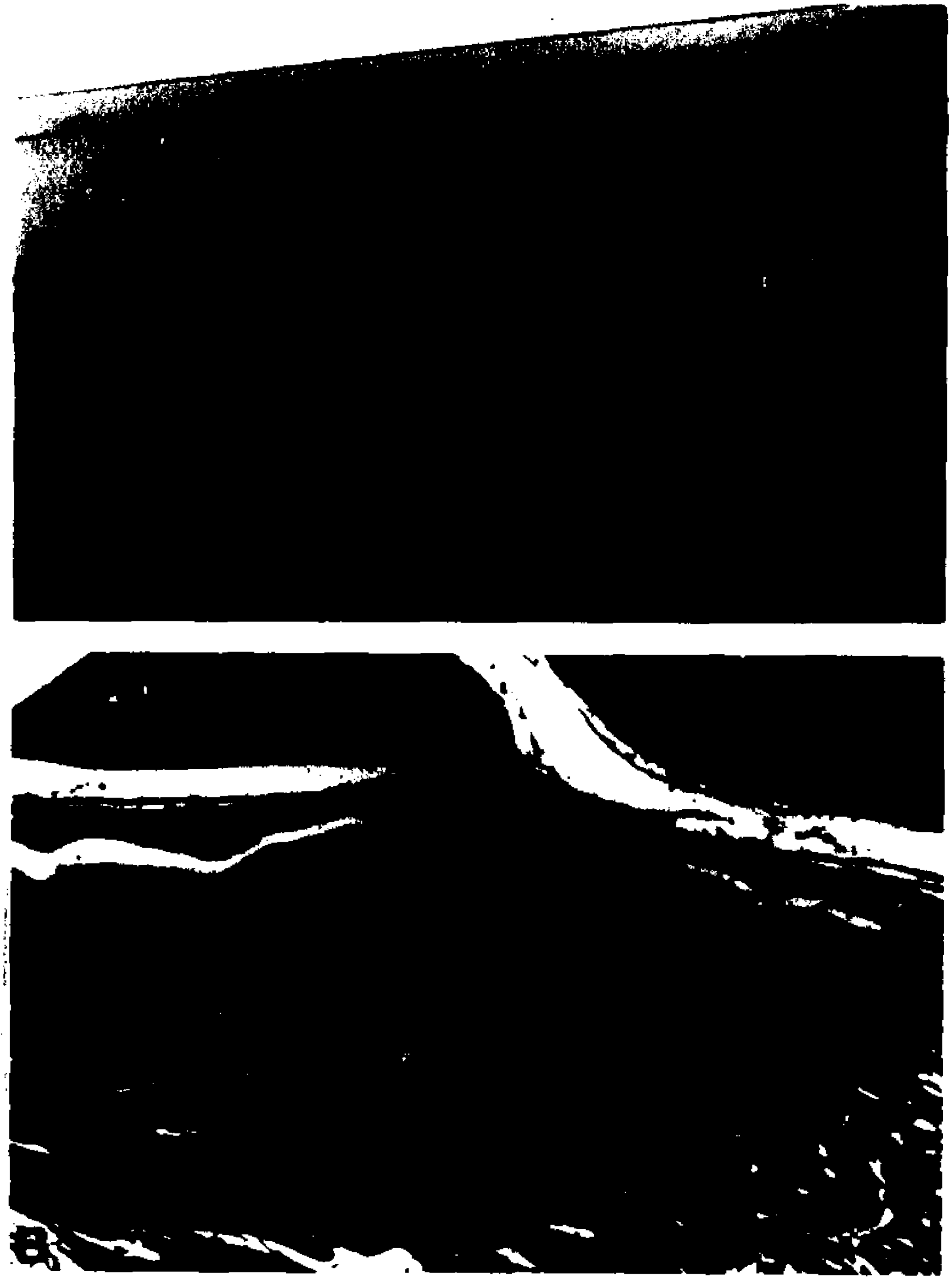

B.

Figure 32: Histological sections through the normal spheno-occipital synchondroses of a 40 day old rat (A) and a 120 day old rat (B). Note the large zone of proliferative cartilage in the 40 day old compared with the relatively narrow strip in the 120 day old. Sections were cut in the midsagittal plane, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis). P = pituitary, S = spheno-occipital synchondrosis.


A
B

Figure 33:

A) Histological section through the midline of the head of a sham operated rat. Note the relatively high position of the larynx (L), with the epiglottis overlapping the soft palate. The sham rat head shows a relatively flat basicranium, a sphenoid sinus (S) located rostral to the pituitary (P), and a large spheno-occipital synchondrosis (SOS). An enlargement of the area indicated by the box is shown below.

B) Close up of the skull base showing the large cartilaginous strip of the spheno-occipital synchondrosis (S) located under the pituitary (P). This section was taken through the midline, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis).

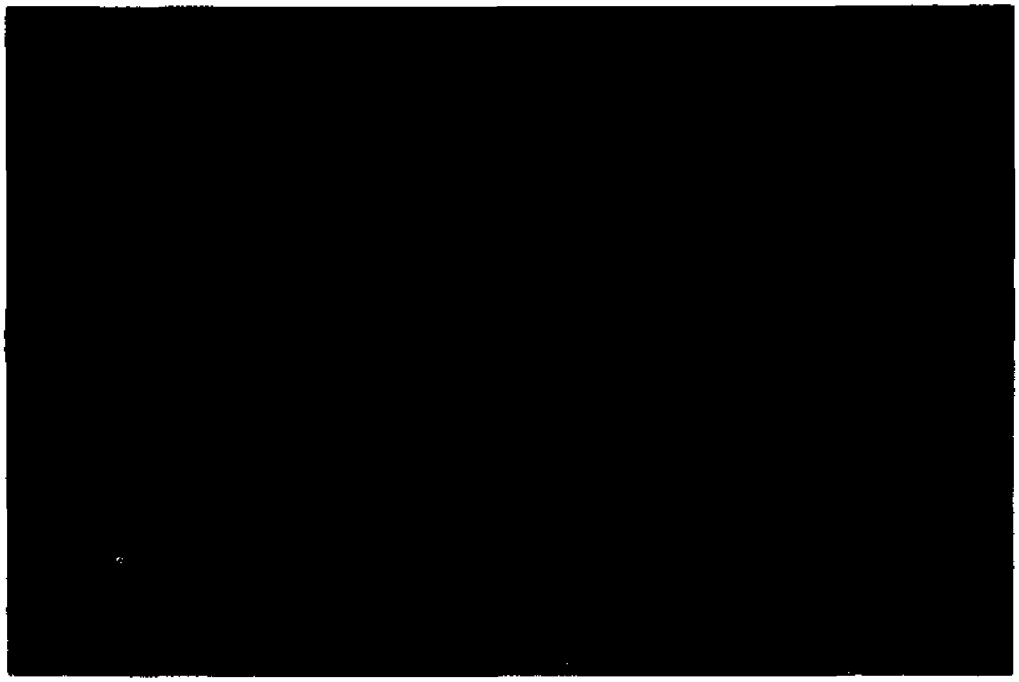


Figure 34: Histological sections through the basicranium of "partially" drilled experimental rats (Group P experimentals). A) Note the distortion of the ventral portion of the spheno-occipital synchondrosis with a relatively normal dorsal portion. B) Note the complete ossification of the ventral portion of the spheno-occipital synchondrosis and presence of distorted cartilage in the dorsal region. In addition, there is an elevated ridge forming on the dorsal surface just posterior to the pituitary. Also, note the eroded contour of the ventral surface of the basioccipital bone. These sections were taken through the midline, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis). P = pituitary, S = remaining cartilage of the spheno-occipital synchondrosis.

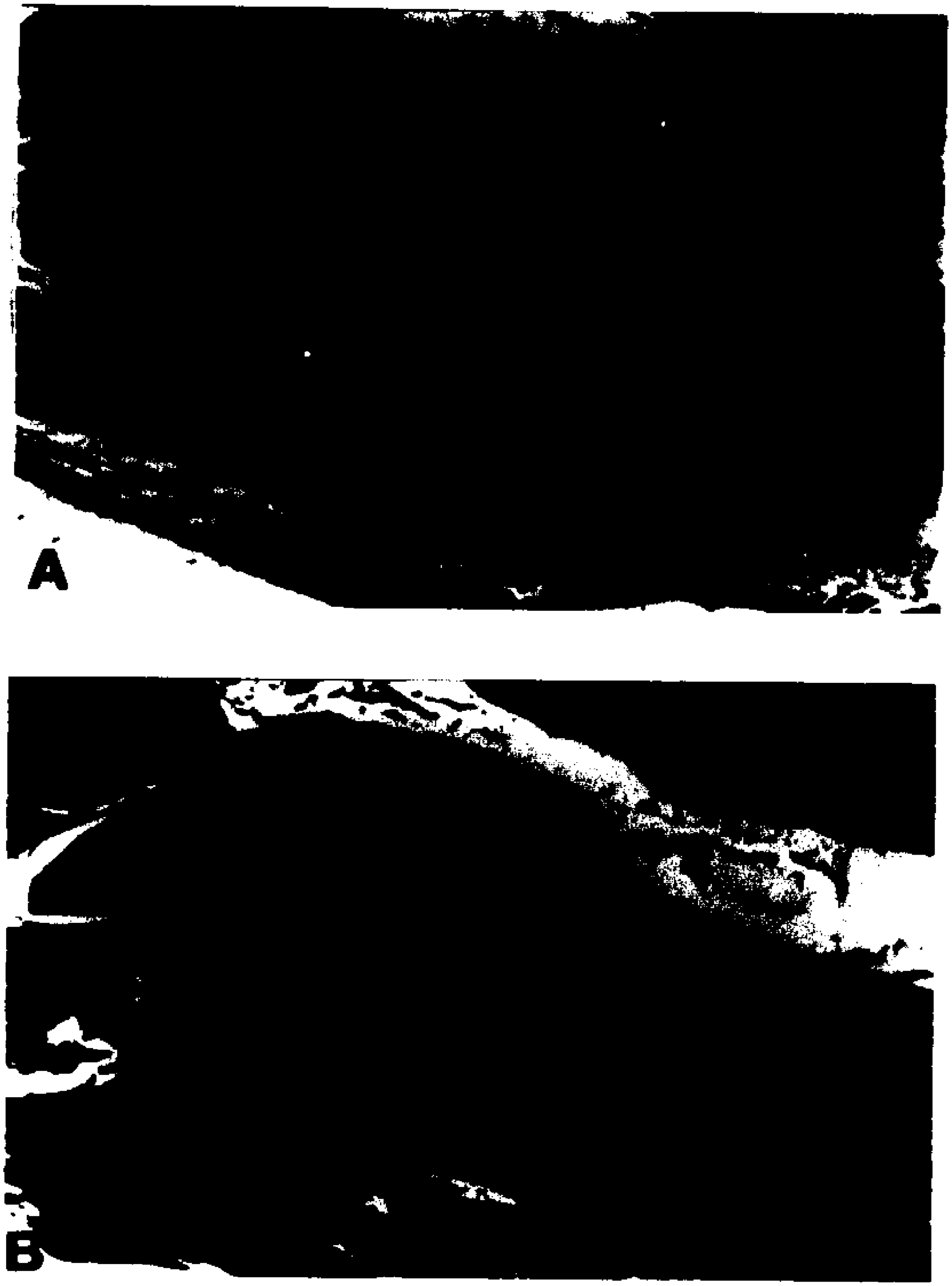

A
B

Figure 35:

A) Histological section through the head of an experimental rat. Note the low position of the larynx (L), absence of a spheno-occipital synchondrosis, erosion of bone at drilling site (arrow), flexed basicranium, and location of sphenoid sinus (S) directly under the pituitary (P).

B) Close up of another experimental rat skull base shows no spheno-occipital synchondrosis. The sphenoid sinus is placed under the pituitary. This section was taken through the midline, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis). P = pituitary, S = sphenoid sinus.

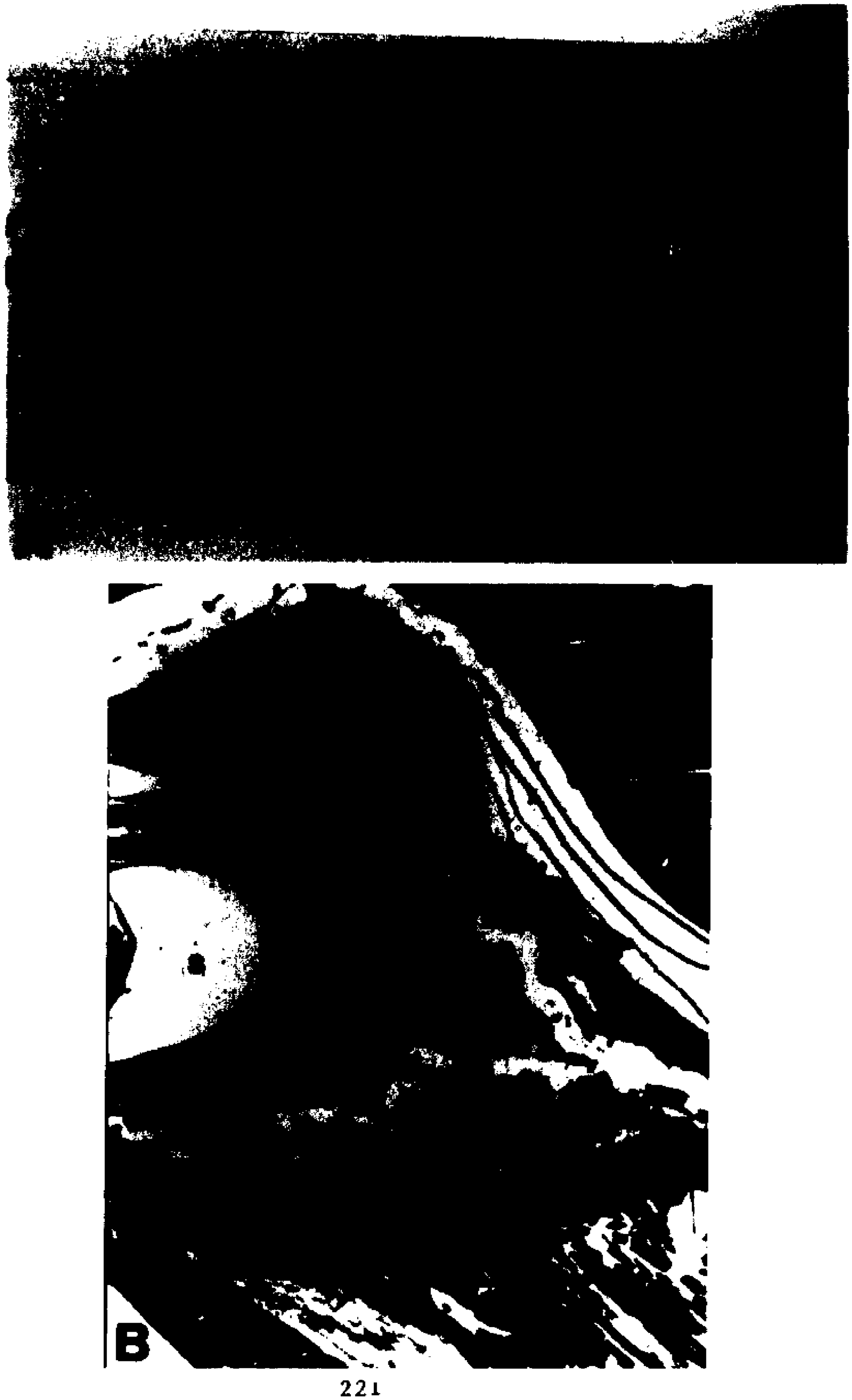
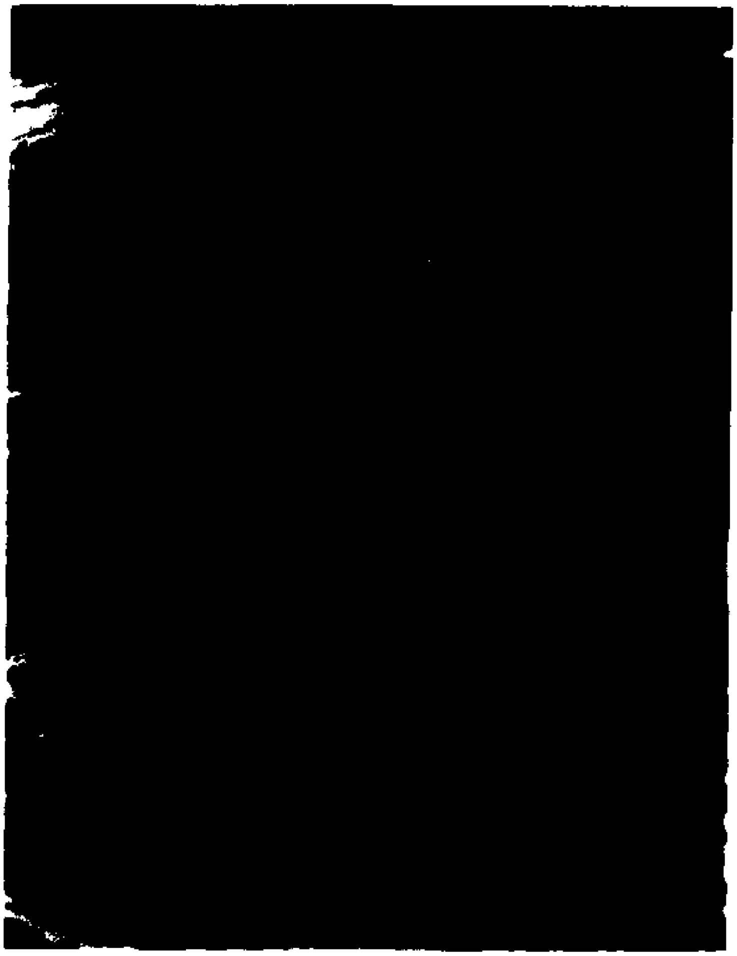

B

Figure 36: Skull base of an experimental rat, showing no spheno-occipital synchondrosis. Note the position of the sphenoid sinus (S) under the pituitary (P), and thickened bone on the ventral surface of the basicranium (B). The posterior surface of the basicranium is steeply sloped, resembling the clivus of the human skull. This section was taken through the midline, as indicated by the presence of all three divisions of the pituitary (pars nervosa, pars intermedia, and pars distalis).

Figure 37: Skull base of an experimental rat showing similarities to the dorsum sellae and clivus of the human skull. Note peaked dorsal surface and steeply sloping posterior region. The spheno-occipital synchondrosis is not present. This section was taken through the midline, as indicated by the presence of all three divisions (pars nervosa, pars intermedia, and pars distalis) of the pituitary (P).



Further statistical analysis

Based upon the histological results, it appears that the experimental group is actually composed of two subgroups: one group in which the drilling successfully removed the synchondrosis, and another group in which only partial destruction of the synchondrosis was accomplished. In light of these results, the experimental group (E) was subdivided into two groups: group T (synchondrosis totally destroyed) and group P (synchondrosis partially destroyed), and all statistical analyses were performed again.

Statistical results of this analysis is presented in table 18, and correlation graphs are presented in figures 38-49. ANOVA statistical results were identical to those obtained previously, and are thus not presented here. The Tukey test, however, revealed results which further clarified the differences between groups based upon operation. In this test, group P was found to be intermediate between the shams and group T for angles S, H, and L. For angle S, group T had the smallest mean (i.e., most flexed skull base), followed by group P, then the shams, while the normals had the largest mean (i.e., the flattest skull base). For angle H, group T had the largest mean (i.e. the lowest hyoid), while the normals had the smallest mean (i.e.

the highest hyoid). Group P and the shams were grouped together as intermediate between group T and the normals, thus indicating no significant difference between group P and the shams. For angle L, group T had the largest mean (i.e., the lowest larynx), group P had the next largest mean, the shams had a mean smaller than group P, and the normals had the smallest mean (i.e. the highest larynx). Pearson correlation coefficients were then calculated for experimental groups T and P separately. For group P, $r = -0.363$ ($P < 0.0037$) for angles S vs. H, and $r = -0.500$ ($P < 0.0001$) for angles S vs. L. For group T, $r = -0.503$ ($P < 0.0001$) for angles S vs. H, and $r = -0.422$ ($P < 0.0007$) for angles S vs. L. These results indicate significant negative (inverse) correlation.

Figure 38: Graph of angle S (skull base) versus angle H (hyoid) for normal, sham, and experimental groups P and T. There is a negative slope, indicating an inverse linear relationship between angle S and angle H. Note that normals are clustered at the upper left, indicating large skull base angles (i.e. flat skulls) and small hyoid angles (i.e. high hyoid positions). The experimental group T rats are clustered in the lower right, indicating small skull base angles (i.e. flexed skulls) and large hyoid angles (i.e. low hyoids). Shams and group P experimentals are positioned in the intermediate ranges, with shams closer to normals and group P closer to group T.

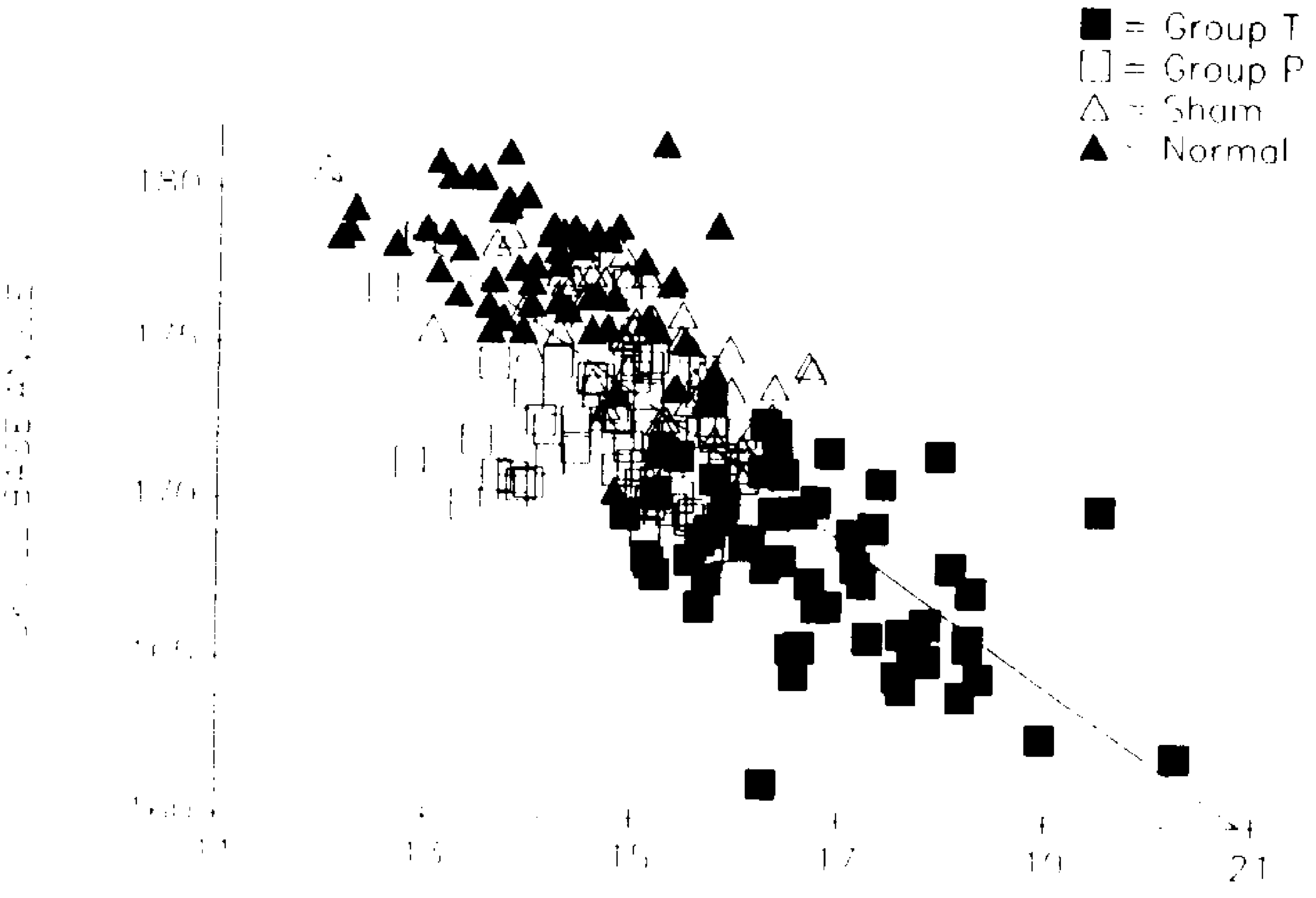


Figure 38

Figure 39: Graph of angle S (skull base) versus angle H (hyoid) for normals.

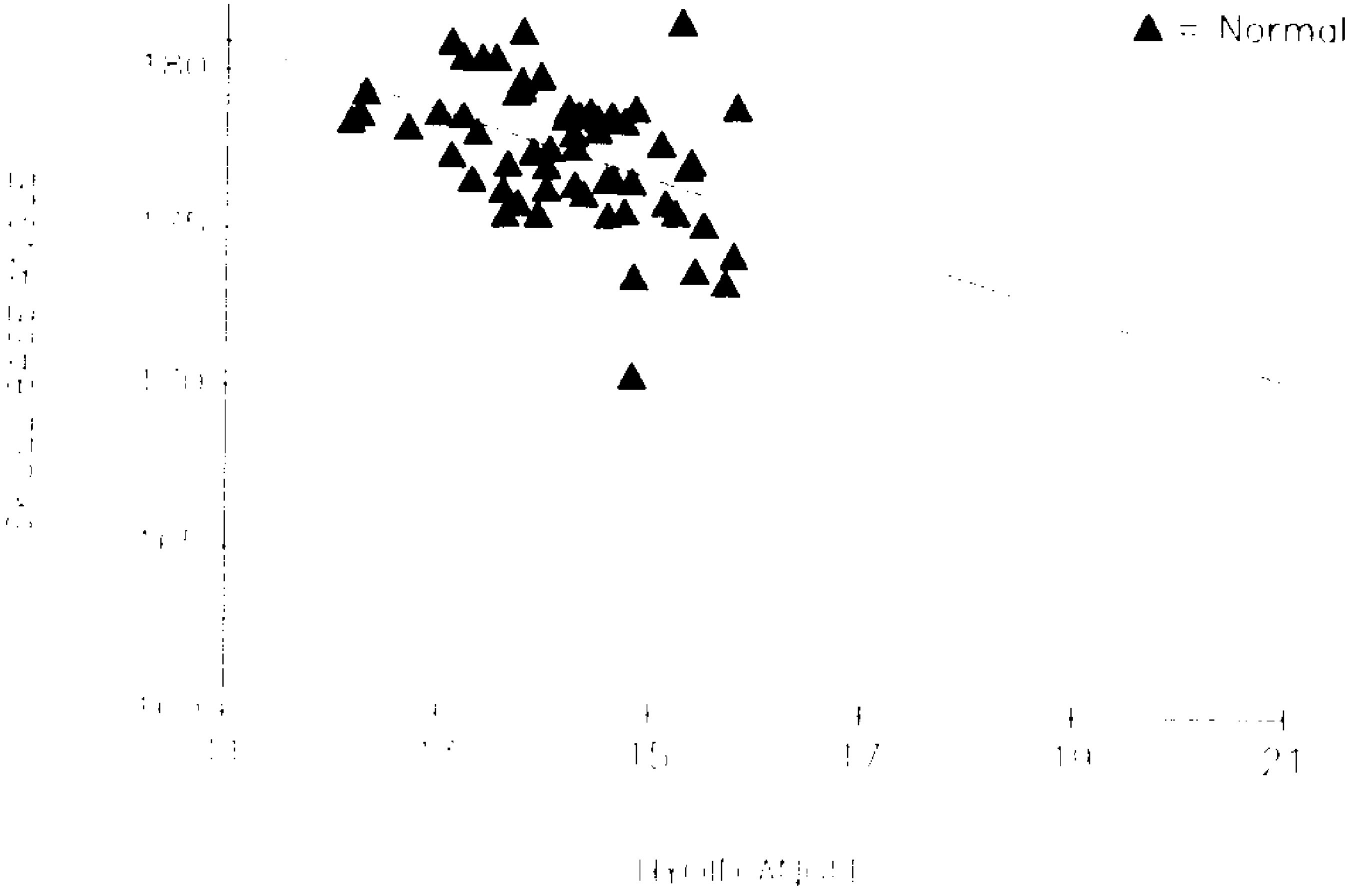


Figure 39

Figure 40: Graph of angle S (skull base) versus angle H (hyoid) for shams.

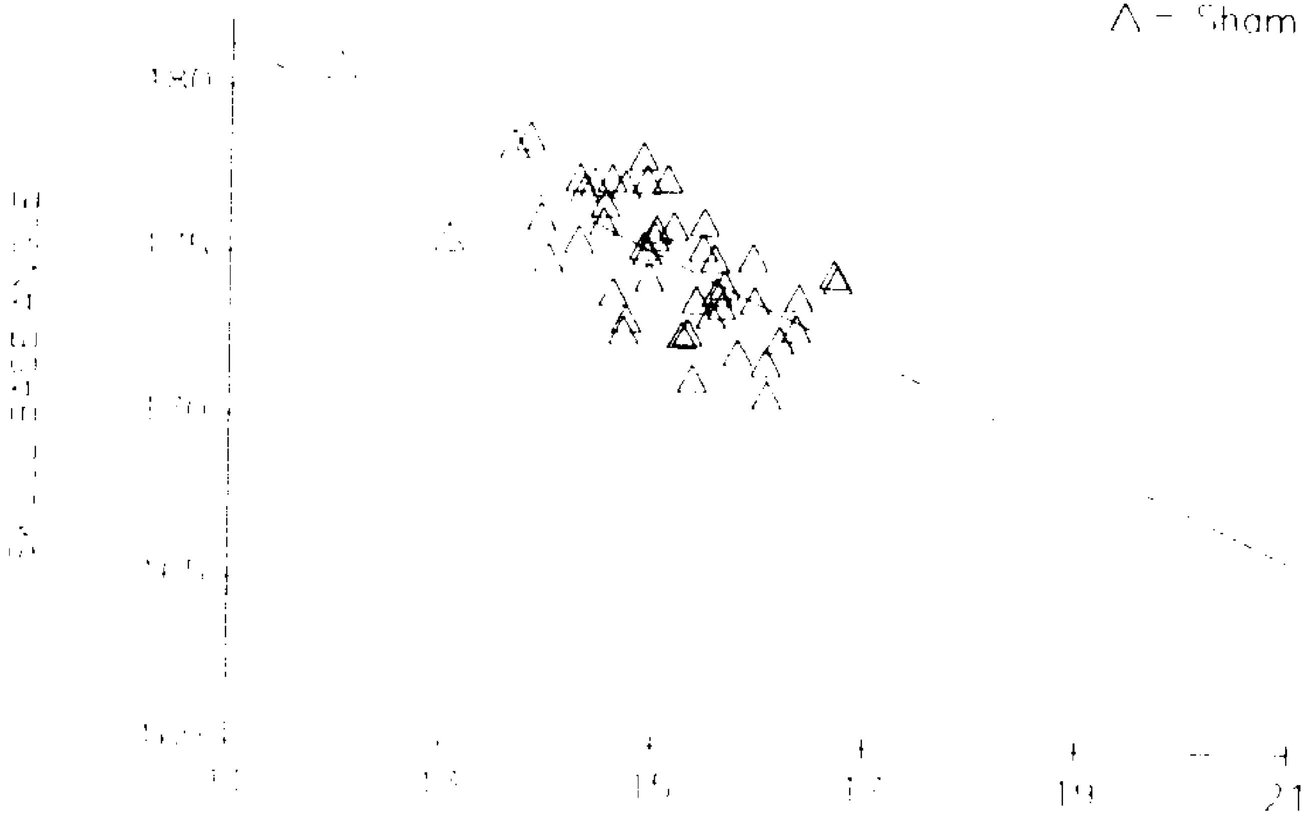


Figure 40

Figure 41: Graph of angle S (skull base) versus angle H (hyoid) for group P experimentals.

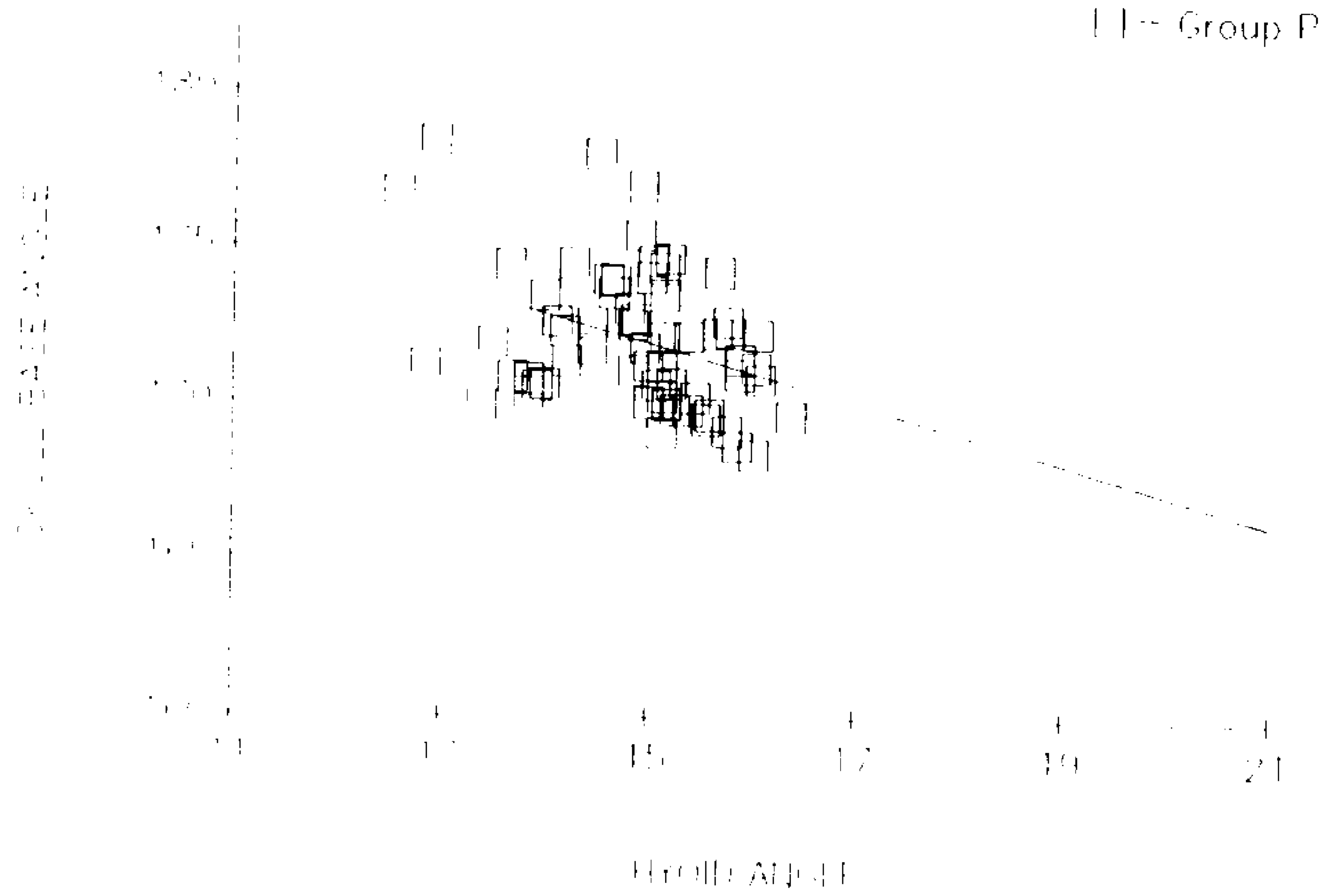


Figure 41

Figure 42: Graph of angle S (skull base) versus angle H (hyoid) for group T experimentals.

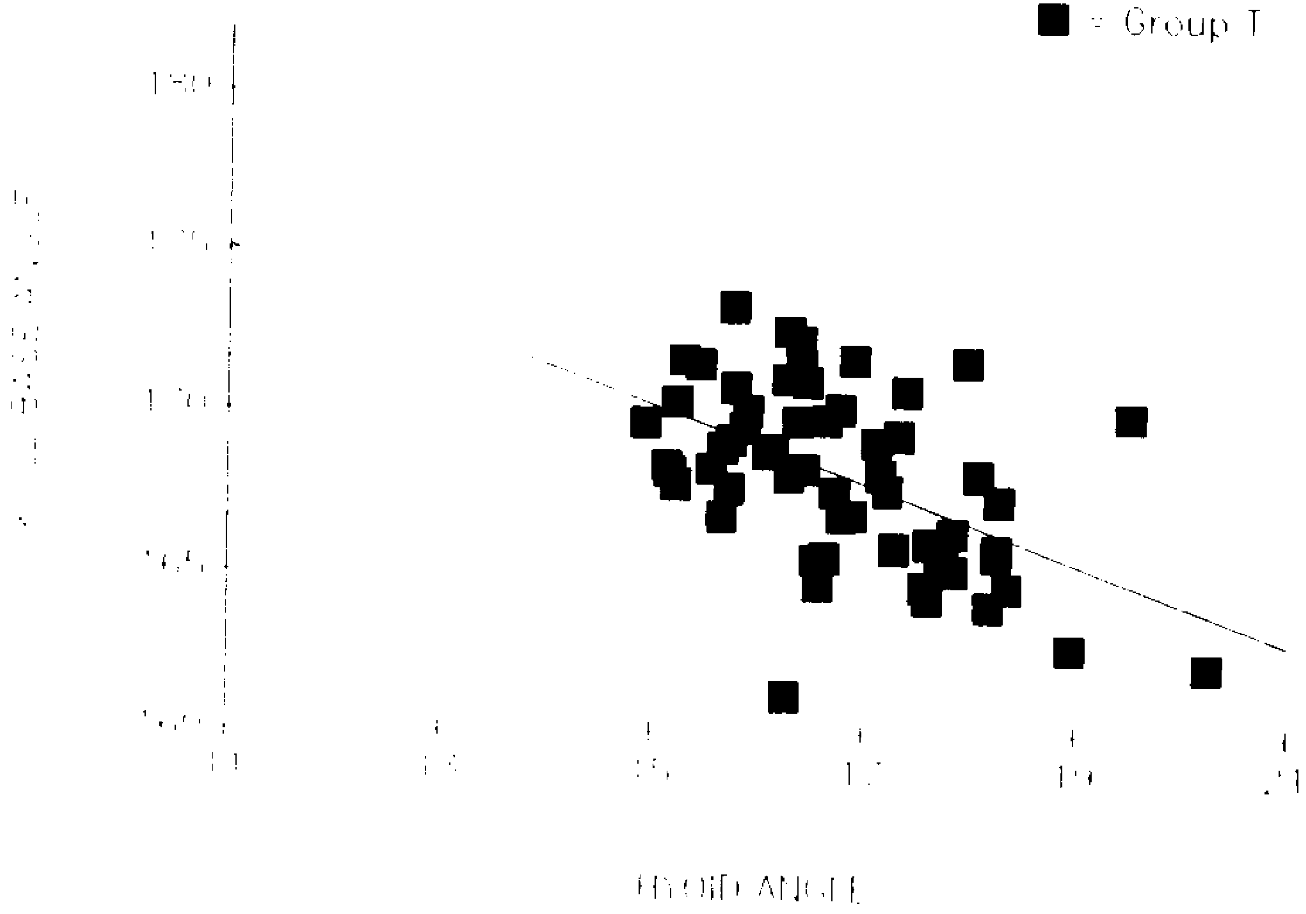


Figure 42

Figure 43: Graph of means (one mean shown for each age group: 40, 60, 80, 100, 120 days) for angle S (skull base) versus angle H (hyoid) for normals, shams, group P experimentals, and group T experimentals.

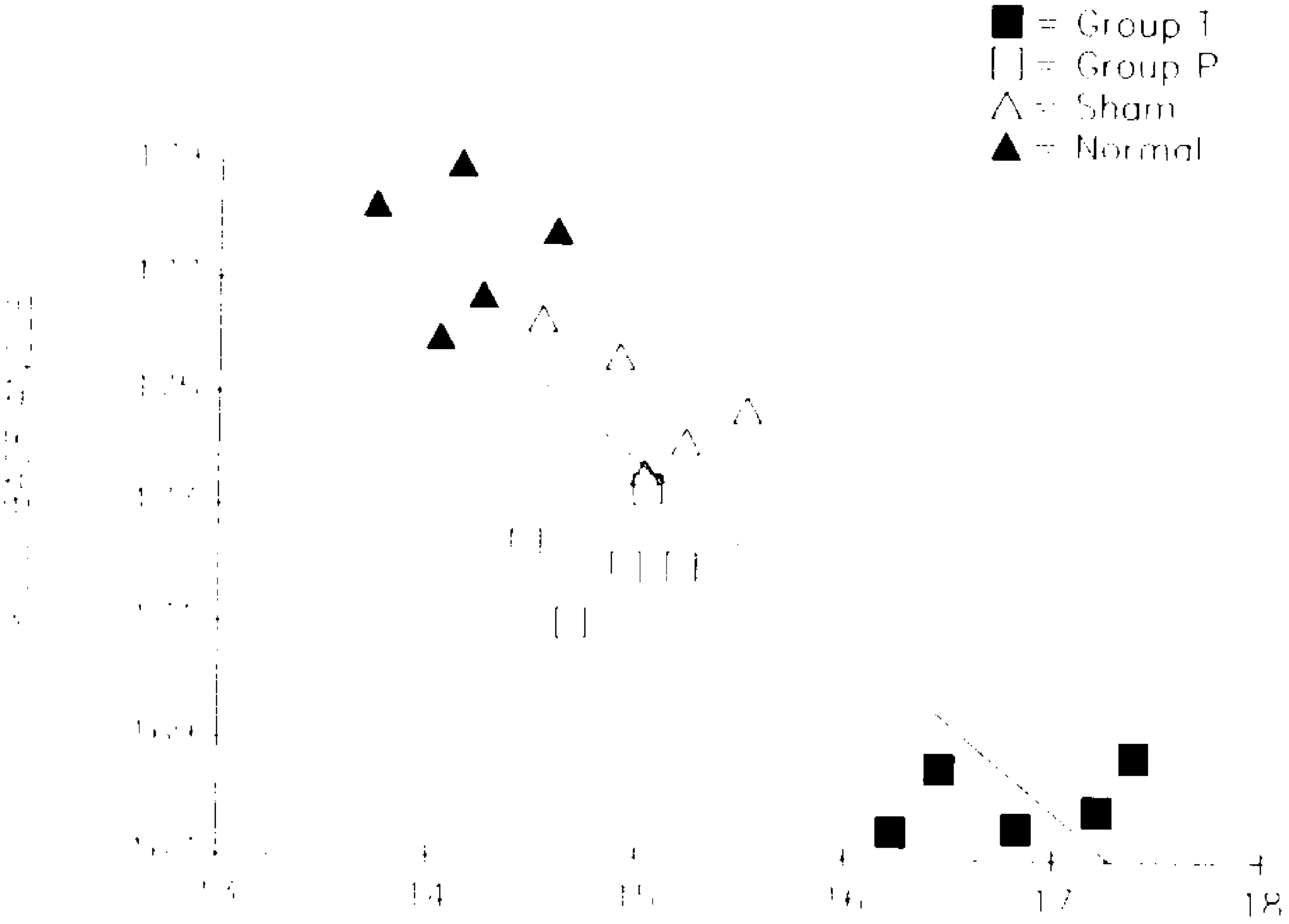


Figure 43

Figure 44: Graph of angle S (skull base) versus angle L (larynx) for normal, sham, and experimental groups P and T. There is a negative slope, indicating an inverse linear relationship between angle S and angle L. Note that normals are clustered at the upper left, indicating large skull base angles (i.e. flat skulls) and small larynx angles (i.e. high larynx positions). The experimental group T rats are clustered in the lower right, indicating small skull base angles (i.e. flexed skulls) and large larynx angles (i.e. low larynges). Shams and group P experimentals are positioned in the intermediate ranges, with shams closer to normals and group P closer to group T.

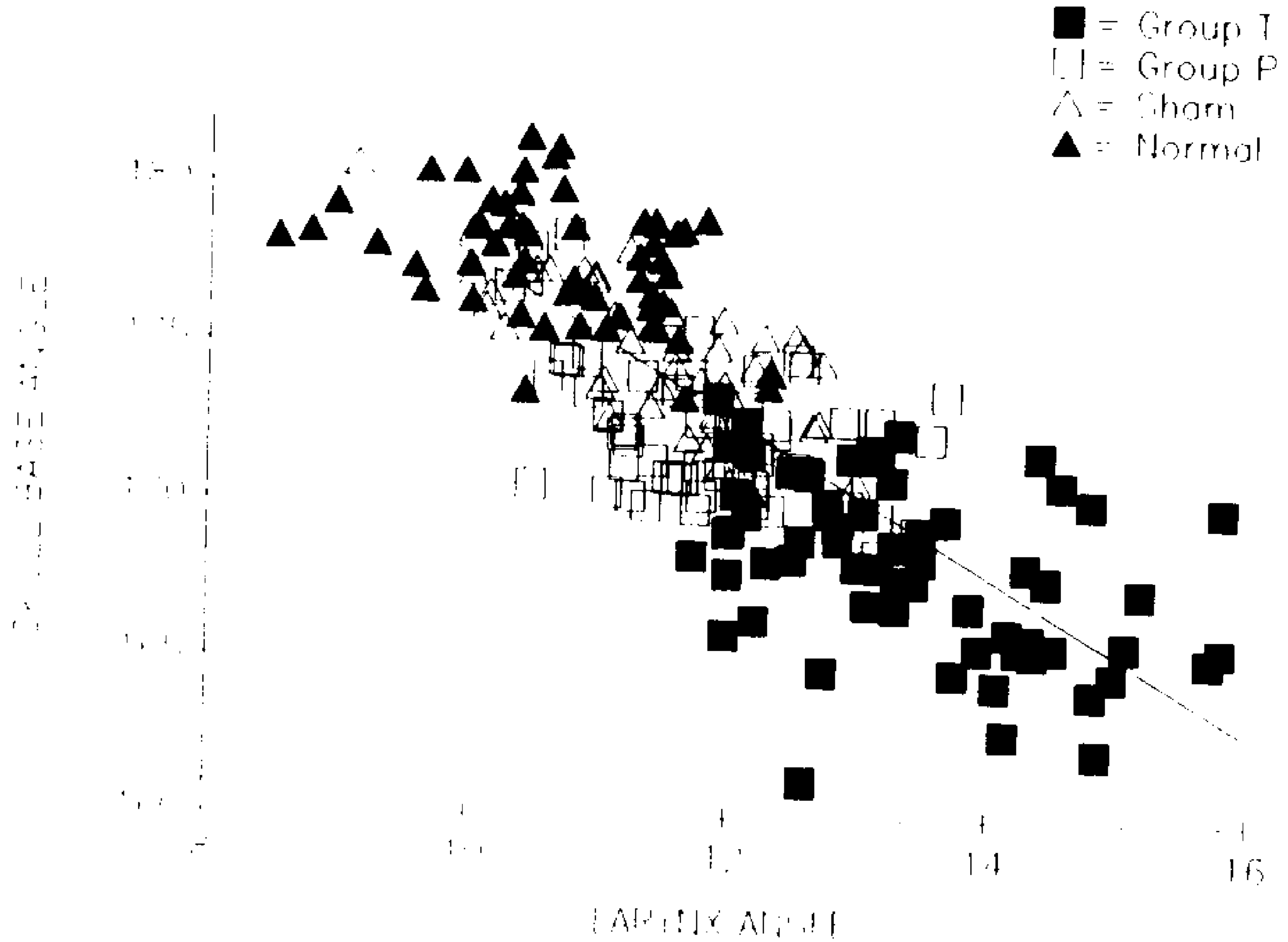


Figure 44

Figure 45: Graph of angle S (skull base) versus angle L (larynx) for normals.

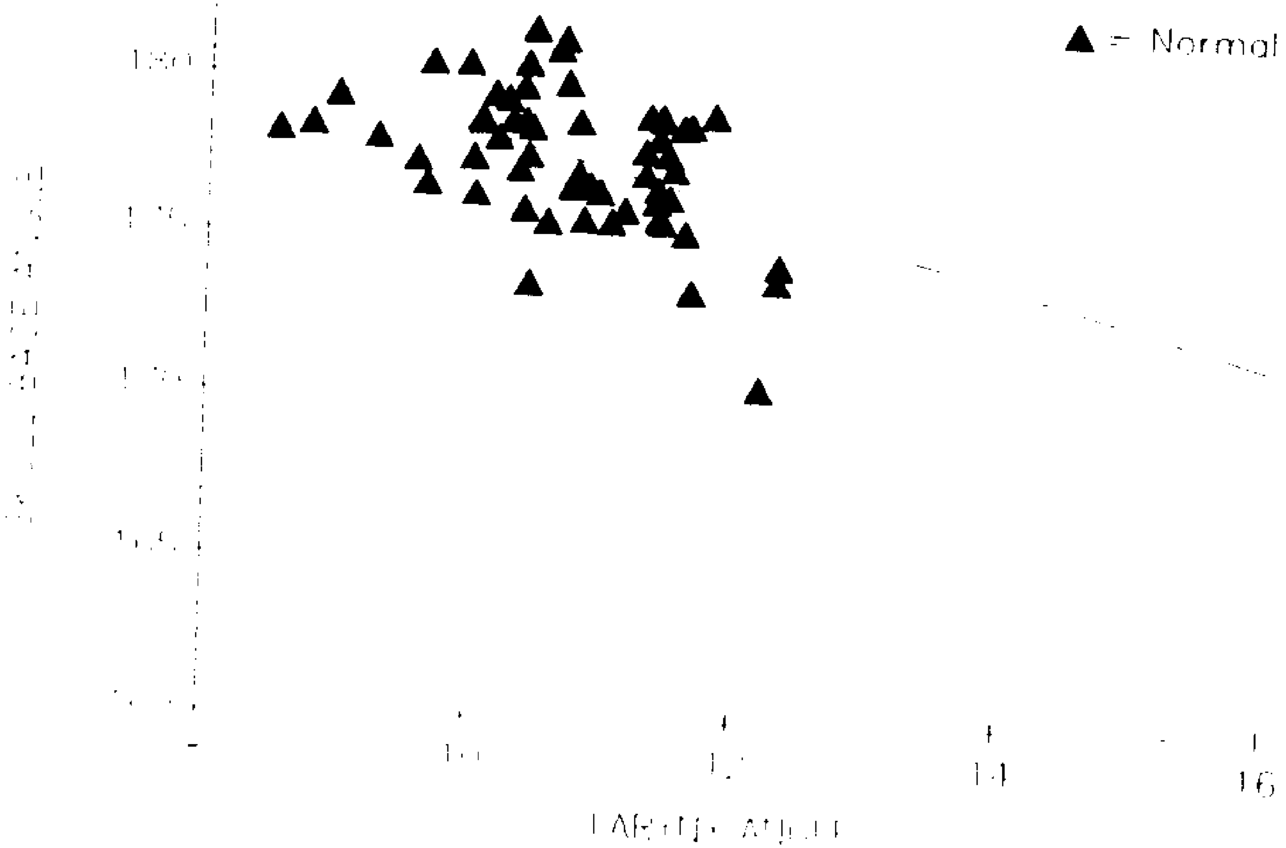


Figure 45

Figure 46: Graph of angle S (skull base) versus angle L (larynx) for shams.

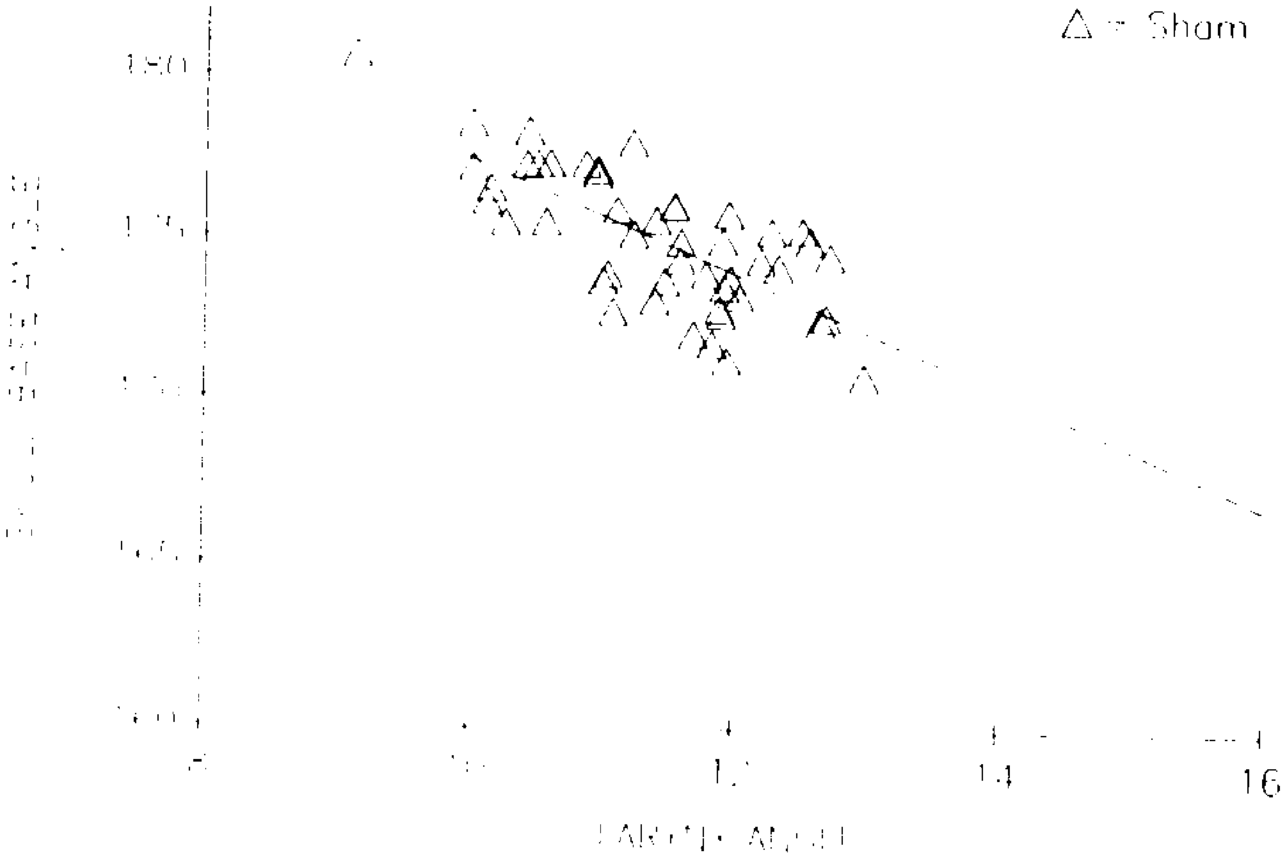


Figure 46

Figure 47: Graph of angle S (skull base) versus angle L (larynx) for group P experimentals.

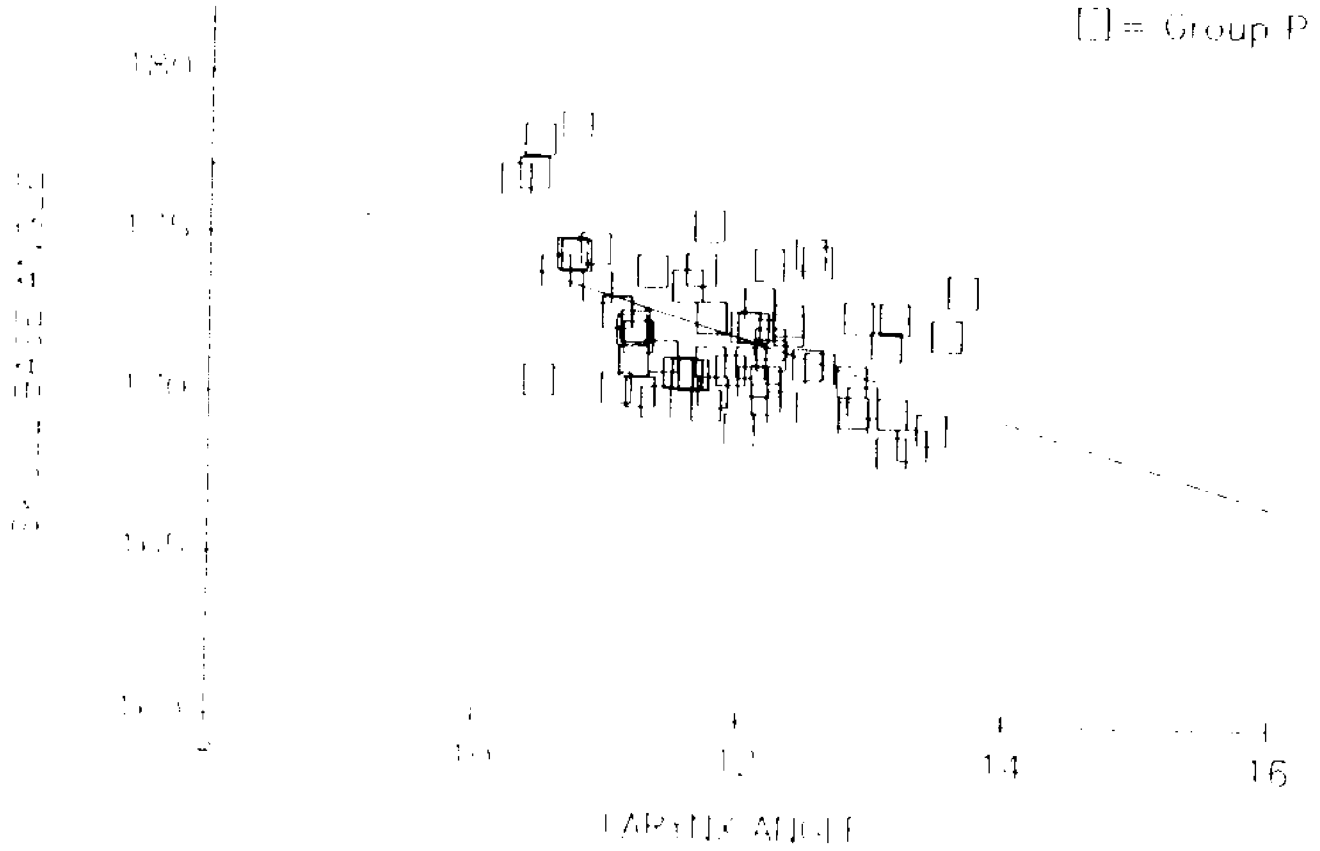


Figure 47

Figure 48: Graph of angle S (skull base) versus angle L (larynx) for group T experimentals.

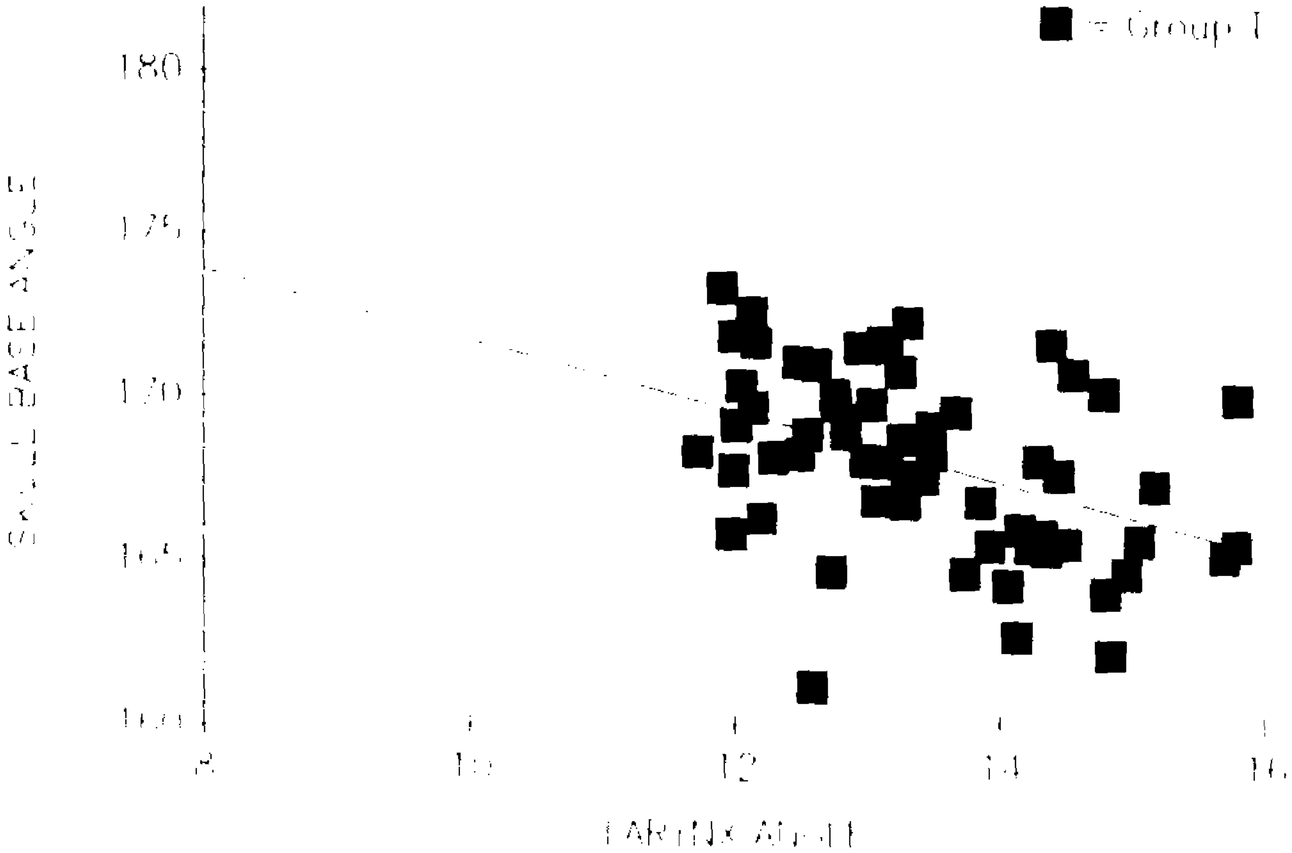

■ = Group I
SMALL BASE ANGLE
180
175
170
165
160
8
10
12
14
16
LARYNX ANGLE

Figure 48

Figure 49: Graph of means (one mean shown for each age group: 40, 60, 80, 100, 120 days) for angle S (skull base) versus angle H (hyoid) for normals, shams, group P experimentals, and group T experimentals.

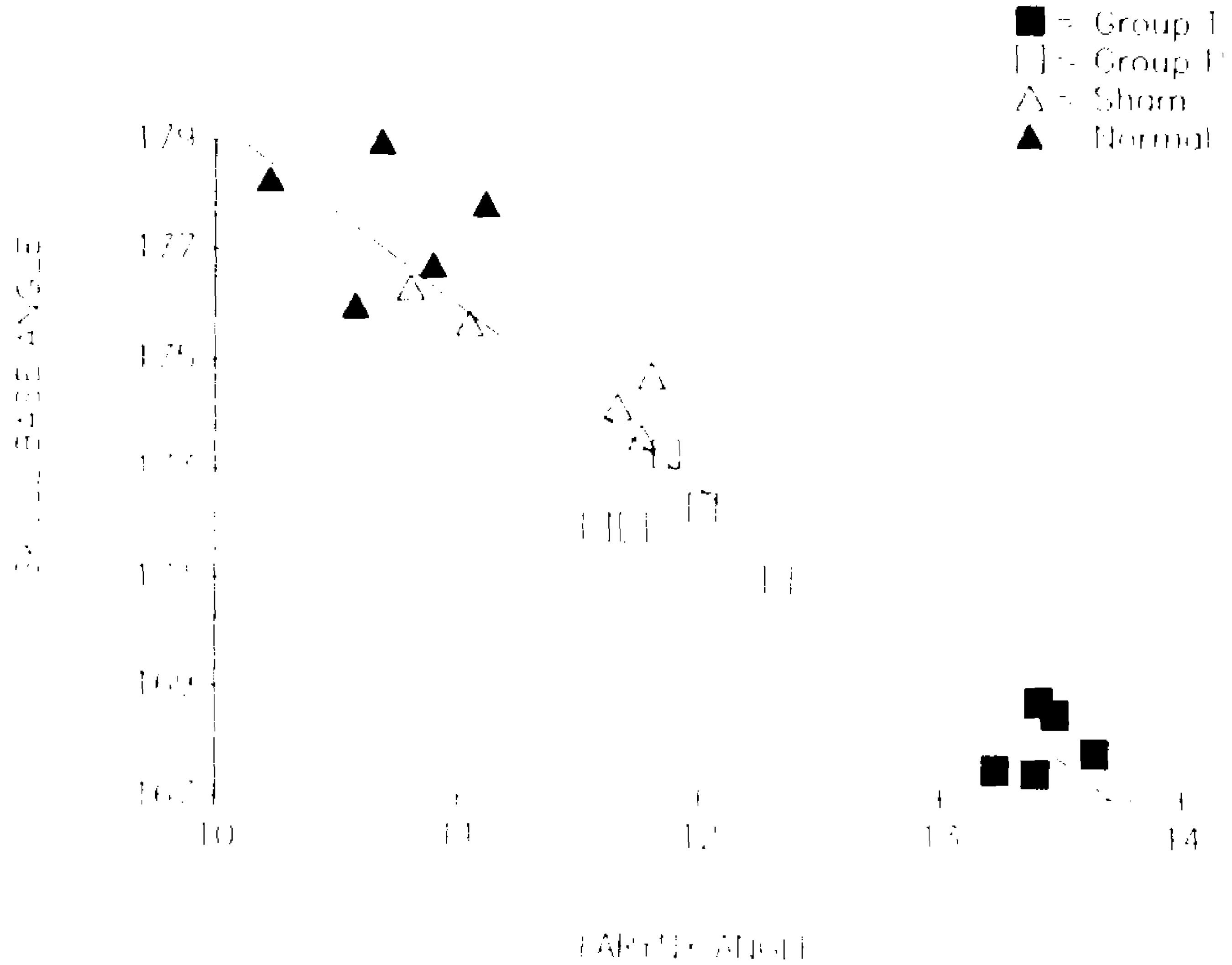


Figure 49

CHAPTER 6

DISCUSSION

Changes in the skull

Destruction of the spheno-occipital synchondrosis resulted in premature fusion (synostosis) of the basisphenoid and basioccipital bones. By eliminating this important cartilaginous growth center, basicranial elongation was stunted in the experimental rats. The remainder of the skull did not exhibit arrested growth, however, as it was not directly affected by this surgery. Although the calvaria continued to grow, its normal flat shape was slightly distorted by its ventral attachment to the shortened skull base. Instead of elongating in a horizontal direction, the skull vault of adult experimentals exhibited an abnormally domed appearance (convex dorsally and concave ventrally), similar to the juvenile condition in sham and normal controls. As the calvaria continued to grow in a curve directed ventrally, the plane of the foramen magnum was pushed from its normal vertical plane to an oblique plane midway between vertical and horizontal. (See also Reidenberg and Laitman, 1988.)

Since the skull vault is anchored to the skull base through the occipital bone posteriorly and temporal bones laterally, continued growth of the calvaria also affected the shape of the basicranium. The calvaria "overgrew" the shortened basicranium, pushing its posterior portion inferiorly, thus resulting in an artificially induced flexion of the skull base. In addition, continued growth of the brain (including the meninges and blood vessels) appears to have influenced the skull base to bend inferiorly. The combined expansion of both the brain and the cranial vault may exert a mechanical force upon the skull base, causing it to bend ventrally. As a result, a significant kyphosis of the skull base was observed.

Statistical analysis showed that skull base angles for experimentals, shams, and normals were significantly different from each other. The mean for experimentals was less than the mean for shams, and the mean for shams was less than the mean for normals. This relationship indicates that the skull base of experimentals was the most flexed (i.e., angle S was the smallest), while the skull base of the normals was not flexed (i.e., angle S was the largest). The skull base of the shams was only mildly flexed (i.e., angle S was larger than in experimentals, but smaller than in normals). Although the shams displayed smaller basicranial angles than the normals, their angles were closer to those of the normals than the experimentals.

It is interesting to note that the shams differ significantly from the normals. This result indicates that there is some effect of the surgical approach alone on flexion of the skull base. As the spheno-occipital synchondrosis was not removed from the shams, the most likely explanation for this mild flexion is the effect of scraping the longus capitus muscle from the skull base in order to visualize the synchondrosis. It is likely that scraping the muscle caused some damage to the synchondrosal cartilage, thereby resulting in a slight impairment of basicranial growth. Alternatively, the change in forces resulting from damage to, and healing of, the insertion of the muscle fibers may have remodeled the basicranium externally, thereby producing a thickened ventral surface. In measuring angle S, this thickening would be interpreted as increased flexion if it places the point basion more ventrally.

In light of these observations in the shams, it was suspected that the experimentals may also have differing degrees of damage to the synchondrosis. Histological analysis of the skull base confirmed that the experimentals are actually composed of two groups: 1) rats in which the synchondrosis was totally removed (group T), and 2) rats in which the synchondrosis was partially removed (group P). The surviving cartilage in group P was found almost exclusively on the endocranial surface of the basicranium, thus indicating incomplete drilling.

Statistical analysis showed that the mean of angle S for group T experimentals was significantly smaller than the mean of angle S for group P experimentals. These results showed that the group P experimentals exhibited less flexion than the group T experimentals, thus indicating a direct linear relationship between the degree of synchondrosal destruction, and the degree of basicranial flexion. In confirmation of this relationship, statistical analysis showed that the mean of angle S in shams was significantly larger than the mean of angle S in group P experimentals. As expected, the normals had the largest mean for angle S (i.e., the flattest basicranium), while the group T experimentals had the smallest mean for angle S (i.e., the most flexed basicranium).

In experimental rats with marked kyphosis, the posterior skull base exhibited an abnormal triangular elevation above the spheno-occipital synostosis. This bony ridge appeared to cradle the pituitary on its anterior (rostral) surface. Its posterior (caudal) surface was directed inferiorly (ventrally). It is interesting to note that this arrangement resembles the dorsum sellae of the sella turcica, and the steeply sloped clivus of the adult human skull. Although there is no definitive explanation for the appearance of such an elevation, it may be related to mechanical forces within a flexed skull base. Perhaps this structure provides some support, such as strutting or buttressing, which makes its presence architecturally advantageous or necessary when the skull base is kyphosed.

Position of the larynx and hyoid bone

The mechanical interactions between the skull base and the larynx occur through the muscles, ligaments, and connective tissue which suspend the hyoid bone, pharynx, and larynx from the skull base. It logically follows that any change in the surface to which these structures attach should be reflected as a corresponding change in their positions. In this study, the rat skull base was artificially flexed (kyphosed) in a controlled experiment. The hyoid bone and the larynx were found to descend lower in the neck in rats with surgically induced cranial alterations resulting in skull base kyphosis.

Statistical analysis showed that there were significant differences between the experimentals, shams, and normals for the means of angles H and L. The experimentals had the largest means for angles H and L, and thus the lowest positions for the hyoid and larynx. The normals had the smallest means for angles H and L, and thus the highest positions for the hyoid and larynx. The shams were intermediate between the experimentals and the normals. The experimentals were then divided into the two groups based upon histological analysis of the degree of synchondrosal destruction (group T for totals, group P for partials). Statistical analysis showed that group T experimentals were significantly larger than the group P experimentals for the means of angles H and L, thus indicating lower hyoid and larynx positions in rats with complete removal of the spheno-occipital synchondrosis.

There is significant negative (inverse) linear correlation between the degree of skull base flexion and the positions of the larynx and hyoid bone. As the skull base becomes more flexed (i.e., angle S gets smaller), the hyoid and larynx descend lower into the neck (i.e., angles H and L get larger). The group T experimentals have the most flexed skull bases, the lowest hyoid and larynx positions, and total removal of the spheno-occipital synchondrosis. Group P experimentals have moderately flexed skull bases, moderately low positions of the hyoid and larynx, and partial destruction of the spheno-occipital synchondrosis. The shams had only slightly flexed skull bases, slightly lowered larynx and hyoid positions, and little or no disruption of the spheno-occipital synchondrosis. The normals had the flattest skull bases, the highest larynx and hyoid positions, and no damage to the spheno-occipital synchondrosis. Statistical analysis of these results indicate significant negative (inverse) correlation between angle S and angle H, and between angle S and angle L.

The results of this study reveal that there is indeed a mechanical relationship between the contour of the skull base and the position of the larynx and hyoid bone. Since corresponding changes in laryngeal and hyoideal positions were documented, this study has shown that the degree of flexion of the skull base plays an important role in determining the position of the mammalian larynx and hyoid bone. Changes in basicranial shape thus affect the position of these structures by functionally interacting with the upper respiratory tract through a direct, mechanical interaction.

Necropsy

Elevated mortality rates in experimentals relative to shams during the surgery may have been due to the effects of stress induced by the drilling procedure. As the drilling occurs ventral to the pons, care was taken not to puncture the dura and thus injure it. Nevertheless, vibration or pressure from the drill may have disrupted the pons' ability to control the pneumotaxic center and vasopressor regions of the brain, which regulate apnea and arterial blood pressure, respectively (Lambertsen, 1980; Milnor, 1980). In addition, the effects of stress from the trauma of drilling on the cardiopulmonary system may have weakened the experimental rats more than the shams, thus making them more susceptible to respiratory depression from the anesthesia.

Seventeen experimental rats which survived the surgery, were found dead of pneumonia before being weaned. It is not clear, however, whether the primary cause of the pneumonia was bacterial or viral infection, or aspiration of milk. It is possible that the effects of increased skull base flexion on laryngeal position were present earlier, or to a greater degree, in these rats than in those that survived. If the larynx was positioned lower than in the other experimental rats, then the epiglottis may have had difficulty maintaining contact with the soft palate. As a result, these rats may have had difficulty breathing and swallowing simultaneously, and thus may have aspirated milk while attempting to suckle and breathe. This situation would lead to death by one of three mechanisms: 1) blockage of airflow by a bolus of milk, 2) severe chemical pneumonitis resulting from inspired milk, or 3) mild chemical pneumonitis followed by a fatal secondary infection.

CHAPTER 7

CONCLUSION

This study has investigated the relationship between basicranial shape and laryngeal and hyoidal position. This relationship is important in the understanding of both normal and pathological growth and development, functional interactions, and the evolution of the skull and respiratory tract.

Biomedical Applications

The most central application of this study concerns what it adds to the understanding of the biological mechanisms which determine the developmental relationship between the skull and soft tissue structures. More specifically, this study has addressed questions concerning how changes in the shape of the basicranium affect the anatomy of the upper respiratory tract.

One area in which this information may be directly applicable is in the study of craniofacial growth and development. A number of craniofacial pathologies which affect basicranial growth, for example, are often associated with respiratory problems. The degree to which these basicranial shape changes influence respiratory function is, however, still unclear. For example, human infants and children with hydrocephaly, microcephaly, and anencephaly, have difficulty breathing, swallowing, and vocalizing (Gabriel and McComb, 1985). These conditions also result in a prematurely kyphosed basicranium. It has not been determined, however, to what extent this basicranial shape change may affect respiratory function. Although increased or decreased neuromuscular tone probably plays some role in control of these functions, the mechanical effect of the deformed, prematurely flexed basicranium on laryngeal and hyoidal position should not be overlooked.

Some respiration, deglutition, and phonation problems may involve a combination of both malpositioning of the larynx and poor neuromuscular coordination. Sudden infant death syndrome (SIDS), is one example of a class of diseases which may involve such anomalies. SIDS accounts for between one sixth and one fourth of all infant mortality in the United States, making it the most common manner of death between ages one month to twelve months, corrected for gestation (Behrman and Vaughan, 1983; Mellins and Haddad, 1983). Despite this, the etiology of SIDS is currently poorly understood (see Hasselmeyer and

Hunter, 1975; Peterson, 1980; Tildon et al., 1983; Golding et al., 1985; McKenna, 1986 for reviews on SIDS). One possible explanation for SIDS is respiratory arrest due to anatomical anomalies of the upper respiratory tract, perhaps associated with functional disorders of central nervous system control of respiration (Bosma and Showacre, 1975). Descent of the larynx, aquisition of oral tidal respiration, basicranial flexion, and central nervous system maturation are all interrelated changes which occur during infant maturation (Laitman and Crelin, 1980a; Laitman, 1986b). Anomalies in the anatomy or coordination of these developmental changes may contribute to the occurrence of SIDS (see discussion in Laitman, in press).

A better understanding of the relationship between basicranial shape and the position of the larynx and hyoid may aid in the diagnosis and treatment of many craniofacial disorders which affect the upper respiratory tract. Data from this study - showing that basicranial shape is both structurally and mechanically inseparable from the location of upper respiratory structures - will hopefully add new data to further our understanding of the relationship between abnormal skull growth and the frequently associated upper respiratory tract disorders.

Paleoanthropology:
human speech and respiratory patterns

A topic of great interest in the field of human evolution is the question of when and how our early ancestors acquired the abilities for speech and language. Central to these investigations have been studies which began to focus on interpreting what the upper respiratory, or "vocal", tract anatomy of the early hominids may have been like. Since only the skeletal anatomy remains as fossilized evidence, reconstructions of soft tissue structures depend upon an appreciation of the anatomical and functional relationships between these soft and hard tissues. The relationship between the upper respiratory tract and the skull base has thus emerged as an important element in the understanding of what this region was like in our ancestors. Indeed, ranging from the early works of Lieberman and Crelin on Neanderthals (Lieberman and Crelin, 1971; Lieberman et al., 1972; also see reviews in Lieberman, 1984; Crelin, 1987), to the more recent studies by Laitman and his colleagues examining changes in a wide range of hominids (Laitman, 1977; 1983; 1986; in press; Laitman and Heimbuch, 1982; Laitman et al., 1978; 1979), the relationship of the skull base and larynx has proven to be pivotal.

Although controversy continues concerning the interpretation of what the anatomy of the fossil hominid respiratory/vocal tract was like, and how this area has changed during the course of evolution, these studies have broken new ground in an attempt to reconstruct this region. The work of Laitman and his colleagues, for example, has contributed much to our understanding of the relationship between the skull base and laryngeal position. Using comparative anatomical data, reconstructions have been made on fossil hominids ranging from Plio-Pleistocene australopithecines through late Pleistocene members of our own species.

While observations on mammalian anatomy have enabled these upper respiratory reconstructions to be made, questions still remain concerning the functional interactions between the basicranium and contiguous upper respiratory structures such as the larynx. What the present study adds to the search for reconstructing the upper respiratory anatomy of our ancestors, is the additional dimension of understanding the mechanical relationships which are at work in this area. As our knowledge of the functional interactions in this region grows, so too will our ability to approach the central questions of how the anatomy was changing during the course of human evolution. It will only be through an indepth appreciation of the basic biology of this region that we will ever be able to comprehend the mechanisms involved in the evolutionary changes of the human upper respiratory tract, skull base and, indeed, our unique modes of breathing, swallowing, and vocalizing.

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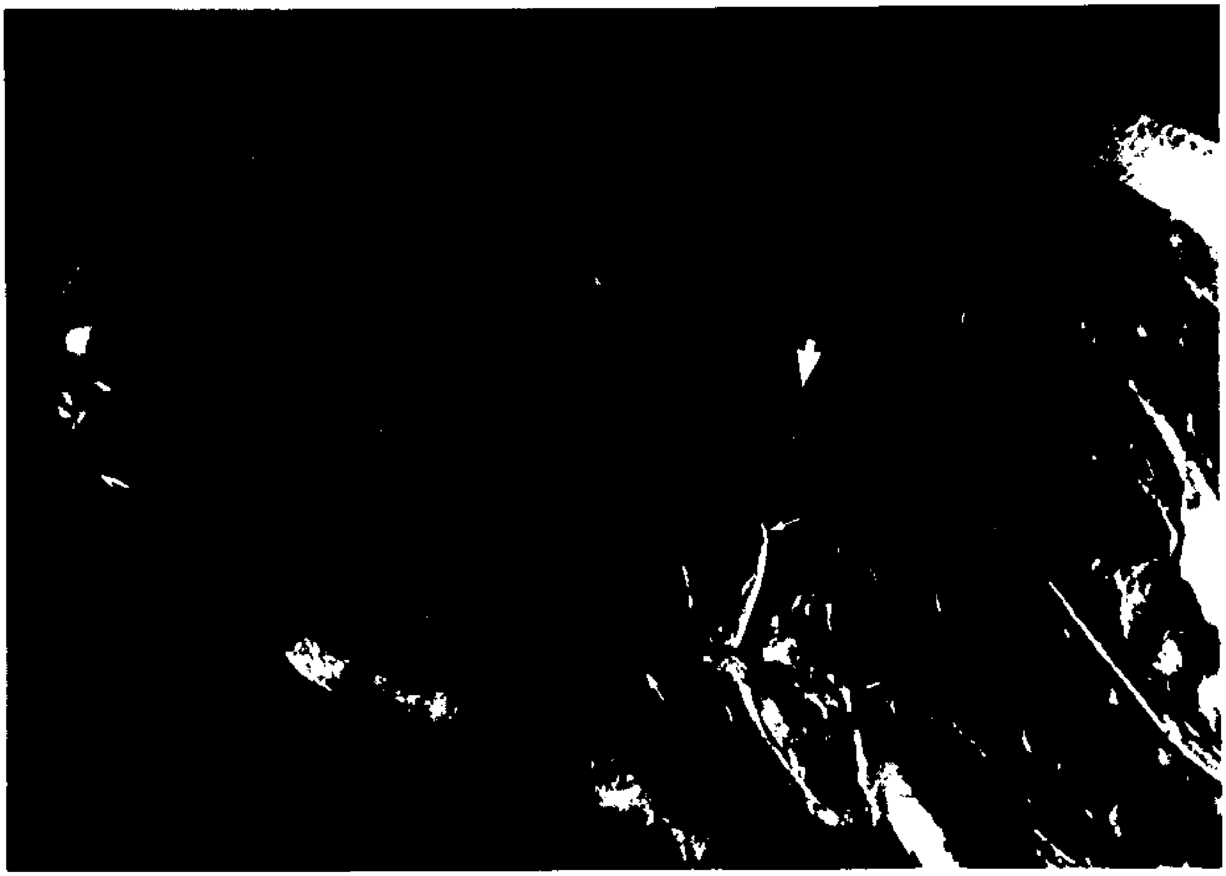

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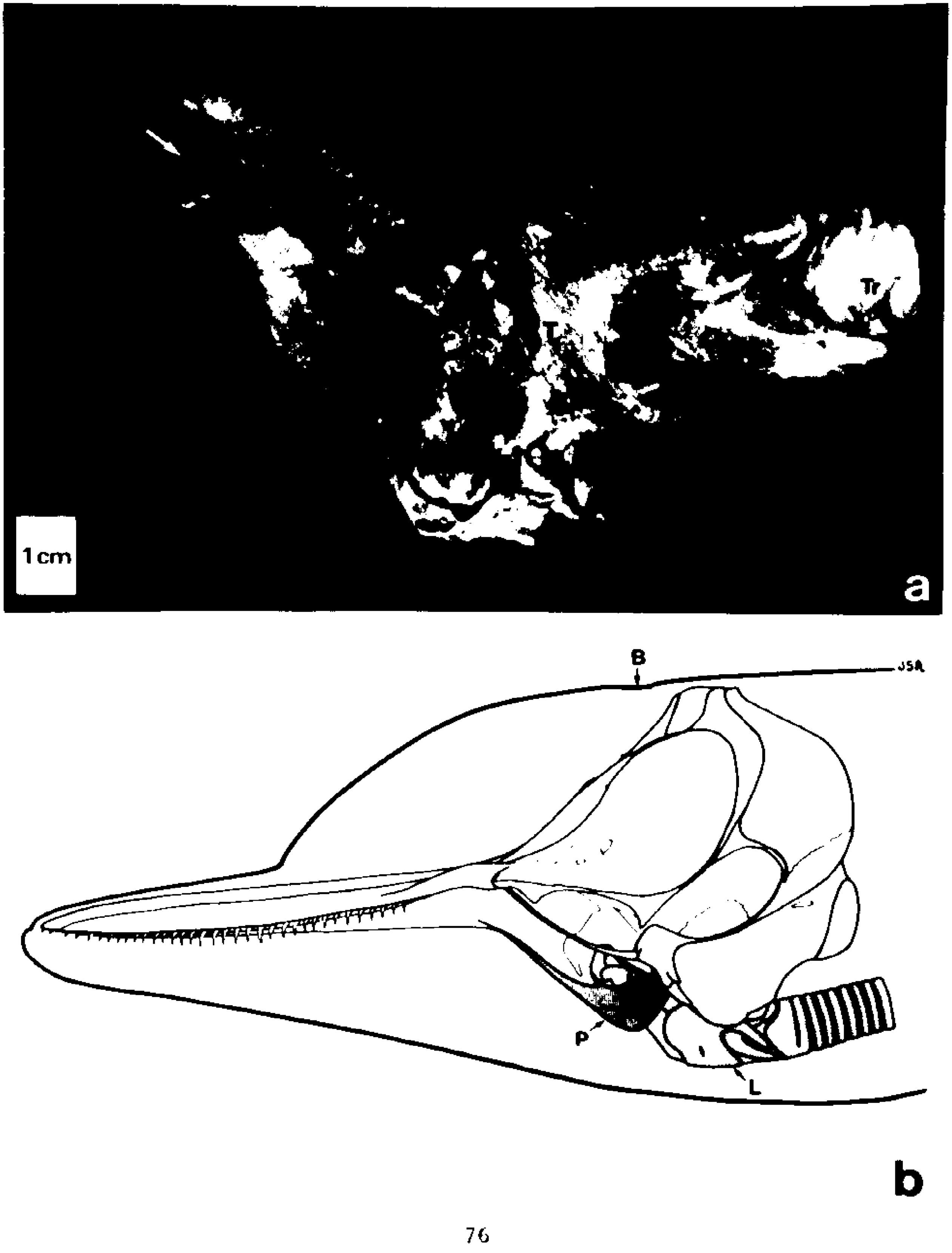
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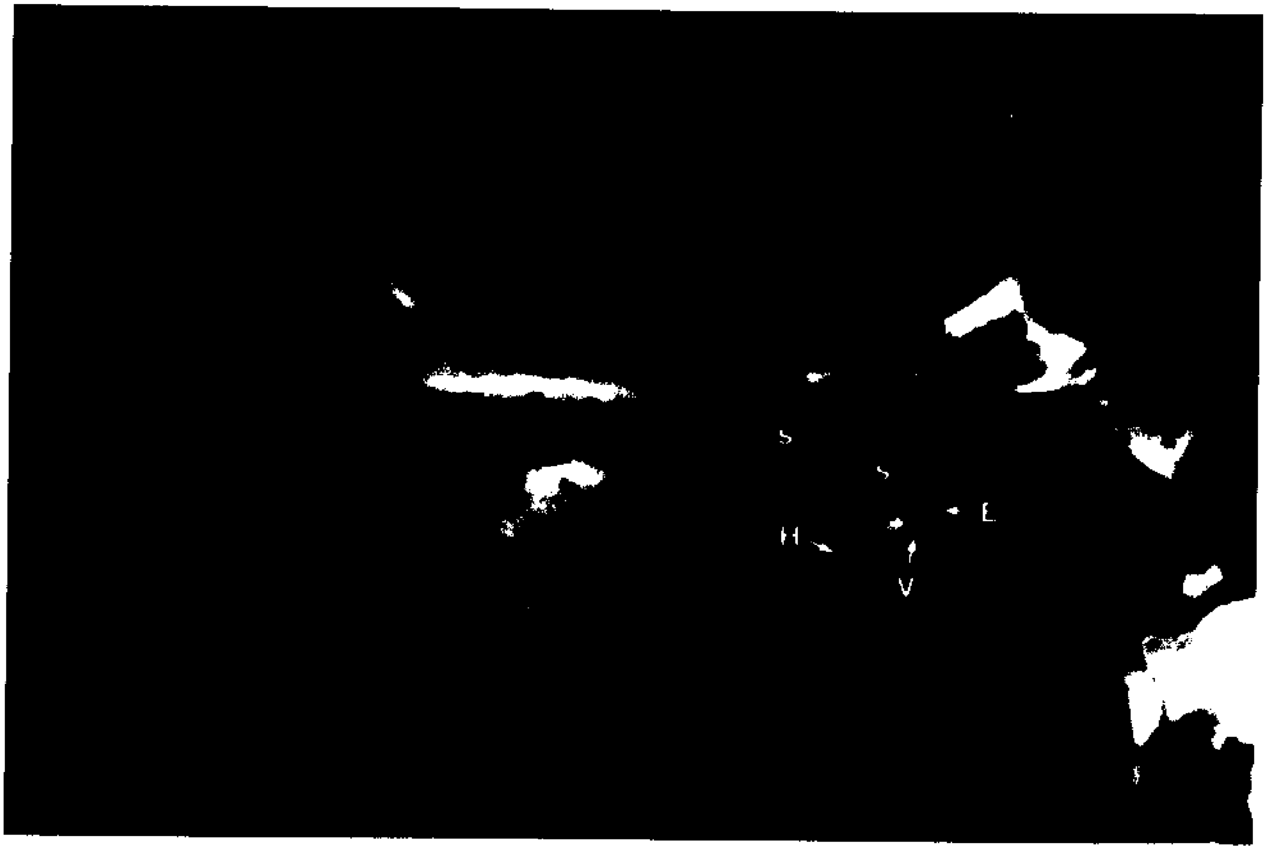
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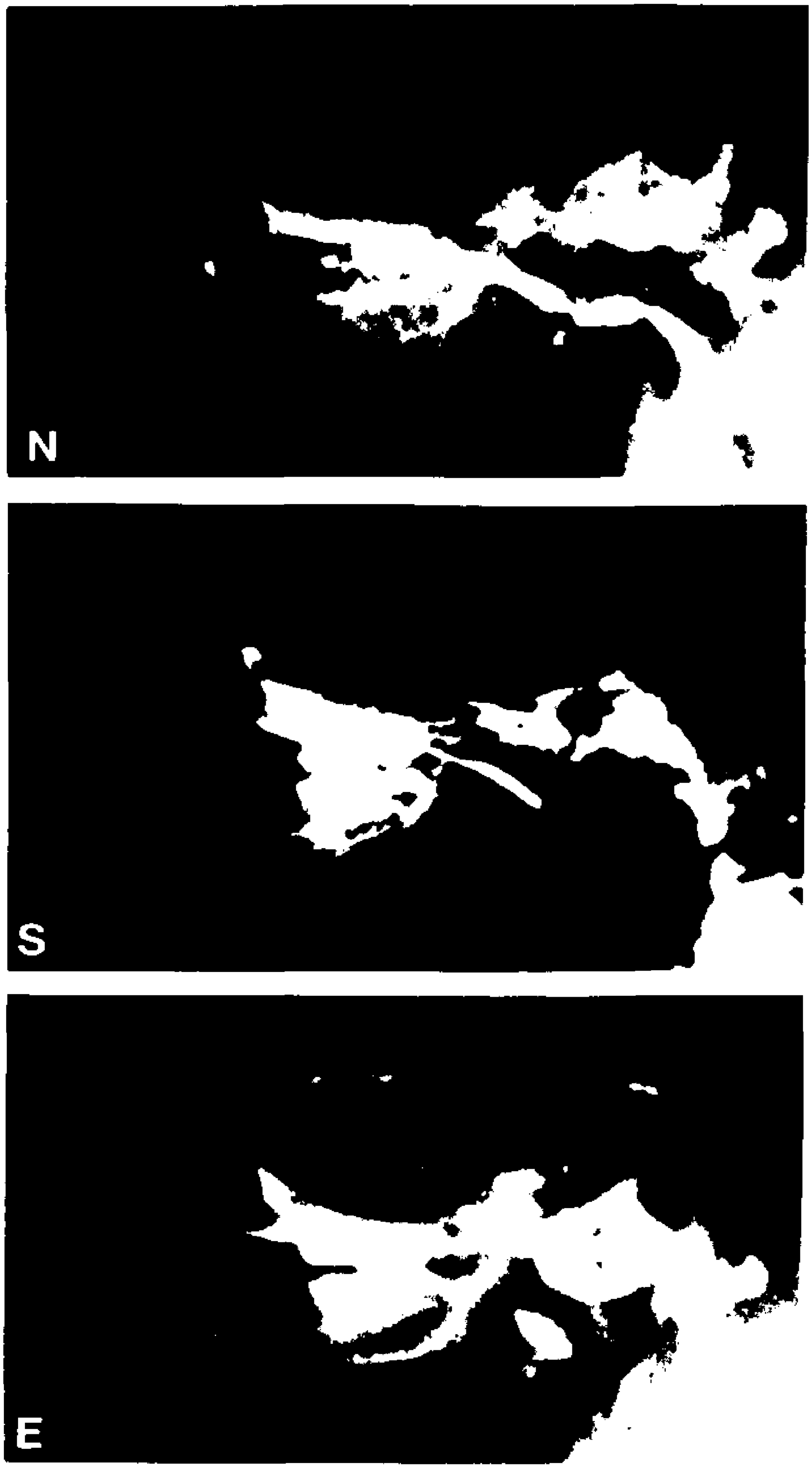

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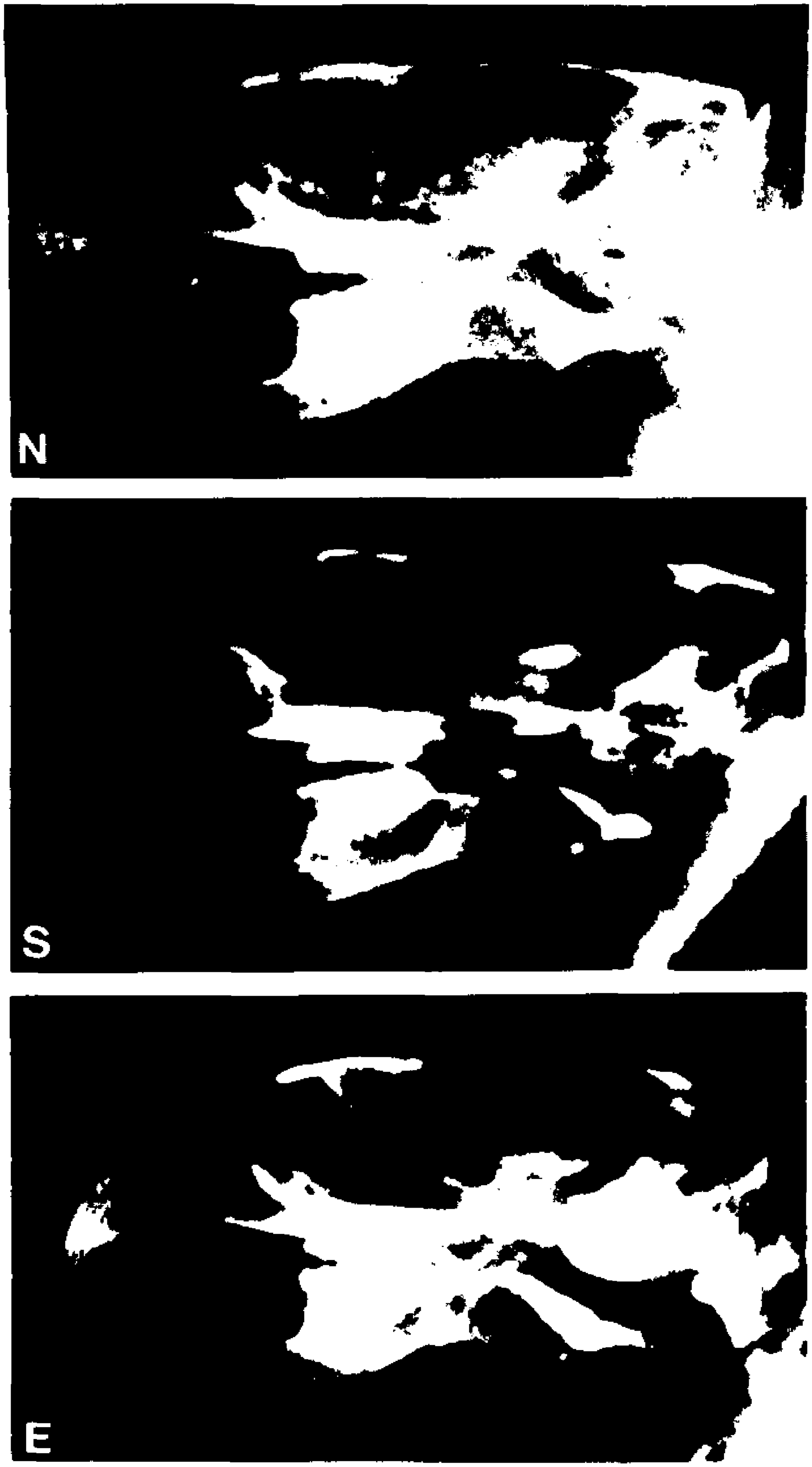

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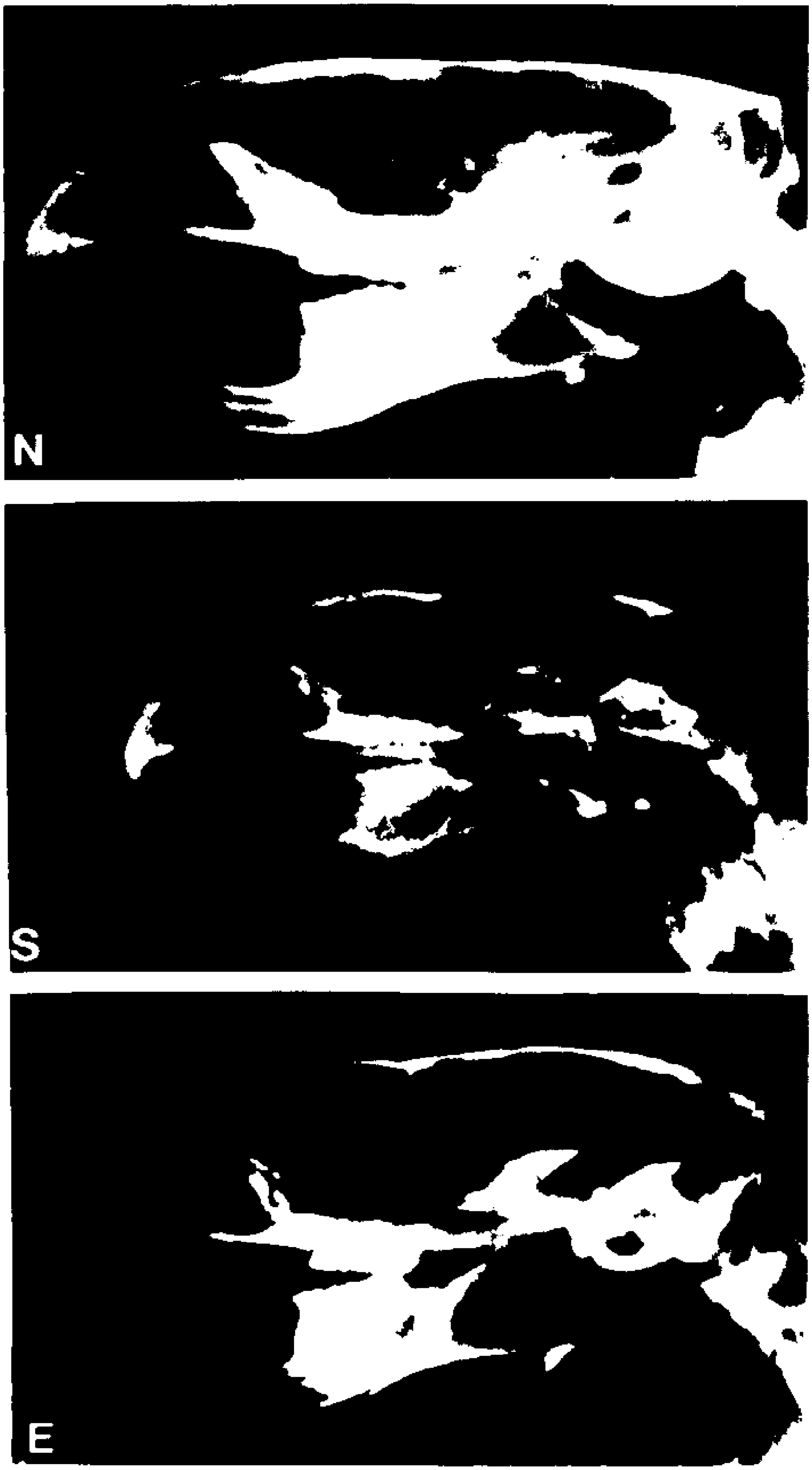

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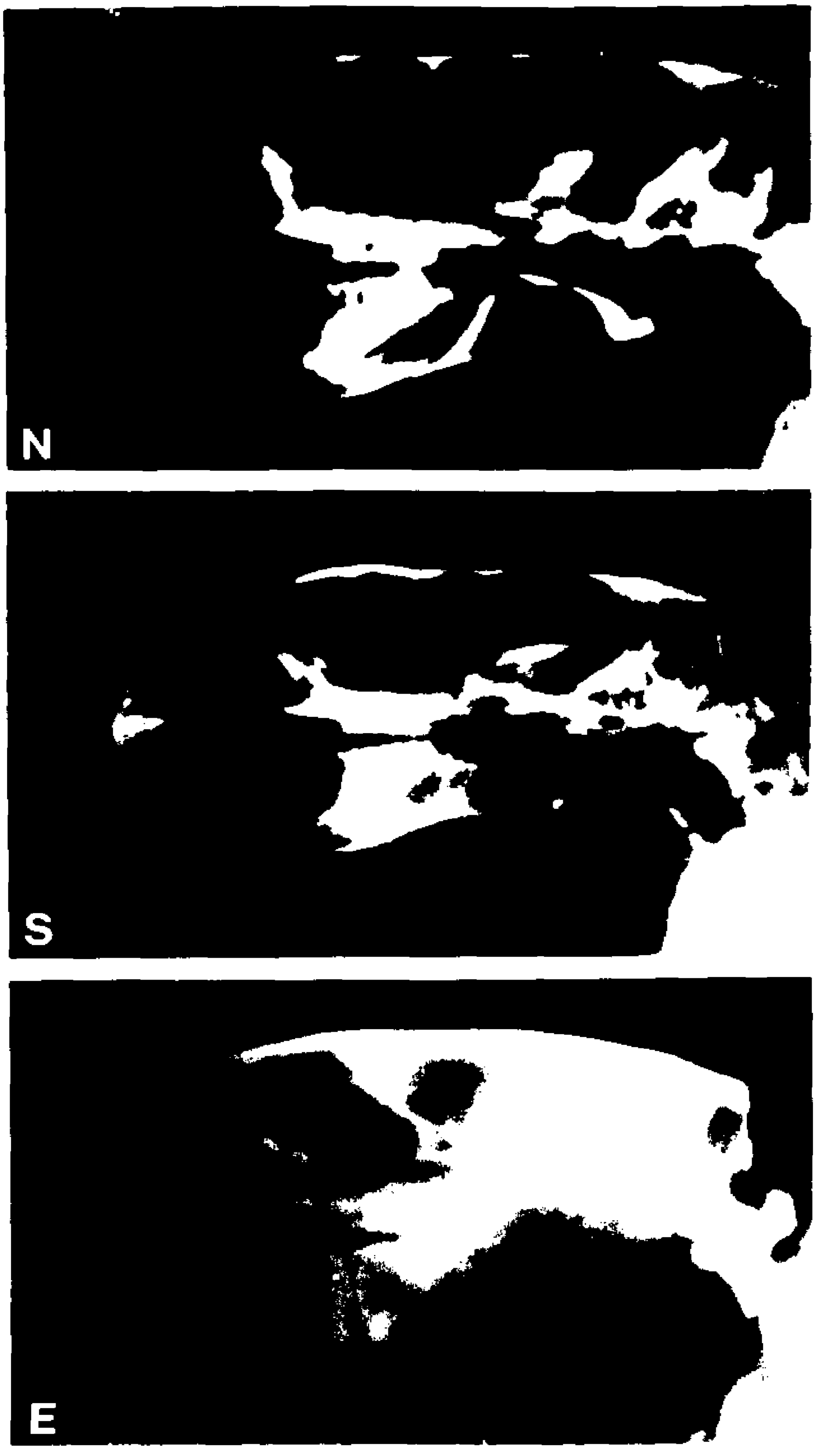

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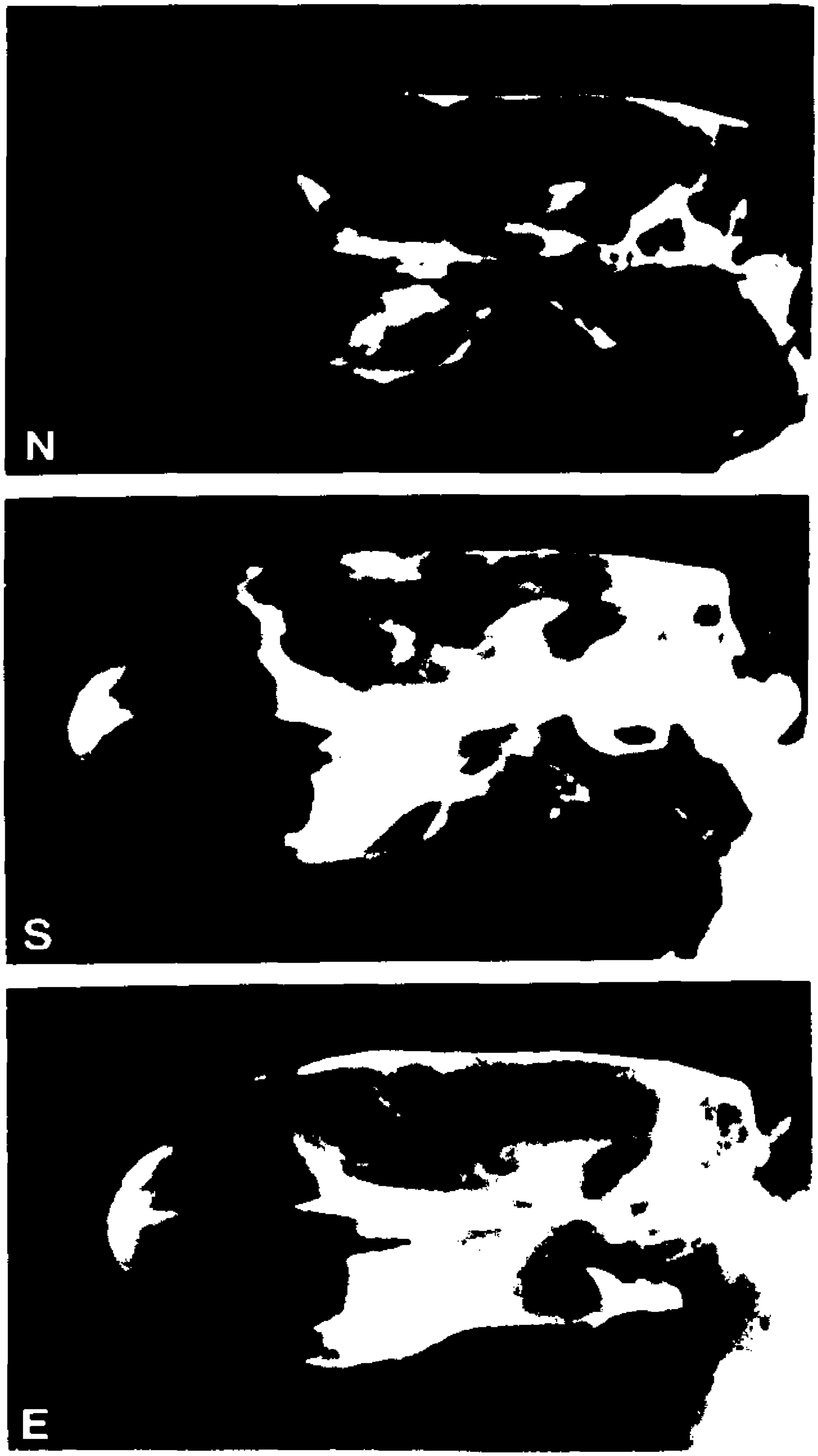

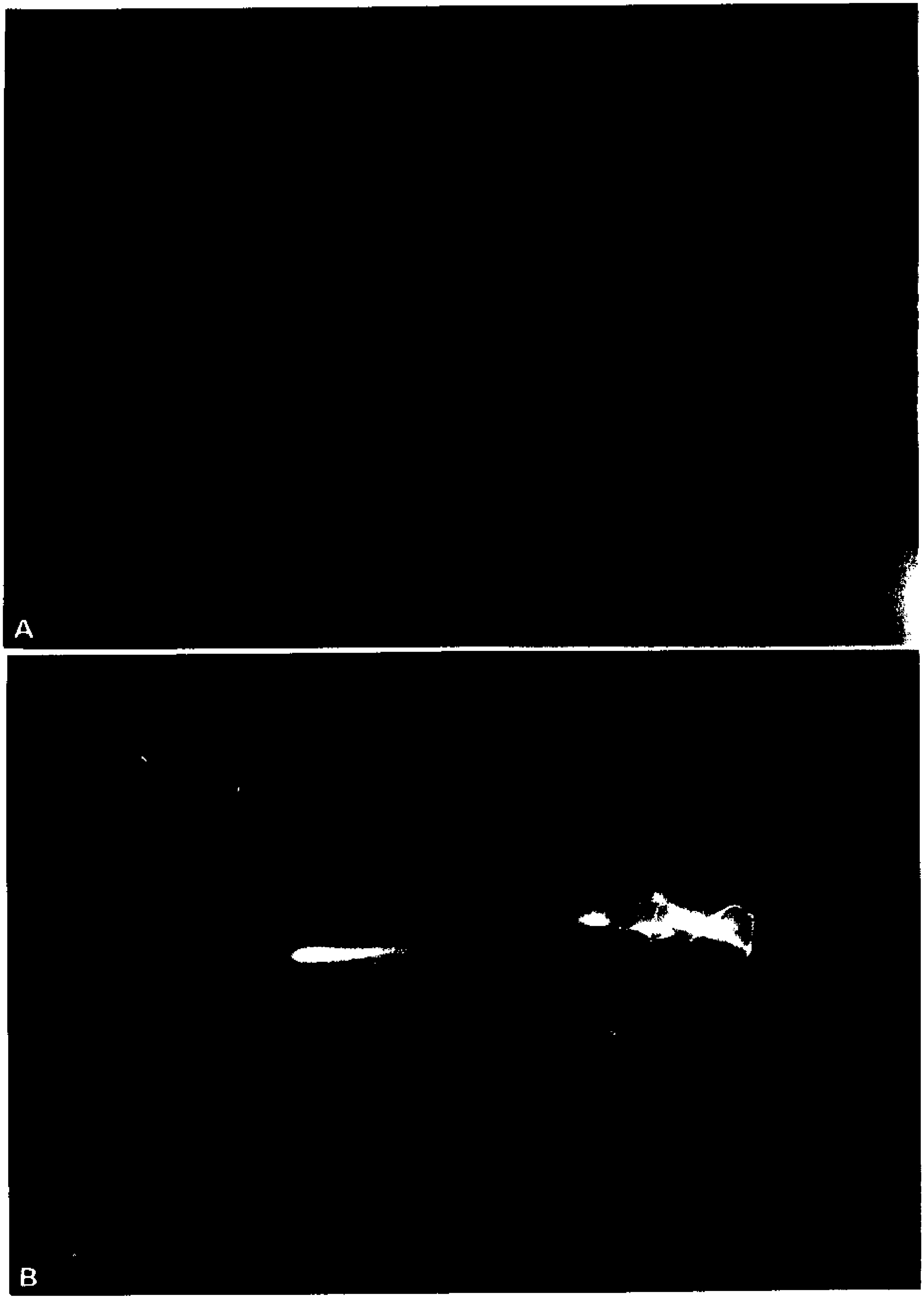
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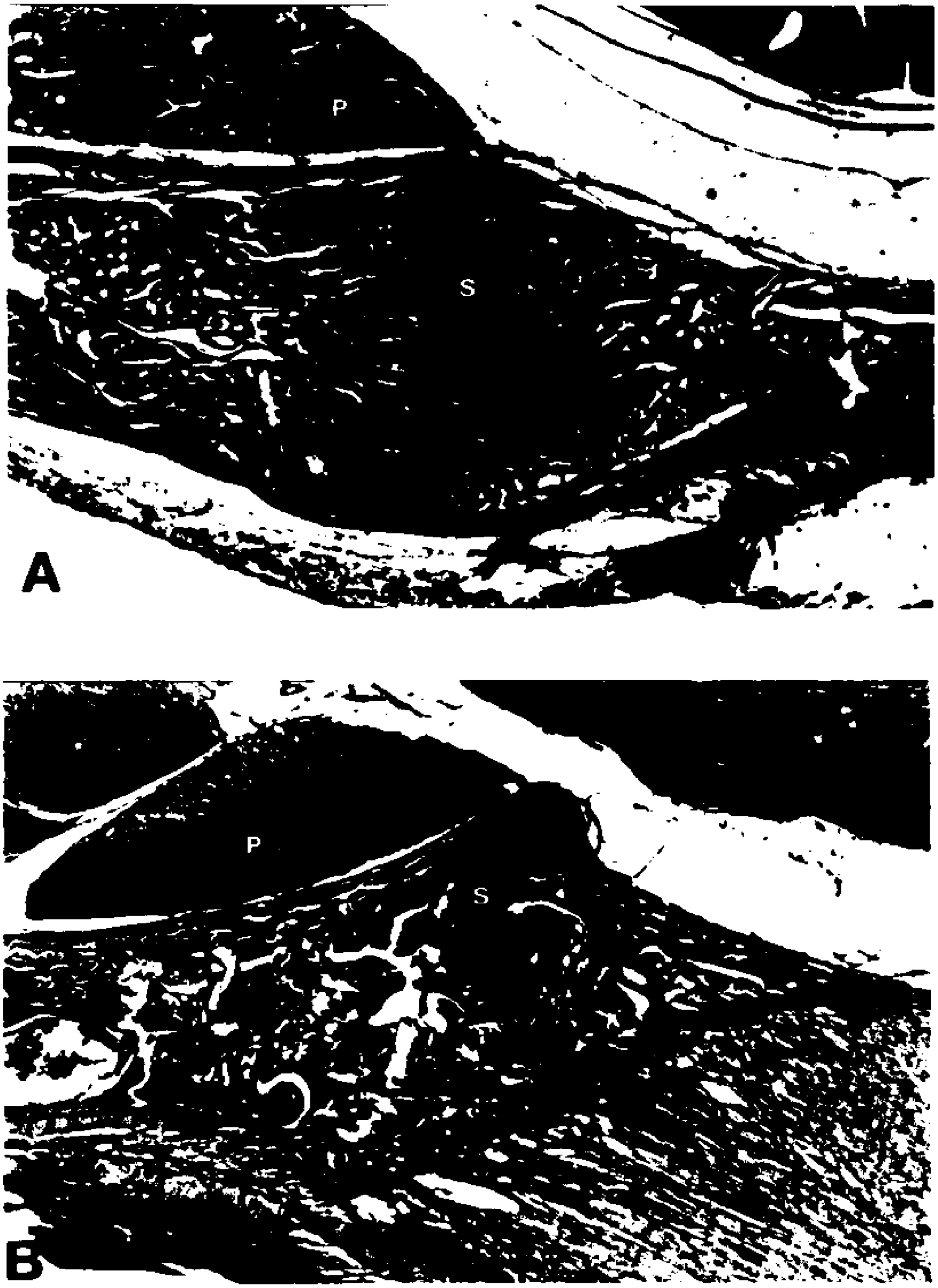
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