The Effect of Single and Fractionated Dose Radiation on Craniofacial Growth in Rats

by

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Abstract

Bone marrow transplant (BMT) preparative regimens for children usually include total body irradiation in combination with chemotherapy. Abnormal growth and endocrine deficiencies have been observed in children after BMT (Sanders 1988). Although the detrimental effects of localized high dose irradiation on craniofacial growth and development are well documented, little is known of the effects of low dose irradiation.

The purpose of this study was to determine the effect of single and fractionated dose irradiation on craniofacial growth in rats. Eighty seven male Sprague-Dawley rats were randomly assigned to seven experimental groups. Two groups (S1, S2) received single dose irradiation ranging from 200 to 500 cGy at two days of age. Four groups (F1, F2, F3, F4) received six fractionated irradiation doses ranging from 250 to 600 cGy, administered between two and four days of age. The seventh group (control), received sham irradiation. Weekly weight, length and cephalometric radiographs were taken of each animal from week one to week eight, and again at twenty-one weeks when the animals were killed. Craniofacial growth changes were determined by measurement of sequential lateral cephalographs. Post-mortem mature skulls were measured by metrographic techniques.

Both single and fractionated dose irradiation significantly affected body weight, while only high single dose irradiation influenced body length. Longitudinal data derived from cephalometric radiographs demonstrated that in general the high single dose group was significantly different from the control group in all measurements except neurocranial length. No significant differences were seen between control and low fractionated groups in any measurements except neurocranial height. Cross-sectional analysis of mature skulls using metrographic measurement techniques demonstrated significant differences in cranial length, viscerocranial length, mandibular width, bizygomatic width and height of the cranial vault between control and high single and fractionated dose (S1, S2, F4) irradiation, and between high (F4) and low

fractionated dose (F1, F2, F3) irradiation.

In conclusion, fractionated low dose irradiation has less significant effect on craniofacial growth than high single dose irradiation, and viscerocranial growth was more affected by irradiation than neurocranial growth.

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CHAPTER 1

Introduction

The effects of irradiation on craniofacial growth are discussed through a review of the literature. This is presented in the following order: incidence of childhood cancers, preparative regimens and side-effects of bone marrow transplantation, the use of the rat as a model for craniofacial growth studies and measurement of craniofacial growth through cephalometric and metrographic techniques.

1.1 Childhood cancers

Cancer is a leading cause of death in children between one and 15 years of age, second only to accidents (Hsu 1992). Advances in treatment have been made in the past few decades, significantly increasing the survival of children with malignancies. Larson et al (1990) estimated that by the year 2000, one in every 1,000 20-year-old adults will be a survivor of childhood cancer. Birch (1988) stated childhood cancer may affect one in six hundred and fifty children by the age of 15 years. He also noted that the survival rate has increased from 21% to 49% over the past 3 decades. The delayed consequences of therapy are thus of major concern.

Bone marrow transplantation (BMT) has become a well established form of treatment in children with nonmalignant and malignant hematologic disorders. Marrow transplant preparative regimens are designed to suppress the immune system and eradicate the underlying disorder through the use of cranial irradiation, total body irradiation (TBI) or total lymphoid irradiation (TLI), with or without chemotherapy. The irradiation may be administered as single or fractionated dose irradiation.

Bone marrow transplantation has a history spanning approximately 50 years; beginning first as a concept of treatment and since the mid-1950's, when the Major Histocompatibility Complex was identified, as an increasingly active treatment option. Before 1969 only a few

patients receiving bone marrow transplantation for treatment of hematological disorders survived. Advances in immunobiology, histocompatibility testing, immunosuppressive preparative regimens and support care have resulted in improved survival. The success of marrow transplantation has continued to improve since the 1970s and has resulted in the use of this technique for an ever increasing number of children, with the numbers of long-term survivors also continuing to increase (Sanders et al 1988).

The 1988 report from the International Bone Marrow Transplant Registry (IBMTR) stated that 15,000 allogeneic transplants were performed from 1957 to 1986. More than 50% of these transplants occurred between 1984 and 1986, with more than 3,000 in 1986 alone. Prior to 1980, 75% were for non-malignant diseases. However more recently more than 75% of transplants were for the treatment of malignancies - with leukemia the leading malignancy in children (Gale et al 1989). Long term survivors of malignancy are defined as those patients who are disease free for at least 5 years, and off therapy for at least 2 years. Survival rates were determined by IBMTR for acute lymphoblastic leukemia in 1988. Of 236 high risk patients (adults and children), the 5 year probability of survival was 46 +/-9%. Studies analyzed by Cheson et al (1989) involving autologous BMT found response rates of 60% to 80% for leukemia and lymphomas and responses of 30% to 80% for solid tumors. Deeg (1990) reported long term survival 55-60% in patients with acute non-lymphoblastic leukemia when given HLA identical transplants in chemotherapy-induced remission. Similarly, in his cohort study 30% of patients with acute lymphoblastic leukemia transplanted in 2nd remission became relapse free survivors. Long term survival of children with aplastic anaemia ranges from 40 to 70% percent after BMT (Deeg 1990). As increased numbers of patients undergo BMT there is a growing concern and related research into the short and long term effects of the treatment regimens on the survivors.

1.2 Preparative regimens and principles of bone marrow transplantation

The bone marrow is the major hemopoietic organ of the body and is responsible for production of the major blood cell lines. Conditions such as aplastic anemia, thalassemia and Chediak-Higashi syndrome involving malignant transformation or failure of production of one or more of the cell series are examples of disorders treated by BMT. The principles of treatment in BMT consist of conditioning of the patient for two major purposes:

1. To eliminate malignant clones of cells in the body.
2. To prevent graft rejection.

Specifically the conditioning treatment usually consists of high dose chemo/radiotherapy followed by BMT. An immunosuppressive agent and antibiotics are given post-operatively. The actual treatment regimens vary from center to center and are dependent on the condition involved and the disease stage.

Total body irradiation has had a long history but was not used routinely until the 1970s (D'Angio 1983). It was first suggested in 1905 and first used in 1925. However its use was limited in the subsequent forty years. Large field irradiation (TBI or TLI) did not become popular and was eclipsed until the late 1960s by the advent of the chemotherapy era . The concept of dose fractionation arose in the first quarter of this century (Evans 1983), subsequent to early therapists using irradiation like a scalpel with massive single doses resulting in few cures and considerable normal tissue morbidity. The rationale for fractionated regimens developed as repair and repopulation were considered. Fractionated TBI was introduced in an effort to improve leukemic cell kill while retaining or possibly improving the degree of normal tissue tolerance and its use refined and developed over the years. The type of irradiation administered can vary greatly. An example of a typical BMT treatment regimen may range from a single dose of 1200 cGy to 6 fractions of 200 cGy. Prior cranial/spinal irradiation doses, for treatment of leukemia may range from 1800-2400 cGy.
Studies with hyperfractionation of the daily dose of 2-2.5 Gy (200-250 cGy) into two or three

fractions have demonstrated that there is little loss in the probability of tumor control and associated improved sparing of normal tissue, specifically skin and mucosa (Kotalik 1981).

1.3 Side-effects of bone marrow transplantation

Complications following bone marrow transplantation can be divided into three general categories:

1. Those related to the transplant itself -for example, acute or chronic graft versus host disease or infections.
2. Those due to preparative chemo/radiotherapy - for example, abnormal growth development and growth hormone deficiencies have been observed in children after bone marrow transplantation (Griffin et al 1980, Sanders et al 1986 and 1988, Borgstrom and Bolme 1988, Johnson et al 1988, Bushhouse et al 1989).
3. Those arising from original disease - for example, recurrent malignancies.

Irradiation may directly impair hypothalamic, pituitary, thyroid and gonadal function while cytotoxic chemotherapy may damage the gonads (Shalet et al 1988). Side-effects may not be fully manifested until many years after therapy and include short stature, increased weight for height, altered cognitive development, and craniofacial abnormalities such as microcephaly, mid-facial hypoplasia and mandibular retrognathia with arrested dental development (Schunior 1990). Shalet et al 1988 reported complications post TBI therapy such as failure to undergo normal pubertal development, precocious puberty, hypothyroidism, thyroid tumors and infertility.

Sanders et al (1986) found a 50% decrease in the incidence of cataracts occurring in children from 1.5-6 years post transplant when fractionated dose TBI was used instead of single dose irradiation. They also indicated that children receiving fractionated TBI demonstrated more

normal growth curves after transplantation, compared with children undergoing single dose irradiation. Bushhouse et al (1989) observed that children undergoing TBI had subsequent growth suppression whereas children receiving total lymphoid irradiation (TLI) did not. The authors suggested that TLI avoids exposure to the long bones, the head, thyroid, gonads and most of the liver, while TBI includes all these structures. Wells et al (1983) asserted that cranial irradiation rather than chemotherapy was related to a decrease in the growth in children post-transplant. Sanders et al (1988) noted growth hormone deficiency and abnormal growth velocity in children who received cranial irradiation. Shalet et al (1976) estimated that the threshold for impairment of growth hormone production was more than 30 Gy (3000 cGy) irradiation to the pituitary. There is some evidence to suggest that fractionation of the irradiation dose may spare some of the endocrine effects associated with the irradiation. Sanders et al (1983a) found that the few reports on the return of ovarian function in the human female after TBI were usually associated with fractionation.

Both irradiation and chemotherapy may affect the growth sites of bone. The mechanisms of growth alteration may be varied. Larson et al (1990) postulated that the retardation of the growth of bones and soft tissues could occur either centrally or directly. The central effects result from irradiation of the pituitary or thyroid gland. The direct effects result from irradiation of bone, soft tissues and blood vessels and has the greatest effect at times of growth spurts - in children less than 6 years old and those undergoing puberty. The damaging effect of therapeutic irradiation on growing bone has been an important source of morbidity and a major dose-limiting factor in the radiotherapeutic management of pediatric malignancies. Retrospective clinical reviews have qualitatively related the degree of growth arrest to dose, daily fraction size and age at the time of treatment. In young children, significant growth arrest may be incurred with fractionated doses of 15 Gy (1500 cGy) or greater and, in children under 1 year of age, with doses as low as 10 Gy (1000 cGy) (Sanders et al 1986 and Eifel 1988). Sanders et al (1986) noted that the type of TBI administered did not appear to affect height until

three or more years after transplant. Those who received fractionated TBI were observed to grow significantly taller than those who received single exposure TBI. Similar results were found by Logghe et al (1988) who studied 88 long-term survivors of acute lymphoblastic leukemia who had been treated with three different irradiation regimens. The severity of epiphyseal, metaphyseal and diaphyseal injury to developing bone appears to increase with increasing irradiation dose, lengthening post-irradiation interval, and younger age at the time of treatment (Sanders et al 1986). Goldwein (1991) described the long-term results of these injuries as suspension or retardation of chondrogenesis and osteogenesis with premature closure of epiphyseal plates and ultimately termination of bone growth. He noted that microscopic changes have been demonstrated with single fractions as low as 200 cGy (200 rads) and clinically measurable growth disturbance may be produced with doses below 500 cGy (500 rads).

The effects of high dose localized irradiation on craniofacial growth and development have been well documented (Guyuron et al 1983, 1987). Irradiation of the flat bones of the face and skull can lead to the most profound abnormalities in children including severe hypoplasia of the affected bones and subsequent facial deformities (Goldwein 1991). Eruption of teeth can be delayed or suspended as a result of mandibular or maxillary irradiation treatment and these effects have been associated with doses as low as 400 cGy (400 rads). Malocclusions, temporomandibular joint fibrosis and severe cosmetic deformities can likewise result from treatment to the craniofacial (CF) area. In a review of late effects of 50 pediatric patients who were treated for head and neck cancers with radiotherapy and chemotherapy, Larson et al (1990) noted the most severe effects on the primary side of the head and neck receiving radiotherapy. These included bony hypoplasia of the jaw, orbit or hemi-face. There was also significant functional impairment including varying degrees of blindness, trismus, constricting neck fibrosis and a variety of dental abnormalities. Clayton et al (1987) measured head size of 38 patients who had undergone craniospinal or cranial irradiation. Their results suggested that

previous cranial irradiation rather than growth hormone deficiency was associated with restricted head growth. Postulated mechanisms for this included disruption of the growth plates of the skull with premature closure of the sutures or damage to the underlying brain tissue leading to secondary stunting of skull growth. Sonis and co-workers (1990) found the severity of dentofacial-developmental abnormalities secondary to antileukemic therapy 5 years after successful treatment, were related to both the age of the patient at the initiation of treatment and the use of cranial irradiation. Ninety percent of the patients receiving cranial irradiation before 5 years of age developed craniofacial abnormalities. Mean cephalometric values of this group showed significantly deficient mandibular development. The field of irradiation in these patients included a portion of the ascending ramus and the entire condyle of the mandibles, the arrested development of the mandible was also seen in the patients younger than 5 years who received 2400 cGy (2400 rads) of cranial irradiation. Likewise Jaffe et al (1984) reported dental and maxillofacial abnormalities as a result of localized maxillofacial irradiation in 68 long-term survivors of childhood cancer. They also found the abnormalities were more severe in those patients who received irradiation at an earlier age and at a higher dose. Nwoku and Koch (1975) suggested that surgery should be more frequently considered in the treatment childhood tumors, since the minimal localized irradiation dosage that causes growth retardation in infants and children was not established.

Savostin-Asling and Silverman (1978) studied the microstructure of the adult human mandible after therapeutic irradiation (6000-7200 rads/cGy) for intraoral cancer and found early cessation of osteogenesis and later cessation of resorption. They also noted microfractures in the irradiated bone which had not healed, and postulated the osteoprogenitor cells were damaged by the irradiation therapy.

Previous studies have considered the effect of either single dose or large fractions of irradiation on growth in general, or craniofacial growth. There have been few reported observations of the effects of low dose irradiation on the craniofacial region in humans or animal models to date.

Eifel (1988) studied the effect of multiple irradiation fractions per day on tibial bone growth in weanling rats and this effect will be discussed further.

1.4 Rat as a model for craniofacial growth studies

The rat has frequently been used as a model for craniofacial growth in humans. A number of factors such as the animal's brief life span, size, ease of handling and breeding, the rapid rate of growth and development and the broad similarity to man physiologically have contributed to its choice as a model. The life span of a rat is 2 - 3.5 years. Rats are weaned at 21 days (weight 25 - 55g), and reach sexual maturity at day 40 - 65 (80 - 100g), (Weihe 1987). Maximum growth occurs from 3 - 15 weeks. In the Sprague-Dawley strain, normal growth curve for the skull precedes that of body weight gain. Hughes and Tanner (1970) followed multiple growth parameters for black hooded rats from 3 to 210 days. They noted the basic growth rate curve consisted of a rise from early in life to a peak, followed by a gradual decline. The peak was reached at or before 23 days post copulation (PC) by skull length, 45 days PC by nose-rump length and 55 days PC by tail length. Body weight has its major peak at about 60-70 days, with males a little later than females. Nose-rump and tail length show a smaller rise in velocity from 23 to 30 days followed by a fall to 40 days then a larger rise. The decline in velocity shows a relative check (or growth spurt) occurring at or a little after puberty and may correspond to the pubertal acceleration seen in many primates. In the rat there is no actual acceleration, only a lessened deceleration. Two stages of CF growth have been identified and these will be discussed in greater detail in the following sections. The early growing stage (0 - 20 days post-parturition), in which the maximum growth rate occurs, has a predominance of neurocranial growth. In the late growing stages (20 - 44 days post-parturition), viscerocranial growth predominates (Moss 1956).

Spence (1940) found that the first bones of the head to calcify were the endochondral bones at

the base of the skull. He selected the basisphenoid as a relatively fixed point for measurement as its position is comparable to that of the sella turcica in man, the sella being absent in the rat. The outline of the brain case appeared to have reached its full dimension by the 70th day. He suggested the sites of growth were at the naso-frontal suture and other sutural junctions of the facial bones and at the appositional surfaces. Baer (1954), using a staining technique involving the dye alizarin red S, further elucidated the growth sites and the extent of new bone formation at different ages in the albino rat. He found that the growth pattern of the rat skull is the result of two basic systems of growth : (a) early rapid expansion of the brain case in conjunction with the growth of the brain and (b) slow growth of longer duration, resulting in elongation of the cranial base and the face. This growth is achieved by incremental addition at the margins of each center of ossification. Proportional changes in the brain case and the face are affected by differential growth at the sutures and at the synchondroses joining the individual bones. This necessitates spatial reorientation and changing accommodation among individual bones. Neither resorption from the surface of the cranial vault nor differential apposition accounted for the radical changes in the relationship of the vault bones in the first 100 days of life.

Moss and Baer (1956) confirmed that simultaneous changes in absolute size and relative proportions occur with increasing age in their study of rat skull growth during the first 280 days of life. They identified the differential changes of the growth gradients in the dorsal and basal skull surfaces which were correlated with the change from neural to facial skull growth. The age at which this shift occurred was found to exhibit rat strain differences. They also found sexual differentiation in the skull was due to differences in growth potential rather than to differences in proportionality of parts.

Moore (1966) was the first to quantify the relative changes in the proportions of the rat skull. He used measurements from monthly (1-5 months) radiographs of the rat skull. During the five month period he noted the braincase increased in size on average by only 7%, whereas the

corresponding figure for the facial skeleton was 25%. In both regions the amount of growth in length exceeded that in breadth and height. This pattern of growth was also shown to be basically similar to that already established for the primate skull. He further broke down the growth increases as follows: by the age of one month, the neurocranium attains 93% of its adult size while at the same age the face and mandible have attained only 75% of their fifth month size. By the end of the fourth month the face and mandible almost complete their growth by attaining 97% of their fifth month size.

Vilmann (1969) studied the growth of the cranial base in Wistar rats between the ages of 14 and 60 days. He confirmed Spence and Moss' assertion that the position of the fossa hypophyseos remained stable during growth and therefore supported the accuracy of the theory concerned with the stability of the fossa in phylogeny and ontogeny of the rat. He also demonstrated that the cranial base in the rat changes from a dorsally convex to a dorsally concave structure with increasing age. The change in the cranial base is attributed to a combination of an elevation of the occiput and an elevation of the cribriform plate. Vilmann suggested the rat skull developed from a clinorhynchal type in which the facial skeleton has a ventral declination relative to the neural (subcerebral) to an orthocranial type in which the facial skeleton lies directly before the neural (precerebral). He postulated this is due to a marked stability of the angle between the nasal bone and the cribriform plate in combination with the changes in angulation between the bones of the cranial base.

Cleall et al (1969) theorized that since the rat is a long snouted animal it would be logical to expect a considerable amount of growth in the growth sites whose long axes are in an anterior-posterior direction. Such areas as the palatal sutures, nasal bones, frontonasal suture and cranial base synchondroses all fall into this category and were found to grow at a rapid rate in his craniofacial growth experiment.

Vilmann and Moss (1980 and 1987) have further identified the changes in the viscerocranium of the rat skull both up to 14 days and from 14 to 150 days. They noted that the process of orthocephalization in the period between birth and 14 days postnatal was primarily a result of an upwards rotation of the palatine bone relative to the cranial base. They also demonstrated that the zygomatic arches and basisphenoid bone of the rat skull in the period from 14 to 150 days maintain a positional stability while the peripheral and internal components of the rat facial skull and posterior part of neural skull are transforming and actively growing. The length axis of the skull (the line connecting opisthion with the anterior nasal spine) also maintains a stable position relative to the basisphenoid bone and the zygomatic arches.

Pucciarelli (1978) defined the centers of rotation of the bony complexes in the skull during the movement towards orthocranial. He characterized three cranial regions and their rotations: the anterior region (frontal-ethmoidal-facial complex) with trigonometric rotation; the mid-region (parietal-sphenoidal complex) remained stable, and the posterior region (interparietal-occipital complex) with clockwise rotation.

Measurements of the changes in the growth and form of the craniofacial region require a reliable measurement technique. Cephalometric radiographs are well suited and have been used in number of different species including the rat. They have the advantage of permitting an analysis of normal and abnormal changes in growth over an extended period of time and throughout the duration of an experiment without sacrificing the animal. This also has the effect of reducing statistical variability. In 1940 Spence used serial cephalograms of the living rat for the purpose of establishing the normal development of the head and dentition of Wistar rats. Lateral cephalometric methods to determine craniofacial growth changes (in the rat) have been used extensively by Engström et al (1982, 1985), Kiliaridis et al (1985), Persson et al (1989). Cephalometric analyses have also been used for detailed description of longitudinal changes in craniofacial morphology during the growth of the rat by Asling and Frank (1964),

Moore (1966), Vilmann and Moss (1980 and 1987), Moss et al (1987).

The head irradiated rat has been used in research since the late 1950's. Factors which may account for the different effects observed in humans and rats after cranial irradiation are the amount and method of administration of the dose of irradiation, and the relative radiosensitivities of the species. Clemente and Yamazaki (1960) demonstrated that irradiation limited to the head of the neonatal rat produced stunting of body weight. They also observed that sensitivity to irradiation decreased after the 1st week of life, and suggested most cranial irradiation of rats should be completed by 2-4 days of age.

Mosier and Jansons (1967) irradiated two day old Long Evans rats with a range of single doses (250 - 1000 rads/cGy) and monitored body weight, tail length and tibial length. They found doses of 350 rads (350 cGy) or greater caused significant reduction of body weight. Two hundred and fifty rads produced no significant stunting. Seven hundred and fifty rads (750 cGy) resulted in death of most of the animals and 1000 rads (1000 cGy) was lethal to all rats. In order to determine the effect of single dose irradiation on growing bone, Engström et al (1981) exposed the left hind legs of 30 day old rats to 50, 200, 500 and 800 rads (50, 200, 500 and 800 cGy). They found alkaline phosphatase activity was decreased in tibial metaphysis of the rats on the first day after irradiation with all doses. There were no differences in enzyme activity between the control and the irradiated metaphyses 30 days after irradiation.

Mosier (1988) in a later study used the head irradiated rat as a model for observing catch-up-growth. Bilateral irradiation of the head of the 2 day old rat with doses of 3.5 Gy (350 cGy) or greater resulted in growth retardation which was dose and sex related. He found the stunted head-irradiated rat is capable of undergoing catch-up growth acceleration, but has defects in the two following respects. The link between the control of catch-up growth and growth hormone (GH) secretion is disrupted and the reference point for body size for age (set

point) is reset. This contrasts with children who, showing growth failure after cranial irradiation for leukemia, may or may not respond to GH therapy. A single dose irradiation experiment of 2, 5 and 8 Gy (200, 500 and 800 cGy) on the neurocranium of Sprague-Dawley rats in the late growing stage, has indicated an initial effect on growth regions within the field. The metabolic changes seen at these growth sites appear to be transient, however the study demonstrated that the impaired growth within the field had secondary effects on other regions of the skull (Engström et al, 1985).

The effect of localized single dose irradiation on the growth of mandibular bone and molar eruption was evaluated using morphometric methods by Ubios et al (1992). Twenty Gy irradiation (2000 cGy) was given to the molar zone of rats at 5 days of age. Results showed alterations in mandibular growth (especially longitudinal growth) and tooth eruption. Histologic findings included odontoblastic atrophy, alveolodentary ankylosis and meager or no root formation.

Experiments involving the use of fractionated irradiation are few in number. Eifel (1988) studied the effect of single and twice-daily fractions of irradiation on the arrest of growth in femoral and tibial epiphyses of 22 day old Sprague-Dawley rats. Tibial length was significantly greater in the legs treated with the fractionated compared to single dose irradiation. This appeared to result from a continuously greater rate of growth during the first 40-50 days following fractionated compared to single dose irradiation. From this study, Eifel concluded that hyperfractionation provided a means of reducing growth deficits in children when skeletal growth centers must be included in the irradiated volume. In a similar study, Hartsell et al (1989) investigated the effect of hyperfractionation of irradiation dose on bone growth in 22 day old Sprague-Dawley rats. They found the animals receiving smaller doses per fraction (1.0 or 1.25 Gy) showed significantly more growth of the vertebral bodies in the treated fields than animals given larger incremental doses (1.5 or 1.8 Gy). They concluded that

hyperfractionation of irradiation had a protective effect on bone growth of vertebral bodies. Schunior et al (1990) used a rat model to determine the adverse effects of central nervous system chemotherapeutic therapy on growth and craniofacial proportions. Single dose cranial irradiation of 1000cGy (1000 rads) with and without prednisone and methotrexate was administered to 17 and 18-day-old Sprague-Dawley rats. Animals subjected to cranial irradiation exhibited microcephaly, whereas those who received a combination of irradiation and chemotherapy demonstrated altered craniofacial proportions in addition to microcephaly. For all irradiation groups there was a permanent suppression of weight gain with no catch-up growth or adolescent growth spurt. The authors concluded from these results that cranial irradiation was a major factor in the growth failure in exposed rats, but chemotherapeutic agents contribute significantly to the outcome of growth and craniofacial dimensions.

In summary, it would appear from the preceding studies that the rat is an appropriate model to study the effect of irradiation on craniofacial growth since the pattern of growth is basically similar to that established for the primate skull. The greatest effect of irradiation on CF growth is found if irradiation occurs in the first week of life. Maximum growth of the skull has occurred by 44 days post-parturition, therefore monitoring this time period allows determination of craniofacial growth changes.

1.5 Cephalometrics

Cephalometric radiography is an anthropometric technique which standardizes magnification and related distortions of the x-ray image. A cephalometric analysis is a collection of numbers intended to compress much of the information from the cephalograph into a usable form for diagnosis, treatment planning and/or assessment of treatment effects. As mentioned previously cephalometric analyses have been used for detailed description of longitudinal changes in craniofacial morphology during the growth of the rat. These measurements are made on tracings of cephalometric radiographs taken specifically for this purpose.

A radiograph is produced when a beam of x-rays is directed on to an appropriate part of the body (the skull in this case), and while some of the beam is absorbed (or loses its energy) as it passes through the subject, part of the beam passes right through the tissues and then interacts with a photographic emulsion on the film. This may then be processed to produce a radiograph which should have the property of so recording detail as to enable an observer to distinguish between objects in close spatial relationship. This is known as the resolution or resolving power and is made up of several phenomena such as edge sharpness, grain size and grain distribution within the emulsion. Resolution is difficult to specify in quantitative terms (Smith 1980). Films of the highest quality may be used to improve the contrast and definition of the radiograph. A conflict exists between irradiation control and film quality in the choice of films, in human studies exposure reduction is of primary importance.

Minor distortions can arise if the film is not flat, this may be compensated by adequate support behind the film and checked by exposing a test grid which will reveal any serious lack of flatness of the film. Processing of the exposed film must be carried out under controlled and standardized conditions if high quality radiographs are to be produced (Houston 1983).

Errors of the cephalometric measurement technique may be systematic or random. Systematic errors occur when a particular measurement is persistently over- or under-recorded. These may occur in the cephalometric system when the geometry of the system varies with no compensation made, when several observers participate in the measurement, a single observer's practice changes with time or experience and by subconsciously weighting results - avoided with double-blind experiments. Random errors may arise as a result of positioning the subject in the cephalostat, variations in film density and sharpness and difficulties in identifying landmarks. The total measurement error is the combined effect of errors due to the projection of the object on to the film, landmark identification, landmark registration and

measurement techniques. The resolution of the radiographic system will affect landmark identification and registration and therefore indirectly the accuracy of any measurement. Since errors may jeopardize the diagnostic yield of cephalographic examinations it is important to analyze the effects of different sources of cephalometric errors (Ahlqvist et al 1988). The appearance of anatomical features may be affected by radiographic techniques. Projection errors emanate from the projection of a three dimensional object on to the two-dimensional film, primarily due to the comparison of lengths without compensating for variations in lateral divergence (Houston 1983). Projection errors may also arise due to misalignment between the different components of the cephalographic equipment and/or misalignment of the patient (Ahlqvist et al 1988). Relations between the focal spot, the cephalostat, and the film should be constant, however they may be affected by the following factors:

-the focal spot, the cephalostat, and the film may be linearly displaced in relation to each other.

-the cephalostat, and the film may be rotated with respect to each other and/or the central ray of the beam.

-the subject may be linearly displaced and/or rotated in relation to the cephalometric system (Ahlqvist et al 1983).

To achieve standardization of the films the subject is positioned in a craniostat - this device enables the subject's head to be held steady in the sagittal plane at a known angle (90°) to the central ray and parallel to the film plane. The x-ray tube is positioned a standard distance from the center of the craniostat, i.e. the sagittal plane of the subject when a true lateral radiograph is being taken. The emerging x-ray beam is cone shaped and the x-rays therefore divergent. The further the x-ray tube is from the subject the smaller the solid angle subtended on to the head by the focal spot, under these conditions the x-rays are nearly parallel and so magnification and distortion are kept to a minimum. The tube-subject distance may be increased however the output of the tube must be increased to compensate as a consequence of the inverse square relationship between incident irradiation and distance from source (Smith

1980). On the side of the craniostat away from the x-ray tube the film holder is positioned at a constant distance so that the magnification which does occur remains constant. The use of a long focus to film distance and a short object to film distance has been recommended (Salzman 1964).

The craniostat has two arms (each has an earplug at the end with a radio-opaque ring attached) which may be moved in or out around the midpoint. The subject is positioned so that the earplugs can be placed in the external auditory meati, this holds the head in the true lateral position and the exposure is made with the earplugs in the center of the film. The radiographic shadows of the earplugs will be then superimposed one over the other if the head is in the true lateral position to the tube when the exposure was made (Smith 1980). A metal scale of known length is positioned at the midsagittal plane to provide permanent evidence of the enlargement of each radiograph (Houston 1983). Errors arising from the orientation of the subject in the cephalostat have been studied. Gron (1960) found that a change in rotation of 5° in either direction only made a difference of 0.8% to linear measurements. Shaw (1977) concluded that the changes due to rotation of the skulls were small. Mitgard (1974) found errors between repeated subject positioning to be of minor importance. Thus the radiographs are taken with the distance between the x-ray source, the subject and the film standardized and with the head fixed in a true lateral position thereby producing reproducible results. This allows comparison between radiographs over time in the same subject and between different subjects.

Landmarks are points serving as a guide for measurement. An ideal landmark is located reliably on the skull and behaves consistently during growth (Smith 1980). Many cephalometric landmarks have been defined for convenience of identification and reproducibility rather than on grounds of anatomic validity. This is often unavoidable, and Houston (1983) states that no better alternative may be available. The reliability of a landmark is affected by the quality of the cephalograph, the experience of the tracer and confusion with other anatomic shadows.

Cephalometric points and landmarks are of the following kind: true anatomic points, implants, extremal points (on extremity of the curvature of bone), intersections of edges of regression and intersections of constructed lines. Landmarks are marked on the tracing paper and planes derived by joining the appropriate points. Measurements whose points lie in a plane parallel to the film are not distorted, however where this does not occur linear measurements may be affected (Houston 1983).

Analysis in the lateral projection is based on presumed skeletal symmetry including the external auditory meati. The need for a craniostat and a true lateral position is apparent when considering that most landmarks chosen are bilateral and superimposition of these is required to achieve reliable results. If the two landmarks of either side of the skull are not exactly superimposed, then the point is drawn midway between the two tracings of the landmarks on either side of the skull - difficulty may arise in cases where there is a marked bilateral asymmetry affecting both the validity and the reproducibility of the measurement. Because anatomic definitions lack precision, one of the greatest sources of random errors is difficulty in identification or imprecision in their definition.

The most important contributions to improvement in landmark identification are experience and calibration especially when more than one measurer is involved (Houston 1983). The precision of the landmark tracing may be improved by tracing in a darkened room with a black surround placed on the radiograph to cut down background illumination - thereby facilitating landmark identification (Sandler 1988).

A number of studies have utilized a digitizer to measure and facilitate recording of cephalometric data. A digitizer is a device that transforms graphical data into planar coordinate information that can be read and understood by a computer - these coordinates are usually presented as X and Y coordinates based on the position of a cursor on the surface, or the platen,

of the digitizer. The cursor has a crosshair for alignment with the traced landmark and is coupled electrically to a position sensing device which provides the positional information to the computer (Carau 1978). The resolution and accuracy of the model 9874A Hewlett-Packard digitizer are 25μm and ±125μm respectively. The accuracy value is a composite that includes the drive system, the platen and the cursor. Carau (1978) states that tests have demonstrated an operator can repeatably position the cross-hair to within 50μm of the known position. Some other inherent problems that affect the overall accuracy of the system include the use of conductive media which can significantly alter the position measured by the cursor. Graphite pencil markings can range from high to fairly low impedance, depending upon the hardness of the graphite lead, the width of the line, the length of the line and the area covered. Paper can change dimension as humidity and temperature change, additionally the electrical resistivity of the paper also changes with humidity. An extreme example is cited in which the linear dimension of a document changes 1.25mm due to humidity and temperature variability. Another significant effect is dimensional variations caused by bending or folding of the source document, for mylar the variation from a 90° bend of small radius (2.5mm or less) can be as much as 100 to 150μm. Many digitizing errors arise because digitizing is an operator-intensive function, hence user-fatigue is thought to be the main source of error (Carau 1978).

1.6 Metrograph

The Reflex Metrograph (H.F. Ross, Salisbury, Wiltshire, England) is defined by MacLarnon (1989) as a non-contact measuring instrument permitting accurate, direct measurement in three dimensions of relatively small objects. It is simple to use, requiring no expertise beyond the stereoscopic vision possessed by 99% of people with two working eyes, no particular training and no additional equipment apart from a personal computer. Standard software provides for immediate calculation of distances, areas and volumes. The basic principle underlying the Reflex metrograph is as follows. An object placed in front of a semi-silvered mirror can be seen by an observer as a virtual image behind the mirror, with all the three-

dimensional characteristics of the object. A small light spot (measuring mark) placed behind the mirror can be moved by the observer in three dimensions without obstruction until it coincides with a point on the objects' image. It appears to the observer that the light spot 'floats' through the object. By optoelectronic means, individual positions of the measuring mark can be monitored and the three-dimensional coordinates of any particular position can be digitized as required by pressing a foot-switch. The coordinates may then be combined using standard or specially written computer software to provide immediate readout variables such as linear distances and for other applications such as calculations of the areas of irregular shapes. The Reflex Metrograph does not magnify the object, although the observer may wear binocular magnifiers.

The Reflex Metrograph has a measuring range of 300mm in all three axes (x,y,z) and a minimum root mean square error on single pointing of 60-80μm. The operator's head may be moved relative to the object increasing depth perception and permitting observation beneath overhangs and around obstructions. The precision of the measurement does not only depend on the Reflex instrument itself. It also involves a combination of the visual acuity of the observer, which may vary in especially depth perception and the precision with which target points may be identified. Additionally errors of precision in the measurement of dimensions increase as the number of individual points required to calculate a dimension increase. Speculand et al (1988) tested the accuracy of the Reflex Metrograph and reported under-measurement of up to 0.67%. This study was carried out on a machined metal cube using the corners as targets. The authors speculated that the shiny surfaces and the lack of sharp definition of the corners which were used as targets may have contributed to this figure. Takada et al (1983) determined that metrographic landmarks or points may be measured with an accuracy of 0.1mm.

The Reflex instruments offer considerable advantages over measurement from radiographs for quantitative research according to MacLarnon (1989). These include the absence of irradiation

of the subject and the avoidance of errors inherent in radiographs, such as distortion of the film, indistinct definitions of the images and superimposition of the left and right sides of the image (if lateral radiographs are used). When compared with calipers the Reflex instruments are more precise, especially for measurements of small dimensions. They can be used to measure inaccessible dimensions and as they are non-contact instruments are less likely to cause damage to the object. There is no problem with variable orientation of objects relative to the instruments because the measurement axis system can always be defined by points on the object.

Chapter 2

Statement of the Problem

Hsu (1992) noted Statistics Canada has reported cancer to be a leading cause of death in children between one and 15 years of age, second only to accidents. Bone marrow transplantation (BMT) has become a well established form of treatment in children with nonmalignant and malignant hematologic disorders. Marrow transplant preparative regimens are designed to suppress the immune system and eradicate the underlying disorder through the use of cranial irradiation, total body irradiation or total lymphoid irradiation, with or without chemotherapy. The irradiation may consist of single or fractionated dose. As a result of the increase in numbers of patients undergoing BMT there is a growing concern and related research into the short and long term effects of the treatment on the survivor population.

Side-effects of irradiation may not manifest themselves fully until many years after initial therapy. These include persistent short stature, increased weight for height, altered cognitive development, and craniofacial abnormalities including microcephaly, mid-facial hypoplasia and mandibular retrognathia with arrested dental development (Schunior 1990). Clayton et al (1987) found previous cranial irradiation rather than growth hormone deficiency was associated with restricted head growth. There have been few reported observations of the effects of low dose irradiation on the craniofacial region in humans or animal models to date (Engström et al 1981 and 1985, Schunior 1990).

The rat has frequently been used as a model for craniofacial (CF) growth in humans. The pattern of CF growth of the rat is basically similar to that established for the primate skull. The greatest effect of irradiation on CF growth is found if irradiation occurs in the first week of life. Maximum growth of the skull has occurred by 44 days post-parturition, therefore monitoring this time period allows determination of craniofacial growth changes.

Cephalometric analyses have been used for detailed description of longitudinal changes in craniofacial morphology during the growth of the rat. Cross-sectional study of the morphology of mature skulls may be facilitated by the use of a metrograph.

The overall goal of the present work was to determine the effects of irradiation on craniofacial growth. The Sprague-Dawley rat was used as a model for this investigation. Two null hypotheses were posed:

1. No differences in craniofacial measurements between irradiated and control groups.
2. No difference in craniofacial measurements between single and fractionated dose groups.

CHAPTER 3

Materials and Methods

3.1 Animals

In this experiment, Sprague Dawley rats (Charles River Laboratories) were bred and reared from birth to 172 days. Litters were sexed at birth. The male pups were then randomly assigned to one of three experimental groups and marked daily for identification for three weeks. Ear-tags were placed with unique identifying numbers after this age. The litters were culled to 6 pups/dam, as evenly divided to sex as possible. At 21 days, the pups were weaned and all females were discarded. The remaining male pups were randomly housed in pairs in clear plastic cages with pine shaving bedding and given tap water and standard formula Purina Rat Chow ad libitum. hey were reared in a controlled environment with an ambient temperature of 25 ± 3°C, a relative humidity of 55 ± 10% and light cycles maintained as 12 hour light/dark periods. A total of 87 rats were reared to 172 days old.

3.2 Experimental Design

The pups were randomly assigned to three treatment groups on day 2. These three groups were: control, fractionated and single dose irradiation. The fractionated group was divided into 4 subgroups of irradiation dose. The single dose group was divided into 3 subgroups. All animals were irradiated on day 2 with the fractionated group having the total dose split into 6 equal fractions, delivered twice a day (six hours apart) on days 2, 3 and 4. The composition of each group and subgroup is shown in Table 3.1 and the irradiation protocol in Table 3.2. The specific range of irradiation doses were determined by converting the average dose given to a two to five year old child to one appropriate for two day old rats. A single dose of more than 600 rads (600 cGy) is known to be lethal to the two-day old rats (Mosier 1988) and this was therefore set as the upper limit of the irradiation dose. Group size was determined using a power statistic as discussed in the statistical methods.

The pups were exposed four at a time to irradiation. Each animal was restrained and shielded by a 2mm lead box with a rubber dam collar. This device was placed over the body and neck so as to expose only the skull and to minimize gastrointestinal mucositis. The irradiation dose was delivered by a lateral beam of x-rays produced by a Phillips X-ray machine operating at 250kVp. Control animals were similarly restrained, shielded and sham-irradiated.

3.3 Measurements

3.3.1 Growth Profile

To determine the effect of the treatments on overall body growth and proportions, weight to the nearest gram, body length - tip of snout to base of tail - (to 0.5mm) were measured at 7, 14, 21, 28, 35, 49, 56, 63 and 172 days. For these and the cephalometric procedures the rats were anesthetized with pentobarbital (Sombutal, MTC, USA) at a dose of 25mg/kg, ip.

3.3.2 Cephalometry

In order to achieve a longitudinal profile of the craniofacial growth of the rats sequential weekly lateral radiographs were taken from one to 9 weeks and at 24 weeks. This was done by placing each individual anesthetized animal into a specially constructed cephalostat. The points of fixation were the external auditory meati with the mid sagittal plane of the skull vertically oriented. The ear-rods were non-metallic, with the exception of a locating device within, to avoid superimposition of anatomical structures. The locating device consisted of two metallic rings, one of a smaller diameter than the other, and was designed to ensure a vertical orientation of the skull perpendicular to the radiograph and irradiation source (Fig 3.5). The cephalostat included a source-to-subject distance of 65 cm, subject-to-film distance was 55 mm. A standard dental x-ray machine was used with an exposure time of 0.9 secs. Determination of the appropriate kilovoltage and time (seconds) for each age of the subjects was made using a trial procedure. Qualitative assessment of the optimal contrast and resolution of radiographs was decided by a number of examiners. The kilovoltage depended on the age of the

animal, ranging from 80kV (days 7 and 14) to 90kV (days 21 and older), while milliamperage was set at 15. An identification number was attached to the outside of each film to aid identification of the radiograph. A standard cephalometric scale positioned in the mid-sagittal plane of the skull (10mm) was reproduced on the x-ray film to correct for magnification differences between radiographs (Fig 3.5). All radiographs in this study were handled in a standardized manner. They were processed under standard accepted darkroom lighting conditions and placed into an automatic processor sequentially with the identifying raised dot placed uppermost in the end of the film entering the processor last, ensuring standardized roller contact.

Each radiograph was secured to the surface of a variable illumination viewing box and a sheet of semi-matt fine grade tracing paper was taped over the radiograph. Tracing was carried out in a darkened room, and a black surround was placed over the radiograph to reduce background illumination. The tracing was performed using a 0.5mm lead pencil and the location of each of the landmarks (both anatomical and extremal points) indicated by a single fine pencil dot. Landmarks were digitized using a Hewlett Packard 9874A model digitizer. Linear measurements were calculated and calibrated with reference to the scale bar by the computer and stored on disc for later use. Twelve selected landmarks and seven linear measures were traced according to the definitions and criteria by Engström et al (1982), as seen in Tables 3.3, 3.4 and Fig 3.1.

3.3.3 Metrographic measurements

At 172 days of age the rats were decapitated and the skinned heads were prepared for skeletal analysis using carnivorous beetles (Dermestes vulpinus). Direct skeletal measurements of the ventral and dorsal surfaces of the skull were made using a Reflex Metrograph (H. F. Ross, Salisbury, Wiltshire, England). Landmarks, as defined by Nonaka and Nakata (1988), were recorded three-dimensionally (±0.1mm) from each skull. The following selected dimensions

were then calculated (Table 3.5): dorsal cranial length, dorsal neurocranial width, dorsal bizygomatic width (anterior and posterior) (Fig 3.2), ventral intermaxillary width, ventral bizygomatic width, ventral viscerocranial length (Fig 3.3), intercondylar width and intergonial width (Fig 3.4).

3.4 Statistical Analysis

The growth, cephalometric and metrographic data were statistically analyzed using one way analysis of variance, Bonferroni t-test and the Student-Newman-Keuls test. The null hypotheses were rejected at the 0.05 level of significance.

3.5 Error Determination

In order to evaluate the methodological error of the cephalometric measurements, repeated radiographic registrations and cephalometric analyses were performed on two of the 172 day old skulls. The magnitude of the different components of variation (tracing, radiographic and digitizing) in the cephalometric technique was estimated by variance analysis.

The number of animals needed for this experiment was determined by calculating the power statistic. Using the standard deviations in the experiment involving the use of cephalometrics (Engström et al 1982), the number of subjects per group required to determine significance was calculated to be 10 animals per group.

Table 3.1 Experimental Groups

Group	Subgroup	cGy	n
Control	C	0	11
Single Dose	S1	200-300	12
	S2	400-500	13
Fractionated Dose	F1	250-300	10
	F2	350-400	12
	F3	450-500	14
	F4	550-600	15

Table 3.2 Irradiation Protocol

Group	Total Dose (cGy)	Day (am/pm) 1	2	3
C	0	0 (sham)		
S1	200-300	200-300		
S2	400-500	400-500		
F1	250-300	41-50	41-50	41-50
		41-50	41-50	41-50
F2	350-400	58-66	58-66	58-66
		58-66	58-66	58-66
F3	450-500	75-83	75-83	75-83
		75-83	75-83	75-83
F4	550-600	91-100	91-100	91-100
		91-100	91-100	91-100

Table 3.3 Definitions of Cephalometric Landmarks

Point	Definition
Po	The most posterior point on the cranial vault
Ba	The most posterior and inferior point of the occipital condyle
E	The intersection between the frontal bone and the most superior-anterior point of the posterior limit of the ethmoid bone
A	The most anterior point on the nasal bone
Iu	The most prominent point between the incisal edges of the upper incisors
So	The intersection between the posterior border of the basisphenoid and the tympanic bulla
Bu	A point on the premaxilla between jaw bone and the lingual surface of the upper incisors
Mu	A point on the intersection between the maxillary bone and the mesial surface of the upper first premolar
Ml	A point on the intersection between the mandibular alveolar bone and the mesial surface of the first premolar
Bl	A point on the intersection between the lingual surface of the lower incisors and the most anterior part of the lingual alveolar bone
Ii	The most prominent point between the incisal edges of the lower incisors
Go	The most posterior point of the angular process of the mandible

Table 3.4 Linear Cephalometric Measurements

Total skull length	Po - A Ba - A
Neurocranial height	Po - Ba
Neurocranial length	Po - E Ba - E
Viscerocranial height	A - Iu
Viscerocranial length	E - Iu

Figure 3.1 Cephalometric landmarks used in the cephalometric analysis.

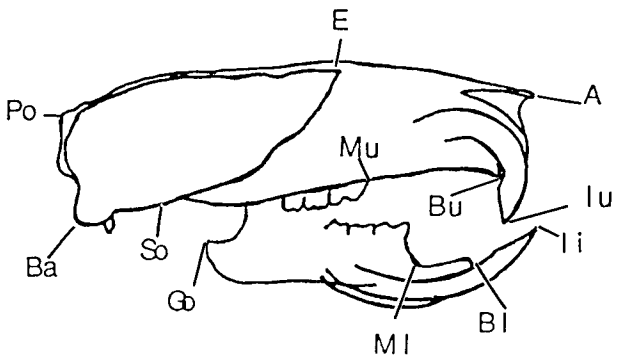


Table 3.5 Definitions of Metrographic Dorsal Cranial Measurements

Surface	Measurement	Definition
Dorsal	Cranial length	Anterior tip of nasal spine at midline to interparietal/supraoccipital suture at midline
	Neurocranial width	Distance between the lateral points of the lambdoidal suture
	Bizygomatic width-P	Maximum distance between posterior surfaces of right and left zygomatic arch spaces
	Bizygomatic width-A	Maximum distance between anterior surfaces of right and left zygomatic arch spaces

Figure 3.2 Metrographic landmarks - dorsal surface of skull.

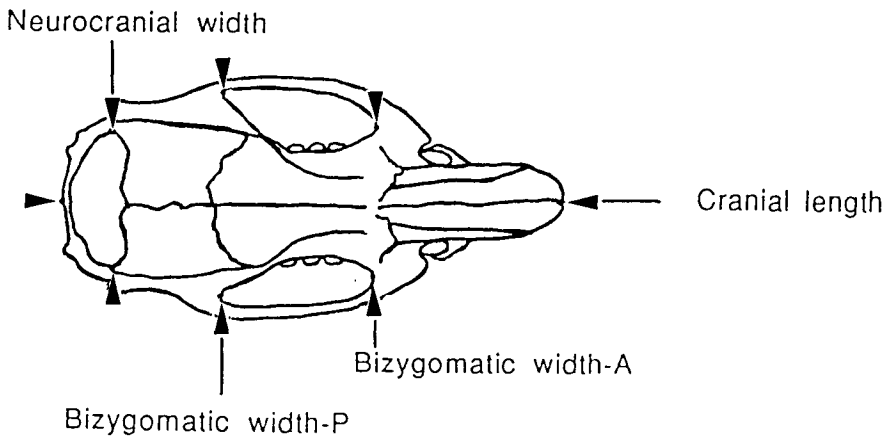


Table 3.6 Definitions of Metrographic Ventral Cranial Measurements

Surface	Measurement	Definition
Ventral	Viscerocranial length	Distance from anterior tip of nasal spine at midline to spheno-occipital synchondrosis
	Bizygomatic width	Maximum distance between lateral surfaces of right and left zygomatic processes of temporal bone
	Intermaxillary width	Maximum distance between anterior surfaces of right and left first molars

Figure 3.3 Metrographic landmarks - ventral surface of skull.

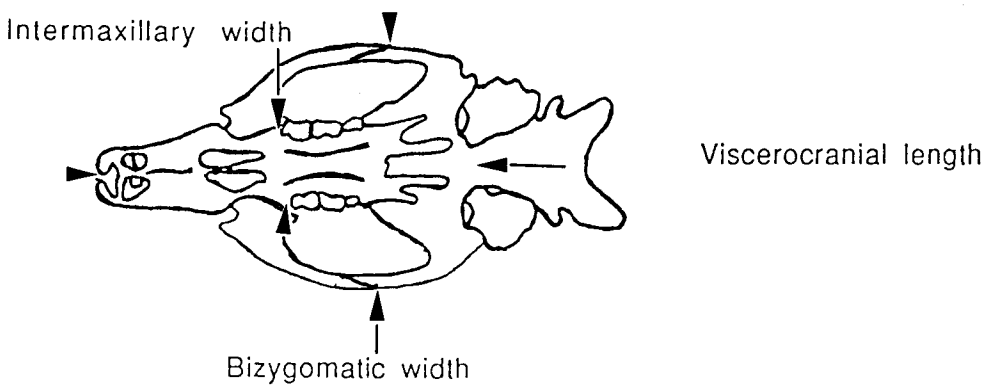


Table 3.7 Definitions of Metrographic Mandibular Measurements

Surface	Measurement	Definition
Mandibular	Intercondylar width	Transverse distance between most posterior, inferior surface of the two condylar heads
	Intergonial width	Transverse distance between gonial angles

Figure 3.4 Metrographic landmarks - mandible.

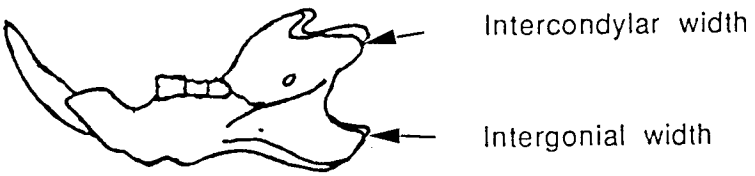
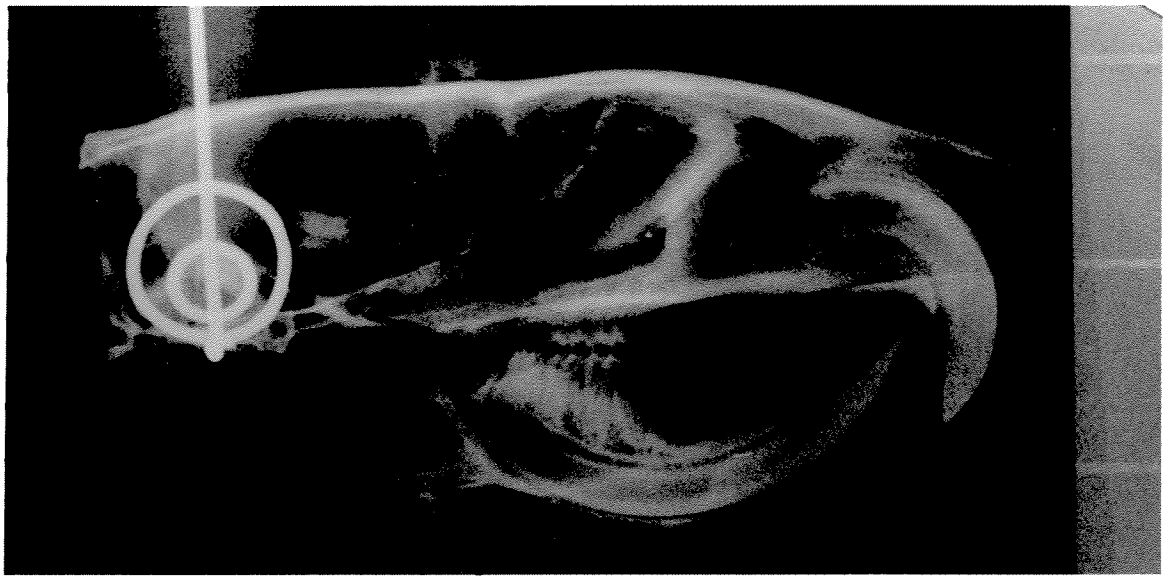
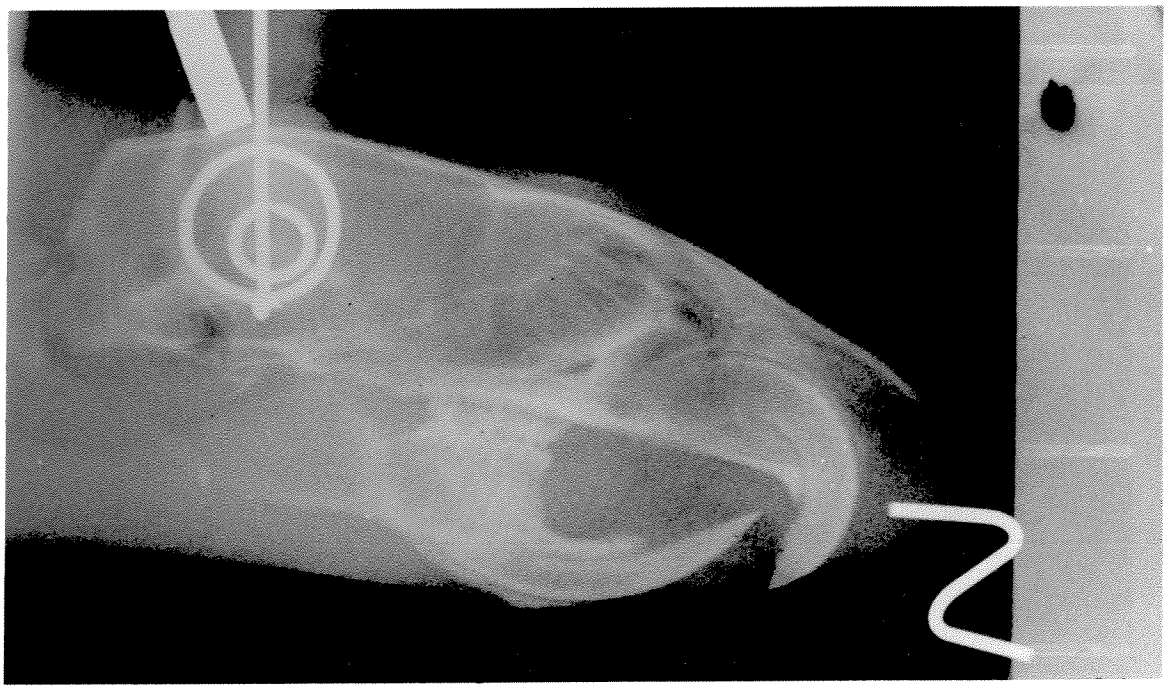


Figure 3.5 Lateral radiographs of 172 day old (A) and 56 day old (B) rats. Note the cephalometric scale, ear-rods with locating devices and identification number (B).

A



B



Chapter 4

Results

4.1 Growth Profile

One hundred and thirty rats were bred and assigned to the 7 experimental and control groups. Forty-three of these rats died, 87 grew to maturity (172 days old). The mortality was spread evenly over all of the three main groups (control, single and fractionated) and throughout the range of doses (Table 4.1a and Table 4.1b). No animals given the single irradiation dose of 600 rads (600 cGy) survived beyond 28 days, the majority died soon after irradiation. The majority of the animal deaths were be attributed to the use of the pentobarbital as a sedative agent. Weekly sedation from 7 to 56 days had a significant cardiotoxic effect on the rats. A range of side-effects from the irradiation treatment was also seen, including the development of cataracts, poor righting responses and tremors in the rats. These side-effects were generally associated with a high irradiation dose and often contributed to an earlier mortality either through direct effects or by being attacked by the other rats.

The statistical data is presented in tabular form (Tables 4.2 to 4.40). The analysis of variance statistic (F) and the associated p value are displayed beside each table. The Bonferroni t-test statistic (t) and the Student-Newman-Keuls statistic (q) are displayed in the tables when these are significant.

The growth profile for body weight (Fig 4.1) follows the expected growth curve for rats. Although a plateau is not evident (at 60-70 days), this may be due to the length of time between the two final measurements (56 and 172 days). At 172 days (Table 4.2) significant differences were evident between the control and all irradiated groups (F1, F2, F3, F4, S1, S2). They were also evident between high single dose (S2) and fractionated and low single dose groups (F1, F2, F3, F4, S1). No statistically significant differences were seen between the

groups at 21 days or less.

The growth profile for body length (Fig 4.2) shows a plateau at maturity (90-100 days). Statistically significant differences (Table 4.3) were evident between high single dose (S2) and all other groups (C, F1, F2, F3, F4, S1) at 172 days. Differences between the groups were not seen in animals 21 days old or less.

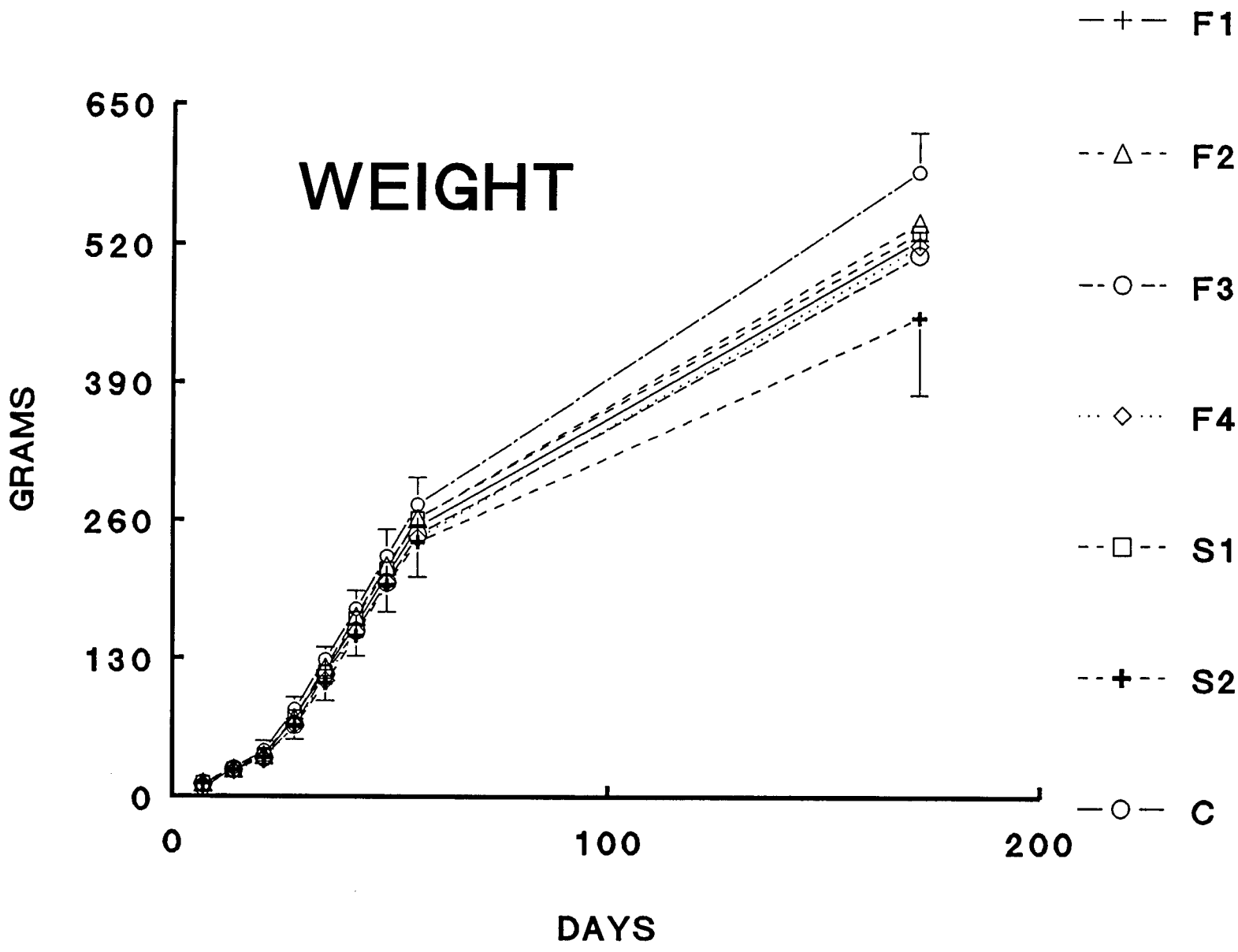


Fig 4.1 Growth profile for body weight

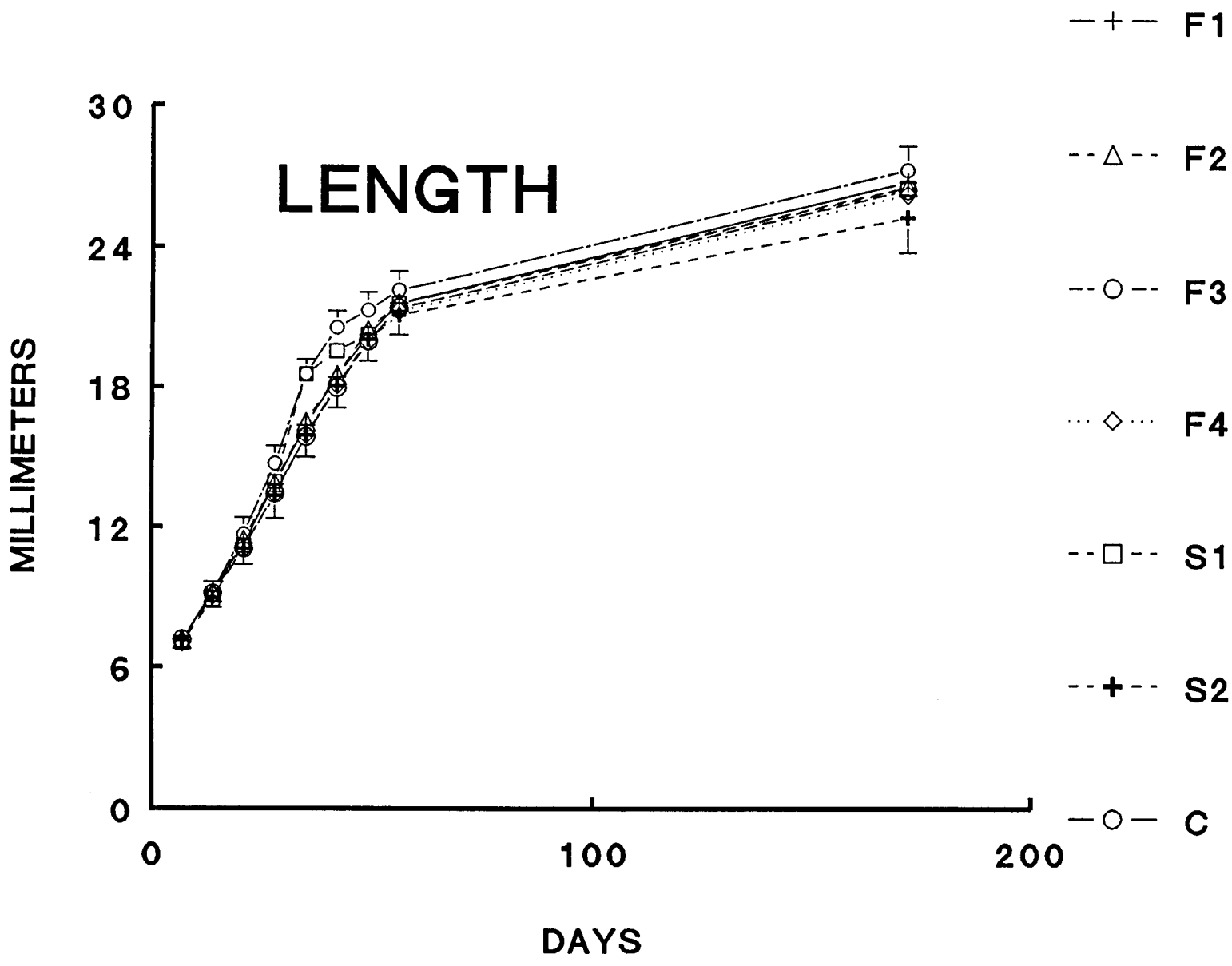


Fig 4.2 Growth profile for body length

Table 4.1a Mortality table: animal deaths by dose

Group	Dose (cGy)	Age (days)	Number Died
Single	200	56	1
	300-350	7	1
		14	1
		21	1
		42	1
	400	7	1
		28	1
	500	42	1
		56	2
	600	7	1
		28	1
Fractionated	250-300	7	2
		14	1
		21	2
		28	1
		35	1
		42	1
		56	1
		140	1
	400-500	7	3
		21	1
		28	1
	550-600	7	3
		14	1
Control	0	7	4
		14	2
		21	2
		35	1
		49	1
		56	2

Table 4.1b Mortality table: animal deaths by age

Age (days)	**Number Died**
7	15
14	5
21	6
28	4
35	2
42	3
49	1
56	6
140	1

Table 4.2 Body weight at 172 days

	C	S2	S1	F4	F3	F2
F1	t=ns q=3.814	t=3.414 q=4.828				
F2	t=ns q=2.938	t=4.402 q=6.226				
F3	t=3.539 q=5.005	t=ns q=4.266				
F4	t=3.171 q=4.484	t=3.526 q=4.987				
S1	t=ns q=3.451	t=3.858 q=5.456				
S2	t=5.941 q=8.402					

F= 6.67

p= 0.001

Table 4.3 Body length at 172 days

	C	S2	S1	F4	F3	F2
F1		t=3.621 q=5.12				
F2		t=3.316 q=4.69				
F3		t=ns q=4.379				
F4		t=ns q=3.653				
S1		t=ns q=4.369				
S2	t=4.514 q=6.386					

F= 4.09

p= 0.002

4.2 Cephalometric Measurements

Longitudinal data derived from the cephalometric radiographs are shown in figures 4.3 - 4.9. The statistical tables following the graphs are from 14, 28, 56 and 172 day data. Data at 172 days will be referred to in the text because the differences between the groups are generally more distinct at this age compared to earlier age stages. In general the cephalometric linear measurements all follow the growth profiles seen in section 4.1. The cephalometric measurements indicate several general trends. The high single dose group (S2) was significantly different from the control group in almost all measurements (except neurocranial length Ba-E). There was in general no significant difference between control and low fractionated groups in all measurements except Po-Ba (neurocranial height).

Total skull length Po-A (Fig 4.3 and Tables 4.4 - 4.7) data indicated a significant difference ($p=0.001$) between the control (C) and high fractionated and single dose (F4, S1, S2) groups at all time points (14, 28, 56 and 172 days). Ba-A (Fig 4.4 and Tables 4.8 - 4.11), a diagonal cranial length measurement, indicated a similar significant difference between control and high fractionated and single dose groups at 172 days. Similar trends were seen in both the 28 and 56 day data, but not however in the 14 day data. There was no significant difference between the control and low fractionated groups for either the Po-A or Ba-A measurement.

Neurocranial height measurements Po-Ba, (Fig 4.5 and Tables 4.12 - 4.15), indicated a highly significant difference between the control group and all irradiated groups at 172 days. There were no significant differences observed between any of the irradiated experimental groups. This trend was evident at all age stages. Neurocranial length Po-E (Fig 4.6 and Tables 4.16-4.19) measurements indicated a significant difference only between control (C) and the high single dose group (S2). Earlier stages (14 and 28 days) indicate the high single dose group was significantly different from low single dose (S1) at 14 days, and control and fractionated groups (C, F1, F2, F3, F4 and S1) at 28 days. The data at 56 days was similar to

172 days. For Ba-E (Fig 4.7 and Tables 4.20-4.23), a diagonal measurement of neurocranial height including cranial base, no significant differences were demonstrated between any of the groups at 172 days (p=0.054). Statistical analysis at 14, 28 and 56 days showed significant differences only at 28 days between high single dose and control and low fractionated groups, and at 56 days between high single dose and control groups.

Viscerocranial height A-lu (Fig 4.8 and Tables 4.24-4.27) data indicated a statistical difference between high single dose (S2) and low fractionated groups (F1, F2, F3) and similarly between control (C) and high fractionated and single dose groups (F4, S1, S2). There were no statistical differences found between control and low fractionated groups (C, F1, F2, F3). Statistical difference between the control and all other groups was seen at 28 days, and between the high single dose and all other groups at 56 days. No significant differences were seen at 14 days. Viscerocranial length, E-lu, (Fig 4.9 and Tables 4.28-4.31) data indicated significant differences between control and high and single dose groups at 172 days. No significant differences were seen between control and low fractionated dose groups. Between 14 and 172 days the general tendency was for significant differences between control and all other groups. Additionally at 28 and 56 days there were significant differences between high single dose and other irradiated groups.

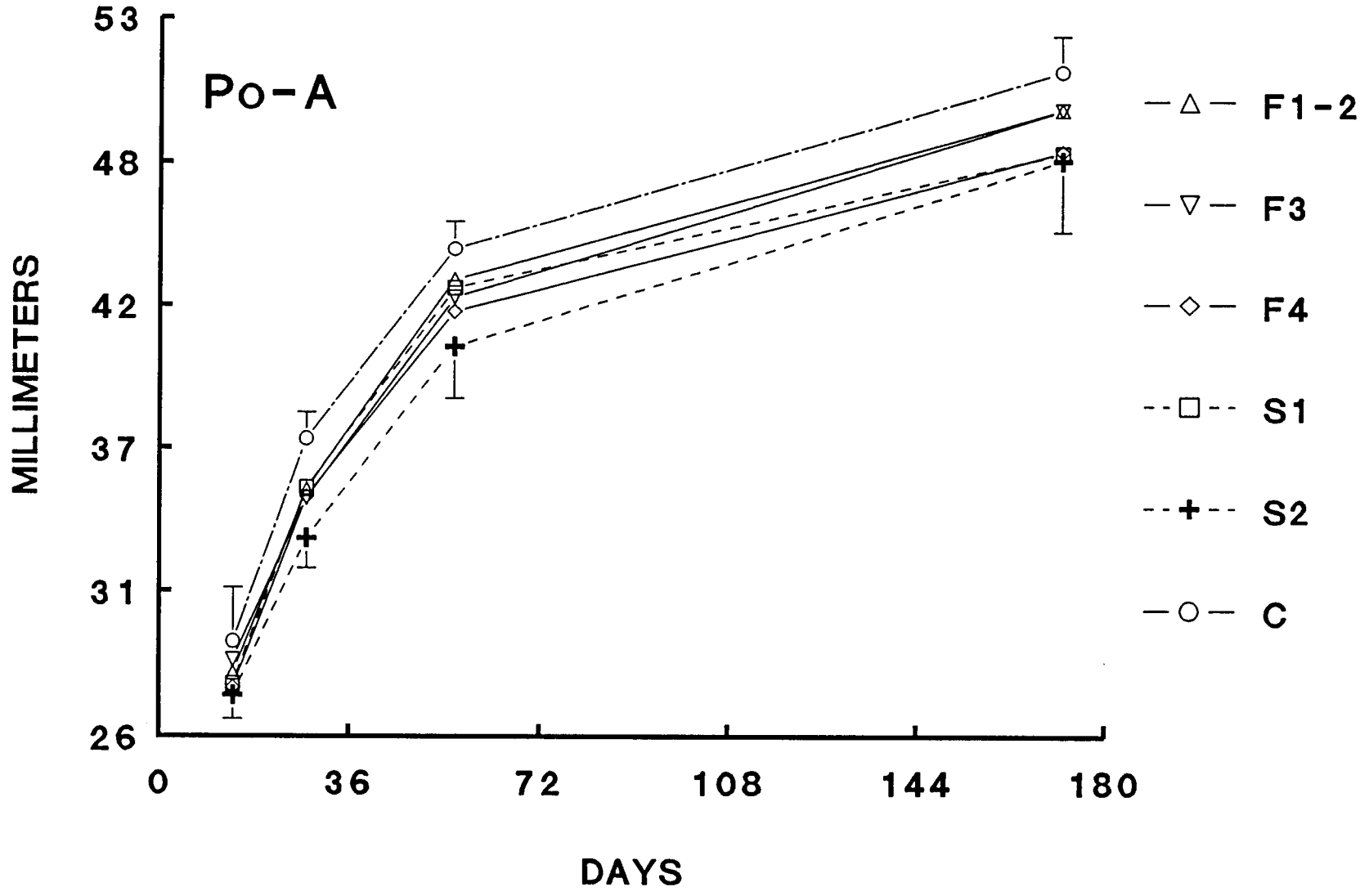


Fig 4.3 Lateral Cephalometric Measurements Po-A

Table 4.4 Lateral Cephalometric Measurement Po-A at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4	t=3.37 q=4.766					
S1	t=ns q=4.260					
S2	t=3.714 q=5.252					

F= 3.31

p= 0.006

Table 4.5 Lateral Cephalometric Measurement Po-A at 28 days

	C	S2	S1	F4	F3	F2
F1	t=4.44 q=6.28	t=4.146 q=5.863				
F2	t=4.643 q=6.567	t=4.588 q=6.489				
F3	t=5.446 q=7.701	t=3.766 q=5.325				
F4	t=5.481 q=7.751	t=4.056 q=5.736				
S1	t=4.473 q=6.326	t=4.763 q=6.736				
S2	t=8.934 q=12.635					

F= 13.59

p= 0.000

Table 4.6 Lateral Cephalometric Measurement Po-A at 56 days

	C	S2	S1	F4	F3	F2
F1		t=4.075 q=5.762				
F2		t=4.82 q=6.817				
F3	t=3.392 q=4.798	t=3.521 q=4.98				
F4	t=4.533 q=6.411	t=2.595 q=3.669				
S1	t=ns q=3.863	t=4.199 q=5.938				
S2	t=6.688 q=9.459					

F= 8.78

p= 0.000

Table 4.7 Lateral Cephalometric Measurement Po-A at 172 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4	t=4.161 q=5.884					
S1	t=4.090 q=5.785					
S2	t=4.362 q=6.169					

F= 5.37

p= 0.001

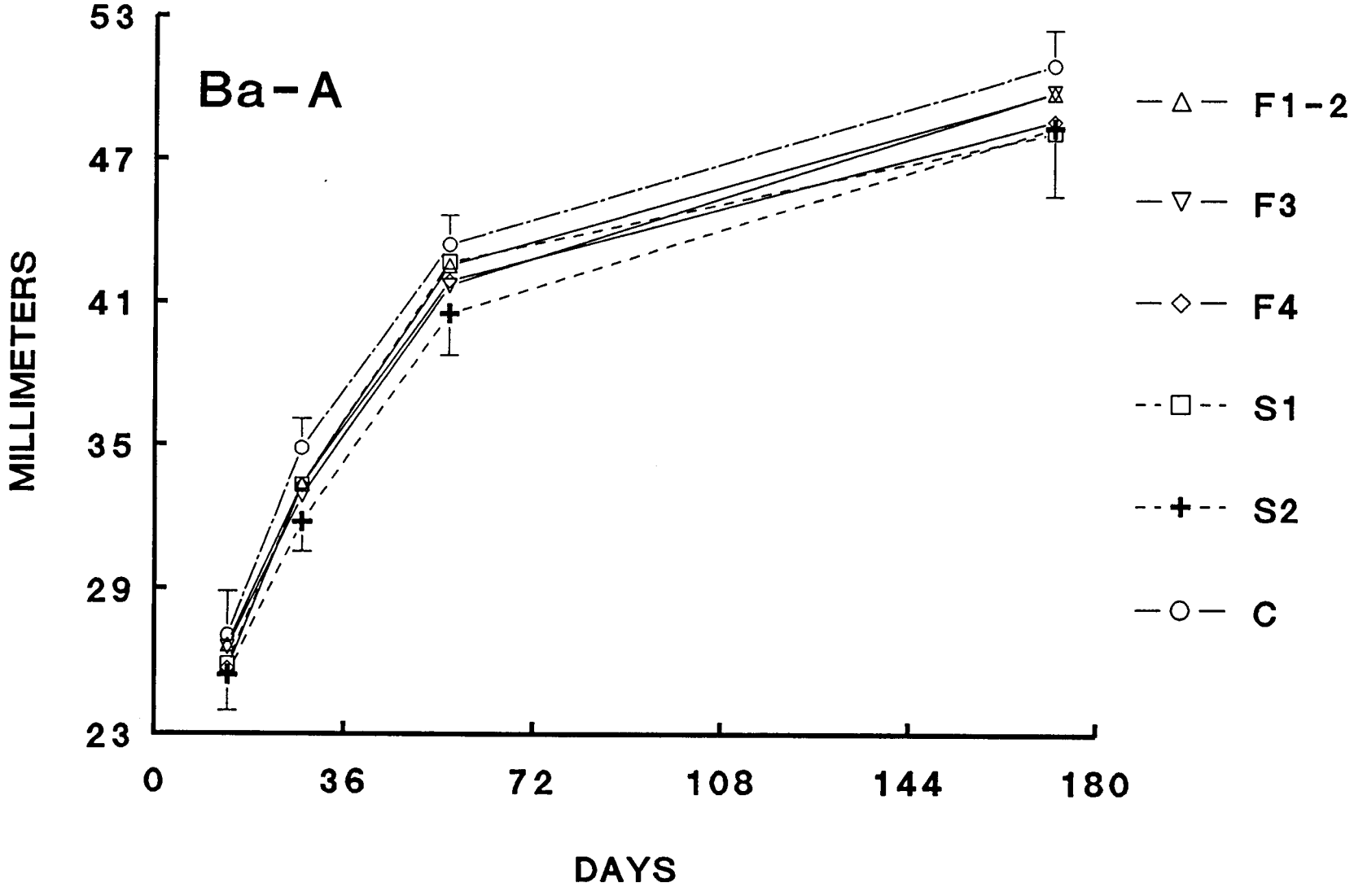


Fig 4.4 Lateral Cephalometric Measurement Ba-A

Table 4.8 Lateral Cephalometric Measurement Ba-A at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2						

F= 2.45

p= 0.032

Table 4.9 Lateral Cephalometric Measurement Ba-A at 28 days

	C	S2	S1	F4	F3	F2
F1	t=ns q=3.884	t=ns q=4.074				
F2	t=ns q=3.756	t=3.401 q=4.809				
F3	t=3.858 q=5.456	t=ns q=3.065				
F4	t=ns q=4.423	t=ns q=4.429				
S1	t=ns q=4.195	t=ns q=4.359				
S2	t=5.851 q=8.275					

F= 6.01

p= 0.000

Table 4.10 Lateral Cephalometric Measurement Ba-A at 56 days

	C	S2	S1	F4	F3	F2
F1		t=ns q=4.38				
F2		t=3.832 q=5.42				
F3						
F4						
S1		t=3.584 q=5.068				
S2	t=4.565 q=6.456					

F= 4.69

p= 0.000

Table 4.11 Lateral Cephalometric Measurement Ba-A at 172 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4	t=ns q=4.359					
S1	t=3.602 q=5.094					
S2	t=3.269 q=4.623					

F= 3.71

p= 0.003

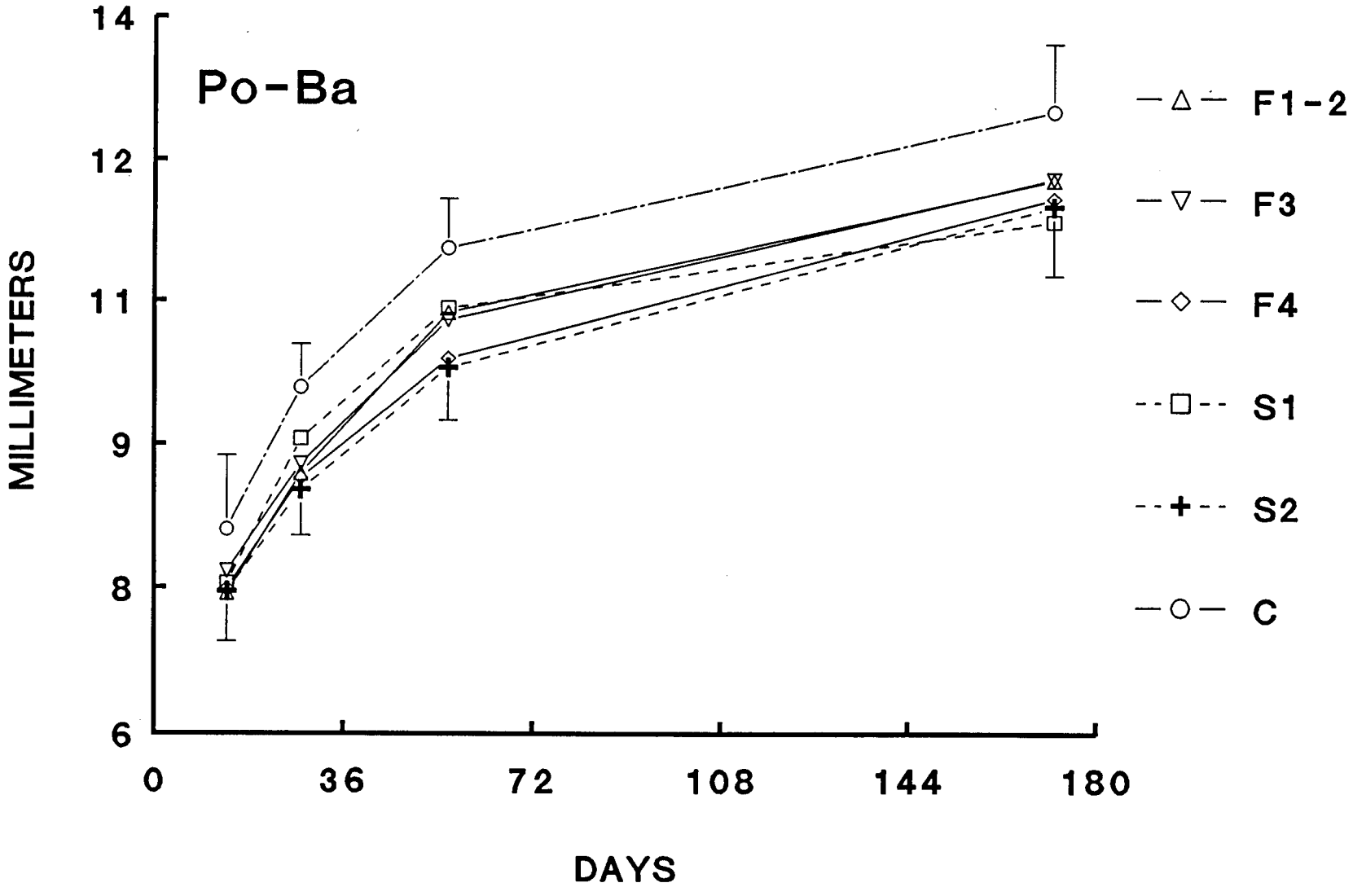


Fig 4.5 Lateral Cephalometric Measurement Po-Ba

Table 4.12 Lateral Cephalometric Measurement Po-Ba at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2						

F= 1.76

p= 0.119

Table 4.13 Lateral Cephalometric Measurement Po-Ba at 28 days

	C	S2	S1	F4	F3	F2
F1	t=3.859 q=5.457					
F2	t=ns q=3.782					
F3	t=3.666 q=5.185					
F4	t=4.505 q=6.371					
S1	t=ns q=3.538					
S2	t=4.819 q=6.814					

F= 5.29

p= 0.000

Table 4.14 Lateral Cephalometric Measurement Po-Ba at 56 days

	C	S2	S1	F4	F3	F2
F1	t=ns q=3.897					
F2	t=3.686 q=5.213					
F3	t=3.281 q=4.640					
F4	t=5.194 q=7.345					
S1	t=ns q=3.895					
S2	t=5.319 q=7.522					

F= 6.28

p= 0.000

Table 4.15 Lateral Cephalometric Measurement Po-Ba at 172 days

	C	S2	S1	F4	F3	F2
F1	t=ns q=3.777					
F2	t=3.454 q=4.884					
F3	t=ns q=4.001					
F4	t=3.724 q=5.266					
S1	t=4.556 q=6.443					
S2	t=3.853 q=5.449					

F= 4.14

p= 0.001

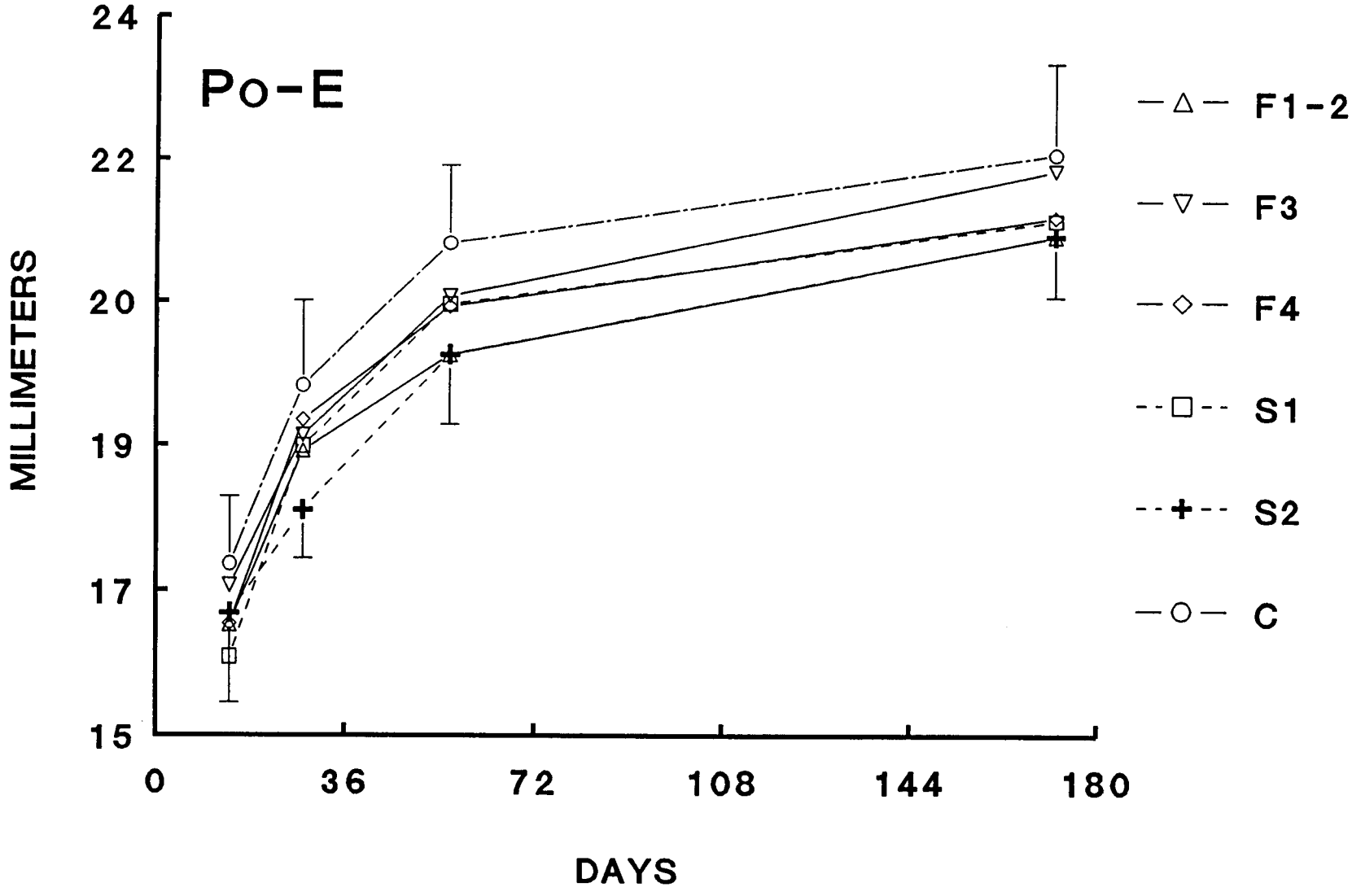


Fig 4.6 Lateral Cephalometric Measurements Po-E

Table 4.16 Lateral Cephalometric Measurement Po-E at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1	t=ns q=4.36					
S2						

F= 2.04

p= 0.071

Table 4.17 Lateral Cephalometric Measurement Po-E at 28 days

	C	S2	S1	F4	F3	F2
F1		t=ns q=3.647				
F2		t=3.183 q=4.502				
F3		t=3.442 q=4.867				
F4		t=4.331 q=6.125				
S1		t=ns q=4.188				
S2	t=5.511 q=7.793					

F= 5.66

p= 0.000

Table 4.18 Lateral Cephalometric Measurement Po-E at 56 days

	C	S2	S1	F4	F3	F2
F1						
F2		t=3.296 q=4.662				
F3						
F4						
S1						
S2	t=4.409 q=6.235					

F= 3.74

p= 0.003

Table 4.19 Lateral Cephalometric Measurement Po-E at 172 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2	t=ns q=4.335					

F= 2.85

p= 0.015

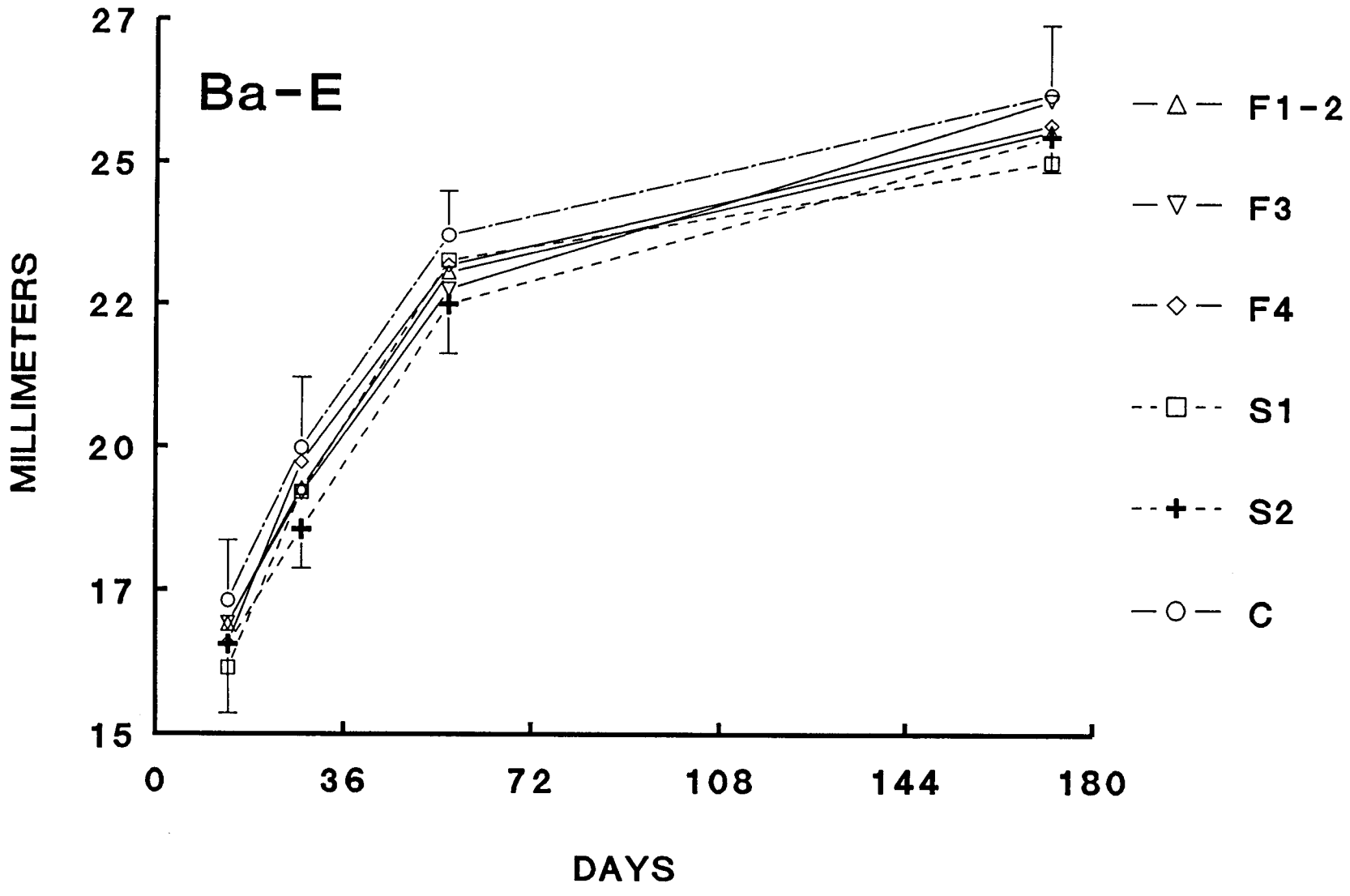


Fig 4.7 Lateral Cephalometric Measurement Ba-E

Table 4.20 Lateral Cephalometric Measurement Ba-E at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2						

F= 2.03

p= 0.072

Table 4.21 Lateral Cephalometric Measurement Ba-E at 28 days

	C	S2	S1	F4	F3	F2
F1						
F2		t=ns q=4.013				
F3						
F4		t=3.611 q=5.107				
S1						
S2	t=4.034 q=5.705					

F= 3.54

p= 0.004

Table 4.22 Lateral Cephalometric Measurement Ba-E at 56 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2	t=3.356 q=4.746					

F= 2.70

p= 0.020

Table 4.23 Lateral Cephalometric Measurement Ba-E at 172 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2						

F= 2.19

p= 0.054

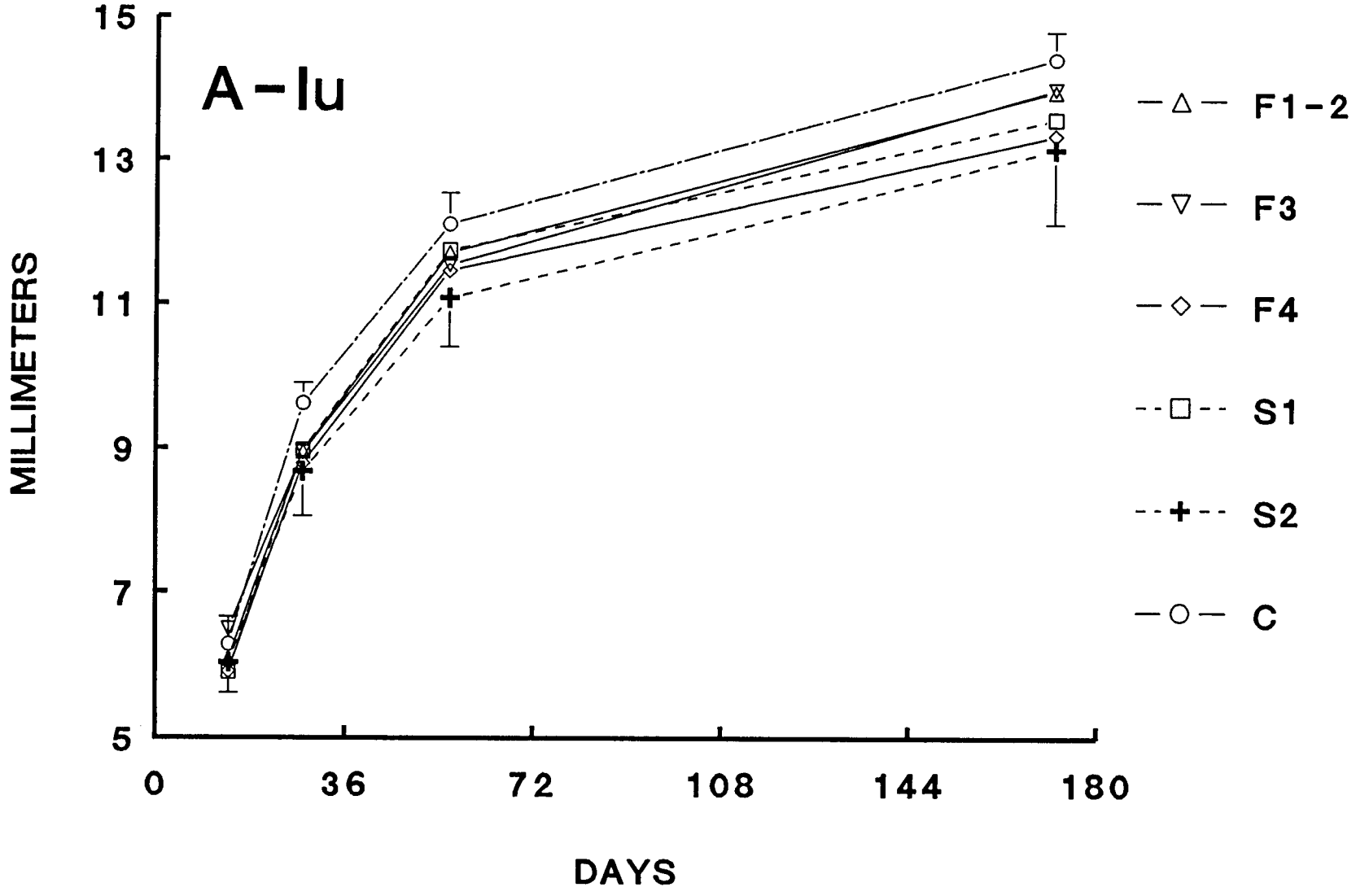


Fig 4.8 Lateral Cephalometric Measurement A-Iu

Table 4.24 Lateral Cephalometric Measurement A-Iu at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3			t=3.205 q=4.533	t=3.269 q=4.623		
F4						
S1						
S2						

F= 2.67

p= 0.021

Table 4.25 Lateral Cephalometric Measurement A-Iu at 28 days

	C	S2	S1	F4	F3	F2
F1	t=3.239 q=4.58					
F2	t=3.696 q=5.227					
F3	t=3.751 q=5.305					
F4	t=4.735 q=6.696					
S1	t=3.53 q=4.993					
S2	t=5.081 q=7.186					

F= 5.23

p= 0.000

Table 4.26 Lateral Cephalometric Measurement A-Iu at 56 days

	C	S2	S1	F4	F3	F2
F1		t=ns q=3.966				
F2		t=3.738 q=5.287				
F3						
F4	t=4.009 q=5.669					
S1		t=3.211 q=4.541				
S2	t=4.716 q=6.67					

F= 4.67

p= 0.000

Table 4.27 Lateral Cephalometric Measurement A-Iu at 172 days

	C	S2	S1	F4	F3	F2
F1		t=ns q=3.917				
F2		t=ns q=4.321				
F3		t=ns q=4.374				
F4	t=4.009 q=5.669					
S1	t=ns q=4.314					
S2	t=4.467 q=6.317					

F= 5.08

p= 0.001

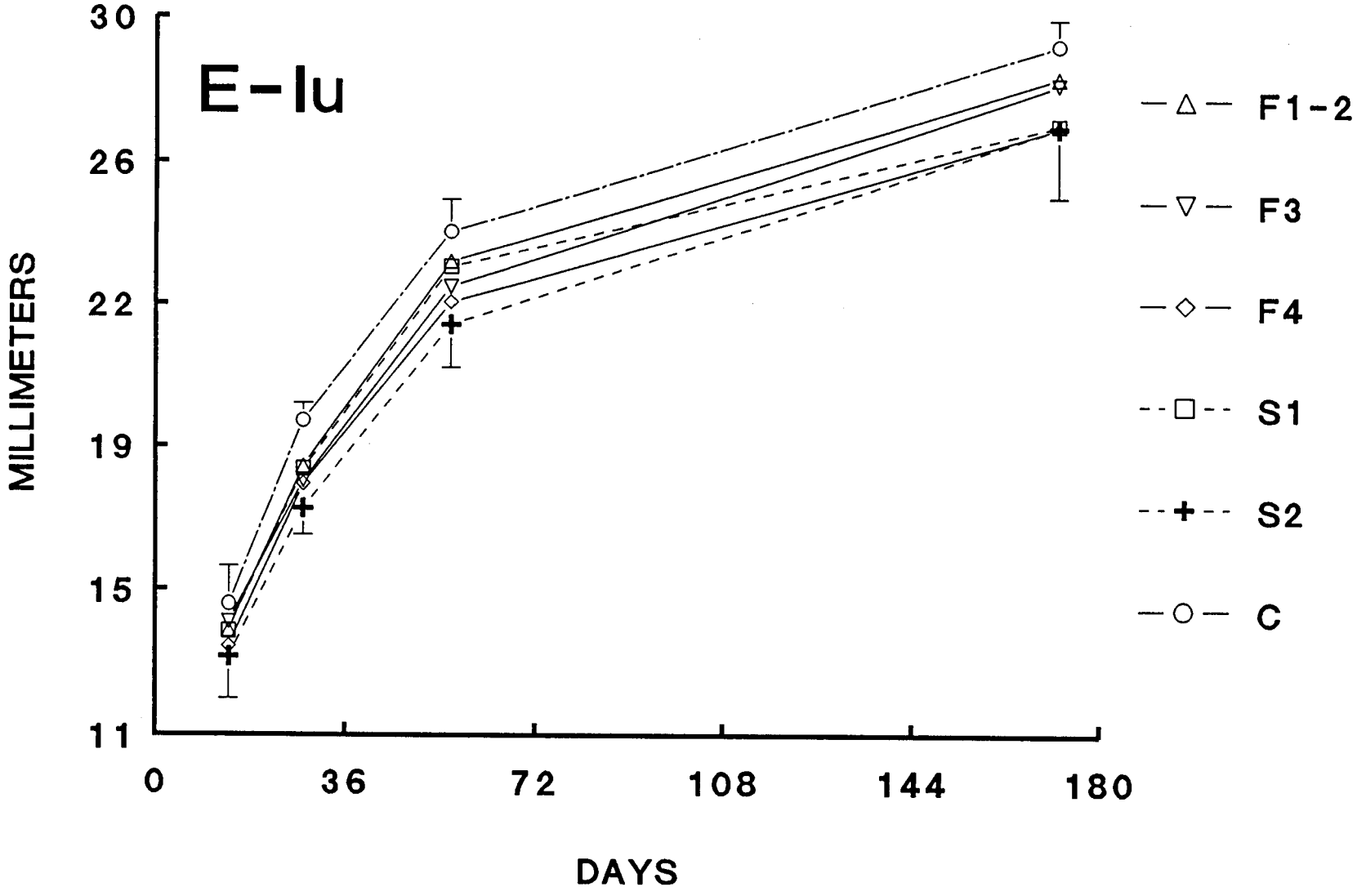


Fig 4.9 Lateral Cephalometric Measurement E-lu

Table 4.28 Lateral Cephalometric Measurement E-lu at 14 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4	t=3.558 q=5.031					
S1						
S2	t=4.191 q=5.927					

F= 3.84

p= 0.002

Table 4.29 Lateral Cephalometric Measurement E-lu at 28 days

	C	S2	S1	F4	F3	F2
F1	t=4.578 q=6.474	t=4.296 q=6.076				
F2	t=5.154 q=7.289	t=4.378 q=6.192				
F3	t=6.483 q=9.168	t=ns q=4.264				
F4	t=7.037 q=9.952	t=ns q=3.933				
S1	t=5.194 q=7.346	t=4.337 q=6.133				
S2	t=9.233 q=13.058					

F= 15.66

p= 0.000

Table 4.30 Lateral Cephalometric Measurement E-lu at 56 days

	C	S2	S1	F4	F3	F2
F1		t=4.419 q=6.249		t=ns q=4.240		
F2		t=4.392 q=6.211		t=ns q=4.056		
F3	t=4.062 q=5.745	t=ns q=4.022				
F4	t=5.365 q=7.587		t=ns q=4.014			
S1		t=4.364 q=6.172				
S2	t=6.698 q=9.472					

F= 9.92

p= 0.001

Table 4.31 Lateral Cephalometric Measurement E-lu at 172 days

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4	t=4.748 q=6.714					
S1	t=4.421 q=6.252					
S2	t=4.499 q=6.362					

F= 6.24

p= 0.001

4.3 Metrographic Measurements

Cross-sectional statistical analysis of mature skulls (172 days old) are shown in Tables 4.32-4.40. In general, statistically significant differences are seen between:

a. control and all other irradiated groups

b. control and high irradiation (fractionated and single) doses

c. high single dose and low irradiation doses in most measurements

d. high and low fractionated doses

The tests for statistical significance were at $p<0.05$, however most of these were significant at $p<0.001$, indicating a very low likelihood of these results being "false positive" (type I statistical error).

The dorsal cranial measurements included one length and three width measurements. Cranial length (Table 4.32) data indicated significant statistical differences between control and all irradiated groups, and between high single and low fractionated and single dose groups. No statistically significant differences were demonstrated in neurocranial width (between lambdoidal sutures) data (Table 4.33) between any of the experimental and control groups ($p=0.267$). Dorsal surface distances between the zygomatic arches - anterior and posterior, demonstrated differences between groups. The posterior interzygomatic distance data (Table 4.34) indicated significant differences between control (C) and high fractionated and single dose (S2, F4) groups as well as between high single (S2) and low fractionated and single dose groups (F1, F2, F3, S1). No statistically significant differences were observed between control and low fractionated and single groups (F1, F2, F3, S1). The anterior interzygomatic distance data (Table 4.35) likewise indicated differences between control (C) and high dose fractionated and single dose groups (F4, S2). Unlike the previous measurements no significant differences were seen between high single (S2) dose and low fractionated dose groups. An unexpected result was seen in the difference between the control and low fractionated (F1) dose groups.

Ventral cranial metrographic measurements included one length and two width measurements. Viscerocranial length (Table 4.36) data indicated differences between control and all irradiated groups (p=0.001). Statistically significant differences were also seen between high single dose (S2) and low fractionated and single dose groups (S1, F1, F2, F3) and unexpectedly between F2 and F4. Bizygomatic width (Fig 4.37) showed several statistically significant differences. The control groups were different from all radiated groups. High single dose (S2) was different from low fractionated and single dose groups (F1, F2, F4, S1) and differences were also seen between high (F4) and low (F1, F2) fractionated doses. Intermaxillary width data (Table 4.38) indicated significant differences (p=0.031) between control and high single dose (S2) only. Significant differences were not observed between control and all other radiated groups.

Intercondylar width data (Table 4.39) indicated significant differences between control and all irradiated groups (except the low fractionated group- F2). Differences were also seen between the low fractionated group (F2) and the high single (S2) and high fractionated dose groups (F3, F4). Unexpectedly, no significant differences were seen between F2 and S1, nor between C and F2. Intergonial width data (Table 4.40) demonstrated significant differences (p=0.001) between control and all radiated groups. Also differences were seen between high fractionated (F4) and low single and fractionated groups (S1, F1,F2), however not between these groups and high single dose (S2).

Table 4.32 Metrographic Measurement - Cranial length at 172 days

MEASUREMENT: **Cranial Length (mm)**

Group	Mean	Std Dev
F1	48.61	1.51
F2	48.81	0.71
F3	48.19	1.05
F4	47.31	0.76
S1	48.48	1.35
S2	46.43	2.19
C	50.89	0.76

F= 14.01

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=4.065 q=5.748	t=4.037 q=5.709				
F2	t=3.881 q=5.489	t=4.631 q=6.549		t=ns q=4.266		
F3	t=5.22 q=7.382	t=3.559 q=5.033				
F4	t=7.025 q=9.934					
S1	t=4.497 q=6.36	t=3.989 q=5.641				
S2	t=4.631 q=11.992					

Table 4.33 Metrographic Measurement - Neurocranial width at 172 days

MEASUREMENT: Neurocranial Width (mm)

Group	Mean	Std Dev
F1	12.21	0.79
F2	12.21	0.86
F3	12.01	0.69
F4	12.23	0.88
S1	12.24	0.94
S2	11.94	0.91
C	12.83	0.92

F= 1.30

p= 0.267

Table 4.34 Metrographic Measurement - Posterior bizygomatic width at 172 days

MEASUREMENT: Bizygomatic Width - Posterior (mm)

Group	Mean	Std Dev
F1	18.41	0.91
F2	18.43	0.61
F3	18.29	0.53
F4	17.89	0.37
S1	18.42	0.71
S2	17.41	1.18
C	19.12	0.87

F= 5.81

p= 0.001

	C	S2	S1	F4	F3	F2
F1		t=ns q=4.389				
F2		t=3.326 q=4.704				
F3		t=ns q=4.218				
F4	t=4.045 q=5.721					
S1		t=3.294 q=4.658				
S2	t=5.449 q=7.707					

Table 4.35 Metrographic Measurement - Anterior bizygomatic width at 172 days

MEASUREMENT: Bizygomatic Width - Anterior (mm)

Group	Mean	Std Dev
F1	11.15	0.74
F2	11.53	0.73
F3	11.48	0.58
F4	10.86	0.60
S1	11.45	0.50
S2	10.96	0.95
C	12.09	0.29

F= 4.90

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=3.270 q=4.625					
F2						
F3						
F4	t=4.710 q=6.661					
S1						
S2	t=4.193 q=5.929					

Table 4.36 Metrographic Measurement - Viscerocranial length at 172 days

MEASUREMENT: Viscerocranial Length (mm)

Group	Mean	Std Dev
F1	36.58	1.16
F2	36.94	0.65
F3	36.36	0.84
F4	35.84	0.39
S1	36.64	0.85
S2	35.18	1.66
C	38.07	0.49

F= 11.01

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=3.608 q=5.102	t=3.521 q=4.980				
F2	t=ns q=4.050	t=4.651 q=6.578		t=ns q=4.249		
F3	t=4.490 q=6.350	t=3.241 q=4.584				
F4	t=5.943 q=8.405					
S1	t=3.308 q=4.678	t=3.858 q=5.457				
S2	t=7.463 q=10.555					

Table 4.37 Metrographic Measurement - Bizygomatic width at 172 days

MEASUREMENT: **Bizygomatic Width (mm)**

Group	Mean	Std Dev
F1	22.21	1.41
F2	22.17	0.51
F3	21.75	0.58
F4	20.97	0.71
S1	22.37	1.08
S2	20.88	1.53
C	23.73	0.67

F= 11.69

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=3.532 q=4.995	t=3.211 q=4.540		t=ns q=4.362		
F2	t=3.795 q=5.367	t=3.272 q=4.627		t=ns q=4.449		
F3	t=4.990 q=7.057					
F4	t=7.060 q=9.984	t=3.670 q=5.191				
S1	t=3.308 q=4.678	t=3.779 q=5.345				
S2	t=7.064 q=9.990					

Table 4.38 Metrographic Measurement - Intermaxillary width at 172 days

MEASUREMENT: Intermaxillary Width (mm)

Group	Mean	Std Dev
F1	6.57	0.38
F2	6.44	0.38
F3	6.49	0.18
F4	6.44	0.27
S1	6.49	0.19
S2	6.22	0.32
C	6.65	0.33

F= 2.46

p= 0.031

	C	S2	S1	F4	F3	F2
F1						
F2						
F3						
F4						
S1						
S2	t=3.537 q=5.002					

Table 4.39 Metrographic Measurement - Intercondylar width at 172 days

MEASUREMENT: Intercondylar Width (mm)

Group	Mean	Std Dev
F1	16.51	1.10
F2	17.03	0.63
F3	16.21	0.44
F4	16.13	0.62
S1	16.71	0.76
S2	15.89	1.09
C	17.49	0.45

F= 6.61

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=ns q=4.193					
F2		t=3.764 q=5.323		t=ns q=4.344	t=ns q=3.896	
F3	t=4.199 q=5.938					
F4	t=4.528 q=6.404					
S1	t=ns q=3.493					
S2	t=5.162 q=7.301					

Table 4.40 Metrographic measurement - Intergonial width at 172 days

MEASUREMENT: Intergonial Width (mm)

Group	Mean	Std Dev
F1	19.22	1.32
F2	19.06	1.03
F3	18.57	1.05
F4	17.74	0.92
S1	19.52	1.29
S2	18.37	1.67
C	20.68	1.23

F= 7.31

p= 0.001

	C	S2	S1	F4	F3	F2
F1	t=ns q=3.851			t=ns q=4.179		
F2	t=3.163 q=4.473			t=ns q=3.928		
F3	t=4.268 q=6.036					
F4	t=6.036 q=8.537		t=3.746 q=5.297			
S1	t=ns q=3.203					
S2	t=4.596 q=6.499					

4.4 Error Determination

Results from the analysis of the precision of the cephalometric measurement technique demonstrate:

1. Overall the standard deviation and variance values for both of the subjects and within each of the measurements are low. The measurement method would therefore appear to be appropriate for determining changes in cephalometric dimensions.
2. Each of the components contribute to the standard deviation, with digitizing being the smallest contributor followed by tracing and digitizing then the combined tracing, digitizing and radiography. Combined average values were 0.09, 0.21 and 0.28 respectively. Values for the normalized data were 0.08, 0.19 and 0.25.
3. The coefficient of variation for individual cephalometric measurements 1-5 within one of the error stages, for example the radiography component of subject A, shows relatively consistent values (eg. 0.031- 0.028) despite the range of values of 20-50mm. The standard deviation however is not consistent which would appear to suggest a relative rather than a linear error.

Takada et al (1983) determined that metrographic landmarks or points may be determined with an accuracy of 0.1mm. They further noted that in order to avoid interoperator error the same operator must carry out the measurements. In this study the measurements were made by a single operator.

CHAPTER 5

Discussion

5.1 Discussion of the Methods

5.1.1 Growth Measurements

This study was restricted to male rats in order to reduce the variability, maximize the absolute size of the measurements and allow comparison with previous studies. Measurements of both body length and weight were made in order to assess whether a permanent deficit in body size and weight occurred as a result of the irradiation treatment. Measurements (weight and body length) were taken on relaxed, anesthetized animals since Hughes and Tanner (1970) have shown this technique to be more reliable than those made on conscious animals. They found a 5% difference in duplicate measurements when using conscious animals compared to a 1% difference in anesthetized animals.

Tail length measurements were recorded but not used in the analysis of irradiation effects as they were found to be unreliable. Some animals suffered trauma to the tail at both early and late stages. To assess bone growth outside of the treatment field Engström et al (1981), Hartsell et al (1989) and Schunior et al (1990) measured the length of the femur or tibia. This step was not done in this study, but may have been a valuable adjunct to the growth measurements.

5.1.2 Cephalometric Measurements

The extensive use of the lateral cephalometric measurement technique in craniofacial morphometric studies of the rat, from Spence in the 1940s up to Kiliaridis et al in the 1980s, would indicate that it is a practical and reliable technique. The determination of significant craniofacial growth changes requires either large differences at each growth stage or the error of the measurement method must be significantly less than the differences between the stages. It was therefore important to evaluate the precision of the measurement stages involved in the cephalometric analysis used in this study.

In order to determine and quantify the precision of the cephalometric measurement method, an error analysis study was included in this experiment. Investigators of studies involving cephalometric techniques have used different methods of determining the error. Engström (1982), Houston (1983), Kiliaridis (1985) and Sandler (1988) all used or advocated error of the method or a measure of the error variance as proposed by Dahlberg in the 1940s:

$$S_e = \sqrt{\Sigma(x_1 - x_2)^2 \ / \ 2(n-1)}$$

This calculation involves a repeated measure of a number of skulls, hence x_1 is the magnitude of the variable on the first measurement, x_2 is the magnitude of the variable on the second measurement and n is the number of pairs of radiographs. While Dahlberg's analysis produces a confounded error value (which encompasses both random and systematic errors), the method used in this study allowed some quantification of the errors associated with each of the measurement stages (radiography, tracing and digitizing).

The standard deviation of 0.28mm for all measurement stages compares well with Kiliaridis (1985) who used a similar cephalometric measurement system and reported a range in values of 0.32-1.63. The standard deviation value is also in agreement with the results of Sandler (1988) who found standard deviations for his two groups were less than 0.3mm for linear measurements.

Houston (1983) suggested the validity of measurements made during a cephalometric analysis may only be determined by comparing direct measurements from a series of skulls with the radiographic measures. He also noted that measurements whose points lie in a plane parallel to the film are not distorted, however when this does not occur, linear measurements may be affected. In this study the landmarks were selected following the definitions of Engström et al (1982). Although the validity of the radiographic landmarks and subsequent measurements was

not specifically analyzed, two measurements (cranial and viscerocranial length) were repeated using both the cephalometric and cross-sectional (metrographic) analyses. The measurements were similar with the metrographic analysis tending to demonstrate more significant differences than the cephalometric analysis at 172 days. Therefore both of these cephalometric measurements appear to be valid according to Houston's (1983) criteria.

5.1.3 Metrographic Measurement

In this study the cross-sectional analysis involved measurements directly from the rat skulls (aged 172 days). This method of examination was similar to that of both Eifel (1988) and Schunior et al (1990). In contrast to the use of the metrograph, as used in this study, both of the studies involved the use of calipers to measure the bone directly. Schunior et al (1990) determined a 1% error in their technique and Eifel (1988) specified a resolution of ±0.2mm for her tibial measurements. The resolution of ±0.1mm for the metrograph measurements compared well to these studies.

5.2 Discussion of the Results

5.2.1 Growth Data

Mortality and morbidity results for this experiment were comparable to those found by Mosier and Jansons (1967) despite the different breed of rat used. All animals irradiated with 600 cGy (600 rads) in this experiment died within a short time of the irradiation treatment. As part of the pilot study for this experiment several animals were irradiated with 750 cGy (single dose) and died within hours. It was subsequently decided not to extend the irradiation range beyond 600 rads. Mosier likewise found 750 cGy resulted in death of most of their animals. In a more recent paper Mosier (1988) stated that a single dose of 600 cGy was known to be lethal to rats and he subsequently set this as his maximum dose. Our results concurred with this.

In general the growth curves for the rat (both weight and length) follow those demonstrated by Hughes and Tanner (1970). They found the rate of increase of body weight peaked at 60-70 days. Our results for all the groups demonstrated a similar peak. The clear and statistically significant differences in body weight at 172 days are of interest. The marked separation of body weight into high single dose, control and all other irradiated groups suggested fractionation and dose have a marked effect on body weight. This confirms the work of Clemente and Yamazaki (1960) who found both stunted weight associated with general irradiation treatment of rats and suggested rats are most sensitive to irradiation in the first week of life. The rats in this study were all irradiated from 2 to 4 days of age and the lowest irradiation dose to affect body weight was 250 cGy. Similar results were noted by Mosier and Janson (1967) who found a decrease in body weight associated with a irradiation dose of 350 rads (350 cGy) or greater. Mosier (1988) also noted growth retardation with 350 rads (350 cGy) or more. The suppression of weight gain in the irradiated groups in our study confirmed the results of Schunior et al (1988). In their study the rats were exposed to single dose cranial irradiation and showed a similar failure to achieve the weight and body length of the control group.

The body length profiles reported in this experiment in general correlate with clinical observations in children undergoing single, high dose or cranial irradiation. Body length was significantly shorter in high single dose groups than in all other groups, including control. Sanders et al (1986) observed more normal growth curves with fractionated than with single dose irradiation in children. The results in this study show similar trends.

5.2.2 Cephalometric Data

The general trends in the cephalometric results confirm the work of Engström et al (1985), Eifel (1988), Schunior et al (1988) and Hartsell et al (1989). The observation that the high single dose group (S2) was significantly different from the control group in almost all

measurements was in accordance with the data of Engström et al (1985) who described significant differences in craniofacial growth with localized high dose irradiation. The finding of no significant differences in general between control and the low fractionated dose irradiation groups also validates the work of Hartsell et al (1989) who suggested that increased fractionation of irradiation dose resulted in increased bony (vertebral) growth. Eifel (1988) suggested that the increased tibial bone growth in her study with fractionated irradiation resulted from a sparing effect on the bone. Hence she suggested rats given fractionated dose would be expected to recover from the irradiation faster and therefore show increased bone growth (length or width) compared with the single dose groups, which incur more direct damage to the bone, with a consequent delayed growth. The overall trends of the cephalometric data tended to confirm this, with the control group demonstrating consistently greater values for all cranial dimensions measured. The general cephalometric results are in agreement with Schunior et al (1989) who found microcephaly associated with high single dose cranial irradiation. They however made their observations from the cross-sectional analysis of mature skulls, in contrast to this study where measurements were made both longitudinally and cross-sectionally. The overall trend of smaller craniofacial measurements with high dose irradiation correlates with the findings of Clayton et al (1987) who noted a smaller head size in human subjects who had previous cranial irradiation.

Observations made by Baer (1954), Moore (1966), Cleall et al (1969) and Vilmann and Moss (1980) of the overall growth of the skull and the sites most likely to be affected by irradiation are confirmed by this study. The only cranial dimension not affected by irradiation at 172 days was the neurocranial height measure (Ba-E). This was expected since this measure incorporates the cranial base/basisphenoid region which is assumed to remain fairly constant in the rat - similar to sella turcica in man (Spence 1940). No differences were noted for either of the skull length measurements (Po-A and Ba-A) between control groups and low fractionated dose groups, however a difference was noted between control and high single dose

groups for both measurements. These findings are consistent with other studies. Based on studies by Cleall (1969), Moore (1966) and Eifel (1988), the viscerocranial components contributing to the cranial length would be affected to a greater extent by the single rather than the fractionated dose irradiation therefore leading to reduced longitudinal growth of the skull.

Both measurements involving the viscerocranium use the upper incisor tip (Iu) as a landmark (A-Iu, E-Iu). This was also used by Engström et al (1982). The choice of this landmark is open to some criticism since the rat incisor is continually erupting and subjected to continual attrition, hence the landmark may be viewed as unstable. In defence of this however, all animals were treated in a standardized manner (similar diet and bedding) thus attrition was assumed to be similar throughout all groups. Any effect on the dental development would therefore be a consequence of the irradiation therapy. Both height and length viscerocranial measurements demonstrated differences between high dose and low dose fractionated irradiation or control. Studies by Baer (1954) and Vilmann and Moss (1980, 1987) offer an explanation for these results since they show that the continuous and rapid growth of the viscerocranium normally occurs throughout the period when irradiation was given to the rats in this study, thus this region may be relatively more sensitive to irradiation effects than the neurocranium. Vilmann and Moss (1980, 1987) also noted both the facial skull and the posterior of the neurocranium grew rapidly in the first 5 months. Hence the demonstrated sensitivity to irradiation and consequent differences in growth dimensions in the 2 measurements involving the posterior neurocranium (Po-E and Po-Ba) were as expected.

5.2.3 Metrographic Data

In general, the metrographic measurements of the 172 day old dry skulls appeared to be a more sensitive measure of growth response to irradiation dose and delivery. This in part is due to the greater number of craniofacial dimensions involving the viscerocranium in this analysis than in the cephalometric measurement analysis. As noted in the previous section, the work of Baer

(1954), Vilmann and Moss (1980) and Moore (1966) strongly suggest the viscerocranium, which is undergoing rapid growth during the period of irradiation therapy, is more susceptible to growth alterations than the neurocranium. Ubios et al (1992) noted altered mandibular length and height as a result of localized irradiation. This effect was confirmed for two mandibular measurements (intercondylar and intergonial width) in this study.

5.3 Future Directions

The results of this study demonstrated that both low and fractionated dose irradiation had a sparing effect on craniofacial growth changes in the Sprague-Dawley rat (in comparison to single and high dose irradiation). This was seen most clearly in the viscerocranium which is more sensitive to irradiation than the neurocranium.

This studies may be further developed in several directions. The first of these may involve more detailed analysis of the growth of the rat skull, utilizing larger sample sizes, confining the irradiation to low and fractionated doses and incorporating more cephalometric and metrographic measures. This would have the advantage of further defining the lowest doses and fractions of irradiation to have an effect on craniofacial growth in the rat model.

A retrospective survey of changes in craniofacial growth changes in children who have undergone irradiation therapy (TBI, TLI or cranial irradiation) may further clarify the effect of irradiation on craniofacial morphology. In addition, a prospective survey of children scheduled to undergo BMT may enable specific measurements (such as serial radiographs, photographs and three-dimensional analysis of the facial shape) to be recorded, thus determining the effect of the irradiation on craniofacial morphometric changes in humans.

CHAPTER 6

Summary

Bone marrow transplantation (BMT) has become a well established form of treatment in children with nonmalignant and malignant hematologic disorders. Marrow transplant preparative regimens involve the use of cranial irradiation, total body irradiation or total lymphoid irradiation, with or without chemotherapy. The irradiation may consist of single or fractionated dose irradiation. As a result of the increase in numbers of patients undergoing BMT there is growing concern and related research into the short and long term effects of the treatment on the survivor population. Side-effects may not fully manifest themselves until many years after initial therapy. These effects include persistent short stature, increased weight for height, altered cognitive development, and craniofacial abnormalities including microcephaly, mid-facial hypoplasia and mandibular retrognathia with arrested dental development (Schunior 1990). Few reported observations of the effects of low dose irradiation have been made on the craniofacial region in humans or animal models to date.

The aim of this investigation was to determine the effects of irradiation on craniofacial growth using the rat as a model. Eighty-seven Sprague-Dawley rats were bred from birth through to maturity (172 days old). Cephalometric analyses were used to describe the longitudinal changes in craniofacial morphology during the growth of the rat skull and metrographic techniques were used to facilitate the cross-sectional study of mature skulls. Two null hypotheses were posed:

1. No differences in craniofacial measurements between radiated and control groups.
2. No difference in craniofacial measurements between single and fractionated dose groups.

Conclusions from this study were as follows:

1. The growth profiles for body weight and length follow the expected growth curves for rats. Both single and fractionated dose irradiation significantly affected body weight, while only

high single dose irradiation influenced body length.

2. Longitudinal data were derived from cephalometric radiographs. In general the high single dose group was significantly different from the control group in all measurements except neurocranial length. Significant differences were not demonstrated between control and low fractionated groups in all measurements except neurocranial height.
3. Cross-sectional statistical analysis of mature skulls using metrographic measurement techniques demonstrated significant differences between:
   a. control and all other irradiated groups
   b. control and high irradiation (fractionated and single) doses
   c. high single dose and low irradiation doses in most measurements
   d. high and low fractionated doses
4. Single dose irradiation had more effect in general on craniofacial growth than fractionated dose.
5. High dose irradiation had more effect in general on craniofacial growth than low dose.
6. In general viscerocranial growth was more affected by irradiation than neurocranial growth.

Results from this study on the rat model suggest that there may be value in investigation of fractionated dose irradiation therapies on craniofacial growth in humans.

CHAPTER 7

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