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A Radiographic Study of Ossification in the Spine
and Limbs of the Human Fetus

by

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A Doctoral Thesis

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CHAPTER I

INTRODUCTION

INTRODUCTION

Until recently relatively little has been known about factors which affect growth and development in early life and which have a fundamental influence on later adult life. Thus pre- and post-natal growth and development must be studied as a continuum for they have a profound effect on ultimate physical and mental development. However, legal and ethical problems governing the availability of human fetal material diminish the prospects for assembling large comprehensive surveys. Because of this, there is often inaccuracy and inconsistency in many reports of human fetal development and, in particular, the study of certain regions has been considerably neglected. Ossification of the human fetal spine and limbs is one such example.

The initial purpose of the present study was an attempt to increase knowledge in an area where there appears to be substantial gaps. As the study developed it became obvious that the research should be extended into other relevant areas and an attempt has been made to correlate various findings so that a more comprehensive understanding of growth as a whole in this region might be obtained. In particular, study of the fetal spine cannot be isolated from the study of fetal movement.

An important feature of the present study has been the construction of "standards" for human fetal growth and development, the data being presented graphically and in tables. These allow comparisons to be made with single or serial measurements of other fetuses and they may assist in establishing the presence of abnormal development, for example, spina bifida, anencephaly and congenital scoliosis. The standards might also form the basis for comparisons with the influence of various factors such as sex, ethnic groups, environmental changes, nutrition and disease which are known to affect growth and development.

It is hoped that the present study, by adding to existing data concerning fetal growth and development, will promote assessment of fetal "well

being", maturity and age. This is particularly important for the paediatrician and obstetrician especially where circumstances indicate that premature delivery of the child may be necessary or where there are signs indicative of fetal ill-health.

The results of earlier investigations of pre-natal, neonatal, and post-natal growth need to be interpreted with caution in the light of the increased accuracy of techniques available for measuring and analysing fetal growth. Indeed earlier growth curves may need to be revised. The results of the present study might also assist in establishing the age, sex and size of whole or part of a skeleton and might therefore be useful in disciplines such as forensic medicine, physical anthropology and archeology.

CHAPTER II

REVIEW OF LITERATURE

The fetal skeleton "in toto" is a complex subject for research. Although its very complexity creates many possible areas for research, individual topics can be isolated for investigation. However, when investigating any particular items, the findings must be viewed in relation to the entirety of the spine and limbs and not in isolation.

FETAL AGE

During recent years there has been increasing interest in developing new methods for estimating the gestational age of the human fetus. These methods have recently been extensively reviewed (Casaer and Akiyama, 1970).

Traditionally, but nevertheless inaccurately, fetal age has been estimated from the date of onset of the Last Menstrual Period (L.M.P.) (Chan, Ang, and Soo, 1972; Birkbeck, Billewicz, and Thomson, 1975). An earlier worker (Mall, 1918) referred to this as the Menstrual Age but it has been called Postmenstrual Age by later workers (Casaer and Akiyama, 1970). A well-defined landmark such as this clearly has its attraction as a basic method of ageing fetuses and indeed has been used (Brewer and Jones, 1947; Birkbeck et.al., 1975). Treloar, Behn, and Cowan (1967) suggest that the L.M.P. age can be used as one of several methods for estimating the age of a conceptus. However, it must be remembered that life actually commences with fertilisation of the ovum (Patten, 1953; Anson, 1966) and the age of the fetus is really the time which has elapsed after conception (Hess, 1917; Hamilton and Mossman, 1972). Unfortunately, the date of conception cannot be accurately determined in the human and this has led to the rather unsatisfactory situation which exists at the present time.

Keefer (1965) in comparing ovulation with ejaculation, observes that it is surprising that ovulation should be so unobtrusive when ejaculation is so obvious. Warwick and Williams (1973) state that fetal age derived from the L.M.P. is approximately 14 days in excess, since ovulation occurs around the 14th day following the onset of menstruation and there is a limited viability of the female gamete. Guerrero and Florez (1969) using a change of basal body temperature as an indication of ovulation were more precise in their determination of this Ovulation Age and found that the interval between L.M.P. and the date of ovulation has a mean of 16.5 days and a standard deviation of 3.8 days which accords with a similar but earlier study by Greulich, Morris,

and Black (1943). Shabaan and Klopfer (1973) provided further evidence of a variation in the pre-ovulation interval by determining mid-cycle hormonal peaks and demonstrated that the interval between L.M.P. and mid-cycle oestradiol peak varied from 8 to 17 days, whilst Allen, Pratt, Newell, and Bland (1930) after recovering unfertilised ova and studying the associated corpora lutea, concluded that ovulation occurred approximately on day 14 of a 28 day cycle. Brewer and Jones (1947) in a review of the available literature offer evidence which supports the view that ovulation occurs during mid-cycle and report that earlier investigators who examined cervical mucus concluded that ovulation takes place on the 9th to 19th day of the cycle. They further report on a vaginal smear method which found that ovulation occurred most often on days 11, 12 and 13 with a range from 7 to 17 days, and a method which measured sodium pregnandiol glycuronidate excretion in urine which indicated that ovulation occurred during mid-cycle. These results contrast sharply with the views of earlier workers at the turn of the century when, as Mall (1910) reports, the separate studies of a number of investigators including Bischoff, Dalton, Williams, Reichart, Arnold, Leopold, and Ravano, showed conclusively that ovulation and menstruation were usually synchronous. Patten (1953) considers this to be one of the main reasons for the erroneous retention of Menstrual Age as an indication of conception age. There was evidence however, that menstruation could take place in the intermenstrual period (Mall, 1910). Similarly, there was a time when conception was thought to be most frequent during the week after the end of menstruation (Mall, 1918) but this was very firmly rejected by a study of the sexual habits of orthodox Jewish women (Park, 1968). The above examples illustrate the complexity of this region of research particularly in terms of investigation and emphasise how comparatively recent is much of the available information. Both Bell and Lorraine (1965) and Keefer (1965) state that there are no useful criteria which allow the day of ovulation to be predicted and Brewer and Jones (1947) attempting to summarise the available data, state that ovulation takes place during

the mid-cycle with a range from 8 to 19 days in women with cycle lengths of 26 to 30 days. In their survey they introduced the additional problem of irregular cycle length which directly affects the relationship between any two points in the cycle.

Chiazzi, Brayer, Macisco, Parker, and Duffy (1968) confirm that there is a wide variation in the cycle length and Gunn, Jenkin, and Gunn (1937) remark that the only regular feature about the monthly menstrual period is its lack of regularity. Arey (1939) is very critical of several studies which suggest that a very high percentage of women have regular 28 day cycles or even menstruate regularly and he suggests that only data from actual calendar records of responsible women are acceptable for such studies. From 20,000 such records he calculates that the modal length for both pubertal girls and adult women is 28 days whilst the average length is 33.9 days for girls and 28.4 days for women which is in close agreement with the study of Gunn et.al. (1937). He stresses that the variation in cycle length decreases as women get older but on average an adult woman can expect one-third of all her cycles to depart more than 2 days from her mean cycle length. In no case was an example of perfect menstrual regularity noted over any significant period of time although many individuals declared themselves to be the acme of invariability. Arey (1939) concludes that it seems improbable that menstrual regularity, in any true sense of the word, will ever be encountered over a significant length of time because there is as yet no report indicating perfect regularity for even one individual. Beazley and Underhill (1971) confirm this irregularity in cycle length and suggest a correction for the calculation of duration of human pregnancy from Naegele's rule if the cycle is regular but greater than 28 days. Brewer and Jones (1947) reporting on earlier workers who studied the histology of the endometrium, of the ovarian follicle, or of the recent corpus luteum emphasise the importance of the menstrual cycle length by putting forward evidence which indicates that ovulation takes place about

14 days BEFORE the next menstrual period and perhaps their evidence emphasises the uncertainty associated with this particular area of research in addition to the difficulties encountered when attempting to inter-relate two specific times in what may be an extremely flexible cycle. Perhaps even more significant is the fact that the duration of one interval is not necessarily influenced by the duration of its preceding cycle (Gunn et.al., 1937).

It therefore appears that following any given L.M.P. date ovulation in one female could easily occur as long as one week later when compared with another female (Birkbeck et.al., 1975) and this could lead to a considerable error when estimating the Age of the human fetus using ovulation as the criterion.

In addition to this pre-ovulation interval, must be added the undetermined interval from ovulation to conception in order to estimate the true fertilisation Age of the fetus, i.e. from the time of fertilisation. Conception does not follow immediately after insemination (Hess, 1917) and thus there may well be a time lag between intercourse and ovulation, (in which an ovum is fertilised) or between ovulation-and-intercourse of \pm 2 days (Finnstrom, 1972). The viability of the gametes has long been a subject for much research and Cary (1936) noted that normal morphology and migratory power were only two of several criteria that could be used to assess the viability of the male spermatozoa. Several investigators report the findings of living male spermatozoa in the reproductive tract of women at varying time intervals after copulation (Hubner, 1925; Moench, 1934) although Cary (1936) concludes that most spermatozoa are dead within 4 days. Hamilton and Mossman (1972) suggest that there is good reason to believe that human spermatozoa, like those in most mammals, have a short life in the female genital tract and that secretions found in the vagina destroy the fertilising power of the sperm after a short period. This is in close agreement with the work of both Vignes and Boros (1934) and Belonoschkin (1934) who conclude that the survival of the sperm probably does not exceed 48 hours but they attribute this to

the relatively high temperature of the female pelvis when compared with that in the male scrotum. Similar extensive work has been completed regarding the female ovum and from animal experiments it is known that the ovum is capable of being fertilised for only a short time after ovulation. If fertilisation does not take place within a limited period after shedding (rabbit 12 hours, guinea-pig 26 hours, ferret 30 hours) then the ovum undergoes degenerative changes (Hamilton and Mossman, 1972). Thus there is an additional inherent error between the use of Ovulation Age and the true Fertilisation Age and this error, although small, can be variable.

From the date of a single coitus which has led to conception a Coital Age can be determined and the actual Fertilisation Age associated with this cannot be much less because of the limited viability of the gametes (Patten, 1953). Particular use of this has been made in some animal studies (Hughes and Tanner, 1970). The difference however, could still be several days and this is a highly significant interval in the earlier stages of embryonic development (Warwick and Williams, 1973). As an extension to this, Finnstrom (1972) in a study of 34 women who could accurately supply the date of a single coitus leading to pregnancy, calculated the time lag between L.M.P. and intercourse to be 12.6 days with a standard deviation of 3.8 days, results which agree very well with a similar study undertaken by Patten (1953). If this sample was representative of a randomly selected, N-distributed population, the interval would be 12.6 ± 7.6 days for 95% of women. This interval combines the variations from several sources and represents a rather large source of potential error in an accurate estimation of Fertilisation Age of the fetus. In theory, coital age would be fairly accurate as an estimate of true fetal age but the problems associated with determining the actual coitus leading to conception eliminate this method of ageing human fetuses as being of general use.

Other problems which present when trying to use L.M.P. dates include the incidence of anovulatory menstruation. Hartman (1932) described anovulatory menstruation in the Macacus Rhesus monkey and Rock, Bartlett, and Matson

(1939) in a study of adult fertile human females found evidence that bleeding indistinguishable from normal menstruation may occur from a non-secretory endometrium. This was interpreted as indicating an anovular cycle. They found that 4% of mature women regularly had anovular cycles and that 9% had occasional ones. Moreover Hamilton and Mossman (1972) observe that anovular menstruation occurs more frequently between puberty and 18 years and after 35 years. Whilst anovular menstruation may be uncommon, it may nevertheless confuse the use of L.M.P. dates in attempts to assess the age of the human fetus.

Perhaps of more significance is the work of Arey (1939) who found not surprisingly, that for many women, memory alone is completely unreliable as a method of recollecting L.M.P. dates and Beazley and Underhill (1971) reported as many as 22% of women who did not remember L.M.P. dates. Thus the L.M.P. is often completely unknown or very unreliable (Hooker, 1952; Windle, 1970; Birkbeck et.al., 1975) and, if used, must be checked very carefully (Finnstrom, 1972).

When aborted material is being examined, a further error may occur in estimating its age. There may be an indefinable period between cessation of growth and possibly death of the fetus and the actual recovery of the conceptus (Patten, 1953; Warwick and Williams, 1973) and Campbell and Dewhurst (1971) quote two instances of such variation; one where death occurred 10 days after growth had stopped, and the other where there was a 4 week period of growth stasis. This might lead to a considerable error in the estimation of fetal age but with the recent changes in legislation concerning abortion, more fetuses have become available for study and this source of inaccuracy might be less important. Patten (1953) in fact, suggests that the best data are to be obtained by using normally growing embryos which have been removed at hysterotomy.

For most of the menstrual cycle the female is unaware of events except for the period of menstrual bleeding. It is therefore hardly surprising that this landmark is used by the clinician to estimate the age of the human fetus.

It is one of only two easily recognised occasions which may be recalled by the expectant Mother which give an indication of the actual cycle in which conception was achieved. Trolle (1948) suggests that the simplest way for the clinician is to ask about coitus or L.M.P. but emphasises that in the early conceptus it makes a considerable difference whether age is calculated from copulation or menstruation. Park (1968) suggests that our ideas have changed little in 100 years and that we may well have neglected a basic principle by continuing to use Menstrual Age. He records that it is now accepted that ovulation most frequently happens on or about the 14th day of the menstrual cycle and suggests that the length of a pregnancy should be defined as the interval of the time between conception and delivery and should not include a week when pregnancy is unlikely and a further week when it is almost impossible. He stresses that no matter how usual or convenient the previous definition is, it is false and he derides the common practice of discussing, for example, a 20 week fetus, meaning 20 weeks from conception, for a mother who is 22 weeks pregnant, a criticism repeated by Johnson and Odell (1973). He suggests that the present inaccuracy could be overcome by referring to "full term" as being 39 weeks, instead of the usual 41 weeks Menstrual Age. In this way 39 weeks pregnant would be a valid statement.

Thus the error involved in estimating the gestational age of the human fetus from L.M.P. dates may amount to at least ± 1 week even if the dates for L.M.P. are accurate (Finnstrom, 1972). This represents a definite error and perhaps to underline its relative significance, it is disconcerting to find that conception is possible throughout the whole menstrual cycle (Hollenweger-Meyer, 1950) and, in fact, Arey (1949) found that there was no day in the menstrual cycle upon which conception had not been proved to be possible!

Not infrequently when fetuses become available for study there is no clinical history available and therefore even a gestational age based upon the L.M.P. dates of the Mother cannot be calculated.

Many studies have been undertaken with the aim of providing a method by which the gestational age can be determined from measurements of a particular dimension or from the degree of differentiation of the fetus (Scammon and Calkins, 1923; Hill, 1939) and Birkbeck et.al. (1975) present a detailed review of the various parameters that may be involved.

Crown-Heel length (C.H.) has been used extensively as a standard measurement in attempts to relate body length and pre-natal age. Scammon and Calkins (1923) report that previously published empirical formulae are not in accord with present day figures and criticise many of the formulae for being too complicated. In 1923 and 1929, Scammon and Calkins gave mean post-menstrual ages of fetuses of specified C.H. lengths using data quoted from Mall (1910). They report that Mall's observations approximate a parabola and indicate a formula for estimating the post-menstrual age in months from the C.H. length in centimetres. Birkbeck et.al. (1975) have subsequently observed that the lengths at each age are, on average 15 to 20 mm shorter than their own average values. Similarly the mean values of C.H. length reported by Schultz (1926) are slightly higher than those reported by Mall (1910) but are approximately 10 mm shorter when compared with the corresponding age groups reported by Birkbeck et.al. (1975). On the other hand Wich (1972) has reported C.H. lengths even higher than those of Birkbeck et.al. (1975) who themselves suggest that the ages reported by Wich may be unreliable.

Crown-Rump length (C.R.) has also been extensively used as a standard measurement and Streeter (1927) gives C.R. lengths of several hundred fetuses for many of whom post-menstrual ages were recorded. A comparison of his work with the data of Birkbeck et.al. (1975) reveals that the mean lengths for a given age compare quite well although there is a much greater variability in Streeter's data. This suggests that many of the ages must have been very inaccurate and this is emphasised by Schultz (1926) whose data agree fairly closely with Streeter's and

was also derived from the Carnegie Institute Collection. The C.R. lengths reported by Wich (1972) show differences similar to those already reported for C.H. length and it is interesting to note that unpublished data from Japan reported by Birkbeck et.al. (1975) indicate mean C.R. lengths which are appreciably shorter than their own at any given gestational age but which have a similar degree of variability.

An extension of the normal methods used for measuring C.R. length involves the modern technique of ultrasound which allows various fetal measurements to be made in utero. Using this method, Robinson (1973) claimed that measurements of C.R. length in fetuses aged from 6 to 14 weeks post menstrual age, show very little scatter when plotted against age and that such measurements accord well with measurements of the same fetuses made directly after delivery.

Similarly Bi-Parietal Diameter measurements (B.P.D.) are commonly taken using ultrasound techniques (Willocks, 1963; Willocks, Donald, Duggan, and Day, 1964; Willocks, Donald, Campbell, and Dunsmere, 1967; Campbell, 1968, 1969; Ylostalo 1974; Ylostalo and Jarvinen, 1974). Hellman, Kobayashi, Fillisti, and Lavenhar (1969) and Campbell and Newman (1971) have reported in utero measurements of B.P.D. from as early as 12 weeks gestation. Campbell (1970) claims that there is good agreement between ultrasound and direct measurements over the age range 13 to 25 weeks although the averages presented for each week of gestation by Campbell and Newman (1971) are slightly below the regression line of Birkbeck et.al. (1975) whose material consisted of 187 fresh fetuses in whom L.M.P. dates had been very carefully checked. The variability claimed by Hellman et.al. (1969) and Campbell and Newman (1971) is lower than that reported by Birkbeck et.al. (1975) whilst the regression line reported by Hellman et.al. (1969) is considerably below that of Birkbeck et.al. (1975) and clearly diverges from it with increasing age. Wich (1972)

as before, gives B.P.D. measurements of formalin-fixed fetuses which are, in general, considerably larger than those reported by Birkbeck et.al. (1975) for a given gestational age.

Complications concerning the factors affecting growth have resulted in some authors using mean foot length as their measurement whilst others have used the maximum length of whichever is the larger foot (Trolle, 1948). Birkbeck et.al. (1975) used the length of the right foot in 55% of their cases and the left foot in the remainder and they justified this method by stating that no systematic difference was apparent between the lengths of the right and left feet where both could be measured. Their results agreed well with the values reported by Streeter (1920).

Another traditional measurement of fetal maturity has been on the basis of birth weight (Pitkin, 1969) and this principle has been extended into the fetal period so that several studies report a relationship between fetal weight and gestational age (Stockland and Marks, 1961; Ylostalo, 1974; Ylostalo and Jarvinen, 1974). Streeter (1920) recorded the weights of formalin-fixed fetuses and added 5 per cent to estimate fresh weight. His corrected average values agree very well with the data of Birkbeck et.al. (1975) whilst the values reported by Wich (1972) are considerably higher than those reported by Birkbeck et.al. (1975).

Other measurements which have been used to estimate fetal gestational age include various limb lengths (Scammon and Calkins, 1929; Krogman, 1941; Olivier, 1969) and X-ray techniques which have been advocated include fetography (Daw, 1973), the length of the fetal spine on a roentgeogram (Fagerberg and Roonemaa, 1959; Chang, Woesner, Nakamoto, and Sanders, 1971), the calcification of fetal teeth (Lemons, Kuhns, and Poznanski, 1972) the measurement of fetal long bones (Brandfæss and Howland, 1967; Martin and Higginbottom, 1971; Owen 1971), and the detection of ossification centres (Adair and Scammon, 1921; Christie, Martin, Williams, Hudson, and Lanier, 1950; Schreiber

Nichols, and McGarity, 1963; Chan and Khoo, 1965). However Kelly (1974) records that since none of these techniques is consistently reliable and all entail some degree of risk, many radiologists have transferred their interests to the application of ultrasound.

Irrespective of the fetal dimensions selected for measurement a problem common to all is the accuracy of the techniques used for measuring the body profiles. Schultz (1926) in a very detailed review suggests that fetal measurements should correspond, wherever possible, to those in general use on adults and his measurements are based on this principle. Schultz (1929) poses two questions which should influence the measurements to be used: What is to be measured? How is it to be measured? He suggests that end-points should be determined in an accurate unequivocal manner and stresses that the publication of measurements without adequate definition of the methods used is a waste of time. He reports that this is, unfortunately, a very frequent occurrence and adds weight to his criticism by noting that no measurement is of any interest by itself and is intended for comparison either with the measurement of the same structure in another animal or with other measurements in the same animal. Trolle (1948) suggests that the lengths must be well-defined, simple, and easy to measure although he does not conform to this practice even in his own writings. Schultz (1929) supports the development of unification of measurements in physical anthropology by international agreement and stresses the importance of such work, for example, by Hrdlicka(1920). The measurements developed by Hrdlicka(1920) however, were mainly designed for studying man after birth and for pre-natal growth certain minor alterations, which were unavoidable, were made and reported by Schultz (1929). Reicher (1923) reporting on the differences in techniques used by several authors comments that such differences mean that the data cannot be compared, whilst in addition he also criticises many studies for possessing too little material.

Schultz (1929) suggests that the definition of a particular measurement

must include some reference to the orientation of the specimen and stresses that all specimens should be in the same position. With particular reference to the fetus, Schultz (1929) records that the limbs can never be completely extended and this is particularly true of the leg and the thigh which cannot be brought completely into line. On such material therefore, it is impossible to measure the total length of the limbs or the height of the body and, on this basis, Schultz (1929) is very critical of the measurement of C.H.length. Schultz (1929) also suggests that all measurements should be taken on the same side in case of any systematic discrepancy and suggests the adoption of the left side for this procedure.

No measurement should be considered as final until the investigator has controlled the accuracy by measuring the same specimen repeatedly until his measurements are identical (Schultz,1929). This is particularly applicable to the fetus for after preservation there is no problem of movement during measurement and therefore it will be thought possible that landmarks can be determined with rather greater accuracy than in the living subject. Scammon and Calkins (1925) confirm this approach by noting that the error of measurement for a living specimen is greater than that for a fresh specimen, whilst that for preserved material is least.

A certain proportion of the discrepancies between repeated measurements taken on fetuses is due to the effects of the method of preservation which may swell or shrink the delicate fetal tissues to a certain, albeit limited, extent (Jackson,1909; Schultz, 1919). Scammon and Calkins (1929) also examined the effects of formalin on fetal dimensions and found that they may vary according to the dimension measured, also with the method of fixation, and with time, but Reicher (1923), and Birkbeck et.al. (1975), bearing these results in mind, suggest that they are minor with respect to the dimensions under discussion. Schultz (1926) suggests that the use of relative lengths would overcome this problem.

Unfortunately however, the data from all these various types of studies suffer from the difficulty of equating fetal dimensions or degrees of differentiation with the actual time of conception which can rarely, if ever, be exactly established. Perhaps even more unfortunate is the fact that most of these studies do not give any statistical evaluation of their results (Scammon and Calkins, 1929) and, in particular, very few investigators include a value for the standard deviation about the regression line they have calculated thus making it impossible to establish confidence limits. This particular criticism unfortunately must be levelled at most of the early investigators such as Friedenthal (1914), Arey (1949), and Hamilton and Mossman (1972), whose graphs and tables have been repeatedly reproduced in many general text books on this subject. As valuable as their work has been, meaningful estimates of fetal age cannot be made unless indications of the deviation or error involved are also available. An example of earlier work where confidence limits are expressed is to be found in the modification of Boyd's monograph produced by O'Rahilly and Meyer (1956) where the unusual confidence limits of 82% are expressed which represent a deviation of ± 25 days at 150 mm Crown-Rump length. This study also demonstrates that the deviation about the regression line is not constant for the range of material studied (Willocks, Donald, Duggan, and Day, 1964; Campbell, 1969; Campbell and Newman, 1971). Several more recent papers include statistical data in their contents (Birkbeck et.al., 1975).

In some studies comparisons have been made between various methods for estimating gestational age on the basis of the more commonly calculated 95% confidence limits. This type of comparison however is only valid where the age distributions of the two sets of material are identical (Finnstrom, 1972). This is because the breadth of the confidence limits is dependent both on the age distribution of the material studied and the correlation coefficient between the two variables (Finnstrom, 1972). Thus great care must be taken when evaluating

the various methods of determining gestational age.

In some studies the confidence limits have also been wrongly interpreted (Brody, 1960; Kirschaum, 1962). These studies claim to have reached the uppermost limit of precision in estimating gestational age since their 95% confidence limits for estimating age (from the mean value of fetal haemoglobin) are close to the 95% confidence limits for the duration of pregnancy. The problem however is that these two confidence limits actually refer to different phenomena and so cannot be compared. One set of confidence limits reflects the precision of the method for estimating gestational age, whilst the other set represents the natural range for the duration of pregnancy (Finnstrom, 1972).

Finnstrom (1972) in a review of the available literature concerning methods available for estimating the gestational age in new born infants, compared and contrasted the 95% confidence limits of 16 methods which incorporated either a single variable or a combination of variables. The range extended from:

1) B.P.D.

$\bar{\pm}$ 34 days

to

2) External characteristics and neurological examination + head circumference + femoral epiphysis + motor conduction velocity of ulnar nerve

$\bar{\pm}$ 19.5 days

The values he gave for the combination of methods were those in which the variables entered the multiple regression at significant F-values ($P < 0.05$). In this way he was able to decrease his confidence limits by $\bar{\pm}$ 9.4 days by using the combination of the five methods above instead of birth weight alone which had the lowest confidence limits of the single variable methods with $\bar{\pm}$ 28.9 days. He reduced his confidence limits still further by either selecting only those infants above the 10th percentile by weight or by using only males or females. Finnstrom added that it was difficult to evaluate the results of most of these studies because there is always a tendency to over-estimate the accuracy of

determining gestational age. He stressed the need for studies in which different methods for estimating maturity are applied to the same material which would thus make it easier to compare different methods.

Although many graphs based on large numbers of observations have been constructed in order to provide averages, the absolute size of neither fetus nor embryo gives a reliable indication of its true age or stage of structural organisation (Warwick and Williams, 1973). The relatively wide biological range of variation in normality limits the accuracy of prediction models (Finnstrom, 1972; Robinson, 1973). There is every reason to believe that there is true variation in development among infants of the same gestational age and O'Rahilly and Meyer (1956) criticise measurements of fetal length because it is apparent from the publications of Streeter (1951) that there is considerable variation in length among human fetuses of the same developmental stage. Studies on Motor-Conduction velocities and neurological development in particular, in infants whose mothers could state the probable day of conception, support this (Blom and Finnstrom, 1971). The extent of this variation is not known but experiments such as those mentioned above and experiments concerning the development of twins, suggest that it corresponds to at least ± 1 week (Cope and Murdoch, 1958; Finnstrom, 1972).

Taking the error inherent in the estimation of gestational age from the dates of L.M.P. together with that attached to natural biological variation it appears that a prediction model for estimating fetal age which is based upon a single variable will probably not permit precision in the estimation of ± 2 weeks or even less, as has been claimed by several authors. (Kirschaum, 1962; Falkner, 1966; Dubowitz, Whitaker, Brown, and Robinson, 1968; Dubowitz, Dubowitz, and Goldberg, 1970). A single variable prediction model which can be empirically shown to estimate gestational age to within 3 weeks for all but a few infants can be regarded as satisfactory (Finnstrom, 1972). Results of tests however have indicated that it is possible to obtain a precision in the age estimation of

+ 2½ weeks for 95% of the fetuses but only when using a combination of variables (Finnstrom, 1972). This is supported by Kelly (1974) who suggests that with several methods available it is always best to rely on a battery of tests rather than only one or two. Evidence which gives some indication of the problems in this area is provided by Trolle (1948) who reported that tables purporting to represent similar estimates were not quite parallel and the discrepancies must be representative of the variabilities involved.

Fetal growth is the final common expression of the interaction of many different forces. It is only in recent years that the various systems and mechanisms involved have begun to be fully appreciated and several factors have been revealed which can directly affect the progress of fetal growth (Anson, 1966). These must therefore be kept in mind when collecting fetuses for study. If fetal weight is taken as an indicator of fetal growth then it becomes possible to compare studies which have investigated these factors, although it must be realised that at present, many of these studies have been restricted to the perinatal period.

Environmental factors can include ethnic origin and the mean birth weights can vary to an extraordinary degree between different populations (Schultz, 1926; Birkbeck et.al., 1975). Even within populations, marked secular changes can occur (Ounsted and Ounsted, 1973) and this may be related to the consistent finding that pregnancy at high altitude substantially slows the rate of intra-uterine growth (Ounsted and Ounsted, 1973). In our own British society, social class, which is often considered as a factor affecting growth, tends to be an inconstant variable within a family and has been shown to be markedly unstable over the generations. Therefore it is now considered a doubtful measurement for biological studies (Ounsted and Ounsted, 1973).

Maternal factors per se can effect fetal growth and disease and illness may be particularly important. Although the prevalence of diabetes mellitus is low nevertheless it has been shown that its presence usually enhances the growth rate of the fetus (Ounsted and Ounsted, 1973). Using ultrasound however it has

been shown that with the fetuses of diabetic mothers the normal standards for B.P.D. and gestational age and rate of growth hold true through to the 37th week of pregnancy and it is only beyond this time that the greater size and rate of increase makes the correlation less reliable (Kelly 1974). Cardell (1953) suggests that this difference only becomes apparent in fetuses delivered after a gestation length of 28 weeks or more and this is supported by Hsia and Gellis (1957). Cardell (1958) has shown that fat, oedema, and size of body organs are not important considerations. With hypertension and pre-eclamptic toxæmia, Kelly (1974) reported that the B.P.D. as estimated by ultrasound was retarded in 50% of the fetuses although opinion on this matter varies greatly (Ounsted and Ounsted, 1973). Among other maternal agents which may affect fetal growth is the habit of smoking and there are many studies which have shown that smoking by a pregnant woman reduces the growth rate of her fetus (British Medical Journal, 1973; Ounsted and Ounsted, 1973). Regarding age and parity Ounsted and Ounsted (1973) believe that any positive associations between fetal growth rate and maternal age per se may well be spurious although the Perinatal Mortality Survey Data (Butler and Alberman, 1969) clearly show that second and subsequent babies grow faster than the first. A number of suggestions have been put forward to explain this increased rate of fetal growth rate with increasing parity and they include differences in fetal environment such as enhanced efficiency of uterine circulation in second and subsequent pregnancies (Ounsted and Ounsted, 1973) coupled with a change in immunological factors whereby the mother becomes sensitised to paternal antigens (Ounsted and Ounsted, 1969). Warburton and Naylor, (1971) however confuse the issue by reporting that after a paternal change there was no increment in birth weight or placental weight. Furthermore increasing the parity after the first birth did not appear to have much effect upon fetal growth rate (Ounsted and Ounsted, 1973). Several studies have shown a positive association between maternal stature and birth weight (Ounsted and Ounsted, 1973) but this factor might well

be incorporated as a genetic factor which may be associated with normal biological variation. The presence of a multiple pregnancy has also been shown to dramatically influence the growth of the individual fetus and in addition the gender and zygosity of fetuses in a multiple litter may have a subtle influence on the growth rate (Ounsted and Ounsted, 1973).

An important factor affecting the rate of growth in the fetus is its sex. Male fetuses have been found to grow faster than females and on average weigh 150g. more at birth (Ounsted and Ounsted, 1973), whilst Finnstrom (1972) was able to reduce his confidence limits by using only males or females for his regression equation. Surprisingly however Trolle (1948) who determined the sex of 158 cases of spontaneous abortion found that after the third month significantly more male than female fetuses are aborted. In addition, many congenital abnormalities can influence fetal growth rate and although they may be comparatively rare, there are many syndromes in which the incidence of children with at least one major malformation evident at birth or soon after, (stillbirths included), is of the order of 15/1000 total births (Ounsted and Ounsted, 1973). More often however it is the less dramatic but nevertheless equally effective infections such as rubella or syphilis which may reduce fetal growth rate (Ounsted and Ounsted, 1973).

Thus, there are many factors whether they be environmental, maternal, or fetal which can effect the growth of the fetus in utero and consequently have to be borne in mind when selecting a sample upon which to base measurements of various fetal parameters.

The complications surrounding the accurate ageing of the human fetus has led to some studies, such as Forbes (1951) and Moss, Noback, and Robertson (1955), stressing the importance of relative growth between bones and the development of differential growth analysis in the study of human growth, although Schultz (1929) argues that few proportions or indices are of interest by themselves. Similarly Noback and Robertson (1951) noted the times of appearance of

ossification centres in terms of a body dimension as they considered this to be the most accurate estimate of fetal age possible. This method of ageing has a great deal to commend it in terms of simplicity and accuracy. Streeter (1951) made an excellent attempt to overcome the problem of ageing and developed his now famous Horizons in Human Embryos where he abandoned the idea of chronological ageing and assessed the maturity of the embryos, planning to establish 25 stages of development or "horizons". He later decided that stage 23 would mark the ending of the embryonic period because the end of this stage is clearly defined by the onset of marrow formation in the humerus. These "horizons" have been used extensively in embryonic work (Gardner and O'Rahilly, 1968; Gray and Gardner, 1969; Windle, 1970) and it is unfortunate that no similar system exists for the fetal period since this would reduce many of the forementioned problems.

THE SUITABILITY OF RADIOGRAPHY AS A METHOD OF STUDYING

FETAL SKELETAL DEVELOPMENT

The prenatal development of the human skeleton has been described, illustrated, and recorded in considerable detail by many authors using various techniques; these include:-

- 1) Clearing of the soft tissue (Mall, 1906; Horand, 1908) followed by staining with alizarin-red S (Augier, 1931; Teissandier, 1944; Noback and Robertson, 1951; Felts, 1954).
- 2) Radiography (Jonata, 1938; Hill, 1939; Flecker, 1942) preferably after silver impregnation (Sullivan, 1930; O'Rahilly and Meyer, 1956; Kjar, 1974).
- 3) Serial sectioning - used to a limited extent for skull, vertebrae, and limbs. (Zawisch, 1953; 1954; Gray, Gardner, and O'Rahilly, 1957; Gray and Gardner, 1969; Babler, 1975).
- 4) Gross dissection.

Many of the earlier investigators ranked the techniques in the following order of usefulness:-

- 1) Serial sectioning.
- 2) Clearing, with alizarin-red staining.
- 3) Radiography.
- 4) Gross dissection.

(Noback and Noback, 1944; Noback and Robertson, 1951; O'Rahilly and Meyer, 1956) and they based their judgement on the length of time between the first appearance of a centre of ossification and when it could first be recognised by a particular technique. Radiography, in particular, did not rate very highly in the opinion of the early investigators. For example, Mall (1906) using the alizarin-red technique was able to demonstrate the presence of the parietal bone at Crown-Rump Length (C.R.) 31 mm whilst Hill (1939) using radiography was only able to demonstrate its presence at C.R. 194 mm. Whilst this is possibly an extreme example, Hess (1917) reported that the time difference in detecting the first

appearance of an ossification centre using a serial histological sectioning technique as compared with radiography was approximately one week and he obtained these results using the two different methods on the same fetuses. He did not condemn radiography outright however, and suggested that:-

"the roentgenographic method is undoubtedly capable of much greater refinement by further studies and observations." (Hess, 1917).

Before 1956, radiography was considered to produce unsatisfactory results during early fetal life because of the lack of sufficient radio-opacity, although the method was simpler and quicker than either clearing or serial sectioning. The current feeling was that radiological estimation of fetal maturity was virtually useless and that an estimate was correct to within only 5 or 6 weeks. Hartley (1957), who reports the popular feeling of that time, was however at variance with such opinion and conducted experiments using a high standard of radiography which was designed to reveal fetal morphology in great detail. He was able to show clearly that a very real and steady pattern of bony and epiphyseal development does exist in the human fetus and that this development is easy to recognise, a fact confirmed by Noback and Robertson (1951) and O'Rahilly and Gardner (1965). This is in contrast to the studies of Stevenson (1924) who warned against the use of radiographs because they were merely;

"a confusing medley of shadows" (Stevenson, 1924).

Hartley (1957) stresses that a definite pattern of fetal skeletal development is only demonstrable in radiographs produced specifically for this purpose, and that a precise estimate of fetal maturity from radiographs taken for other purposes will often prove impossible. This reservation must be applied particularly when the removal of different parts or organs is contemplated since Noback and Noback, (1944) reported striking differences in the relative sensitivity with clearing before radiographing and O'Rahilly and Meyer (1956) found that the centrum T₉ became apparent radiographically after removal of the viscera.

Hodges (1953) thought that the density differences throughout the whole fetus are so slight that there is a risk of error in recognising the earlier ossification centres. Since the 1920's his Department had been employing heavy metal salt impregnation to increase the radiological density of various tissues and, having discarded lead nitrate, after several trials they selected silver nitrate as being the more suitable. Hodges (1953) records that after 2 or 3 days immersion in 0.5% aqueous silver nitrate, there was partial replacement of the calcium by silver in the terminal portions of the primary ossification centres, peripheral portions of the epiphyseal centres, and eventually throughout all parts of the calcified skeleton, with only a little silver being deposited in the soft tissues when immersion was continued still longer. His illustrations of the results of this technique demonstrate the quite dramatic improvements in the radiological image which can be obtained and the technique has the added attraction of not involving the destruction of the soft tissues. However, Cameron (1930) concludes that silver nitrate is not a test for calcium or phosphate. His enquiries into the histological tests which are commonly supposed to indicate calcium salts, showed that there is intense reduction of the silver throughout the bony areas, a reaction which completely disappeared with decalcification. He found that the test only gives a demonstration of a deposit of inorganic material which is in fact in most instances composed of calcium phosphate or carbonate, with the black reaction being given by a variety of solid deposits of which the anion is more important than the metal. This lack of specificity has been acknowledged more recently by O'Rahilly and Gardner (1972) and Cook (1974) although Pearse (1953) notes that the test is usually regarded as being specific for calcium because the insoluble phosphates and carbonates that are present are nearly always those of calcium, and Bancroft (1967) suggests that this must always be borne in mind.

Cameron (1930) similarly criticised the method of alizarin-red S staining. He found that alizarin is very nearly a specific stain for calcium which reacts readily in vivo and in vitro with recently deposited calcium phosphate or carbonate,

normal or pathologic, but which often fails to stain older deposits, thus limiting its usefulness. Cameron (1930) comments that the method is fairly selective for the staining of the bones which constitute the most important depots for calcium salts, and records that the alizarin reaches the deposit by way of the tissue fluids where it unites with any calcium that is available. However, he acknowledges that most areas of calcification are extremely complex in structure and that calcium itself makes up only a portion of the deposit. This lack of specificity for the demonstration of calcium is again reported by O'Rahilly and Gardner (1972).

To assess the discrepancies found between the various techniques used to study the onset of prenatal ossification, Meyer and O'Rahilly (1958) used three different methods on the one specimen; clearing followed by staining with alizarin-red S, radiography after silver impregnation, and serial sectioning. Four human fetuses were used and these were bisected in the median plane, one half being cleared and stained with alizarin-red S whilst the other half was impregnated with silver and radiographed. Also this latter half was subsequently sectioned and stained. Thus the onset of ossification could be compared using different techniques on the assumption that the rate of ossification is similar on the two sides of the body. As had been anticipated, histological examination of the serial sections yielded results superior to the other two methods because bone as a tissue can be most critically defined using histology. It is interesting to note that the photomicrographs showed that silver deposits had occurred not only in the circumferential osseous areas but also in the cartilage in the centre of the shaft which presumably had been calcified. The deposit of silver in the ossified tissue was found to be confined to the bone first laid down and, therefore, there was a silver-free osseous area, which stained blue, in a sub-periosteal position around the middle of the shaft. This blue area covered the silvered area but also extended further both proximally and distally. Their results using the alizarin-red S technique appeared to be slightly superior to those of the radiographic method

with several middle phalanges of the hand being made visible, a result which agrees well with their previous work (O'Rahilly and Meyer, 1956). Kjar (1974) however, used a higher concentration of aqueous silver nitrate (10%) and found that all mineralised tissue showed silver impregnation whilst there was no impregnation found in non-mineralised tissue. These results are contrary to those of Meyer and O'Rahilly (1958) and suggest that this technique requires further investigation in view of the many factors which may influence it as discussed by McGee-Russell (1958). Meyer and O'Rahilly (1958) also consider the type of ossification that is demonstrated by each technique. The initial osseous deposit around the cartilage of the long bones is intramembranous and becomes the periosteal collar. After a delay of between 1 and 5 weeks in the hand (Gray, Gardner, and O'Rahilly, 1957) and sometimes more in the foot, vascular invasion of the cartilage by the periosteal bud takes place and it is only after this event that endochondral ossification commences, a result confirmed by Babler (1975). The results of the histological examinations of their sections by Meyer and O'Rahilly (1958), to determine which had undergone vascular invasion, showed that alizarin and radiography do not demonstrate the onset of endochondral ossification. Rather their first positive reaction was found to coincide fairly closely with the laying down of the periosteal collars and Meyer and O'Rahilly (1958) consider that in the strictest sense this should not be regarded as a centre of ossification.

Very few writers commit themselves as to what they mean by ossification centre and Zawisch (1956) considered the terms "centre" and "nucleus" as applicable only in the case of endochondral ossification. This interpretation is confirmed by Ham (1957):

"When the osteogenic cells, osteoblasts, and capillaries of the periosteal bud reach the interior of the mid-section of the cartilage model, they are said to constitute a centre of ossification."

Meyer and O'Rahilly (1958) therefore believed that only with the advent of the

onset of endochondral ossification may a CENTRE of ossification be said to be present and they concluded that histological examination is necessary in order to detect this phase.

From a clinical point of view, only radiography can be applied to study the onset of ossification on the living fetus, but unfortunately radiography of a fetus in the first half of pregnancy is not common. Hartley (1957), Bishop (1965), and Russell (1973) record that the fetus is not visible radiologically until the 10th week of pregnancy and until the 13th week it cannot be consistently identified. The parts which can be identified at this time are skull-base, spine, and the lateral margin of the rib cage but the centres can easily be obscured for example by maternal features such as fecal material and Bishop (1965) therefore believes that negative findings must be interpreted with caution. By the 14th week Russell (1973) states that the fetus should be readily recognised and at this time the skull vault and long bones are also identifiable. Between the 14th and 26th weeks a subjective assessment of fetal maturity can be made from its radiological appearance (Hartley, 1957) although Russell (1973) does not suggest the use of any particular standards to be used for this assessment.

Antenatal radiography, however, carries with it certain hazards (Stewart, Webb, Giles, Hewitt, 1956; Bishop, 1965; Schwarz, 1968) some of which may be very serious, for example an increased risk of leukaemia or other abnormalities (Russell, 1973). However, it is only in obstetrics that the use of diagnostic X-rays has been materially curtailed because of fear of fetal damage (Hartley, 1957) although it has been realised for a long time that radiation may cause tumours, particularly of the skin. On the other hand, Russell (1973) and Russell and Pritchard (1975) report that since the original paper of Stewart et al. (1956) improvements in radiographic techniques have led to a 30-fold reduction in the fetal dose and, although the risk of genetic damage from antenatal radiography is normally small, it does not add appreciably to the hazards to which man's

chromosomes are already subjected. On theoretical grounds however, there is an increased genetic risk present from irradiation in early pregnancy, including the time around conception, which has caused the development of the "10-day" rule in the radiographic examination of women (Schwarz,1968). Furthermore, it has been shown that irradiation of animal fetuses during early pregnancy may result in a failure of normal development of the fetus, and it has been clearly demonstrated that the abnormalities which occur are related to the stage of development of the fetus at the time of exposure (Bishop,1965; Schwarz,1968). Russell (1973), summarising the available evidence, concludes that the hazards of antenatal radiography seem acceptable provided radiographic examination is clearly indicated.

Carmichael and Berry (1976) conducted a survey of the current practice of radiology in late pregnancy and in neonates in the United Kingdom. They found that up to 34.8% (average 23%) of all pregnancies were still X-rayed as well as up to 10% of neonates, and warned that time has dulled the earlier warnings of Stewart et.al. (1956). Russell (1976) shared the desire of Carmichael and Berry (1976) to reduce the fetal-radiography-rate but stressed that it is often only in retrospect that radiography is found to have been unnecessary. Russell (1976) records that using poor technique, one film could represent an X-ray exposure forty times larger than need be used and suggests the introduction of low-dose techniques. Hobbins and Taylor (1976) report that fetal-irradiation is less than 9% in the Yale-New Haven Hospital and stress the desirability of avoiding all irradiation during pregnancy. In its place they advocate the development of ultrasound techniques but record that it is virtually unavailable in Britain except for a small number of centres, a fact supported by Swinhoe (1976).

PRE-NATAL OSTEOGENESIS

Mineralisation is only one of the stages in the process of ossification and is a process that is fundamentally similar wherever it occurs (Gardner,1971). It has been the subject of many investigations and Gardner (1971) gives a comprehensive summary of the relevant literature and a very lucid description of the stages involved.

All bones begin as mesenchymal condensations and where these condensations are mainly fibrous or membranous, the intramembranous ossification that occurs forms "membrane bones". Many bones, especially long bones, however, pass through a stage of chondrification which produces a cartilage model, before periosteal and endochondral ossification finally takes place and results in the production of "cartilage bones". Gardner (1971) believes that it is the environment which determines the type of ossification that occurs.

Bick (1952) believes that a proper understanding of the progress of events occurring during osteogenesis of the human vertebra has become increasingly desirable but has not, as yet, received adequate attention. His study involved the investigation of the histology of the developing human vertebra ranging from a 14 week old fetus to a 90 year old female, but in his report he does not say which particular vertebrae he investigated. Sensenig (1949) considers this to be a very important point because the somites form in a craniocaudal direction and because of this there is an axial gradient of vertebral differentiation, which is already under way in the cervical region before the caudal somites are developed. Because of this gradient Sensenig (1949) emphasises that in a single embryo it is possible to study in a craniocaudal direction successive stages of development of the vertebral column.

In a 14 week fetus, Bick (1952) found that the body of the vertebra was composed chiefly of early connective tissue with cartilage cells forming the centre

of the area. Both the nucleus pulposus and annulus fibrosus of the vertebral discs were found to be already in their proper positions relative to the vertebra and the anterior and posterior longitudinal ligaments, which presumably will play an important role in the maintenance of vertebral stability were also found to be visible and well developed. In a 15 week fetus, Bick (1952) found that the cartilaginous field had enlarged and calcified with the first evidence of formation of osteogenic tissue being found at the centre around advancing blood vessels, an association which he particularly stresses. The cartilage mass was found to have expanded but still occupied only about half of the vertebral body. By 18 weeks, the osteogenic centre was found to be well formed with the ossified area beginning to exhibit signs of trabeculation but still possessing the irregularity and greater vascularity of primary bone. In a 25 week fetus the ossification centre had extended to the edge of the body on each side of the vertebra, but, as yet, no appositional bone had appeared from the periosteum or perichondrium of the vertebra to supplement the endochondral growth. At this time columns of cartilage cells also made their first appearance at the extremities of the ossifying mass forming what Bick (1952) described as true diaphyseal plates which were synonymous with epiphyseal plates. In mammals Bick (1952) explained that these diaphyseal plates are the equivalent of epiphyseal cartilage in a typical long bone, such as the femur, the difference being functional and not histological. Bick (1952) found that neither at this stage nor any other does an epiphysis form beyond the diaphyseal plate in the human vertebra. In a 3 day old post-natal male Bick (1952) found that the ossifying centre was well-formed with the trabecular structure assuming a predominantly vertical orientation which he believes to be in anticipation of the impending assumption of orthograde posture. Furthermore he believes that this must of necessity be the result of stresses and strains produced by normal fetal muscle tone and relates the progressive increase in trabeculation by 3½ years of age to locomotor activities such as sitting up, walking

and running.

Ossification of long bones in the limbs and development of the intervening joints has been the subject of many investigations (Gardner and Gray, 1950; Gray and Gardner, 1951; Gardner and Gray, 1953; Gray, Gardner, and O'Rahilly, 1957; Gardner, Gray, and O'Rahilly, 1959; Gardner and O'Rahilly, 1968; Gray and Gardner, 1969; Gardner and Gray, 1970; Gardner, 1971; O'Rahilly and Gardner, 1972).

Gray and Gardner (1969) realised that a detailed report of the histogenesis and ossification of the human humerus from the time of its first appearance to birth was not available although a thorough search of the literature had revealed fragments of the total picture. They therefore studied 40 pairs of humeri from a series of human fetuses which ranged from 26 mm to 342 mm C.R. length and radiographed each bone. They silverized all the right humeri and then radiographed these again but because the radiographs did not show endochondral ossification until 69 mm C.R. length, longitudinal sections were used to aid the identification and also to measure the length of the ossified shaft. By the end of the embryonic period, they found that the shaft is surrounded by a bony collar which was present in their youngest specimen of 26 mm. In their 27 mm fetus this primary bone collar had extended to include $\frac{1}{3}$ rd of the length of the humerus and this collar was not found to be visible radiographically. At 34 mm C.R. length the erosion of the bony collar by the periosteal bud marked the end of the embryonic period according to Streeter's (1951) classification and endochondral ossification was found to have begun by 37 mm. Until about 150 mm C.R. length, the periosteal bone formation was found to extend approximately 1 mm. beyond the zones of cartilage destruction and after 150 mm the extent of both periosteal and endochondral ossification was found to be the same. In their smallest specimen of 69 mm C.R. length in which ossification had been noted radiographically, nearly one half of the humerus was found to be occupied by the endochondral bone and somewhat more of the shaft was found to be ossified when extensions of the periosteal bone as seen histologically were considered. This fraction increased until term, when almost $\frac{4}{5}$ consisted of bone.

Gardner and Gray (1970) in another paper of their extensive series of observations on fetal ossification, subjected the human femur to an investigation similar to that for the humerus. The major differences appear to be that the humerus can precede the femur at times in its state of development and that the femur has been studied by more investigators than is the case with the humerus. Gray and Gardner (1950) found that a primary bone collar was forming in the femur of an embryo of 20mm C.R. length whilst O'Rahilly, Gray, and Gardner (1957) and Gardner and O'Rahilly (1968) found it to be present in Streeter's stages 22 and 23 (1951), having changed their method for fetal ageing. Gardner and Gray (1970) found that their smallest specimen in which ossification was noted radiographically was 61 mm. and Gardner and Gray (1950) noted that this ossification has almost reached the neck of the femur by 85-95 mm C.R. length.

Several factors are known to affect ossification but the studies upon which the conclusions are based are mainly concerned with postnatal life. The literature on factors affecting antenatal ossification appears to be comparatively limited.

Pryor (1906) demonstrated that the bones of the female ossify in advance of the male and Pryor (1923,1933), continuing his earlier studies, examined 140 fetuses ranging in age from 10½ weeks to 38 weeks which included 71 males and 69 females. He found centres of ossification in the distal epiphyses of the femur surprisingly as early as 25-30 weeks in females and in the rather later stages of pregnancy (30-40 weeks) in males and therefore concluded that in the embryo and throughout intra-uterine development the bones in the female are in advance of the male. Furthermore he concluded that this difference increases progressively from days to weeks to months and that such advances are taking place even before external sex-differentiation can be determined. Hill (1939) states that it is in the seventh fetal month that skeletal differences between male and female fetuses can be observed and Flecker (1942) notes that the priority of the female fetus is clearly marked. After birth there is abundant evidence that ossification

in the female is in advance of that in the male (Menees and Holly, 1932; Dunham, Jenss, and Christie, 1939; Christie, Dunham, Jenss, and Dipple, 1941) with perhaps the most conclusive evidence being shown by Cope and Murdoch (1958) who found in a study of twins sharing the same period of gestation that the female is more advanced than the male. Warwick and Williams (1973) summarising the available evidence conclude that in all studies up to that date the female antedates the male and that the difference, although apparently trivial, and perhaps insignificant before birth, becomes progressively greater thereafter, whilst Gardner (1971) suggests that there might well be a sex difference in the onset of ossification but this remains to be determined.

Garn, Burdi, and Babler (1974) however, record that in earlier work they found male advancement in primary dentition rather than the anticipated female advancement. Intrigued by this finding and realising that, during the first trimester, female advancement is not definitely acknowledged possibly because of lack of sex specific embryological data, Garn et.al. (1974) looked at the developing hand. They used two series of embryos all between 15-75 mm C.R. length and all clearly identifiable as to sex by histological examination of gonadal development. Their results showed that when comparing hand development of male and female embryos of the same size, male advancement was seen to be a consistent feature, primarily in the 15-30 mm C.R. range, and they concluded that there is a rather large sex difference in early development and developmental timing of the hand skeleton. Their findings are contrary to other postnatal findings but are consistent with palatal closure and early prenatal dental development. The significance however is primarily between 15-30 mm C.R. length and although there is a similar trend between fetuses of 30-70 mm C.R. length it is less significant.

Several studies have shown that factors other than sex, can affect ossification. Dunham et.al. (1939) and Christie et.al. (1941) conclusively demonstrated that not only sex but also race is of the utmost importance in the

appraisal of the osseous pattern of new born infants. Francis and Werle (1939) have shown that epiphyseal ossification is a more sensitive index of constitutional health than is progress in height or weight and also that ossification shows the greatest retardation with severe or prolonged illness, although Hill (1939) has suggested that the protection against nutritional deficiency and illness as afforded by prenatal existence may lead to the variability in the dates of appearance of the ossific centres not being as marked as in infancy. Cope and Murdoch (1958) have shown conclusively in a study of twins that an individual variation in appearance exists and other studies have stressed that heredity, race, sex, nutrition, endocrine secretions, and disease may all be important factors affecting the timing of the initial appearance of ossification centres (Shelton, 1931; Sidhom and Derry, 1931; Clark, 1936; Noback, 1944; Elgenmark, 1946; Christie, 1949; O'Rahilly and Meyer, 1956; Ryan and Berry, 1972; Warwick and Williams, 1973). O'Rahilly and Meyer (1956) have suggested that when attempting to relate these factors to antenatal life a major obstacle is the difficulty in obtaining sufficient information and data for a particular fetus.

MECHANICAL FACTORS AFFECTING OSSIFICATION

The capacity of living bone to adapt its structure to meet mechanical demands has been known for a very long time (Bassett, 1966) and according to the classical theory of functional structure of bone which was developed by Wolff (1892) bone is laid down in its gross form as well as in minute architecture in accordance with a "maximum-minimum" law. This law states that as a result of functional adaptation maximum efficiency is achieved with a minimum of material, but several variations of this law are reported (Felts, 1959; Bassett, 1971). Jansen (1920) stated the law simply by saying that "the form of the bone being given, the bone elements place or displace themselves in the direction of the functional pressure" and Bassett (1966) reminds us that the law is in constant use in orthopaedics where it has been realised that skeletal growth can be influenced by the appropriate application of external forces. Bassett (1966) concludes that this adaptive mechanism allows the organism to achieve harmony with its surroundings so that it is not limited to a skeletal shape or size pre-determined by inflexible genetic and hormonal factors.

Felts (1959) acknowledges this concept of skeletal plasticity and dependency of organisation upon the functional environment, but considers that the concept of environmental influence has been over-stressed in relation to skeletogenesis. He believes that the differentiation and early development of the skeleton is dependent as much if not more upon hereditary factors than upon functional circumstances and his beliefs have been upheld by many investigations (Murray 1936; Fell 1956; Amtmann 1971).

Fell (1956) reporting on her earlier work and that of other investigators recorded that skeletal tissue was one of the earliest to be cultivated in vitro in an organised state but at first she was only able to develop nodules of cartilage in axial mesoderm, and these nodules bore little resemblance to the normal limb skeleton. In later work she was able to record the first observation

of periosteal bone in culture and reports on other workers who were able to cultivate bone rudiments already defined in shape to such an extent that they grew and became calcified. Since these early experiments techniques have improved greatly and have led to even further development of cartilage and bone in vitro. All these culture experiments suggest that the gross form of limb elements is the result of self-differentiation, and that each developing element is under genetic control (Murray, 1936). However, the results also suggest that these factors alone are not sufficient to produce a functional skeleton (Murray, 1936).

Fell (1956) concluded that when undifferentiated chondrogenic and osteogenic tissues are grown in culture they acquire an almost normal histological structure. She also concluded that when entire primordia are cultivated, although they too develop their characteristic shapes to a surprising degree, they always deviate from the normal in some respects. Fell (1956) based her conclusions on a series of experiments since the 1920's which have involved the study of skeletal elements of the chick and she was able to demonstrate that skeletal primordia will develop in vitro even when the explant consists merely of an apparently undifferentiated condensation of mesoderm in which there is no sign of joint development or of the individual bone rudiments. Felts (1959) concludes that the same appears to be true for any skeletal structure preceded by a cartilage skeletal model. More recently, mammalian skeletal primordia have been cultured in vitro with comparable results (Chen, 1952). Amtmann (1971) reports on earlier studies which demonstrated that the femora of homozygous luxoid mice were able to maintain their typical luxoid features when cultured as explants. These studies also demonstrated that the femur anlagen of non-mutant animals, when grown in vitro with the normal environment lacking, still develops into normal chondral skeletal elements. Thus, formation of structure appears to occur in the absence of normal mechanical stimuli. However, Amtmann (1971) suggests that mechanical forces are in fact generated in vitro

within the isolated anlage of the femur as a result of intrinsic forces which develop within growing tissue. Amtmann (1971) suggests that this could influence the development of form, a view which is shared by Kummer (1962). Fell (1956) describes experiments in which rudiments of long bones have been successfully cultivated to the extent of the development of models of human fetal long bones. In these experiments however, the very curious fact was noted that whilst explants from a young embryo (6½ weeks) developed in vitro in the normal organotypic way, those from older fetuses only did so if enveloped by epidermis. Thus, all of these studies demonstrate that the general form of embryonic long-bones represent the inherent pattern of development of the skeletogenetic tissue (Felts, 1959).

Joints also have been developed in culture (Fell, 1956). The development of the articular surfaces in the early stages of joint formation has been shown to occur in the absence of muscles, nerves, and blood supply although the conditions in vitro have been found to be inadequate for the completion of the process, particularly the formation of the capsule (Fell, 1956). Drachman and Sokoloff (1966) commenting on the experiments of Fell (1925), Murray (1926), Murray and Selby (1930), Fell and Canti (1934), and Hamburger and Waugh (1940) concluded that the joints were neither complete nor perfect with adjacent elements often fused and the details of the articular surfaces being poorly sculptured. They suggested that certain factors, such as immobility of the limb, growth in an abnormal environment, or lack of innervation might account for this and proposed that a joint, which is a structure so specifically adapted for motion, might logically require movement in order to perfect its development. Their experiments involved various degrees of elimination by paralysis of skeletal muscular contractions in chick embryos and they found that the outstanding abnormality in the paralysed joints was the marked impairment of cavity formation. Their results showed that all the preparatory changes proceed normally up to the point when cavitation should occur and they concluded that it required the mechanical action normally provided by the embryo's own skeletal muscle to administer the "coup de grace" which converts a potential space into a

joint cavity. Furthermore, Drachman and Sokoloff (1966) believed that the maintenance of the articulation also required muscular movements. These results are consistent with the previous observations of Pain (1965) whose experiments involved the destruction of skeletal muscle in mice during gestation. The affected, presumably paralysed, mice also showed failure of joint cavity formation.

The concept of mechanical factors, such as the forces produced by skeletal muscle, affecting the development of the skeletal system has been mentioned previously in the discussion of Wolff's Law. Its relative importance is summarised by Murray (1936) who believes that it is difficult to accept that intrinsic factors alone could produce a normal skeleton however unfavourable the extrinsic factors might be. Furthermore he believes the importance of the extrinsic factors increases in the later stages of embryonic life when the gross skeletal model is being refined and perfected and he presents evidence which suggests that the various grooves and prominences of the late embryonic skeleton are probably produced in reaction to extrinsic and presumably mechanical factors. Fell (1956) suggests that while the general shape of the cartilaginous skeleton may develop in response to intrinsic factors, extrinsic factors are concerned in providing the right conditions for the normal expression of inherent potentialities and for maintaining normal structure once it has developed. To support her suggestion she records that skeletal primordia do not complete their anatomical development in culture and that certain structures, such as the mandible, require appropriate mechanical conditions for their normal development. That environmental factors are responsible for refining the characteristic shape of a skeletal rudiment is provided by experiments which demonstrate that explanted primordia develop a comparatively normal form during the first few days in vitro but lose much of their characteristic shape on more prolonged cultivation and become increasingly distorted. Fell (1956) suggests it is probable that not only an effective blood circulation is required for the maintenance of normal anatomical structure but also functional activity once differentiation has

occurred. Chen (1952, 1953) however, showed that even certain features of the cartilaginous skeleton seem to be determined by extrinsic factors. His experiments on segmentation of the mouse sternum showed conclusively that the segmental arrangement of the ossification centres is imposed upon the sternal rudiment by the association of the ribs, but Fell (1956) believes that the gross morphology of bone probably depends much more upon environmental influences than does that of cartilage. On the other hand, if additional mechanical stimuli are introduced experimentally, structural reactions appear to be evoked. For example Hall (1967) was able to evoke cartilage formation in the quadrato-jugale of a chicken in vitro by experimentally introducing intermittent compressive and tensile stresses where normally in the absence of such stimuli, bone is formed.

It is only in recent years that some light has been able to be shed upon the actual mechanisms by which bone is able to respond to the stimuli presented by mechanical forces. In 1951, Johnson postulated that since bone is a multicrystalline structure it might possess piezo-electric properties. Bassett (1968) defines piezo-electricity as electricity which results from pressure on crystals and, in its purest sense, the term is restricted to single, inorganic crystals that lack a centre of symmetry. Apparently when such materials are deformed charges of opposite polarity appear on opposite faces of the crystal and in the mid 1950's the postulate was confirmed independently in Japan and America by the demonstration that electrical potentials were developed by bone when it is deformed (Fukada and Yasuda, 1957; Bassett, 1957). Fukada and Yasuda (1957) demonstrated not only a direct piezo-electric effect (the production of electric charge by deformation) but also an indirect or converse piezo-electric effect (the production of mechanical strain or deformation by the application of an electric field). Becker (1961) demonstrated an electric phenomenon during limb-bud regeneration in salamanders and this stimulated

Bassett and Becker (1962) to perform further experiments on both living and dead moist bone. They were able to demonstrate charge separation in both these conditions and, significantly, their specimens which were mounted as cantilevers, routinely became negative on the concave side and positive on the convex. Considerable controversy exists over the origin of the electrical response in bone, but there appears to be no dispute concerning its existence. (Shamos and Lavine, 1964,1965; Bassett,1968).

Bassett and Becker (1962) proposed that these electrical charges, generated by strain or stress in bone, were biologically active and suggested that piezo-electricity could affect the alignment and aggregation of extracellular macromolecules and influence cells. Bassett and Hermann (1961), Bassett (1962), and Bassett (1964) were able to demonstrate that mesenchymal cells engaged in osteogenesis were sensitive to changes in the microenvironment, particularly physical changes, and Bassett, Pawluk, and Becker (1964) were able to demonstrate that direct continuous currents in vivo can also affect osteogenesis. These results were then incorporated into a negative feedback concept which attempted to explain the mechanisms behind Wolff's Law (Bassett, 1968).

In this closed-loop system the mechanical strain or stress is thought to be initially converted into an electrical stimulus by piezo-electric elements within the bone. The electrical stimulus affects not only the alignment and the aggregation pattern of the extracellular macromolecules, but also the behaviour of the cells including their migration, nutrition, by-products, and possibly their proliferative rate. Through these cellular and extracellular influences the piezo-electrically generated stimulus results in a change in osseous architecture, which is appropriate to resist the deforming force and shut off the initiating signal (Bassett, 1968). Epker and Frost (1965) also believed that such a controlling stimulus controlled cell function in bone. Bassett (1968) further believed that the rate of repetition, the direction of application of force and the duration of the force may also be critical in determining whether a

biologically active stimulus will result. From the results of their experiments, Becker and Murray (1967) proposed that a "trigger" stimulus may initiate the train of cellular events because they found an optimal range of current and a distinct "cut-off" point which affected the cellular activity.

Both clinically and experimentally it has been known for many years that the concave aspects of a bone under pressure will be buttressed with new bone whilst the convex aspects will have bone removed (Murray, 1936). Recent experiments however have added to this by showing that bone formation occurs in electronegative regions which are generally concave surfaces under compression, whilst bone resorption takes place in electropositive regions which are usually regions under tension and generally convex (Pawluk and Bassett, 1970). In addition, if the force is directed along the axes of pre-existing bone structures, the alterations may involve only an increase in bone mass, whilst if the force produces shear, the modifications will involve re-alignment (Bassett, 1966).

It is now established that piezo-electric behaviour is an inherent property not only of bone, but a whole spectrum of connective tissues including tendon, ligaments, fascia, cartilage and arteries (Bassett, 1971; Zengo, Pawluk, and Bassett, 1973). Concentrating specifically on cartilage, Bassett and Pawluk (1972) were able to demonstrate that when cartilage is deformed it becomes electrically polarised and, although they believed its significance to be obscure, they commented that regardless of origin, mechanically induced electrical polarisation possibly exerts a major influence on the behaviour of cells, ions, and charged macromolecules. Furthermore, they suggested that growth and regeneration could be regulated electrically.

Bassett (1966, 1968) discusses the mechanical stresses which might affect bone mass and orientation of bone trabeculae and concludes that the cardiovascular system, gravity, voluntary muscle action, contact with the environment, and the

continuous activity of cell motion all constitute deforming forces. If the structures that are deformed by these forces are constructed of long-chain, piezo-electric, biopolymers electric potentials are developed (Bassett 1968). Conversely the effects of inhibition of mechanical stresses have also been studied and Bassett (1968) records the results of experiments concerned with bed rest, weightlessness, disuse, and ageing which demonstrate that mechanical forces are of importance in the maintenance and development of skeletal tissues, a result which Bassett (1964) had already shown in tissue culture experiments.

Regarding osteogenic induction, attention in the past has been directed towards chemical agents which might influence cells to become osteoblasts, but now there is increasing evidence to suggest that electric factors may be involved. Saxen and Toivonen (1962) suggested that it may be more propitious to consider induction conditions rather than specific induction substances and, in a similar manner, Urist (1968) postulated two mechanisms by which bone formation could occur. One mechanism incorporated the chemical induction principle whilst the other involved the bone induction principle but at present it is impossible to identify precisely which of these two postulates is correct and their relative contribution (Bassett, 1971).

Attempts have been made to associate the concept of Wolff's Law directly to the vertebral column and Gooding and Neuhauser (1965) specifically studied the growth and development of the vertebral body in the presence and absence of normal stress. They emphasised that man's erect posture has produced stresses on the vertebral column which differ from those of the quadruped and they believe that, phylogenetically, these stresses have resulted in shortening of the vertebral column, a relative decrease in the height of the individual vertebrae, and an absolute decrease in the number of vertebral bodies. They report that some children who never develop the capacity to stand erect have

increased vertical growth of the vertebral bodies and they were able to demonstrate that in the absence of the usual pressures, longitudinal overgrowth of the vertebrae occurs. Gooding and Neuhauser (1965) believe that both intrinsic and extrinsic factors are involved in determining the ultimate size and form of an adult vertebra but appreciate that all the forces are not known. They report that in growing children, the vertebral response to lack of normal stress is most marked in the lumbar area because these vertebrae bear most of the weight in the erect posture and they suggest that in the absence of normal vertical stress, these vertebrae have the greatest capacity to respond by increasing in height. The authors report that they have seen this most frequently in children who have not walked for a significant portion of the time during which vertebral growth occurs but, interestingly, they noted that decalcification or disuse atrophy of the involved vertebrae was not observed regardless of how inactive the child had been. They compare their findings to the condition of scoliosis and conclude that growth is inhibited on the concave side whilst being unrestrained on the convex. Gelbke (1951) suggested that the findings of vertebrae with a relative increase in height is a reversible phenomenon during the growth period of the spine and was able to demonstrate that if normal stresses and pressures begin to appear then the affected vertebrae will resume their normal properties.

THE PATTERN OF OSSIFICATION

For almost 2000 years the pre-natal development of the human skeleton has been the subject of much research. Galen (ca. 130-200 A.D.) described the presence of 7 ossification centres in the sternum and two in the mandible, whilst Fallopius in 1561 is reported to have described 5 centres in the pre-natal sphenoid bone, 3 centres in each vertebra, 4 centres in the axis, and 8 centres in the newborn sternum. The problems surrounding the number and location of the ossification centres of each bone, of the pre-natal developmental anatomy of each bone, and of the congenital anomalies have been the primary topics of the work of Bardeen (1910), Bryce (1915), de Beer (1937), Flecker (1942), Noback (1944), Noback and Robertson (1951), and Hartley (1957).

It is generally agreed that a typical vertebra develops from 3 ossification centres; one in each half of the vertebral arch and one in the body. The primary centres in the vertebral arch appear at the roots of the transverse processes from where the ossification spreads backwards into the laminae and spine, forwards into the pedicles and postero-lateral portions of the body, laterally into the transverse processes, and cranio-caudally into the articular processes. The exceptions are:-

1. The atlas, which although ossifying from 3 centres has one centre in each lateral mass which appears before birth and a third in the anterior arch which appears approximately 12 months after birth.
2. The axis ossifies from 7 centres, 5 of which are primary and two of which are secondary. The vertebral arches each develop from one centre which appears in intra-uterine life and the centrum develops from one centre which also appears in intra-uterine life. The dens, which is really the centrum of the atlas, is ossified from two laterally placed centres which appear during intra-uterine life.
3. Cervical vertebra 7 (C₇) has costal processes which usually ossify from

separate centres. These may remain separate and grow laterally and forwards as cervical ribs. Such centres have also been found on C₄, C₅, and C₆ (Warwick and Williams, 1973). O'Rahilly and Meyer (1956) found that 11 specimens out of a total of 77 possessed cervical ribs, and Flecker (1942) records their presence in fetuses of 165 mm. (female) and 134 mm (male) C.R. length. Noback and Robertson (1951) similarly report 34 specimens from a total of 136 fetuses as having cervical ribs and Shanks and Kerley (1971) discuss the many variations of cervical rib that are to be found.

This account of vertebral ossification is very clearly confirmed by Schinz and Tondury (1942) who conducted an exhaustive anatomical and radiographic study of the early ossification of the vertebrae in the human fetus. They produced a series of excellent photographs which demonstrate clearly the relative positions of the ossification centres in the individual vertebrae.

However, there is some variation in the pattern of ossification of the vertebral body (Cohen, Currarino, and Neuhausser, 1956) and three principal theories have been proposed. The first, reiterated in most textbooks, postulates a single centre for each segment (Mall, 1906). The second theory has been put forward to explain hemivertebrae or "butterfly" vertebrae and states that ossification begins in paired lateral centres. The third theory describes a dorsal and ventral centre for each vertebral body with subsequent joining. Specimens representing all three theories have been reported but their incidence is relatively unknown, although it appears that the third theory possibly applies to about 5% of cases (Cohen et.al., 1956) and Schinz and Tondury (1942) reported 4 cases with the double ossification centre. Similarly Ward (1965) regards ossification from two laterally placed centres as a well recognised variation and as early as 1911, Poirier and Charpy recognised that coronal cleft vertebrae as described in the third theory, could be present. Hartley (1957) was actually

convinced that determination of the sex of the fetus was only possible where multiple coronal cleft vertebrae could be identified with the ratio being 4:1 in favour of the fetus being a male.

The actual appearance time of the ossification centres has been the subject of many studies (Hess, 1917; Camp and Cilley, 1931; Hodges, 1933; Noback, 1944; O'Rahilly and Meyer, 1956; Hartley, 1957). However, Flecker (1942) notes that there are very striking discrepancies regarding the ages at which different ossification centres are said to appear and Noback (1954) suggests that although the voluminous literature gives the impression that there is a wealth of accurate information available, this is deceptive for there are only scant original data which have been critically documented and accurately defined. Noback (1954) cites Mall (1906), Angier (1931), Teissandier (1944), and Noback and Robertson (1951) as being the only four papers available at that time in which direct observations had taken place of more than a few centres in many specimens, and concludes that many of the figures which are said to show the general times of appearance have been copied and re-copied from sources which represent essentially vague estimates or which are based on poor evidence, a criticism shared by Flecker (1942). Nevertheless, the general concensus appears to be that most of the primary ossification centres appear before the end of the 4th month and practically all of them appear between 7-12 weeks (Hill, 1939). Noback and Robertson (1951) note how early in life and over what a short space of time these centres differentiate and record that those centres which ossify during the first 5 months of fetal life do so in a definite and orderly sequence but with exceptions occasionally occurring. Hess (1917) finds it particularly disconcerting that ossification of the vertebral column may occasionally be delayed whilst being normal in other parts of the body.

All such sequences, however, are necessarily speculative because no direct evidence is available. The evidence that has been obtained is from

observations of the appearance of centres in a serial study of fetuses throughout prenatal life and, in this respect, a true longitudinal study is not possible (Noback and Robertson, 1951). Longitudinal growth charts, on the other hand, are statistically more valid than cross-sectional data and, as such, are to be preferred (Tanner, 1962; Campbell and Newman, 1971).

Concentrating upon the vertebral column, the centra are reported to first appear in the lower thoracic and upper lumbar regions at about 9-10 weeks of intra-uterine life. The first centres to appear are those for T₁₀, T₁₁, T₁₂ and L₁ (Hill, 1939; Noback and Robertson, 1951; O'Rahilly and Meyer, 1956; Hartley 1957; and Warwick and Williams, 1973). Apparently the other centra then appear both cephalically and caudally from this area and in a short time all the centra from C₂ to S₃ are present, there being some delay before C₁, S₄, and S₅ ossify. Some exceptions to this orderly sequence have been noted and Noback and Robertson (1951) report one such exception where T₇ was absent but T₅ through to L₄ were present.

Disagreement, however, surrounds the developmental pattern of the neural arch ossification centres, with most general text books reporting that the neural arches appear in a cephalo-caudal direction (Warwick and Williams, 1973; Davis and Dobbing, 1974). With the exception of an observation by Mall (1906), all the data before 1944 consisted of findings of either no neural arches being present or at least nine, and since the development of neural arch ossification centres from T₂ was thought to be cranio-caudal, the assumption was made that the uppermost neural arches also appeared cranio-caudally. Noback (1944) compared the specimen of Mall (1906) to a specimen he encountered and suggested that the cervical and upper thoracic neural arch centres do not follow this cranio-caudal development. He further suggested that the centre for T₁ is possibly the first to appear and that probably there is no systematic order for appearance of the 9 most cranial arches. He acknowledged that more data would be

needed before a definite opinion could be given and Noback and Robertson (1951) were able to discuss this phenomenon in more detail having found five more specimens with less than 9 neural arches present in addition to having the work of Teissandier (1944) as a supplementary source. However, they reached the same conclusion as Noback (1944) and suggested that more data are needed in order to be definite.

Only a few authors discuss the question of whether it is the centra or neural arch ossification centres which appear first in the sequence of development. Hess (1917) records that the neural arch ossification centres appear in the 9th week and it is only in the 10th week that the centra become evident, a result which agrees very closely with Barson (1974).

Since human fetal material is so difficult to acquire, several investigations have been made of the pattern of ossification in laboratory animals such as the rat (Strong, 1925), mouse, (Johnson, 1933) and pig (Hodges, 1953).

Using the albino rat, Strong (1925) found that the neural arch ossification centres appear first in the arches of the cervical vertebrae and first two lumbar vertebrae. The centres were more developed in the cervical vertebrae, especially in the more cephalic ones, and they were particularly advanced in the lateral portions of the atlas. The centra ossification centres were found to arise later than the neural arch ossification centres and appeared in a series beginning with the centre for T₄ and ending with the last lumbar. In both neural arches and centra, the ossification centres then appeared progressively cephalad and caudal to this series.

Johnson (1933) stained his albino mouse material but frequently found it difficult to decide whether a centre was truebone or merely calcified cartilage. He found that the ossification centres for the vertebral arches were present in all cervical vertebrae, the first two and last four thoracic vertebrae, and the the first four lumbar vertebrae at a very early stage, with the first two thoracic

vertebrae showing least ossification, each arch being represented by two extremely small areas of bone which fused in a short time to form a single centre. At a similar stage, Johnson (1933) found that the last eight thoracic and all the lumbar centra ossification centres were present, and using the data of Mall (1906) and Strong (1925) he found it interesting to compare the relative rates of skeletal development and order of appearance of the ossification centres in mouse, rat, and man. He showed that most of the primary ossification centres appear in the mouse during the last 25% of the gestation period, in the rat during the last 28.5%, and in man during the latter half of the first trimester. Therefore, although ossification progresses extremely rapidly in the mouse and rat, once it has started, it appears that the relative time required for ossification is greater than in the human. Strong (1925) however, suggests that ossification begins very soon after differentiation has started, regardless of the length of the gestation period, which implies that man has attained the same degree of ossification at the end of one-third of the total period of gestation as the mouse and rat have at the time of birth.

Hodges (1953) used the fetal pig as his laboratory animal and radiographed his specimens after previously staining them with silver nitrate. He described the order of ossification for all of the fetal bones and found that ossification of the vertebral bodies proceeds from at least two and possibly three loci, the first locus being at T_{12} , the second, which he was only able to demonstrate in two fetuses, in the upper thoracic region, and the third, which lagged behind the others, being found at C_2 . Regarding the vertebral arches, Hodges (1953) found that their ossification begins in two loci, the first being at T_6 or T_7 , and the second being at C_1 or C_2 . He uses the data provided by Noback and Robertson (1951) to compare his findings with similar data for human fetuses, and concludes that there are some differences but also similarities in the sequences.

The patterns of ossification for the vertebral column discussed so far do not mention the occurrence of asymmetry in development between the two neural arch ossification centres of each vertebra. Pryor (1906) records that regardless of the normal variations, the ossification within the body is bilaterally symmetrical but several authors do not agree. Flecker (1942) notes that no detailed study has been made concerning the possibility of any appreciable difference of one side as compared with the other and, moreover, he considers it doubtful that any appreciable difference exists. However, he does think that it is conceivable that the different function of one side, particularly after birth, compared to the other might lead to some variation in the times for appearance of the ossification centres, but he feels that this will not affect the spine. Similarly, Mences and Holly (1932) in a study of 11 infants with asymmetrical development of certain epiphyseal centres, found that in 9 cases the preferred hand coincided with the side on which extra centres occurred and they found it difficult to account for such an occurrence on the basis of endocrine or of any other general disturbance. Noback and Robertson (1951) conducted a more detailed study of the problem and recognised that when one of a pair of bilateral centres is observed, it is during the time when these centres normally appear and found that such an occurrence is not observed after the time of their normal appearance. From these observations they suggest that the absent centre of each of the bilateral pairs noted could normally be assumed to appear shortly afterwards. Noback (1944) in an earlier study reached a similar conclusion and suggested that although asymmetrical appearance of bilateral centres does occasionally occur, the appearance of bilateral centres is essentially symmetrical.

To overcome this possible variation, Francis and Werle (1939) examined only the left side for development and assumed that for practical purposes, both sides are alike. Christie (1949) restricted his observations to the right side only and made the observation that any asymmetry in the development of any given

centre is insignificant. O'Rahilly and Meyer (1956) recognised that variation might be present, and decided that a specimen would be recorded as having an ossification centre present irrespective of whether it occurred unilaterally or bilaterally.

THE SPINAL CURVATURES

The adult vertebral column is said to have four antero-posterior curvatures, one for each of the spinal sections; cervical, thoracic, lumbar, and sacral (Romanes, 1972; Warwick and Williams, 1973; Snell, 1973). During the first month of embryonic development the whole spine acquires a marked ventral flexion (Bardeen, 1910) and therefore the thoracic and sacral curves are recognised as being "primary" for they are concave ventrally during fetal life and retain the same curvature after birth. However, in contrast, the cervical and lumbar curves are concave dorsally in adult life and are termed "secondary" or "compensatory". These curves are said to be the result of differences in the antero-posterior thickness of individual vertebral bodies and also to result from differences in the shape of intervertebral discs (Basmajian, 1970). The primary curvatures are caused mainly by the shape of the vertebrae (Romanes, 1972) and when it is in the bones that are chiefly concerned, it has been stated that the curve is more likely to be permanent (Basmajian, 1970). The secondary curvatures, which develop in the cervical and lumbar regions, are said to be mainly due to the shape of the relatively thick discs that these regions possess and therefore can be temporarily abolished by appropriate movement of the spine (Basmajian, 1970; Romanes, 1972, Passmore and Robson, 1973). In old age, the intervebral discs shrink and the vertebral column tends to revert to its original primary curvature (Romanes, 1972; Sinclair, 1973). In the upright position of the adult, the alternating curves of the vertebral column have been suggested to provide for absorption of vertically transmitted forces which are not transmitted straight through the column but are absorbed by the intervertebral discs and by the slight bending of the curvatures which is resisted by the muscles and ligaments (Romanes, 1972).

Whilst Warwick and Williams (1973) do record that the cervical curvature actually appears late in intra-uterine life many anatomical text books (Hamilton and

Simon, 1958; Basmajian, 1970; Romanes, 1972; Passmore and Robson, 1973; Caffey, 1973; Sinclair, 1973) deny its presence until the child begins to raise its head in the first few months after birth, when Warwick and Williams (1973) suggest that the curvature is merely accentuated. Thereafter the cervical curvature is convex ventrally, being least marked in the atlas and reaching as far as the level of T₂ (Romanes, 1972; Warwick and Williams, 1973). Hamilton and Simon (1958) however illustrate with radiographs that the cervical curvature in the adult depends upon the degree of flexion, with the vertebral bodies being in alignment when the individual is at rest, but with a certain amount of gliding movement being possible on full flexion or extension of the neck. Romanes (1972) summarises the situation by stating that the cervical curvature is least marked and is undone as the neck is flexed.

The thoracic curvature is always concave ventrally, and extends from T₂ to T₁₂ and is thought to be formed by the greater depths of the posterior parts of the vertebral bodies (Warwick and Williams, 1973).

The lumbar curvature is said to begin to develop when the baby sits up at 9 months and there is a compensatory exaggeration of the curvature when the baby starts to stand up and to walk (Bardeen, 1910; Sinclair, 1973; Passmore and Robson, 1973; Caffey, 1973) so that the centre of gravity of the body will not lie in a plane anterior to the hip joints during the sitting or standing positions (Basmajian, 1970, Warwick and Williams, 1973). Thereafter the lumbar curvature is convex forwards, stretching from T₁₂ to the lumbosacral angle (Romanes, 1972), with the convexity of the lower three vertebrae being greater than that of the upper two. In the adult the lumbar curvature is most pronounced when standing upright, a position in which the vertebral bodies come to be relatively close to the anterior abdominal wall (Romanes, 1972).

The sacral curvature stretches from the lumbosacral joint to the apex of the coccyx and during the first 2-3 months of intrauterine life is fairly

straight (Bardeen, 1910). Subsequently at about the 5th month of intrauterine life a marked dorsal flexion of the lumbosacral border takes place (Bardeen, 1910; Hamilton and Simon, 1958) and an additional curvature appears "in order to accommodate the pelvic viscera." (Romanes, 1972).

In addition to curvatures in the sagittal plane, most adults possess a slight lateral curvature of the thoracic region accompanied by curvature of the lumbar region in the opposite direction (Hamilton and Simon, 1958; Warwick and Williams, 1973). The direction of this curvature is thought to show some relationship to "handedness" with a slight asymmetry affecting both the proportions and movements of the body. For example, one limb may be shorter by $\frac{1}{2}$ " than its fellow, and breathing movements may be more extensive on one side than the other (Hamilton and Simon, 1958). In the right-handed person the thoracic part of the vertebral column may show a slight convexity to the right with the right clavicle tending to be shorter, stouter, and more horizontally placed than the left one. The converse asymmetry is usual in the left-handed person (Hamilton and Simon, 1958).

Regarding a sex difference being present in spinal curvatures, two contradictory statements have been made. Trotter (1929), who studied the vertebral column in both white and negro Americans, found a general tendency of female columns to show less curvature than those of males in both races, whilst Basmajian (1970) clearly states that the normal curvatures of the column are rather more pronounced in the female than in the male.

Barnett and Nairn (1965) define the normal fetal attitude of the spine as one of general moderate flexion, with the limbs being well flexed and close to the body, although they realise that there are definite numbers of variations. They suggest that instead of normal moderate flexion the spine may well exhibit local or general hyperflexion, local or general deflexion, and lateral curvatures. Tager (1952) suggests that the curvatures of the fetal spine

are significant to the extent that they reflect fetal well-being and that fetal death in utero is shown on radiographs by collapse of the fetal spine and deepening of the curvature in the lumbosacral segments and sharp angulation of the neck on the thorax. He attributed the loss of normal spinal curvature in the dead fetus to be due to loss of tone in the muscles.

MEASUREMENTS OF THE LIMBS AND VERTEBRAL COLUMN

Direct measurements of the lengths of ossifying bones in fetal limbs have been the subject of many radiographic studies. Mehta and Singh (1972) measured the lengths of the diaphyses of both the humerus and femur in 50 apparently normal fetuses, ranging from 65-290 mm. in C.R. length. They found a significant correlation between diaphyseal length and C.R. length and they were able to observe various growth phases. No appreciable difference was found between the lengths of the humerus and the femur when the fetus was less than 85 mm. in C.R. length and the difference in length at greater C.R. lengths was attributed to the comparatively slower growth of the humerus during the later period of pregnancy. They were able to calculate that the diaphyseal growth rates of the humerus and femur are 0.18 mm. and 0.21 mm. respectively for every 1 mm. increase in C.R. length and they compared their results to those of previous workers.

Brandfass and Howland (1967) criticised various methods which have been devised to estimate fetal maturity or weight in utero and they included in their criticism the work of Hodges (1937) who had correlated the length of the calcified femur with the week of gestation. In approximately 100 human fetuses between 16-38 weeks gestation Hodges claimed that growth in length of the femur occurred at a regular rate for each week of gestation. Their criticism was that these methods had not withstood the test of general clinical use for various reasons, including problems with radiological technique, complicated formulae, or unavailability of charts and tables, and therefore they proposed a series of measurements of long bones in limbs which would be the most accurate indicators of a weight of 2250 g. or more, which weight they suggest is one of the most commonly accepted criteria of pre-maturity. Brandfass and Howland (1967) evaluated various techniques to improve the detail of the films to achieve more accurate measurements and they argued that a single radiological measurement

may be inaccurate because of the fetal position, maternal weight variation, or indistinct outlines due to fetal movement and they therefore selected the long bones of the fetal limbs as being the only visible portion of the fetus with sufficient components to overcome these problems. They excluded the fibula from their measurements and gave a series of 10 measurements from which to estimate fetal maturity with the recommendation that if any of the bones were found to equal or exceed in length one of the reference measurements, then it could be concluded that the fetus weighed more than 2250 g .

Owen (1971) continuing the theme of estimating fetal maturity from measurements of the long bones of the limbs from radiographs made approximately 600 measurements from 150 films of subjects between 12 weeks and full-term. Only those bones which appeared to be parallel to the film were measured. The mean measurement for each week was determined and plotted against duration of pregnancy. Separate graphs were drawn for each bone . Owen (1971) comments that a tracing from one of the limb graphs was found to fit the other five almost exactly by moving the base-line slightly up or down.

Martin and Higginbottom (1971) emphasised the need to estimate fetal age in utero but stressed that at present only ultrasound appears to give accuracy sufficient for present day needs. They realised that ultrasound is expensive and therefore not generally available and proposed a method for assessing fetal age from radiographs of the human femur. They measured the thigh length in the newborn child of 30-43 weeks gestation from the lateral prominent part of the tip of the greater trochanter to the line of the knee-joint and found that the rate of growth was 3 mm. per week. They also found no detectable difference between the two sexes. They next measured the length of the ossified femur between the convex ends of the radiopaque shaft from radiographs taken in utero up to 8 weeks before the baby was born. To this they added 3 mm. for every week that the pregnancy progressed and ultimately the thigh was measured in the newborn infant to find out if the radiological measurement was correct. From this they

found it possible to give an estimate of gestational age by direct measurement of the femur on the radiograph. They realised that foreshortening of the limb might well occur in vivo and endeavoured to overcome the problem by measuring the femur on each side and then used the measurement of the longest femur since this was thought to give the more realistic measurement. Furthermore they calculated that until the amount of abduction or adduction exceeds 15° from the horizontal plane, then the difference in measurement will not exceed 3 mm. or the equivalent of 1 week of gestational age.

Russell, Mattison, Easson, Clark, Sharpe, and McGough (1972) discuss the work of Owen (1971) and Martin and Higginbottom (1971) and note particularly that Owen (1971) reported that the femur grows at 1 mm./week in the last weeks of pregnancy whilst Martin and Higginbottom reported growth of 3 mm/week. Russell et.al. (1972) therefore used 217 prone radiographs of pregnancies and measured the ossified femoral lengths in a manner similar to Martin and Higginbottom (1971). They found a poor correlation between fetal maturity and femoral length and found that its rate of growth was approximately 1.5 mm./week, with a femoral length of 77 mm. indicating maturity within only 1 Standard Deviation corresponding to a gestational age of 30-42 weeks. This range they felt was far too wide to be of any practical use.

The lengths of human fetal long bones have also been studied by methods other than radiology. Hesdorffer and Scammon (1928) reviewed earlier work on growth in length of the human tibia and included measurements of total body length, total length of tibia, length of tibial diaphysis, and combined lengths of superior and inferior cartilaginous tibial epiphyses. They calculated regression formulae between each of these measurements and found that the empirical formulae for the total length of tibia and for the length of the tibial diaphysis, with respect to the total body length, have negative second constants whilst for the epiphyses, the empirical formulae have positive second constants. They therefore concluded that while the length of the tibia as a whole and of its diaphysis are becoming relatively, as well as

absolutely, greater the epiphyses are becoming relatively shorter although absolutely longer. Furthermore they differentiated their expressions for lengths of tibial structures with respect to time and found that all parts of the bone decline in absolute rates of lineal growth in the latter part of prenatal life. Similarly, Felts (1954) did not use radiological techniques but instead stained with alizarin red 53 whole femurs from fetuses and infants between 31-485 mm. C. R. length. He made no selection as to the side from which the femurs were taken and found that the total length of the femur increases by 0.21 mm. for every mm. increase in C.R. length and that length of the ossified part of the shaft increases very rapidly relative to the total length of the shaft.

In addition to the direct measurements of length, human fetal long bones have been studied by other techniques. In particular, Moss (1954,1955) and Moss, Noback, and Robertson (1955) applied the concepts of differential growth. Moss et.al. (1955) commented that the qualitative descriptions of the changing proportions of the various parts of the developing organism tend to be excessively descriptive and are seldom reinforced by actual qualitative or quantitative histological or gross anatomical observations. Moss (1955) notes that the growth of the human fetus is characterised by great changes in the relative proportions of different parts of the body which accompany increases in absolute body size and suggests that these changes are not haphazard but occur in an orderly sequence which can be simply expressed by using differential growth techniques. Differential growth is related to the concept of self multiplication of growing tissues and therefore there is some doubt as to its applicability to bone growth which is accretionary rather than interstitial. Moss (1954) however explains that if bone is viewed as the result of self-multiplicative processes of the surrounding osteogenic tissues, then the differential growth analysis concept is applicable not so much to overall growth of bone itself as to self-multiplication in the osteogenic tissue. Moss (1955) studied 119 alizarin stained

fetuses between 14 mm. and 175 mm. C.R. length and measured the lengths of all the long bones, clavicle, and 6th rib. The differential growth relationships between all of these dimensions were then plotted and they were also compared with log. C.R. length and log.time. The results showed that fetal skeletal growth was capable of a simplified quantitative expression by the allometric growth formula and also showed that there was a critical developmental horizon for all of the skeletal system at the end of the first trimester. This horizon was represented by a change in the value of the growth ratio k in the equation

$$y = bx^k$$

and this critical period was found to be correlated with many morphological events in neighbouring tissues and structures, particularly the onset of endochondral ossification. The ratio of specific growth rates of any combination of bone lengths was also found to remain constant throughout the period of growth. Moreover, the bones of both distal limb segments grew relatively faster than those of the respective proximal segments. Furthermore each segment of the lower extremity grew relatively faster than the corresponding segment in the upper extremity. When each of the osseous dimensions was plotted against log. C.R. length or log.time a change in linear slope or "interphase" was noted with the interosseous ratio remaining constant both before and after the interphases. The value of the interphase was essentially the same for each of the osseous dimensions and Moss (1955) concluded that some common factor must have intervened simultaneously in the growth of all the postcranial skeletal bones. Moss et.al. (1955) calculated the value of this interphase to be approximately 12 weeks of menstrual age and from their calculations of growth rate (dw/dt) of the osseous shafts were able to conclude that the 12th week is the time of greatest activity for all the dimensions they measured.

A confusing picture is presented by the literature regarding the comparative development of the right and left sides of the body. Schultz (1923)

measured the total length of left and right humeri in 100 fetal cases in addition to foot length and ear height. He stressed that precise instruments are required and measurements need to be taken immediately after dissection so that the cartilaginous parts have had no chance to shrink during drying. His results indicate that human fetuses show a high incidence of asymmetry at least after the beginning of the 4th month, the right humerus being longer in over 50% of cases. He found a similar proportion for 105 adults and therefore concluded that the asymmetries in the adult must rarely be due to function or right-handedness. Schultz (1923) also found that many more asymmetries exist in other parts of the fetal body such as length of the clavicle and position of the nipples. Schultz (1926) reported on the available literature and highlighted the apparently contradictory statements to be found where, on the one hand, asymmetries were stated to appear at the earliest during childhood, whilst, on the other hand, other studies had shown that young human embryos exhibit marked asymmetry. Konig and Kornfeld (1927) measured the lengths of the ossified shafts of fetal humeri between 4 months and term and found asymmetries in approximately one-third of their specimens whilst their measurements of ossified femoral shafts showed that only 10% of paired femurs were asymmetrical between 4 months and 6 months. These femoral asymmetries decreased in number in older fetuses and disappeared at term. Flecker (1942) realised that no detailed study had yet been made concerning the possibility of any appreciable difference of one side as compared with the other but felt that it was doubtful whether any definite difference exists although he thought that it was conceivable that the different function of one side compared with the other might well lead to some variation of the times of appearance of the ossification centres. More recently Burwell, Coates, Jackson, and Piggot (1974) and Burwell and Dangerfield (1975) have studied asymmetry in the upper limbs of children and have attempted to compare their results to similar measurements taken on children suffering from scoliosis. Many of their 510 scoliotic patients were

found to have asymmetry in length of the upper limbs with the asymmetry being related to the side of the spinal curve in that the upper limb was longer on the convexity of the lateral spinal curvature. These results have been compared to a study of normal boys and girls where various upper limb measurements were taken and the findings suggest that the velocity of growth in the upper limbs may not be symmetrical during growth in all normal individuals.

The picture surrounding the measurement of fetal long bones may well be clouded by a subtle difference in measurements between the two sexes, but here also the available literature is confusing. Although Adair and Scammon (1921) and Pryor (1923) found that the tibia ossified earlier in females than in males, Hesdorffer and Scammon (1928) found no significant sex differences in the rates of growth of the bone as a whole or in any of its parts, even though their graphs show the male to be in advance of the female. On the other hand, Halonen (1929) using fetuses between 100-500 mm. C.H. length reported the percentage length of the humerus occupied by the ossified part of the shaft and claimed to have found sexual differences in the extent of diaphysial ossification with the female being more advanced.

There is only a very restricted literature which discusses the growth of the various regions of the vertebral column. Bardeen (1910) reports that Aeby (1879) measured the length of various regions of the spinal column at different ages and included the height of the constituent vertebrae with the thickness of the intervening intervertebral discs. He found that in young embryos the cervical region is relatively much longer than in the adult and the lumbar region is relatively much shorter. These results were confirmed by Ballantyne (1892). Bardeen (1905) studied embryos between the 2nd and 3rd month of development and concluded that if the length of the thoracic region is taken as 100, then the length of the cervical region is 60, the lumbar between 40 and 50, and the sacral between 33 and 42.5. He compares his results to a similar study

in adults where the length of the cervical region is 41.7-47.5, the lumbar 56.3-71.6, and the sacrococcygeal 61-68. Stockland and Marks (1961), having found the existing methods of fetal weight determination unsatisfactory, attempted to develop a more accurate method which involved measurement of the fetal spine. This was just one of several measurements taken from radiographs of the fetus in utero and involved placing one point of a plastic flexible ruler at the level of the coccyx and measuring to the dens along the contour of the spine. These measurements were limited to fetuses near to term and no specific standards were presented for the vertebral column as a single item.

Some investigators have recognised the potential of the lumbar region of the vertebral column as an indicator of fetal maturity. Fagerberg and Roonemaa (1959) criticised other methods of ascertaining fetal age based on various fetal measurements because of the difficulties in obtaining exact projections of the fetus in utero. They argued that since it is usually easy to measure the length of the lumbar spine in utero, then it would seem logical to study the correlation between this and fetal length. The length of the lumbar spine was measured from the upper edge of the first lumbar vertebral body to the lower edge of the fifth lumbar vertebral body following the curvature of the spine. However, Fagerberg and Roonemaa (1959) state that there is uncertainty sometimes in determining the points for measurement. Nevertheless they were able to give a regression line for lumbar length against C.H. length and concluded that their method permits a good approximation of fetal length, although they restricted the accurate measurement of fetal lumbar length to the latter half of pregnancy with the patient in the prone position. Following this, Chang, Woesner, Nakamoto, and Sanders (1971) recognised the reliable results in the correlation between fetal lumbar length and total fetal length presented by Fagerberg and Roonemaa (1959). They therefore attempted to determine fetal age from measurements of the fetal lumbar spine on a radiograph utilising a specially constructed fetometer.

The fetal lumbar spine was measured from the upper end plate of L₁ to the lower endplate of L₅ and the fetometer was made of flexible material in order to accommodate the curve of the fetal lumbar spine. Fetal age could then be estimated by measuring the fetal lumbar spine with the age being read directly from the fetometer. Chang et.al. (1971) subsequently made estimations of fetal age between 17 and 40 weeks of gestation but found that the method was less accurate during the earlier periods of gestation.

FETAL MOVEMENT

There have been only a very limited number of recorded observations on human fetal movements and adequately controlled studies did not begin to appear until the work of Minkowski (1920), whose studies, between 1920 and 1946, covered 75 fetuses and whose results have been based on dictated notes made at the time that the fetal reflexes were seen ex utero. Hooker (1952) reported on the work of earlier investigators who, unlike Minkowski (1920), had made little effort to maintain a normal condition of the fetuses during their observations and argues that their results cannot be evaluated satisfactorily. The most extensive and carefully documented accounts of human fetal reflexes appear to be those of Hooker himself between 1932 and 1956 when he reported on a total of 159 fetuses basing his reports almost entirely upon motion picture records. He also studied 20 premature infants and 7 embryos below the age at which the earliest reflex had been noted (Fitzgerald and Windle (1942) at 20.0 mm.C.R.; Hooker (1952, 1954, 1958) at 20.7 mm). Hooker published many papers and among them was "A Preliminary Atlas of Human Fetal Activity" which unfortunately was a limited edition (100 copies) published privately in 1939. Illustrations from this and other unpublished records of Hooker have been widely used in scientific publications and in more general articles and Gesell (1945) includes, amongst others, a life-size illustration of the earliest recorded fetal movement taken by Hooker which is reported to be of a fetus of C.R. 25 mm. or $8\frac{1}{2}$ weeks menstrual age. A fine hair was stroked across the right cheek and the long muscles of the neck and trunk on the opposite side (left) contracted causing a contralateral body flexion. The shoulder muscles also contracted, causing the arms and hands to move backwards and in 3 pictures taken over approximately $\frac{1}{2}$ second the fetus was observed to return to its original attitude. Similarly for a fetus of $11\frac{1}{2}$ weeks menstrual age Gesell (1945) illustrates that stimulation of the maxillary-mandibular nerve

area by lightly stroking the right cheek caused the head to be extended slightly and flexed contralaterally. The arms and hands meanwhile moved backwards and downwards on both sides of the body and the hands moved towards the midline. The whole of this action occurred within approximately $\frac{1}{2}$ second. For a fetus of 14 weeks menstrual age, Gesell (1945) illustrates its response to tactile stimulation applied to the lumbar region and extending to the neck along the paravertebral line. The fetus responded by extending its head, opening its mouth, and arching its back whilst rotating the right side of its trunk forwards. This response was accomplished in one second and in one further second the fetus had returned to its original posture.

All of Hooker's films have been recorded on black and white film at a speed of 16 frames per second and the aim was to record as accurately as possible those movements that occur in response to a known stimulus during the short time available before the oxygen supply of the fetus becomes so depleted that all reflex activity ceased. All observations were made with the fetus in an isotonic bath containing normal mammalian saline or Tyrode's solution, usually at or near normal body temperature and the stimuli were evoked by hair esthesiometers. These were calibrated to provide maximum pressures of 10, 25, 50, 100 mg, 2, 5 g, although the actual pressure exerted by a light stroke was far less than the recorded value.

Hooker (1952) reports that until about the middle of the 7th week of menstrual age, the human embryo appears to be incapable of any type of reflex activity and stresses that there is certainly no area of integument sensitive to exteroceptive stimulation before this time. He found, however, that the musculature of the human embryo may be electrically stimulated to contraction beginning with the latter part of the 6th week but records that the contractions are sharply localised. Between the 7th and 8th weeks of menstrual age Hooker (1952) found that stimulation by stroking the upper or lower lip or nose led to a typical contralateral flexion of the neck and uppermost trunk with little or no

participation of the upper extremities and certainly none by any other part of the body. From his Pittsburgh studies which involved 131 fetuses ranging in menstrual age from $6\frac{1}{2}$ weeks to 45 weeks (post mature), Hooker (1952) notes two typical cases.

At $8\frac{1}{2}$ weeks Hooker (1952) records that the typical response consisted chiefly of contralateral flexion of the neck and trunk, although two ipsilateral flexions are recorded. Extension of the arms at the shoulder was also typical although this was without any separate movement of the elbow, wrist, or fingers. There was also rotation of the pelvis towards the contralateral side and all these movements were simultaneous although, as the fetuses got larger, there was a short period of delay before the pelvic rotation followed the trunk flexion. The relaxation of the movement and return to normal position was found to be accomplished first by the arms and then by the neck, trunk, and rump, although an exact return to the original position did not always occur. Hooker (1952) found that a series of such responses may be elicited at short intervals for as long as three to four minutes after the beginning of placental separation.

At $7\frac{1}{2}$ weeks Hooker (1952) found that the area of skin most sensitive to stimulation is restricted to the area about the lips and alae of the nose, but by $8-9\frac{1}{2}$ weeks this region of sensitivity had extended to include the chin and more lateral parts of the mouth and nose. By $11\frac{1}{2}$ weeks the entire face is said to be sensitive in all fetuses and Hooker (1952, 1954) connects these findings to the development of the trigeminal nerve which supplies these areas and which has long been known to be the cutaneous sensory nerve which first becomes functional in mammals. It appears that in Man it has been impossible to distinguish with certainty whether the skin area supplied by the maxillary or mandibular divisions becomes sensitive first even though stimulation of the ophthalmic division has been shown to cause reflex responses only later in development (Hooker 1954).

The axial reflexes which first appear at about $7\frac{1}{2}$ weeks are restricted to the muscles of the neck at their inception and later they extend caudally to involve all the trunk and limb girdle musculature (Hooker 1954). The extent

and pattern of the axial responses appear to be so closely related to age that it has been possible to age embryos and younger fetuses solely on the basis of the pattern of their reflexes (Hooker 1954). At $7\frac{1}{2}$ weeks, exteroceptive stimulation with a fine hair over the perioral cutaneous area causes a contralateral flexion of the neck and although ipsilateral responses do occur at slightly older ages, they are rare and none was ever seen at $7\frac{1}{2}$ weeks either by Fitzgerald and Windle (1942) or by Hooker (1954). By 8 weeks there is sufficient caudal progression of neuromuscular development that the upper trunk muscles become involved in the response and by $8\frac{1}{2}$ weeks, exteroceptive stimulation in the perioral region causes primarily a contralateral flexion of the neck and trunk. By $10\frac{1}{2}$ weeks axial extension has begun to replace lateral flexion.

Hooker (1952) discusses three factors which might modify the validity of his results and his arguments might well apply to many similar studies regarding fetal movements. The progressive hypoxic condition resulting from placental separation occurs 1-2 $\frac{1}{2}$ minutes before the stimulus is applied and must have a major influence on the fetal responses it evokes. This hypoxic factor cannot be eliminated unless the situation arises allowing examination of the fetus in utero merely by incising the gravid uterus (Fitzgerald and Windle, 1942). Similarly maternal anaesthesia may well affect any maternal factor(s) involved in the production of "normal" fetal movements and if the anaesthetic itself crosses the placental barrier then the fetal movements themselves may well be curtailed. Amongst the physical factors which might modify fetal movements is the change in pressure following delivery of the fetus. However, Hooker (1952) believes the effect is minimal having observed no actual difference between movement of embryos within the intact amniotic sac, where the pressures differ little perhaps from those in utero, and after their release from the sac. Older fetuses however will gain a freedom of action once they are delivered, particularly of their extremities, which is denied within the uterine walls (Hooker 1952).

The relative temperatures of the uterus and waterbath might also dramatically affect fetal responses and although this factor can be controlled to some extent it can never be completely eliminated particularly at the moment of transfer. Another factor that is impossible to eliminate involves the manipulation of the fetus and its membranes during hysterotomy. No matter how careful removal is performed there must always be some effect upon the fetus and therefore it appears that results from studies such as Hooker (1952, 1954) must always be viewed with some caution.

A few motion pictures have also been made by Windle and Fitzgerald (1942) using pregnancies terminated for medical reasons which allowed a brief opportunity to examine unasphyxiated embryos with the placental circulation intact. Unfortunately they do not appear to have been made available to others (Humphrey 1970) nor have the illustrations ever been published. Fitzgerald and Windle (1942) observed the first somatic movements about 8 weeks menstrual age. By tapping on the exposed amniotic sac of an 8 weeks fetus they were able to observe a quick movement of the fetal arm, and touching of the oral region resulted in head movement to the opposite side and backwards, a result very similar to that reported by Hooker (1952, 1954). When similar experiments were carried out on older fetuses movements of the trunk and legs appeared but always, after placental separation, the discrete character of the movements was lost although head extension and mass movements of the trunk were still obtainable. Fitzgerald and Windle (1942) criticise other investigators whose examination of fetuses was comparatively delayed when, presumably, only those reflexes which are most resistant to asphyxia remain.

Little information is available concerning the origin and sequence of development of the various components of the human brain. Although gross features of the developing nervous system were established many years ago Windle (1970, 1971) reports that very few new details have been added since the turn of the

century. Even so, more appears to be known about the development of structure than of function since only fleeting glimpses of movements or reflexes have been observed and although there are several accounts of early behavioural development there is a lack of correlation between structure and function and, more particularly there is a lack of interpretation of the observations made on human fetuses.

The legal and ethical problems associated with experimental attempts to use human fetal material has led to the development of comparative studies using animal fetuses where the appearance of the earliest somatic movements have been correlated with the development of certain intrinsic spinal cord and medullary structures (Windle 1970, 1971). The embryos of cats and other mammals have provided the basis for much of our present knowledge although Windle (1971) found that the fetuses of cats were at first rather more responsive, and spontaneous movements were occasionally seen in the older fetuses. After clamping the umbilical cord he noted specific changes in the response to stimulation. For example extension gave way to flexion or ipsilateral movements and were interspersed with contralateral movements. As asphyxia occurred all responses to stimulation stopped, mass movements being the last to disappear. Structurally too, human embryos closely resemble other mammals in that only minor species differences in the pattern of neurofibrillar differentiation were encountered in specimens comparable with human embryos of between 4 and 7 weeks of menstrual age (Windle 1970).

The development of effective pathways is said to begin in the rhombencephalon and upper part of the spinal cord and then proceeds both rostrally and caudally (Windle 1970) with elements of primary efferent and secondary or interneuron systems being recognisable at 4 weeks (Windle 1971). Movements induced in the 8 week old human fetus, particularly those of the forelimbs, were thought to be simple reflexes involving 2-3 neurons and the principle question now centres around the relationship between the development of the embryonic brain

and the concepts of behavioural development. Windle (1970) states that the fetal brain certainly becomes concerned with both sensory input and motor output but it is hard to say just when either of these functions has its beginning. The first sign of muscle contractions appears not to be a very worthwhile landmark because even though there are no intrinsic neural elements present, contractions of the human heart muscle begins at 3-4 weeks of menstrual age and, furthermore, other types of muscle have been shown to be capable of contraction before their nerves have reached them, e.g. smooth muscles of the intestine by 7 weeks and skeletal muscle before 8 weeks (Windle 1970). The principal neural elements required for reflex arcs are present at 6-7 weeks of menstrual age and histological examination of the spinal cord stained with silver has shown that they consist of primary afferent and efferent neurons with second order neurons inbetween. The primary afferent axons however do not have collateral branches to connect them with inter-neurons or the primary efferent neurons until the end of the 7th week (Windle 1970). These early responses to stimuli could not be proved to be reflexes however because the formation of synapses had not been demonstrated. Bodian, Melby, and Taylor (1966) however demonstrated their presence in non-human primates using the electron microscope. There is therefore no longer doubt that the responses elicited by stimulation at 8 weeks by Windle and Fitzgerald (1942) and Hooker (1952, 1954) were indeed reflexes. As gestation develops from 8 to 12 weeks these simple reflexes appear to become more complex, but one feature which emerges is that the first connections appear to be so organised that ipsilateral rather than crossed reflexes occur (Windle 1971). However, Gesell (1945) illustrates the first fetal movements using photographs from Hooker's studies and these clearly show the first movements to be contralateral activity of the trunk with bilateral activity of the limbs.

Windle (1970) continued his neural observations and found that at 8 weeks there is a region between the tracts ascending from the spinal cord and

rhombencephalon and those descending from the forebrain where the neurofibrillar development is sparse and he concludes that it is therefore unlikely that the rostral parts of the brain exert any influence upon the lower centres during this period. Windle (1970) suggests that the reflex mechanisms once they are initiated are not restrained but later on, with older fetuses, an inhibition is brought about with the descent of tracts from the extrapyramidal centres and much later on from the cerebral cortex. Windle (1971) gives evidence from several species to illustrate that the responses of a fetus to stimulation become more difficult to elicit at a certain stage of development and from such findings it has been assumed that this coincides with the time when neurons carrying inhibitory impulses descend from the higher centres of the brain. Evidence to show that the cerebrum exerts little influence on the developing centres in the medulla oblongata is provided by rabbit fetuses which have been decapitated in utero. They have continued to grow and the Central Nervous System (C.N.S.) was found to be relatively normal in appearance. The fetuses, however, were found to be much more responsive to stimuli than control fetuses and their movements were found to be more rapid and lasted longer (Jost 1967, 1969), and possibly this illustrates failure of development of an inhibiting mechanism.

A theory has been proposed that the initiation and maturation of function in the nervous system is dependent upon the formation of myelin sheaths (Windle 1971). In general, functional development and acquisition of myelin by certain fibre tracts do appear to be related but it has been clearly shown that there is much well-organised activity present within animal fetuses such as the cat before there is any myelination. That myelination increases conduction velocity has been shown by Grafstein (1963), and Huttenlocker (1970) has suggested that before myelination nerve fibres might also possibly fatigue rapidly thus creating some sort of conduction block. These facts suggest that with myelination the nature of the

fetal responses might change and this is supported, although made more complicated, by recognition of the possibility that myelination is governed to some extent by the onset of neural function. This has been shown by Gyllensten and Malmfors (1963) who showed that the development of myelin sheaths on the optic nerve fibres is retarded in mice who have been reared in total darkness.

Regarding the peripheral nervous system, nerves and ganglia have been shown to be present in human embryos of 4-5 weeks menstrual age (Windle 1971), but actual motor and sensory nerve endings are not found until much later. The nerve endings in the organs for balance, hearing and olfaction develop before those for taste and vision (Windle 1971) and little is known about the time when the sense organs become capable of functioning although inferences have been drawn from the responses produced by various stimuli. Buller (1969) showed that muscle spindles and motor end plates begin to form in human fetuses at 3-4 months of menstrual age and the fact that muscle contractions can be elicited at 2 months, indicates that a simple form of motor response can take place before any specialised nerve endings are present and long before the peripheral axons become myelinated. No spontaneous myogenic activity has been shown to occur in mammalian striated muscle except cardiac although whether or not such muscle may be mechanically or electrically excited to contraction without the intervention of motor nerves is not yet settled (Hooker, 1952). Bare nerve fibres with bulbous varicosities at their growing tips have been shown to be present among the myoblasts of the 7 weeks old human fetus (Windle 1971) and by 8 weeks they have been shown to be capable of transmitting impulses to the young muscle fibres. By the 3rd and 4th month the specialised sensory endings of the human skin have been developed although nothing appears to be known about their function in utero. Windle (1971) doubts whether pain, touch, or temperature exist in pre-natal life as we know them and there are no indications that the fetus possesses any positional sense. Windle (1971) suggests that these functions presumably lie dormant until

the fetus becomes exposed to the external environment but illustrates their potential by recording that the taste buds have been shown to be active in utero through experiments involving sweetening of the amniotic fluid. Similarly the olfactory mechanism is also believed to be capable of functioning and, although the neural mechanisms for vestibular function and hearing are laid down by the 5th to 6th week they apparently do not become active in pre-natal life (Windle 1971). Several reports are available however which clearly illustrate that the older fetus can respond to loud sounds, apparently in a manner similar to an individual in deep sleep (Sontag and Wallace, 1934). The autonomic nervous system, which regulates an individual's response to his internal environment, is presumably required very little in utero. Some functions, however, such as response to an increase in carbon dioxide, have been shown to exist (Windle 1971) and these have been related to the need of the fetus to be prepared for action should birth occur (Windle 1971).

Hooker (1952) suggests that behaviour is a fundamental characteristic of all animals and that structure and function are directly and inseparably related. He believes that when any organ reaches a level of development where its differentiation is consonant with function, then the organ will begin to function provided the environment is appropriate. He draws attention to the fact, however, that the functioning of a developing organ may not be the same as that exhibited when development is complete and he draws attention to the fact that all organs do not reach a functional state in their development at the same time. Therefore the behaviour of an organism attains its adult form only when it approaches maturity (Hooker 1952). Gesell (1945) records that as the fetal body grows so too does behaviour and, from a functional aspect, the fetus can be regarded as a growing action system. Hooker (1952) notes that just as an embryo develops morphologically in an orderly sequence, so it also does in its behaviour. Windle (1971) continues this line of thought and attempts to organise the movements

observed into some sort of pattern.

Sucking, swallowing, grasping, righting, locomotion, and breathing are to be regarded as basic fetal movements and Windle (1971) considers the earliest fetal movements as "avoidance" mechanisms in which movement is made away from the stimulus. Later in fetal life, touching the oral region may become a component of feeding which requires movement towards the stimulus with opening of the mouth. Windle (1971) however believes that the action of mouth opening might also be necessary for "gaspings". Evidence for Windle's beliefs is to be found in experiments which have shown feeding reactions to be adversely affected by asphyxia with sucking becoming abolished and swallowing impaired.

From his animal experiments, Windle (1971) believes that as animals of all species need to acquire the ability to right themselves and engage in locomotion sometime during their maturation, then "righting" and "locomotion" would presumably be more developed during fetal life in "herd" animals than in "nest" animals. On this basis, these actions would be imperfectly represented in the human infant at birth and Windle (1971) believes that they can hardly be considered essential for survival, and this interpretation does explain the differences one might expect to find between species when observing fetal movement. Windle (1971) believes that respiration is the most immediately essential movement required for survival and it is not surprising therefore to find that the motor components for breathing develop very early. The first reflexes to be elicited at 8 weeks of age appear to Windle (1971) to be utilised in the "gasp" reflex, the motor components being those of head extension accompanied by forelimb flexion. Gasping appears to be the most resistant reflex of all to asphyxia because although most of the fetal reflexes succumb quickly after placental separation, Windle (1971) has found that gasping (or, in early fetuses, the neck components which are later incorporated into gasping) is the last to disappear.

Similarly, many investigators believe that preparations are made early

in pre-natal life for obtaining and assimilating nourishment after birth (Preyer, 1885; Davis and Potter, 1946; Windle 1971). They believe that since the need of food is secondary only to that of air, it is not surprising to find evidence of swallowing and gastrointestinal movements in young human fetuses. From experiments which showed the presence of squamous epithelial cells and lanugo hair in the contents of the digestive tract, it has long been inferred that the fetus swallows amniotic fluid. Preyer (1885) in fact suggested that amniotic fluid is a food for the fetus and that the proteins in particular that are swallowed may contribute to fetal nutrition. Windle (1971) however believes that the nutritional value of the proteins must be slight even though radioactive tracer experiments have shown that the normal fetus swallows approximately 500 ml of fluid/day. To support this Windle (1971) observes that anencephalic fetuses do not swallow and there is no evidence to suggest that deprivation of these amniotic fluid proteins greatly retards their growth. By using radiography to follow the progress of ingested Thorotrast in human fetuses obtained from therapeutic abortions, Davis and Potter (1946) showed that fetal swallowing can occur when only 12 to 13 weeks old.

There is considerable argument concerning the development pattern of the various reflexes. Humphrey (1970) records that the spinal nerves in the cervical region innervate the muscles of the neck which cause contralateral bending of the head seen at 20.0 mm. (Windle and Fitzgerald 1942) and 20.7 mm (Hooker 1952, 1954) C.R. length (7.5 weeks of menstrual age). She believes that the reflex pathways available at this time probably only includes trigeminal nerve fibres, the ventral white commissure at cervical levels of the spinal cord, the ventral roots of cervical spinal nerves, and the spinal accessory nerve with additional neuronal pathways being necessary as the reflexes become more complex. Therefore, although they are limited in the amount of muscle contraction they involve, these earliest reactions are believed by some investigators to

constitute "total pattern reflexes" in the sense used by Coghill (1929) and later by Hooker (1952, 1954), for they involve all the neuromuscular mechanism sufficiently mature at that particular time to react. For human fetuses Humphrey (1970) records that these total pattern reflexes soon include contraction of all axial trunk muscles and shoulder and pelvic girdle muscles as well. The whole body is then involved in the contralateral flexion reflex with the arms pulled back or extended at the shoulders and the rump being contralaterally rotated. At 8½ weeks of gestation the mouth first begins to open as part of a total pattern reflex (Humphrey 1970) and therefore the earliest mouth opening is not an isolated event but part of a reflex involving head, trunk, and extremities. Humphrey (1970) has found that it is only much later that mouth opening can be elicited without either head or extremity movements occurring at the same time. This approach is disputed by Windle (1971) who criticised investigations of fetal movements involving some delay after removal of the fetus from the uterus. He found that when the placenta separated, the discrete character of the responses was lost, although head extension and mass movements of the trunk were still obtainable. The situation is complicated by the fact that when the oxygen supply of the fetus is exhausted, reflex activity ceases (Angulo 1932). Humphrey (1970) interprets this as meaning that only when oxygen is unrestricted can the most recently developed reflexes be detected and consequently as the carbon dioxide accumulates and the oxygen diminishes only those reflexes that developed early in fetal life are retained.

Associated with all work on fetal movement is the philosophical question of whether fetal movements can occur spontaneously or are indeed reflex activities. Hooker (1952) defines behaviour as:

".....the sum total of the adjustments made by the organism to changes in its internal or external environment. The activities of the mechanisms involved in restoring the dynamic balance of the organism as a consequence of the environmental changes give rise to bodily activity."

Using this definition, it would appear that there are no spontaneous fetal movements but Humphrey (1970) records that for a few reflexes, the nature of the stimulus is unknown and that traditionally such activity has been referred to as "spontaneous". Windle (1970) has found that some fetal movements occur spontaneously although Gesell (1945) believes that within the environment of the amniotic sac the fetus is well protected from shocks and distorting stresses with perhaps only gravity exerting its moulding influence ceaselessly and evenly. Gesell (1945) also reports that the early actions of the fetus must be presumed to occur in response to internal promptings but then fails to elaborate on this. However, Sterman (1971) found that the spontaneous movements of the human fetus show two periodicities from 21 weeks of gestation to term; one being between 30 and 50 minutes which appears to be the same as the innate Basic Rest-Activity Cycle (B.R.A.C.) of the infant after birth; the other being between 80 and 110 minutes which appears to correspond to the B.R.A.C. of the mother.

There have been reports of fetal breathing in utero for many years but the spontaneous nature of this activity has often been doubted (Boddy and Mantell, 1972). Ahlfeld (1888) described certain maternal abdominal wall movements which he attributed to fetal breathing. His observations have not been generally accepted because there was no direct evidence that the movements reflected those of the fetal respiratory muscles and studies in laboratory animals suggested that fetal breathing in utero was not normally present (Boddy and Mantell 1973). Dawes, Fox, Leduc, Liggins, and Richards (1970) however, thought that the absence of respiratory movements in the normal fetus would be surprising in view of the remarkable competence of the respiratory musculature to maintain prolonged hyperpnoea after birth and in their experiments on fetal lambs between the fractions 0.3 and 0.9 of term, which were delivered under maternal epidural anaesthesia into a warm saline bath, they were able to observe fetal respiratory movements. Dawes et.al. (1970) were able to detect similar movements from

intratracheal and amniotic pressure recordings from indwelling catheters in 14 fetal lambs in utero between the fractions of 0.68 to 0.95 of term and, after recovery from the operation, Dawes et.al.(1970) found that the irregular rhythmic respiratory movements were always present up to half the time over several days. A surprising finding was that there was no obvious correlation with fetal carotid blood gas values and that the fluid movement within the trachea was very small and insufficient to clear the "dead" space presumably because of the high viscosity and density of the fetal pulmonary fluid. These findings would explain the results of experiments where radio-opaque contrast medium has been introduced into the amniotic cavities of animals and of man and has resulted in the contrast medium reaching the gastrointestinal tract but not the bronchial tree. (Boddy and Mantell 1972). Fetal breathing movements have been used as an indication of good health in fetal lambs (Boddy and Mantell, 1972) and the first sign of impending disaster appears to be the diminution of or alteration in the characteristics of these movements (Boddy and Dawes, 1975). In fetal lambs there is normally fetal breathing for about 40% of the time (Boddy and Mantell 1973) and there appear to be two distinct types of movement; the first type consists of rapid irregular movements of 1-4 Hz only discernible during electrocortical signs of Rapid Eye Movement (R.E.M.) sleep; the second type consists of episodes of slow, 1-4/min., relatively deep respiratory efforts for 5% of the time described as gasps or sighs by analogy with such phenomena after birth (Boddy and Dawes, 1975).

The recent development of ultrasonic techniques for monitoring fetal development has included a method for monitoring fetal respiratory movements (Boddy and Mantell, 1973; Boddy and Dawes, 1975). Such a method involves locating the fetal heart echo on an A-scan display, when the fetal chest wall is identified and wall movements recorded (Boddy and Dawes, 1975). Using this method human fetal chest wall movements in utero have been detected as early as 11 weeks of gestation (Boddy and Dawes 1975) and these results correlate well with Windle's

beliefs (1971). The breathing movements are easier to identify by 13-14 weeks of age but even then they are very irregular. This irregular pattern is present up to 20 weeks but in many fetuses over 36 weeks, fetal breathing movements have become regular, as occur normally after birth at full term (Boddy and Dawes, 1975). The incidence of movements appears to be rather greater in man than in sheep, being present in the region of 55-90% of the time and recent evidence (Boddy and Dawes, 1975) suggests that the incidence falls near the onset of parturition. As in sheep, the movements are often episodic and irregular but are clearly distinguishable from "hiccoughs" (Windle, 1971) and from movements of the limbs, with a normal frequency of between 30 and 70/min. There is also evidence of diurnal variation in the proportion of time during which fetal breathing movements are normally present (Boddy and Mantell, 1973; Boddy and Dawes, 1975) and there is also evidence to suggest that hypoxaemia, hypocapnia, hypoglycaemia, and respiratory depressant drugs reduce fetal breathing, whilst hypercapnia increases its rate and depth as in sheep (Boddy and Mantell, 1973).

In many women, particularly multiparae near term, fetal breathing movements have been seen occasionally on the abdominal wall (Boddy and Dawes, 1975) and in many antenatal patients, localised abdominal movements have been seen which can be attributed to fetal breathing in utero. Boddy and Mantell (1972) identified fetal breathing from maternal abdominal movements using the positions of the fetal parts because they found that usually an intervening fetal limb appeared to transmit the breathing movements to a small area.

There is a fairly high correlation between measurements of fetal thoracic movements in utero using ultrasound A-scan and their detection using a force transducer placed on the maternal abdominal wall (Boddy and Mantell, 1972) and similar experiments involving the placing of a force transducer on the maternal abdominal wall have been undertaken to investigate the incidence of more general fetal movements. Trials involving subjective measurements of maternal appreciation have also been used to investigate the incidence of more general fetal movements (Mathews, 1973) but there appear

to be many factors limiting the value of such experiments (Liley, 1972).

Reinold and his colleagues (1971(a), 1971(b), 1971(c), 1972, 1973, and 1976; Reinold and Geortiadis 1974; Reinold and Kucera, 1975) proposed methods for observing active fetal movements in utero using ultrasonic equipment. With the aid of an ultrasonic sectional imaging unit (VIDOSON) it was found possible to continuously follow the course of fetal movements. In addition to recording fetal cardiac action, Reinold (1971(c)) was able to monitor active fetal movements and these he considered to be of greater importance than the heart movements. He found it possible to demonstrate these movements successfully from the 8th week of pregnancy onwards and in an attempt at quantitation he describes these movements as being either lively and very frequent or slow and with considerable intervals between them lasting from 1 to 5 minutes (Reinold 1971(a)). In particular he was unable to demonstrate in any of the cases a cause which might have triggered-off the movements (Reinold 1971(c)). He stresses that uterine contractions might lead to passive movements of the fetus which need to be clearly distinguished from the active movements. Reinold (1971(a)) compares the early fetal movements to the darting movements of a fish and suggests (Reinold, 1973) that the fetal body is kicked away from the wall of the amniotic cavity and then continues to swim and slowly resettle again into its original position. He suggests that the touching of the amniotic wall may induce a new movement which starts with a strong and sudden action resulting in the whole body of the fetus moving and changing position in the amniotic cavity. The other type of movement which Reinold (1973) detected is described as being slow and inert, there being no strong component in the movement. The position of the fetus in this situation is changed either only slightly or not at all and it appears that only parts of the body, such as the extremities, are moved. Using this method, Reinold (1973) suggests that it is possible to predict when the fetus is at risk, particularly when spontaneous fetal movements are absent.

Gesell (1945) suggests that the behaviour of the fetus is not as random nor even as reflex as it might seem. He encourages people to suppress the image of the text book fetus on the grounds that many are based on post-mortem, frozen sections which exaggerate the crumpled and confined attitudes of the fetus, too often being pictured in a stereotyped sedentary posture. He advocates picturing the fetus in prone, supine, and oblique orientations which it must variously assume realising that even later in gestation the fetus still has scope for body movements and variations in position.

CHAPTER III

METHODS AND MATERIALS

METHODS AND MATERIALS

Three different sets of radiographs were used in the study and each set was obtained from a different source; Nottingham, London, and Southampton.

Nottingham Material

One hundred and ninety-five human fetuses were obtained from the Department of Human Morphology of Nottingham University Medical School. The fetuses were the products of induced abortions and had been acquired over a period of approximately four years during which time they had been fixed and preserved in formalin. Scammon and Calkins (1929) showed that preservation in formalin does not significantly alter such specific dimensions as C.R. length but O'Rahilly and Meyer (1956) criticised formalin for being a decalcifying agent either alone or in conjunction with other substances. Ham (1957) however, states that mineral deposits are not materially affected by aqueous fixing solutions such as formalin unless they specifically contain an acid and therefore this latter criticism is inapplicable in this case.

Unfortunately no clinical history was attached to any of the fetuses and therefore no determination could be made of any of the factors known to affect growth and development or ossification.

The fetuses were initially graded by a visual assessment of their approximate C.R. length and were then marked by attaching a small piece of numbered Dymo tape to either the left ankle for the larger fetuses or to the neck for the smaller fetuses. In this way the fetuses were catalogued for future reference in an approximate order of C.R. length. Such a procedure also showed that all of the fetuses were whole and none showed any sign of external abnormality. On this basis, none of the fetuses was initially excluded from the study.

Several external measurements were taken of the individual fetuses;

a) Crown-Rump Length

The fetus was allowed to take up its "fixed" position in a lateral plane and the greatest distance from the vertex to the buttocks was measured to the nearest 0.5 mm, using the anthropometric calipers shown in Fig.1. This position was easily obtained in those fetuses that were truly "fixed" but in several fetuses the neck was "loose" and a subjective estimate of a "normal" fetal position had to be applied before the measurement could be made. Coupling this with the apparently normal range of biological variation in the fixed fetal position, there is a possibility that the potential variance of such a measurement is going to be quite large.

b) Crown-Rump Extended Length

With the help of a second observer, each of the fetuses was held in an extended position in a lateral plane and the greatest distance from the vertex to the buttocks was measured to the nearest 0.5 mm using the anthropometric calipers. (Fig.2). This position is more objective in terms of measurement than that of C.R. length and because of this does not possess the variation associated with "normal" fetal posture. Quite clearly it might also be an entirely different measurement to that of C.R.length (Bagnall, Jones, and Harris, 1975).

c) Bi-Parietal Diameter (B.P.D.)

With the fetus held carefully in the left hand, the greatest distance between the parietal bones of the skull was measured to the nearest 0.5 mm.(Fig.3). During this procedure considerable variation was noted between fetuses regarding their relative state of dehydration with several of the fetal heads showing a distinct shrivelled or shrunken appearance whilst others displayed a more full, rounded shape. It would appear that although formalin has been shown not to significantly alter such specific dimensions as C.R. length (Scammon and Calkins, 1929) it might well affect the measurement of such lengths as B.P.D. A further



Figure 1. The Measuring of Crown Rump Length.



Figure 2. The Measuring of Crown Rump Extended Length.

variation was noted concerning the shapes of several of the fetal skulls which appeared to be outside the normal range of biological variation. The majority of the fetuses possessed an approximately spherical skull whilst others possessed a distinctly conical-shaped skull, and it was realised that this might lead to a sizeable source of variation. Perhaps an explanation for a conical skull, which is apparently normal in all other respects, is to be found in either the mechanics and circumstances of the abortion from which the fetus came or the position and method of storing, in addition to the possibility of normal variation.

d) Weight.

The fetuses were carefully dried in a paper towel and then weighed on a torsion balance to the nearest 0.1 g. It was realised that the weight of a fetus will fluctuate according to how much fluid has been absorbed as well as how much material is included in the measurement and it was therefore appreciated that weight is by no means an ideal measurement, only a convenient one. As Tanner (1970) states:

"It lumps together too many different tissues".

However, the simplicity and convenience of weight as a measurement must weigh heavily against any criticism of its accuracy.

e) Sex

Using the criteria set out by Hamilton and Mossman (1972) an attempt was made to determine the sex of each of the fetuses. Hamilton and Mossman (1972) report that up to about 50 mm. C.R. length the external genitalia in the two sexes are essentially similar and it is only after 50 mm. C.R. length that sex can be determined from external characteristics without error. However, even with the aid of a 4 diopetre lens, this proved to be a most difficult task especially with the smaller fetuses and therefore there were several fetuses in whom the sex was not determined.

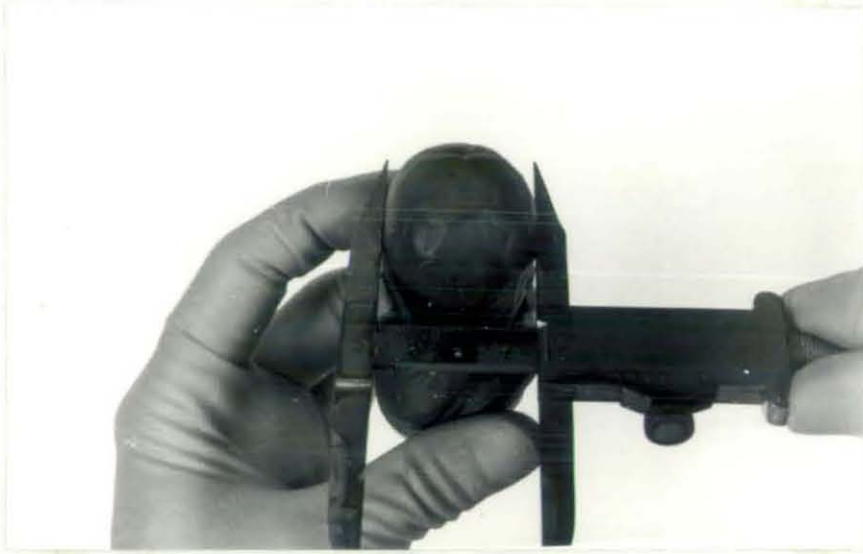


Figure 3. The Measuring of Bi-Parietal Diameter.



Figure 4. The Arrangement of the Apparatus for the Lateral Radiograph.

To eliminate inter-observer error of measuring, the same observer made all of the measurements and in order to check the accuracy of these vital external measurements, the distances of B.P.D. and C.R.E. length were taken "blind" for a second time approximately three weeks after the initial measuring. The results of this second occasion were compared with those obtained initially and any discrepancies were checked.

The close inspection of the individual fetuses that the determination of the external measurements required, failed to reveal any fetus which exhibited an obvious external abnormality and therefore all of the fetuses were included to the latter part of the study.

No attempt was made to determine the traditional Crown-Heel length of the fetuses owing to the difficulty of placing the fetuses in the required position for such a measurement. When the fetuses are fixed it is extremely difficult to satisfactorily straighten the flexed hip and knee joints.

Photographs

A black and white, 35 mm. photograph was taken of each individual fetus in a lateral position. A corresponding catalogue number and a scaled marker were also placed in the field of view. The photographs made easier the identification of the fetuses for future discussion and it also allowed a fetus to be correlated with a similar radiograph which was to be taken later in the study. The scaled marker enabled life-size photographs to be reproduced.

Radiography

Hartley (1957) showed that a high standard of radiography which has been specifically designed in order to reveal fetal morphology must be used when

attempting to estimate fetal maturity from radiographs. Logically, the pattern of fetal development will only be shown clearly when the radiographs are produced with this end in view and therefore a specific technique was planned and carried out.

A comprehensive view of as many as possible of the bones in the human fetus was required and therefore methods for obtaining both a lateral and an antero-posterior (A.P.) view of each of the fetuses were considered. It was hoped that this combination of views would eliminate those problems encountered when there is superimposition of one centre upon that of another.

A framework of lead sheeting was constructed so that it provided both a permanent base and a surround for the radiographic film envelope. (Fig.4). The lead base would absorb the penetrating X-rays and so prevent a back scatter. This would aid in increasing the definition on the radiographs. The lead surround was arranged so that the radiographic film envelope could be placed in a fixed position and was of double thickness so that pieces of single lead could be placed on the envelope as masks whilst at the same time maintaining the same overall thickness.

The fixed fetuses were very resistant to any movement of position and so a rig was built upon which the fetuses could be placed and held in any required position whilst being radiographed. To determine the most suitable radio-translucent material from which this rig could be made, several materials including wood, cork, thin sheet-steel, and aluminium were radiographed and it was found that a transparent $\frac{1}{8}$ " perspex sheet was the most suitable material in terms of both radio-translucency and application.

The lateral view presented few problems because the fetuses could be positioned quite easily on their side and if any variation from a true lateral position was encountered this could be corrected by suitable placings of non-opaque pads.

The A.P. view however presented several problems because the fixed position of the fetuses was not conducive to an easy positioning of the limbs and uncurling of the body. With this in mind several methods were considered which consisted of holes being drilled into the perspex and tape-loops being formed to position the fetuses. The poor radiograph which resulted from these translucent holes eliminated this idea and a method using Velcro tape was adopted. (See Figure 4 and Figure 5). Two strips of "positive" Velcro tape were glued approximately 12" apart on the perspex and this enabled various-sized strips of ordinary white cotton binding tape with small strips of "negative" Velcro tape attached to the ends, to be stretched between them. The Velcro tape was able to generate sufficient force to allow the white cotton tapes to hold the fetuses quite firmly in any required position so that an A.P. radiograph could be taken with the minimum of radio-opacity. This arrangement worked very well and after a short while became very easy to use particularly when it was realised that if the first tape applied was one across the fetal trunk followed by one across the fetal head, then a great deal of both effort and time were saved. (See Figure 5).

The lateral view, therefore, was taken with the fetuses lying on their left side in a true lateral position with the right shoulder and right iliac bone being directly above their counterparts on the left side. No attempt was made to straighten the fetal trunk or limbs from their fixed positions.

The A.P. view was taken with the fetuses in a supine position on the perspex sheet and the fetal trunk was straightened using the cotton tapes. The head was held in the Frankfort plane and the shoulders and hips were both placed in a horizontal plane. The limbs were positioned as shown in Figure 5 and care was taken to ensure that both the upper and lower limbs were held in the same horizontal plane, parallel to that of the film.

In both views lead numerals which corresponded to the number on the

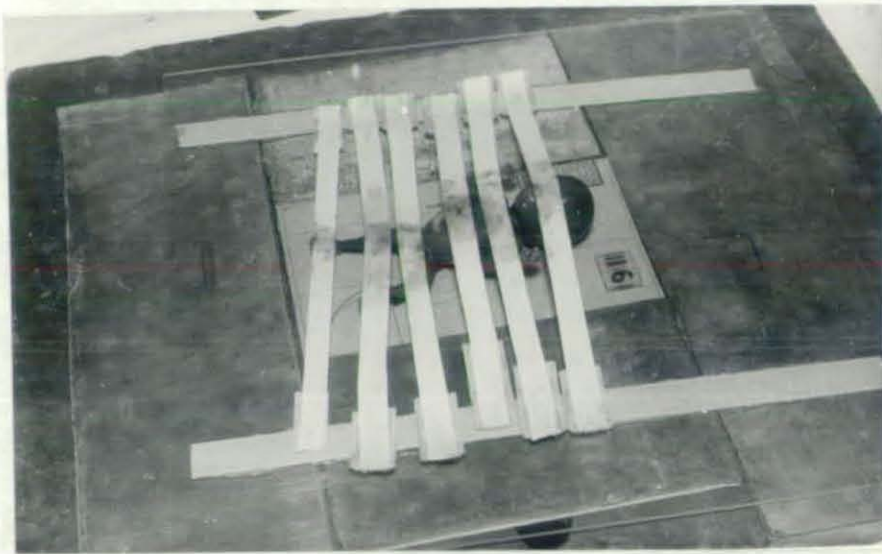


Figure 5. The Arrangement of the Apparatus for the Antero-Posterior Radiograph.

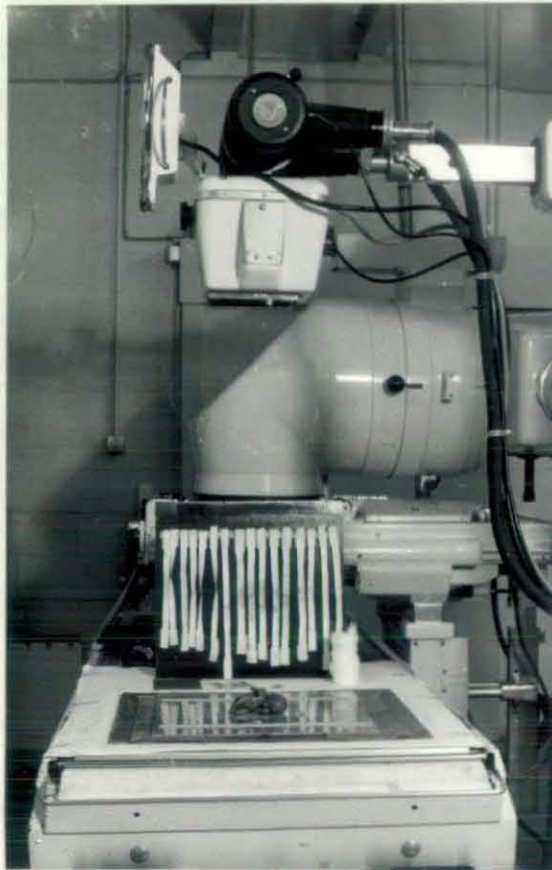


Figure 6. The Arrangement of the Apparatus.

fetal Dymo tag, were placed on the perspex sheet so that the radiographs could be catalogued and correlated with the external measurements of the individual fetuses.

To obtain radiographs of high quality and definition several experiments were made with combinations of relevant factors, which established the following points:

- a) The radiographic film yielding optimum quality was Kodak Industrex C which is a fine-grain, high contrast, non-screen film specially designed for the radiography of morbid specimens where fine detail is required.
- b) 180 mAs.
- c) 42 kV for the smaller fetuses.
55 kV for the larger fetuses.
- d) The anode-film distance was kept constant at 100 cm. For all practical purposes this distance minimised any magnification factor involved with the X-ray beam.
- e) The centre of the radiation field was determined in relation to the light field from the light beam diaphragm and from this the central ray was always positioned in the centre of the section of the film that was to be exposed. The fetus was then positioned so that it too was centred on the film before any exposure was given.
- f) The radiographs were developed in Kodak DX80 developer for 4 minutes at 20°C.

Figure 6 illustrates very clearly the arrangement of the apparatus that was used whilst taking the radiographs and Figure 7 is an example of the type of radiograph produced, both in a lateral view and in an A.P. view.

The radiographs were then catalogued with their corresponding photographs and external measurements of the fetus.

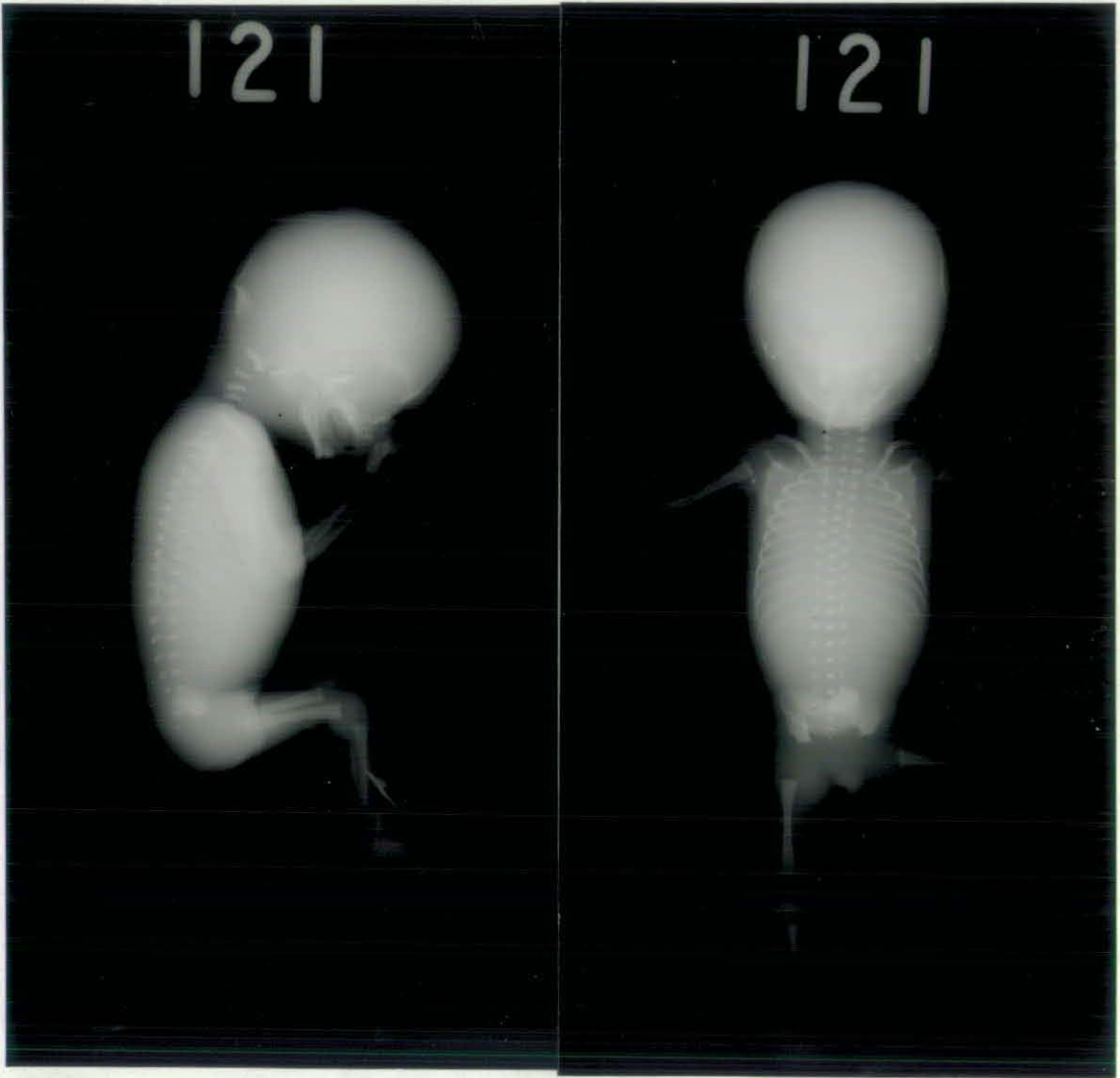


Figure 7. An Example of the Types of Radiograph Produced.

London Material

Radiographs of 236 fetuses were borrowed from the Department of Growth and Development, Institute of Child Health, University of London. The fetuses were products of spontaneous abortions in the late 1950's and early 1960's and had been measured and radiographed whilst still fresh. Because of this, the fetuses were still very flexible and no restraining apparatus was required to position them. For the radiographs the fetuses had been positioned in a similar manner to that previously described for the Nottingham material but unfortunately only an A.P. view had been taken. The anode-film distance had been kept constant at 36" (96 cm) and the radiographic film that was used was Ilford 'ILFEX' non-screen film which is no longer being manufactured. For each of the fetuses several external measurements were presented and these included an assessment of the fetal sex in addition to the measurement of Crown-Rump Extended length. Several of the fetuses also had a brief clinical history attached relating to both the maternal and fetal health.

Southampton Material.

From the Department of Child Health, Medical School, Southampton University, radiographs were borrowed of 297 fetuses. The fetuses were the products of both spontaneous and induced abortions and recordings had been made of both the Crown-Rump Extended length and sex of each fetus whilst it was fresh. Prior to the radiography each fetus had been immersed in a 0.5% solution of silver nitrate for approximately two days and then had been positioned using pins and cellophane on a piece of cork. Care had been taken to place the limbs in a plane parallel to that of the film but unfortunately no attempt had been made to align the hips and shoulders so that the vertebral column invariably had a marked lateral curvature. The anode-film distance had been kept constant at 36" (96 cm.) for

all of the radiographs but only A.P. views had been taken.

The total number of fetuses for this study amounted to 728; 195 from Nottingham, 236 from London, and 297 from Southampton.

In order to define the various regions of the vertebral column, several lines were drawn where possible on the A.P. radiograph of each of the fetuses. These lines were drawn very carefully in pencil and were determined as follows: (See Figure 8).

- a) The number of ribs that were present was counted and the neural arches and centrum associated with the 12th rib were located. The line A was drawn midway between the line of the neural arches and centrum of T_{12} and the line of the neural arches and centrum of L_1 .
- b) This preliminary inspection of the ribs enabled a record to be made of vertebral anomalies, such as the presence of lumbar or cervical ribs.
- b) Line B was drawn and connected the centres of the ossification centres of the neural arches for C_1 .
- c) The position of the right neural arch of T_1 was located by counting the neural arches from line A, and line C was drawn midway between the centre of the right neural arch of T_1 and the centre of the right neural arch of C_7 .
- d) The position of the centrum of T_1 was located by counting the centra from line A and line D was drawn midway between the centre of the centrum of T_1 and the centre of the centrum of C_7 .

At first it was surprising to note that line D was not a continuation of line C and that several millimetres separated the two lines. This prompted investigation of whether or not the positioning of the X-ray affected the relative positions of the centra and neural arches. Accordingly several radiographs were taken of the same fetus, each time varying either the position of the fetus (A.P. or P.A.) or the position of the central ray of the X-ray beam. (At the head or toes). In all the radiographs the

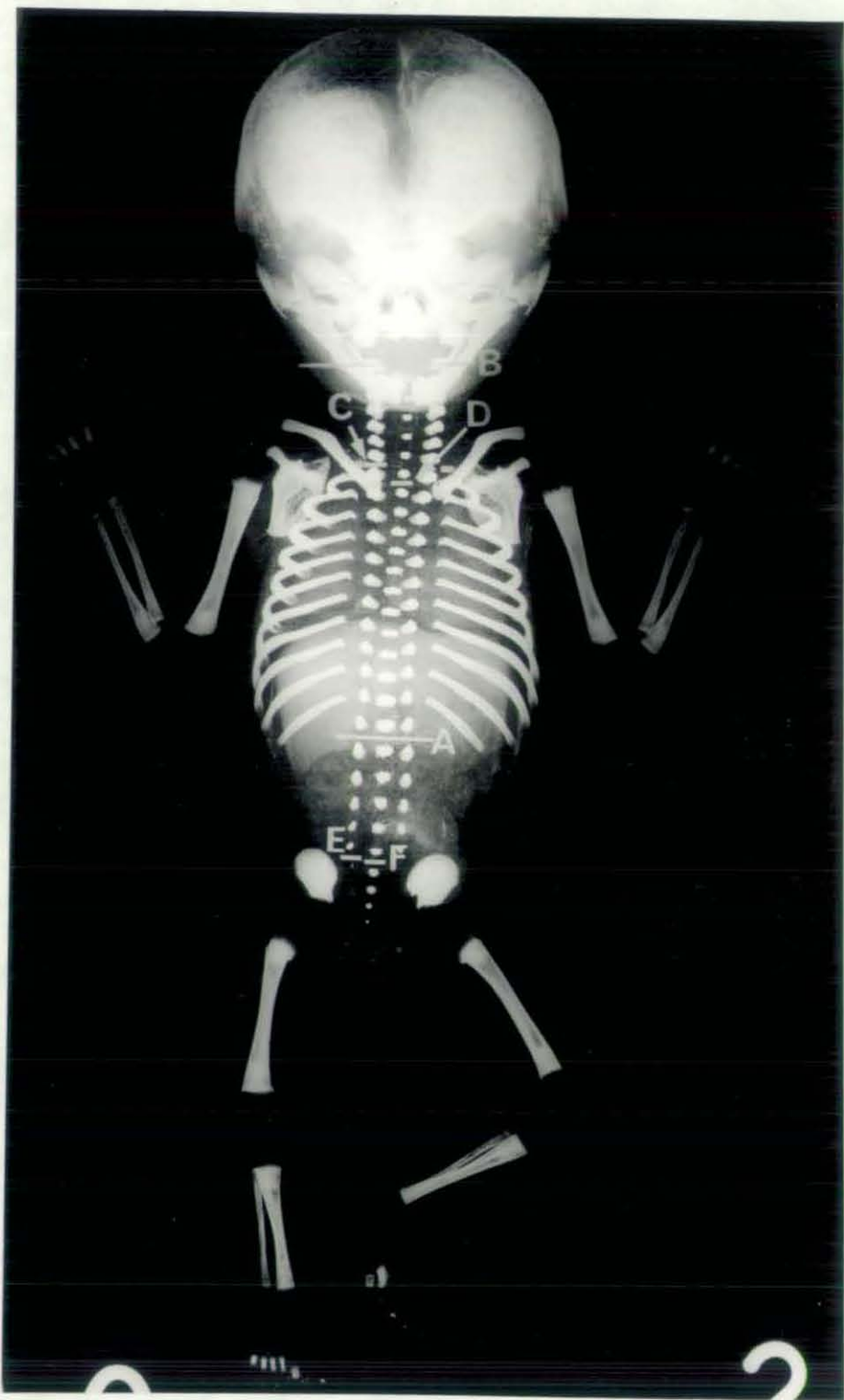


Figure 8. The Positioning of the Identification Lines on the Radiograph.

vertebral column had the same appearance with an apparent discrepancy between the relative positions of the neural arches and centrum for each vertebra in the upper thoracic region. This apparent anomaly is brought about by a difference in the relative positions of the centres of ossification for the vertebrae in this region but nevertheless does mean that different measurements can be made for apparently the same regions depending upon whether the centra or the neural arches are used as boundaries to define the regions.

- e) The right neural arch corresponding to L_5 was identified by counting the neural arches from line A, and line E was drawn midway between the centre of the right neural arch of L_5 and the centre of the right neural arch of S_1 .
- f) The position of the centrum of L_5 was determined by counting the number of centra from line A, and line F was drawn midway between the centre of the centrum of L_5 and the centre of the centrum S_1 .

These lines therefore were used to define the various regions of the vertebral column but, as can be seen for each region two measurements can be taken depending upon whether the centra or neural arches are used for identification

Using the centra the complete regions were defined as follows:-

- a) Cervical - from the centre of the centrum for C_1 to the line D.
- b) Thoracic - from line D to line A.
- c) Lumbar - from line A to line F.
- d) Sacral - from line F to the centre of the centrum for S_5 .

Using the neural arches, the complete regions were defined as follows:-

- a) Cervical - from line B to line C.
- b) Thoracic - from line C to line A.
- c) Lumbar - from line A to line E.
- d) Sacral - from line E to the centre of the neural arch for S_5 .

No definition for the coccygeal region was made because no ossification centres for this region develop during the period under study and therefore no

boundaries can be determined radiographically.

The determination of the boundaries for the regions was accomplished by observing each of the radiographs through a 4 dioptré lens. This lens was used throughout the study wherever measurements were taken from the radiographs and was especially useful in determining whether or not a particular ossification centre was present.

Several measurements were then taken from each of the radiographs, each for a specific purpose, but not all of the measurements were able to be taken from all of the radiographs;

1. Numbers of Ossification centres and their positions.

The number of ossification centres that were present for the vertebral column were noted as follows:-

- a) The number of neural arches on the right side.
- b) The number of neural arches on the left side.
- c) The number of centra.
- d) The total number of centres.
- e) The number of cervical centra.
- f) The number of thoracic centra.
- g) The number of lumbar centra.
- h) The number of sacral centra.
- i) The number of cervical neural arches.
- j) The number of thoracic neural arches.
- k) The number of lumbar neural arches.
- l) The number of sacral neural arches.

All of these measurements were taken from the A.P. radiographs but if there was any doubt as to whether or not a centre was present, the lateral radiograph was used to help in the decision. In the literature, cases have been reported of bilateral centres of ossification being present for the centra and this

situation was accounted for by only allocating a score of one even if two centra ossification centres were observed for one vertebra. This is particularly relevant when the centrum for the dens is considered because this normally ossifies from two centres which only merits an allocation of a score of one.

The number of ossification centres that were present for the 12 long bones of the fetal limbs was also noted.

In addition to recording the numbers of ossification centres that were present for the vertebral column and long bones of the limbs the positions of these centres were also noted so that patterns of ossification, if they existed, could be determined.

2. Absolute Measurements.

Several absolute measurements were taken from the A.P. radiographs of each of the fetuses using the radiographic calipers described by Tanner and Whitehouse (1955). (See Figure 9). These calipers allowed each of the measurements to be made to the nearest 0.1 mm. and included:

a) Longitudinal lengths of the vertebral column

These measurements were designed to represent the lengths of the various regions of the vertebral column and used the ossification centres of the vertebrae as landmarks. It was appreciated that the regions include within their boundaries the intervertebral discs and the lines A, D, and F that were drawn on the radiographs previously were considered to represent the midlines of the corresponding discs. Therefore, the regions of the vertebral column as shown by the centra were considered to have boundaries midway between the centres of the appropriate centra which corresponded to the midline of the intervening intervertebral disc. Similar boundaries for the regions as shown by the neural arches were considered simply to be midway between the appropriate neural arches.

The upper border of the cervical region, however, in both the neural arches and the centra cannot conform to this criterion because there is no



Figure 9. The Radiographic Calipers.

additional vertebra by which to assess the upper limit. It was decided therefore that the upper border of the cervical region would be determined by a line drawn through the centre of the corresponding ossification centre for C₁, and the lower border of the sacral region was defined by a line drawn through the ossification centre corresponding to S₅. These definitions also eliminated the small error that would have been encountered had the line been drawn along the superior and inferior borders of the respective ossification centres. Growth in length would now only be recorded when actual growth of the vertebra took place and not when there was merely growth of the ossification centre within its cartilaginous mass.

Another problem arose when regions were encountered where not all of the vertebrae had commenced ossification and the length of the complete region of the vertebral column could not be determined. One solution would have been to ignore these situations entirely and concentrate solely upon completed regions of the column but it was felt that these "partial" lengths had some value. Therefore, whenever an incomplete region was encountered and the complete region could not be determined a line was drawn midway between the centres of the two most superior or inferior ossification centres and represented the border of that particular "partial" region. This line was related to the lines that had been drawn previously and allowed for an estimate to be made of the length of a complete number of specific vertebrae. Using this method, one more vertebra would be added to the length of a particular region as each ossification centre developed and this would continue until all of the vertebrae for that region were ossified in addition to the first centre of the consecutive region when the intervening border line could be constructed. A problem arose however when the ossification centres for C₁ and S₅ developed because they represented the ends of the column as a whole and the total lengths of the cervical and sacral regions could be defined. The lengths of approximately $1\frac{1}{2}$ vertebrae were therefore added

to the lengths of both the cervical and sacral regions instead of the usual one vertebra when the ossification centres for C_1 and S_5 appeared. This was borne in mind when assessing the results. Coupled with the times for appearance of the ossification centres of the vertebral column it was thought that the method described would allow the growth of the various regions of the vertebral column to be followed continuously.

The measurements of the longitudinal lengths of the vertebral column involving the centra were;

i) Cervical region.

If the ossification centre for C_1 was present, from the middle of this centre, to line D.

If the ossification centre for C, was not present, from the most superior intervertebral disc line that could be established to the line D.

ii) Thoracic region.

If the complete region could be identified, from line D to line A.

If the complete region could not be identified, from line A to the most superior intervertebral disc line that could be established.

iii) Lumbar region.

If the complete region could be identified, from line A to line F.

If the complete region could not be identified, from line A to the most inferior intervertebral disc line that could be established.

iv) Sacral region

If the ossification centre for S_5 was present, from line F to the inferior border of this centre.

If the ossification centre for S_5 was not present, from line F to the most inferior intervertebral disc line that could be established.

The measurements of the longitudinal lengths of the vertebral column involving the neural arches were taken on the right side only and consisted of;

i) Cervical region.

If the ossification centre for C_1 was present, from the line B to line D.

If the ossification centre for C_1 was not present, from the most superior intervertebral disc line that could be established to line D.

ii) Thoracic region.

If the complete region could be identified, from line D to line A.

If the complete region could not be identified, from line D to the most inferior intervertebral disc line that could be established.

iii) Lumbar region

If the complete region could be identified, from line A to line F.

If the complete region could not be identified, from line A to the most inferior intervertebral disc line that could be established.

iv) Sacral region.

If the ossification centre for S_5 was present, from line F to the inferior border of this centre.

If the ossification centre for S_5 was not present, from line F to the most inferior intervertebral disc line that could be established.

The measurements were taken parallel to the central axis of the fetus and perpendicular to the horizontal lines that had been drawn. This was particularly relevant to the measurements involving the neural arches because there are lateral curvatures of the neural arches in all regions of the column. It was thought that if measurements were taken in this manner then the direct contributions of each of the regions to growth in functional length of the fetus, could be established.

b) Inter-neural arch distance of the vertebral column.

Having completed the measurement of the longitudinal growth of the vertebral column, attention was directed towards developing a method for measuring the width of the vertebral column in the coronal plane. To accomplish this a series of A.P. radiographs which demonstrated the range of development of the

vertebrae, was selected and studied in order to find landmarks that were present and easily identifiable throughout fetal life and which would be representative of horizontal growth of the vertebrae.

The landmarks that were selected were two circles, one for each side of each vertebra, and these were found to be constant features not only throughout fetal life but also to each vertebra. (See Figure 7 and Figure 8). Their presence has been noted previously and commented upon by Barson (1965). These circles represent the junction between the pedicles and laminae of the vertebrae and are present throughout fetal life because this is the site for the commencement of ossification of the neural arch. They are easily recognizable and the horizontal distance between them for each vertebra is indicative of the growth of the vertebral canal.

The actual point that was used to define the landmark for measurement purposes, was the centre of each circle as this point would remain constant regardless of growth in thickness of either the constituent lamina or pedicle, and would represent "true" growth in width of the vertebral arch. It was also appreciated that by using this method the actual width of the vertebral canal might become less without any growth being recorded in the distance measured because of growth in the constituent pedicles or laminae without any corresponding growth in the actual vertebra.

Therefore, the inter-neural arch distance for every identifiable vertebra was measured on each of the A.P. radiographs for each of the fetuses. The horizontal distance measured was that between the centres of two corresponding easily identifiable circles which represented the perimeters of the periosteal ossification at the junction of the laminae and pedicles of both sides of each vertebra. (See Figure 10).

Measurement of the growth in width of the vertebral column in the sagittal plane was also considered, but from a selected series of lateral radiographs it was apparent that the ribs, in particular, obscured the view of

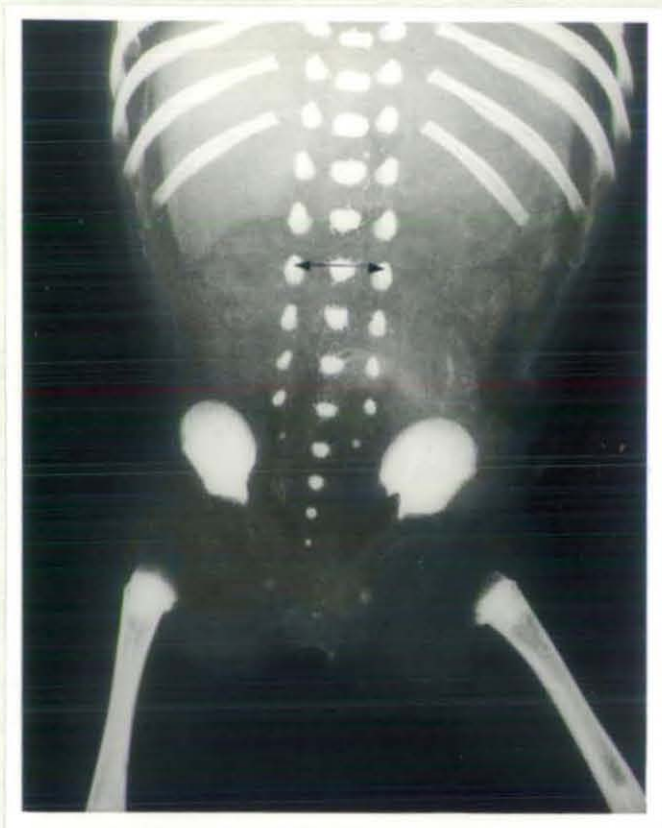


Figure 10. The Measuring of the Vertebral Inter-Neural Arch Distance. (Only one vertebra has been selected as an example).

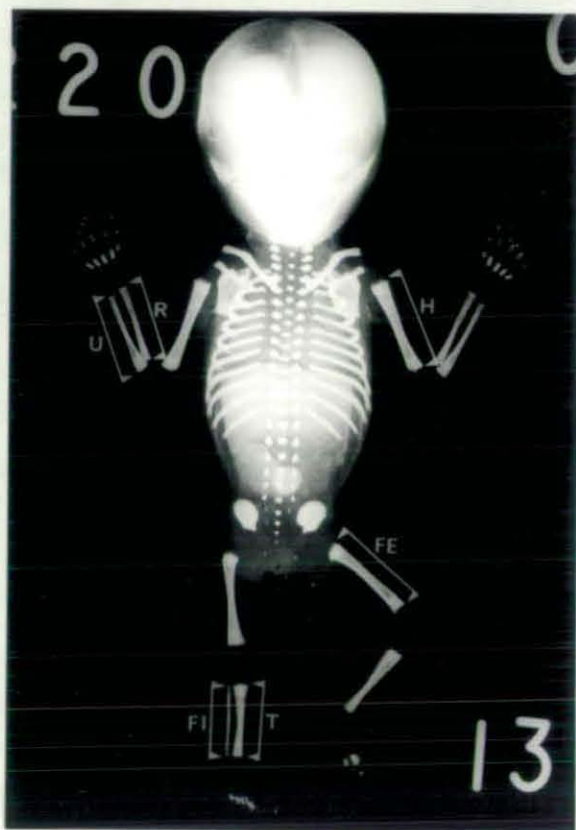


Figure 11. The Measuring of the Length of Ossified Shafts of the Long Bones of the Limbs. (Only one bone of each pair has been selected as an example).

the column and that unless the lateral view was perfectly true, the misaligned neural arch ossification centres merely confused the picture. Growth of the vertebral column in this dimension therefore, was not attempted.

c) Lengths of the ossified sections of the long bones of the limbs.

A method for measuring the ossified lengths of the long bones of the limbs was devised which would be applicable throughout fetal life. In order to appreciate the changes that would occur with fetal growth a series of radiographs was selected which illustrated the development of the fetal limbs.

From a study of these radiographs it was apparent that the periosteal and endochondral ossification developed together, with the end of the ossified length being built up from a mixture of the periosteal collar, primary ring, and the edge of the endochondral ossification area. It was also noted that the primary ring of the periosteal collar projected further than the lateral edge of the endochondral ossification area but sometimes did not project further than the centre of this area particularly when it was convex or contained a slight protuberance as it often did in later fetal life. This situation was found to exist for all of the long bones and therefore it was decided to measure the maximum length of ossified bone, whether it be defined by endochondral or periosteal bone. Since this work was completed Birkbeck (1976) has published descriptions of the growth and development of the long bones of the limbs as seen radiographically. He has used several landmarks in development as stages in a system for estimating skeletal maturity and the changes described are suitable for the measurements taken in this study.

The method devised consisted of drawing a line along the bone parallel to its long axis and measuring the maximum length of ossified section along this axis between two more lines which had been drawn at 90° to this axis and which represented the two ends of the ossification whether they be represented by endochondral or periosteal bone. (See Figure 11).

Such a measurement would take into account any longitudinal rotation of the limb and would be applicable throughout fetal life. Therefore, using this method, the lengths of the ossified sections of the long bones in both right and left limbs of each fetus were measured.

3) The Curvatures of the Vertebral Column

From the lateral radiographs the presence or absence of curvature in the four regions of the vertebral column was noted in addition to the direction of any curvature. From these results it was hoped to establish the growth and development of the curvatures in the sagittal plane of the vertebral column.

Determination of any lateral curvature in the vertebral column was also contemplated but because of the rigid position of the fetus during the A.P. radiography this was considered to be a very artificial situation and was consequently not attempted.

Reliability

Before any absolute measurements were taken for the study, whether they were external measurements taken directly from the fetus or measurements taken from the radiographs, they were practiced in order to reduce the intra-observer error. To estimate the reliability of these measurements, a representative for each of the definitions given was selected and measured a total of 10 times, with the Coefficient of Variation being calculated for each measurement.

Being satisfied with the reliability of the absolute measurements a check was then made on the reliability of positioning for the A.P. radiographs. This involved radiographing the same fetus 5 times in an A.P. position as defined previously and calculating the Coefficients of Variation for each of the measurements.

All the results of the study were written directly onto computer

data sheets to avoid any error of copying and the computer cards that were punched from these sheets were interpreted and verified again in an attempt to reduce any error of copying. In addition to this, computer print-outs were made of the data cards and these were checked with the original data sheets. Several of the computer programmes that were used also contained scatter plots in their design and from these any large errors would be clearly apparent. Therefore, all in all, it was thought that the results which were fed into the computer for final analysis were free from any errors.

Silver Nitrate Staining

The effect on the assessment of fetal ossification after staining with silver nitrate, was considered. The fetuses from Southampton had been immersed for approximately two days in a 0.5% solution of aqueous silver nitrate before radiography and therefore an attempt was made to assess the extent of any changes that this might make.

One of the fetuses from Nottingham, for whom all of the external measurements and radiographs had been taken, was immersed in a 0.5% solution of aqueous silver nitrate. It was kept in this solution in a darkened refrigerator at 6°C for several days and at regular daily intervals it was radiographed in an A.P. position. The radiographs that were produced were then compared with the original radiographs of the same fetus that were taken for the study before it was stained. In this way an assessment was made of the effect of silver nitrate staining on the appearance of ossification centres by radiography. The fetus was allowed to remain in the silver nitrate for longer than two days in order that a more general assessment of the method could be made.

Fetal Ageing

There are numerous methods available for estimating the age of the human fetus but when such things as L.M.P. dates or coital dates are not available, the fetus is given an age based upon its characteristics, usually some form of external measurement. Several external measurements that were available for each of the fetuses were considered for this purpose and it was decided that the best measurement available from an anthropometric point of view was Crown-Rump Extended length. The selection of this measurement also meant that it was possible to assign an age to all of the fetuses based upon the same parameter.

The measurement of C.R. length is common and there are Tables available in many text books which profess to being able to assign an age to a fetus based on this measurement. Several of these Tables were consulted and their results plotted on the same sheet of graph paper. It quickly became apparent that there were large discrepancies between many of these graphs and the age that could be assigned to a particular fetus was dependent upon the graph that was selected, with large differences being present between the estimates of age for the same fetus using different graphs. This situation was entirely unsatisfactory and therefore a new Table, designed specifically for this study was constructed and was able to give an estimate of age to each of the fetuses based upon the measurement of C.R.E. length.

The records of 256 human fetuses were obtained from the Institute of Child Health, University of London, and included the date of the last menstrual period of the Mother, the sex of the fetus, and several external measurements including C.R.E. length. Unfortunately, the records of 127 of these fetuses contained information which suggested that the growth of the fetus might be abnormal in some way or that the L.M.P. dates of the Mother were unreliable. The records of the remaining 129 fetuses were subjected to polynomial regression analysis and an equation was calculated which would allow an estimate of fetal

age to be made based upon the measurement of C.R.E. length. Prior to this analysis an allowance of 14 days was made upon the L.M.P. dates so that the age obtained could be considered as an estimate of the true conceptual age. The age was also expressed as a decimal of a year.

Other methods and other families of curves besides polynomial were considered in an attempt to improve upon the method of fetal age assessment and the records of the 127 'abnormal' fetuses were also subjected to analysis in order to assess their degree of abnormality.

Radiography in Obstetrics.

The radiography of pregnant women is common in current clinical practice although its use is restricted because of the recognition of the dangers from X-rays. To assess the direct relevance to the clinical situation of a study which provides information on the development of the fetal vertebral column and limbs a questionnaire was sent to a sample of Radiology Departments in Hospitals throughout Great Britain. The questionnaire asked specifically for information about the incidence of fetal radiography and also asked for information about the methods used for estimating fetal maturity from radiographs. An example of the questionnaire is shown in Figure 12.

Fetal Movement

As the study developed the importance and relevance of fetal movements in utero became apparent but a search of the literature revealed that no method for observing fetal movements in utero existed. The information that was available concerning fetal movements had been gleaned from very artificial situations ex utero and there was a suspicion that these movements that were described might not truly represent the movements that the fetus actually performed in utero. Therefore

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DEPARTMENT OF HUMAN SCIENCES

3rd March, 1975

Dear Sir,

My Ph.D. study is concerned with the osseous development of the human foetal skeleton and in order to view the maturation pattern, I have taken radiographs of human foetuses "ex utero".

I was wondering just how applicable my study is to the "in vivo" situation because I am placing particular emphasis on the estimation of gestational and developmental age.

Therefore, I would be very grateful if you would briefly describe the method used in your department for the estimation of gestational age from radiographic examination, and if you would also kindly indicate the number of radiographic examinations per year of the gravid uterus where specific requests for foetal age have been made.

Your help would be greatly appreciated.

Yours faithfully,

Keith M. Bagnall

Figure 12. The Questionnaire concerning Fetal Radiography.

a method was devised to monitor fetal movements in utero using ultrasound.

The apparatus consists of a transducer which emits very high frequency sound waves and receives any returning echoes which are then displayed on a series of screens. (See Figure 13.)

Whenever the ultrasound beam crosses an interface between two substances of different densities an echo is produced which is sent back to the transducer and a white spot is produced on the B-scan screen. In this way, if the transducer is moved over the abdomen of the pregnant woman a two-dimensional, cross-sectional picture of the abdomen will be produced on the B-scan screen although in practice it takes several scans to produce a fully composite B-scan picture. A permanent record of the pictures produced on the B-scan screen can be made by using a polaroid camera which can be brought down in front of the B-scan screen. An example of the type of photographs that are produced is shown in Figure 14.

In order to help identify specific fetal parts a series of both longitudinal and transverse scans were made of an aborted fetus placed in a tank of water (See Figure 15) and these were repeated for several fetuses of different size.

Actual fetal movements however are continuous and therefore another technique called "Persistence Scanning" was used to monitor the movements. In this technique the picture that is produced by a single scan is allowed to remain on the B-scan screen for only a few seconds before it fades. If repeated scans are made across the same section of maternal abdomen then those parts that do not move will remain as constant features on the screen whilst those parts that do move will become new features. Using this technique it has been found possible to continuously monitor fetal movements in utero even from a very early age.

In order to check that the interpretation of the movements seen was correct, simulated fetal movements were performed by an aborted fetus placed in a tank of water. (See Figure 16).

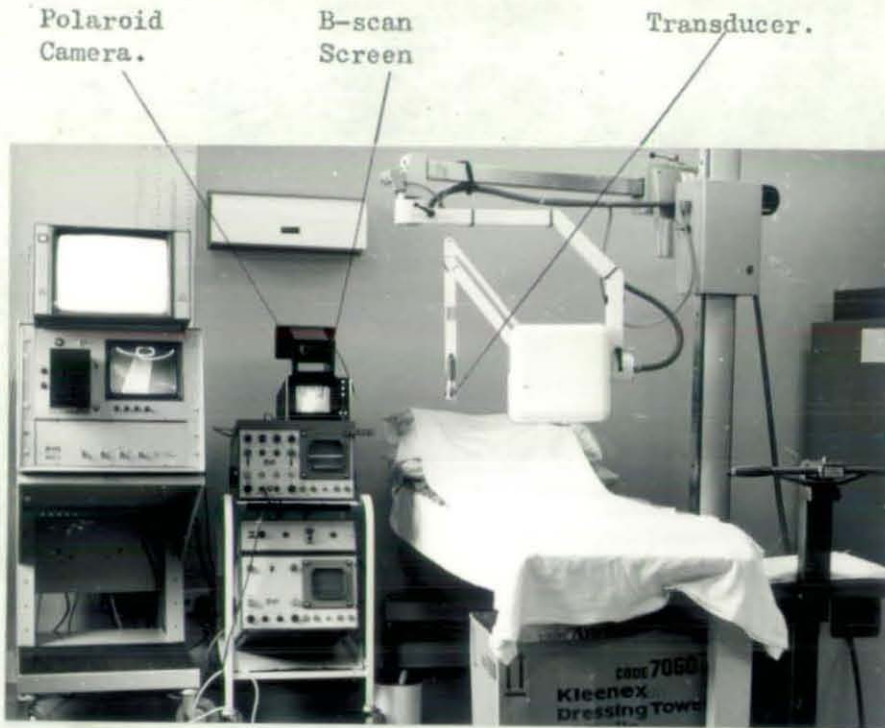


Figure 13. The Ultrasound Apparatus.

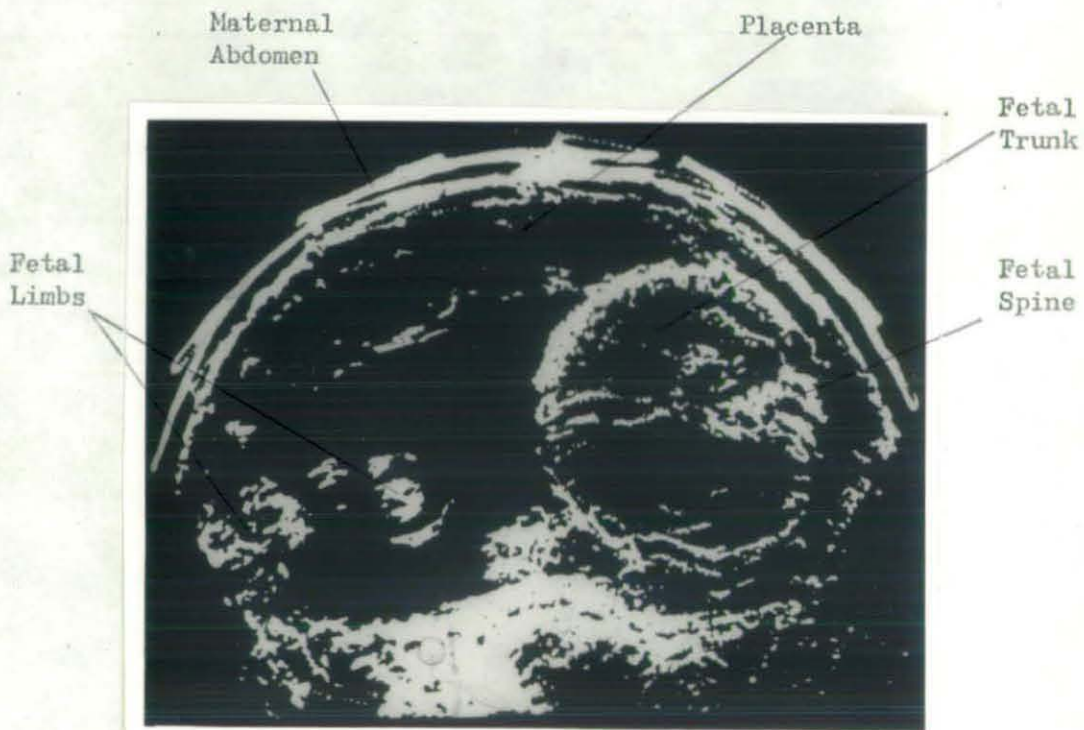


Figure 14. An Example of a Compound B-Scan Picture.

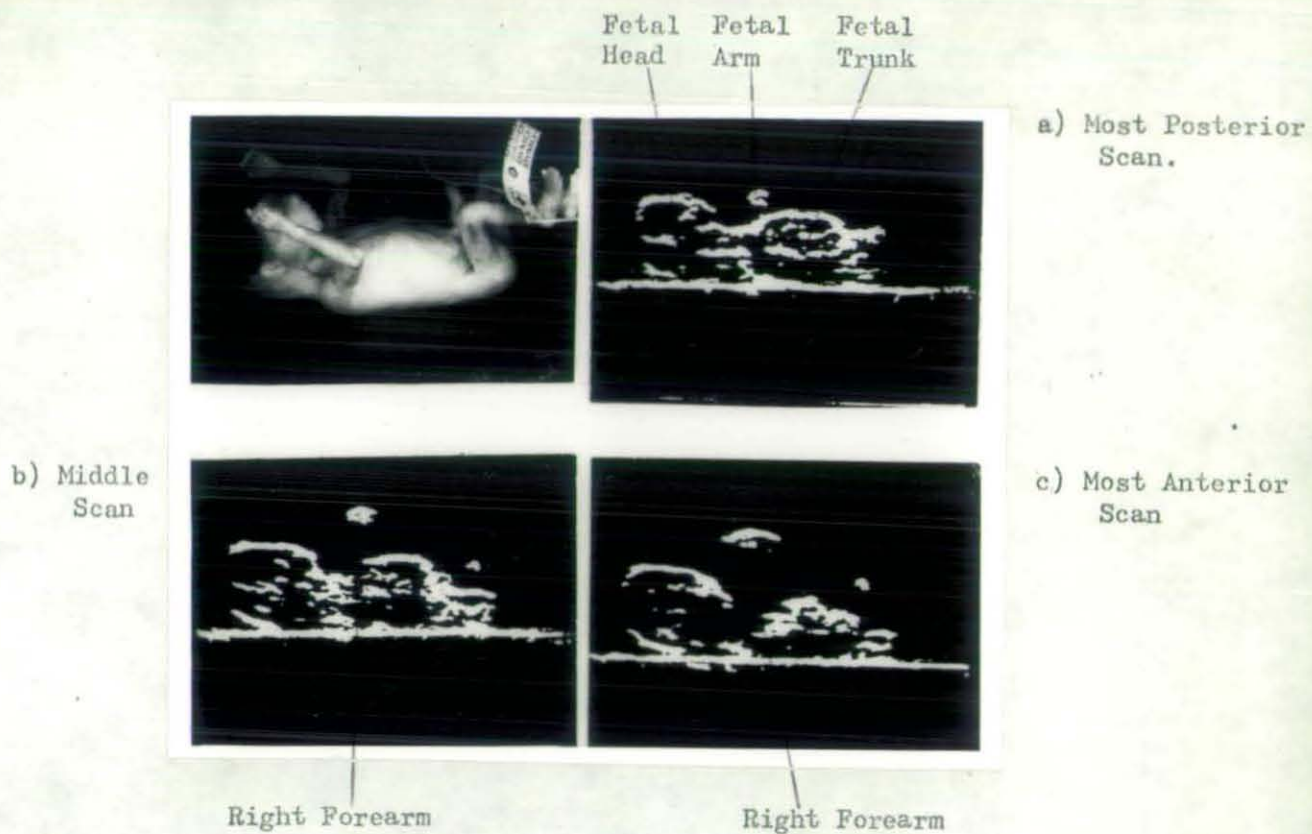


Figure 15. The Identification of Fetal Parts using the Ultrasound Apparatus.

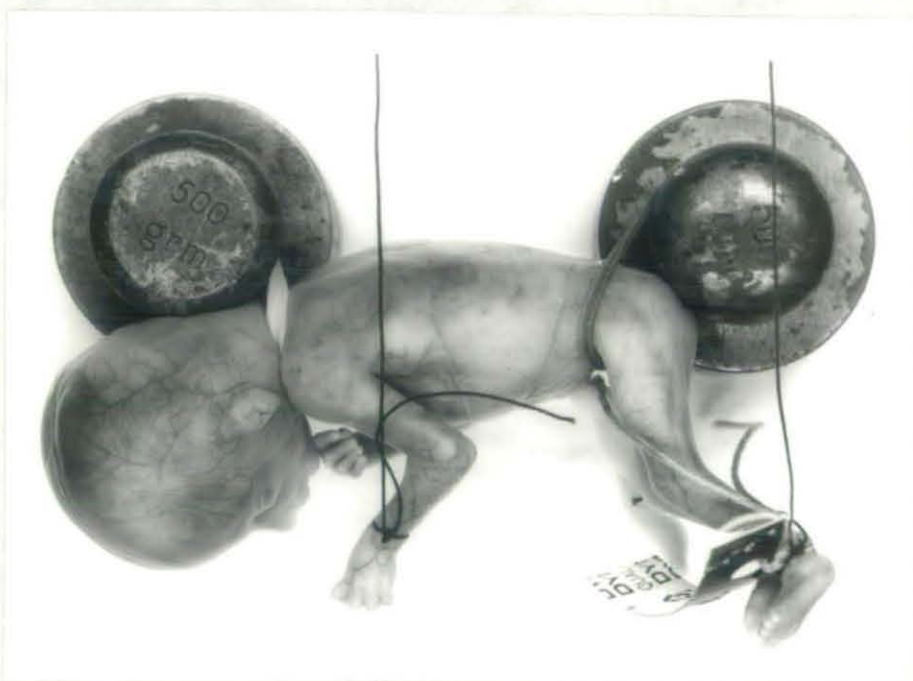


Figure 16. The Fetal Puppet.

The fetus had pieces of cotton attached to both its wrists and ankles. By running these pieces of cotton under a series of metal hooks that were sited around the edge of a wooden base this fetal "puppet" was able to "perform" prescribed movements e.g. flexion of the elbow. Several of these movements were engineered and their progress was followed using the Persistence technique. In this way the validity of the movements seen in utero was checked.

CHAPTER IV

RESULTS AND DISCUSSIONS.

COMPUTER WORK

To help with the lengthy calculations, a computer was used throughout the results section. Wherever possible standard computer programmes were used, but in several parts of the study such programmes were not available and suitable programmes were written in Fortran IV language. This was particularly time-consuming because the results were calculated partly at Loughborough and partly at Manchester. Unfortunately the input programmes written for the Loughborough University computer were incompatible with the Manchester computer and this necessitated much re-writing.

THE METHODS USED

1. The Accuracy of the Measurements taken.

The results in Table 1 show that the coefficient of variation ($\frac{S.E.E.}{\bar{Y}} \times 100$) is very low for each of the external measurements taken from the Nottingham fetuses. This means that the reliability of these measurements taken using the anthropometric calipers shown earlier to the nearest 0.5 cm., is very high.

The low coefficient of variation for the C.R. length means that for each individual fetus that has been fixed, this measurement is reliable but the differences in degree of flexion that are observed between fetuses even of the same size suggests that this measurement is not reliable between different fetuses. Similarly, although the measurement of C.H. length is very reliable for each individual fetus that has been fixed, it does not necessarily mean that the measurement is reliable between different fetuses of the same size. In fact the awkwardness of obtaining this measurement from fetuses that have been fixed suggests that this is indeed the case, particularly when the variable extension of the hip, knee and ankle joints is taken into consideration.

The objectiveness and low coefficient of correlation calculated for C.R.E. length lend themselves very well to the argument for basing fetal age estimations upon this measurement.

Table 2 shows that the measurements taken from the radiographs using the radiographic calipers shown in Figure 9 to the nearest 0.1 mm are very reliable. The low coefficients of variation for each of the measurements illustrates that the measurements are clearly defined and objective in their use.

For Table 3 the same fetus was radiographed in an A.P. position following the procedure laid down earlier. The low coefficients of variation for each of the measurements taken from the radiographs show that the technique

Table 1 Reliability of the external measurements taken from a representative fetus . Measurements were taken to nearest 0.5 cm.

MEASUREMENT	MEAN mm of 10	STANDARD DEVIATION mm	COEFFICIENT OF VARIATION %
Crown Rump Length	87.8	0.253	0.29
Crown Rump Extended Lgth	89.6	0.237	0.27
Crown-Heel Length	120.3	0.514	0.43
Biparietal Diameter	24.8	0.267	1.08

Table 2 Reliability of the measurements taken from a representative radiograph Measurements were taken to the nearest 0.1 mm.

MEASUREMENT	MEAN mm of 10	STANDARD DEVIATION mm	COEFFICIENT OF VARIATION %
Ossified Humeral Shaft	17.8	0.13	0.7
Inter Neural Arch of T1	6.0	0.1	1.7
Longitudinal length of Thoracic Neural Arches	28.2	0.11	0.4
Ossified Femoral Shaft	17.7	0.18	1.0

Table 3 Reliability of positioning a representative fetus for the A.P. radiographs

MEASUREMENT	MEAN mm of 5	STANDARD DEVIATION mm	COEFFICIENT OF VARIATION %
Ossified Humeral Shaft	17.4	0.15	0.9
Inter Neural Arch of T1	6.0	0.13	2.2
Longitudinal length of Thoracic Neural Arches	27.2	0.3	1.1

of positioning is very reliable although, on comparison with the same measurements shown in Table 2, there is a slight increase in variation due to positioning of the fetuses. The results of Table 3 however, show that this increased variation is negligible providing care is taken during the positioning procedure.

Using similar triangles, the theoretical value for the magnification factor of the radiographic technique was calculated. The object-film distance (O.F.D.) for each of the structures that were measured did not exceed 3 cm. With an anode-film distance (A.F.D.) of 100 cms, the magnification factor with an O.F.D. of 3 cms. was calculated to be 1.03. This represented the magnification factor for the Nottingham material and for comparison with the Southampton and London material, similar calculations were carried out for an O.F.D. of 1" and an A.F.D. of 36". In this case, the magnification factor was also calculated to be 1.03 and therefore all of the measurements taken, no matter what their source, were considered to be comparable. The slight magnification factor involved with the radiographic technique, which had a maximum value of 3%, was considered negligible and no corrections were subsequently made to any of the measurements taken.

2. The Effect of Silver-staining

The radiographs from Southampton were of fetuses that had been stored in an aqueous solution of 0.5% silver nitrate which is known to affect the picture of ossification that is produced. Unfortunately, the available literature which would help to assess the difference this would make to any measurements that were

taken, is sparse although the procedure has often been used. Therefore an investigation of this procedure was carried out. The results can be seen in Figure 17.

The fetus took up the silver very quickly and apparently this was restricted to the areas of ossification. The longer the fetus remained in the solution, the more silver appeared to be deposited particularly around the edges of the ossification centres. Certainly, with silver nitrate staining the ossification centres are much more apparent and where there had been difficulty in establishing the presence of a centre, the ossification was now more clearly marked. Meyer and O'Rahilly (1958) using photomicrographs showed that the deposit of silver has occurred not only in the circumferential osseous areas but also in the cartilage in the centre of the shaft, which is presumably calcified. They also found that the deposit of silver in the osseous tissue was confined to the bone first laid down and therefore a silver-free osseous area, which stained blue, was observed in a subperiosteal position around the centre of the shaft, covering the silver area, but which extended further both proximally and distally.

Standards for the changes in appearance of the ossification centres during fetal life are not available, but the silver nitrate staining did not appear to alter significantly the presentation of the ossification centres. However, as more silver became deposited the penetration (kV) of the X-rays had to be increased in order to obtain the same density value.

A difference was observed in the apparent rate at which the different ossification centres took up the silver and this was particularly important for the vertebral column because the vertebrae appeared to be the last centres to become affected, especially the centra. This suggests that a specific approach needs to be made depending upon which area of fetal ossification is to be studied.

For the practical purposes of this study the measurements that had been taken from the radiograph of the unstained fetus were compared with the same

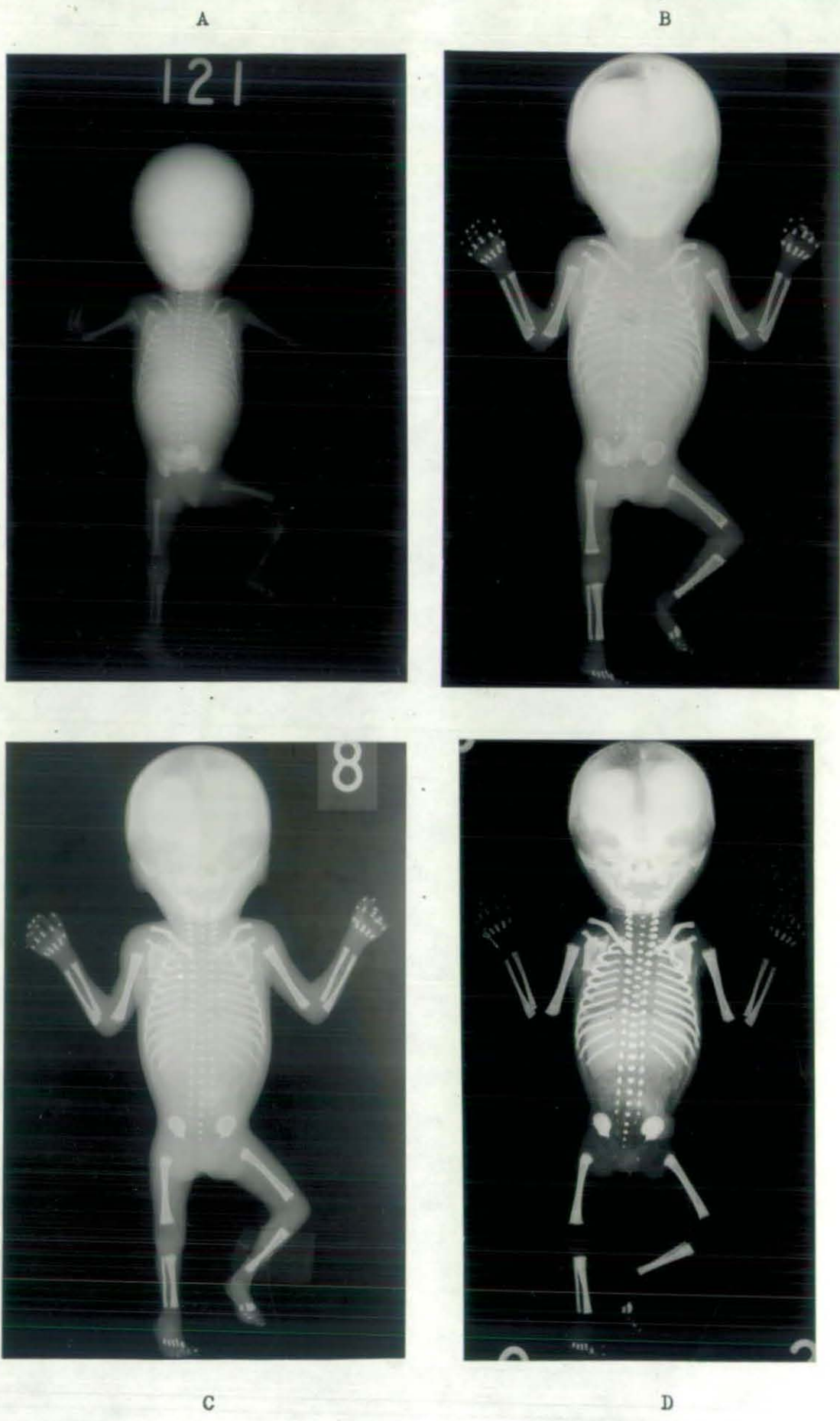


Figure 17. The results of silver nitrate staining.

- | | | | |
|---|-----------------|---|------------------|
| A | No staining | C | 8 days staining |
| B | 2 days staining | D | 14 days staining |

measurements taken from the radiograph of the stained fetus.

There was no significant difference ($p < 0.05$) between the measurements of the unstained shafts of the long bones of the limbs and those that had been stained. This suggests that the deposition of the silver was confined to the area of ossification that could be carefully determined previously, although the boundaries of the centres could be determined much more easily. In addition, there was no significant difference between stained and unstained longitudinal and horizontal lengths of the vertebral column but this is not surprising because these measurements are independent of the actual size of the ossification centres.

It was thought that the improvement with staining in visualisation of the ossification centres might lead to an increase in the total number of ossification centres that could be seen, particularly in the vertebral column. In Figure 17 however, it can be seen that the centrum for C_2 and the neural arch for S_2 are the most caudal ossification centres of the vertebral column that can be seen in both stained and unstained radiographs, although the appearance of these centres is much more apparent when the fetuses have been stained. Correct use of the 4 dioptré lens, in addition to practice, can therefore be said to compensate for the clarity of appearance of the ossification centres in radiographs taken of fetuses that have been stained with silver nitrate.

For all practical purposes of this study, the radiographs from Southampton of fetuses that had been stained with silver nitrate were considered to be comparable to the radiographs of unstained fetuses from Nottingham and London. It was appreciated however that care had to be taken in the measurements, especially in identification of the most recent ossification centres, and the use of the 4 dioptré lens was particularly stressed when examining the radiographs from Nottingham and London.

This investigation of the effects of silver nitrate staining is by no means exhaustive and was undertaken merely to assess the potential of the

Southampton radiographs. Future investigations of this highly promising procedure might well contemplate studying:

1. Changing the solution at regular intervals.
2. Altering the strength of the solution.
3. Varying the temperature of the solution.
4. Varying the length of time of immersion.
5. Variability due to the size of the fetus.
6. Specific criteria dependent upon the particular centre to be studied.
7. The long term effects on the fetal material particularly if it has eliminated the fetuses from further studies.

PETAL MOVEMENT

An association between ossification and mechanical force is well known (Bassett, 1971). In this study an attempt was made to relate the ossification that was seen in the fetal radiographs to the mechanical forces which impress themselves upon the fetus in utero. A search of the literature however revealed that very little is known about fetal movements in utero and it was thought that these movements might well play a major role in determining the actual force applied to the fetus as it is developing. A method using ordinary compound ultrasound scanning techniques was therefore developed to monitor fetal movements in utero.

It is easier to analyse and comprehend the specific movements of older fetuses because the larger fetal parts facilitate easier observation. An example of the type of movement that has been able to be monitored in a larger fetus is shown in Figure 18. It must be appreciated that photographs such as these are merely glimpses of a continuum of movements that can be seen on the ultrasound machine and fetuses of all ages have been seen twisting and turning, flexing and extending their limbs as they develop in their liquid world. To aid the analysis of these movements, an extremely flexible child's doll was used because as Liley (1972) points out, it is perhaps a little naive to feel that we ourselves can demonstrate the more peculiar positions that the fetus is capable of assuming. Of particular importance is the fact that fetal movements have been observed whilst their mothers have been totally unaware of any fetal movement and gross movements have even been identified as early as 6 weeks conceptional age (see Figure 19) although the identification of particular parts at this age is obviously much more difficult. Nevertheless twisting movements have been recognised at a very early age although specific identification of parts is limited. These twisting movements involve all parts of the fetal body and it appears that the

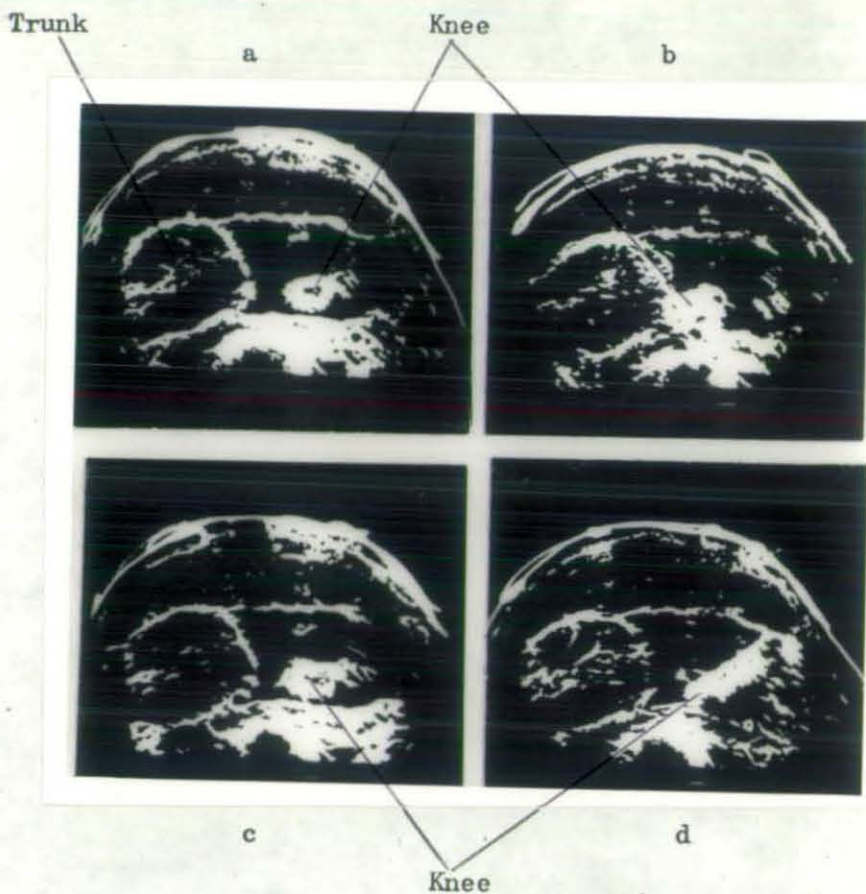


Figure 18. A 30-week-old fetus drawing its right knee up to its trunk and then extending the lower part of the limb, which causes the knee to move away from the trunk.

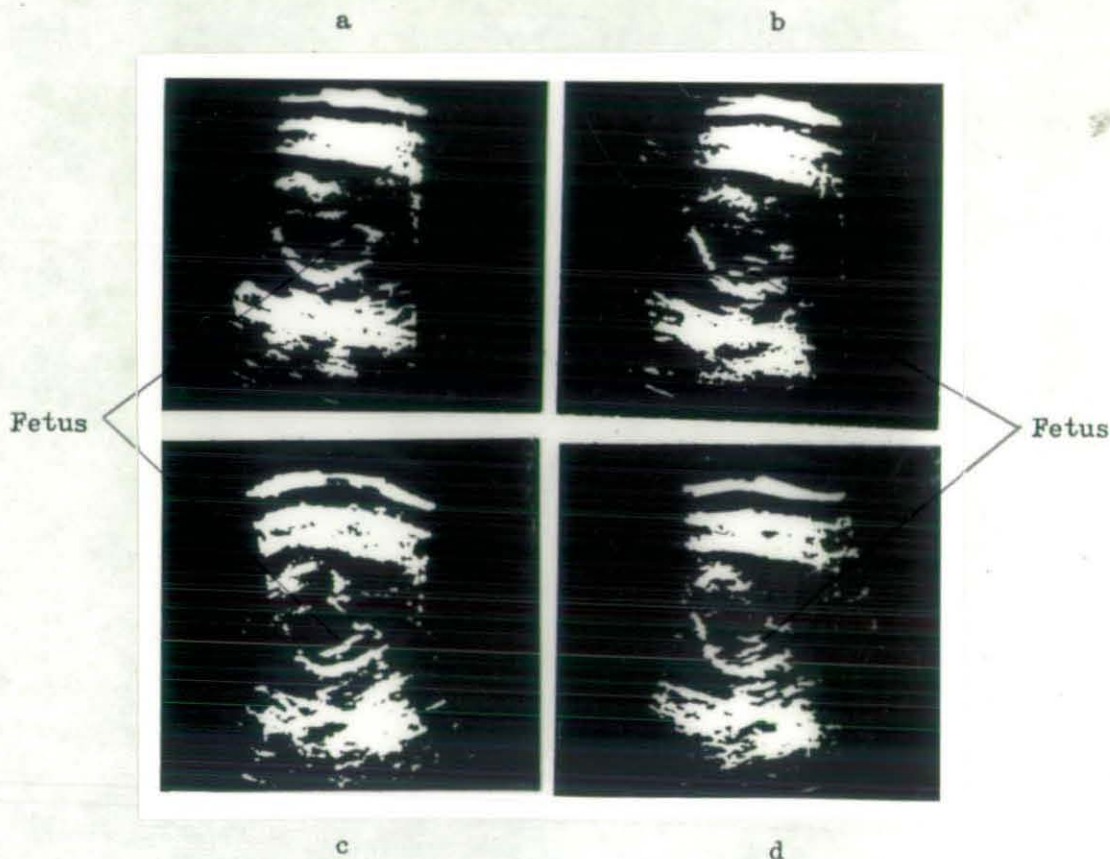


Figure 19. A 6-week-old fetus demonstrating its ability to move within the amniotic sac, which is in the centre of each picture.

head, trunk, and limbs are all capable of individual movement from a very early age.

Similar results to these have been recorded by Reinold (1971, 1973). Although Reinold (1971, 1973) uses ultrasonic apparatus in his method, the arrangement is different to that from this study, consisting of a rotating directional emitter and a mirror optic system which results in 15 to 16 cross-sectional pictures per second becoming visible on the screen. This rapid build up of each picture must considerably reduce the clarity and it is significant to note that Reinold (1971, 1973) does not include any pictures in his work representative of fetal movements. Nevertheless his descriptions of the movements seen accord well with the results of this study. He reports that from a fetal age of about 6 weeks, which corresponds to the 8th week of pregnancy, a suggestion of the embryo in the amniotic cavity can be established and as soon as the "shadow" of the embryo is visible on the oscillograph screen, clear embryonic movements can be observed. He describes the movements as being "jerky" movements which are reminiscent of the movements of the tail of a swimming fish and often result in the fetus acquiring a completely different position. Between the 10th and 20th week of pregnancy Reinold (1973) was able to observe two different types of movement; one type began with a strong movement leading to a change of position of the whole fetal body within the amniotic cavity; the second type of movement was slow and inert confining itself to only part of the body with the position of the fetus being altered only slightly or not at all.

The question of whether or not ultrasound stimulates fetal movements remains unanswered. As there are no standards available which quantify fetal movements in utero, no comparative test can be performed, but several other tests have been carried out to examine this question. Fetuses of different ages were selected at random and when the fetus remained still the ultrasonic beam was concentrated on varying parts of the fetal anatomy for periods up to 30 seconds.

If ultrasound does stimulate fetal movement it was thought that the fetus would subsequently move, but in most cases the fetus remained still and appeared to be unaware of the beam. There are many other factors involved in obtaining an answer to this question and therefore a satisfactory and conclusive answer will require a more detailed study.

It is unfortunate that the movements of the early fetuses as seen by ultrasound cannot be more explicit because then a better comparison could be made between them and those movements seen in similar but aborted fetuses by Windle and Fitzgerald (1942) and Hooker (1954). It would be surprising if the pattern of movements seen by ultrasound were similar to those seen in aborted fetuses because in utero the emphasis would be on normal development rather than survival in the anoxic conditions which surround aborted fetuses.

The ability to view and analyse fetal movements in utero introduces the possibility of a whole new range of fetal monitoring with important scientific and clinical implications. No other non-invasive method exists at present and for further study several important questions present themselves. Which parts of the body move first and do some parts move more than others? Do movements occur regularly or irregularly? Do movements of different parts increase in frequency with motivation? Are certain patterns of movement associated with particular stages of development or with specific fetal abnormalities or other complications? How do maternal states or various methods of treatment or investigation of the mother affect fetal life as expressed by movement? Many of these questions are stimulated by observations noted in the literature.

Montagu (1964) reports on experiments involving a loud noise and a vibrator placed on the maternal abdomen which resulted in demonstrating a classic example of a conditioned reflex in the fetus. The fetus can therefore learn before birth and the potential of this finding is enormous. Montagu (1964) also reports that a sharp, loud sound close to the fetal head results in a sudden move

which might be representative of a startle reflex. The question is asked whether or not a child is capable of an emotional response before birth? Preyer (1937) argues that the fetus lies with crossed, drawn-up legs and arms crossed upon the breast because every other position requires more space. The fetus takes up the minimum amount of space which has the smallest amount of pressure and will keep returning to this position. Preyer (1937) also discusses the behaviour of anencephalic fetuses and, because of their apparently normal activity in utero, finds them very interesting because they show how little brain activity is necessary for development and for movement before birth. Windle (1942) in histological studies of the development of the nervous system suggested that simple unilateral reflexes developed first with more complicated bilateral reflexes only occurring later after collateral neurons have developed. Gesell (1945) in a series of photographs showing the earliest movements observed in aborted fetuses ex utero clearly shows bilateral movements of the arms rather than unilateral as might be expected. More recently Pearson and Weaver (1976) have developed a daily fetal movement count (D.F.M.C.) using maternal appreciation of fetal movements for the counting system and have described a threshold value of 10 below which there is a danger that the fetus is at risk. It might be equally postulated that an upper threshold value might also represent fetal danger in terms of anoxia or a similar distressing situation. Sontag and Wallace (1934, 1935; and 1936) and Sontag and Richards (1938) measured the fetal response to changes in various maternal and environmental factors by recording fetal heart rate with a stethoscope. Possibly the extent of these responses can now be related to the whole fetal period rather than being restricted to near term.

Certainly routine obstetrical ultrasound scanning could provide a new dimension in terms of the study of motor activity before birth and an illustrative atlas of fetal movements could be constructed. Just as there are well-recognised "milestones" in motor development after birth in the infant and

young child, so it may be possible to establish equally important antenatal milestones in the developing fetus, and the concept of antenatal paediatrics might well become a practical proposition.

At present this work is being continued and a method for quantifying fetal movements is being developed in order that a longitudinal study of fetal movements may be carried out.

SUMMARY

1. Movements of the human fetus in utero have been observed as early as 6 weeks of conceptual age.
2. These movements consist of twisting and turning movements of both the whole body and its separate parts.
3. As the fetus becomes older identification of specific fetal parts becomes easier and specific movements can be identified.
4. Ultrasound does not appear to stimulate the fetus to move although it was not possible to prove this conclusively.
5. There are many important scientific and clinical implications of this method for monitoring fetal movements in utero.

Part of this work on Fetal Movements has already been published:

1. Higginbottom J., Bagnall K.M., Harris P.F., Slater J.H., and Porter G.A. 1976.

Ultrasound monitoring of fetal movements -
a method for assessing fetal development?
Lancet 1, 719-721.

2. Bagnall K.M., Harris P.F., and Higginbottom J. 1976.

Analysis of Human Fetal Performance.
Paper read by K.M.B. to Symposium on Analysis of
Human Performance. April 1976.

3. Bagnall K.M., Harris P.F., and Higginbottom J. 1976.

Analysis of human fetal performance.
Symposium of Analysis of Human Performance. (In Press)

FETAL AGEING

The data obtained from the 129 normal fetuses belonging to the Department of Growth and Development, Institute of Child Health, University of London were subjected to polynomial regression analysis in an attempt to construct an equation from which the conceptual age of a fetus, expressed as a decimal of a year, could be determined from a measurement of its Crown-Rump Extended (C.R.E.) length. Equations up to and including the power of 3 were attempted using computer programmes (See Appendix A and Appendix B) and as each successive term was added the consequent reduction in sum of squares was tested against the mean square remaining. In this way, the power of the selected equation was determined by the significance or non-significance at the 5% level of the corresponding F-values. This method produced the following quadratic equation:-

$$Y = 0.124671 + 0.001053X + 0.000002X^2$$

where Y = Conceptual Age in Decimal Year

X = Crown-Rump Extended Length in mms.

The Standard Error of the estimate (S.E.E.) = 0.019 yr. (7 days)

Multiple Correlation Coefficient R = 0.967

Other computer programmes were constructed which enabled a polynomial regression equation to be drawn upon a set of axes in addition to the original data points being plotted. (See Appendix C and Appendix D). This allowed the corresponding graph to be drawn and the results of this can be seen in Figure 20.

This fetal material, therefore, provided a means by which the 728 fetuses of this study could be aged based upon the measurement of their C.R.E. length. Another computer programme was devised which allowed the fetuses to be aged and arranged in chronological order (Appendix E). On the Cyber 72 Computer System a further computer programme was used which drew histograms showing the frequency distribution of the ages of the 129 fetuses in the original data (Figure 21) and

TANNERS DATA CHECKED FOR ACCURACY 129 FETUSES

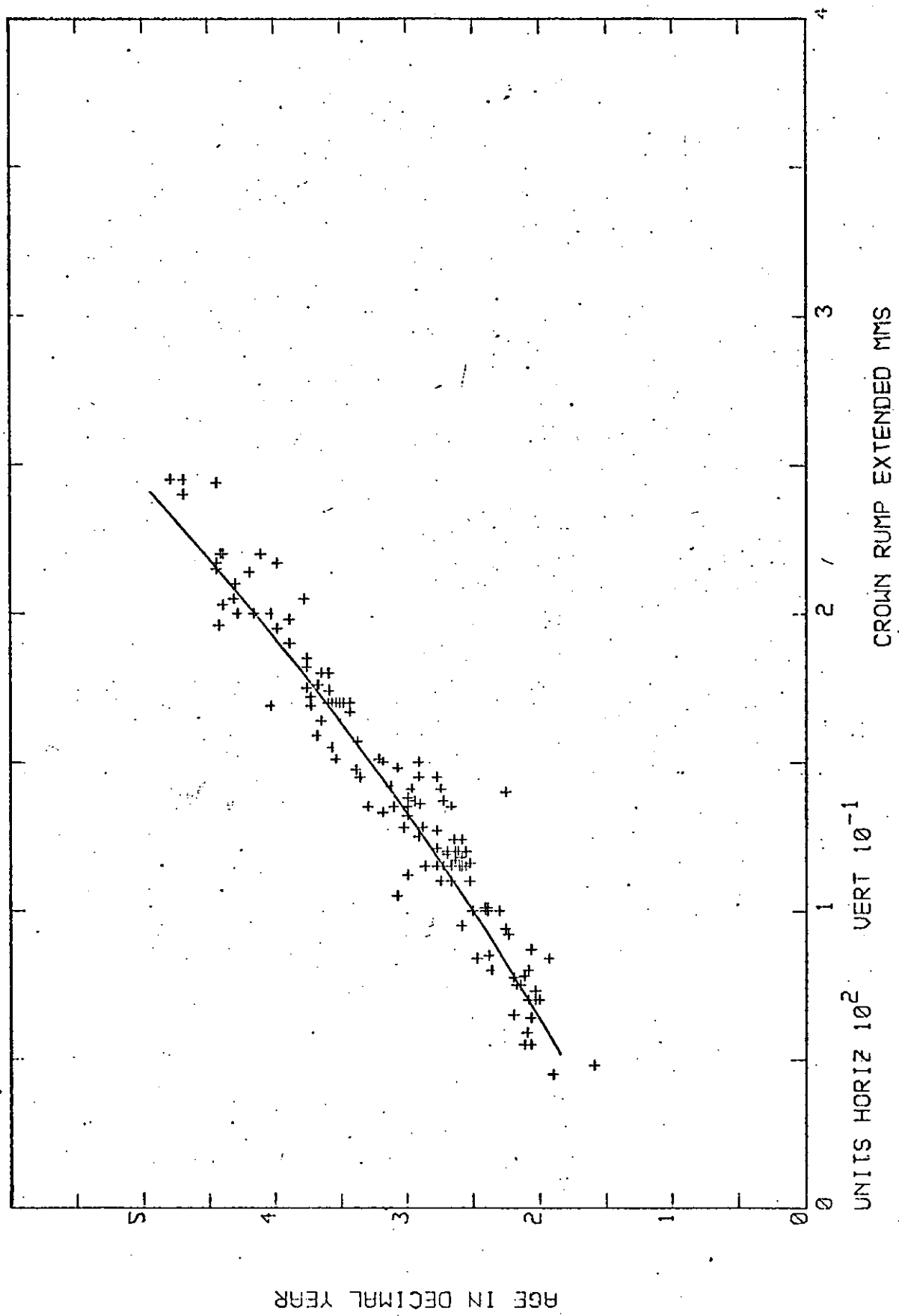


Figure 20. To estimate fetal age from measurement of C.R.E. length.

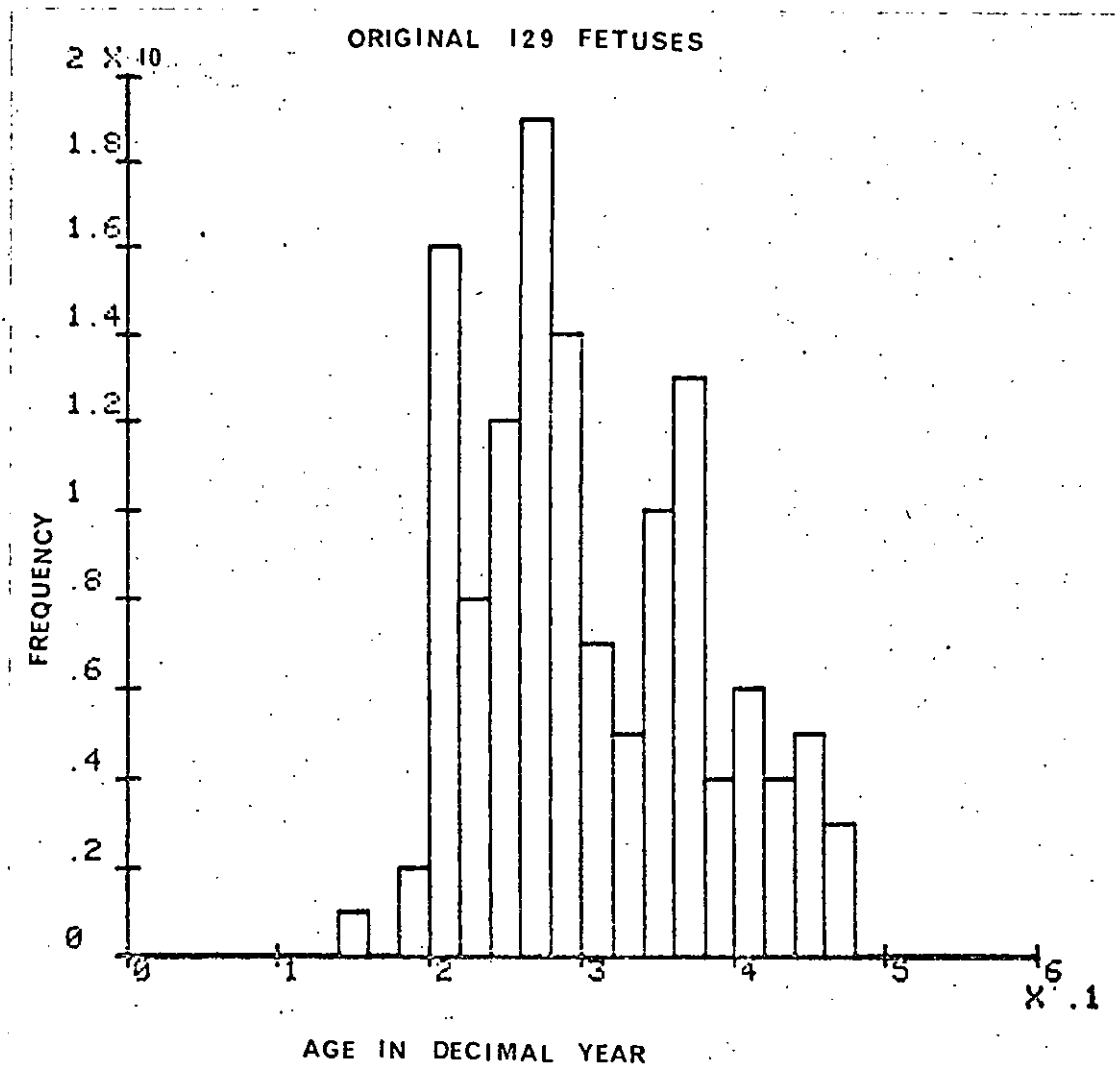


Figure 21. The distribution of the 129 fetuses for whom the measurements of C.R.E. length and fetal age were available.

the consequent ages of the 728 fetuses that formed the material for this study (Figure 22) . These histograms show that the age range for the original 129 fetuses was similar to that for the 728 fetuses of this study and justifies the use of this equation in ageing the fetuses (Ryan and Berry , 1972).

The 95% confidence limits (2 S.D.) which were established using the S.E.E. allowed the estimate of conceptional age to be correct only within 0.038 yr. (14 days). Coupling this to the inherent variation of $\bar{+} 0.019$ yr.(7 days) which is automatically attached to the use L.M.P. dates when attempting to estimate conceptional age, it becomes apparent that the estimate of true conceptual age is only accurate to within 0.057 yr.(21 days). On the other hand, the square of the multiple correlation coefficient ($R^2 = 0.93$) allows us to calculate that of the total sum of squares of y (Age), only about 7% ($1-0.93 = 0.07$) is still present after the prediction of fetal conceptual age from C.R.E. length. This means that the predictive value of C.R.E. length is high.

Originally it had been hoped to age the fetuses of the study by comparing their crown-rump (C.R.) lengths with published data (Arey, 1965; Hamilton and Mossman, 1972) relating fetal length to fetal age. However, it quickly became apparent that different estimates of age could be made for the same fetus depending upon which particular Table or graph was selected and, therefore, it was decided to investigate further this confusing situation.

Eight Tables and graphs relating fetal C.R. length to fetal age were selected from standard Anatomical sources and were plotted on the same set of axes for comparison (Mall, 1910; Streeter, 1920; Patten, 1953; Olivier and Pineau, 1958; Arey, 1965; Hamilton and Mossman, 1972; Robinson, 1975; Birkbeck et.al; 1975) (See Figure 23). To this graph was also added another equation determined by this study and called "Tanner's equation" for descriptive purposes. All of the graphs, except for Arey (1965), used the menstrual age of the fetus and therefore 14 days were subtracted from their descriptions so that the conceptual age was

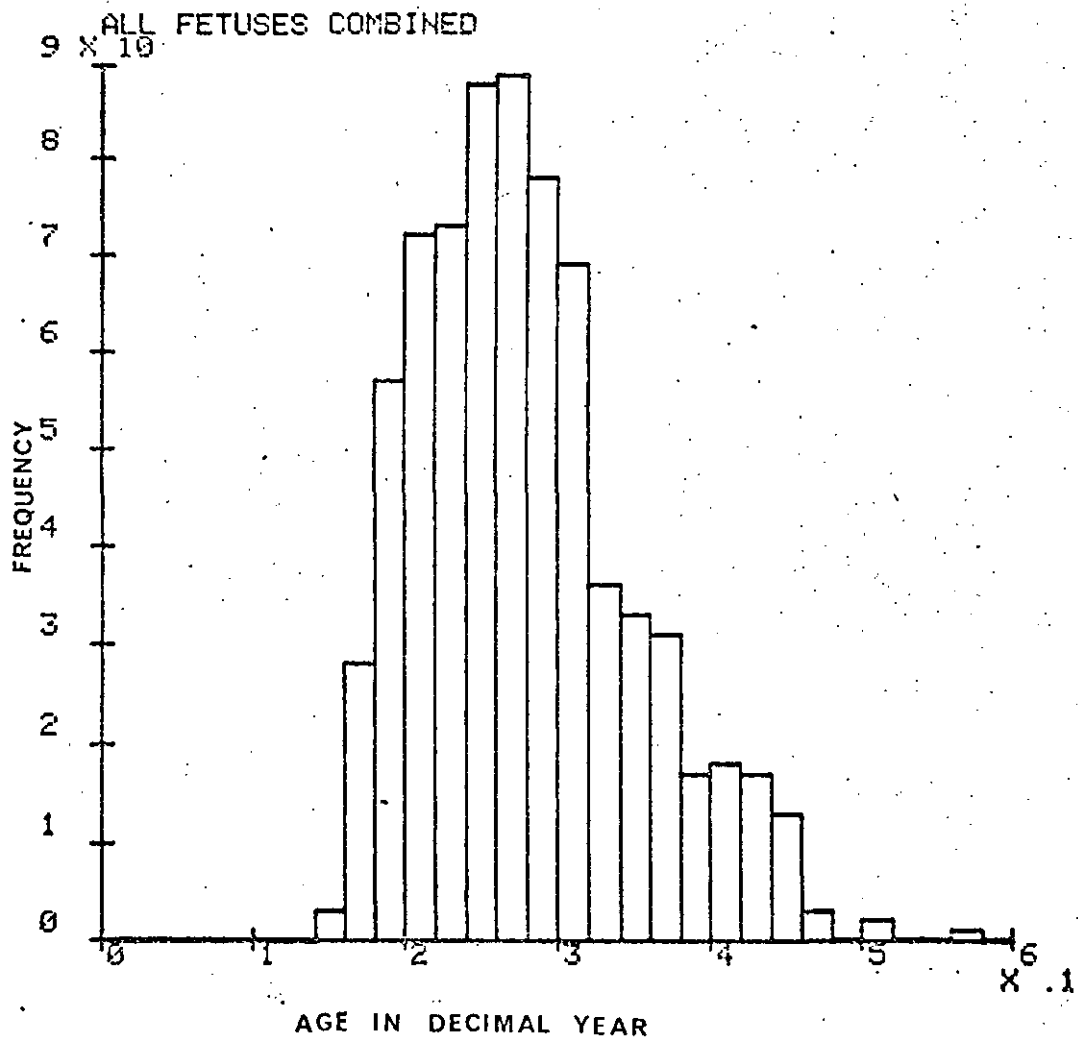


Figure 22. The distribution of the 728 fetuses from this study.

COMPARISON OF FETAL GROWTH STANDARDS.

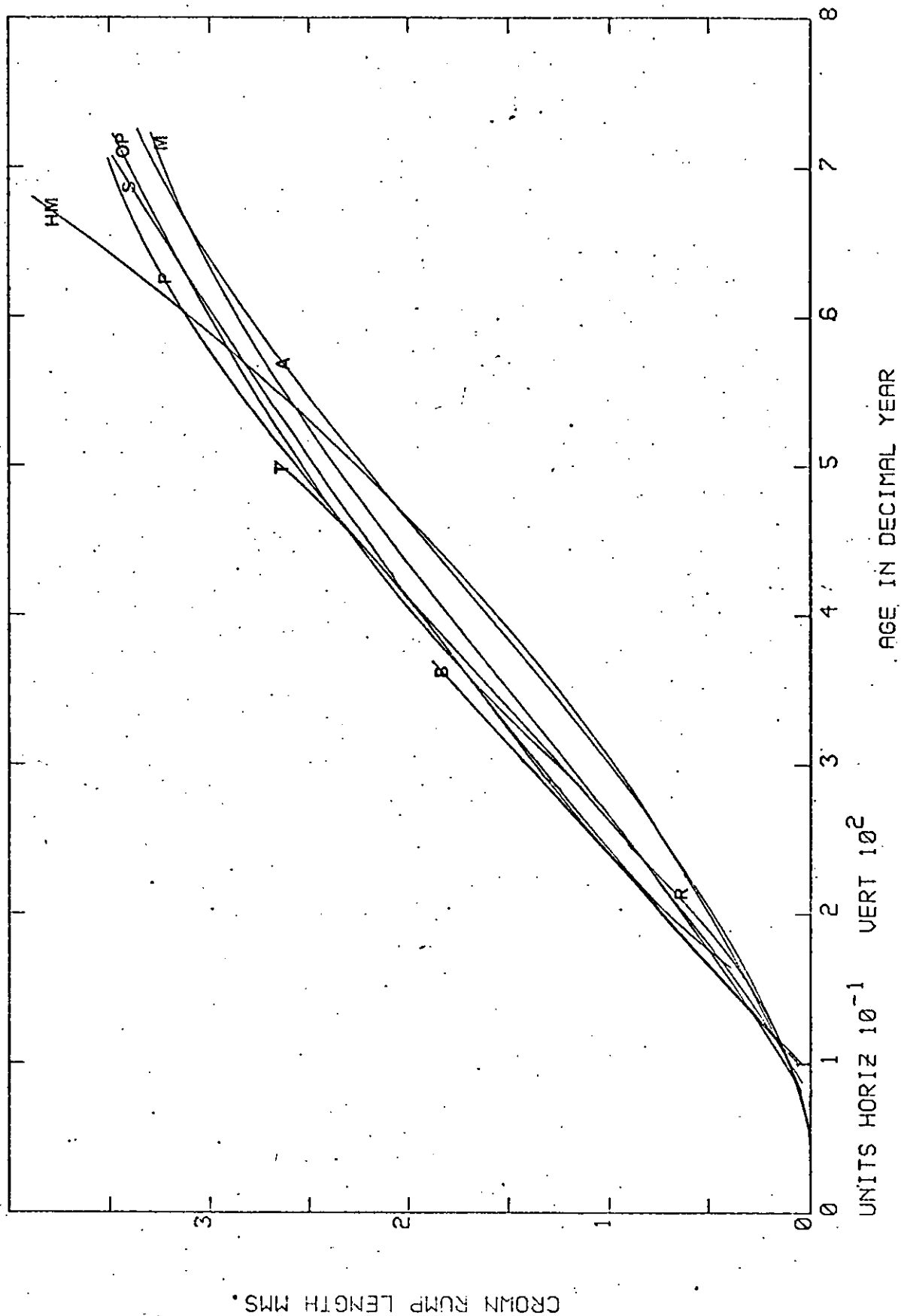


Figure 23. A comparison of published fetal growth standards.

- | | |
|-------------------------------|----------------------------------|
| A = Arey (1965) | HM = Hamilton and Mossman (1972) |
| M = Mall (1910) | B = Birkbeck et. al. (1975) |
| S = Streeter (1920) | P = Patten (1953) |
| OP = Oliver and Pineau (1958) | T = Tanner's Equation |
| R = Robinson (1975) | |

used, which was the same as for Tanner's equation. Arey(1965) already used the conceptual age of the fetus and the graph had been constructed on the basis of conception being 14 days after L.M.P. which again, was in line with Tanner's equation.

In Figure 23, Tanner's equation is designed to estimate C.R.E. length from fetal age and not fetal age from C.R.E. length as before. The equation was derived by submitting the data from the original 129 fetuses from the University of London to polynomial regression analysis with the minimising of the differences between the actual values and the estimated values being performed along the length (y) axis and not the age (x) axis. This procedure produces a different equation to the previous equation of this study which was designed to estimate fetal age from fetal length. This new equation, which estimates fetal C.R.E. length from fetal age is:

$$Y = -190.650772 + 1969.062961X - 4131.019163X^2 + 4026.072186X^3$$

where $Y =$ C.R.E. length in mms.

$X =$ Conceptual Age in Decimal Year.

S.E.E. = 12.1 mm. $R = 0.968.$

Figure 23 shows that there is considerable difference between the standard tables and graphs that are available to describe the growth of fetal crown-rump length with age. There are several reasons which might explain this difference.

Each of the investigators has studied a separate sample of fetuses from which has been produced a growth standard. It is conceivable that each of the samples does in fact come from an entirely separate population from the other samples and that there really is a difference in fetal length for a particular age. These separate populations could stem from several sources not least of which might well be a predominant ethnic influence particularly when it is realised that Olivier and Pineau (1958) used a French population, Mall (1910) and Streeter (1920)

used an American population, Robinson (1975) used a Scottish population, and Tanner (1976) used a London population. This thought is given support by Birkbeck et.al.(1975) who reported that unpublished data by Nishimura from Japan indicated mean C.R. lengths appreciably shorter than their own at any given gestational age but with a similar degree of variability. It is difficult however to see how an ethnic difference would manifest itself in Figure 23 because all of the sources apparently use fetuses of Caucasian origin which would considerably restrict any ethnic effect.

Another source of variance which might explain the differences between the graphs of Figure 23, could be a secular change in fetal length, presumably consisting of an increase due to the better nutrition and understanding of prenatal life nowadays. The fetal material used in Figure 23 spans 65 years from Mall (1910) to Birkbeck et.al. (1975) and in this time there might well have been a secular increase in fetal length with age similar to that which has been shown to have taken place in postnatal life (Sinclair,1973). However, if this was so Figure 23 would be expected to be arranged in chronological order which it clearly is not, although other factors such as ethnic difference might well be camouflaging this effect.

Before 1968, the only material available for fetal study came from spontaneous abortions and therefore automatically carried all the disadvantages associated with such material. Presumably attempts were made to eliminate from these studies those fetuses with obvious abnormalities, although this is not always stated, but nevertheless such material will always contain some degree of increased variation and irregular development which is in addition to the normal variation. In this respect, it is interesting to note that the equation of Birkbeck et.al.(1975) in Figure 23 gives the greatest C.R. length for any particular fetal age and this is significant because his material is fresh from pregnancies that have been terminated for non-pathological indications. One would expect that in pregnancies

where pathological conditions existed the growth of the fetus would most likely be inhibited and that for a given fetal age the C.R. length would be less than that for a normal fetus. Birkbeck et.al.(1975) however have compared their results to previous studies which involved fetal material from spontaneous abortions and have declared that the average relationships they calculated, agree remarkably well with the results reported many years ago. The differences that they did find, they suggested might be partly attributable to the effects of preservation of specimens in formalin.

Schultz (1919) found that marked changes in size can be noted after being in formalin and these changes may vary according to the different parts of the body involved and the duration of the preservation. These changes may involve increases or decreases and Schultz (1919) found that C.R. length decreases on average by -2.54% (range + 1.8 to - 6.7%) after 36 weeks of preservation in 10% formalin. He further concluded that the absolute size of the fetus does not seem to have any noticeable influence upon the relative change in C.R. length and this applied also to the state of the specimen. The decrease was found to be most marked in the first week, and a t-test applied to his results showed a significant decrease at the 5% level for preservation in 10% formalin for 36 weeks. Scammon and Calkins (1929) added to the work of Schultz (1919) but their results showed that the changes following preservation in 10% formalin are relatively slight. After 6 months their average decrease in C.R. length was only -0.4%. Only rarely do studies reveal whether or not their specimens were preserved in formalin prior to measuring and in Figure 23 it is significant to note that both Tanner's equation and the equation of Birkbeck et.al. (1975) would give a greater C.R. length for a particular fetal age than any of the other equations. The material for Tanner's equation was measured whilst the fetuses were still fresh and Birkbeck et.al.(1975) took scaled photographs of their fetuses within 1 hour after delivery and used these

photographs to obtain the C.R. lengths. If formalin preservation does decrease the C.R. length, then Tanner's equation and the equation for Birkbeck et.al.(1975) would be expected to represent the maximum length obtainable at any particular age, which apparently they do. The information available concerning formalin preservation however is not clear. The results of Schultz (1919) and Scammon and Calkins (1929) are conflicting regarding whether or not the changes due to preservation in formalin are significant, but even so in Figure 23 part of the differences between the graphs might well be explained on the grounds of differences in preservation procedure. With the necessary information unavailable however, the extent of this effect is impossible to determine.

The most significant factor producing the differences between the graphs of Figure 23 concerns the way in which the measurement of C.R. length was taken. Only if all of the fetuses were measured in the same way would the graphs be expected to be similar, and because they are spread out suggest that the fetuses were, in fact, measured in different ways. This is exclusive of those situations in which different observers made the measurements and which would result in increased variation due to inter-observer error. Unfortunately in many studies of fetal growth the method by which the fetuses were measured is often either absent or very badly worded resulting in a great deal of confusion.

The maximum C.R. length that can be obtained from a fetus is one in which the fetal back has been straightened, the head has been placed in the Frankfort plane and the thighs have been placed at 90° to the trunk. The distance measured is from the vertex to the buttocks and in this study has been called Crown-Rump Extended Length. This name was so assigned to distinguish this measurement from the more usual Crown-Rump length which has been taken to mean the distance between the vertex and the buttocks with the fetus in a slightly flexed position, similar to that which it takes up after it has been fixed.

Many studies do not make this distinction between measurements and consequently the expression Crown-Rump Length has, in the past, included a variety of definitions of measurements.

In Figure 23 Olivier and Pineau (1958), Birkbeck et.al. (1975), and Tanner (1976) have all measured C.R.E. length, although both Olivier and Pineau (1958) and Birkbeck et.al.(1975) call it C.R. length. They have therefore measured the maximum C.R. length possible and it is significant that for any given age these three graphs would give the largest estimates of C.R. length. All of the other graphs in Figure 23 either clearly have not measured the maximum C.R. length (Robinson, 1975) or there is considerable doubt surrounding the measurement they actually made (Mall, 1910; Streeter, 1920; Patten, 1953; Arey, 1965; Hamilton and Mossman, 1972). This doubt hinges upon the descriptions of the measurements taken but nevertheless all of the investigators have called their measurement C.R. length.

Mall (1910) describes the measurement as "sitting height" but the diagram he includes illustrates a well-flexed head, certainly not in the Frankfort plane. Patten (1953) also uses the description "sitting height" but again the diagram used to illustrate the measurement shows a fetus with its head not in the Frankfort plane and even includes flexion of the fetal trunk. Arey (1965) also measured "sitting height" but his diagram is similar to Patten (1953) and illustrates the distance measured as being from the vertex to the breech of a flexed embryo. Hamilton and Mossman (1972) are very brief in their description and simply mention the measurement of sitting height from the vertex of the skull to the breech. All of the definitions which include the measurement of sitting height can be criticised because sitting is an action that the fetus has never performed and it is difficult to visualize an extension of the postnatal sitting height measurement described by Tanner, Hiernaux and Jarman (1969) into antenatal life. Streeter (1920) in his description of the

measurement of C.R. length mentions that the body can be safely straightened for C.R. lengths down to 35-40 mm. but does not include any description for the head position. It is conceivable therefore that the original data for these 5 graphs include measurements that are different to C.R.E. length and because C.R.E. length is the greatest C.R. length possible, then it would be expected that the graphs would give a smaller estimate of C.R. length for a particular fetal age. From Figure 23 it can be seen that this is so and this suggests that there is variation amongst investigators as to the precise definition and anthropometric technique for C.R. length. The fact that the graphs are so diverse highlights the fact that combinations of flexed and extended fetal heads and trunks can lead to a variety of definitions.

In Figure 23 the graph of Robinson (1975) supports the idea of differences of fetal position during measurement. Robinson (1973) attempted to estimate fetal age during the first trimester in utero by measuring the C.R. length using ultra-sound techniques. On the basis that the fetus in utero assumes a flexed attitude, Robinson's (1973) C.R. length measurement is different to that of C.R.E. length and Robinson (1973) confirmed his measurements on 20 fetuses after they had been aborted. He made a direct measurement of the C.R. length with the fetus in what was thought to be a "normal" degree of flexion and his error was sufficiently small to allow him to assume that the sonar measurement was indeed C.R. length. However, it is difficult to understand how Robinson (1973) was able to assess a "normal degree of flexion" because the fetus moves a great deal (Robinson, 1973; Higginbottom et.al., 1976) and in doing so will alter its position. Perhaps there is an attitude to which a fetus always returns on relaxing after moving but this has yet to be shown and even so, this attitude would probably vary from fetus to fetus and would alter during the course of time. Similarly it is not certain that the actual length Robinson (1973) was measuring in utero was the same as the C.R. length measurement ex utero

Robinson (1973) has shown that his measurements using ultrasound are indeed reliable but whether or not they are valid is very difficult to prove. Nevertheless, the fact that Robinson (1973) has described his measurement as C.R. length and inferred that there is some degree of flexion in this measurement, highlights that the definition of C.R. length is not universal amongst investigators of fetal growth. The definition of C.R. length used by one investigator does not necessarily mean that it is the same as that used by any other investigator.

Compared with the other studies included in Figure 23, it is significant that Robinson's (1975) graph lies below that of Olivier and Pineau (1958), Birkbeck et.al. (1975), and Tanner (1976) because these investigators measured C.R.E. length which would give a greater estimate of C.R. length for any particular fetal age than Robinson's (1975) graph, which used a flexed fetal position in its measurements. Furthermore it is significant that the graphs of Arey (1965) and Hamilton and Mossman (1972) lie below that of Robinson's (1975). Both of these authors leave considerable doubt concerning the precise distance that was measured and perhaps they used a rather more curled position in their estimation of normal fetal posture than Robinson (1973) found to be the actual case in utero. It would appear also that Patten (1953) has used a position similar to that of Robinson (1975) and this flexed attitude agrees with the diagram he includes in his study to aid his definition of the measurement. Streeter (1920) on the other hand suggested that the fetal body should be straightened before measuring although he does not mention the head position and, therefore, not suprisingly, his estimate lies very close to those studies which used C.R.E. length as the basis for measuring.

Until recently when it has perhaps become more accessible, the human fetus has remained in a transition zone between two well established academic areas of research and study, namely embryology on the one hand and postnatal

paediatrics on the other. Perhaps an explanation for the various measurements used to define C.R. length is to be found in this situation. "If an embryologist extends his work to include fetal life then, quite naturally, he might take with him the mannerisms and habits of embryology including the measurement in length of a curled embryo, which embryologists call C.R. length. On the other hand, a paediatrician in the same situation might well define his measurements in a manner similar to that suggested by Tanner, Hiernaux, and Jarman (1969) for postnatal life and develop an objective measurement based upon the concept of maximum dimensions. It is here that a difference in meaning, albeit with the same terminology, might well be found and it is not surprising therefore to notice that Arey (1965) demonstrates C.R. length as a curled embryo and Hamilton and Mossman's (1972) work is entitled "Human Embryology" whilst both these studies produce graphs which suggest that a flexed fetal attitude has indeed been used.

A search of the available literature concerning fetal C.R. length development shows that only rarely are revealed the means by which the data collected are treated statistically to produce the growth standard. From those studies which do give details of the data handling, it is apparent that several different methods are employed. The more common methods include arranging the fetuses into convenient intervals and calculating the mean values for the intervals (Mall, 1910; Streeter, 1920; Patten, 1953; Olivier and Pineau, 1958; Arey, 1965; Hamilton and Mossman, 1972), simple graphic representation (Schultz 1926) and regression analysis (Wich, 1972; Robinson, 1973; Birkbeck et.al., 1975). This variance in the underlying models which are used to describe fetal growth will in themselves, lead to variance in the graphs that are produced as standards. (See Figure 25). In Figure 23, therefore, some of the variance between the represented graphs may be attributable to the different procedures involved in the data handling.

Along similar lines, the statistical methods that have been used in the production of the growth standards will determine to a large extent their future

use. When the data for two related parameters are condensed so that a single estimate for Y from a given value of X may be made, then the condensation of the data ought to take place in the Y-direction. It is assumed that the variance of the data is to be found in the Y-variable, with the X-variable possessing little or no variance. Following this procedure, the graph produced by condensing the data in the Y-direction in order to be able to estimate Y from X might well be different to that produced by condensing the same data in the X-direction in order to be able to estimate X from Y. This means that when fetal age is required to be estimated from C.R. length the data should be condensed along the age-axis. Similarly, when fetal length is required to be estimated from fetal age the condensation of the data ought to take place along the length-axis. These two situations are entirely separate and yet a search of the literature revealed only one study, Birkbeck et.al. (1975), in which separate attempts had been made to solve this problem. All of the other studies involved in Figure 23, apart from Robinson (1975) and Tanner (1976), merely produced a fetal age accompanied by a measurement of C.R. length with no indication of the direction in which condensation of the data had taken place. The implication from these studies was that the data were interchangeable and for comparative purposes they were all superimposed on the same set of axes in Figure 23, but as Birkbeck et.al. (1975) and this study have shown, this implication has no sound basis and there are in fact two entirely separate situations; one where age is estimated from length and the other where length is estimated from age.

The regression of Birkbeck et.al.(1975) for predicting fetal age from C.R. length is shown in Figure 23. Their regression equation for predicting C.R. length from fetal age is shown in Figure 24. In their study Birkbeck et.al. (1975) restricted their fetal age range to between 50 and 150 postmenstrual days (0.096 yr. - 0.370 yr. conceptual age) and stated that care should be taken not to extrapolate the regressions beyond the ranges of the observations. In Figure 23

COMPARISON OF REDUCED TANNER, NORMAL TANNER, AND BIRKBECK.

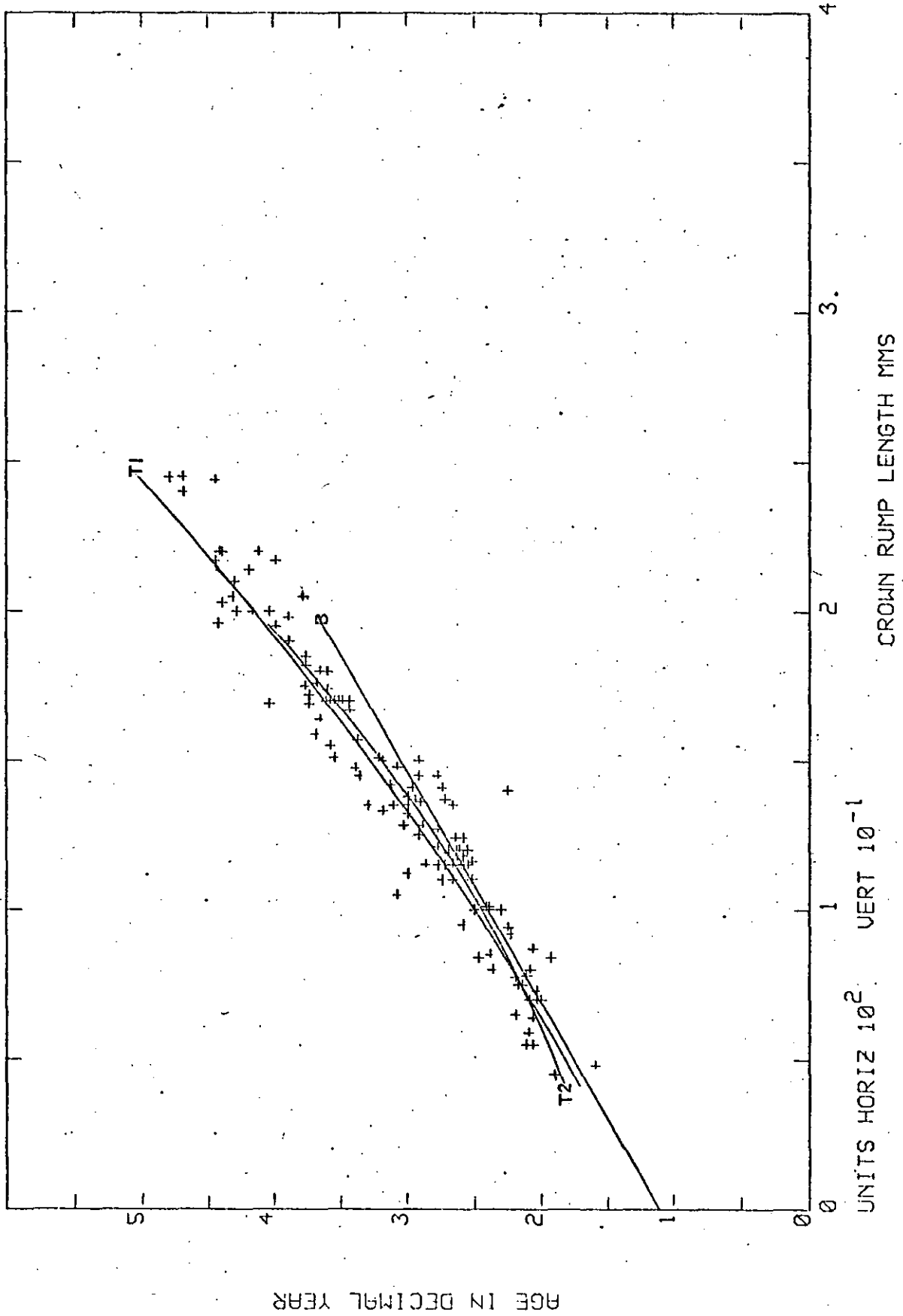


Figure 24. The development of fetal age based upon fetal length.

- B = Birkbeck et. al. (1975)
- T1 = Tanner (1976) (n = 129)
- T2 = Tanner (1976) (n = 101)

the determination of the borders of the regression line based upon the age range is easy to accomplish but in Figure 24 the regression line represents a negative length when the fetal age is 0.096 yr. which is obviously wrong. The borders for the regression equation in this situation ought to be expressed in terms of C.R. length because it is quite meaningless to express them in terms of fetal age.

For comparative purposes the original data from the 129 fetuses supplied by the University of London were reduced to 101 fetuses by selecting only those fetuses between the conceptual ages of 0.096 yr. and 0.370 yr. These were subjected to polynomial regression analysis in order to be able to predict fetal age from C.R. length and the results are shown as "Reduced Tanner" in Figure 24. To complete the picture the original regression equation from the original 129 fetuses was also included in addition to the 129 data points.

Bearing in mind that in Figure 24 it is difficult to establish the exact end-points of the regression line for Birkbeck et.al. (1975), it can be seen that the three regression lines shown are very close together particularly when the C.R. length is small. This is not surprising since all three lines are based upon the measurement of C.R.E. length although it is difficult to explain the divergence of the graphs with the increase in C.R. length. Perhaps another factor influencing the situation is that Birkbeck et.al. (1975) only attempted linear regression analysis and in both of the situations involving the material from the University of London a quadratic equation was found to be the best fit when polynomial regression analysis was applied. At the extremes of the analysis perhaps the influence of the quadratic equation is most felt.

Table 4 shows that the error involved in the estimate by using the regression equation of Birkbeck et.al.(1975) is similar to both of the equations involving Tanner's material. This means that there is an equal spread in terms of age around the regression line of Birkbeck et.al. (1975) as for the regression

Table 4

Conceptual Age in Decimal Year Estimated from Several Sources using C.R. Length

SOURCE	EQUATION	S.E.E.	CORRELATION	n
Birkbeck et. al. 1975	$Y = 0.0012876X + 0.1113151$	0.019 (7 days)	0.933	98
Tanner 1976 Reduced	$Y = 0.156053 + 0.000486X + 0.000004X^2$	0.018 (7 days)	0.937	101
Tanner 1976 Normal	$Y = 0.124671 + 0.001053X + 0.000002X^2$	0.019 (7 days)	0.967	129

Table 5

Comparison of Different Mathematical Models on the Data from London University

SOURCE	EQUATION	S.E.E.
Polynomial Regression	$Y = 0.124671 + 0.001053X + 0.000002X^2$	0.019 (7 days)
Logistic Equation	$Y = 0.1489.1.005^X$	0.020 (7 days)
Allometric Equation	$Y = 0.01399X^{0.627982}$	0.045 (17 days)

lines of this study. Along similar lines, it is interesting to note that there is a slight increase in the S.E.E. with the inclusion of the older fetuses in Tanner's material. If the biological variation of C.R. length in fetuses of the same age increases with age as the individual aspects of fetal growth become more effective, then this slight increase in error of the estimate would be expected, but the extent of this effect is difficult to assess because it is camouflaged by the errors inherent with the use of L.M.P. dates.

The polynomial family of curves is only one of several that can be applied to growth and it was thought possible that polynomial regression might not be the most suitable for analysis of the data. Consequently a study of different underlying models was attempted to see if a more accurate estimate of fetal age could be obtained from C.R. length. This involved subjecting the original data from the 129 fetuses from the University of London to various procedures.

The logistic equation is of the form:

$$Y = A.B.^X$$

If logarithms are taken for both sides of the equation, we obtain;

$$\log.Y = \log.A + X \log.B.$$

Therefore, if the log. of fetal age and the corresponding C.R. length are subjected to linear regression analysis the intercept value obtained will be equal to log.A and the slope of the line will be equal to log.B. In this way a logistic growth curve can be drawn.

The allometric equation is of the form;

$$Y = A.X^B$$

If logarithms are taken of both sides of the equation, we obtain;

$$\log.Y = \log.A + B \log.X.$$

Therefore, if the log. of fetal age and the log of C.R. length are subjected to linear regression analysis, the intercept value obtained will be equal to log.A and the slope of the line will be equal to B. In this way an allometric growth curve can be drawn:

If the data are arranged in classes with 10 mm. C.R. length as the class-interval, another growth curve can be formed by plotting the mean age-value for each class against the mid-interval C.R. length value for the same class.

The original data from the 129 fetuses from the University of London were subjected to each of the three procedures outlined above. The resultant graphs were drawn on the same set of axes for comparative purposes and in addition the original data points were also plotted as well as the polynomial regression equation that was used in the study to age the other fetuses (Figure 25) Table 5 gives the details of the individual graphs.

The graph which was drawn on the basis of arranging the fetuses into groups of 10 mm. C.R.E. intervals is shown in Figure 25 merely as a single line. An assessment of the error involved was attempted but in order to be accurate required the application of complicated mathematical procedures. An assessment of the error involved is therefore not included and the graph is included in Figure 25 merely for comparison with the others. This "interval" graph was not considered as a practical possibility for the study because each of the intervals contained different numbers of fetuses. This meant that equal emphasis was being placed upon unequal numbers of fetuses and it was thought that such a procedure would bias the resultant graph. Nevertheless, from Figure 25, it can be seen that such a procedure produces a graph which is very similar in appearance to those of the other three methods.

In Figure 25 it can be seen that all of the procedures produce graphs which are very similar in appearance although there is increased difference towards both ends of the range of fetal length. This is only to be expected because of the lack of data beyond these points and highlights the dangers associated with placing too much emphasis on estimates which are from the ends of the data range.

COMPARISON OF TANNER ESTIMATES

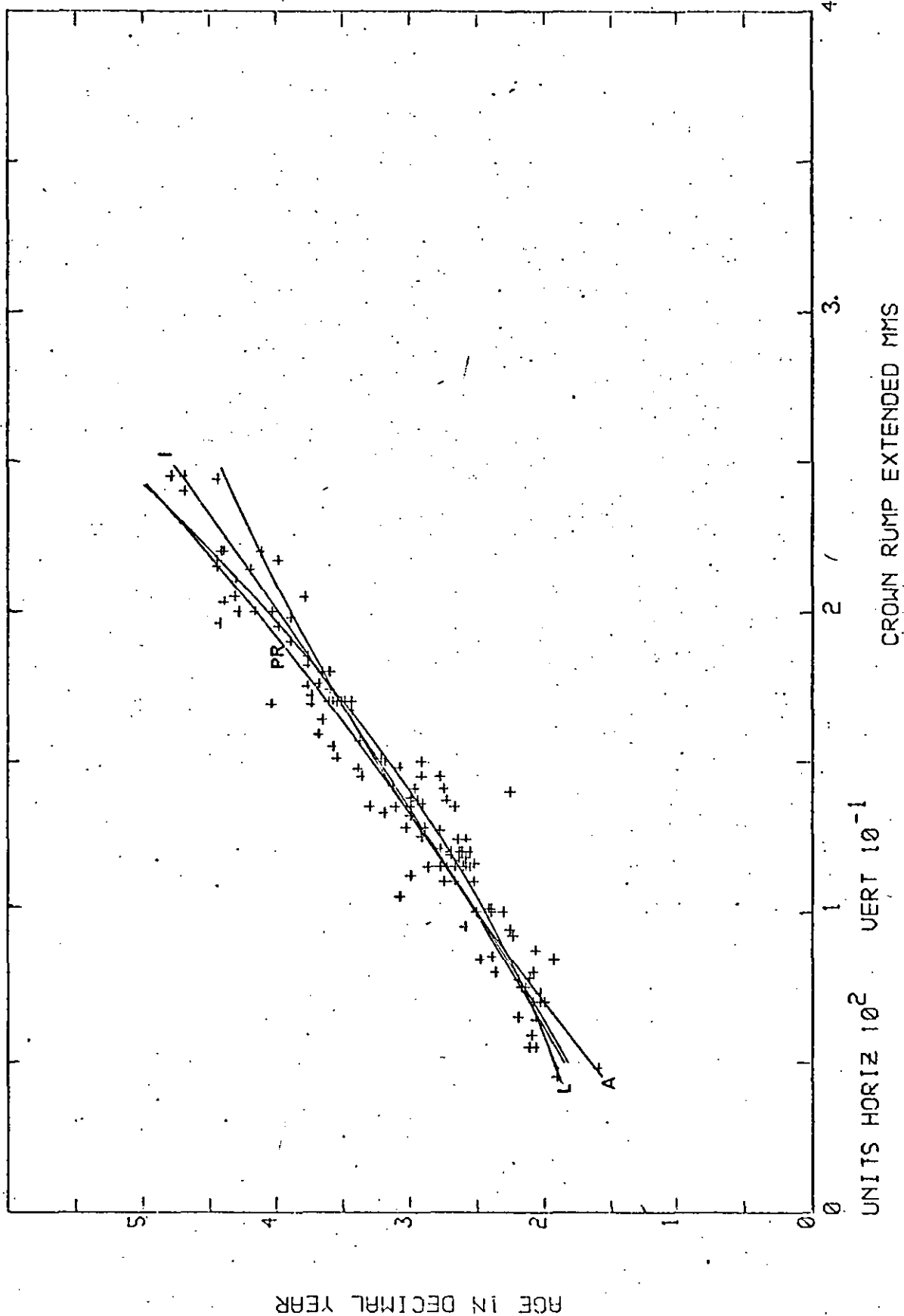


Figure 25. A comparison of different underlying models applied to the data from London University.

- PR = Polynomial Regression Equation
- L = Logistic Equation
- A = Allometric Equation
- I = Interval Equation (10mm C.R.E. length)

Table 5 shows that each of the methods is a good predictor of fetal age from fetal C.R.E. length but the relatively high S.E.E. associated with the allometric equation (0.045 yr.) would appear to eliminate it from any practical consideration.

Moss et.al. 1955 found an interphase to be present when they attempted to relate the allometric growth equation to C.R. length and this means that the relationship of

$$Y = A.X^B$$

is not suitable to describe C.R. development. It is therefore not surprising to find that the error involved with such a model is high.

Table 5 also shows that the error involved in using the polynomial regression analysis (0.019 yr) is similar to that found when using the logistic equation (0.020 yr) and although the difference does not appear to be significant, it suggests that the use of the logistic equation in further studies might be considered.

In addition to studying different mathematical models which might be used to describe the growth of fetal C.R. length, consideration was also given to other fetal measurements from which fetal age might have been estimated.

Crown-heel (C.H.) length was considered as a substitute for C.R. length and the data of the fetuses from the University of London contained details of the C.H. length, which allowed polynomial regression analysis to be carried out (See Figure 26). The correlation coefficient for the data of Figure 26 shows that C.H. length is a good predictor of fetal age and the S.E.E. of 0.018 yr (7 days) demonstrates that the error involved in the prediction is slightly lower than that involved when using C.R. length and polynomial regression. Even so, the errors involved with the two methods are very similar and Birkbeck et.al.(1975) in a similar study found a similar situation with the error involved using C.R. length to be 7.2 days whilst that for C.H. length was only

TO AGE FETUSES USING CROWN HEEL LENGTH

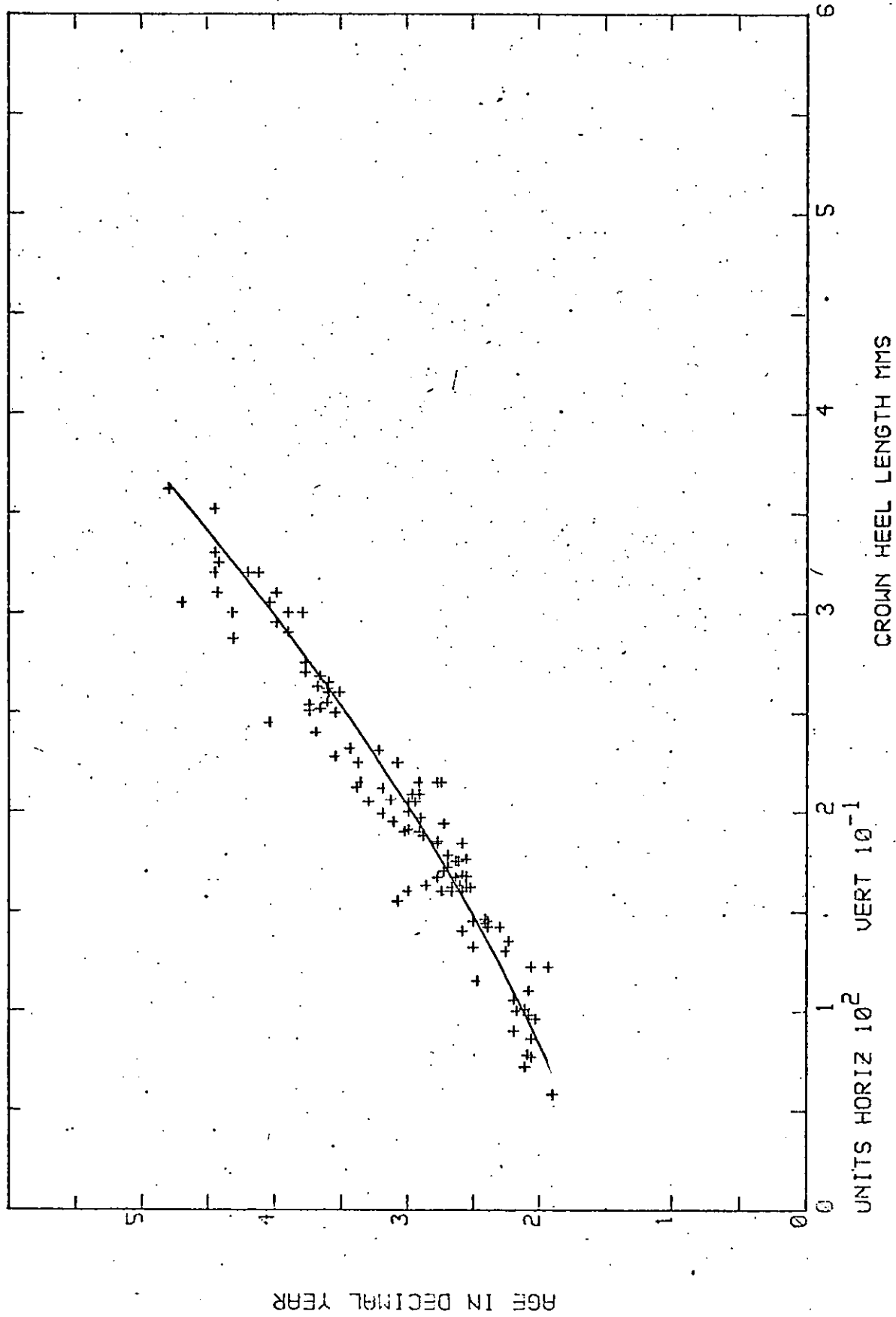


Figure 26. To estimate fetal age from crown-heel length.

6.9 days. On this basis it may be assumed that the measurements have almost the same value of predictive accuracy although C.H. length must be considered as being slightly more accurate. On the other hand, C.H. length is a more difficult measurement to take than C.R. length, particularly when the fetus has been fixed and is in a flexed attitude with the knees drawn up to the chest. There is often great difficulty encountered when trying to straighten the fetal lower limbs and there is the possibility of greater error becoming involved as the variable positions of the knee and ankle joints are included.

Finnstrom (1972) was able to reduce considerably the error involved with the estimate of age in human neonates by using a combination of five measurements. It is anticipated that a further study of fetal age estimation might well be able to reduce the error involved by using a similar combination of measurements, for example C.H. length in conjunction with C.R. length. Biparietal diameter (B.P.D.) might also be included for consideration for the future because this method is now receiving particular attention on a longitudinal basis in utero by the use of ultrasound. This suggests that it might well become the most accurate method of fetal age estimation from a single measurement and the longitudinal approach means that a continuous growth pattern can be obtained rather than a cross-sectional picture which has been the only method available so far. Unfortunately, however, the underlying effects of the error involved with using L.M.P. dates mean that a perfect correlation coefficient of 1.0 between fetal length and fetal age is extremely unlikely, unless a more accurate method of estimating true gestational age becomes available. Even if such a method were found, the natural biological variation that is thought to exist in terms of length at any particular age, would still tend to destroy a perfect correlation, but perhaps the true extent of this variation could then be calculated.

The accuracy of any method involved with the estimation of fetal age is influenced to a great extent by the normality of the original fetuses. In this study, 127 fetuses were eliminated from the original total of 256 fetuses

from the University of London because they were considered to have some abnormality which would affect fetal growth in their records. Fetuses were eliminated therefore on the basis of, for example, toxæmia, nephritis, diabetes mellitus, previously threatened abortion, twins, diseased placenta, and ethnic origin if other than Caucasian. There is very little literature available regarding causes of fetal growth retardation and therefore every attempt was made to eliminate all "abnormal" fetuses and include only the "normal". The fact that all of these fetuses however were the products of spontaneous abortions undermined this principle but it is impossible to determine exactly the extent of this problem, although it is thought to be only slight. If any doubt had been expressed concerning the accuracy of the L.M.P. dates, the particular fetus was automatically eliminated. The results of these endeavours can be seen in Figure 20.

For comparison the data from the 256 fetuses which consisted of a combination of the "normals" and "abnormals" were subjected to polynomial regression analysis (See Figure 27). Surprisingly the graph in Figure 27 is almost identical to that in Figure 20 and even more surprisingly the error involved in the estimate of fetal age increases only slightly from 0.019 yr. (7d) to 0.022 yr. (8d) when the abnormal fetuses are included in the calculations. This suggests that perhaps the factors known to affect fetal growth towards the end of pregnancy are not so pronounced earlier on in fetal life and the parasitic action of the fetus with regards to the mother might well over-ride an interference particularly one concerning solely maternal health. In order to study this further, the 127 abnormal fetuses were classified into groups based on their abnormality and the data were compared to the equation calculated for this study from the normal fetuses. Unfortunately the lack of numbers for many of the groups made impossible the justification of any conclusions and therefore only two abnormal groups are presented here although all abnormal groups were studied.

Ethnic origin is a factor known to affect skeletal growth and development

ALL OF TANNERS FETUSES REGARDLESS OF PROBLEMS 256 FETUSES

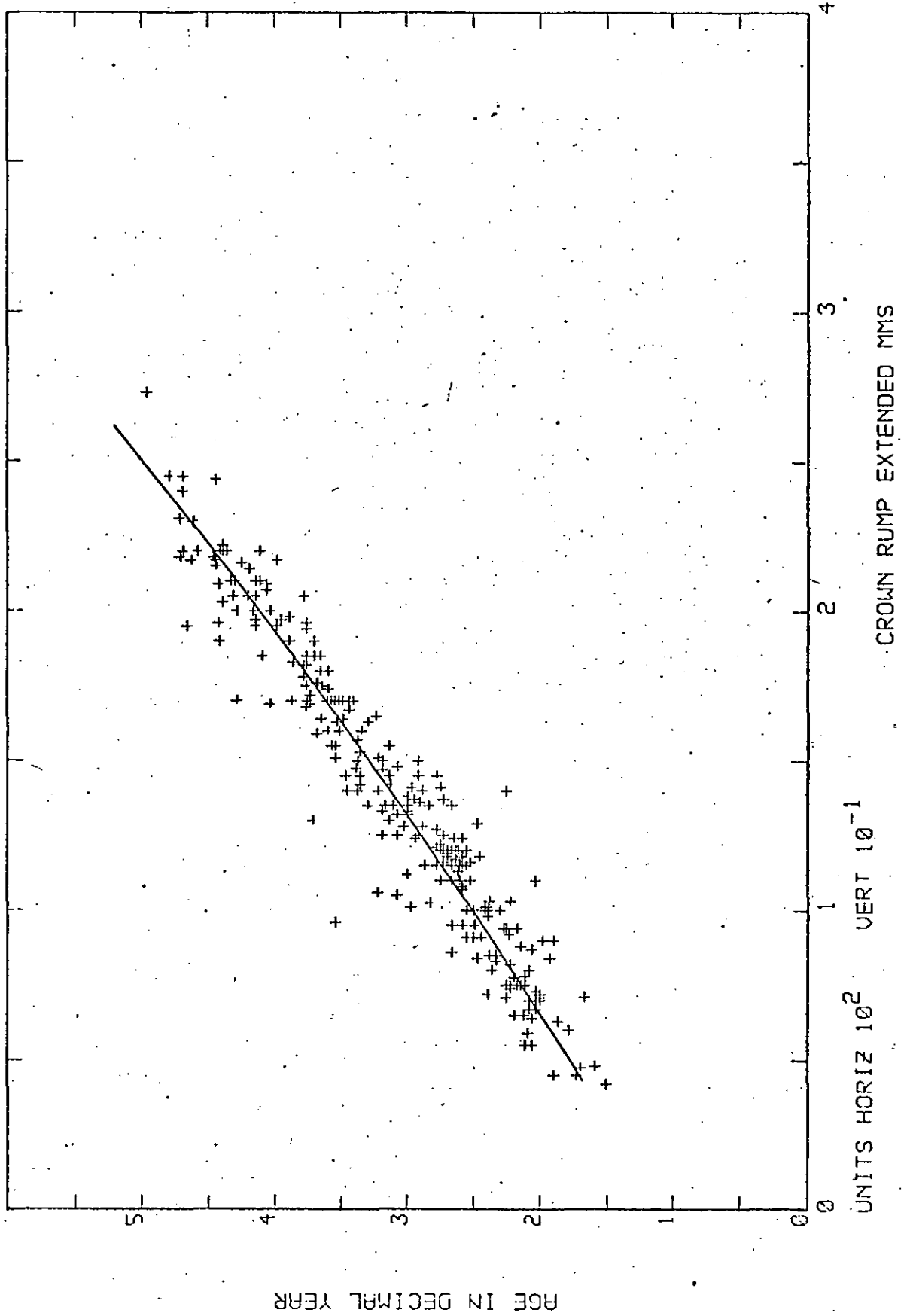


Figure 27. The development of fetal age based upon C.R.E. length regardless of "abnormality".

particularly during childhood. (Christie et.al., 1950). Therefore, those fetuses for whom at least one parent was not Caucasian were eliminated from the data as a group and their co-ordinates were plotted on graph paper in addition to the equation of this study $\pm 2.S.D.$ (See Figure 28). Figure 28 shows that the influence of ethnic origin on C.R. length is nearly normal over the age range for this study although it must be said that there is considerable variety in these fetuses regarding parental origin. Perhaps a more specific racial group similar to that studied by Nishimura (quoted by Birkbeck et.al.1975) in Japan, would produce a more consistent difference.

A similar procedure was applied to the data of 10 sets of twins and in Figure 29 their data points can be seen superimposed upon Tanner's equation (1976). The fact that four of the sets have different C.R. lengths is, in itself, very interesting in terms of the biological variation in C.R. length involved with fetuses of the same age but presumably a more detailed study which included the determination of monozygous or dizygous twins would be needed before any firm conclusions could be reached. Figure 29 shows that the growth in C.R. length of human twins does not appear to be abnormal between the range of 60 - 230 mm. although it is known that singleton neonates are larger than twin neonates (Ounsted and Ounsted, 1973). It is conceivable that the retarding influence on twin fetal growth might manifest itself only when there is restriction in terms of space. In early fetal life, the twin fetuses might well have sufficient room to grow normally and it is only in the last trimester, when available space is at a premium, that growth retardation might take place. This certainly requires further investigation.

In a further attempt to improve the estimate of fetal age from C.R. length and to investigate the possibility of there being a sex difference in C.R. length between fetuses of the same age, the data from the original 129 fetuses were separated into groups of males and females. Both the 71 males

ETHNIC ORIGIN

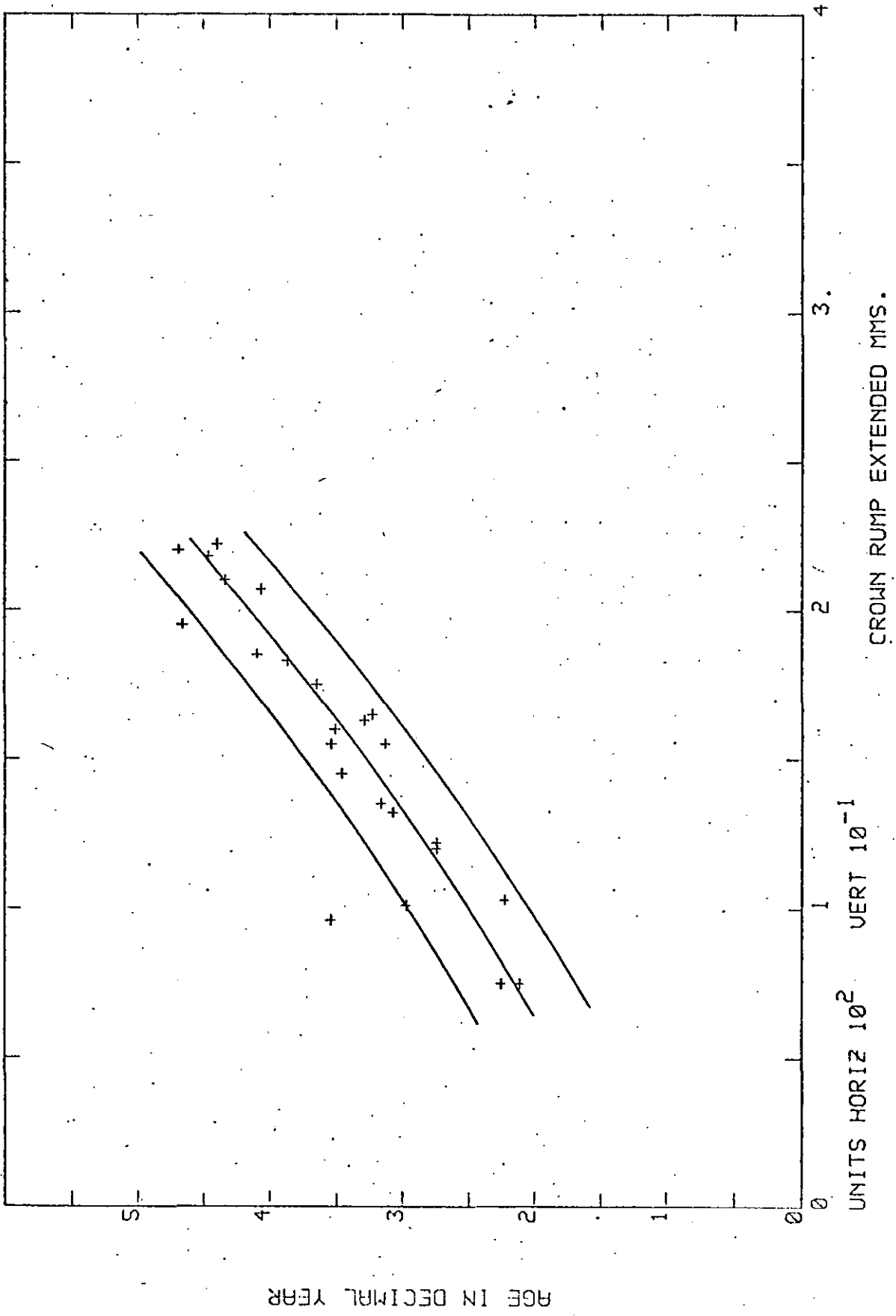


Figure 28. Assessment of the effect of ethnic origin on fetal growth.

TWINS

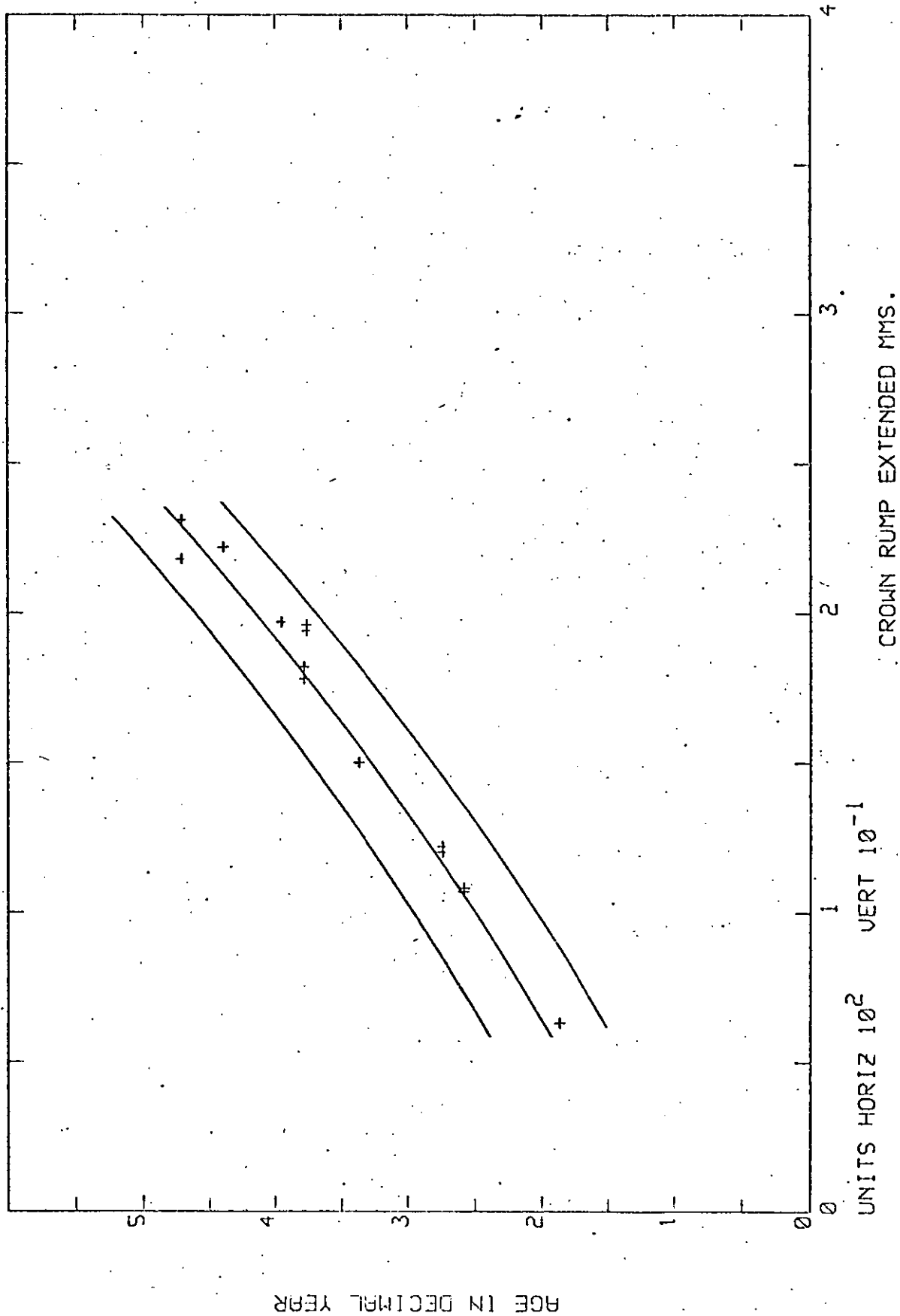


Figure 29. Assessment of the effect of the presence of twins on fetal growth.

and 58 females were subjected to polynomial regression analysis and Figure 30 shows that the resultant equations are very similar in appearance. No sex difference is therefore suggested in the development of C.R. length and the similarity of the errors involved with the equations suggests that the variance about the lines is of the same magnitude with no improvement of estimate of fetal age being made possible by separation into groups based on sex. These results agree with Birkbeck et.al. (1975) who could not find any evidence of sexual dimorphism in the relation between C.H. length, or weight, and post-menstrual age between 50 and 150 days, and suggested that if such dimorphism is present, it must be of small magnitude. Birkbeck et.al. (1975) however, do not reveal how such an assessment was made.

The use of conceptual age for the fetuses throughout this study is an attempt to be nearer the biological truth than would be the case if menstrual age was used. The life of an individual begins with fertilisation of the ovum, although Birkbeck et.al. (1975) have suggested that it is difficult to precisely define when this occurs. At present it is difficult to determine the actual time of conception but because ovulation usually occurs about the fourteenth day after the onset of menstruation, a simple subtraction of 14 days from the menstrual age allows a reasonable estimate of ovulation age to be obtained. Bearing in mind that the ovum has only a very limited viability, this ovulation age may also be considered as a reasonable estimate of conceptual age, although it must be appreciated that no greater accuracy in ageing is obtained by doing so. The error involved with the use of L.M.P. dates could be considerably reduced by the use of copulatory age because of the limited viability of the male gametes but has to be reluctantly dismissed because of its impracticality. This is unfortunate because the use of "post-copulatory age" rather than "age after birth" for growth studies in rats (Hughes and Tanner, 1970) has led to a considerable reduction in variability of measurements at any given age and so is clearly advantageous.

COMPARISON OF MALE AND FEMALE VALUES

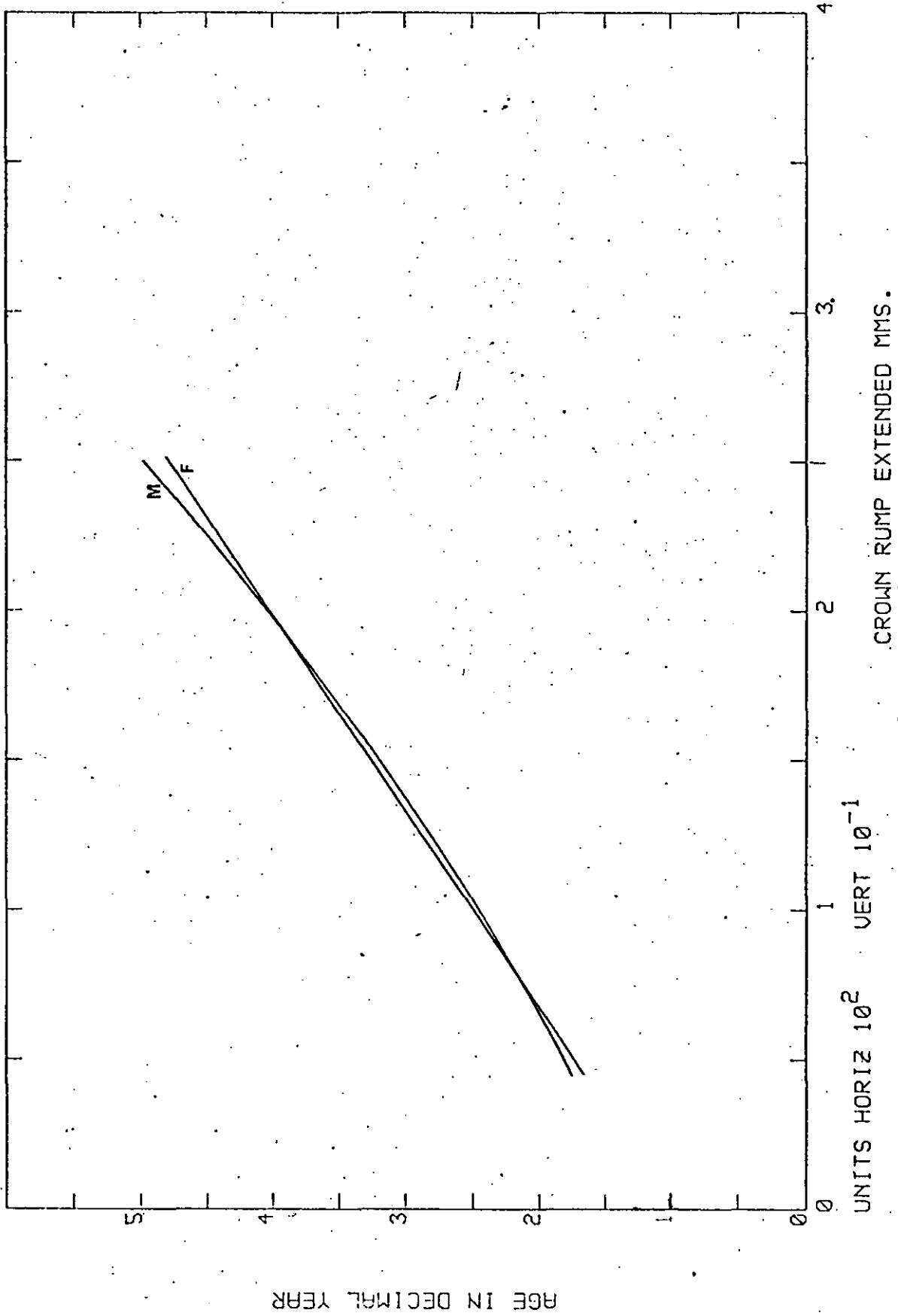


Figure 30. Assessment of the difference between male and female fetal growth.

SUMMARY

- (1) The fetuses were aged based upon the measurement of their Crown-Rump Extended Length.
- (2) Published data relating C.R. length to fetal age was criticised because there was considerable difference between sources. This difference was attributed to:
 - a) Different samples upon which equations were based.
 - b) Secular change.
 - c) Some material being from spontaneous abortion, other material being from induced abortion.
 - d) Preservation of fetuses.
 - e) Imprecise definition of C.R. length, and differing anthropometric techniques.
 - f) Different underlying models of growth.
- (3) C.R.E. length is put forward as a clearly defined objective measurement which lends itself readily to precise measuring.
- (4) It is emphasised that when fetal age is to be estimated from fetal length, the condensation of the variance ought to be along the age-axis.
- (5) When some form of abnormality is present fetal growth of C.R. length might not be affected in the first two-thirds of pregnancy as much as the retardation at term would suggest, particularly where twins and racial difference are concerned.
- (6) There is no difference in growth of C.R. length between male and female fetuses over the age range studied.
- (7) The use of conceptual age is advocated for use in all fetal work. It is closer to the true biological situation of gestational age than the more commonly used menstrual age.

Part of this work on fetal ageing has already been published.

"Bagnall, K.M., Jones, P.R.M., and Harris, P.F.1975.

Estimating the age of the human foetus from crown-rump
measurements.

Annals of Human Biology, 2, 387-390"

The Times for Appearance of the Ossification Centres

Having noted the presence or absence of each of the ossification centres on each of the radiographs, the fetuses were arranged in chronological order (See Appendix E). This made easier the determination of the earliest age at which each ossification centre was seen and also helped to identify the oldest fetus in which the ossification centre was not seen. This difference between these two ages can be considered as the age range over which each particular ossification centre can be said to appear and the results can be seen in Table 6, Table 7, Table 8, Figure 31, Figure 32, Figure 33.

Throughout this study, it has been realised that the vertebra C_1 does not have a body. Its centrum, therefore, has been considered to be the cartilaginous mass which will form the future dens attached to vertebra C_2 . This is in accordance with Gray's Anatomy (Warwick and Williams, 1973).

Great care was taken in the assessment of the presence of an ossification centre, particularly when the fetuses had not been stained with silver nitrate prior to radiography. The initial change in appearance observed when a centre becomes present involves only a very slight change in radiotranslucency and therefore a 4 diopetre lens was used in all cases. Wherever there was doubt as to the presence of an ossification centre in the A.P. view, the lateral view was taken into consideration if this was available.

In order to assess further the validity of combining the three sources of data, especially to include the radiographs of those fetuses that had been stained with silver nitrate, each of the three series of radiographs were subjected to individual determinations of appearance times for each of the ossification centres. The three sets of results were then compared to see if any particular source of data would dominate either the earliest age for appearance of the ossification centres or the oldest fetus in which the ossification centre was not seen. The distribution of these overall values was evenly spread throughout the three series of radiographs and it was therefore concluded that it was perfectly legitimate to combine the three sources of data.

Table 6

Appearance Times for Vertebral Neural Arch Ossification Centres

Skeletal Element	Earliest Age at which Centre was seen	Oldest fetus in which Centre was not seen
C1	0.184	0.196
C2	0.184	0.196
C3	0.184	0.196
C4	0.184	0.196
C5	0.184	0.196
C6	0.179	0.196
C7	0.179	0.195
T1	0.179	0.195
T2	0.179	0.195
T3	0.179	0.200
T4	0.184	0.202
T5	0.184	0.202
T6	0.184	0.202
T7	0.184	0.202
T8	0.188	0.204
T9	0.189	0.202
T10	0.189	0.202
T11	0.189	0.202
T12	0.189	0.204
L1	0.189	0.202
L2	0.189	0.216
L3	0.190	0.218
L4	0.195	0.218
L5	0.202	0.247
S1	0.202	0.397
S2	0.211	0.397
S3	0.250	0.413
S4	0.266	0.453
S5	0.294	0.501

Table 7

Appearance Times for Vertebral Centra Ossification Centres

Skeletal Element	Earliest Age at which Centre was seen	Oldest fetus in which Centre was not seen
C1	0.250	0.441
C2	0.234	0.365
C3	0.212	0.344
C4	0.203	0.291
C5	0.200	0.272
C6	0.195	0.265
C7	0.190	0.246
T1	0.189	0.246
T2	0.189	0.246
T3	0.189	0.246
T4	0.189	0.246
T5	0.188	0.246
T6	0.179	0.246
T7	0.179	0.246
T8	0.179	0.246
T9	0.179	0.246
T10	0.179	0.246
T11	0.179	0.195
T12	0.179	0.195
L1	0.179	0.210
L2	0.179	0.210
L3	0.185	0.210
L4	0.188	0.210
L5	0.189	0.210
S1	0.190	0.233
S2	0.191	0.292
S3	0.203	0.338
S4	0.207	0.386
S5	0.266	0.453

Table 8

Appearance Times for Primary Ossification Centres in the Long Bones of the Limbs

Skeletal Element	Earliest Age at which Centre was seen	Oldest Fetus in which Centre was not seen
Humerus	0.157	0.169
Radius	0.160	0.169
Ulna	0.160	0.169
Femur	0.157	0.169
Tibia	0.160	0.169
Fibula	0.160	0.169

APPEARANCE TIMES FOR NEURAL ARCHES

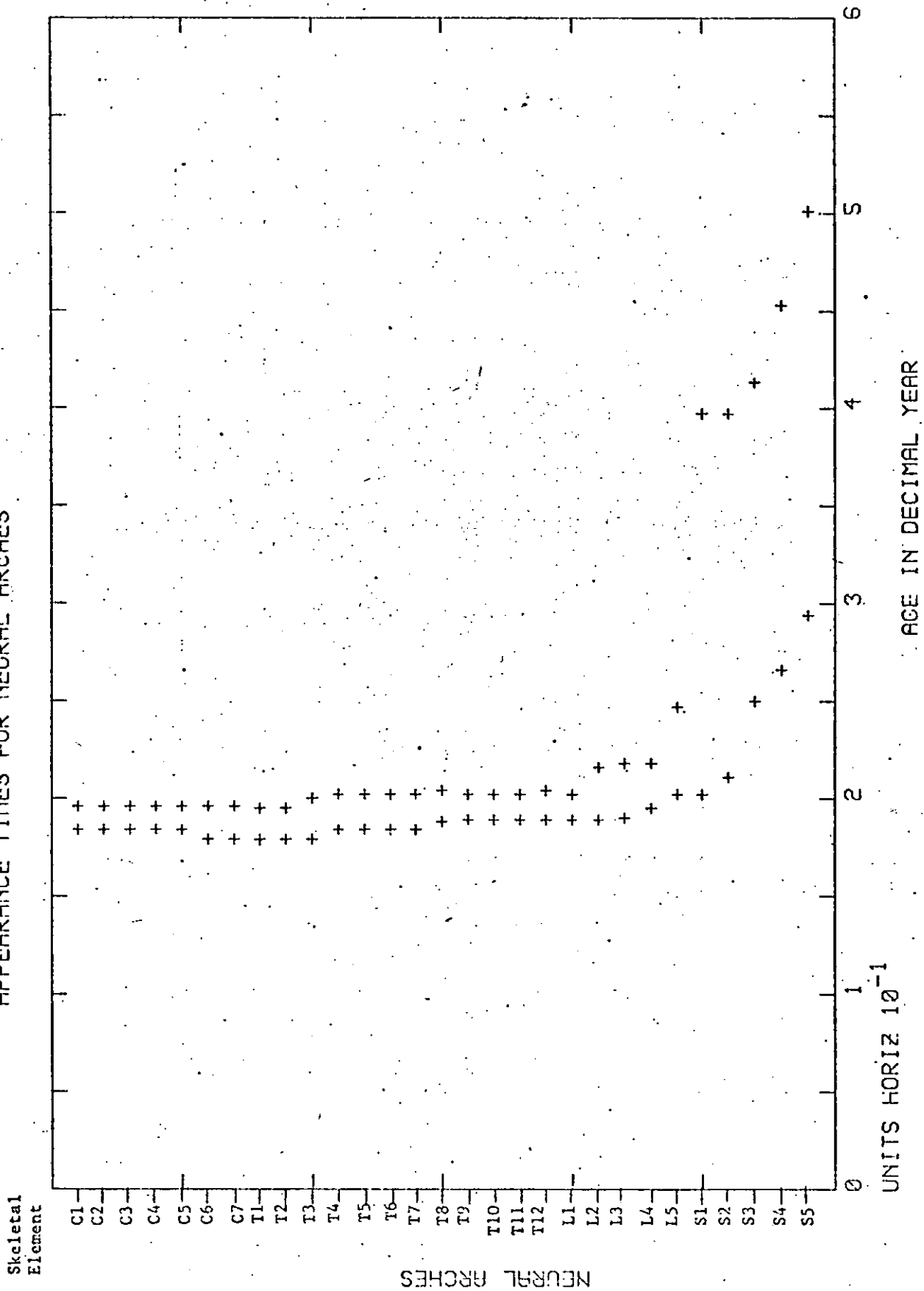


Figure 31. The appearance times for the vertebral neural arch ossification centres. The first cross indicates the age before which the centre was not seen and the second cross indicates the age after which the centre was always seen.

APPEARANCE TIMES FOR CENTRA

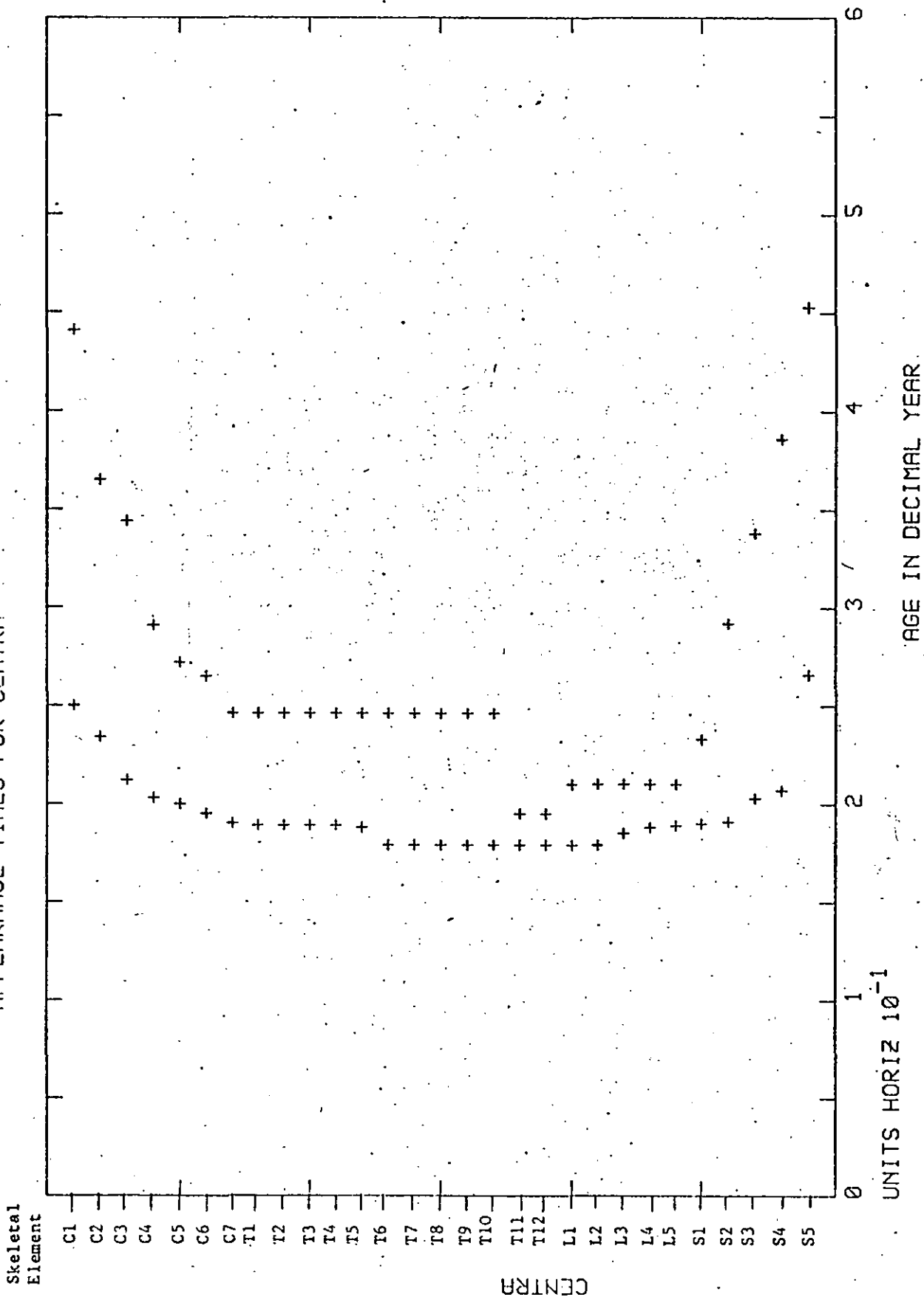


Figure 32. The appearance times for the vertebral centra ossification centres. The first cross indicates the age before which the centre was not seen and the second cross indicates the age after which the centre was always seen.

APPEARANCE TIMES FOR LIMB OSSIFICATION CENTRES

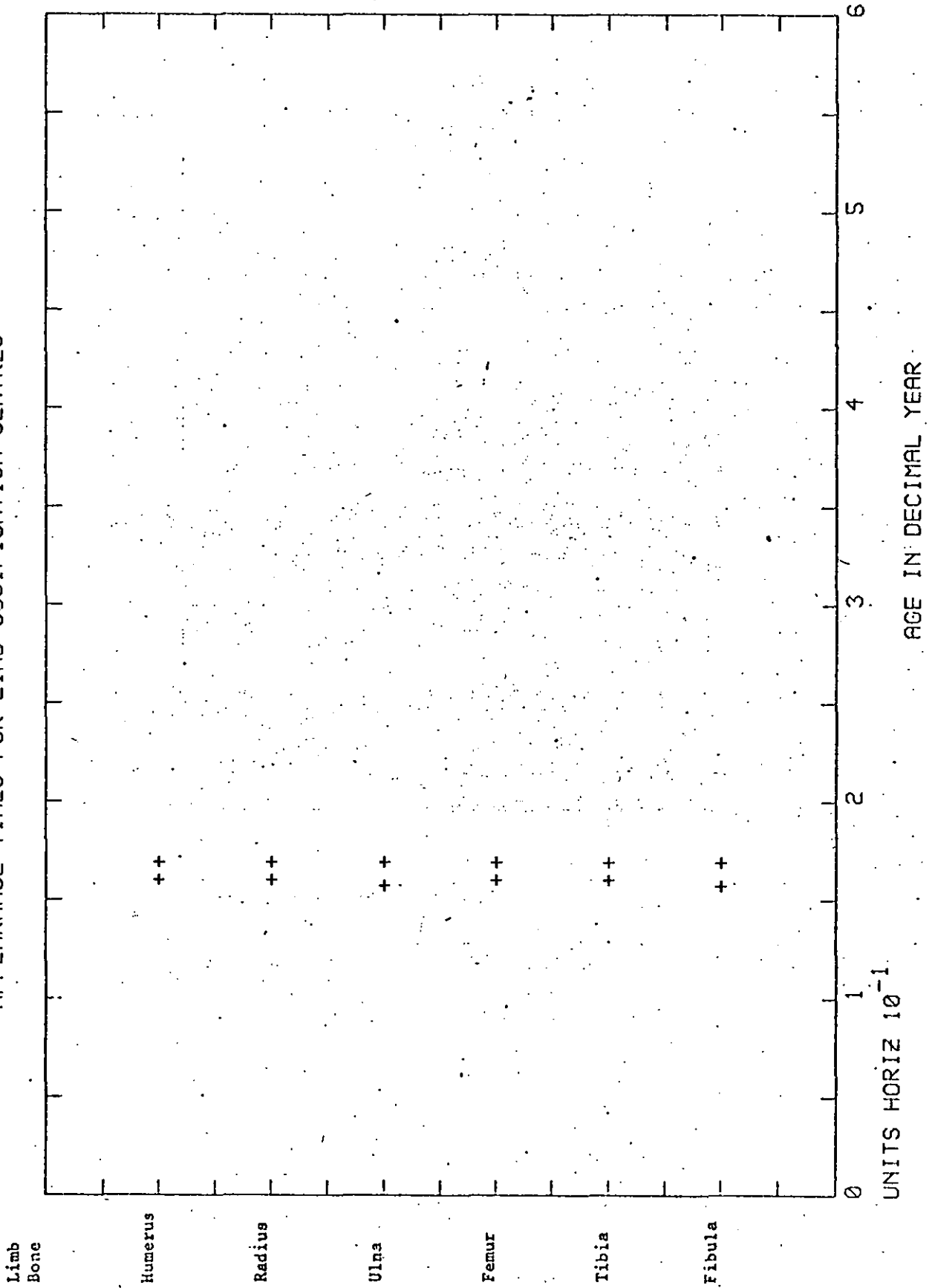


Figure 33. The appearance times for the primary ossification centres of the long bones of the limbs. The first cross indicates the age before which the centre was not seen and the second cross indicates the age after which the centre was always seen.

From Tables 6, 7 and 8 it can be clearly seen that most of the ossification centres for the vertebral column and long bones of the limbs appear over a very short period of time. This is also well illustrated in Figures 31, 32 and 33 which are diagrammatic reproductions of Tables 6, 7 and 8 respectively.

None of the ossification centres for the vertebral neural arches appears before 0.179 yr (9 weeks) and all of them are present after 0.501 yr. (26 weeks). However, all of the centres from C_1 to L_4 are present by 0.218 yr. (11 weeks) and it is only the appearance of L_5 and all of the sacral neural arch ossification centres which significantly increase the range for appearance time.

From Table 7 and Figure 32 it can be seen that none of the ossification centres for the vertebral centra appears before 0.179 yr. (9 weeks) and that all of the centres are present after 0.453 yr. (24 weeks). However, the ranges for appearance time of all of the centra ossification centres are greater than the range for the corresponding neural arch ossification centres, with only T_{11} , T_{12} , L_1 , L_2 , L_3 , L_4 , and L_5 having a relatively short period of time over which the centre appears.

The appearance times for the primary ossification centres in the long bones of the limbs can be seen in Table 8 and Figure 33. All of these centres appear over a very short period of time and they are all present before the vertebral column has commenced ossification.

In order to compare these results to the previous work of Teissandier (1944), Noback and Robertson (1951), and O'Rahilly and Meyer (1956), the ages represented in Tables 6, 7 and 8 have been converted to C.R.E. lengths. Teissandier (1944), Noback and Robertson (1951) and O'Rahilly and Meyer (1956) have all used the fetal C.R. length as their measurement of development and the comparison with the present work can be seen in Tables 9, 10 and 11.

Teissandier (1944) used the Splatehotz method of staining with alizarin red and studied 148 embryos and fetuses, 21 between 17 and 36 mm. in

Table 9

Comparison of Appearance Times for Vertebral Neural Arch Ossification Centres

Ranges For Appearance Times

Skeletal Element	This Study C.R.E. lgth mm	Noback & Robertson 1951 C.R. lgth mm	Teissandier 1944 C.R. lgth mm	O'Rahilly & Meyer 1956 C.R. lgth mm
C1	51.0 - 61.0	45.0 - 49.0	50.0 - 60.0	51.0 - 52.0
C2	51.0 - 61.0	40.0 - 45.0	51.0 - 60.0	51.0 - 51.0
C3	51.0 - 61.0	38.0 - 45.0	51.0 - 60.0	51.0 - 52.0
C4	51.0 - 61.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
C5	51.0 - 61.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
C6	47.5 - 61.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
C7	47.5 - 61.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
T1	47.5 - 60.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
T2	47.5 - 60.0	38.0 - 45.0	51.0 - 60.0	51.0 - 54.0
T3	47.5 - 63.5	40.0 - 52.0	51.0 - 60.0	51.0 - 61.0
T4	51.0 - 65.0	40.0 - 60.0	51.0 - 60.0	51.0 - 64.0
T5	51.0 - 65.0	40.0 - 60.0	51.0 - 60.0	51.0 - 64.0
T6	51.0 - 65.0	40.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T7	51.0 - 65.0	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T8	54.5 - 66.5	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T9	55.5 - 65.0	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T10	55.5 - 65.0	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T11	55.5 - 65.0	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
T12	55.5 - 65.0	45.0 - 60.0	52.0 - 60.0	54.0 - 64.0
L1	55.5 - 65.0	45.0 - 60.0	51.0 - 60.0	54.0 - 64.0
L2	55.5 - 76.0	45.0 - 60.0	51.0 - 85.0	54.0 - 64.0
L3	56.0 - 77.0	45.0 - 68.0	55.0 - 91.0	54.0 - 64.0
L4	60.0 - 77.0	45.0 - 68.0	58.0 - 91.0	59.0 - 82.0
L5	65.5 - 98.0	60.0 - 69.0	60.0 - 93.0	69.0 - 82.0
S1	65.5 - 190.0	65.0 - 76.0	75.0 - 110.0	75.0 - 112.0
S2	72.0 - 190.0	102.0 - 127.0	80.0 - 139.0	104.0 - 143.0
S3	100.0 - 199.0	102.0 - 161.0	135.0 - 170.0	106.0 - 143.0
S4	111.0 - 220.0	135.0 - 161.0	139.0 - 205.0	122.0 -
S5	129.0 - 244.0	163.0 - 173.0	170.0 - 350.0	-

Table 10

Comparison of Appearance Times for Vertebral Centra Ossification Centres

Ranges for Appearance Times

Skeletal Element	This Study C.R.E. lgth mm	Noback & Robertson 1951 C.R. lgth mm	Teissandier 1944 C.R. lgth mm	O'Rahilly & Meyer 1956 C.R. lgth mm
C1	100.0 - 213.5	135.0 - 161.0	165.0 - 195.0	-
C2	88.5 - 172.0	69.0 - 120.0	75.0 - 130.0	81.0 - 123.0
C3	73.0 - 160.0	69.0 - 102.0	75.0 - 105.0	70.0 - 112.0
C4	66.0 - 127.0	57.0 - 85.0	75.0 - 105.0	70.0 - 112.0
C5	63.5 - 115.0	57.0 - 71.0	65.0 - 91.0	70.0 - 82.0
C6	60.0 - 110.0	52.0 - 71.0	65.0 - 80.0	70.0 - 82.0
C7	56.0 - 97.0	52.0 - 68.0	60.0 - 80.0	59.0 - 82.0
T1	55.0 - 97.0	52.0 - 69.0	57.0 - 72.0	59.0 - 82.0
T2	55.0 - 97.0	52.0 - 57.0	57.0 - 65.0	54.0 - 75.0
T3	55.0 - 97.0	48.0 - 57.0	54.0 - 60.0	54.0 - 64.0
T4	55.0 - 97.6	48.0 - 57.0	51.0 - 60.0	54.0 - 64.0
T5	54.5 - 97.0	40.0 - 52.0	51.0 - 60.0	54.0 - 64.0
T6	47.5 - 97.0	40.0 - 52.0	51.0 - 60.0	54.0 - 64.0
T7	47.5 - 97.0	40.0 - 52.0	51.0 - 60.0	54.0 - 64.0
T8	47.5 - 97.0	40.0 - 52.0	51.0 - 60.0	54.0 - 59.0
T9	47.5 - 97.0	40.0 - 52.0	51.0 - 60.0	54.0 - 59.0
T10	47.5 - 97.0	40.0 - 52.0	43.0 - 60.0	54.0 - 59.0
T11	47.5 - 60.0	40.0 - 52.0	43.0 - 60.0	54.0 - 59.0
T12	47.5 - 60.0	40.0 - 52.0	43.0 - 60.0	54.0 - 59.0
L1	47.5 - 71.0	40.0 - 52.0	43.0 - 55.0	54.0 - 64.0
L2	47.5 - 71.0	45.0 - 52.0	43.0 - 55.0	54.0 - 64.0
L3	52.5 - 71.0	45.0 - 52.0	51.0 - 56.0	54.0 - 64.0
L4	54.5 - 71.0	45.0 - 54.0	51.0 - 56.0	54.0 - 64.0
L5	59.0 - 71.0	45.0 - 57.0	51.0 - 65.0	54.0 - 75.0
S1	56.0 - 88.0	52.0 - 65.0	57.0 - 85.0	59.0 - 75.0
S2	56.5 - 128.0	60.0 - 68.0	59.0 - 93.0	59.0 - 95.0
S3	66.0 - 156.0	60.0 - 97.0	59.0 - 93.0	59.0 - 122.0
S4	69.0 - 184.0	84.0 - 143.0	82.0 - 170.0	81.0 -
S5	111.0 - 220.0	135.0 - 175.0+	107.0 - 350.0	122.0 -

Table 11

Comparison of Appearance Times for the Ossification Centres of the Long Bones of the Limbs

Ranges for Appearance Times

Skeletal Element	This Study C.R.E. length mm	Noback and Robertson 1951 C.R. length mm	Mall 1906 C.R. length mm
Humerus	29.0 - 39.0	23.0 - 30.0	18
Radius	32.0 - 39.0	24.0 - 35.0	19
Ulna	32.0 - 39.0	24.0 - 35.0	24
Femur	29.0 - 39.0	23.0 - 35.0	18
Tibia	32.0 - 39.0	23.0 - 35.0	19
Fibula	32.0 - 39.0	29.0 - 35.0	30

C.R. length and 127 between 37 and 400 mm. C.R. length. Noback and Robertson (1951) studied 136 human embryos ranging in C.R. length from 14 to 235 mm. having cleared their specimens with potassium hydroxide and stained their bones with alizarin red. O'Rahilly and Meyer (1956) studied 77 fetuses of undetermined sex and ranging from 49 to 150 mm. in C.R. length. Their fetuses had been fixed in 10% formalin for not more than a few days and had been immersed prior to radiography in a 0.5% aqueous solution of silver nitrate for a period of from 2 to 10 days depending upon the size of the specimen.

Table 9 compares the results of this study concerning the appearance times for the vertebral neural arch ossification centres with the results of Teissandier (1944), Noback and Robertson (1951) and O'Rahilly and Meyer (1956). At first glance it would seem that for most of the ossification centres, Noback and Robertson (1951) have recorded an earlier period for appearance time and this might be immediately attributed to the earlier recognition of such centres by using alizarin red staining in contrast to radiography. However, such an explanation is difficult to reconcile with the work of Teissandier (1944) who also used alizarin red staining and whose results agree more closely with the results of this present study and O'Rahilly and Meyer (1956) than with Noback and Robertson (1951). A more acceptable explanation of the differences between the three studies might well be found in the measurement of C.R. length.

Noback and Robertson (1951) do not include in their work a precise definition of how C.R. length was measured. Instead they present a table which relates C.R. length to fetal age and reference is made to the work of Patten (1946) and Scammon and Calkins (1929). Patten (1953) describes C.R. length as "sitting height" but the diagram he uses to illustrate the measurement shows a fetus with its head certainly not in the Frankfort plane and even includes flexion of the fetal trunk. Scammon and Calkins (1929) on the other hand define C.R. length as being measured as a straight line parallel to the vertical axis

of the body and extending from the crown to the level of soft tissues over the ischial tuberosities. The head is held in an erect position but is not extended, and the thighs are flexed at right angles to the long axis of the trunk. The measurement is taken with a slight but firm pressure being exerted against the soft tissues over the ischial tuberosities in each case. This description by Scammon and Calkins (1929) is similar to the measurement of C.R.E. length as defined by this study but is entirely different to that described by Patten (1953) and therefore considerable doubt surrounds the method by which Noback and Robertson (1951) measured the C.R. lengths of each of their fetuses although it is interesting to note that they call their fetuses "embryos". This illustrates nicely the problems that are to be encountered when attempts are made to compare results with previous fetal work. Descriptions of measurements such as C.R. length are often absent or imprecise and, in particular, often no reference is made to the actual fetal age being used by the author, whether it be menstrual or conceptual age.

In whatever way the fetal C.R. length was measured, the Table relating fetal age to C.R. length presented by Noback and Robertson (1951) can be compared to a similar Table from this study. In Table 12 the C.R. length from Noback and Robertson (1951) corresponding to a specific fetal age can be compared with the equivalent C.R.E. length from this study for the same fetal age. Clearly there is a considerable difference for each fetal age between the C.R. length of Noback and Robertson (1951) and the equivalent C.R.E. length given by this study. If this difference is carried back to Table 9 which compares the appearance times for the vertebral neural arch ossification centres between the results of this study and Noback and Robertson (1951), it becomes apparent that there is no difference between the two studies for the ranges of appearance for most of the neural arch ossification centres. The apparent previous discrepancy has therefore been explained on anthropometric grounds and not methodological. Unfortunately, neither Teissandier (1944) nor O'Rahilly and Meyer (1956) give an indication of

Table 12

Comparison of Differences in C.R. Length and Age between this study and Noback and Robertson (1951)

Conceptual Age in weeks	Equivalent C.R. length from Noback and Robertson (1951) mm	Equivalent C.R.E. length from this study mm
6	12 - 13	-
7	19 - 20	-
8	28 - 30	27.5
9	39 - 41	42.5
10	51 - 53	58.0
11	64 - 66	72.0
12	77 - 79	86.0
13	91 - 93	99.0
14	105 - 107	112.0
15	119 - 121	125.0
16	132 - 134	137.5
17	$\bar{+}$ 147	149.0
18	$\bar{+}$ 160	160.5
19	$\bar{+}$ 173	171.5

how C.R. length was measured in their studies but it is significant to note that the ranges for appearance time for each vertebral neural arch ossification centre are approximately the same for each of the four studies. If Teissandier (1944) measured the equivalent of C.R.E. length for each of his fetuses, then there does not appear to be any significant difference in recognition of vertebral neural arch ossification times between the methods of alizarin red staining and radiography which is specifically designed for this purpose.

In Table 9 it can be seen that the appearance times for the most caudal vertebral neural arch ossification centres have relatively large ranges. It is felt that these large ranges can explain the differences to be found between the three studies for the appearance times of these centres even after allowing for the different measurements of C.R. length. The appearance times for these centres are so variable that with a larger study significant extensions of these ranges might well be found.

Table 10 compares the appearance times for the vertebral centra ossification centres between the results of this present study, Noback and Robertson (1951) Teissandier (1944) and O'Rahilly and Meyer (1956). A similar picture is presented in Table 10 as for Table 9 with no apparent difference between the ranges for appearance time of those ossification centres which appear over a short period of time, once an allowance has been made for the different ways in which C.R. length has been measured. The major differences between the four studies concerns those ossification centres which have a large range for appearance time and it is felt that this in itself is sufficient to explain these differences because of the large variability in appearance times. Again however, it appears that there is little difference in recognition of ossification centres between the method of alizarin red staining and radiography which has been specifically designed for this purpose.

Table 11 compares the appearance times for the ossification centres of

the long bones of the limbs between the results of this study, Noback and Robertson (1951) and Mall (1906). Mall (1906) studied alizarin red stained fetuses and described the measurement of C.R. length as "sitting height" but in his diagram to illustrate the measurement he included a well-flexed fetal head which was certainly not in the Frankfort plane of the C.R.E. length measurement. Making allowances therefore for the differences between the measurements made of C.R. length it would again appear that there is little difference to be found in recognition of the ossification centres between methods where the fetuses have been stained with alizarin red and methods which use high definition radiography.

O'Rahilly and Gardner (1972) note that the main sources of data relating to the first appearance of the prenatal ossification centres are based on studies which have used alizarin red S even though this technique is by no means specific for the demonstration of calcium. They go further and suggest that radiography generally leads to unsatisfactory results owing to the lack of sufficient radio-opacity although the method is simpler and quicker. The results of Meyer and O'Rahilly (1958) showed that the alizarin technique was slightly superior to the radiographic method but they suggest that because bone is a tissue which has to be defined in histological terms, its critical detection is necessarily a function of histological technique. Therefore, as anticipated, their results using histological examination of serial sections yielded results superior to the other two methods. In particular they were able to determine which of the cartilage masses had undergone vascular invasion which they used as the criterion for the approaching onset of endochondral ossification. Their results showed that alizarin and radiography do not demonstrate the onset of endochondral ossification but rather their first positive reaction coincides fairly closely with the laying down of the intramembranous periosteal collar. Therefore, strictly speaking, the alizarin and radiographical reactions do not coincide with the initial appearance of centres of ossification as defined by

Ham (1957). A centre of ossification using the radiographic technique must be defined as a change in radiotranslucency within the cartilage mass and it must be appreciated that this does not distinguish between endochondral and intramembranous ossification and might not even distinguish calcified cartilage from true bone.

Figures 31, 32 and 33 have a direct application because they can be used to assess the maturity of a particular fetus. Recognition of the presence of specific ossification centres of both the vertebral column and long bones of the limbs will allow an assessment of fetal conceptual age to be made. Those ossification centres which appear only over a very short period of time are especially suitable for this purpose because they carry with them a high degree of accuracy. Once an estimate of fetal age has been made it can then be compared with the L.M.P. dates provided by the mother or with an age based upon some other parameter and an assessment of fetal progress can be made. On a similar basis Figures 31, 32, and 33 can also be used as Normal Standards for development of the ossification of the vertebral column and limbs and as such can be used to determine the degree of effect of factors such as ill-health and nutrition which might affect fetal growth. Furthermore, because the ossification centres cover the whole of the fetal body an overall assessment of fetal skeletal maturity can be made and discrepancies between regions of the body can also be noted.

The times of appearance for the ossification centres as presented here, conceal the individual pattern of development. Although Figures 31, 32 and 33 are very useful in their application they fail to reveal in detail the sequence of formation for the ossification centres and in this sense they are only half of the complete story. The question of whether or not a definite order exists in the appearance of the ossification centres must be included and therefore the times for appearance of the ossification centres must not be divorced from their pattern of development.

SUMMARY

1. The range for times of appearance for the ossification centres of the vertebral column and long bones of the limbs are presented in Table and graphic form.
2. These Normal Standards of fetal skeletal development can be used to assess fetal development in both normal and abnormal situations, and they are particularly suitable because they cover the whole body of the fetus.
3. There does not appear to be any significant difference in recognition of the appearance of the ossification centres between radiography that has been specifically designed for this purpose, radiography following silver-staining, and alizarin red S staining. Previous differences that have been reported in the literature can be attributed to either "poor" radiography (bearing in mind the advances in technology that have been made) or differences in the method of measuring C.R. length.

THE SEQUENCE OF DEVELOPMENT OF THE OSSIFICATION CENTRES.

By noting particularly those fetuses in whom only a few ossification centres were present, it was possible to construct a developmental pattern for ossification centres in both the vertebral column and long bones of the limbs.

The fetuses were initially arranged in chronological order and in order of size but no pattern of ossification is evident by this means. It was therefore decided to arrange the fetuses in order of the number of centres present and the results of this can be seen in Tables 13, 14, 15 and 16. In this way a developmental pattern emerges.

From Table 13 it can be seen that whilst there is no exact pattern for the appearance of neural arch ossification centres, in general, a group of centres first appears in the lower cervical/upper thoracic region and this is rapidly followed by a second group in the upper cervical region. The remaining cervical neural arches then quickly ossify and the sequence spreads towards the mid-thoracic region. A third group of centres appears in the lower thoracic/upper lumbar region and ossification spreads upwards to meet centres from the thoracic region which are themselves extending caudally. The lower lumbar and sacral regions develop rather more slowly and in an orderly sequence, the 5th sacral arch being the last centre to appear.

In the study there were 37 fetuses smaller than 51.0 mm C.R.E. length and 8 fetuses greater than 51.0 mm C.R.E. length in whom there were no ossification centres present for the vertebral neural arches.

These results support the suggestion of Noback and Robertson (1951) that there does not appear to be any regular order for the appearance of the 9 most proximal neural arches. Although some authors (Mall, 1906; Noback, 1944) claim to have demonstrated that there is a definite cephalocaudal sequence of differentiation for the thoracic, lumbar, and sacral neural arches, Teissandier (1944) observed that in three fetuses where all cervical and upper one or two

Arranged in Ascending Order of Number of Centres Present

Study No of Fetus	11	14	467	275	425	758	34	18	8	763	32	22	31	17	30	23	464	6	368	332	19	20	643	2
C.R. Length	59.0	55.5	61.0	49.0	60.0	47.5	55.0	55.0	54.5	65.0	59.0	55.5	52.5	51.0	58.0	51.5	55.0	64.0	58.0	53.8	54.5	56.0	55.0	63.5
	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL
C1							XX	XX		XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
C2	X						XX	XX		XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
C3								X		XX	XX	XX	XX	XX	XX	XX		XX	XX	XX	XX		XX	XX
C4										XX	XX	XX	XX	XX	XX	XX		XX	XX	XX	XX		XX	XX
C5										XX	XX	XX	XX	XX	XX	XX	X	XX	XX	XX	XX		XX	XX
C6	X					X			XX	XX	XX	XX	XX	XX	XX	XX	X	XX	XX	XX	XX	X	XX	XX
C7		XX		X		X	XX	X	XX	XX	XX	XX	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX
T1	XX	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
T2			XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX
T3					XX	XX			XX	XX	XX	XX	XX	XX	X	XX	XX	XX	XX	XX	XX	XX	XX	XX
T4					XX	X			XX	XX	XX	XX	XX	X			XX	XX	XX	XX	XX	XX	XX	XX
T5									XX				XX				XX	XX	XX	XX	XX	XX	XX	XX
T6														X		X	XX	XX	XX	XX	XX	XX	XX	XX
T7																X	XX	XX	XX	XX	XX	XX	XX	XX
T8																			XX	XX	XX	X	XX	XX
T9																				XX	XX	X	XX	XX
L10																	XX	XX	X			XX		X
L11																	XX	XX	X			XX		
L12															XX		XX	XX	X			XX		
L13															XX		XX	XX				XX		
L14															X		X	X				X		X
L15																	X	X				X		
S1																								
S2																								
S3																								
S4																								
S5																								

Table 13 The sequence for appearance of the vertebral neural arch ossification centres.

C = Cervical T = Thoracic L = Lumbar S = Sacral
 R = Right L = Left X = neural arch ossification centre

Arranged in Ascending Order of Number of Centres Present

Study No of Fetus	277	13	11	41	66	467	425	23	332	31	464	758	29	51	43	8	25	10	368	763	22	643	34	
C.R. Length	56.0	55.5	59.0	62.0	66.5	61.0	60.0	51.5	63.0	52.5	55.0	47.5	55.5	67.5	65.0	54.5	62.5	65.0	58.0	65.0	55.5	55.0	55.0	
C1																								
C2																								
C3																								
C4																								
C5																								
C6																								
C7																								
V1																								
V2																								
V3																								
V4																								
V5																								
V6																								
V7																								
V8																								
V9																								
T10																								
T11																								
T12																								
L1																								
L2																								
L3																								
L4																								
L5																								
S1																								
S2																								
S3																								
S4																								
S5																								

Table 14 The sequence for appearance of the vertebral centra ossification centres.

0 = centrum ossification centre

Table 15 Appearance of the vertebral ossification centres.
 O = centrum ossification centre
 X = neural arch ossification

Arranged in Ascending Order of Number of Centres Present

Study No of Fetus	277	13	14	275	18	467	11	425	758	17	34	8	31	19	763	23	22	464	32	332	30	368	66	
C.R. mm Length	56.0	55.5	55.5	49.0	55.0	61.0	59.0	60.0	47.5	51.0	55.0	54.5	52.5	54.5	65.0	51.5	55.5	55.0	59.0	63.0	58.0	58.0	66.5	
	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	RL	
C1					XX					XX	XX		XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C2					XX					XX	XX		XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C3					X					XX	XX		XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C4										XX			XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C5										XX			XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C6							X			X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
C7			XX	X	X					X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T1			XX	XX	XX	XX	XX	XX		X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T2				XX		XX				X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T3										X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T4											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T5										X	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T6											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T7											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T8											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T9											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T10											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T11											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
T12											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
L1											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
L2											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
L3											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
L4											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
L5											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
S1											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
S2											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
S3											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
S4											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX
S5											XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XX	XOX	XX	XX

Study No of Fetus	193	207	179	177	180	276	265	189	191	188
C.R.E. mm Length	29.0	39.0	33.5	36.0	37.5	37.0	38.0	32.0	32.5	35.5
Humerus	X	X	X	X	X	X	X	X	X	X
Radius		X	X	X	X	X	X	X	X	X
Ulna		X	X	X	X	X	X	X	X	X
Femur	X		X	X	X	X	X	X	X	X
Tibia			X	X	X	X	X	X	X	X
Fibula								X	X	X

Table 16

Appearance of Ossification Centres in Long Bones of the Limbs.
Arranged in order of Number of Centres Present

thoracic arches were ossified, thoracic arches 3 or 4 down to 8 or 9 were in fact absent, but in the same fetuses the thoracic arches 9 or 10 down to lumbar arches 1 or 4 were present. From his observations, Teissandier (1944) concluded that cervical arches 2 and 7, thoracic arches 1 and 12, and lumbar arch 1 are the first arches to ossify. Noback and Robertson (1951) found two exceptions to the orderly cephalocaudal appearance of the thoracic, lumbar, and sacral neural arches but these did not accord fully with the sequence suggested by Teissandier (1944). In one case the arches of thoracic vertebrae 6 to 8 had not ossified whilst the rest of the centres from cervical arch 1 to thoracic arch 10 were present. In the other case, the centres for thoracic arches 9 to 12 were absent while the other centres from cervical arch 1 to lumbar arch 1 were present. From their observation they concluded that a sequence similar to that suggested by Teissandier may occur but felt that the small number of fetuses in their study could not justify any firm conclusion.

The results of this study from a total of 35 fetuses in whom not all of the neural arches proximal to lumbar arch 2 had appeared, support the conclusion of Teissandier (1944) and suggest that the sequence of neural arch ossification spreads from two basic regions, namely the cervical and lower thoracic/upper lumbar regions. Furthermore it is evident that the suggestion of Noback and Robertson (1951) that there is no regular sequence for appearance of the 9 most cephalic neural arches, can now be extended to include all the thoracic and upper lumbar regions.

These conclusions are contrary to current textbook descriptions of vertebral neural arch ossification development. Current textbooks suggest that the neural arch centres first appear in the upper cervical vertebrae followed by centres in successively lower vertebrae (Warwick and Williams, 1973; Barson, 1974). These published descriptions are based on studies of only small numbers of fetuses and the results of this study show that they are inaccurate

and require revision.

In contrast to the findings for the vertebral neural arch ossification centres, the results shown in Table 14 do substantiate the view put forward by current textbooks that the vertebral centra ossification centres first appear in the lower thoracic/upper lumbar region (Warwick and Williams 1973; Barson, 1974). No single vertebrae could be identified as the starting point for ossification. Instead, several centres were seen to form a specific group involved in the earliest stages of ossification and these were always located in the lower thoracic and upper lumbar region. In one case (No.277) only 3 centra were observed; T₁₁, T₁₂, and L₁. In five other cases 4 centra only were observed; No.13, T₁₁, T₁₂, L₁, L₂; No.11, T₁₀, T₁₁, T₁₂, L₁; No.41, T₁₀, T₁₁, T₁₂, L₁; No.66 T₁₁, T₁₂, L₁, L₂; No.467, T₁₀, T₁₁, T₁₂, L₁. Three centra therefore, were found to be common to all such fetuses and these were thoracic 11 and 12 and lumbar 1. These three vertebrae not only ossify at the same time but are the first to do so. The results also confirm that ossification then spreads in both cephalic and caudal directions, proceeding more rapidly in the cephalic than in the caudal direction, until all the centra are ossified. Within a period of a few weeks all the centra from cervical 3 to sacral 3 are present whilst there is some delay before cervical centrum 2 and sacral centrum 4 are formed, followed closely by cervical centrum 1 and sacral centrum 5. These results compare very well with previous reports and there appears to be no disagreement concerning where centra first appear and in which directions ossification spreads, although Noback and Robertson (1951) have reported 7 specimens with 5 centra present and have suggested that centra for thoracic vertebrae 10, 11, and 12 together with lumbar centrum 1 are the first to appear.

Table 15 emphasises that ossification centres for the neural arches and the centra develop independently with no definite correlation between the patterns of ossification for the two groups of centres. Furthermore Table 15

illustrates that neither centra nor neural arches can be said to develop first, with only 6 fetuses having either only neural arch ossification centres or only centra ossification centres present. This number is far too small to reach any firm conclusion, although Barson (1974) reports that the neural arches are normally the first centres to ossify.

Table 8 and Figure 33 show very clearly that the primary ossification centres for the long bones of the limbs appear over a very short period of time. In cross-sectional studies, which are the only kind available for fetal work, situations such as this present particular problems because the probability of obtaining material which can separate such short periods of time into meaningful intervals is very low. This is shown in Table 16 where out of 727 fetuses only 7 fetuses were found in whom not all of the primary ossification centres for the long bones of the limbs had yet appeared. Such small numbers make it very difficult to determine whether or not a pattern of ossification exists particularly when, as in this case, 5 of the 7 fetuses have 5 of the centres present. Close observations using a longitudinal study by, for example, ultrasound, would allow the accurate determination of whether or not a pattern of ossification exists, but unfortunately at present no such data are available. This situation also applies to any group of centres which appear over a very short period of time, such as the first group of ossification centres for the vertebral centra.

Table 16 shows that there is no regular pattern as a whole for the appearance of the primary ossification centres for the long bones of the limbs. However it would appear that the centre for the humerus is the first to appear and the centre for the fibula is the last, but there does not appear to be any regular sequence for the centres for the radius, ulna, femur, or tibia. Noback and Robertson (1951) were more precise in their description of this sequence and stated that in the upper extremity the sequence is 1) Humerus 2) Radius, and 3) Ulna whilst in the lower extremity the sequence is 1) Femur, 2) Tibia and 3) Fibula. The results of this present study are too few to either confirm or

deny this sequence except to recognise that the centre for the humerus is certainly the first to appear, whilst that for the fibula is the last with possibly the centre for the femur developing before that for the radius. However, it is also interesting to note that the results presented by Noback and Robertson (1951) whilst agreeing with the results of this present study do not substantiate the sequence they themselves present. The findings of more fetuses in whom not all of the primary ossification centres for the long bones of the limbs are yet present would help to clarify the situation.

The absence of one member of a bilateral pair of ossification centres has been reported occasionally and Noback and Robertson (1951) give a very detailed account of these reports which include all regions of the body. The paired vertebral neural arch ossification centres and the bilateral primary ossification centres for the long bones of the limbs are potential sites for such discrepancies. In the present study no unilateral primary ossification centres were present in the long bones of the limbs and no other cases have been reported in the literature, although there have been reports of absence on one side only in neonatal life of the secondary ossification centres for the head of the humerus and the distal femoral epiphysis (Menees and Holly, 1932). The short period of time over which the primary centres of ossification for the long bones of the limbs appear, might camouflage such a situation however and it is conceivable that one of the pair of centres could appear slightly in advance of the other.

Table 13 shows that the absence of one of a pair of vertebral neural arch ossification centres is not uncommon and this is supported by other reports in the literature (Noback and Robertson, 1951). However, it was found that the absence of an ossification centre was always in a region that was undergoing ossification and were in fetuses whose ages were such that the centre would normally appear. Although there is no direct evidence available, this indirect

evidence suggests that the absent centre would have appeared if the fetus had survived longer.

In addition to situations where a vertebral neural arch ossification centre was actually absent, occasionally two paired centres appeared to be at different stages of ossification. This discrepancy was noticeable by differences in both size and radio-opacity but was again confined to centres that had apparently only recently undergone ossification. At present an accurate assessment of this difference is impossible because there are no standards available for stages in skeletal maturity for either the vertebral neural arch ossification centres or the primary ossification centres of the long bones of the limbs, although Birkbeck (1976) has recently published standards for the humerus and stated that stages for the other long bones have been completed. The determination of stages of skeletal ossification for the vertebral column is being undertaken at present (Jones, Ward, Bagnall, and Harris, 1976). It is anticipated that when these stages are completed they will be sufficiently sensitive to assess the degree of asymmetrical skeletal development, if such a situation exists. Certainly in the vertebral neural arch ossification centres there appears to be an occasional discrepancy early on in development although this might be lost later on.

Table 15 shows that the regular, longitudinal, sequential development of the vertebral neural arch and centra ossification centres is occasionally broken with a rogue centre developing apparently out of order (No.34, No.22). These centres were again found to be confined to regions which were in the initial stages of ossification and were in fetuses whose ages suggested that the absent centres might well have developed had the fetus survived longer.

The overall impression given by Table 15 is that the individual vertebrae are at different stages in terms of skeletal maturity and even within the individual vertebrae the state of maturity of the centra could well be out of

phase with that of the corresponding neural arches. Without the availability of standards representative of skeletal maturity for the vertebral column, assessment of the situation is not possible. It might be that individual vertebrae mature at different stages of development at different times. Because of this any short term factor, such as an illness, which affects ossification in particular, could affect different regions of the vertebral column in different ways and have consequent drastic results.

Although descriptions of the sequence of appearance of ossification centres in the vertebral column are well documented there has been little, if any, consideration of possible explanation for the pattern of ossification or its variations. Pyle and Sontag (1943) described the variations in the time of appearance of ossification centres in the epiphyses and short bones of the extremities and Caffey (1973) described similar variations in patients with certain metabolic disorders. Pryor (1939) suggested that this variability is due to genetic factors whilst Noback, Barnett, and Kupperman (1949) felt that endocrine secretions played a large part. Todd (1939) and Francis (1939) found evidence to suggest that the state of health of a person affected the appearance of the ossification centres and Francis (1940) believed that nutritional status might also make a significant contribution.

Pyle and Sontag (1943) found that ossification centres which appear postnatally do so earlier in the female than in the male and this forms the basis for the separate sets of standards for males and females in both the Greulich and Pyle Skeletal Maturity System (1959) and the TW2 method of Tanner, Whitehouse, Marshall, Healy, and Goldstein (1975). In the present study most of the ossification centres that were being studied had appeared by the time it was possible to determine the sex of the fetus by observations made on the external

genitalia (Hamilton and Mossman, 1972). Therefore, although attempts were made to assess whether the ossification centres appeared earlier in the female than in the male, the lack of available centres upon which to base any firm decision made the results inconclusive.

Many of these factors, and more, will undoubtedly affect fetal ossification to varying degrees. The situation is complicated by the presence of the placental barrier which directly affects the fetal environment and which might make observations which were made postnatally, invalid to the antenatal situation. Nevertheless there are many factors which will affect fetal ossification and they form the subject of much research in a great many laboratories. However, there is evidence in this study which suggests that the mechanical action of muscles may have an important influence on the progress of ossification.

Such an association is strongly supported in this study by the observation that the states of ossification of the superior nuchal line and the squamous occiput are relatively advanced and well synchronised with that of the neural arches in the cervical and upper thoracic regions together with associated musculature even at relatively early stages. (Figures 34 and 35). The state of ossification in the arches appears to be particularly advanced when compared with that in the centra. This pattern of ossification in these particular regions could provide firm anchorage for the muscles controlling extension of the head and also movements of the pectoral girdle and upper limbs. From his studies of freshly aborted fetuses, Windle (1971) concluded that these movements are amongst the first that the fetus is capable of performing in a co-ordinated manner and supports his findings by relating them to the most fundamental reflex, the "gasp" reflex, into which these movements will later become incorporated. Gesell (1945) gives an illustrated account of these movements in a 6 week old freshly aborted fetus. The results from the



Figure 34. A lateral radiograph of a fetus of conceptual age 0.190 yr. (10 weeks)



Figure 35. A lateral radiograph of a fetus of conceptual age 0.250 yr. (13 weeks)

investigation of fetal movements in this study are unable to confirm or deny whether the movements so clearly described by Gesell (1945) and Windle (1971) are so well co-ordinated in utero, but they do confirm that the fetal head and upper limbs do move independently from as early as 6 weeks conceptual age. Attention is drawn, therefore, to the close similarity in terms of development between the areas of attachment for the muscles which cause such movements.

Regarding movements of the fetal head it is also of interest to note that the occipital condyles appear to develop in harmony with the squamous occiput and cervical neural arches (See Figures 34 and 35). Any flexion and extension movements of the fetal head will hinge upon the occipital condyles which must be sufficiently rigid for the proposed head movement to take place. Along similar lines it is also of interest to note in Figures 34 and 35, that the neural arches for the second cervical vertebra (the axis) are particularly well developed. Any movements that involve lateral rotation of the head will use this vertebra as a pivot and a prerequisite for such movements must surely be a firm base upon which to work.

A similar case can be advanced concerning ossification in the more distal vertebrae of the lower thoracic and upper lumbar regions. These vertebrae give attachment to the developing flexors of the thigh and as Gesell (1945) and this study have shown, flexion of the hip is one of the first movements performed by the fetus. It is interesting to relate this muscular attachment to the appearance of both the vertebral neural arch and centra ossification centres in this region.

The results of the investigation of fetal movements in utero, showed that the fetal limbs move independently from as early as 6 weeks conceptual age. In this association between fetal movements and ossification sequence, it is therefore interesting to note that the primary ossification centres for the long bones of the limbs are amongst the first centres to appear and these bones will provide attachment for the muscles which produce these movements. Very little

distinction was made between the appearance times for these ossification centres and similarly no distinction has been found in terms of related movements.

Many facts point towards an association between fetal movements and fetal ossification but the subject requires a closer examination than this study is able to afford. At present the association is based primarily on specific movements observed on aborted fetuses although in a general sense they have been confirmed by ultrasound as being performed in utero. Nevertheless only when a more detailed analysis has been carried out of specific fetal movements in utero will the true relationship between fetal movements and fetal ossification be revealed. Furthermore this study has restricted its observations to the regions of the vertebral column and the limbs and there are other facts in other areas of the body which add weight to the association and which would need to be included in such an investigation. For example in Figure 34 the angle of the mandible is virtually absent and yet in Figure 35 it is a prominent feature of the lower jaw. It would be interesting to relate this development to the movements in the oral region described by Humphrey (1970). It would also be interesting to relate the ossification of the ribs which would appear to commence virtually en masse apart from the 12th (O'Rahilly and Meyer, 1956), to the fetal breathing movements described by Boddy and Mantell (1973), Boddy and Dawes (1975) and others. With all these movements there appears to be a close relationship between their onset and the commencement of ossification of the bones that would provide anchorage for the muscles that produce such movements.

The association requires further investigation because it does not appear to be a simple cause and effect relationship with several factors confusing the issue. The major factor which disturbs the arrangement concerns the time lag between the onset of the fetal movements and the associated ossification. In this study the ultrasound experiments showed that the fetus moves its arms, legs, trunk, and head in utero at 0.115 yr. (6 weeks conceptual age) and yet the ossification of the fetal limbs and vertebral column only

commences at 0.179 yr. (9 weeks conceptual age.) If the association is valid, the forces produced by the muscles causing the movements must act primarily on the cartilaginous skeletal model with the ossification being a secondary effect. Any investigation therefore must initially concentrate on the effects of mechanical force on the cartilaginous skeleton.

Another factor which confuses the issue is that the attachment of the muscle that produces the movement is sometimes removed from what would be the resultant ossification centre and this is particularly so concerning the vertebral centra ossification centres which appear at the centre of their cartilaginous mass whilst the attached muscles remain on the surface. However, the precise attachments of fetal muscles and the determination of their functional capacity have yet to be made and it could well be that there is a very different arrangement in fetal life both of muscle attachments and sources of fetal movement as compared with the adult. In addition it might be necessary to make a distinction between endochondral, periosteal, and intramembranous ossification in terms of their association to fetal movements, with periosteal ossification being the most likely candidate to be affected by movements because of its close association to the relevant muscles.

Phylogenetically, it might be expected that the axial musculature would be amongst the first to become functional. If there was a simple relationship between fetal movements and ossification it might therefore be expected that ossification of the vertebral column would be amongst the first to commence but it actually commences later than that for the limbs. Studies of specific fetal movements will clarify whether or not axial movements occur before limb movements but nevertheless, it is an intriguing question and confuses the picture that can be built up to relate fetal movements to fetal ossification.

The association between fetal movements and fetal ossification necessarily involves Wolff's Law of Transformation (1892). This law which deals with the

response of bone to mechanical forces, has been studied from many aspects and it has become generally accepted that the pull of muscles has a significant influence on the morphology of developing bones. Bone responds to extrinsic forces which serve to augment intrinsic or genetic factors acting within the bone itself or the periosteum (Hoyte and Enlow, 1966). Regarding the fetus, Fell (1956) believes that whilst the gross lines of development are determined by evolutionary and genetic factors, there is a degree of flexibility peculiar to each individual by which adjustments are made to unpredictable and fluctuating environments including mechanical stresses.

Although the mechanism by which mechanical stress influences ossification is not completely understood, it is known that in bone mechanical energy is converted into an electrical signal (Piezo-electrical force) and alterations in the electrical environment of mesenchymal cells do have an important influence on their mitotic and functional activity. Bassett (1971) suggests that not only bone but a whole spectrum of connective tissues including tendon, ligaments, fascia, cartilage, and arteries reflect the effects of dynamic physical forces. Thus mechanical stimuli in the form of muscular contractions may control osteoblast and osteoclast activity and in this way might directly influence the sequence of ossification in the vertebral column and limbs in particular.

The origin of mechanical stresses in the fetus which will affect ossification are not restricted to those produced by muscle contractions. The cardiovascular system is an alternative source with bone being deformed continually by both the ballistic shock of cardiac recoil transmitted through the skeleton and the pulsatile deforming force associated with the systolic surge of blood. Gravity too will directly distort the skeleton although its effect will be diminished in fetal life because of the aquatic environment. Firm contact with the environment would also introduce mechanical stresses but again its effect could be limited in fetal life because of the cushioning action of the uterine walls. Finally, the growing fetus could generate mechanical stresses within

itself, particularly within the cartilaginous skeleton, in the form of compressional forces produced by the multiplication and enlargement of developing cells. All of these forces are in addition to those produced by the contractions of muscles and would help to explain those situations in which ossification proceeds in the apparent absence of muscular force. Active fetal movements however, appear to be important for the proper development of fetal bones because in cases of amyotrophia congenita in which the fetus has only rudimentary muscles there is only a very slender bone structure similar to that found in victims of paralytic poliomyelitis (Liley, 1972).

Therefore, there appears to be an association between fetal movements and fetal ossification although the association is not a simple one and is affected by several other factors. It is suggested that movements of the fetus influence the appearance and subsequent development of the primary ossification centres and with its variability of affective factors, such an explanation would not only account for relatively random patterns, but also groupings of centres of ossification developing in harmony.

For further work, there are obvious comparisons to be made with other animals. It is interesting to note that Strong (1925) found a similar sequence for appearance of vertebral ossification centres in the albino rat and Johnson (1933) found a similar sequence for the albino mouse. On the other hand, Hodges (1953) in a study of ossification in the fetal pig found that ossification of the vertebral bodies proceeds from at least two and possibly three loci with the first locus being at T_{12} , the second locus being in the upper thoracic region, and the third locus, which lagged behind the others, being found at C_2 . He found also that the vertebral arches began in two loci with the first being at T_6 or T_7 and the second commencing at C_1 or C_2 . It would be interesting to relate these differing patterns for appearance of the vertebral ossification centres to the corresponding patterns of fetal movement.

SUMMARY

1. Assuming that ossification centres for the neural arches and centra of vertebrae appear in a strict numerical sequence and not as a simultaneously arising group then;
 - 1a The sequence for appearance of the vertebral neural arch ossification centres was found to be different to that described in current anatomical textbooks. A group of centres first appears in the lower cervical/upper thoracic region and is rapidly followed by a second group in the upper cervical region. The remaining cervical neural arches then quickly ossify and the sequence spreads towards the mid-thoracic region. A third group of centres appears in the lower thoracic/upper lumbar region and ossification spreads upwards to meet centres from the thoracic region which are themselves extending caudally. The lower lumbar and sacral regions develop rather more slowly and in an orderly sequence, the 5th sacral arch being the last centre to appear.
 - 1b The vertebral centra ossification centres first appear in the lower thoracic/upper lumbar region and then spread in both cephalic and caudal directions.
2. The appearance of the primary ossification centres for the long bones of the limbs commences with the humerus and finishes with the fibula but no pattern could be determined for the remaining centres because they appear over such a short period of time.
3. The vertebrae develop independently of each other. In terms of ossification each vertebra is at a different stage of development and there is discrepancy between the centrum and neural arches even within a single vertebra. The introduction of any factor which affects growth and development will therefore affect different vertebrae in different ways and even parts of the same vertebra in different ways. It is suggested that this could have relevance in illnesses such as scoliosis.
4. Fetal movements are suggested as being one of the major factors affecting the direction and progress of fetal ossification through the action of piezo-electrical forces.

Part of this work on the sequence for appearance of the
ossification centres has already been accepted for publication:

"Bagnall, K.M., Harris, P.F., and Jones, P.R.M., 1976.

A radiographic study of the human fetal spine:

2. The sequence of development of ossification centres in
the vertebral column."

J. Anat. - In Press.

THE SPINAL CURVATURES

The lateral radiographs taken of each of the 195 Nottingham fetuses were inspected carefully to determine the presence or absence of each of the four spinal curvatures. The assessment was made by drawing a straight line between the centres of the most cephalic and most caudal vertebral centra ossification centres for the region and observing the displacement of the intervening centres about this line. Where there were insufficient vertebral centra ossification centres present to make a decision, the corresponding vertebral neural arch ossification centres were used. The results can be seen in Table 17.

The Cervical Curvature.

The results in Table 17 show that no decision as to the absence or presence of the cervical curvature could be made on 39 of the total 195 fetuses. Of the remaining 156 fetuses a well-defined secondary cervical curvature was present in 130 fetuses (83%), in 18 fetuses (11%) the cervical spine was straight and in 8 fetuses (6%) the cervical spine appeared to be merely a continuation of the primary curvature present in the thoracic region.

Since the position of the head might affect the degree of cervical curvature, lateral radiographs were taken of a single fetus with the head placed in various degrees of extension or flexion (Figure 36) and a note was made of the degree of flexion of the head in each of the radiographs. Of the 18 fetuses who had a straight cervical spine, the majority were classified as having a marked degree of flexion of the head and all of the fetuses in whom the cervical spine appeared to be merely a continuation of the primary curvature also possessed a marked degree of flexion of the head. Combining this with the illustrations in Figure 36 it appears that, as after birth, the degree of flexion of the head directly affects the degree of curvature in the cervical spine. If the fetal head is upright the curvature is more pronounced than when the head is flexed onto the chest, but even in this flexed position the curvature is readily apparent

Table 17

Number of Fetuses Showing the Spinal Curves

Spinal Segment	Straight	Concave Anteriorly	Convex Anteriorly	No Centres To Make Decision
Cervical	18	8	130	39
Thoracic	4	154	0	37
Lumbar	1	150	2	42
Sacral	0	113	0	82

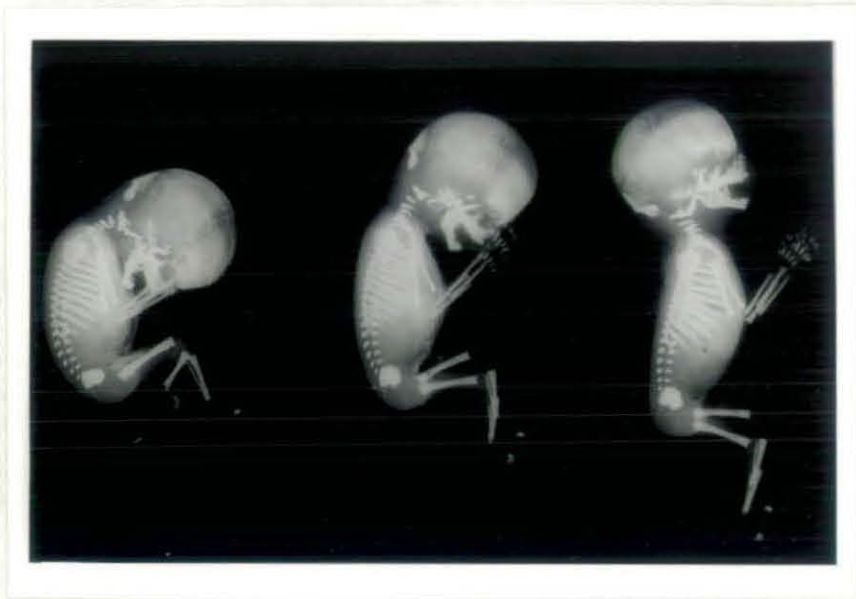


Figure 36. Lateral radiographs of a single fetus with the head placed in various degrees of extension or flexion. Note the cervical curvature.

Figure 37 illustrates the development of the cervical curvature and, in particular, the relative effects of the position of the head. It also shows that the curvature can be clearly recognised as soon as ossification centres for the cervical spine become radio-opaque and that a well-defined "secondary" curvature can be demonstrated from a very early conceptual age, namely 0.184 yr. (9½ weeks). It must be emphasised however, that, as after birth, the degree of head flexion must be taken into account when interpreting the curvature in the human fetal spine. These findings are in complete disagreement with the statements of standard anatomical textbooks.

In almost all textbook descriptions of the developing spine in the human fetus considerable emphasis is made of the essentially flexed position of the fetus, with the developing spine being described as having only one primary curvature. After birth when the young child begins to raise its head and to sit up a secondary curvature is said to develop in the cervical region. A typical description for the development of the cervical curvature is:

"The cervical curve appears late in intra-uterine life and is accentuated when the child is able to hold up its head (at three or four months), and to sit upright (about nine months)"

(Gray's Anatomy - Warwick and Williams, 1973).

In fact this is the only description found in the literature which even acknowledges that the secondary cervical curvature may develop before birth. In most accounts, the secondary curve is said to appear only as a postnatal event.

That the secondary cervical curvature may become particularly evident when the baby begins to hold up its head after birth is not disputed. This study indicates, however, that the curvature is present at a much earlier stage in development than is normally accepted and it is possible that it might develop even earlier than the 0.184 yr (9½ weeks) found. It may, in fact, be present soon after the embryo first acquires a neck and begins to uncurl.

Figure 37. The development of the cervical curvature during fetal life. For each stage two fetuses have been selected, one with the head straight and one with the head flexed, to indicate that the cervical curvature is decreased by this action but is not lost.







From Figure 37, it can be seen that ossification in the occipital bone is well developed at an early age and appears to develop in harmony with the vertebral neural arch ossification centres in the lower cervical/upper thoracic regions. These two regions would provide anchorage for the muscles involved with head movements and it is suggested that the early development of the "secondary" cervical curvature may be related to these movements.

The results of the investigations using ultrasound of fetal movements in utero have established that the limbs, head and trunk of the fetus are all capable of movement at 0.115 yr. (6 weeks) conceptual age and they confirm the results of Windle and Fitzgerald (1937) on the reflex activities of freshly aborted fetuses. Gross movements of the fetus have been observed long before the mother herself has detected them (Campbell, 1975; Higginbottom et al., 1976) because the movements are felt by the mother only when they are sufficiently strong to stimulate the abdominal wall (Liley, 1972), and Robinson (1973) observes that they may be so intense at an early age that they interfere with attempts to measure the fetus using ultrasound.

Respiratory movements are also an important group of spontaneous fetal movements which may be directly related to the development of the secondary curvature in the cervical spine. Many studies have been made of such movements and those concerning the human fetus have utilised ultrasound which raises the possibility of ultrasound stimulating the movements (Boddy and Dawes, 1975). However, similar rhythmical respiratory movements have been demonstrated by intratracheal and amniotic pressure recordings from longstanding indwelling catheters in fetal lambs in utero. In particular, there was no obvious correlation with fetal carotid blood gas levels and this raises interesting questions concerning the source of stimulation (Dawes, Fox, Leduc, Liggins, and Richards, 1970). Nevertheless, although the chest movements reported at 9 weeks conceptual age (Boddy and Dawes, 1975) may be accentuated by ultrasound,

they certainly do occur without such stimulation. Dawes et.al., (1970), observe that these respiratory movements correlate well with the ability of the respiratory muscles to maintain prolonged hyperpnoea after birth and Windle (1971) suggests that respiration is one of the most vital mechanisms necessary for survival at birth. It therefore seems logical that the movements necessary for breathing should develop very early and it is interesting to note that the first reflex to be observed at 6½ weeks is the "gasp" reflex, two important components of which are head extension and forelimb flexion. This is directly relevant to the findings in the present study. The early development of the secondary cervical curvature can be correlated with early ossification in the occipital bone which provides anchorage for the extensor nuchal muscles. Windle (1971) also notes that the gasp reflex in the fetus is the most resistant of all reflexes to asphyxia and whereas most of the fetal reflexes succumb quickly after placental separation, gasping or, in early fetuses, the neck movements incorporated later into gasping, disappears last of all. This suggests that movements of the fetal head are particularly well developed.

Liley (1972) has shown that the fetus in its liquid environment is capable of movements far in excess of those occurring under the damping effect of gravity. There is, therefore, the strongest possibility that a considerable amount of head movement occurs in the fetus during intrauterine life, some of this movement being related to the early development of certain respiratory reflexes. Thus although the secondary cervical curvature may become accentuated after birth when the baby begins to raise its head, it is by a similar mechanism of muscular effort that the secondary cervical curvature can be shown to develop in the very young fetus.

Figure 38 shows a sagittal section of a fetus in utero and was obtained using compound B-scan ultrasound equipment. The vertebral canal can be clearly seen and a secondary cervical curvature is readily recognizable. In this

particular case, there was an excess amount of fluid within the amniotic sac and this allowed the fetus to have relatively more room in which to move. The clearly marked secondary cervical curvature has therefore been produced by active movements of the fetus and confirms the radiographic findings of this study.

The Thoracic Curvature

Table 17 shows that in 37 of the fetuses no decision could be made as to the absence or presence of a thoracic curvature. Of the remainder, 154 (97.5%) possessed a primary curvature and only 4 fetuses (2.5%) possessed no curvature in this region. Again it was found that the curvature was affected by the degree of flexion of the fetal body and this is similar to after birth.

These findings support the descriptions of standard anatomical textbooks concerning the development of the primary thoracic curvature and illustration of its development is shown in Figure 37.

The Lumbar Curvature.

Table 17 shows that no decision could be made about the presence or absence of a lumbar curvature in 42 fetuses. Of the remainder 150 (98%) possess a primary curvature which was merely an extension of the thoracic curvature, 1 (0.7%) possessed a straight lumbar spine, and 2 fetuses (1.4%) possessed secondary curvatures which were in the opposite direction to that of the thoracic region, although these were not particularly well-defined.

These results suggest that the normal development of the secondary lumbar curvature is truly a postnatal event and occurs, as most textbooks state, when the baby starts to stand and walk. In its aquatic environment in utero the fetus does not require the mechanical rearrangements necessary for efficient

movement to take place after birth and so the curvature develops as a primary one. It is conceivable, however, that in an extraordinary situation the movements performed by the fetus in utero could cause the shift in centre of gravity to take place early and in this way a secondary lumbar curvature could develop in utero. Such reasoning might explain the two cases in which a secondary lumbar curvature was found to be present in antenatal life. (See figure 39). It must be emphasised however that the normal curvature to develop in utero in the lumbar region is a primary one as a continuation of the thoracic curvature.

The Sacral Curvature.

Table 17 shows that in 82 fetuses no decision could be made as to the presence or absence of a sacral curvature. This high figure reflects the long time it takes for the vertebral sacral ossification centres to develop and appear on radiographs. On the other hand a primary curvature was observed in all those fetuses in whom there were sufficient centres present to make a decision. This curvature therefore is present from the time when sufficient ossification centres can be seen but its degree of curvature is often only very slight being little more than a straight line. (See figure 37). Furthermore, although the curvature is a primary one, it is not merely a continuation of the thoracic primary curvature, with the development of the sacral promontory being particularly evident (See Figure 37.).



Figure 38. A photograph of an ultrasound B-scan picture taken of a fetus in utero which clearly shows a secondary cervical curvature.

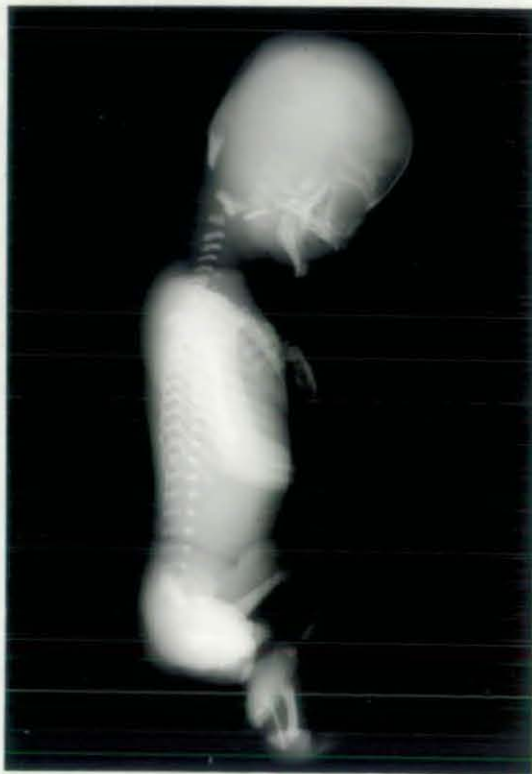


Figure 39. A lateral radiograph of a fetus which shows a secondary lumbar curvature of the vertebral column.

SUMMARY

1. The secondary cervical curvature was shown to be present at a very early age (0.184 yr.). It is present from the time sufficient ossification centres are visible for a decision to be made but its degree of curvature, as after birth, is dependent upon the degree of flexion of the head. Its development has been related to movements of the fetal head in utero. These findings are contrary to descriptions in current anatomical textbooks.
2. Thoracic, lumbar and sacral curvatures also develop in fetal life and all are present from the time when they can be recognized. They all develop in utero as primary curvatures and the secondary lumbar curvature does not normally develop until postnatal life.

Part of this work on spinal curvatures has already been accepted for publication.

"Bagnall, K.M., Harris, P.F., and Jones, P.R.M. 1976.

A radiographic study of the human fetal spine.

1. The development of the secondary cervical curvature.

J.Anat. In Press".

THE DEVELOPMENT OF THE PRIMARY OSSIFICATION CENTRES FOR THE
LONG BONES OF THE LIMBS.

The data collected from the measuring of the longitudinal lengths of the primary ossification centres of the long bones of the limbs were subjected to polynomial regression analysis using computer programmes (See Appendix B). Equations up to and including the power of 3 were attempted and as each successive term was added the consequent reduction in sum of squares was tested against the mean square remaining. In this way the powers of the selected equations for each of the 12 long bones of the limbs were determined by the significance or non-significance at the 5% level of the corresponding F-values. The results of this procedure can be seen in Table 18 and in Figure 40 the equations are represented graphically for comparative purposes.

Figure 40 shows that the general growth curves for each of the 12 ossified shafts of the long bones of the limbs are similar, being represented by a gradually diminishing slope. This similarity in shape of growth curve is borne out by Table 18 where all but three of the lines of best fit are equations to the power two with negative second coefficients. The remaining three, right tibia, right fibula, and left fibula, are all best fitted by an equation to the power three although Figure 40 shows that the extra curve which is produced by the addition of the third term is only very slight, and even though it is mathematically significant to include this term, from a practical point of view it might well be permissible to exclude it.

Simply relating the length of ossified shaft to the fetal age and subjecting the data to polynomial regression analysis produces the graphs shown in Figure 40. The adoption of this particular mathematical model often leads to an increased variability towards the ends of the range of data due to a lack of sufficient points but in this case there were a great many data points through

Table 18

The Development of the Ossified Shafts of the Long Bones of the Limbs in Relation to Gestational Age

Long Bone	No of Data Points	Selected Equation	S.E.E.	R
Left Humerus	687	$Y = -39.293674 + 271.628471X - 201.499577X^2$	1.72	0.99
Left Radius	688	$Y = -32.857206 + 226.730783X - 171.265656X^2$	1.54	0.98
Left Ulna	693	$Y = -35.129187 + 239.736495X - 168.771473X^2$	1.73	0.98
Left Femur	668	$Y = -36.831866 + 244.100112X - 140.135608X^2$	1.96	0.98
Left Tibia	597	$Y = 32.512747 + 210.386409X - 114.557493X^2$	2.2	0.97
Left Fibula	577	$Y = -20.674979 + 80.669707X + 315.80881X^2 - 464.381980X^3$	1.84	0.98
Right Humerus	643	$Y = -39.857409 + 274.814X - 207.464076X^2$	1.82	0.98
Right Radius	655	$Y = -32.884210 + 227.234231X - 172.879002X^2$	1.63	0.98
Right Ulna	656	$Y = -35.642756 + 241.634351X - 169.267815X^2$	1.85	0.98
Right Femur	633	$Y = -38.934088 + 260.127058X - 168.823417X^2$	2.74	0.97
Right Tibia	518	$Y = -21.465753 + 92.490556X + 279.987466X^2 - 423.035967X^3$	2.04	0.98
Right Fibula	501	$Y = -21.862974 + 92.969900X + 273.354522X^2 - 422.251601X^3$	1.92	0.98

COMPARISON OF GROWTH OF LIMB LENGTHS

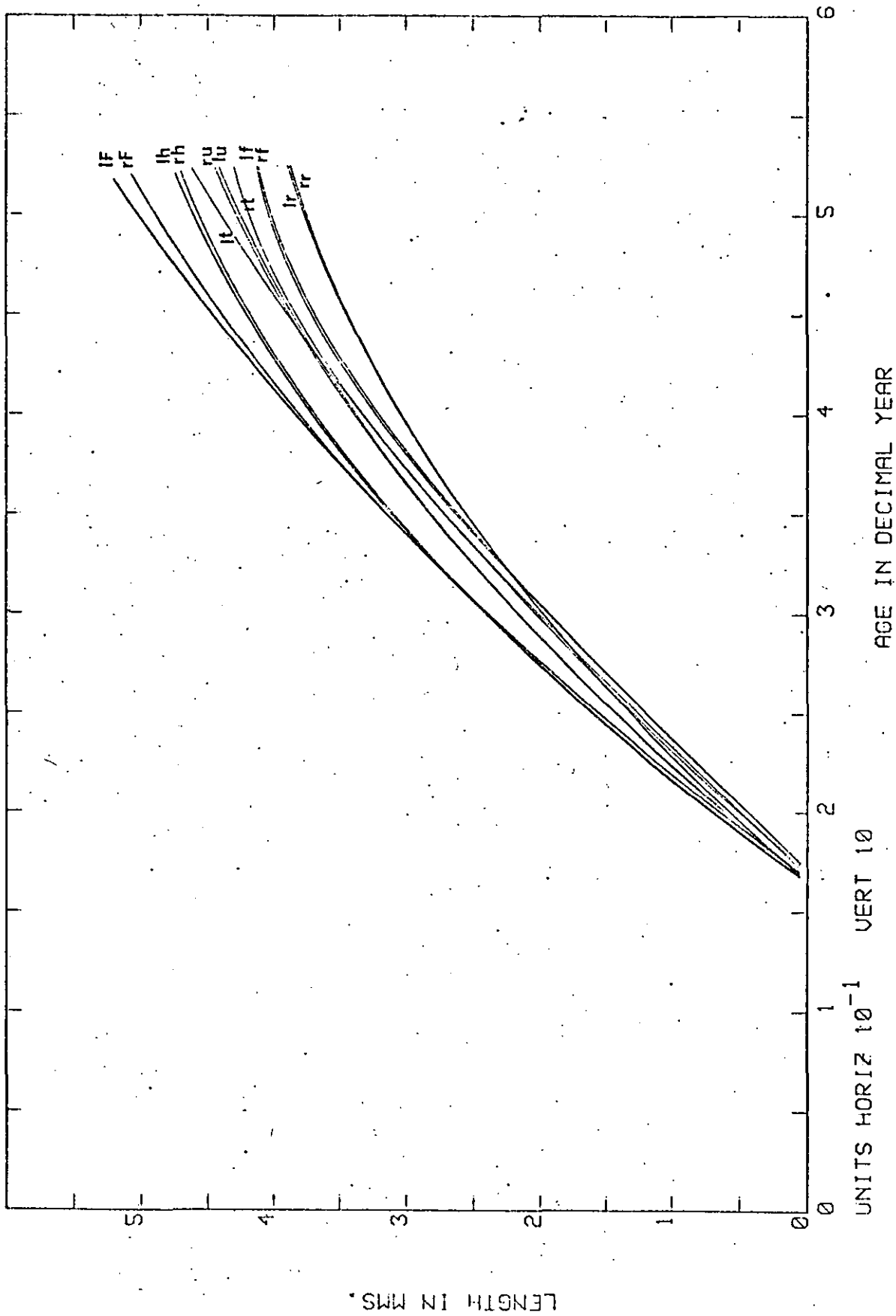


Figure 40. Comparison of growth of the ossified shafts of the long bones of the limbs.

l = left h = humerus F = Femur u = ulna
 r = right r = radius t = tibia f = fibula

the range and in particular towards the beginning of ossification. In this respect it is interesting to note that Figure 40 confirms the results discussed earlier concerning the appearance times of the ossification centres, namely that they all appear over a very short period of time at approximately 0.170 yr. (9 weeks). Figure 40 also suggests that the centre of ossification for the shaft of the humerus is the first to appear whilst that for the fibula is last. The ossification centres for the femur, ulna, and radius appear simultaneously, quickly followed by the centre for the tibia. The progress of growth of each of the centres can also be followed and it can be seen that the ossified shafts of the humerus and femur remain approximately the same until 0.310 yr. (16 weeks) when the femur overtakes the humerus until at 0.5 yr. (26 weeks) it is considerably larger. The fibula is the last of the ossification centres for the long bones of the limbs to appear and remains the shortest in terms of absolute length until 0.310 yr. (16 weeks) when it overtakes the radius, which then remains the shortest of the ossified shafts. Meanwhile the ulna and tibia have followed similar growth patterns with the ulna always in advance of the tibia until 0.420 yr. (22 weeks), when it would seem that they become the same length. Although these absolute lengths have applications in their own right, they are not the complete story because hidden within these data are factors which would affect the length of ossified shaft. For future studies it would be interesting to relate this length of ossified shaft to both the final adult length and also the corresponding length of fetal cartilaginous bone. Each of the long bones of the limbs will eventually have different lengths in adult life and it would be interesting to relate development of the ossification to this adult length and perhaps be able to express the development as a percentage of its maturity. Similarly each of the cartilaginous fetal long bones, of which the portion of ossified shaft forms a part, will have different total lengths and it would be of interest to determine whether or not the cartilaginous model and the area of ossification develop in harmony or whether they are separate entities with

different growth control factors.

When the absolute lengths of ossified shaft are related to fetal age and subjected to polynomial regression analysis the graphs of Figure 40 are produced . These graphs suggest that the growth of corresponding left and right limbs is similar in all long bones of the limbs. Early in fetal life, before 0.400 yr. (21 weeks), it is certainly very difficult to distinguish the graph representing the growth of the left ossified shaft from its counterpart on the right side, although after 0.400 yr. (21 weeks) all of the corresponding graphs tend to diverge. Apart from the ulna shaft, it is interesting to note that this difference is in favour of the left limb being greater in length than the right limb. Although none of these differences would appear to be significant, they are perhaps influenced by a shortage of data at the upper end of the range. It would therefore be interesting to continue this procedure to include fetuses up to term and determine whether or not this trend is maintained.

Although the graphs in Figure 40 are informative they nevertheless conceal individual differences between the lengths of left and right ossified shafts within the same fetus. To study this, 421 fetuses in whom the lengths of all 12 ossified shafts had been measured, were selected and subjected to statistical tests. The differences between the lengths of ossified shafts of corresponding left and right long bones were subjected to a paired t-test. The proportions of fetuses in whom the left ossified shaft was greater than the right ossified shaft, the left ossified shaft was smaller than the right ossified shaft, and the left ossified shaft was equal to the right ossified shaft, were calculated. (See Table 19). It was found that in the humerus, tibia, and fibula approximately 60% of the fetuses had the length of the left ossified shaft greater than the right and that 30% of the fetuses had the length of the left ossified shaft smaller than that of the right. This difference was related to a significance at the 5% probability level of significance and means that a

Table 19

The Results of a series of t-Tests on Data Produced by Subtracting the Length of the Ossified Shaft of a left Long Bone of a Limb from its Corresponding Bone on the Right Side in the Same Fetus

A Comparison of the Proportions of Differences in Lengths of Left and Right Ossified Shafts of the Long Bones of the Limbs

Long Bone	t-Value d.f. = 420	Significance at P < 0.05	This Means	L > R	L = R	L < R
Humerus	6.313 $\bar{X} = 0.215$	s	L > R	270 64.1 %	33 7.8 %	118 28.0 %
Radius	1.322 $\bar{X} = 0.039$	n.s.	L = R	188 44.7 %	54 12.8 %	179 42.5 %
Ulna	1.672 $\bar{X} = 0.059$	n.s.	L = R	186 44.2 %	41 9.7 %	194 46.1 %
Femur	-2.462 $\bar{X} = -0.062$	s	R > L	172 40.9 %	53 12.6 %	196 46.6 %
Tibia	6.573 $\bar{X} = 0.265$	s	L > R	266 63.2 %	36 8.6 %	119 28.3 %
Fibula	4.393 $\bar{X} = 0.133$	s	L > R	230 54.6 %	40 9.5 %	151 35.9 %

s = significant n.s. = not significant

Table 20 To Compare the Difference Between Left and Right Ossified Shafts of the Long Bones of the Limbs

Number of Paired Long Bones

Direction of Difference	0	1	2	3	4	5	6
L > R	8	32	93	123	111	43	11
L < R	30	89	132	97	55	15	3
L = R	255	130	34	2	0	0	0

difference exists in growth of these ossified shafts dependent upon whether they are on the right or left side of the body, with growth apparently being favoured on the left side. With the radius and ulna, equal proportions of fetuses had the length of the left ossified shaft greater than the right and the length of the left ossified shaft less than the right and this was amplified by a non-significant difference which suggests that these bones have equal lengths on both left and right sides of the body during fetal life. Regarding the femur, it was found that 41% of the fetuses had the length of the left ossified shaft greater than that on the right whilst 47% had the length of the left ossified shaft less than that on the right. This reversal in proportions of differences was illustrated further by the production of a negative level of significance which again suggests that there is a difference in growth within the body, related to side and which in this case is favoured on the right side.

These results are difficult to interpret because an explanation for the findings is not obvious, particularly concerning the reversal in difference between the humerus and femur. On a general basis, perhaps the method used for measuring the lengths of the ossified shafts of the long bones of the limbs can be criticised because the measurements for the femurs, tibiae, and fibulae are taken from different views on the A.P. radiograph. (See Figure 7). The definition of the measurement however was designed to be a maximum measurement and allowed for any longitudinal rotation of the limb about its axis. In addition, great care was taken to straighten as much as possible the right lower limb of each fetus and those radiographs were excluded in which it was obvious that this had not been achieved. It was therefore felt that equivalent measurements were in fact being taken on both left and right lower limbs and that the results for the lower limbs shown in Table 19 are indeed valid. This criticism of the method however does not apply to the upper limb where similar

views are seen of corresponding bones and yet a significant difference was still found between the lengths of ossified shafts of right and left humeri.

The results shown in Table 19 came as a surprise and as yet have not been fully investigated. They therefore require further investigation and one factor which might have affected these results and which must be borne in mind is the sequence of measuring the ossified lengths. In this study the sequence was left upper limb, right upper limb, left lower limb, right lower limb with the individual order being humerus, ulna, radius, femur, tibia, fibula. It is conceivable that such a sequence might affect the results of such a study although in this particular case it would be difficult to reconcile this with the reversal in difference between right and left humeri and femurs.

One investigation of this situation that was undertaken, required the counting of the number of different lengths of corresponding ossified shafts within each individual fetus and noting of the direction in which the difference pointed. The results of this are shown in Table 20. It was hoped that such an investigation would clarify the situation but as can be seen from Table 20 there is a wide spread of the results with most fetuses having differences between two, three, or four corresponding ossified shaft lengths. Where these differences are centred and whether there is a relationship between differences in the upper limbs compared with the lower limbs has yet to be established.

Asymmetry in the total length of the limbs has also been found in postnatal life. Burwell and Dangerfield (1975) studied 510 patients with scoliosis and found that many had asymmetry of length of the upper limbs. They related this to the side of convexity of the primary spinal curve in that the upper limb was longer on the side of convexity (shorter on concavity) of the lateral spinal curvature. Hamilton and Simon (1958) also report that in the normal adult one limb may be shorter by $\frac{1}{2}$ " than its fellow and also relate this

disproportion to the lateral curvature of the vertebral column. Furthermore, they associate the lateral curvature of the vertebral column with "handedness" and note that in the right-handed person, the thoracic part of the vertebral column may show a slight convexity to the right with the converse asymmetry usual in the left-handed person. On the basis that more people are right-handed than left-handed, even though it is a difficult concept to define, it would be expected that there would be a predominance of larger right limbs than left limbs. Although such a situation was found in this study only in the femur, nevertheless an inherent "handedness" might well be affecting fetal growth in a complicated way.

Burwell and Dangerfield (1975) compared their findings from the scoliotic patients to a study of normal boys and girls. They took various upper limb measurements and analysed the data according to age, sex, growth of proximal and distal limb segments, and the difference between right and left limbs. Their findings suggested the possibility that the velocity of growth in the upper limbs may not be symmetrical during growth in all normal individuals and such a conclusion is confirmed by the results of this present study.

After birth there is abundant evidence that the female is more advanced than the male in terms of ossification (Menees and Holly, 1932; Dunham, Jenss and Christie, 1939; Christie, Dunham, Jenss, and Dipple, 1941). Pryor (1906, 1923, 1933) was able to demonstrate that this difference could be extended to late fetal life and concluded that the difference progresses from days to weeks to months with such advances taking place even before the external sex-differentiation can be determined. Hill (1939) confirmed these thoughts and stated that it is in the seventh fetal month that differences between male and female fetuses can be observed. If the female is in advance of the male fetus in terms of ossification throughout fetal life, it was thought that this difference would manifest itself in the lengths of the ossified shafts of the long bones

of the limbs. To investigate this the lengths of the ossified shafts of the long bones of the left limbs in 269 male fetuses and 242 female fetuses were subjected to polynomial regression analysis and the results are shown in Figure 41. Not surprisingly all of the graphs are similar to their equivalent combined graphs shown in Figure 40, and it is interesting to note that in early fetal life at least, the corresponding male and female graphs lie very close to each other and only diverge later in fetal life at 0.400 yr. (21 weeks). It is significant that when the graphs do diverge it is the female that is the greater for any particular fetal age, and although none of the differences appears to be significant, it nevertheless suggests that the female fetus is indeed in advance of the male in terms of skeletal maturation [after 0.400 yr. (21 weeks)].

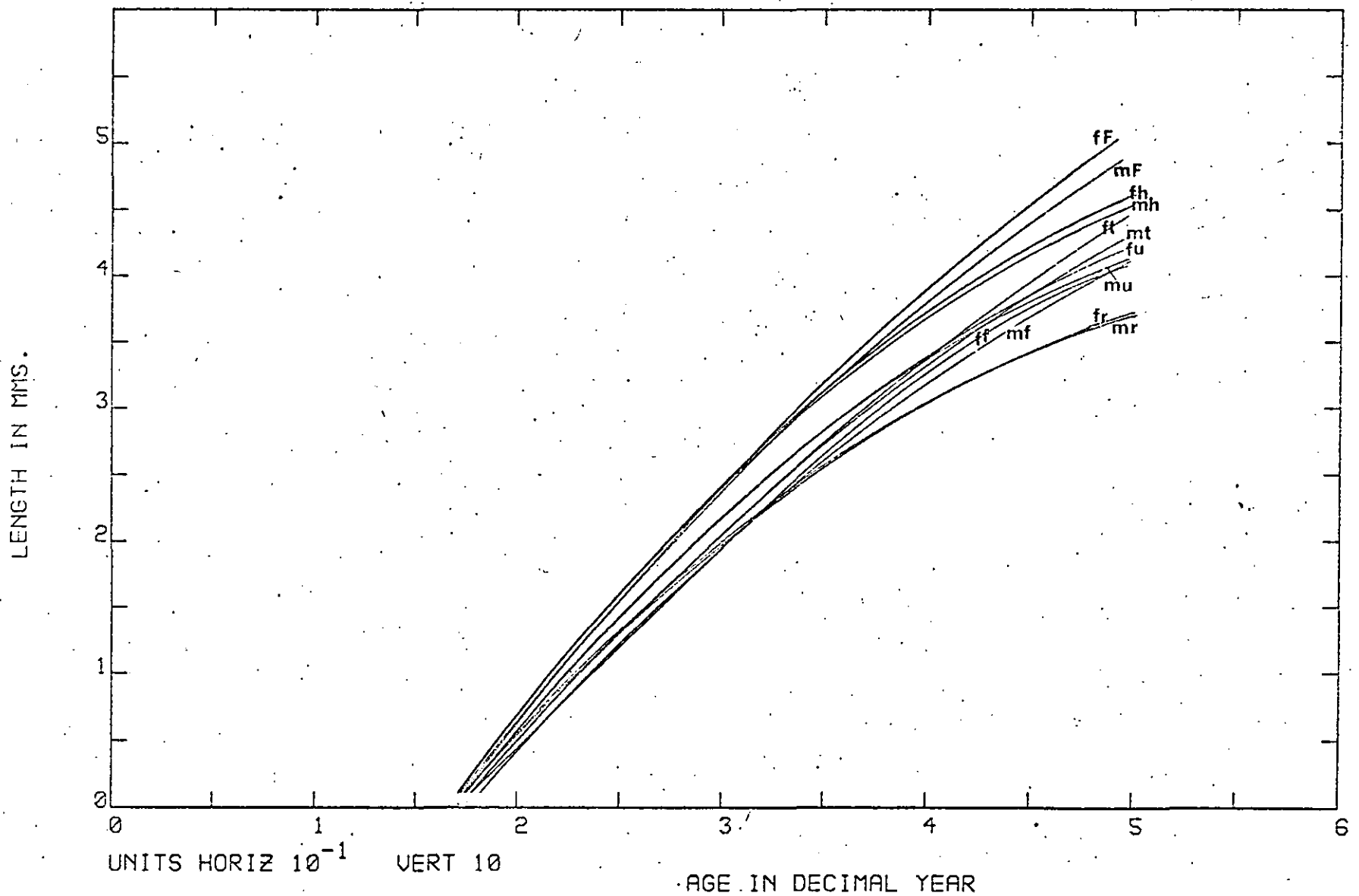
It is also significant to note that the pairs of graphs in Figure 41 diverge to different degrees which demonstrates either that each of the long bones of the limbs is at a different stage of skeletal maturity at any specific fetal age or that the effect of sex upon ossification is not equal throughout the body. If the former is the true case, which seems most likely, then any factor such as illness which affects fetal ossification might well affect each limb bone differently and depending upon the powers of recovery from such an illness, the results might well have different permanent degrees of effect.

In Table 18 the high multiple coefficient of correlation (R) coupled with the low S.E.E. for the lengths of the ossified shafts of each of the long bones, means that an accurate assessment of fetal development can be made using these parameters. Given the fetal conceptual age, based on L.M.P. dates, an accurate estimate can be made of the lengths to be expected for each of the ossified shafts of the long bones of the limbs and using ± 2 S.E.E. as first approximations of 95% confidence limits, any abnormality ought to be easily spotted. Such procedure has application in the study of fetal abnormalities such as anencephaly, spina bifida, and scoliosis, in order that a

COMPARISON OF MALE AND FEMALE LIMB LENGTHS

Figure 41. Comparison of the lengths of ossified shaft in the long bones of the left limbs in male and female fetuses.

m = male
f = female



better understanding of the effects of such abnormalities can be made. For future work the encouragement of the high R value found for each of the long bones might lead to the production of the converse graphs from which an estimate of fetal age based upon measurement of the ossified shafts of the long bones of limbs can be made, although Russell et.al., (1972) found a poor correlation between fetal age and ossified femoral length measured from radiographs taken of fetuses in utero. Even though radiography of pregnant women is rarely undertaken, such graphs would add to the battery of tests available for assessing fetal maturity and as such would have considerable clinical value.

Apparently very little previous work has concentrated on the length of ossified shaft of the long bones of the limbs in early fetal life and no references were found in which such measurements had been taken from radiographs, except for Hodges (1937), Gardner and Gray (1969, 1970) and Owen (1971).

Moss et.al. (1955) studied all the long bones of the limbs from 119 fetuses which had been cleared and stained with alizarin and grouped their fetuses into 10 mm intervals of C.R. length. They used as their measurement for each group the mean overall lengths of the ossified shafts of the long bones of both extremities and estimated the menstrual age of the median C.R. length for each interval from Boyd (1941), although they do not define how the C.R. length was actually measured.

Gray and Gardner (1969) studied the fetal humerus and in 1970 reported similar measurements taken from the fetal femur. In both studies 40 pairs of bones were examined which had been removed from fetuses ranging from 26 - 342 mm. C.R. length. No definition is given of the way in which C.R. length was measured and all the bones were radiographed, silverized and radiographed again. The bones were then decalcified and serially sectioned in frontal, sagittal, or transverse planes and stained with Masson's Trichrome. Such a procedure allowed them to establish that below 69 mm C.R. length no endochondral ossification is

present and therefore longitudinal sections were used to measure the length of the endochondral ossification in the centre of the limb shaft. Above 69 mm C.R. length the radiographs were used to measure the lengths of the bones and the ossified parts of the shafts. Their measurements therefore relate to the lengths of endochondral ossification and not periosteal, although the two are very similar in appearance and the actual difference between the lengths will depend very much on the morphological state (Birkbeck, 1976).

Mehta and Singh (1972) measured the lengths of the ossified diaphyses of both the humerus and femur in 50 fetuses ranging from 65 - 290 mm C.R. length. They measured the C.R. length using an osteometric board and placed the limbs in 5% KOH at room temperature for 2 weeks having detached them from the fetal body. The humerus and femur were then dissected out and cleaned and the cartilaginous ends were removed. The remainder of the ossified shaft was dried at room temperature for 48 hours after which the maximum length of ossified shaft was measured using a sliding gauge. In order to exclude the possibility of crossed asymmetry only the right bones were measured and from the results formulae were derived from which fetal age could be estimated based upon diaphyseal growth.

All of these graphs have been compared with the equivalent graphs produced by this study and the results can be seen in Figures 42, 43, 44, 45, 46, and 47.

It is reassuring to note that each of the graphs in Figures 42, 43, 44, 45, 46 and 47, have basically the same shape which means that the investigators independently found that the lengths of the ossified shafts of the same bones of the limbs grow to a similar pattern. It is significant however to note that each of the graphs is in a slightly different position in each of the Figures which indicates that each investigator has possibly measured different lengths, or assigned different ages to similar-sized fetuses.

Moss et.al. (1955) provide data from which the fetal age can be estimated

COMPARISON OF HUMERI

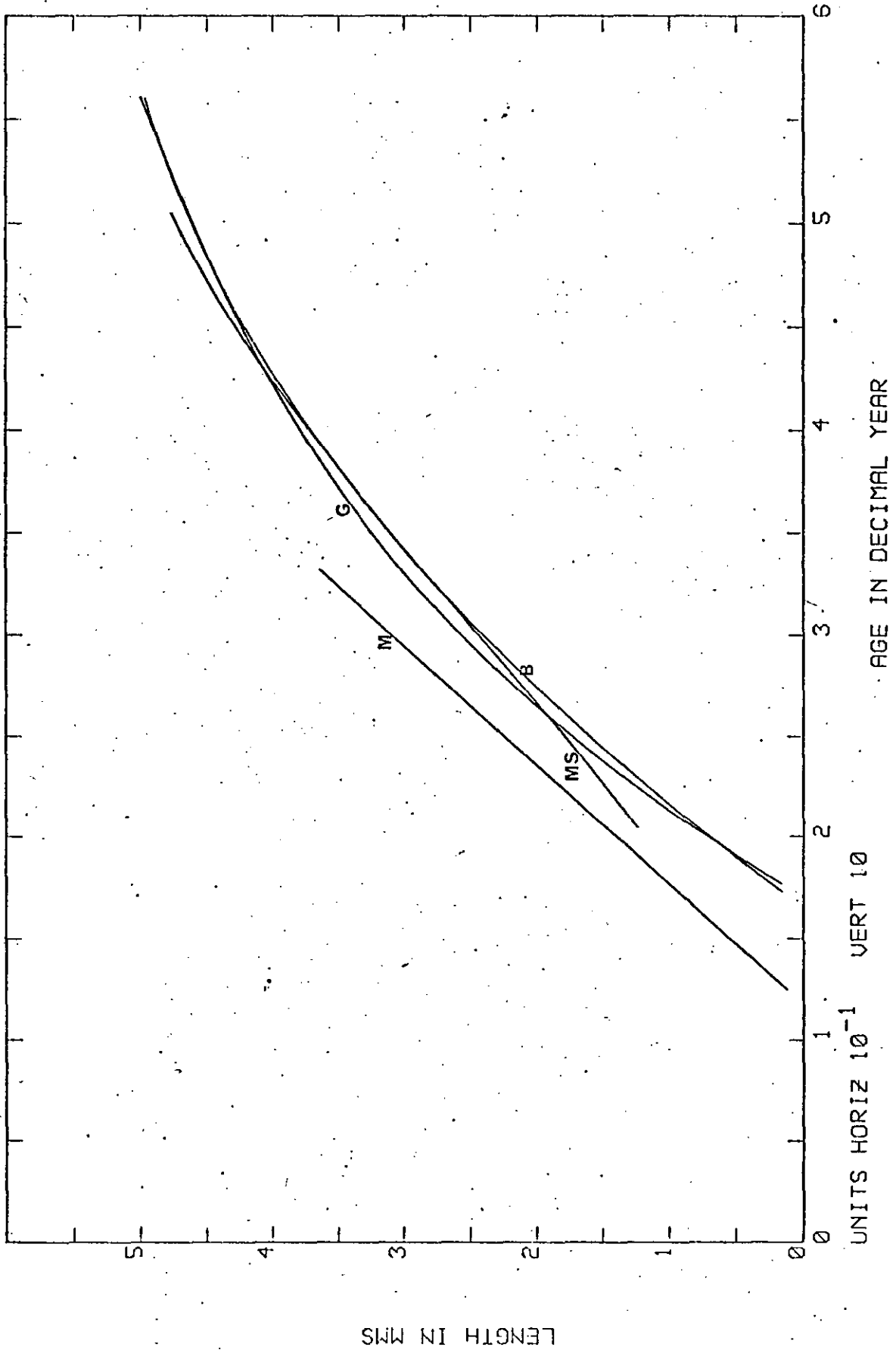


Figure 42. Comparison of published data concerning the length of ossified shaft of the fetal humerus

M = Moss et.al. (1955) G = Gray and Gardner (1969)
 MS = Mehta and Singh (1972) B = This study

COMPARISON OF RADII

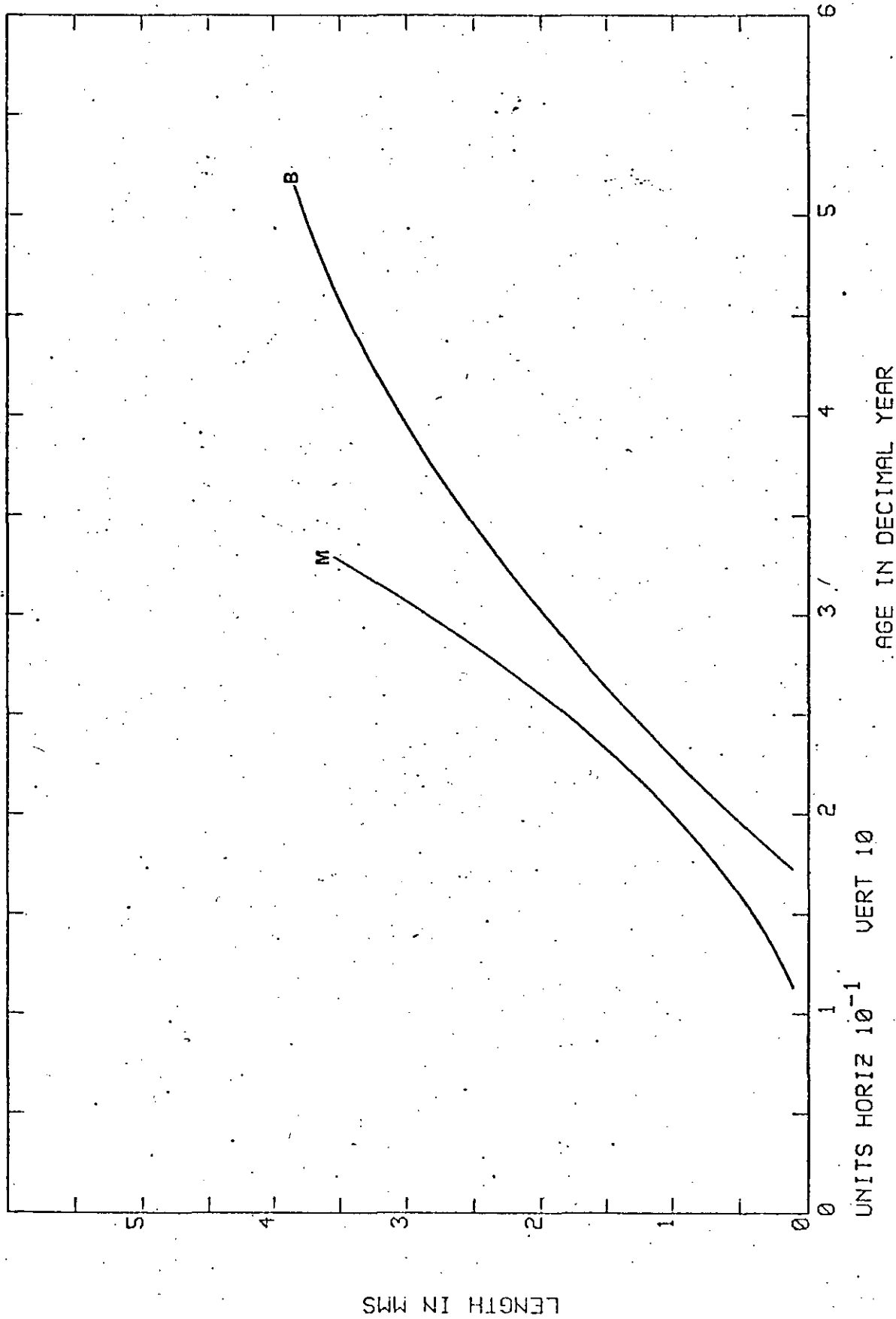


Figure 43. Comparison of published data concerning the length of ossified shaft of the fetal radius.

COMPARISON OF ULNAE

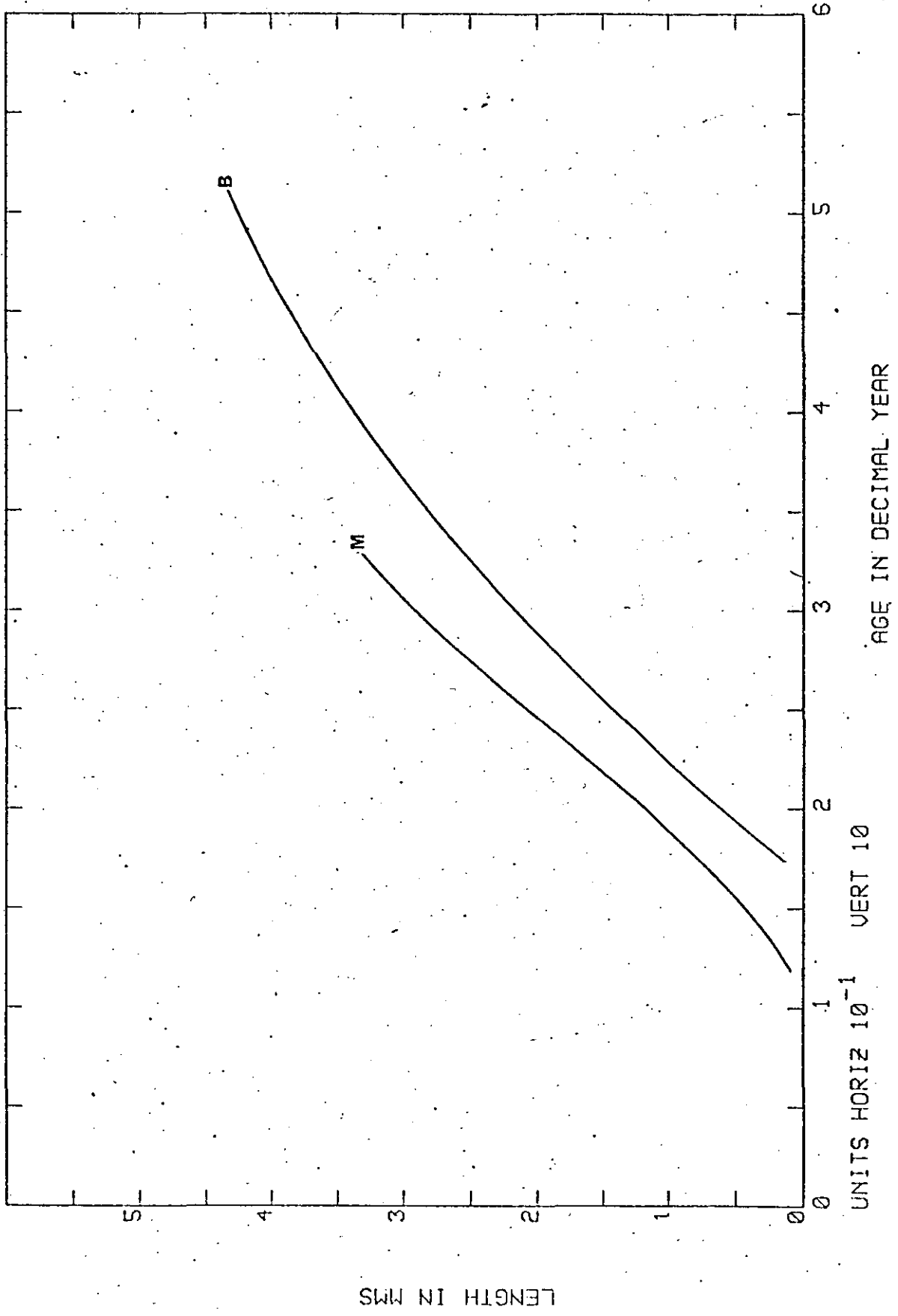


Figure 44. Comparison of published data concerning the length of ossified shaft of the fetal ulna.

COMPARISON OF FEMURS

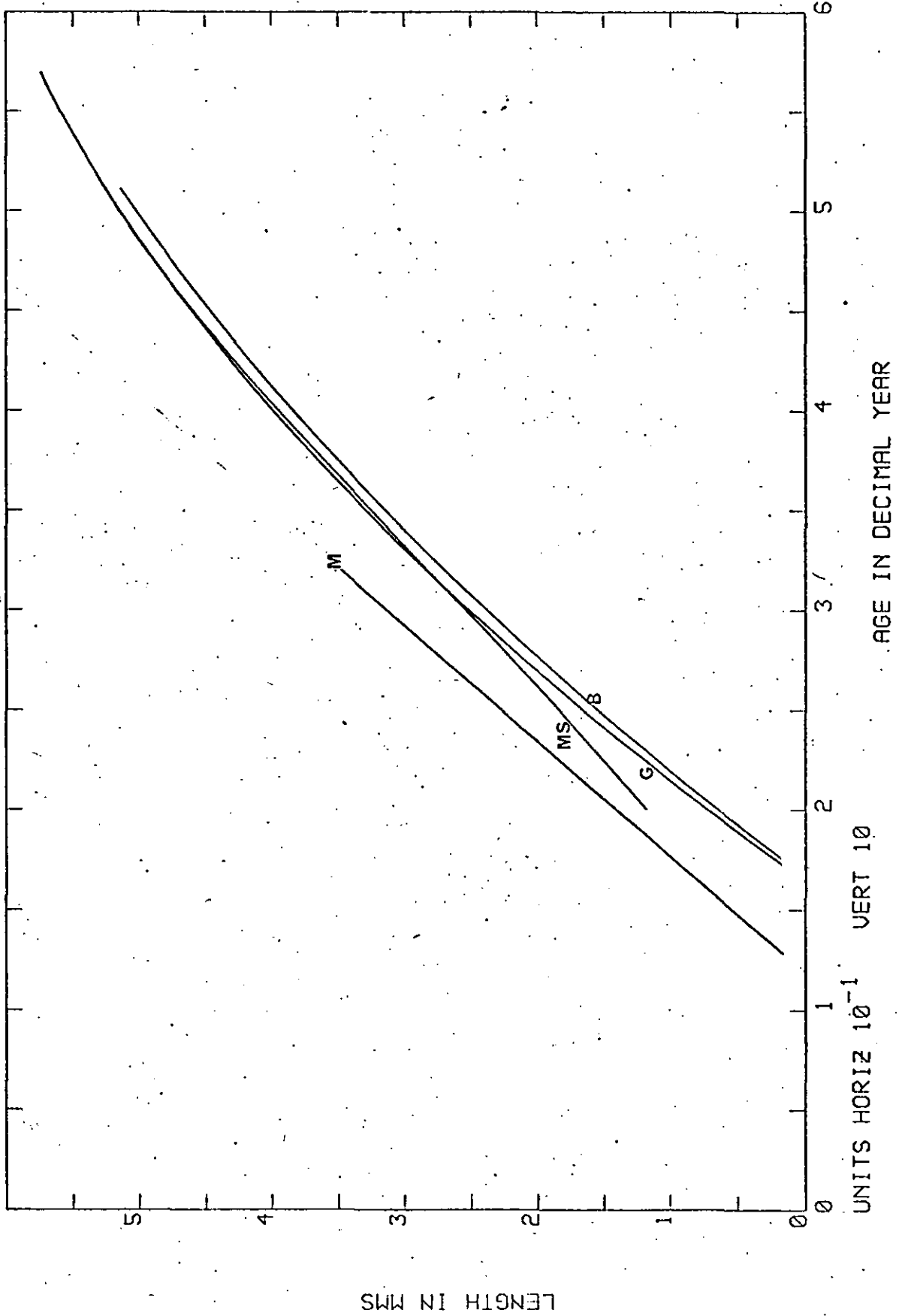


Figure 45. Comparison of published data concerning the length of ossified shaft of the fetal femur.

COMPARISON OF TIBIAE

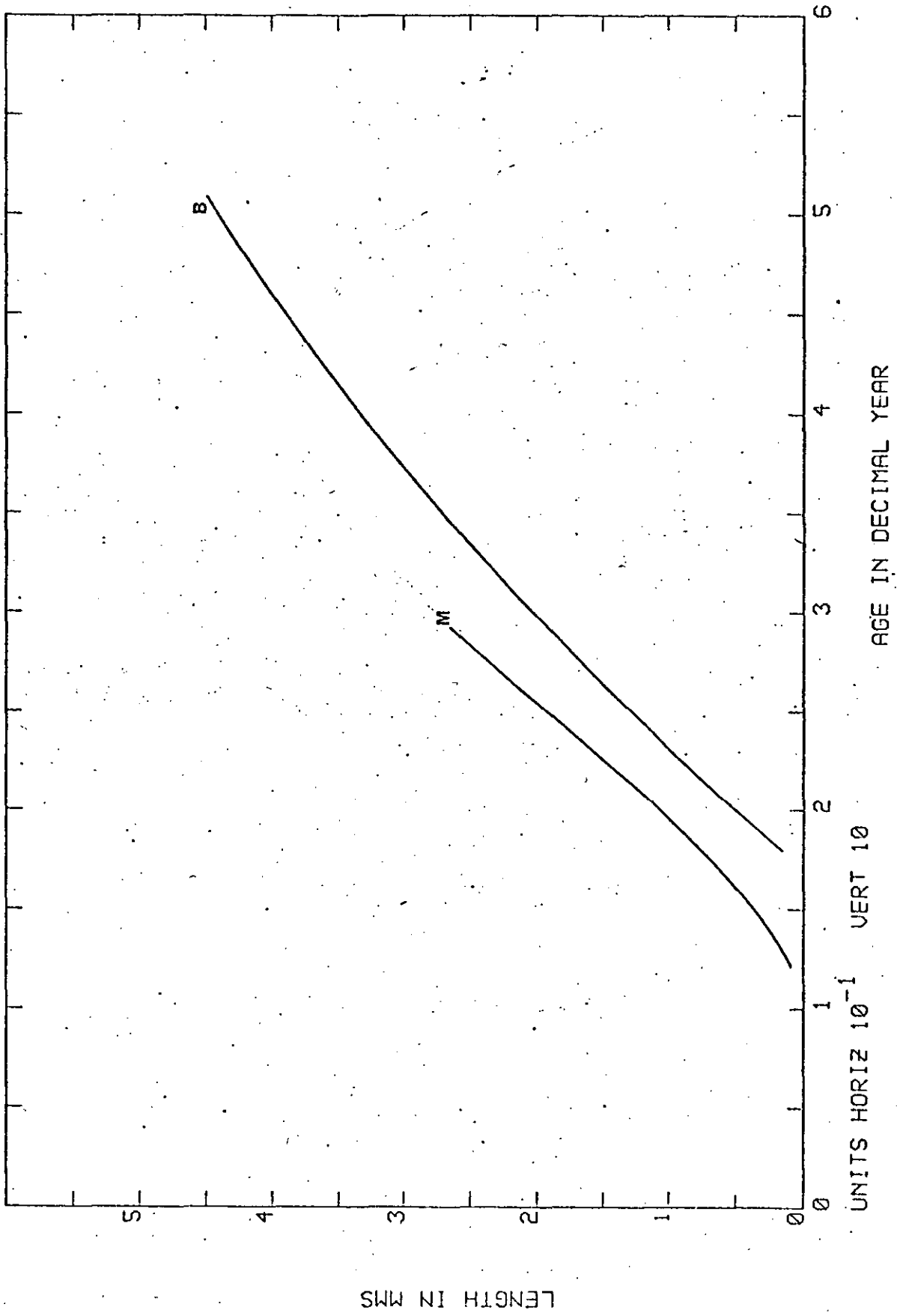


Figure 46. Comparison of published data concerning the length of ossified shaft of the fetal tibia.

COMPARISON OF FIBULAE

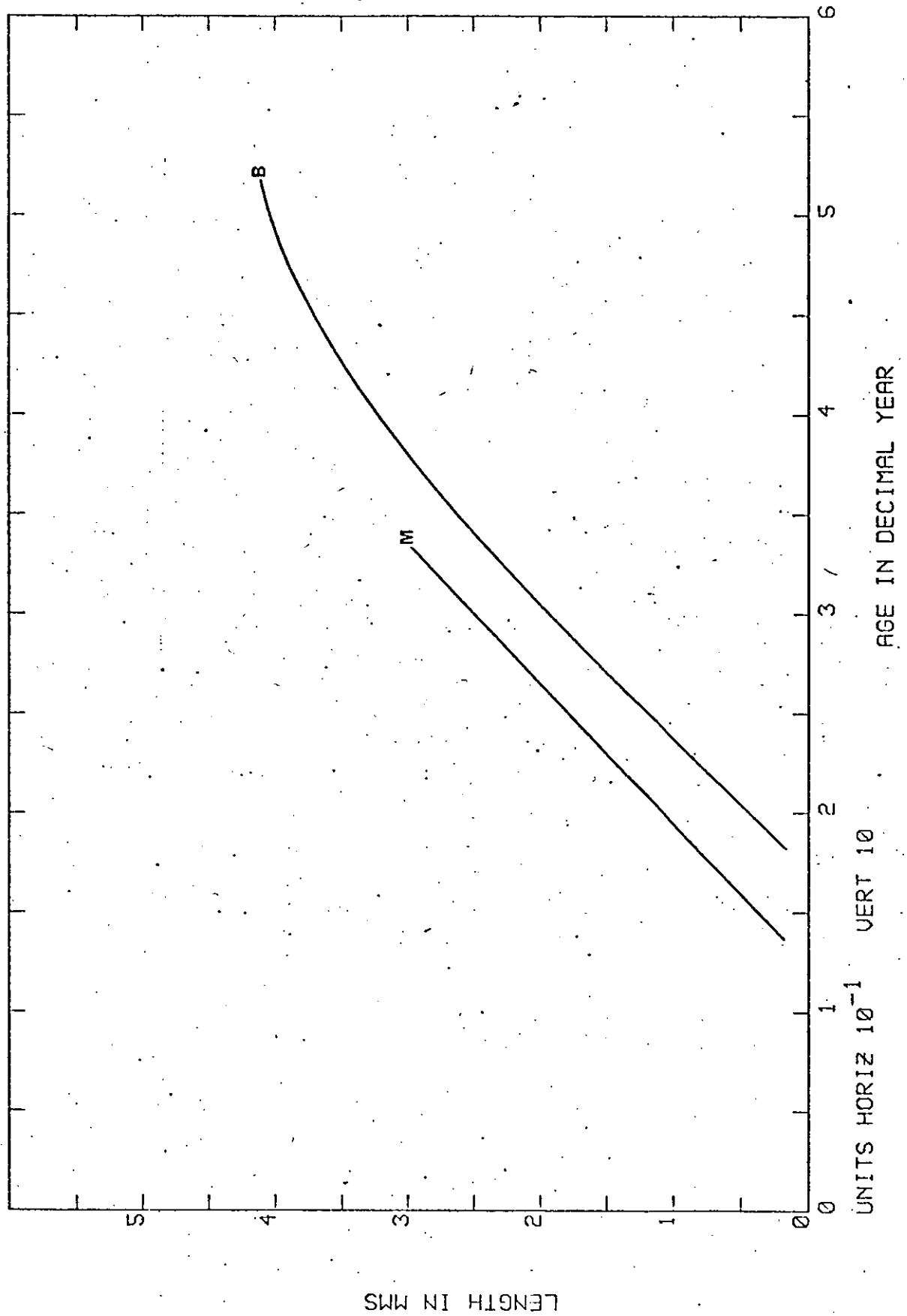


Figure 47. Comparison of published data concerning the length of ossified shaft of the fetal fibula.

from the measurement of C.R. length. Their data have been taken from Boyd (1941) and for comparison have been plotted against similar data from this study. (See figure 48).

Figure 48 shows that for any particular C.R. length, Boyd (1941) gives a considerably smaller estimate of fetal age than either Birkbeck et.al.(1975) or this study. This is surprising because the measurement of C.R.E. length for this study was designed to be the maximum C.R. length possible and therefore means that Boyd (1941) measured either larger fetuses for comparable ages or more than C.R.E. length, which seems improbable. Nevertheless, this difference in estimated age would mean that for a fetus of a particular maturity, Moss et.al.(1955) would assign a considerably smaller age than would the results of this study. Such a situation is in fact represented in each of the Figures between 42 and 47 with the graph for Moss et.al. being considerably different to that of this study. Even after allowing for such a difference however, the graph for Moss et.al. (1955) in each case is in advance of the equivalent graph for this study and therefore it would appear that the method using alizarin-red staining involves measuring greater distances than the radiographic method of this study. Moss et.al. (1955) do not provide a definition of the actual measurement they made and this, coupled with the non-specificity of alizarin-red for calcium, might account for the discrepancies encountered.

Gardner and Gray (1969, 1970) provided data which relate C.R. length to the measurement of the ossified shafts of the respective long bones. It was therefore easy to relate directly their results to those of this study and it is interesting to note that their graphs relate fairly closely to the ones from this study. It is even more interesting to note that after 0.178 yr. (9 weeks) they completed their measurements from their radiographs and although they do not include a definition relating to the measurements they made, it is reassuring to find their graphs are very similar to those from this study.

COMPARISON OF TANNER, BIRKBECK AND BOYD

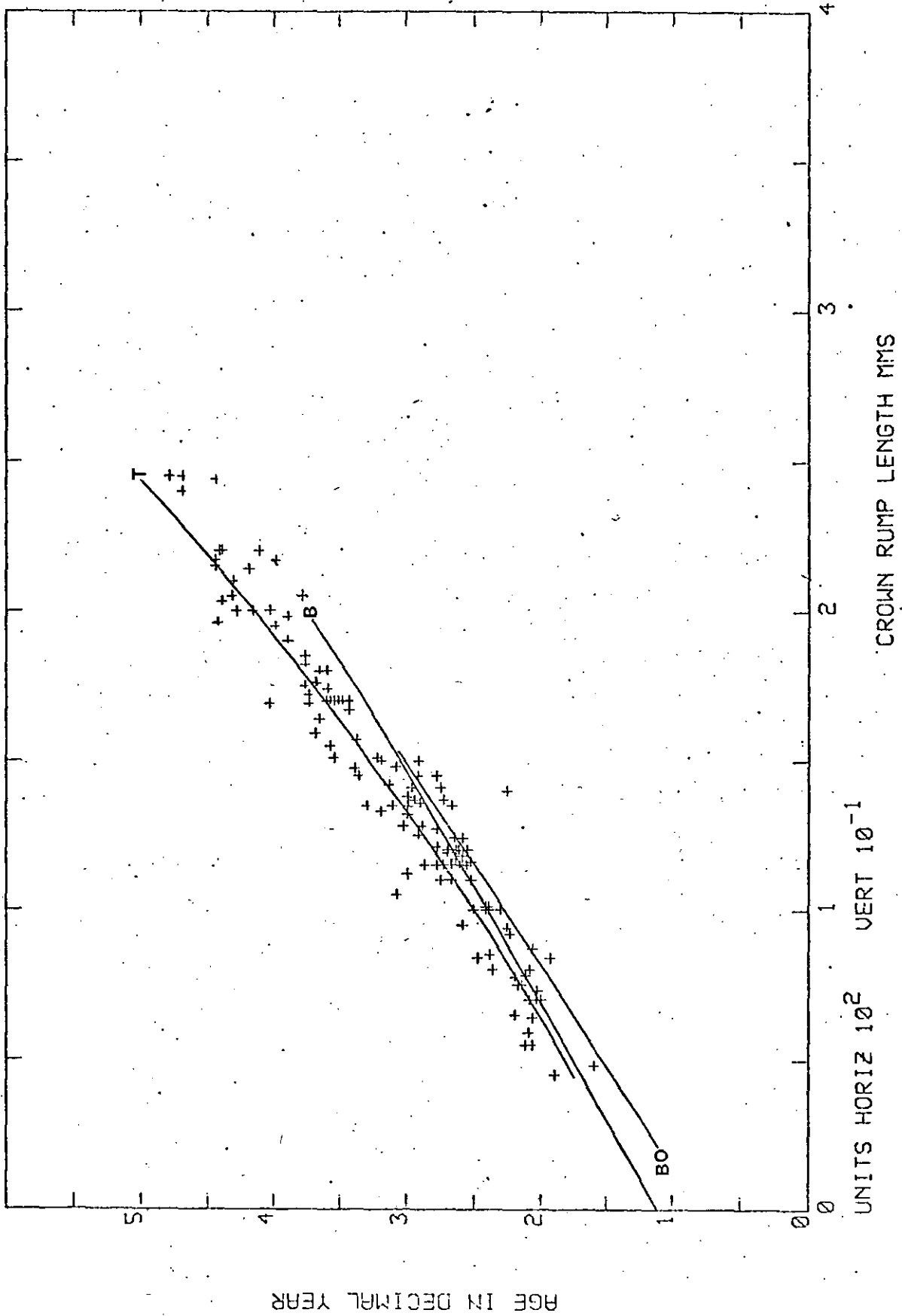


Figure 48. A comparison of the methods employed in assigning ages to fetuses based upon C.R. length.

T = This study BO = Boyd (1941)
 B = Birkbeck et.al. (1975)

It is difficult to assess the extent of alteration the procedure employed by Mehta and Singh (1972) would have had on the length of the ossified shaft of the long bones of the limbs. It is almost certain that the measurements they made were different to those of this study but it is difficult to estimate the size of this difference and even to assess whether or not it would be constant throughout fetal life. Their principle of measuring, however, was similar to that of this study in that a maximum length was employed and in this sense it is again reassuring to find that their graphs accord closely with the similar graphs from this study. The destructive procedures they employ however in the preparation of the ossified shafts restricts their practical application.

It therefore appears that the graphs produced by this study to show the growth of the ossified shafts of the long bones of the limbs are valid and compare very well with other methods of investigating these parameters. They have the added advantage of being applicable to all the long bones of the limbs and have a means by which abnormal growth can be recognised. In addition the actual measurements that are taken are clearly defined and the simplicity of radiography does not lead to destruction of the fetus and allows further investigations of the same fetus to be carried out.

By differentiating the equations shown in Table 18 and plotting the resultant equations in graphic form the growth velocities of the ossified shaft of the long bones of the limbs can be produced. (See Figure 49). The shapes of these graphs are determined by the form of the original equations but nevertheless they are valid even though they have been produced from cross-sectional data which would tend to suppress any changes in velocity of growth that are present (Tanner, 1962). However, there are no means by which confidence limits can be expressed for such graphs because there are no longitudinal data available from which these limits can be calculated. Velocity curves are very sensitive to changes in slope of the distance curves shown in Figure 40. In

COMPARISON OF GROWTH VELOCITIES OF LONG BONE LENGTHS

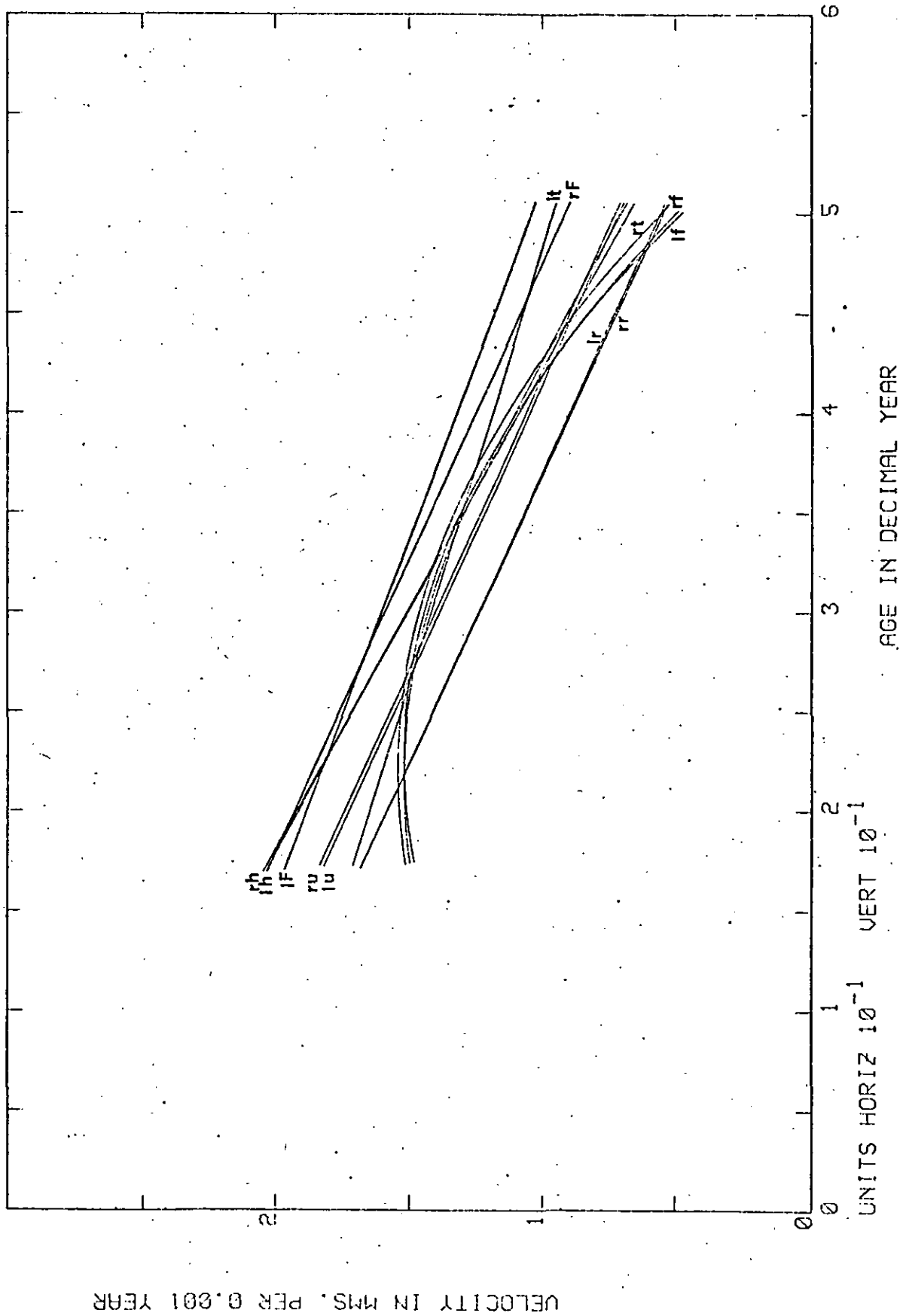


Figure 49. The growth velocities of the ossified shafts of the long bones of the limbs.

ordinary use, velocity curves are more sensitive to abnormalities of growth than are distance curves because the serial measurements required for their use are able to distinguish periods of growth stasis more readily. Because fetal radiography is not carried out on a serial basis, there is no direct clinical application for the graphs of Figure 49 but even so, from an academic point of view, the graphs do provide a great deal of worthwhile information.

Even though nine of the twelve velocity graphs shown in Figure 49 are represented by straight lines, it is significant that they all have negative slopes which shows that the velocity of growth of these ossified shafts is getting less as the fetus gets older. Of the remaining three, all of them are represented by equations to the power two and having passed through a peak velocity at approximately 0.25 yrs. (13 weeks) it is interesting to note that their course follows a similar pattern to the graphs of the other nine shafts. This means that these three ossified bone shafts are delayed in relation to their peak velocity growth time when compared to the other bone shafts and emphasises once again that the ossification of the bone shafts is peculiar to each individual bone. This is further emphasised by the fact that it is the right and left fibula which are delayed and it is these two bones which appear last in the sequence of ossification for the primary ossification centres of the long bones of the limbs. Along these lines, it is difficult to interpret why the growth velocity of the ossified shaft of the left tibia is so different to that of the ossified shaft of the right tibia although it is interesting to note that the growth of the ossified shaft of the right tibia is very similar to that of the ossified shafts of the right and left fibulae. The situation is made even more confusing by the fact that all of the other pairs of ossified bone shafts grow at approximately similar velocities to each other although the differences that do exist indicate that equal symmetrical growth does not exist in the fetal body.

Previous attempts have been made to calculate the velocity of growth of the ossified shafts of the long bones of the limbs. These studies have centred mainly upon radiographs taken of fetuses in utero near term in order to make an assessment of fetal maturity. Owen (1971) measured the lengths of each of the ossified shafts of the long bones of the limbs in a small number of fetuses ranging in age from 12 weeks to term and calculated that the femur grows at 1 mm/week in the last weeks of pregnancy. Martin and Higginbottom (1971) reported that the femur grows at 3 mm/week in the last 8 weeks of gestation and Russell et.al. (1972) found a poor correlation between fetal maturity and femoral length with its rate of growth being 1.5 mm/week with a femoral length of 77 mm. The graphs of Figure 49 show that the velocity of growth for all of the ossified shafts is dependent upon fetal age and therefore it is perhaps wrong to state that the velocity of growth is a constant in the later weeks of pregnancy. Concerning the femur in particular, it can be seen that its velocity of growth at 0.500 yrs (26 weeks) is approximately 0.09 mm/0.001 yr. which is equivalent to 1.8 mm/week. This means that there must be a considerable change in the shape of the velocity curve of the femur between 0.500 yrs. (26 weeks) and term with two possibilities being either a dramatic reduction in the rate of slowing down or that a minimum value in terms of velocity of growth is passed through during this period. This certainly requires further investigation and contradicts the work of Hodges (1957) who stated that the growth in length of the calcified femur occurred at a regular rate for each week of gestation between 16 and 38 weeks.

Mehta and Singh (1972) calculated that the diaphyseal growth rates of the humerus and femur are 0.18 mm. and 0.21 mm. respectively for every 1 mm. increase in C.R. Length. In order to compare their results to those of this study similar equations were calculated which related the C.R. length of the

fetus to the lengths of the ossified shafts of the long bones of the left limbs and the results are presented in Table 21 and Figure 50. Differentiation of the equations in Table 21 provides the equations representative of the velocities of growth of the ossified shafts of the long bones of the limbs in relation to C.R. length. For the femur the velocity of growth is equivalent to 0.25 mm/mm. increase in C.R. length which is very similar to that of Felts (1954) and Mehta and Singh (1972), but for the humerus and all the other long bones the velocity equations are all to the power of two which means that their velocity of growth is dependent on the C.R. length of the fetus. It is therefore wrong in this case to express the velocity of growth as a constant in terms of mm/mm. increase in C.R. length. For comparison the velocity of growth of the ossified shaft of the humerus in relation to C.R. length has a minimum value of 0.166 mm/mm increase in C.R. length when the C.R. length is approximately 200 mm. and a maximum value of 0.24 mm/mm increase in C.R. length when the fetal C.R. length is approximately 50 mm. These values therefore compare well with those of Mehta and Singh (1972) but emphasise that the growth velocity of the humerus is not a constant value.

In addition to being able to estimate the lengths of the ossified shafts of the long bones of the limbs from either fetal age or a measurement of fetal C.R. length it was thought that it would be helpful if the growth of each of the ossified shafts could be related to each other so that an estimate of ossified shaft length could be made based upon the measurement of any other ossified shaft. Accordingly, the measurements taken of the lengths of the ossified shaft lengths of the long bones of the left limbs were subjected to polynomial regression analysis in turn with each other and the results can be seen in Tables 22, 23, 24, 25, 26 and 27 and Figures 51, 52, 53, 54, 55 and 56. These equations and graphs can be used to estimate the length of any of the

Table 21

Equations Relating Growth of the Ossified Shafts of the Long Bones of the left Limbs to the Fetal C.R. Length

Left Long Bone	Equation	S.E.E.	R
Humerus	$Y = -7.547643 + 0.202328X + 0.00051X^2 - 0.000002X^3$	1.793	0.98
Radius	$Y = -6.620488 + 0.168508X + 0.000456X^2 - 0.000002X^3$	1.603	0.98
Ulna	$Y = -6.062699 + 0.146844X + 0.000804X^2 - 0.00000X^3$	1.792	0.98
Femur	$Y = -9.406625 + 0.249335X$	2.010	0.98
Tibia	$Y = -4.979478 + 0.109532X + 0.000971X^2 - 0.000002X^3$	1.926	0.98
Fibula	$Y = -4.929862 + 0.088232X + 0.001081X^2 - 0.000003X^3$	1.830	0.98

COMPARISON OF LIMB LENGTHS

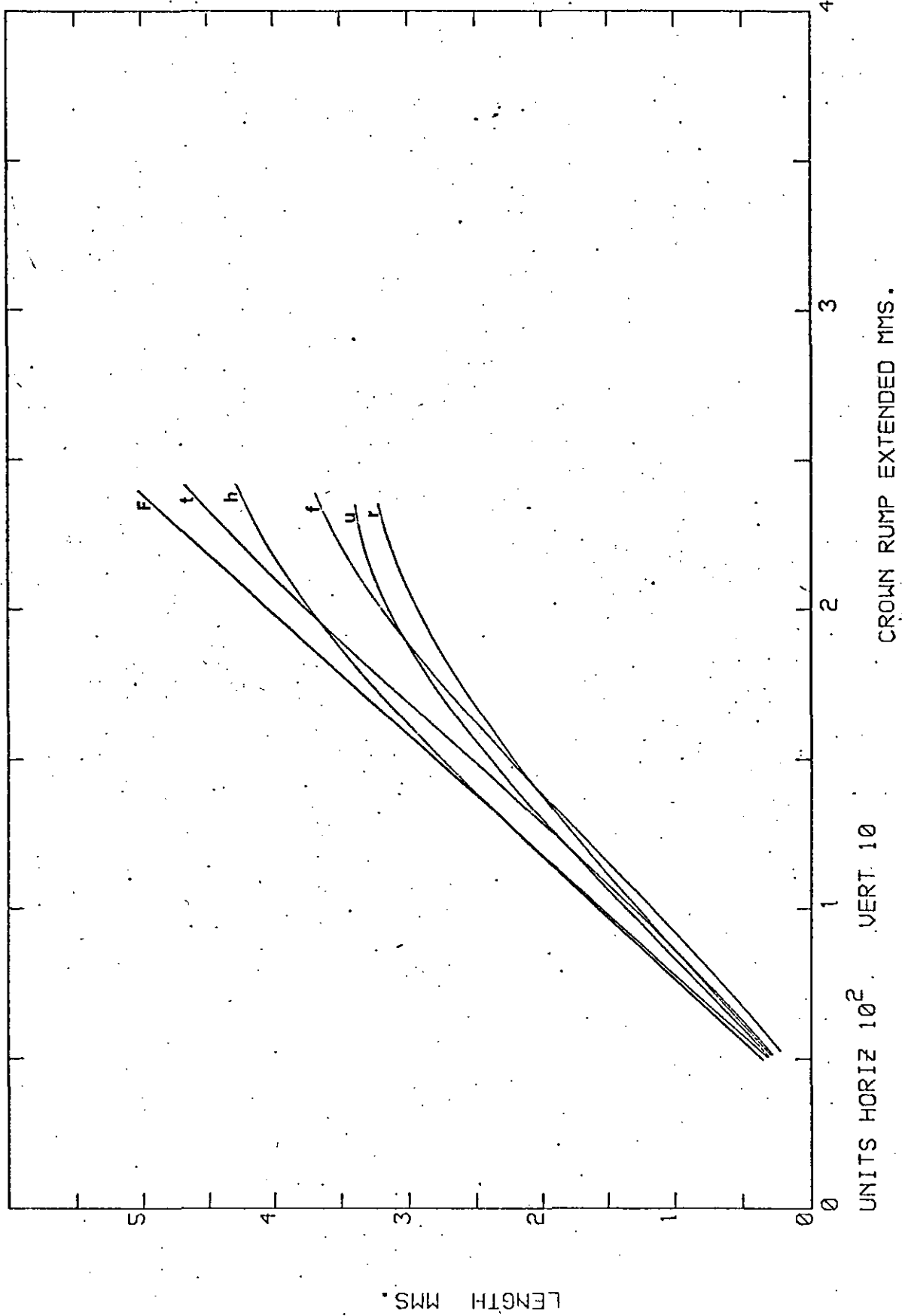


Figure 50. The development of the length of ossified shafts in the long bones of the left limbs in the human fetus related to the C.R. length.

Table 22 Estimates of the Lengths of Ossified Shafts of the Long Bones of the Limbs Based on Measurement of the Ossified Shaft of the Humerus

Long Bone	Equation	S.E.E.	R
Radius	$Y = -0.363950 + 0.857949X - 0.000773X^2$	0.91	0.994
Ulna	$Y = -0.568604 + 0.926919X$	0.90	0.995
Femur	$Y = -0.650001 + 1.031514X - 0.002720X^2 + 0.000089X^3$	0.87	0.997
Tibia	$Y = -0.312583 + 0.760154X + 0.004098X^2$	1.07	0.994
Fibula	$Y = -1.161684 + 0.769840X + 0.003443X^2$	1.00	0.994

Table 23 Estimates of the Lengths of the Ossified Shafts of the Long Bones of the Limbs Based on Measurement of the Ossified Shaft of the Radius

Long Bone	Equation	S.E.E.	R
Humerus	$Y = 0.928260 + 1.060840X + 0.008375X^2 - 0.000143X^3$	1.10	0.994
Ulna	$Y = 0.912635 + 0.827362X + 0.017344X^2 - 0.000294X^3$	0.84	0.996
Femur	$Y = 0.262523 + 1.089989X + 0.005265X^2$	1.26	0.993
Tibia	$Y = 0.727757 + 0.724245X + 0.017430X^2 - 0.000193X^3$	1.19	0.992
Fibula	$Y = 0.120260 + 0.682986X + 0.19555X^2 - 0.000244X^3$	1.07	0.993

Table 24 Estimates of the Lengths of the Ossified Shafts of the Long Bones of the Limbs Based on Measurement of the Ossified Shafts of the Ulna

Long Bone	Equation	S.E.E.	R
Humerus	$Y = 0.810925 + 1.068694X$	0.96	0.995
Radius	$Y = 0.017481 + 0.944647X - 0.001428X^2$	0.75	0.996
Femur	$Y = -0.196296 + 1.142672X - 0.004164X^2 + 0.000118X^3$	1.07	0.995
Tibia	$Y = 0.100803 + 0.837964X + 0.004357X^2$	1.05	0.994
Fibula	$Y = -0.756222 + 0.848472X + 0.003586X^2$	0.93	0.995

Table 25 Estimates of the Lengths of the Ossified Shafts of the Long Bones of the Limbs Based on Measurements of the Ossified Shaft of the Femur

Long Bone	Equation	S.E.E.	R
Humerus	$Y = 0.196212 + 1.072296X - 0.003024X^2$	0.81	0.997
Radius	$Y = -0.201469 + 0.914413X - 0.003130X^2$	0.93	0.994
Ulna	$Y = 0.308823 + 0.865683X + 0.003244X^2 - 0.000079X^3$	0.90	0.995
Tibia	$Y = 0.233939 + 0.739781X + 0.006011X^2 - 0.000071X^3$	0.95	0.995
Fibula	$Y = -0.367403 + 0.705163X + 0.007393X^2 - 0.000096X^3$	0.88	0.996

Table 26 Estimates of the Lengths of the Ossified Shafts of the Long Bones of the Limbs Based on Measurements of the Ossified Shaft of the Tibia

Long Bone	Equation	S.E.E.	R
Humerus	$Y = 0.842001 + 1.242291X - 0.004947X^2$	1.12	0.994
Radius	$Y = 0.357305 + 1.054629X - 0.004779X^2$	1.00	0.993
Ulna	$Y = 0.215385 + 1.144818X - 0.004308X^2$	1.00	0.994
Femur	$Y = -0.132899 + 1.328555X - 0.010241X^2 + 0.000142X^3$	1.07	0.995
Fibula	$Y = -0.787489 + 1.004485X - 0.000710X^2$	0.66	0.998

Table 27 Estimates of the Lengths of the Ossified Shafts of the Long Bones of the Limbs Based on Measurements of the Ossified Shaft of the Fibula

Long Bone	Equation	S.E.E.	R
Humerus	$Y = 1.814678 + 1.235554X - 0.1004434X^2$	1.04	0.994
Radius	$Y = 1.181631 + 1.047980X - 0.004358X^2$	0.93	0.994
Ulna	$Y = 1.102634 + 1.139562X - 0.003828X^2$	0.92	0.995
Femur	$Y = 0.829815 + 1.333360X - 0.010767X^2 + 0.000171X^3$	1.01	0.996
Tibia	$Y = 0.860830 + 0.992300X + 0.000727X^2$	0.67	0.998

COMPARISON OF LIMB LENGTHS

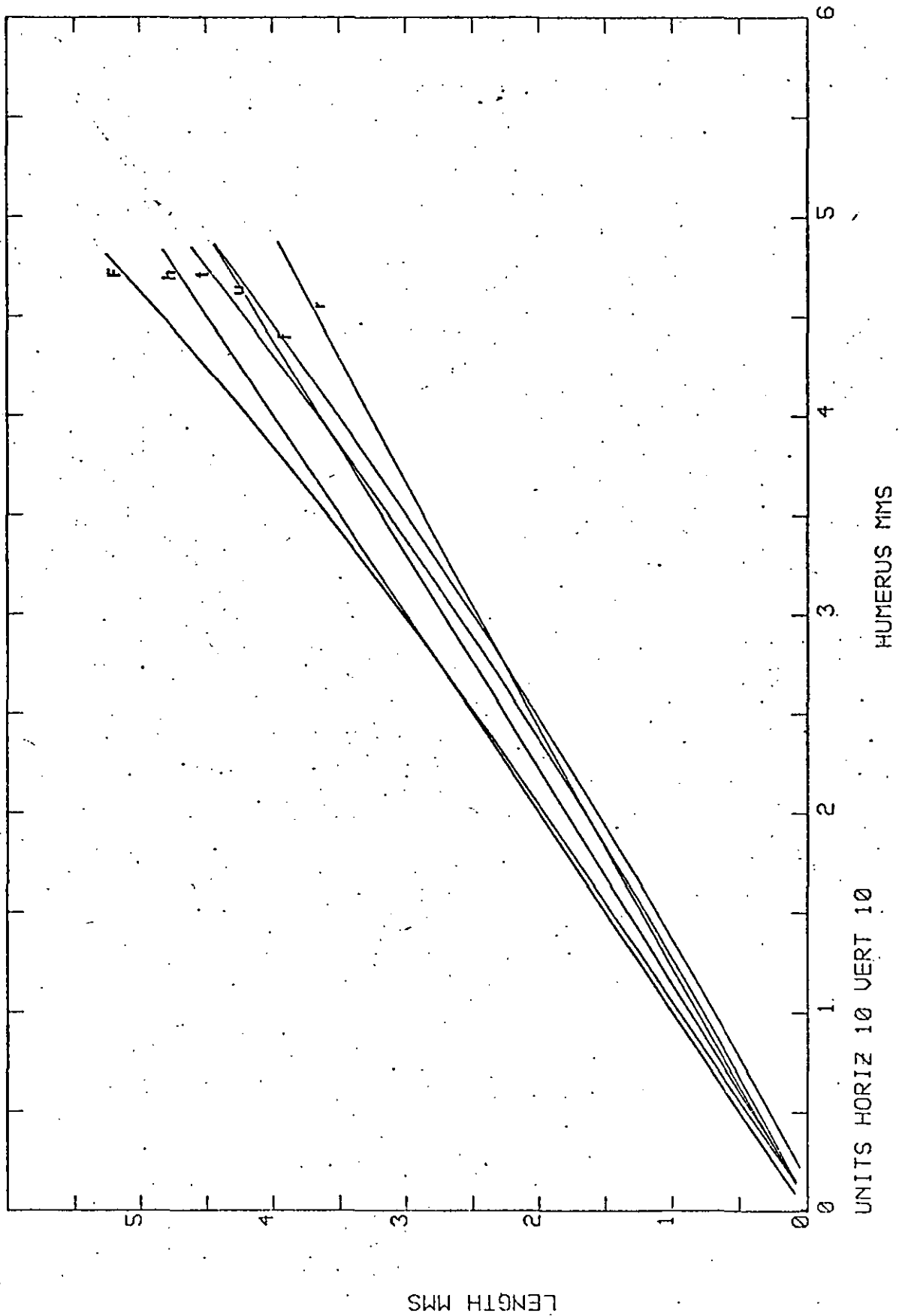


Figure 51. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the humerus.

COMPARISON OF LIMB LENGTHS

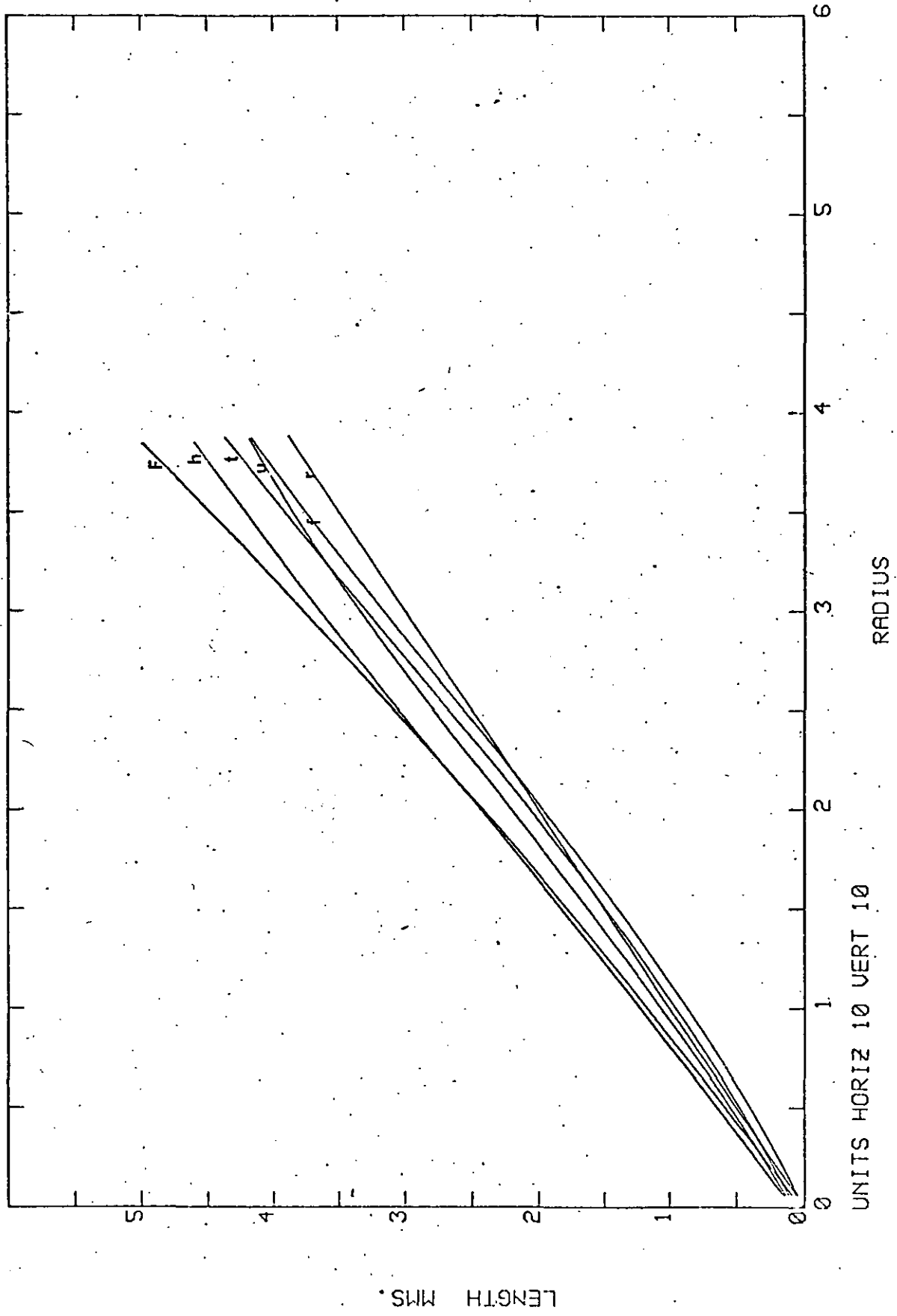


Figure 52. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the radius.

COMPARISON OF LIMB LENGTHS

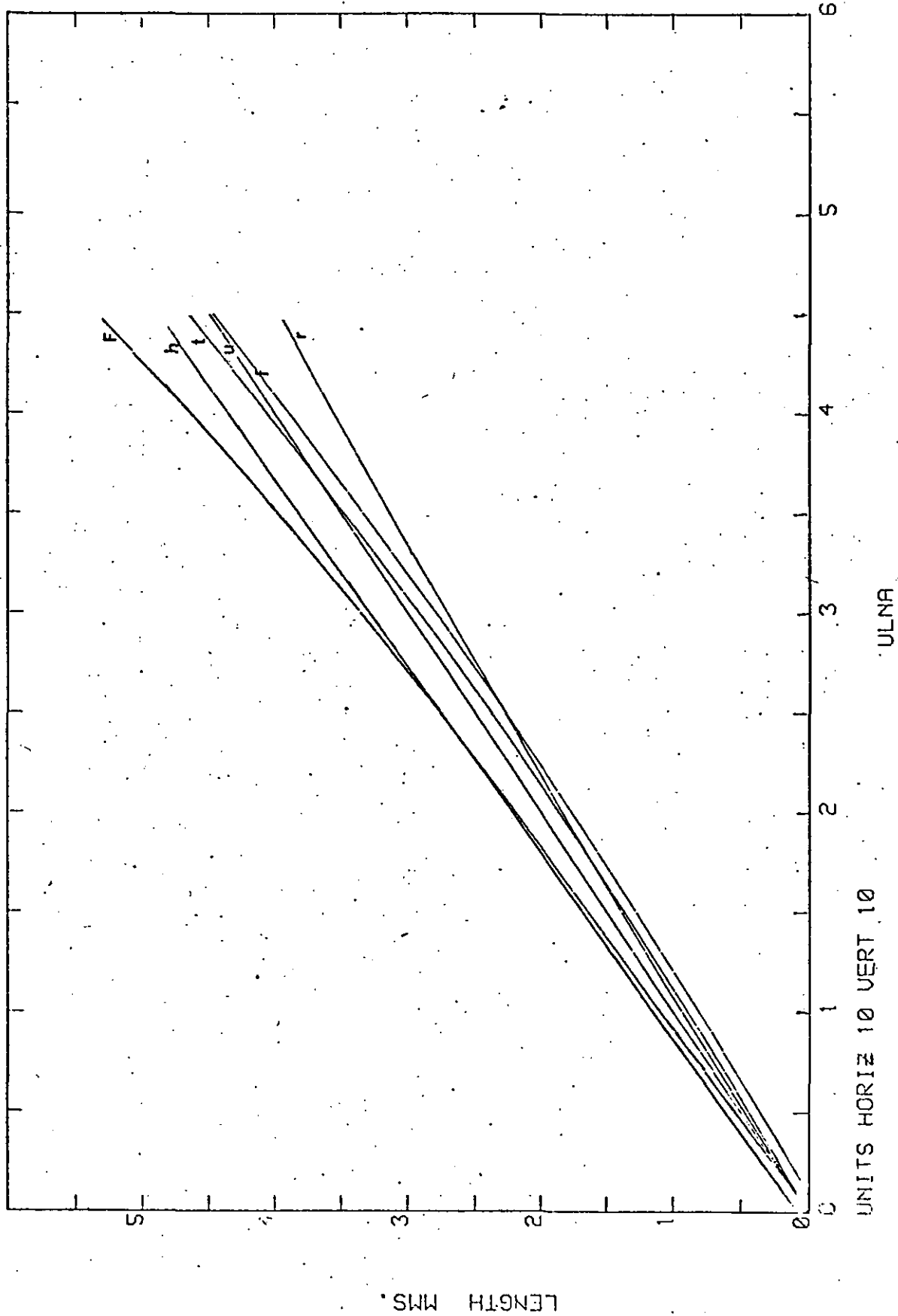


Figure 53. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the ulna.

COMPARISON OF LIMB LENGTHS.

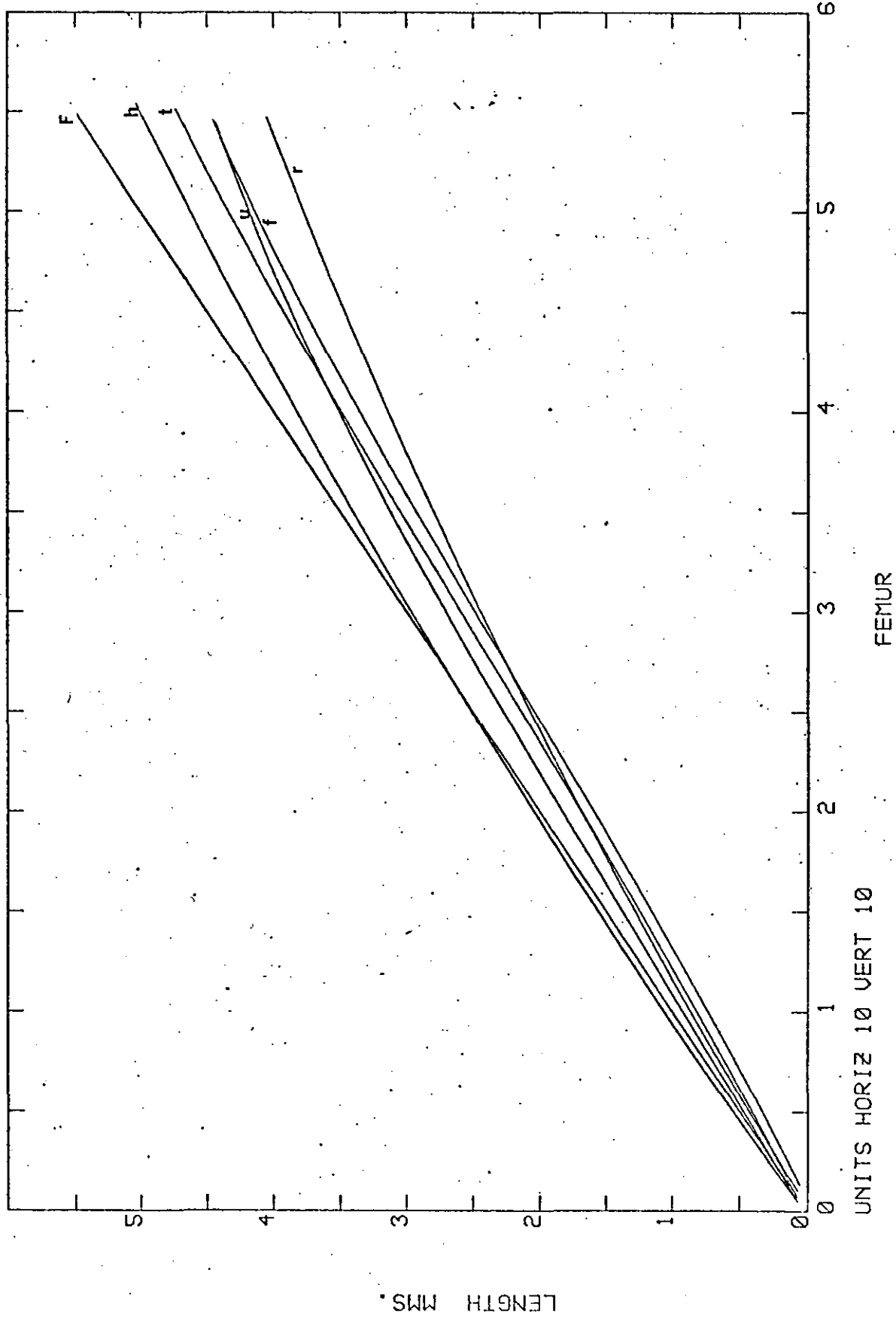


Figure 54. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the femur.

COMPARISON OF LIMB LENGTHS

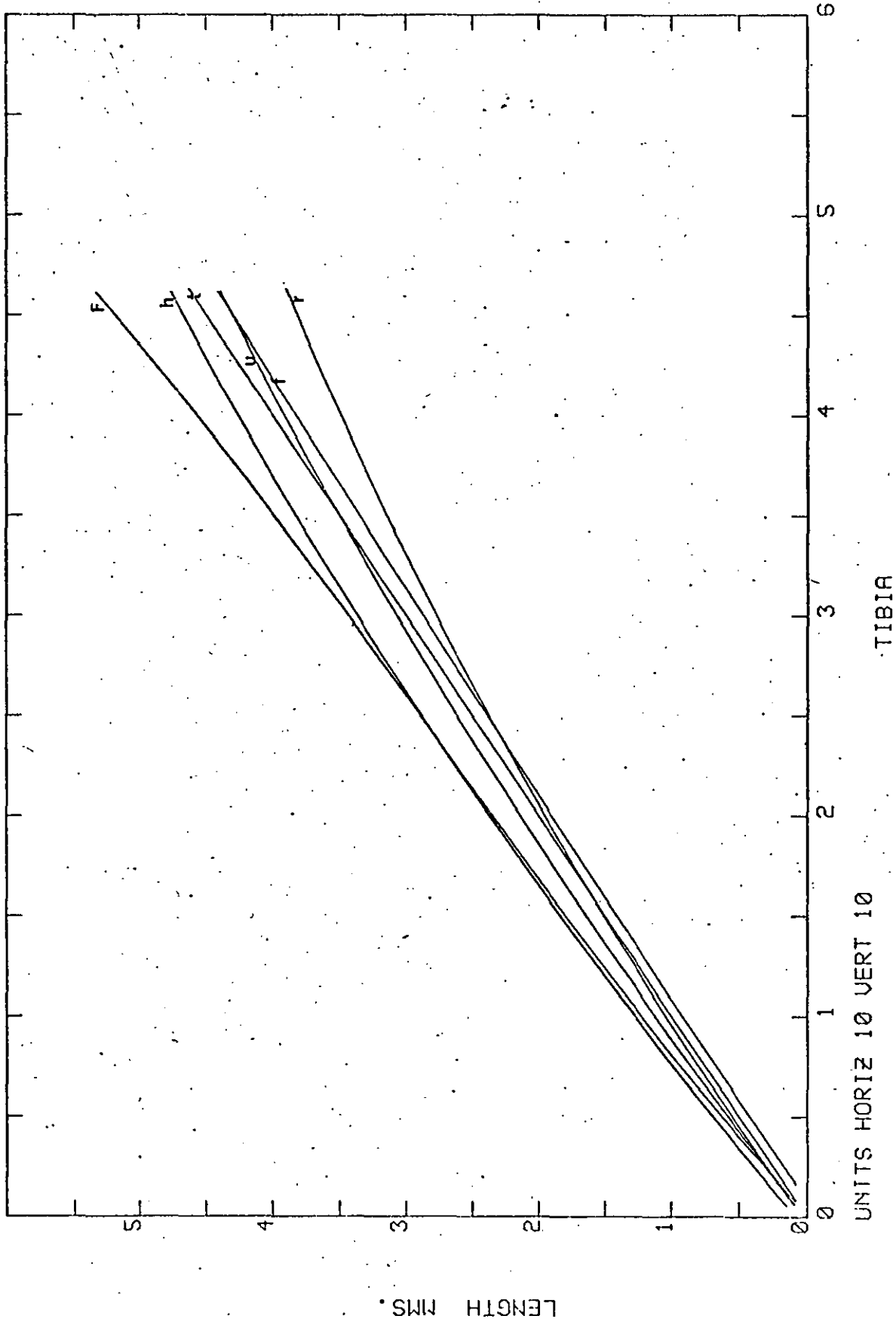


Figure 55. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the tibia.

COMPARISON OF LIMB LENGTHS

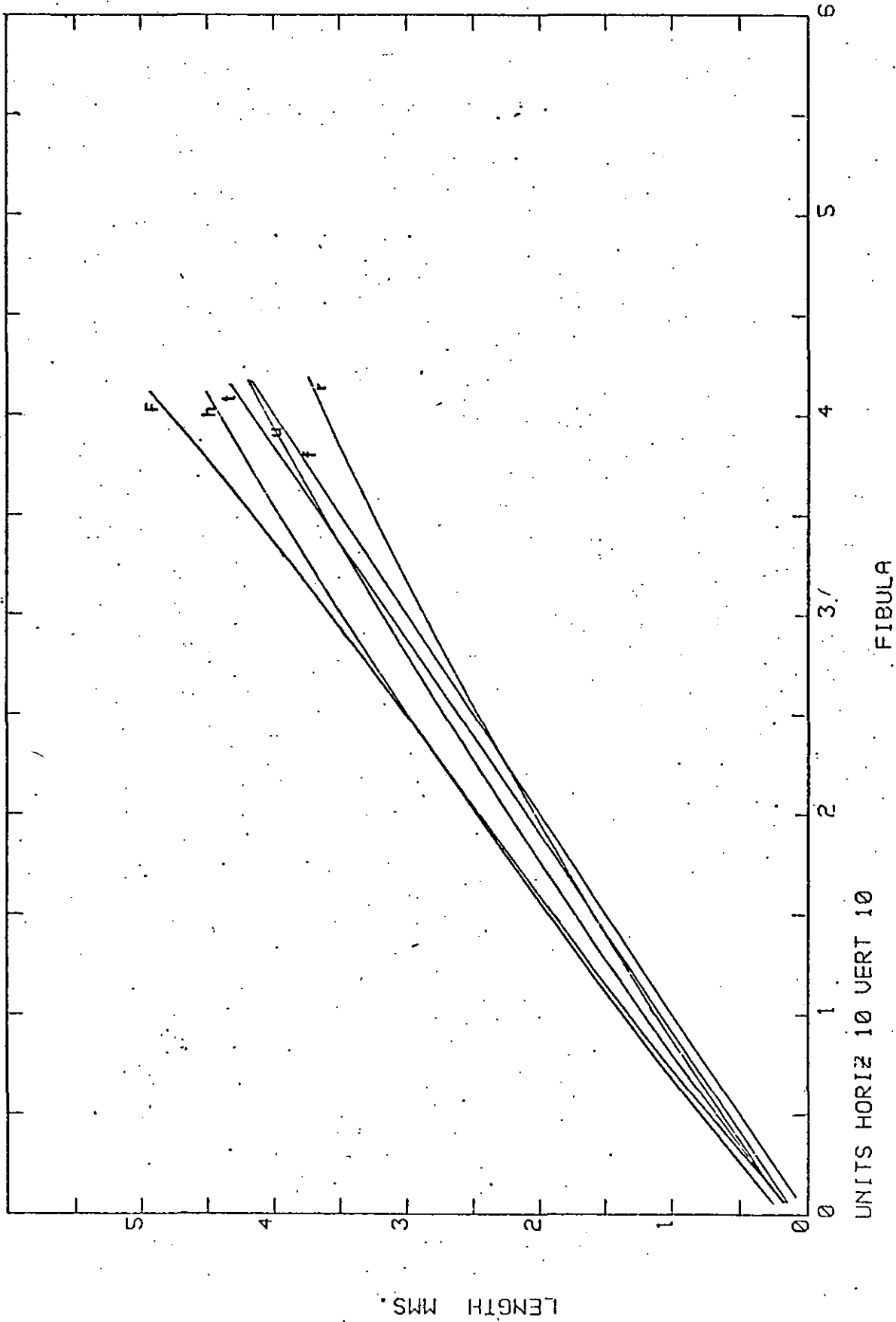


Figure 56. Comparison of the length of ossified shafts of the long bones of the limbs with the length of the ossified shaft of the fibula.

ossified shaft lengths of the long bones of the limbs based upon the same parameters. The high multiple correlation coefficient for each of the equations demonstrates that each is a good fit and the use of the S.E.E. will allow a first approximation to be made in the recognition of any abnormal growth. It is anticipated that these facilities will be particularly useful in studying, for example, cases of anencephaly in which the fetal age is not known and the C.R. length cannot be measured. The apparent lengthening of the limbs associated with this abnormality can now be accurately assessed and whether or not the condition affects all of the limb bones equally can now be determined. A similar application can be made in the study of scoliosis.

Moss (1954, 1955) and Moss et.al. (1955) applied the concepts of differential growth to the ossified lengths of the human fetal long bones, and it was hoped that the results from this study would be able to extend this work. Moss et.al. (1955) judged that there was a characteristic interphase, which occurred at approximately 12 weeks of fetal life, whenever data which related the length of any of the ossified shafts of the long bones of the limbs to the fetal age, were plotted on log./log. paper. They concluded that the two straight lines which could be fitted to the data justified the use of the allometric growth equation and that the interphase between these lines indicated that a common factor must have intervened in the growth of all the long bones. However, when the data from this study were plotted on log./log. axes (See Figure 57) no characteristic interphase was readily apparent for any of the long bones and the form of the datapoints suggested that a curve was more appropriate than two straight lines. The data were subjected to polynomial regression analysis and the resulting cubic equation for the humerus is shown in Figure 57. The mathematical procedures involved with finding the best combination of two straight lines to fit the data shown in Figure 57 are extremely complicated and with the multiple correlation coefficient of the equation shown

ALLOMETRIC GROWTH

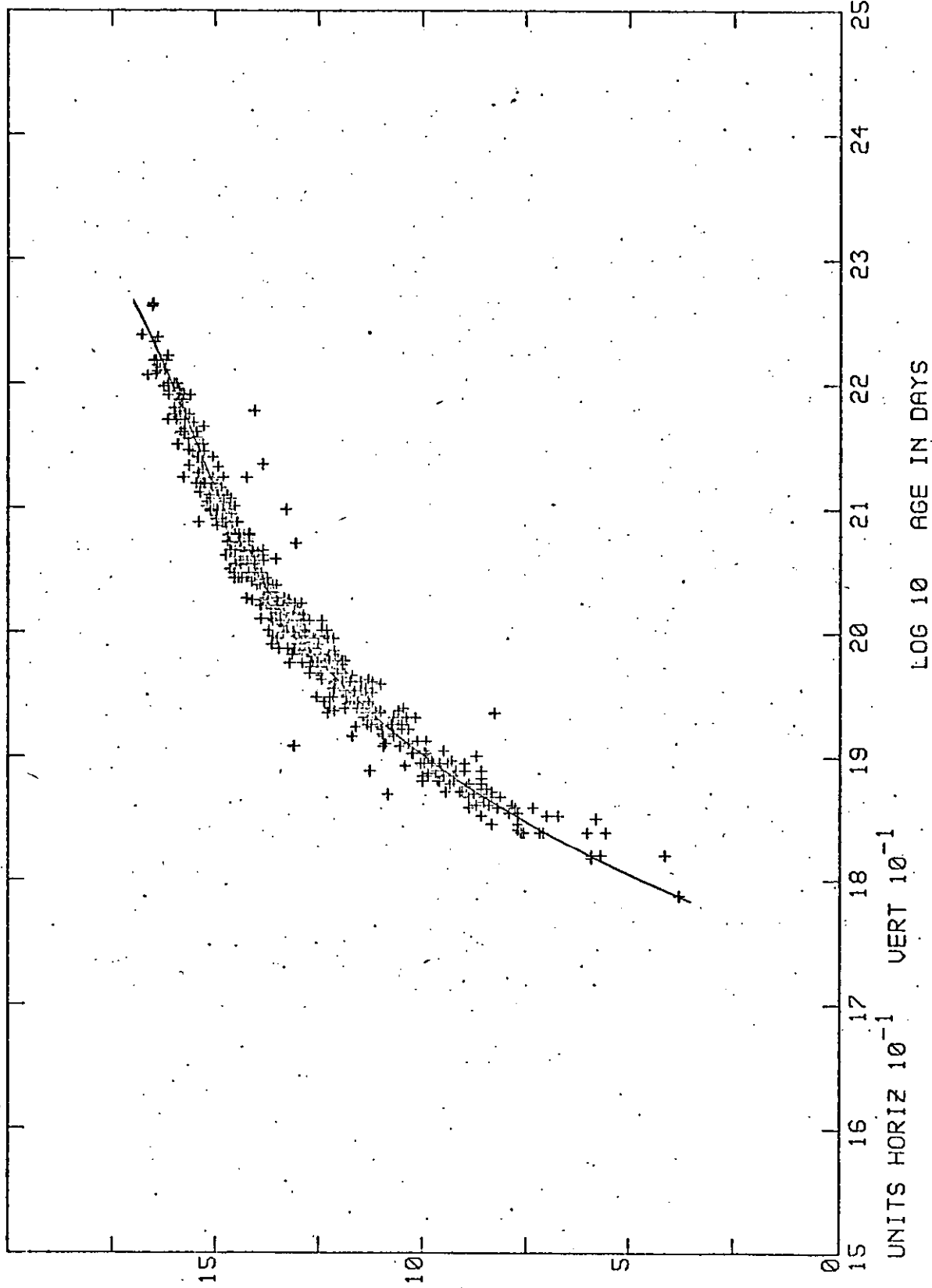


Figure 57. The age of the fetus and the length of the ossified shaft of the humerus plotted on log./log. axes in an assessment of allometric growth.

in Figure 57 being 0.98 it is felt that such a procedure would not reveal any form of better fit. Therefore, it appears that the relative growth of the ossified shafts of the long bones of the limbs are constantly changing throughout fetal life and do not pass through an interphase value as suggested by Moss et.al. (1955).

Similarly Moss et.al. (1955) found that the allometric growth concepts were appropriate when comparing the length of ossified shaft of one fetal long bone with another because they found that a straight line could be passed through their data when they were plotted on log./log. paper. However, when the results from this study were plotted on log./log. axes and the data were subjected to polynomial regression analysis it was found that it was significant to include terms to the powers 2 and 3 rather than limit the line of best fit merely to a straight line. This means that the line of best fit through the data is a curve and that the model of allometric growth cannot be applied to the ossified shafts of the long bones of the limbs. In Figure 58 the data relating the ossified shafts of the radius and humerus have been plotted on log./log. axes. Through the data the best straight line has been plotted and for comparison the line of best fit, to the power 3, has also been plotted. As can be seen the two lines are indeed very close, but nevertheless it is statistically significant to include the curves involved with the cubic equation.

It appears therefore that the addition of mathematical refinements into the application of allometric growth to the lengths of the ossified shafts of the long bones of the limbs has cast doubt on the conclusions reached by Moss et.al. (1955). The tremendous increase in number of observations taken by this study has given a firm base to this doubt but obviously the situation requires further investigation by increasing the number of observations in the lower age range and extending the data in the upper age range.

COMPARISON OF BEST FIT LINES

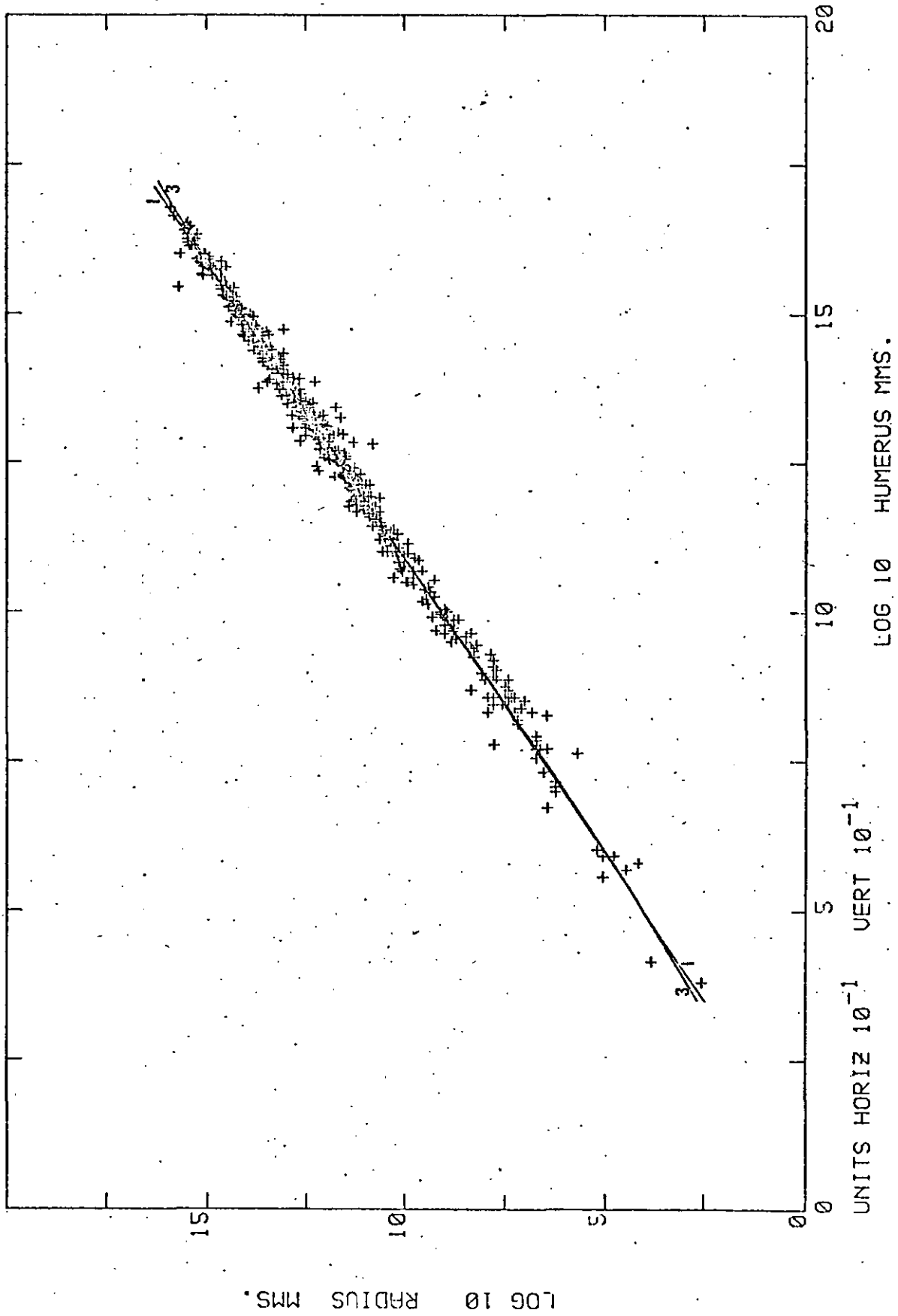


Figure 58. The length of the ossified shaft of the humerus and the length of the ossified shaft of the radius plotted on log./log. axes in an assessment of allometric growth.

- 1 = Regression equation to the power 1 (straight line)
- 3 = Regression equation to the power 3 (best fit)

SUMMARY

1. Graphs and equations are presented from which estimates of the lengths of the ossified shafts of the long bones of the limbs can be made based upon fetal age. Also included is an estimate of the error involved from which it is hoped abnormal growth can be detected.
2. Asymmetrical growth was found to be common within fetuses. For the humerus, tibia, and fibula, growth was found to be significantly favoured on the left side whilst for the femur growth was found to be significantly favoured on the right side. It was suggested that these results might be related to similar postnatal findings.
3. Evidence was found which suggested that the female fetus is in advance of the male fetus in terms of ossification.
4. Criticism is made of those investigators who apply the concept of allometric growth to the ossification of the long bone shafts because considerable doubt arises concerning the validity of this approach when mathematical refinements are applied to the procedure.
5. Graphs and equations are presented which represent the cross-sectional velocity of growth of the ossified shafts of the long bones of the limbs.
6. The relative growth of each of the ossified shafts to each of the other shafts has been calculated and from the respective graphs and equations the relative growth of each of the ossified shafts can be assessed. It is anticipated that such procedures will have particular relevance in clinical situations.

THE LONGITUDINAL DEVELOPMENT OF THE VERTEBRAL COLUMN

The data collected from the measuring of the longitudinal lengths of the vertebral column were subjected to polynomial regression analysis using computer programmes (See Appendix B). Equations up to and including the power of 3 were attempted and lines of best fit were selected as described previously. The results of this procedure can be seen in Table 28 and in Figure 59 the equations are presented graphically for comparative purposes.

When examining Figure 59 it must be remembered that the length shown for each of the graphs can only represent the total length for any particular region when all the ossification centres for that region have appeared. Therefore bearing in mind Figures 31 and 32 which show the appearance times for the centres of ossification of the vertebral column, marks have been placed on the graphs in Figure 58 to show the times after which all the fetuses examined had all of the ossification centres present for that particular vertebral region. After these times, the graphs can be said to represent the length of the whole of a particular vertebral region.

From the morphology of a single vertebra, any point can be selected from which measurements can be taken to determine the distance between one vertebra and another. If the point selected is in the same relative position on each of the other vertebrae then the distance measured between the vertebrae will be the same no matter which point is selected. If however the point selected moves in relation to each vertebra then the distance measured between the vertebrae will be peculiar to the points originally selected. This explains why in Figure 59 there are graphs showing the longitudinal distance between the neural arches for a particular vertebral region which are separate from the graphs representing the same vertebral region but which are based upon measurement between the centra. In different vertebrae the neural arch ossification centres develop in a different position relative to the centrum ossification centre

Table 28

Regression Equations for the Longitudinal Growth of the Regions of the Vertebral Column in relation to Gestational Age

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Centra	97	$Y = -10.284807 + 107.98107X - 67.035127X^2$	1.68	0.89
Thoracic Centra	363	$Y = -28.070603 + 247.673976X - 691.966170X^2$	1.99	0.99
Lumbar Centra	371	$Y = -16.111873 + 133.828985X - 69.931048X^2$	1.20	0.98
Sacral Centra	63	$Y = 1.543283 + 30.906683X$	1.77	0.68
Cervical Neural Arches	391	$Y = -12.241451 + 111.162235X - 75.277101X^2$	1.54	0.95
Thoracic Neural Arches	380	$Y = -32.109091 + 277.583463X - 169.566457X^2$	2.04	0.99
Lumbar Neural Arches	303	$Y = -14.952443 + 130.175581X - 74.730357X^2$	1.51	0.97
Sacral Neural Arches	28	$Y = 0.388824 + 33.653800X$	1.29	0.83

COMPARISON OF GROWTH OF COLUMN LENGTHS

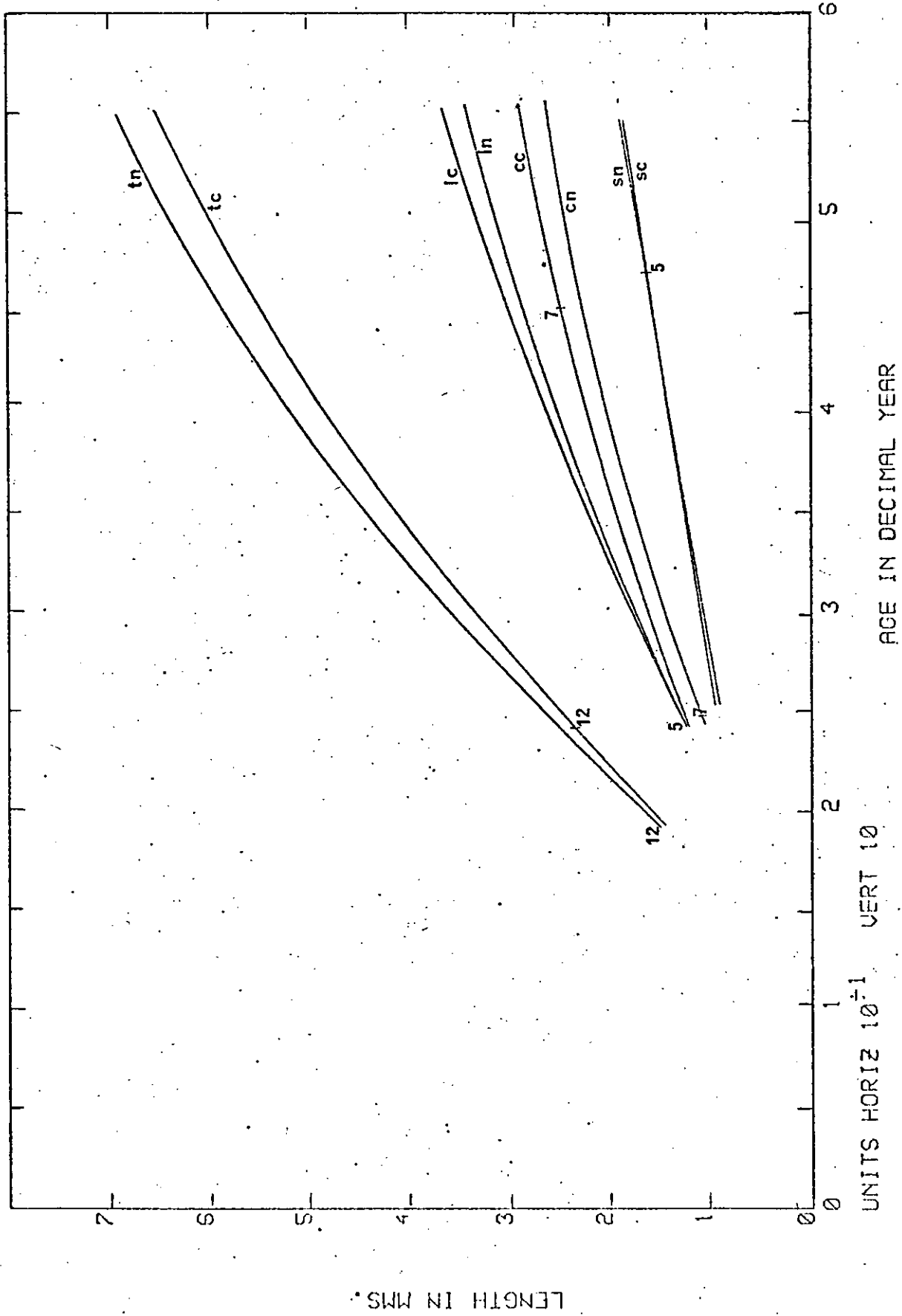


Figure 59. Comparison of the longitudinal lengths of the ossified sections of the vertebral regions.

n = neural arches c = cervical
 t = thoracic
 c = centra l = lumbar
 s = sacral

and this allows different measurements of the vertebral regions to be made. In addition, in any single vertebra it is possible that the relative position between the neural arch ossification centres and the centrum ossification centre changes as the vertebra develops, which would again make any measurement between vertebrae dependent upon the points chosen.

Measurements were taken of the lengths of both the neural arch and centra ossification centres for each vertebral region for another reason also. The ossification centres for the vertebral neural arches and centra develop independently of each other and whilst these centres are appearing the situation might arise where it would be more meaningful to obtain a measurement from either the vertebral neural arch ossification centres or the vertebral centra, particularly if no ossification centres have yet appeared in either the neural arches or centra ossification centres for a particular vertebral region.

Figure 59 shows that the four regions of the vertebral column have different lengths throughout the period of fetal life under investigation. The thoracic region is always the longest region and the sacral region is always the shortest. Intermediate to these are the lengths of the cervical and lumbar regions of which the lumbar is always the largest. In the thoracic region the length of the vertebral neural arch ossification centres is always greater than the equivalent centra measurement whilst in the lumbar and cervical regions the reverse situation is present. In the sacral region the lengths of the vertebral neural arch and centra ossification centres are very similar and this difference in length between the neural arch and centra ossification centres for each region of the vertebral column reflects the change in relative position of the neural arch ossification centres to the centra ossification centres for the vertebrae.

Table 28 shows that the shapes of the graphs in Figure 59 are very

similar with all, except the sacral region, being represented by regression equations to the power two with negative second coefficients indicative of a gradually decreasing rate of growth. The sacral region is represented in both instances by a straight line which indicates a uniform rate of growth and Figure 59 shows that this is compatible with the other equations. The relatively low multiple coefficients of correlation (R) associated with the lengths of the cervical centra, sacral centra, and sacral neural arches reflects the long period of time required for the appearance of all of the ossification centres for these regions. At any particular fetal age there might well be considerable differences in terms of maturity between fetuses and this could manifest itself in the number of ossification centres that are present for a particular region. Therefore, before all of the ossification centres for a particular region have appeared, the length of that region as measured by this study is affected by the skeletal maturity of the fetus and, as such, could considerably increase the variation about any regression line and lower the value of the respective coefficient of correlation.

It is perhaps misleading to directly compare the lengths of the regions of the vertebral column because each region is made up of different numbers of vertebrae. It is therefore worthwhile to compare the vertebral regions using lengths of "average" vertebrae. Table 29 shows the lengths of the various regions of vertebral centra ossification centres for specific fetal ages taken from Table 28. Only those lengths after which all the vertebral centra ossification centres have appeared, are included and accounts for the small number of results for the cervical and sacral centra. Also shown are the lengths of the "average" vertebra for each of the four regions and these were obtained by dividing the lengths of cervical centra, thoracic centra, lumbar centra, and sacral centra by 7, 13, 5 and 5 respectively. For comparative reasons, each of these individual vertebral lengths has been compared to the "average" lumbar vertebra.

Table 29

Comparitive Lengths of Individual Vertebrae

Age Dec Yr	TOTAL LENGTH				LENGTH OF INDIVIDUAL VERTEBRAE				Cerv Lumb	Thor Lumb	Sac Lumb
	Cerv Centra mm	Thor Centra mm	Lumb Centra mm	Sacr Centra mm	Cer mm	Thor mm	Lumb mm	Sacr mm			
0.2			10.4				2.1				
0.225			13.0				2.6				
0.25		25.0	15.4			2.1	2.6			0.80	
0.275		29.3	17.7			2.4	3.1			0.79	
0.3		33.5	20.0			2.8	3.5			0.79	
0.325		37.4	22.1			3.1	4.0			0.78	
0.35		41.2	24.3			3.4	4.4			0.78	
0.375		44.9	26.2			3.7	4.9			0.77	
0.4		48.3	28.2			4.0	5.2			0.77	
0.425		51.6	15.5			4.3	5.6			0.76	
0.45	24.7	54.7	30.0	15.5	3.5	4.6	6.0	3.1	0.589	0.76	0.516
0.475	25.9	57.5	31.7	16.2	3.7	4.8	6.3	3.2	0.584	0.76	0.511
0.5	27.0	60.2	33.3	17.0	3.9	5.0	6.7	3.4	0.578	0.75	0.511

A different picture in terms of development emerges when the lengths of the individual vertebrae are viewed. The typical lumbar vertebra is by far the largest vertebra with the average thoracic vertebra being only approximately three-quarters of its length. The typical sacral and cervical vertebrae are only approximately half the length of the average lumbar vertebra with the cervical vertebra being slightly larger than the sacral. As the fetal age increases it is interesting to note that the lumbar vertebra gets even bigger relative to the other vertebrae which suggests that vertebral growth is peculiar at least to the individual regions. A more detailed study might reveal that the manner of growth is peculiar to the individual vertebra, implying that division of the vertebral column into four distinct regions might well be misleading.

Other investigators of the proportionate regional lengths of the vertebral column include Aeby (1879), Ballantyne (1892), and Bardeen (1905). Bardeen (1905) reports that Aeby (1879) showed that in young embryos the cervical region is relatively longer than the lumbar region but that as growth proceeds there is a constant proportional increase in the length of the latter over the former. Taking the cervical region as 100 for instance he found that in embryos below 10 mm. C.R. length the lumbar region equals 69 while in the adult it is equivalent to 150. Similarly Ballantyne's measurements (1892) show that only in the later parts of fetal life does the growth of the lumbar spine begin to surpass that of the cervical region, so that at the time of birth these regions are not equal in length, but the lumbar is distinctly longer than the cervical. Bardeen (1905) also supports these findings by stating that the length of the cervical region during the second and third fetal months is about 60% that of the thoracic. He also found that the lumbar region is at first less than 40% in length of the thoracic but increases to 50%, whilst the length of the sacral region varies from 33 to 42.5% of the thoracic. These earlier results therefore appear to be supported and extended by the present study even though

precise definitions of their measurements are not included in the respective papers of previous authors.

The high multiple coefficients of correlation (R) shown in Table 28 for the lengths of the thoracic and lumbar vertebral centra ossification centres and the cervical, thoracic and lumbar vertebral neural arch ossification centres means that an accurate estimate of these lengths can be made based upon fetal age. The high correlation for these particular regions is due in part to the short period of time over which the ossification centres for these regions appear. The use of the S.E.E. as a first approximation of normal values will allow any abnormal estimates to be easily recognised and it is anticipated that the graphs and equations will have particular use in studies of spina bifida, anencephaly, and scoliosis. It is hoped that a better understanding of the extent and range of these abnormalities can now be achieved and perhaps the graphs and equations can also be applied to radiographs taken of fetuses in utero particularly in those cases where the radiograph has been taken at a specific angle following a prior use of ultrasound to determine the fetal position.

For future work, the high R-values shown in Table 28 suggest that it would be worthwhile producing similar equations from which an estimate of fetal age can be made based upon the measurement of vertebral lengths and if these proved useful a fetometer similar to that used by Chang et.al.(1971) might be developed, although the reluctance of radiologists to radiograph young fetuses in utero might restrict its application.

By differentiating the equations in Table 28, equations representing the growth velocities of the lengths of the various regions of the column are obtained and are shown in Figure 60. All of the regions of the vertebral column except for the sacral region are slowing down in the development of their length. Whilst the sacral region is growing at a constant rate the lumbar and cervical regions are slowing down at approximately the same rates and the thoracic

COMPARISON OF GROWTH VELOCITIES OF COLUMN LENGTHS

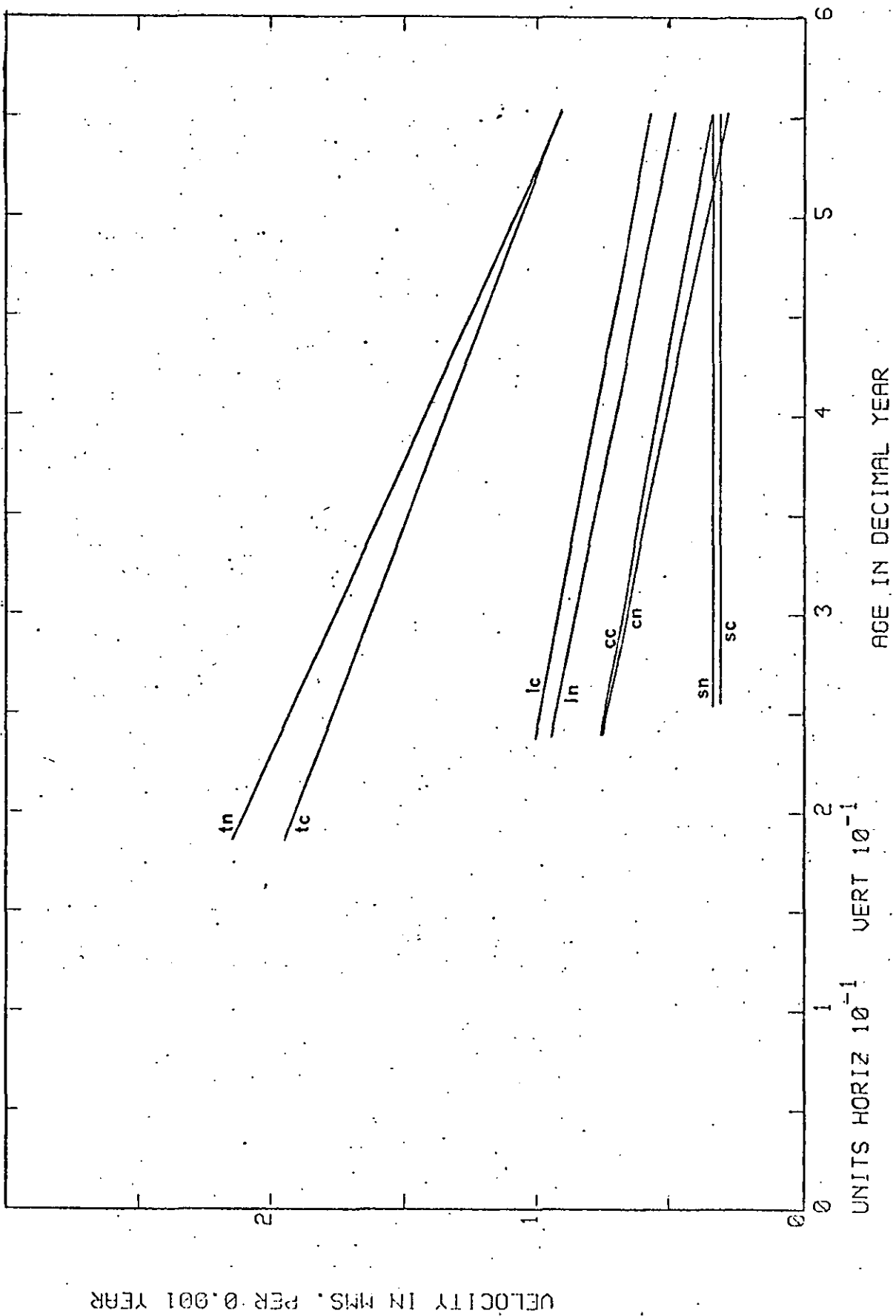


Figure 60. Comparison of the growth velocities of the longitudinal lengths of the various regions of the vertebral column.

region is slowing down at approximately double the rate of either the lumbar or cervical regions. This shows that the thoracic region is getting smaller relative to the other regions whilst the sacral region is getting larger. Relative to each other the cervical and lumbar regions are remaining approximately constant and demonstrates once more that each region of the vertebral column grows independently of the other regions. The growth control factors therefore affect each of the regions of the vertebral column in a different manner and once again a more detailed study might reveal that each individual vertebra has its own particular growth characteristics independent of all others.

Attempts were made to relate the growth of the various regions of the vertebral column to each other and it was thought that this would be especially useful in those cases where fetal age was not known. Accordingly each of the lengths of the vertebral column were related to each other and the data were subjected to polynomial regression analysis. The lines of best fit were then determined as described previously and the results are shown in Tables 30, 31, 32, 33, 34, 35, 36, and 37 with corresponding graphs shown in Figures 61, 62, 63, 64, 65, 66, 67 and 68 respectively. It is anticipated that from such graphs and equations accurate estimates of the lengths of the various regions of the vertebral column can be made from measurements of any of the other vertebral regions although the relatively low multiple coefficients of correlation associated with the cervical and sacral centra ossification centres and the sacral neural arch ossification centres severely restricts their accuracy in these regions. By using the S.E.E. as a first approximation of normal growth it is expected that any abnormal growth can be easily detected and it is in this way that the detailed investigations of abnormalities such as anencephaly, spina bifida, and scoliosis can be carried out. Whether or not such abnormalities affect the longitudinal growth of the vertebral column can now be ascertained and, if it does, whether or not it affects different regions

Table 30 Regression Equations Relating the Length of the Cervical Centra to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Thoracic Centra	97	$Y = 30.294785 - 3.626744X + 0.352060X^2 - 0.006729X^3$	3.25	0.92
Lumbar Centra	97	$Y = -14.905967 + 2.723452X - 0.037890X^2$	2.24	0.89
Sacral Centra	51	$Y = 6.957798 + 0.329561X$	2.01	0.41

Table 31 Regression Equations Relating the Length of the Thoracic Centra to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Centra	95	$Y = 2.369325 + 0.405331X$	1.66	0.89
Lumbar Centra	360	$Y = -1.038516 + 0.563325X$	0.89	0.99
Sacral Centra	63	$Y = 2.191602 + 0.243522X$	1.628	0.73

Table 32

Regression Equations Relating the Length of the Lumbar Centra to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Centra	95	$Y = 4.427131 + 0.666498X$	1.82	0.87
Thoracic Centra	358	$Y = 6.945799 + 0.783852X + 0.059989X^2 - 0.001119X^3$	1.49	0.99
Sacral Centra	63	$Y = 3.636962 + 0.392069X$	1.60	0.75

Table 33

Regression Equations Relating the Length of the Sacral Centra to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Centra	50	$Y = 15.715851 + 0.50727X$	2.51	0.41
Thoracic Centra	62	$Y = -12.562448 + 6.502X - 0.148265X^2$	4.79	0.76
Lumbar Centra	62	$Y = -12.691915 + 4.167931X - 0.095162X^2$	2.96	0.77

Table 34

Regression Equations Relating the Length of the Cervical Neural Arches to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Thoracic Neural Arches	380	$Y = 0.507651 + 2.472425X$	3.83	0.95
Lumbar Neural Arches	303	$Y = 0.728115 + 1.176226X$	2.15	0.93
Sacral Neural Arches	30	$Y = 49.899806 - 3.907323X + 0.103580X^2$	1.62	0.67

Table 35

Regression Equations Relating the Length of the Thoracic Neural Arches to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Neural Arches	380	$Y = 1.025609 + 0.368829X$	1.48	0.95
Lumbar Neural Arches	303	$Y = -0.029815 + 0.485675X$	1.26	0.98
Sacral Neural Arches	30	$Y = 1.744893 + 0.236006X$	1.38	0.77

Table 36

Regression Equations Relating the Length of the Lumbar Neural Arches to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Neural Arches	303	$Y = 4.854693 - 0.022008X + 0.049689X^2 - 0.000965X^3$	1.61	0.93
Thoracic Neural Arches	303	$Y = 15.979528 - 0.686920X + 0.152179X^2 - 0.002688X^3$	2.25	0.98
Sacral Neural Arches	30	$Y = 4.081700 + 0.389837X$	1.48	0.72

Table 37

Regression Equations Relating the Length of the Sacral Neural Arches to the other Regions of the Vertebral Column

Vertebral Region	No of Fetuses	Equation	S.E.E.	R
Cervical Neural Arches	32	$Y = 9.616336 + 0.755756X$	2.96	0.5
Thoracic Neural Arches	32	$Y = 14.283833 + 2.709174X$	4.56	0.8
Lumbar Neural Arches	32	$Y = 5.095808 + 1.470768X$	2.77	0.77

COMPARISON OF COLUMN LENGTHS

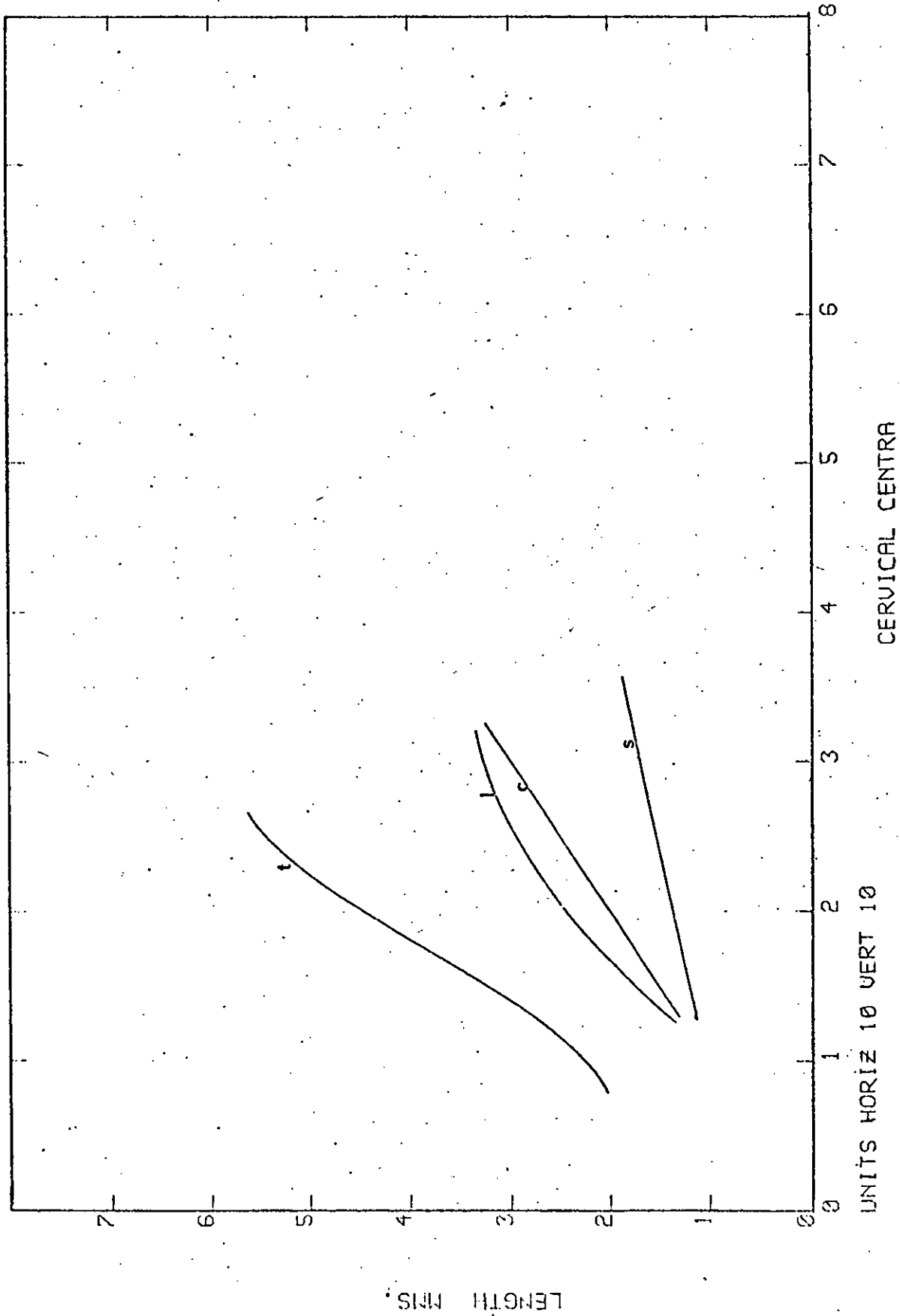


Figure 61. Comparison of the regional lengths of vertebral centra to the length of the cervical centra.

COMPARISON OF COLUMN LENGTHS

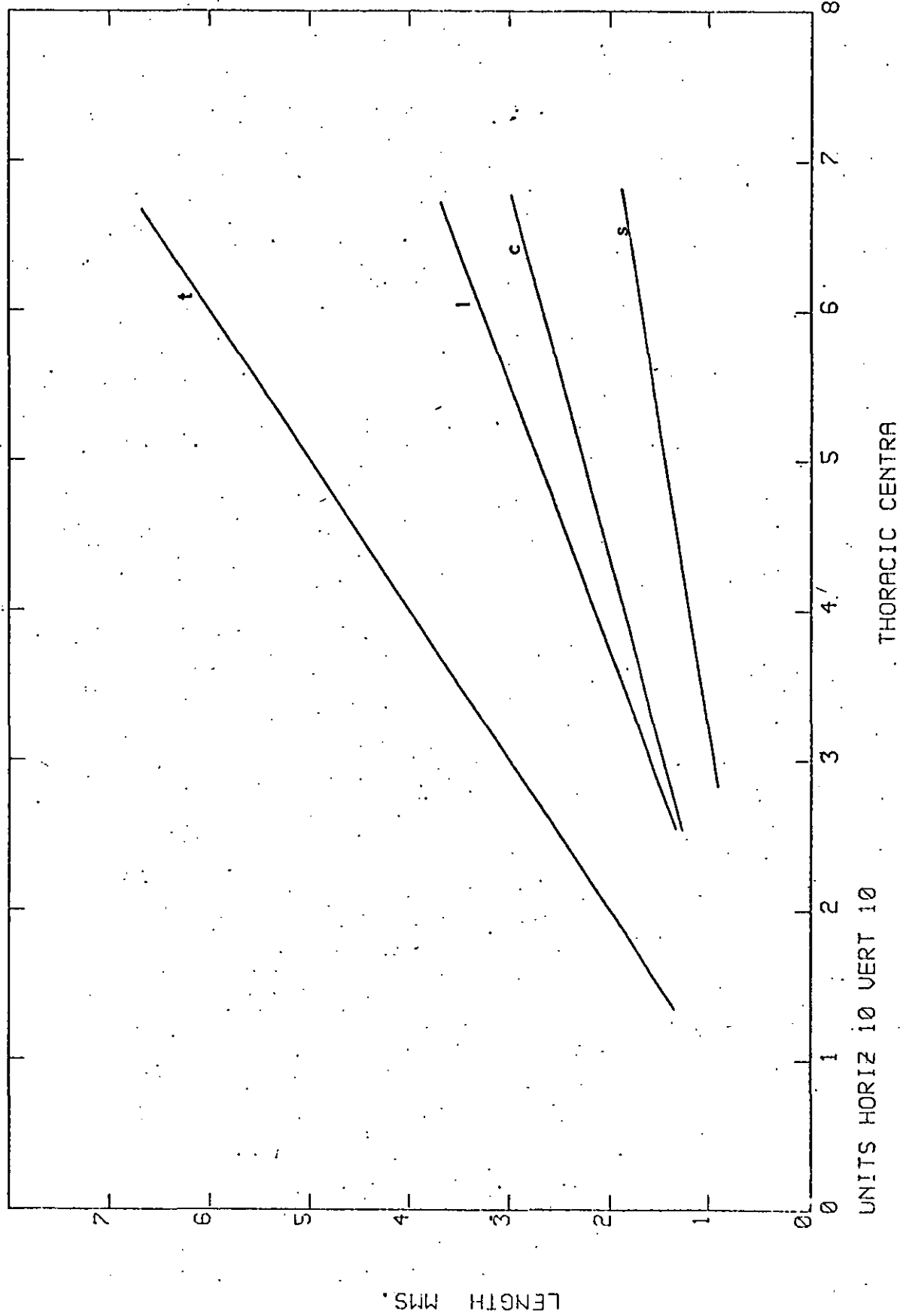


Figure 62. Comparison of the regional lengths of vertebral centra to the length of the thoracic centra.

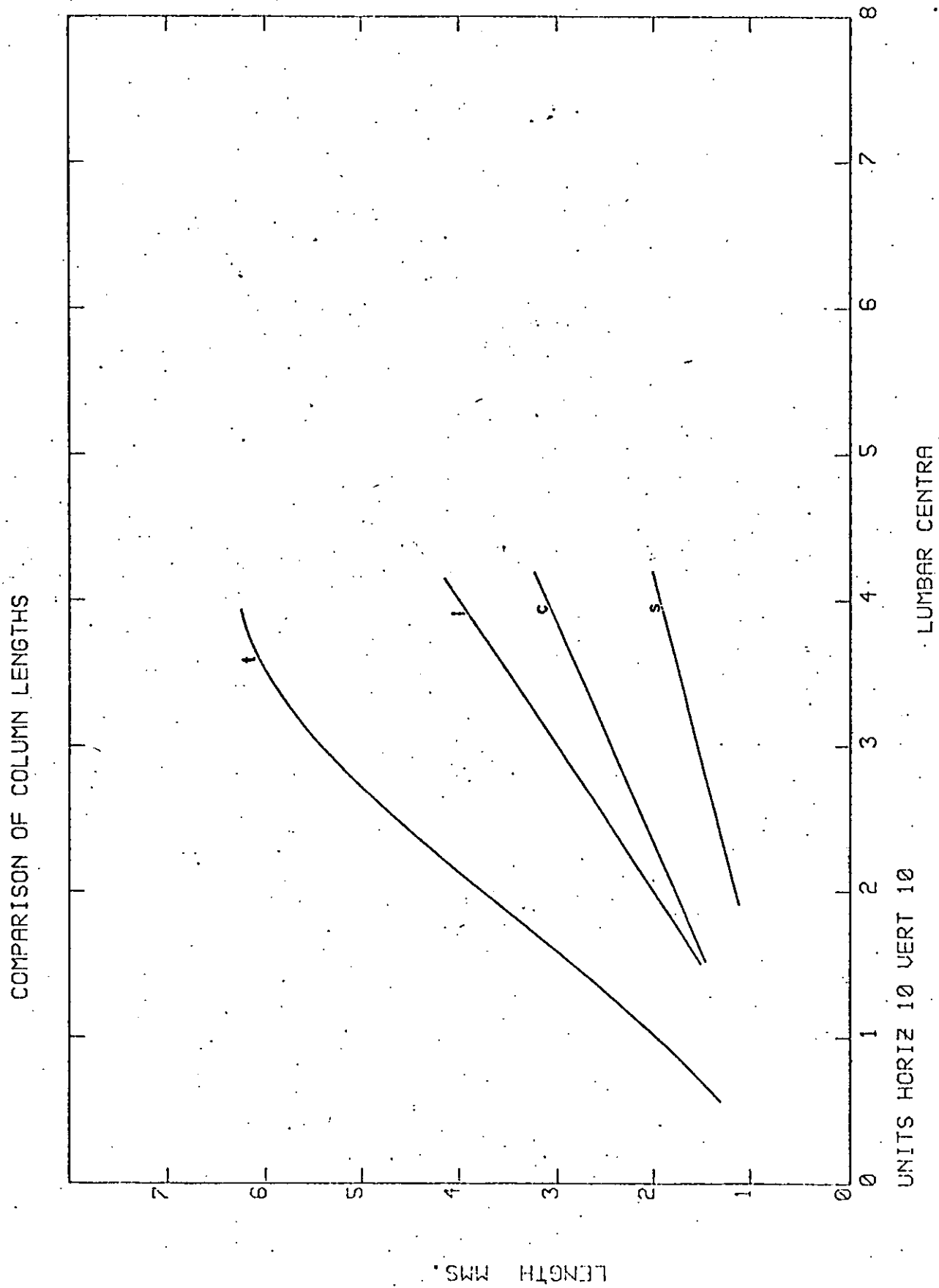


Figure 63. Comparison of the regional lengths of the vertebral centra to the length of the lumbar centra.

COMPARISON OF COLUMN LENGTHS

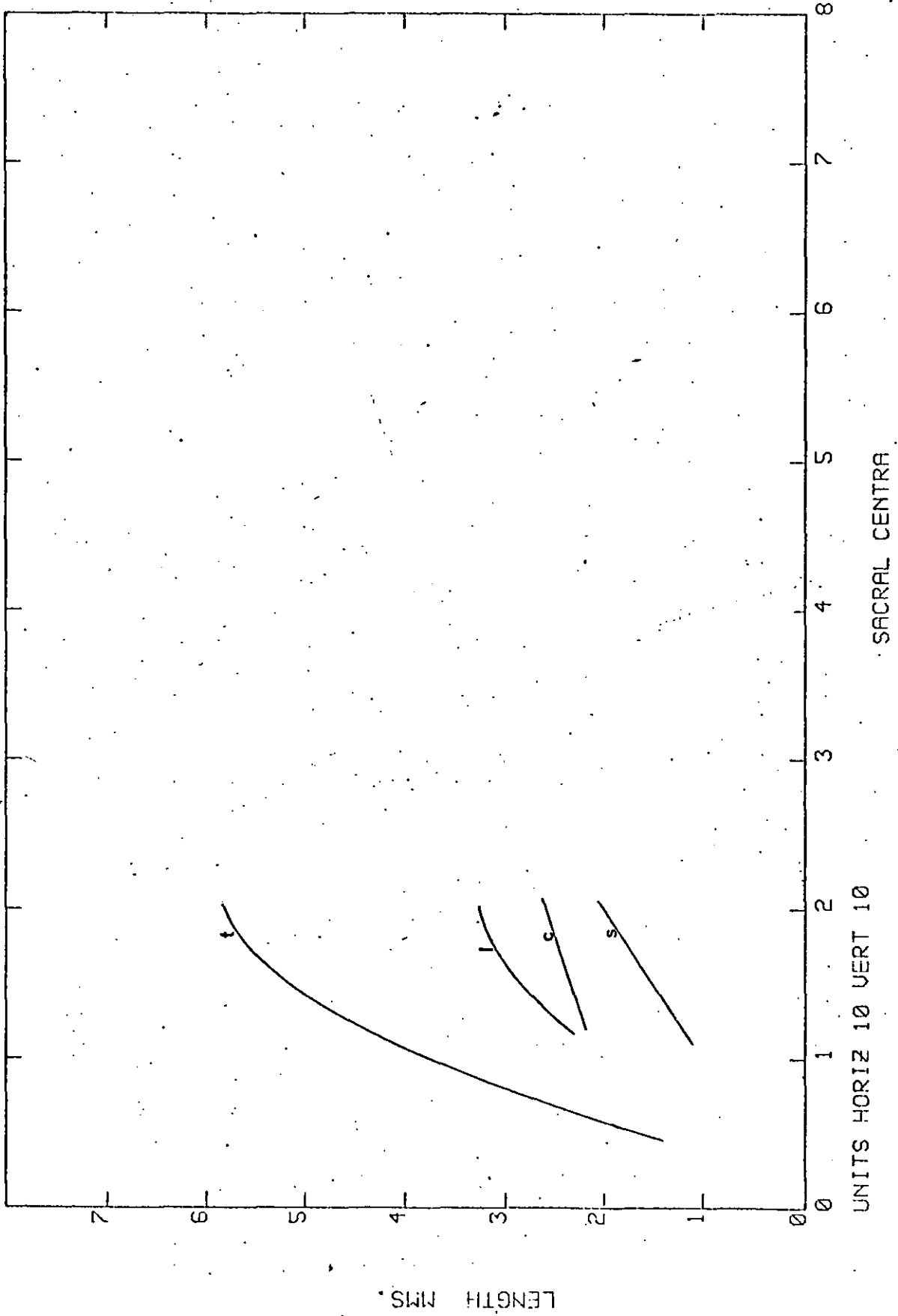


Figure 64. Comparison of the regional lengths of the vertebral centra to the length of the sacral centra.

COMPARISON OF COLUMN LENGTHS

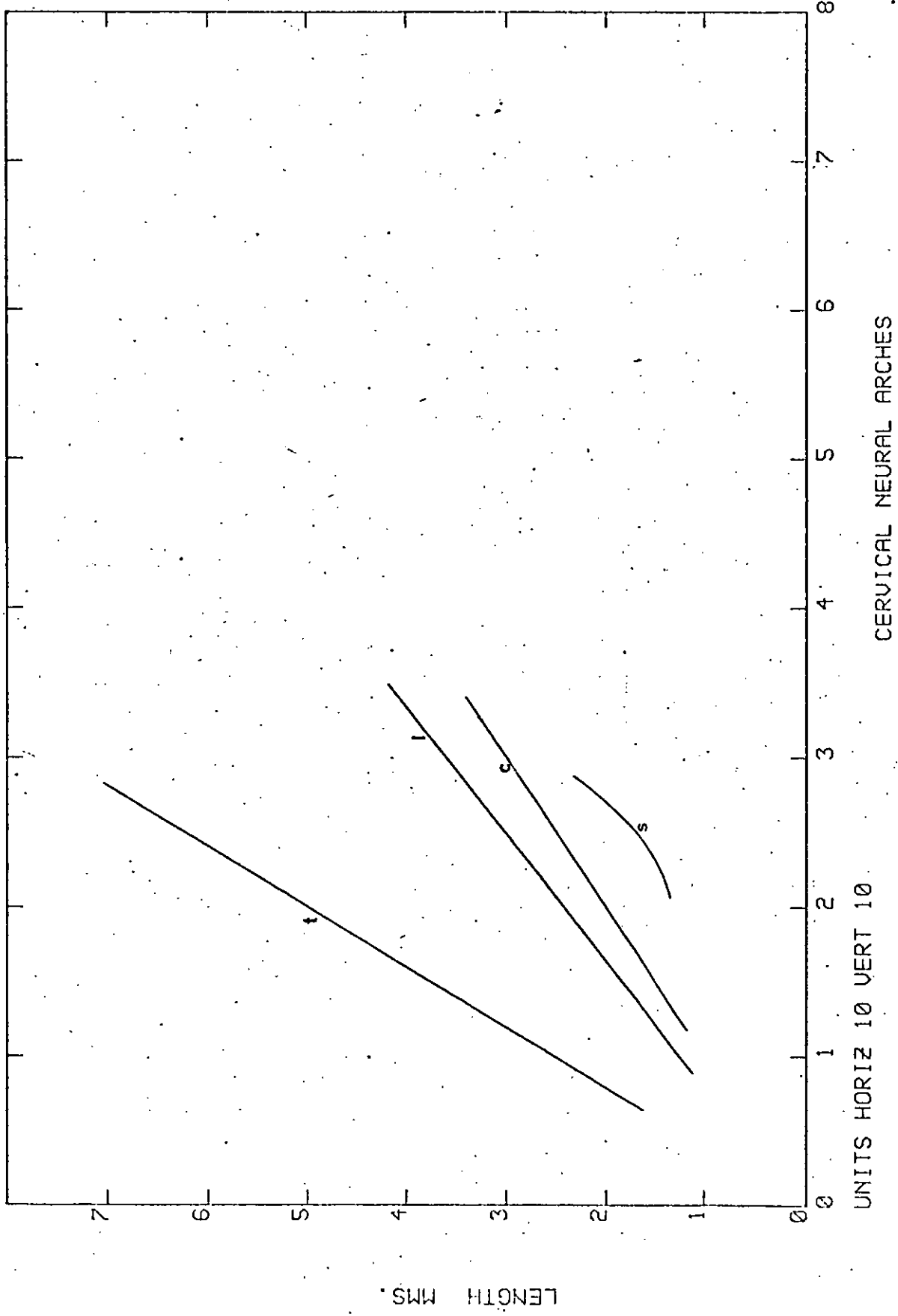


Figure 65. Comparison of the regional lengths of the vertebral neural arches to the length of the cervical neural arches.

COMPARISON OF COLUMN LENGTHS

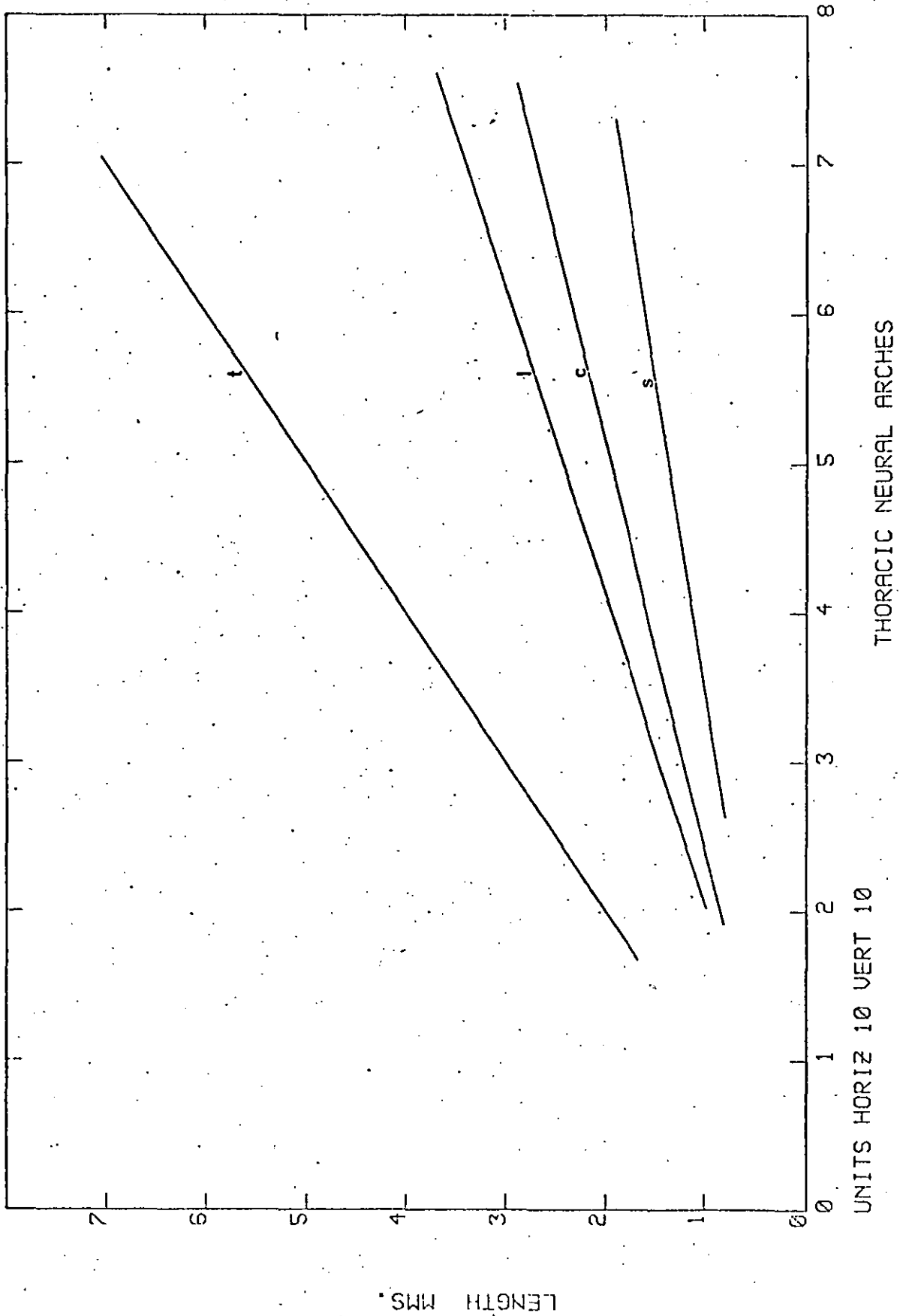


Figure 66. Comparison of the regional lengths of the vertebral neural arches to the length of the thoracic neural arches.

COMPARISON OF COLUMN LENGTHS

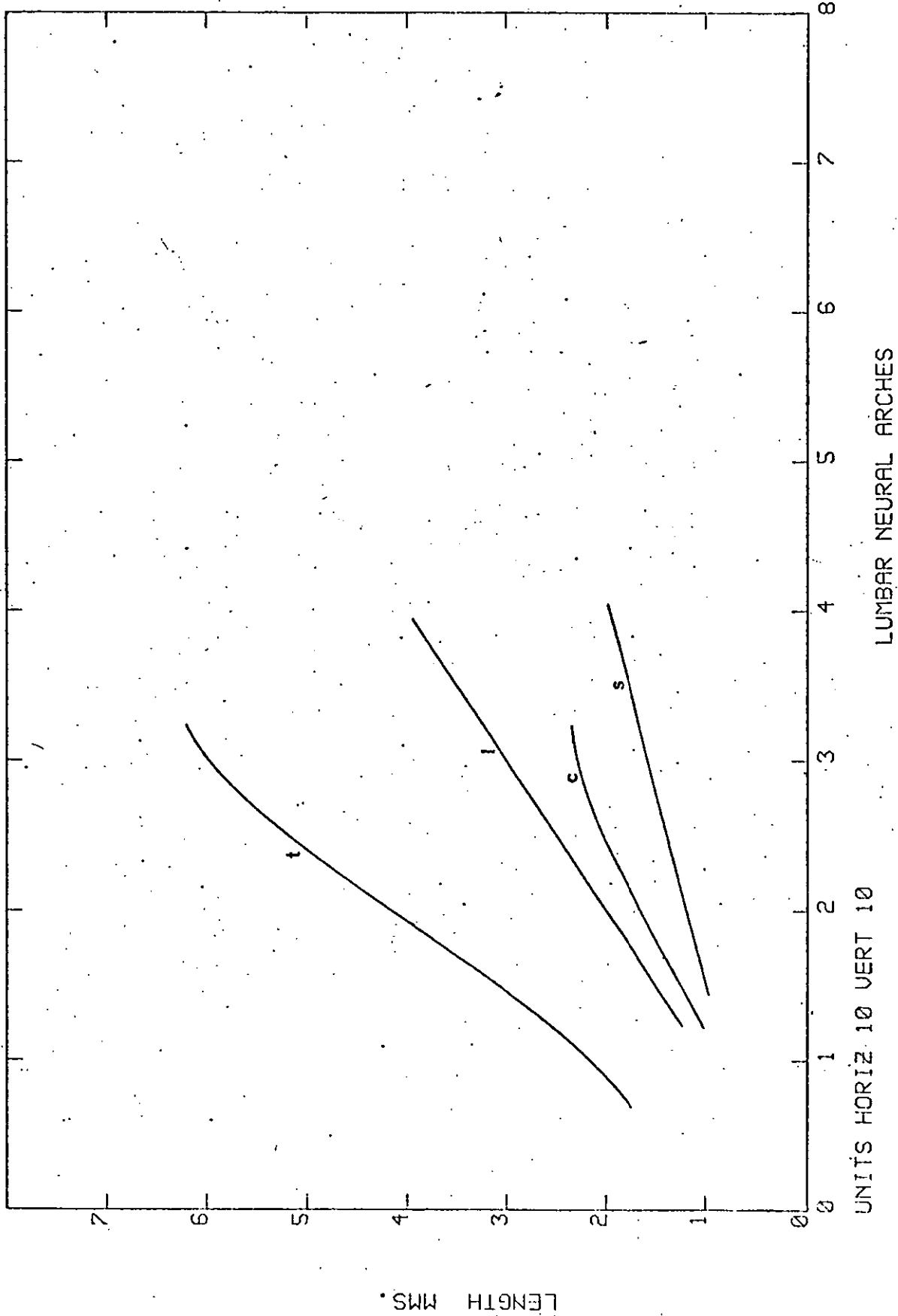


Figure 67. Comparison of the regional lengths of the vertebral neural arches to the length of the lumbar neural arches.

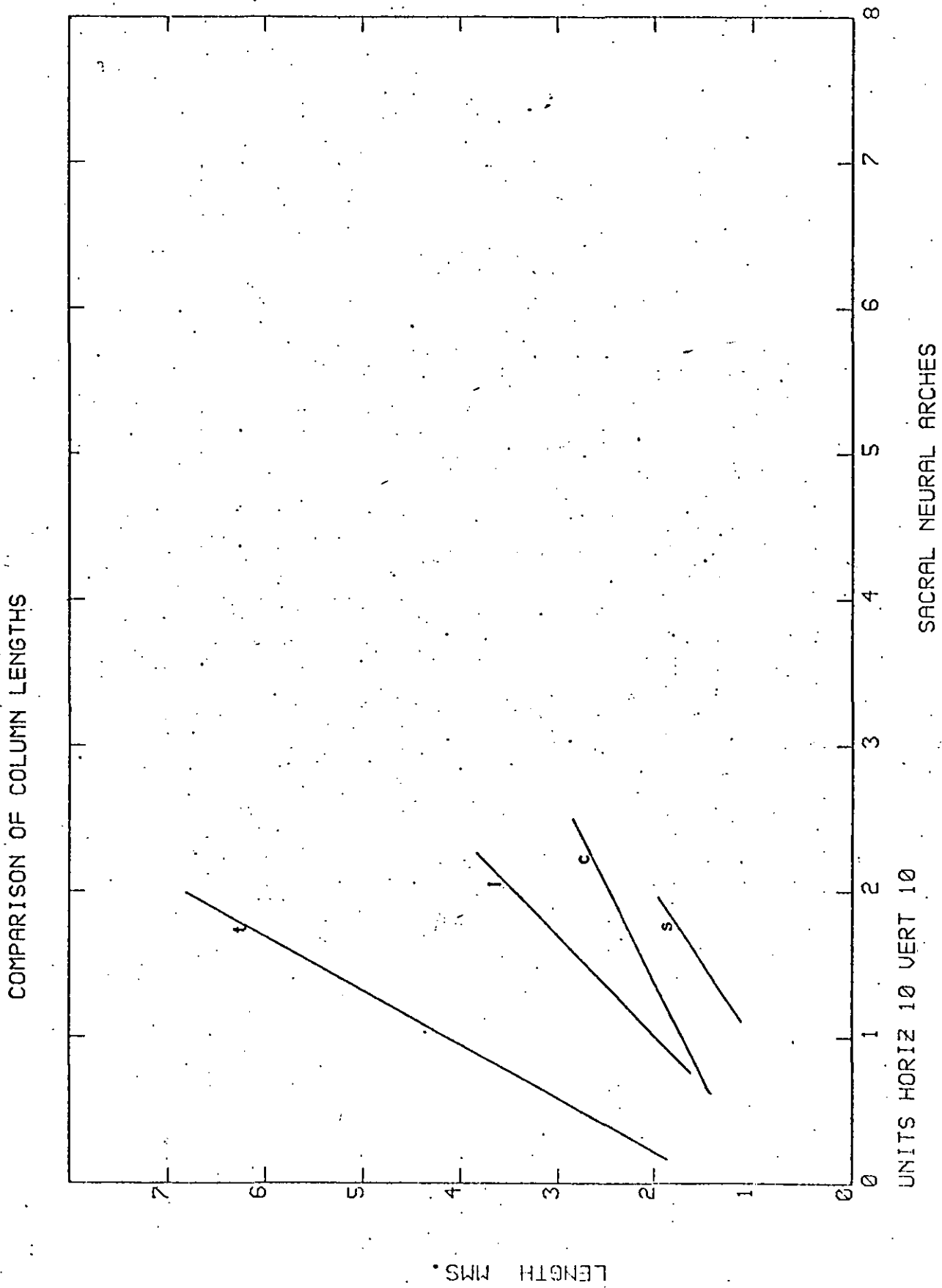


Figure 68. Comparison of the regional lengths of the vertebral neural arches to the length of the sacral neural arches.

equally can also be determined.

Growth of the vertebral column can never be divorced from growth of the spinal cord which develops so intimately with the column. Recently Barson and Sands (1976) have completed measurements on the growth of the spinal cord during fetal life and have produced regression equations which relate the growth in length of the various regions of the cord to fetal age. They measured length in centimetres and menstrual age in weeks but unfortunately combined the lengths of the lumbar and sacral regions. They also attempted to fit several types of curve to their data and found that the allometric equation ($y = AX^B$) and a quadratic equation fitted the data points equally well. These equations are shown in Table 38.

Emerging from this work is a question concerning the selection of appropriate mathematical model to describe the growth of a particular parameter. In the work of Barson and Sands (1976) both allometric and quadratic equations fitted the data points equally well. Using the allometric models however the power of X is always greater than 1 which means that the slope of these curves is always increasing and that the spinal cord growth velocity in all regions is increasing. On the other hand the quadratic models of growth all have negative second coefficients which mean that the slopes of these curves are decreasing and that the velocity of growth of the spinal cord in all regions is getting less. This is a fundamental difference with possibly far-reaching consequences and highlights some of the problems that are encountered when studying cross-sectional data such as is available in fetal studies. Arguments for and against both models can be put forward and it remains to be seen which, if either, represents the true situation.

For comparison with the graphs from this study which show the growth of the regions of the vertebral column, the quadratic equations shown in Table 38 have been appropriately adjusted to being able to estimate length of the cord

Table 38

Regression equations relating to the regions of the spinal cord.
From Barson and Sands 1976

MODEL	REGION	EQUATION
Allometric	Cervical	$Y = 0.0563X^{1.1789}$
	Thoracic	$Y = 0.0931X^{1.1960}$
	Lumbosacral	$Y = 0.1015X^{1.0322}$
Quadratic	Cervical	$Y = 0.6166 + 0.1413X - 0.0005X^2$
	Thoracic	$Y = -1.6095 + 0.2976X - 0.0019X^2$
	Lumbosacral	$Y = -0.7561 + 0.1838X - 0.0014X^2$

in mms. from the fetal age in decimal year. The resulting equations have been drawn on a set of axes in addition to the equations from this study which represent the growth of the total length of the various regions of the vertebral column (See Figure 69).

The vertebral neural arch ossification centres vary in their relative positions according to the individual vertebrae and therefore meaningful comparisons of cord lengths can only be made with equivalent lengths of vertebral centra ossification lengths. In the cervical region it can be seen that the length of the cervical cord is always greater than the length of the cervical vertebrae. Barson and Sands (1976) however included 8 segments in the cervical cord region whereas the measurement of the equivalent column length involved only 7 vertebrae. Furthermore, the boundaries of the vertebral cervical region as used in this study mean that half of the first cervical vertebra is excluded from the measured length. When adjustments are made in Figure 69 to account for both of these facts it becomes apparent that the lengths of the cervical vertebrae and the cervical cord are approximately equal at 0.450 yr. (23 weeks) after which the cord length becomes relatively longer. In particular this would mean that the angulation of the spinal nerve roots in the cervical region would be approximately horizontal at 0.450 yr (23 weeks) but would become gradually more caudally orientated as the fetus developed. In the thoracic region the length of the cord is approximately equal to the length of the vertebrae at 0.250 yr. (13 weeks) but as the fetus develops the length of the cord gradually becomes relatively shorter. Therefore the angulation of the thoracic spinal nerve roots would be approximately horizontal at 0.200 yr. (10 weeks) but would gradually become more caudally orientated as the fetus developed. This would actually be accentuated even more with the addition of the discrepancy between the cervical cord and vertebral lengths as the fetus became older. In the lumbosacral region the length of the lumbar vertebrae

COLUMN VERSUS CORD

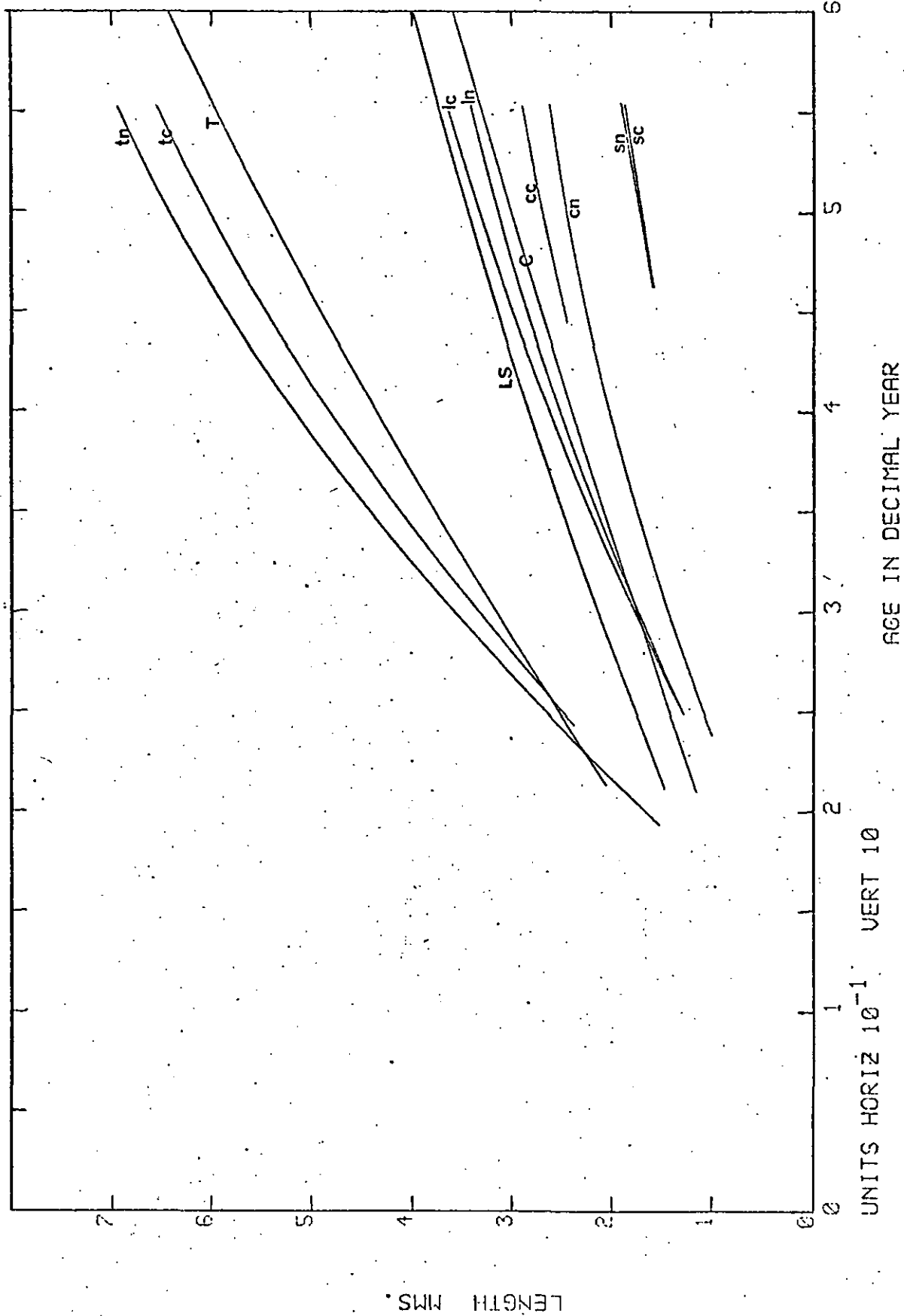


Figure 69. Comparison of growth of the vertebral column with that of the cord during fetal life.

- C = Cervical cord length
- T = Thoracic cord length
- LS = Lumbar Sacral cord length

on their own is approximately equal to the length of the lumbar region of the cord and therefore with the addition of the length of the sacral region of the vertebral column, the lumbosacral cord length is much smaller than the corresponding length of vertebral column even from a very early age. As the fetus gets older this difference gets larger and is accentuated by the displacement of the cord and vertebral column in the more cephalic regions. This means that the angulation of the spinal nerve roots is caudal even from a very early age and becomes even greater as the fetus develops. Obviously assessment of the angulation of the spinal nerve roots by a method such as this must only be very superficial because the actual angulation of each spinal nerve root must be peculiar to itself and its respective intervertebral foramen. However, it is interesting to note that Berry (1956) in a more detailed study describes a similar development as described here for the angulation of the spinal nerve roots during fetal life having based his results on dissection of 10 human fetuses. In this respect, it is reassuring to find that independent workers (Barson and Sands 1976, and this study) investigating related topics can produce comparable results and go a long way to confirming the validity of the measurements taken of the longitudinal lengths of the regions of the vertebral column.

Berry (1956) concludes his study by stating that the spinal cord can be divided into four regions with respect to the angulation of the spinal nerves. These regions are $C_1 - C_4$, $C_5 - T_4$, $T_5 - T_{10}$, and $T_{10} - \text{end}$. He found that the angulation of the individual spinal nerves in these regions follow similar growth patterns and this suggests that it would be worthwhile grouping these sections of the vertebral column and cord together when studying longitudinal growth of the spine rather than the relatively arbitrary division into cervical, thoracic, lumbar, and sacral regions.

SUMMARY

1. Normal standards of growth for the four regions of the vertebral column are presented in both graphic and equation forms. A first approximation for identification of abnormal growth is included and separate standards for neural arch and centra ossification lengths are presented for each region. It is anticipated that such standards will have particular use in the study of abnormalities such as spina bifida, anencephaly, and scoliosis.
2. The lengths of typical vertebrae for each of the four regions of the vertebral column are compared and it was found that the average lumbar vertebra is not only the largest but is growing relatively larger as the fetus develops.
3. The results from this study compare very well with those from other studies which have determined the relative regional lengths from dissection.
4. Growth velocity graphs show that the rates of growth of the regions of the vertebral column are getting less except for the sacral region whose growth velocity remains constant. The growth in length of the thoracic region is slowing down at a rate approximately twice that of both the cervical and lumbar regions and this means that the thoracic region is getting relatively smaller whilst the sacral region is getting relatively larger compared to the other regions.
5. Normal standards of growth are also presented for each of the regions of the vertebral column relative to each other.
6. It was reassuring to find that the results from this study on the growth of the vertebral column compared well with results from a separate study of the spinal cord, and that the conclusions reached by this comparison on the angulation of the spinal nerve roots were confirmed by previous studies.

THE DEVELOPMENT IN WIDTH OF THE VERTEBRAL COLUMN

The distance between the vertebral neural arch ossification centres was measured where possible for each vertebra in each of the fetuses. The data were then subjected to polynomial regression analysis using computer programmes (see Appendix B) and equations up to and including the power of 3 were attempted. The lines of best fit were selected as described previously and the results of this procedure can be seen in Tables 39, 40, 41 and 42. These equations have also been produced in the form of graphs and are presented in Figures 70, 71, 72 and 73 respectively.

The results of this study have shown that the division on morphological grounds of the vertebral column into cervical, thoracic, lumbar and sacral regions might well be misleading when other parameters of vertebral growth are considered. Just as Berry (1956) found that another system of division of the vertebral column was more appropriate when studying the angulation of the spinal nerve roots, so other systems of division may be appropriate with other parameters. With this in mind, the vertebrae were considered to be a continuous series of separate entities when examining the distances between the respective neural arches although for convenience and simplicity they have been presented in the Tables and Figures in groups based upon the normal four regions of the column.

Figure 70 shows that the distance between the neural arch ossification centres for the first cervical vertebra (C_1 - the Atlas) is always considerably larger than for any other vertebra. At 0.200 yr. (10 weeks) it is the largest of the distances and at 0.550 yr. (29 weeks) it is still the largest. This finding is not entirely surprising because the morphology of the atlas is entirely different to any of the other vertebrae with its main function being to provide support for the head. It accomplishes this function by having

Table 39

Regression Equations for the Development of the Cervical Inter-neural Arch Distances in Relation to Gestational Age

Vertebra	No of Fetuses	Equation	S.E.E.	R
C1	476	$Y = -8.371254 + 75.532767X - 57.981121X^2$	0.76	0.97
C2	492	$Y = -6.295763 + 61.248796X - 46.985152X^2$	0.57	0.97
C3	492	$Y = -7.672119 + 69.852301X - 54.596313X^2$	0.56	0.98
C4	492	$Y = -8.113726 + 71.686176X - 54.766347X^2$	0.55	0.98
C5	492	$Y = -8.598049 + 73.476261X - 55.486254X^2$	0.55	0.98
C6	492	$Y = -6.672968 + 55.213353X - 9.894278X^2 - 31.251712X^3$	0.54	0.98
C7	492	$Y = -6.127773 + 48.064891X + 7.391524X^2 - 43.438928X^3$	0.56	0.98

C = Cervical

Table 40 Regression Equations for the Development of the Thoracic Inter-neural Arch Distances in Relation to Gestational Age

Vertebra	No of Fetuses	Equation	S.E.E.	R
T1	501	$Y = -9.766436 + 86.372912X - 128.469263X^2 + 86.076101X^3$	0.53	0.98
T2	500	$Y = -11.261290 + 104.497300X - 203.539379X^2 + 162.335048X^3$	0.49	0.97
T3	495	$Y = -10.379646 + 97.213951X - 191.713924X^2 + 156.558383X^3$	0.48	0.97
T4	490	$Y = 10.633360 + 99.507581X - 200.338831X^2 + 163.072801X^3$	0.43	0.97
T5	486	$Y = -10.83846 + 101.319850X - 206.487523X^2 + 166.397440X^3$	0.42	0.97
T6	485	$Y = -10.802143 + 101.411100X - 209.678376X^2 + 170.968452X^3$	0.42	0.97
T7	480	$Y = -10.621991 + 98.071395X - 196.813425X^2 + 156.088525X^3$	0.42	0.97
T8	473	$Y = -10.738350 + 98.431792X - 197.042227X^2 + 156.774440X^3$	0.42	0.97
T9	474	$Y = -8.879097 + 80.864534X - 144.043720X^2 + 107.730596X^3$	0.44	0.97
T10	478	$Y = -7.746534 + 70.416932X - 112.323778X^2 + 76.910027X^3$	0.43	0.97
T11	482	$Y = -6.818334 + 62.865695X - 93.681955X^2 + 63.920560X^3$	0.46	0.97
T12	483	$Y = -7.676757 + 71.964689X - 120.014923X^2 + 93.034.75X^3$	0.50	0.96

T = Thoracic

Table 41 Regression Equations for the Development of the Lumbar Inter-neural Arch Distances in Relation to Gestational Age

Vertebra	No of Fetuses	Equation	S.E.E.	R
L1	484	$Y = -5.642221 + 48.933453X - 33.447655X^2$	0.50	0.97
L2	473	$Y = -6.369217 + 52.684435X - 36.135931X^2$	0.51	0.97
L3	456	$Y = -6.620423 + 53.552419X - 35.978009X^2$	0.47	0.98
L4	438	$Y = -6.308743 + 51.028698X - 31.989734X^2$	0.48	0.98
L5	423	$Y = -5.718080 + 47.271716X - 25.574375X^2$	0.49	0.97

Table 42 Regression Equations for the Development of the Sacral Inter-neural Arch Distances in Relation to Gestational Age

S1	355	$Y = -4.827216 + 40.793349X - 14.032491X^2$	0.57	0.96
S2	229	$Y = -2.904226 + 25.260915X$	0.79	0.89
S3	167	$Y = -3.386336 + 23.777791X$	0.58	0.92
S4	124	$Y = -4.024220 + 24.520857X$	0.56	0.92
S5	32	$Y = -3.039565 + 20.748957X$	0.72	0.86

L = Lumbar
S = Sacral

COMPARISON OF GROWTH OF CERVICAL INTER NEURAL ARCHES

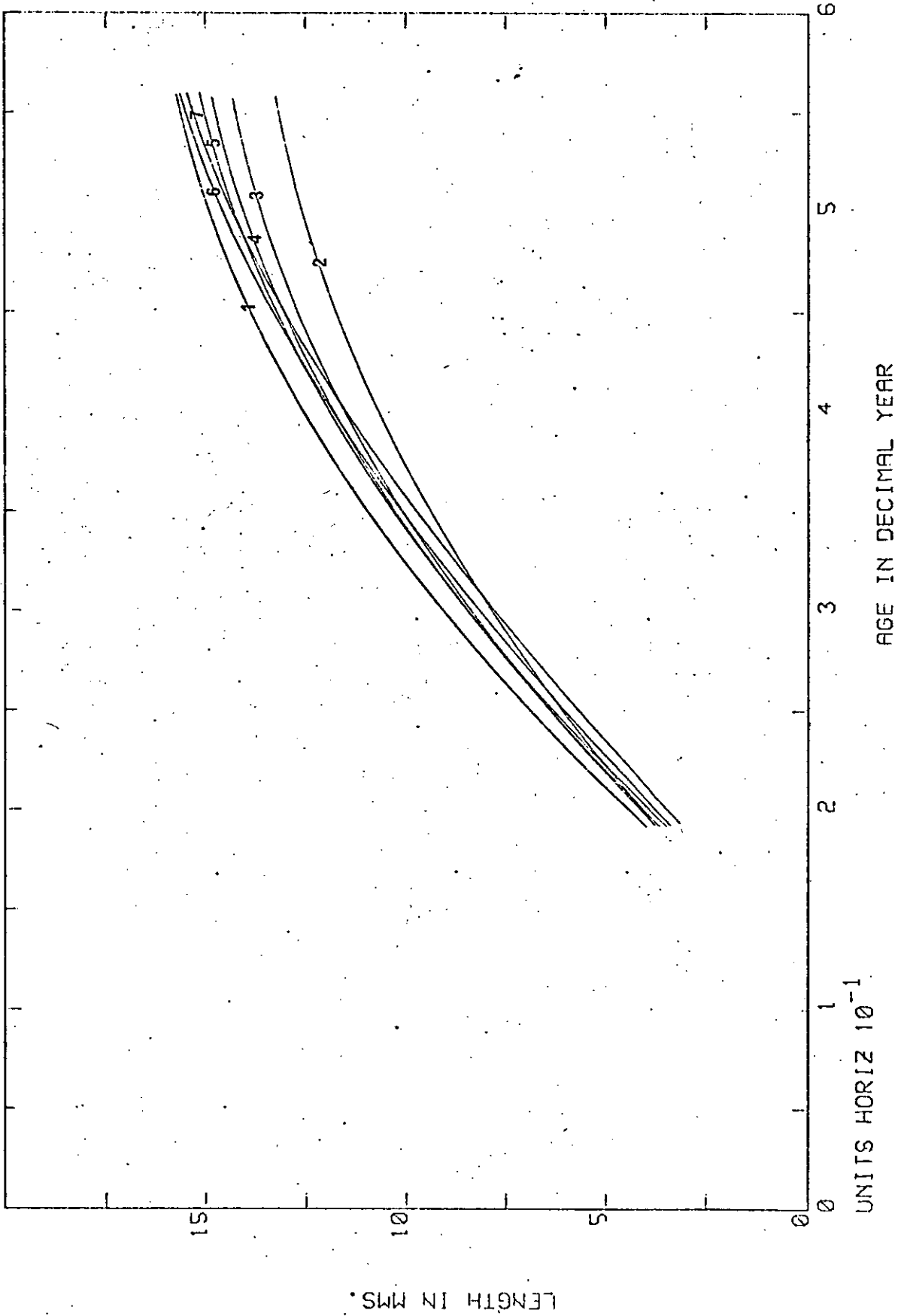


Figure 70. Growth in width of the cervical region of the vertebral column. The numbers refer to the individual vertebrae.

COMPARISON OF GROWTH OF THORACIC INTER NEURAL ARCHES

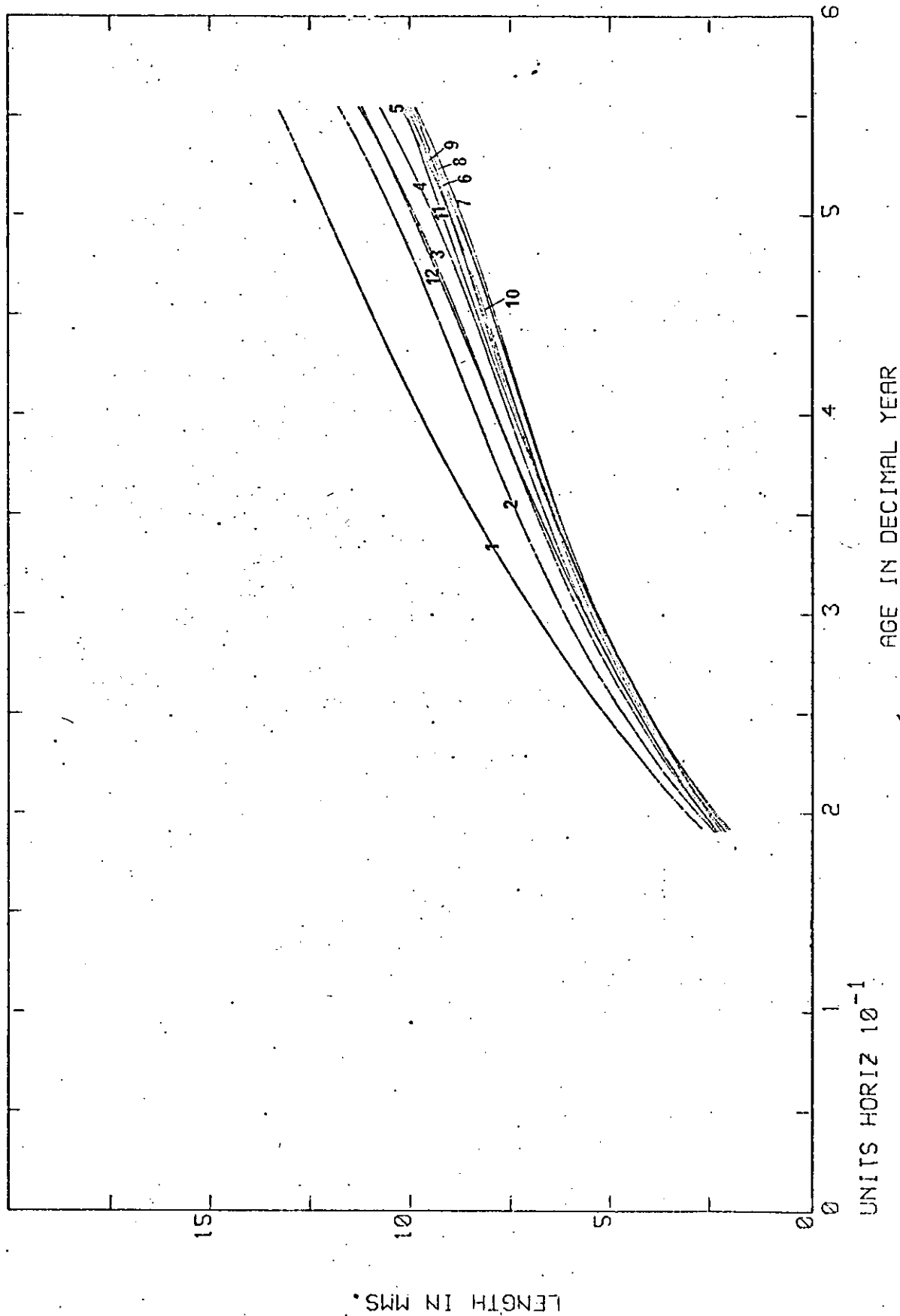


Figure 71. Growth in width of the thoracic region of the vertebral column.

COMPARISON OF GROWTH OF LUMBAR INTER NEURAL ARCHES.

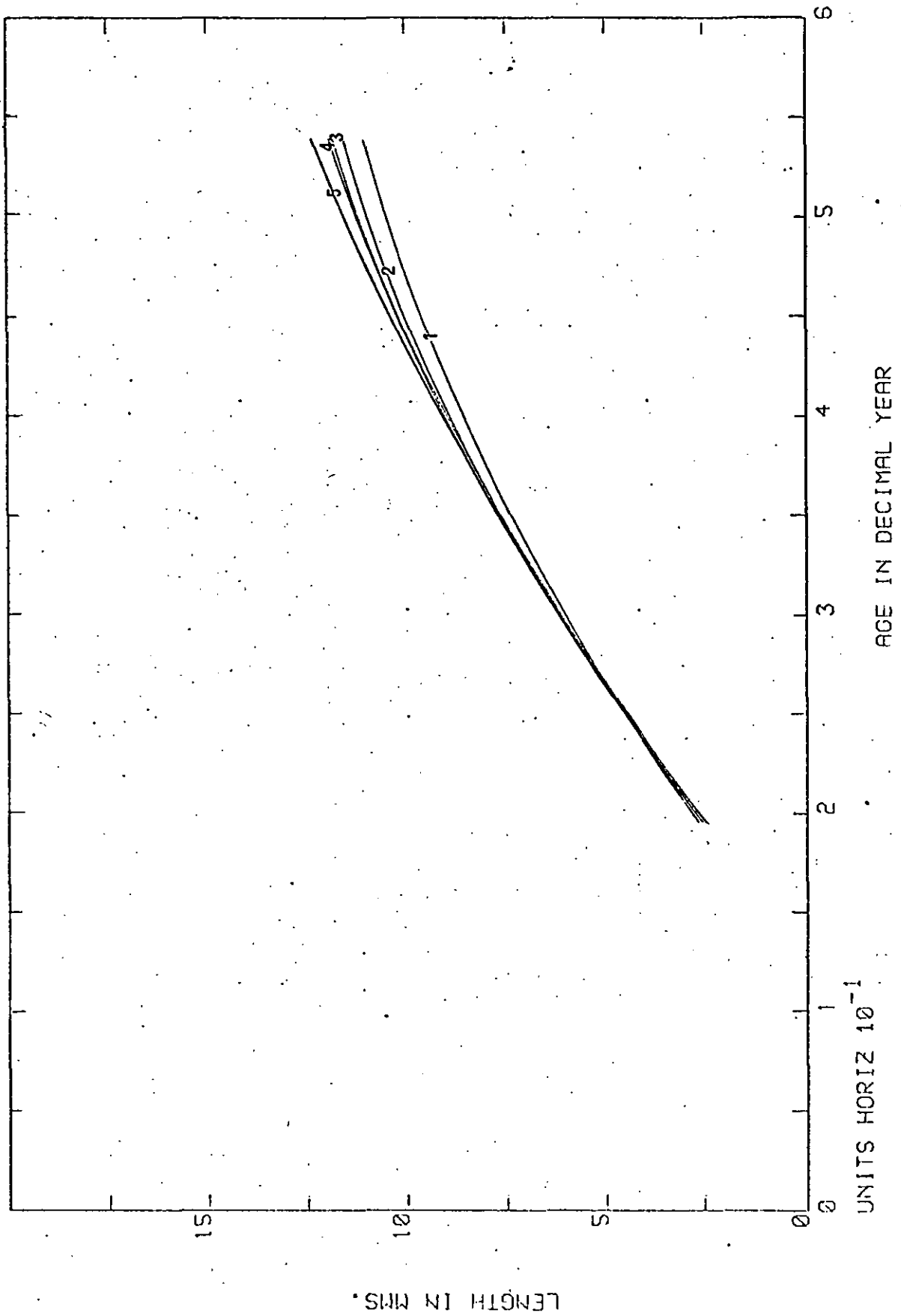


Figure 72. Growth in width of the lumbar region of the vertebral column.

COMPARISON OF GROWTH OF SACRAL INTER NEURAL ARCHES

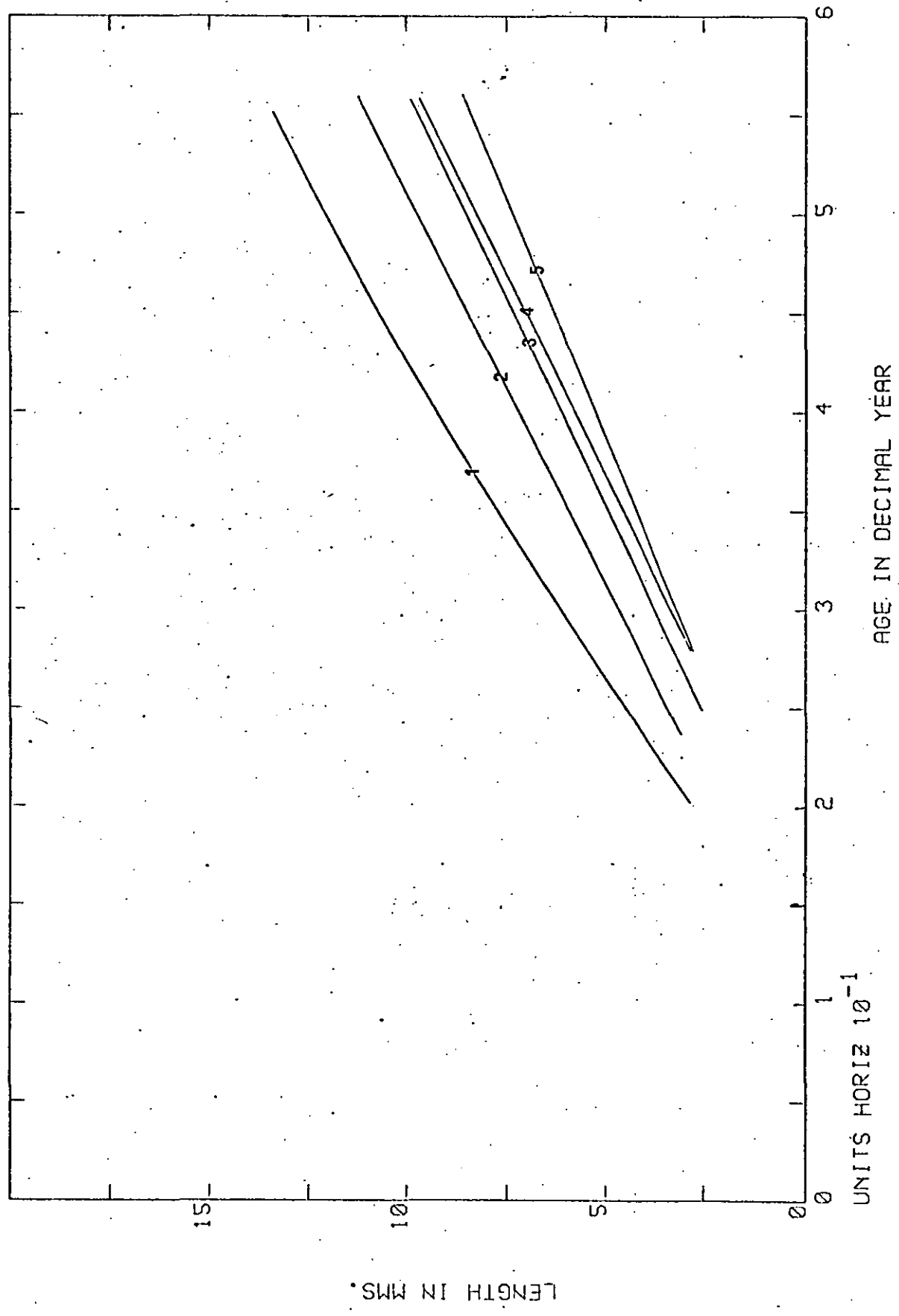


Figure 73. Growth in width of the sacral region of the vertebral column.

contact at its widest part with the occipital condyles of the skull which straddle the foramen magnum and therefore it is expected to have a large width.

At 0.200 yr. (10 weeks) there is a gradual decrease in the width of the vertebral column from the second cervical vertebra (C_2) to the seventh thoracic vertebra (T_7). From the 8th thoracic vertebra (T_8) until the first lumbar vertebra (L_1) there is then a gradual increase in width until at L_1 a width approximately equivalent to that of T_2 is reached and from L_1 to the first sacral vertebra (S_1) the width of the vertebral column remains approximately constant. The width of the vertebral column then becomes progressively narrower until with S_3 , S_4 and S_5 the narrowest parts of the column are reached.

Of particular importance at this time is the absence of cervical and lumbar enlargements similar to those that have been reported for postnatal life (Hamilton and Simon, 1958). This might be associated with the lack of neural development of the spinal cord in these regions at this time, although the general enlargement of the width of the column in these regions suggests that this development is taking place.

By 0.500 yr. (26 weeks) the width of the vertebral column takes on a completely different appearance. The width of C_1 still remains the largest but there is a large decrease in the relative width of C_2 although the absolute width of C_2 has actually increased considerably. There is then a sharp increase in the width of C_3 compared to the width of C_2 and a more gradual increase in the widths of C_4 , C_5 and C_6 . The width of C_7 is then slightly less than C_6 and there is a large decrease in the width of T_1 compared with C_7 . The width of T_1 is now even smaller than that of C_2 . There is another comparatively large decrease in the width of T_2 but then a more gradual decrease in width until the narrowest part of this region of the column is reached with T_7 . There is then a gradual increase in the width of the column until S_1 is reached, when the width has almost regained the value it had at T_1 . There is then a

rapid decline in the width of the column with the widths of S_3 , S_4 , and S_5 becoming the narrowest parts of the column.

It is apparent therefore that over this period of time significant changes have taken place concerning the width of the vertebral column, the most notable of which are the two relative enlargements that have taken place in the cervical and lumbar regions presumably in conjunction with the similar enlargements of the spinal cord as the neural elements in these regions develop.

Perhaps of more significance is that certain groups of vertebrae appear to have similar growth patterns as they develop. Tables 39, 40, 41 and 42 reveal that growth in width of vertebrae C_1 to C_5 are best fitted by equations to the power 2 whilst the growth of vertebrae C_6 to T_{12} are best fitted by equations to the power 3. Similarly the growth of vertebrae L_1 to S_1 are best fitted by equations to the power 2 whilst the growth of the remaining vertebrae are all best fitted by straight lines. This distinction between the growth in width of the vertebrae is slightly more subtle however because the shape of the curve representing growth in width of the vertebrae C_6 and C_7 is different in shape to the curve representing the growth in width of the vertebrae T_1 to T_{12} , even though they are all best fitted by polynomial equations to the power 3. Along these lines therefore the vertebral column can be divided into 5 regions on the basis of patterns of growth in width of the individual vertebrae. These regions would be $C_1 - C_5$, $C_6 - C_7$, $T_1 - T_{12}$, $L_1 - S_1$, $S_2 - S_5$, and this means that those factors which effect growth in width of the vertebral column do so in ways that affect the various parts of the column differently.

This study was concerned with the actual growth in width of the vertebral column and therefore used the centre of the pedicular mass as the point from which measurements were taken. Such a procedure eliminated any false growth in width of the vertebral column which would occur merely by a thickening of the pedicular mass. However, if the mass of the pedicles did increase without any increase in the actual width of the column then the functional width of

the vertebral canal would be diminished and therefore perhaps an equally important measurement that could have been taken, particularly from a functional point of view, is the smallest distance between the pedicles using the outer rims of the neural arch ossification centres as the points from which measurements could be taken. Such a measurement would give details of the functional size of the vertebral canal and has been measured in postnatal life, being called the inter-pedicate distance (Simril and Thurston, 1955; Caffey, 1973). However, assuming that the growth in actual width of the vertebral column increases in harmony with the increase in size of the pedicles, then the functional width of the vertebral canal can be considered to be related to the actual width of the vertebral column and as such the developmental patterns of growth would be very similar. This assumption is supported by the results of Simril and Thurston (1955) who measured the distances between the inner margins of the two pedicles of each vertebra in a large group of normal children between birth and twelve years of age. Their results show a marked similarity to those of this study in terms of relative widths of the individual vertebrae although they note a relative enlargement in both the cervical and lumbar regions compared with the thoracic region between birth and 12 years of age. It would be interesting for the future to compare the relative states of maturity of these enlargements during fetal life and perhaps relate them to the development of the fetal movements as seen by ultrasound. Simril and Thurston (1955) also attempted to measure the A.P. diameter of the vertebral canal but, as was found with this study, no consistently accurate posterior check-point could be determined because of overlapping ribs, transverse processes, and vertebral arches.

The high multiple coefficients of correlation (R) to be found in Tables 39, 40, 41 and 42 mean that accurate estimates of the interneural arch distance can be made for each vertebra based upon knowledge of the fetal age. By using the S.E.E. associated with each equation as a first approximation of normal

growth, it is anticipated that any abnormal growth might easily be detected and it is hoped that such facilities will be useful in studies of abnormal growth of the vertebral column such as are found for example in cases of spina bifida, anencephaly, and scoliosis. Simril and Thurston (1955) suggested that their charts of postnatal growth of interpediculate distance could be used to determine localised regions of abnormal growth and it is hoped that the normal standards produced by this study for prenatal life will be used in a similar manner.

By differentiating the equations in Tables 39, 40, 41 and 42, the growth velocities of the individual vertebral interneural arch distances can be calculated and are shown as graphs in Figures 74, 75, 76 and 77. These graphs highlight many of the findings already mentioned.

The growth velocities of the width of the five most cephalic vertebrae are all decreasing at a uniform rate. Their individual initial velocities at 0.200 yr. (10 weeks) however are different with that for the first cervical vertebra being the largest and an ascending order of magnitude progressing from C_2 to C_5 . This means that from C_2 to C_5 there will be a relative increase in vertebral width in consecutive vertebrae as the fetus develops presumably to accommodate the cervical enlargement of the spinal cord. The growth velocities of the widths of vertebrae C_6 and C_7 are very similar in appearance and confirm that these two vertebrae are under the influence of different control factors than any other vertebrae. Their initial velocities of growth are only comparable to that of C_3 but the decrease in velocity is not as pronounced as for the other cervical vertebrae until 0.450 yrs (23 weeks) when their actual velocity is greater than any other region. This different growth pattern for these two vertebrae produces dramatic changes within the vertebral column and the actual widths of these two vertebrae are now only smaller than the width of the peculiar C_1 . For the thoracic region there is a change of pattern of growth for each of the vertebrae which is different to either of the two groups already

COMPARISON OF GROWTH VELOCITIES OF CERVICAL INTER NEURAL ARCHES

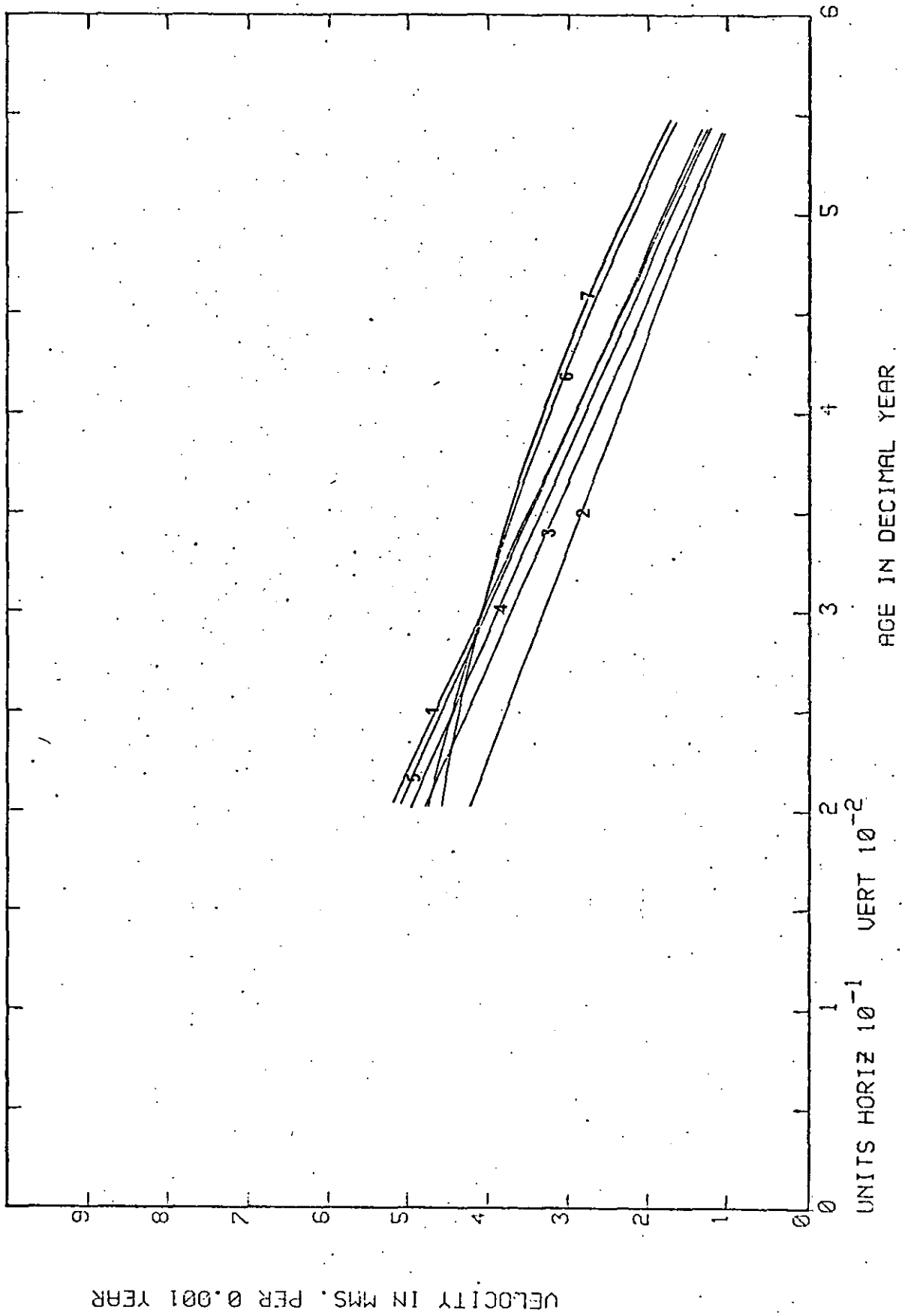


Figure 74. The growth velocities of the width of the cervical region of the vertebral column.

COMPARISON OF GROWTH VELOCITIES OF THORACIC INTER NEURAL ARCHES

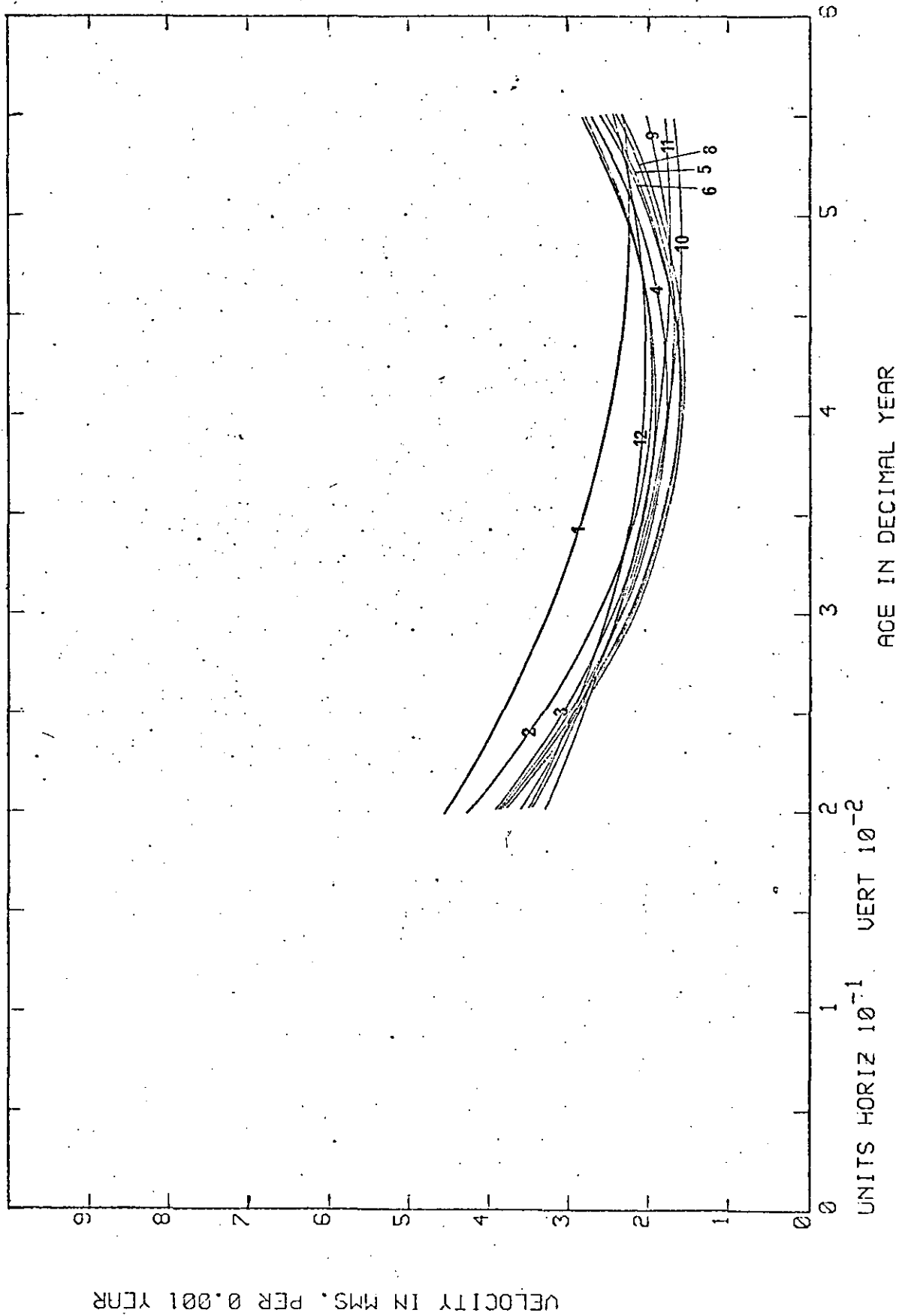


Figure 75. The growth velocities of the width of the thoracic region of the vertebral column.

COMPARISON OF GROWTH VELOCITIES OF LUMBAR INTER NEURAL ARCHES

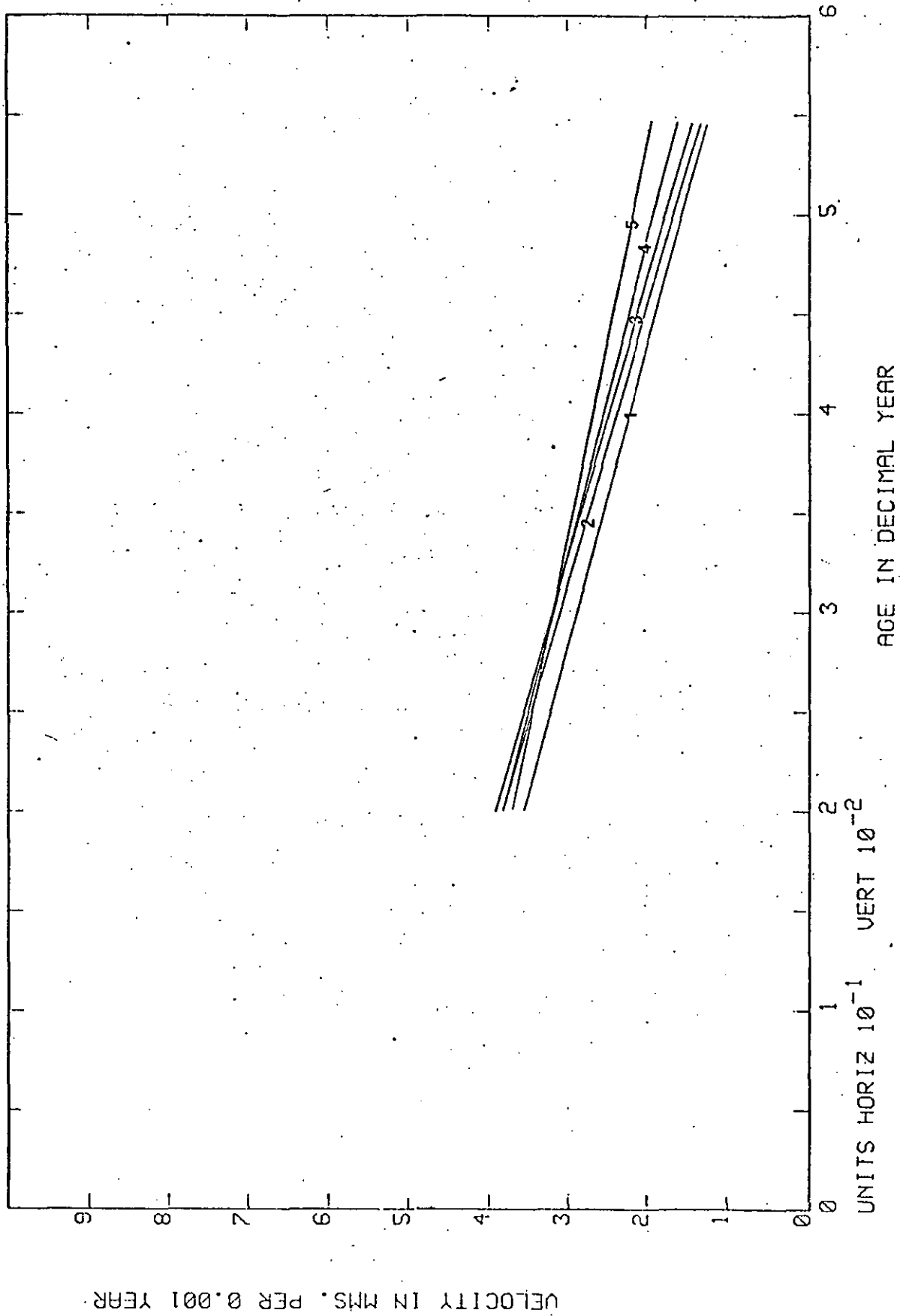


Figure 76. The growth velocities of the width of the lumbar region of the vertebral column.

COMPARISON OF GROWTH VELOCITIES OF SACRAL INTER NEURAL ARCHES

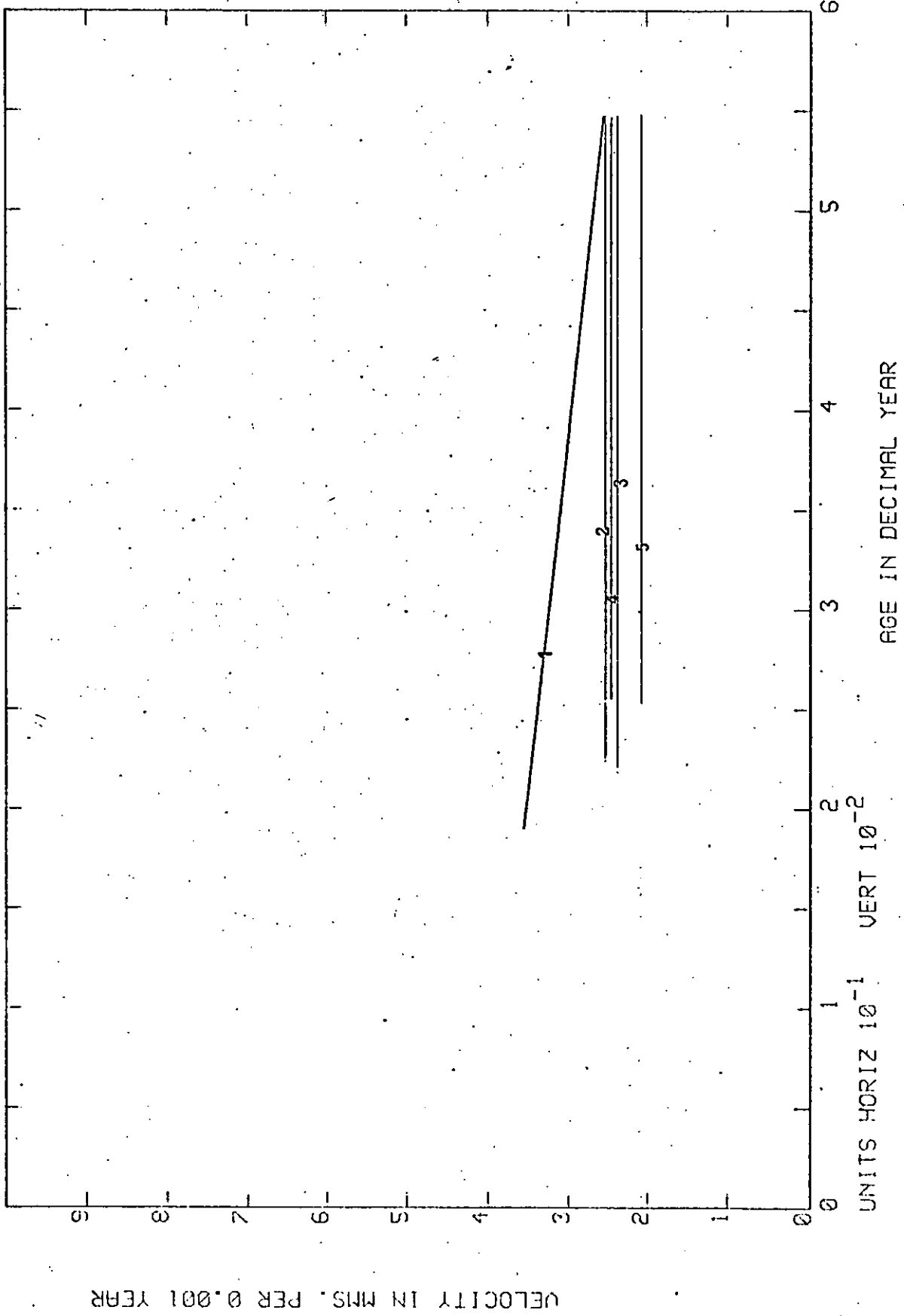


Figure 77. The growth velocities of the width of the sacral region of the vertebral column.

mentioned, with each of the vertebrae passing through a period of minimum growth velocity at approximately 0.375 yr. (19 weeks). This is a very dominant feature of the growth pattern for these vertebrae and it would be interesting to relate the increase in velocity after 0.500 yr. (26 weeks) to the corresponding width of the spinal cord and the development of the respiratory apparatus in the fetus.

In the vertebrae L_1 to S_1 the pattern of growth velocity in terms of width assumes a form similar to that encountered for vertebrae C_1 to C_5 , but the actual decrease in velocity is less in all the vertebrae compared with the vertebrae in the cervical region. Thus the widths of the vertebrae in the region L_1 to S_1 are getting relatively larger than the first five cervical vertebrae and considerably larger than the thoracic vertebrae again, presumably, to accommodate the enlargement in the lumbar region of the spinal cord. The relative maturities of these enlargements in width of the vertebral column is again questioned and the findings suggest that it would be worthwhile attempting to relate these enlargements of the vertebral column to the corresponding enlargements of the spinal cord and integrate both of these with a study of the movements that the fetus performs in utero.

The widths of the four most caudal vertebrae ($S_2 - S_5$) enlarge at a constant rate during the period under study. At 0.200 yr. (10 weeks) their rate of growth is less than any of the other vertebrae and therefore they are getting relatively smaller compared with the other vertebrae. As the fetus develops, however, and the rates of growth of the other vertebrae fluctuate, these vertebrae maintain their progress and eventually become relatively larger than the other vertebrae. Again this emphasises the different patterns of growth that are to be found in the different regions of the vertebral column.

The growth of the vertebral column is in three dimensions and therefore growth in width of the vertebrae might be related to the growth in longitudinal

length of the column. Comparison of Figures 60, 74, and 76 show that the growth in longitudinal length of the cervical and lumbar regions follows a similar pattern to the growth in width. Both aspects of growth are slowing down at a uniform rate. Similarly the longitudinal growth of the sacral region shown in Figure 39 has a uniform enlargement similar to that of Figure 77 which shows the growth in width of the sacral vertebrae. In the thoracic region however Figure 60 shows that the growth velocity does not pass through a minimum period as shown by Figure 75 for the growth in width of this region. This suggests that in some vertebrae, at least, growth in one dimension is under the control of different factors than growth in another dimension. However, an examination of this question in the manner in which it has been undertaken by this study, is only superficial because an accurate examination would require individual vertebral measurements to be taken. Nevertheless, different growth patterns within individual vertebrae have been suggested, and these would have particular relevance in cases where, for example, fetal illness has temporarily affected growth or where an abnormality has affected only part of a vertebra. Thus not only have different intervertebral growth patterns been observed, but also different intravertebral growth patterns.

The relative growth of the width of the vertebral column in the different regions is helpful in understanding the development of the cervical and lumbar enlargements. Four vertebrae were selected as being representative of the vertebrae of their particular region and these were C_4 , T_6 , L_3 , and S_3 . The interneural arch distances of each of these four vertebrae were then related to each other and the results can be seen in Tables 43, 44, 45, and 46 and Figures 78, 79, 80 and 81.

These figures show that relative to the width of vertebra C_4 , the width of L_3 fluctuates, whilst that of T_6 gets progressively narrower. On the other hand the relative width of vertebra S_3 remains constant. Relative to the

Table 43 Regression Equations Relating the Growth of the Inter-neural Arch Distances to that of Cervical Vertebra 4

Vertebra	No of Fetuses	Equation	S.E.E.	R
Thoracic 6	503	$Y = -0.324989 + 0.747340X - 0.008979X^2$	0.38	0.97
Lumbar 3	477	$Y = 1.245544 + 0.083364X + 0.085455X^2 - 0.003073X^3$	0.43	0.98
Sacral 3	171	$Y = 12.637662 + 0.744475X$	0.61	0.91

Table 44 Regression Equations Relating the Growth of the Inter-neural Arch Distances to that of Thoracic Vertebra 6

Vertebra	No of Fetuses	Equation	S.E.E.	R
Cervical 4	503	$Y = 2.393737 + 0.079124X + 0.292728X^2 - 0.017566X^3$	0.62	0.97
Lumbar 3	477	$Y = 2.369634 - 0.602325X + 0.356701X^2 - 0.020339X^3$	0.50	0.97
Sacral 3	171	$Y = -2.125974 + 1.136235X$	0.68	0.88

Table 45

Regression Equations Relating the Growth of the Inter-neural Arch Distances to that of Lumbar Vertebra 3

Vertebra	No of Fetuses	Equation	S.E.E.	R
Cervical 4	477	$Y = 1.871293 + 0.747543X + 0.074234X^2 - 0.003991X^3$	0.52	0.98
Thoracic 6	477	$Y = 0.573193 + 0.821064X - 0.009243X^2$	0.37	0.97
Sacral 3	171	$Y = -1.918996 + 0.890784X$	0.56	0.92

Table 46

Regression Equations Relating the Growth of the Inter-neural Arch Distances to that of Sacral Vertebra 3

Vertebra	No of Fetuses	Equation	S.E.E.	R
Cervical 4	171	$Y = 4.816089 + 1.104312X$	0.74	0.91
Thoracic 6	171	$Y = 2.910791 + 0.684981X$	0.53	0.88
Lumbar 3	171	$Y = 3.040096 + 0.956369X$	0.58	0.92

COMPARISON OF COLUMN WIDTHS

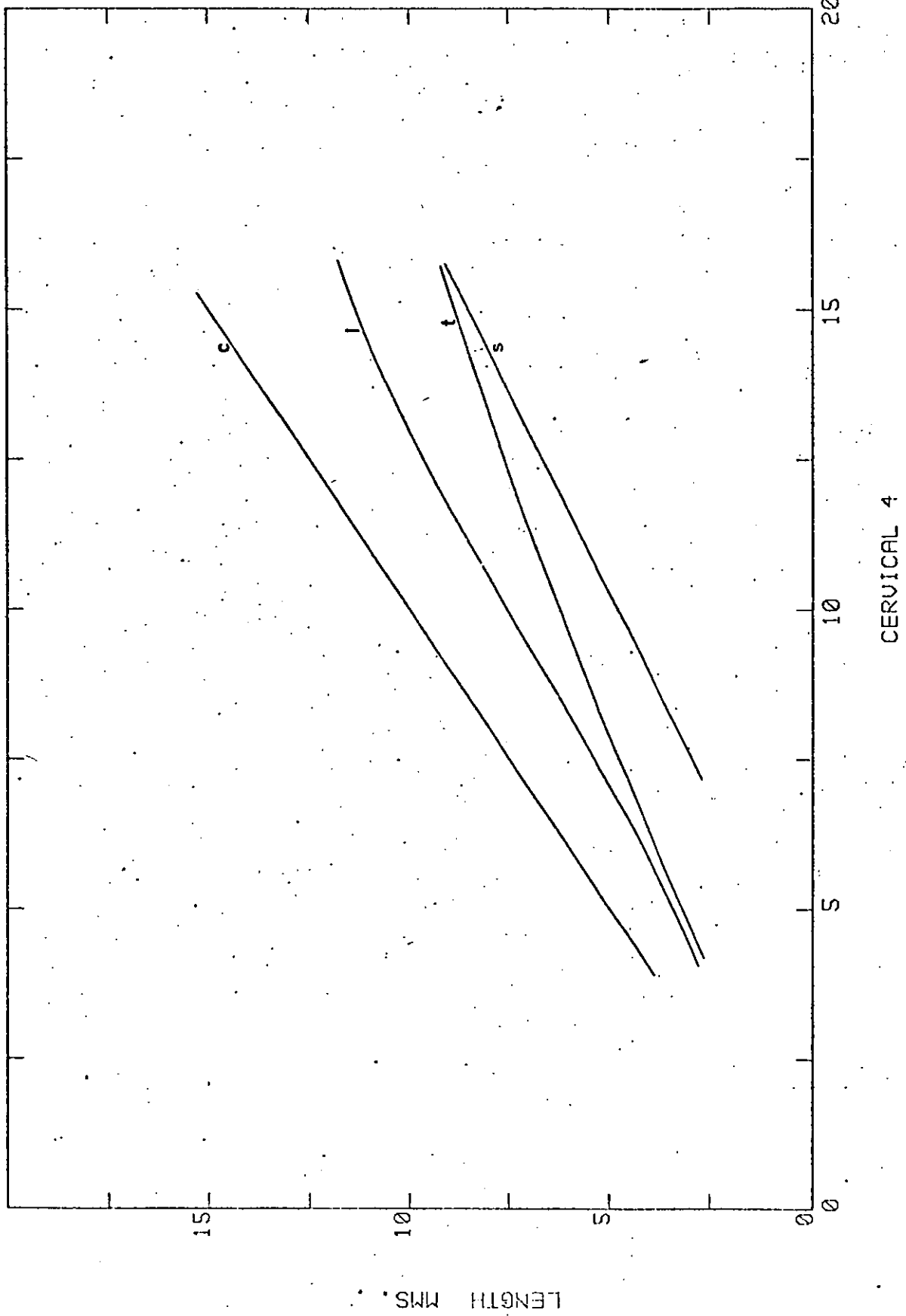


Figure 78. The width of the vertebral column compared to the width of C4.

c = cervical vertebra 4
t = thoracic vertebra 6
l = lumbar vertebra 3
s = sacral vertebra 3

COMPARISON OF COLUMN WIDTHS.

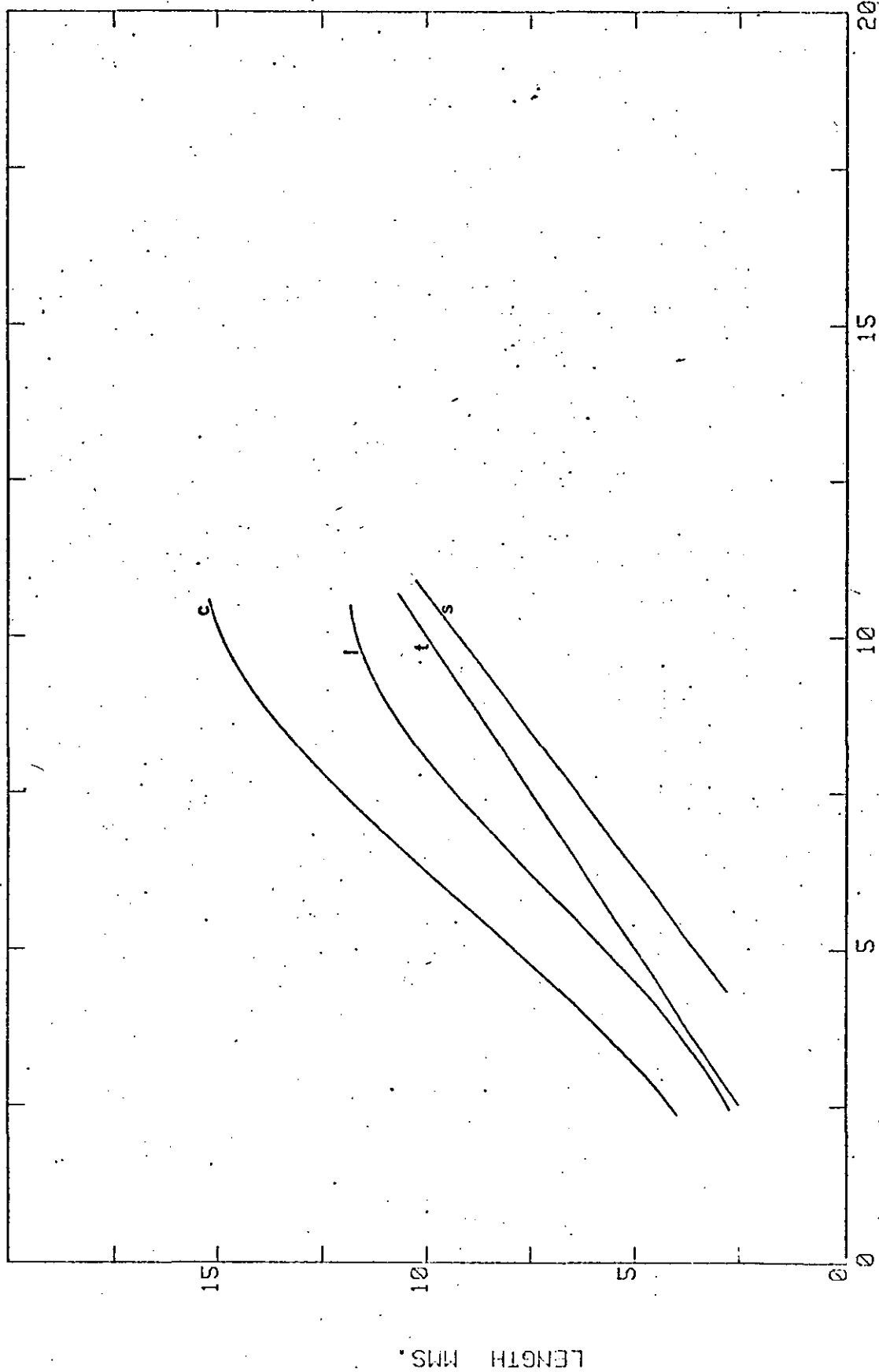
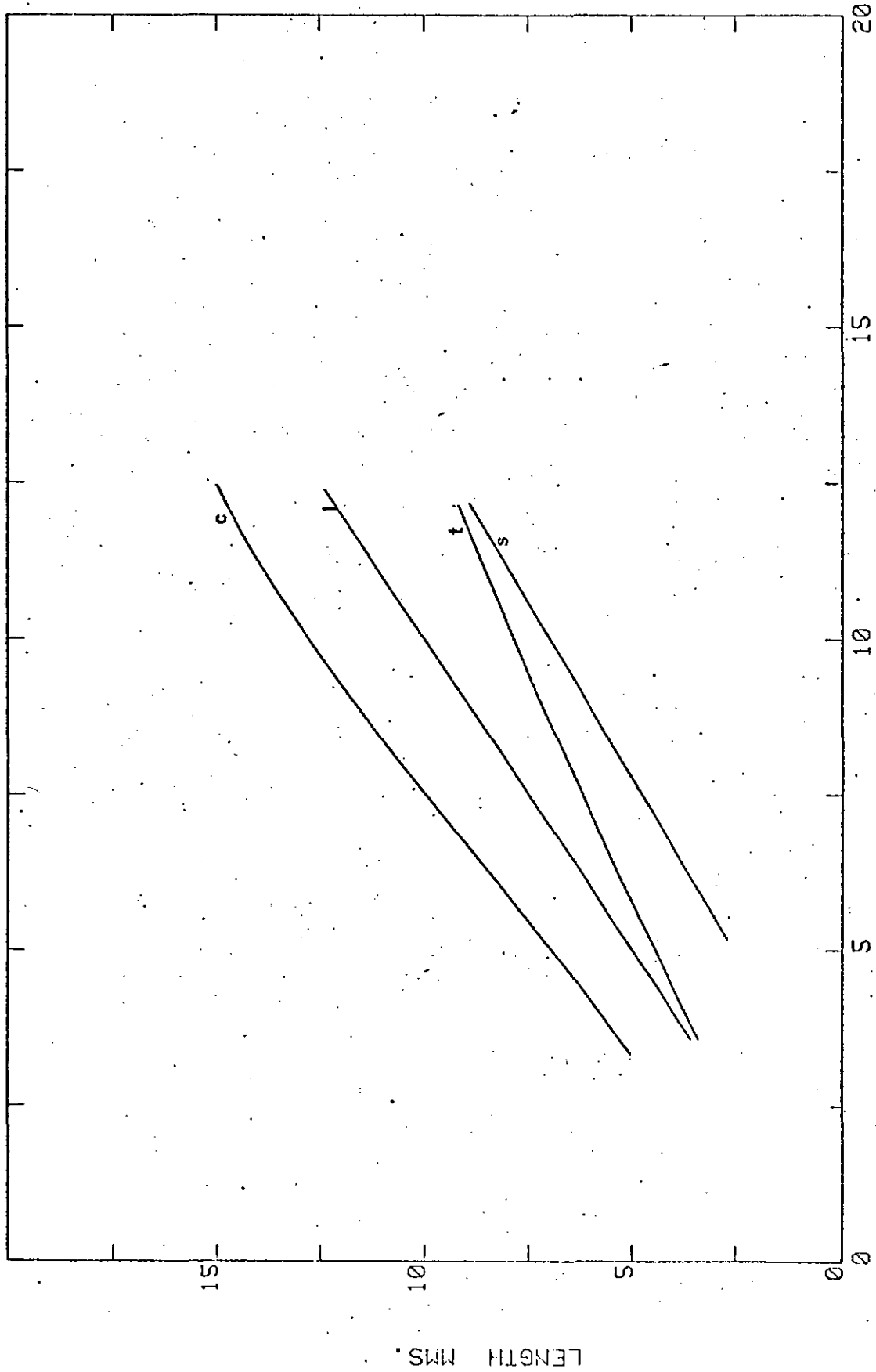


Figure 79. The width of the vertebral column compared to the width of T6.

COMPARISON OF COLUMN WIDTHS



LUMBAR 3

Figure 80. The width of the vertebral column compared to the width of L3.

COMPARISON OF COLUMN WIDTHS

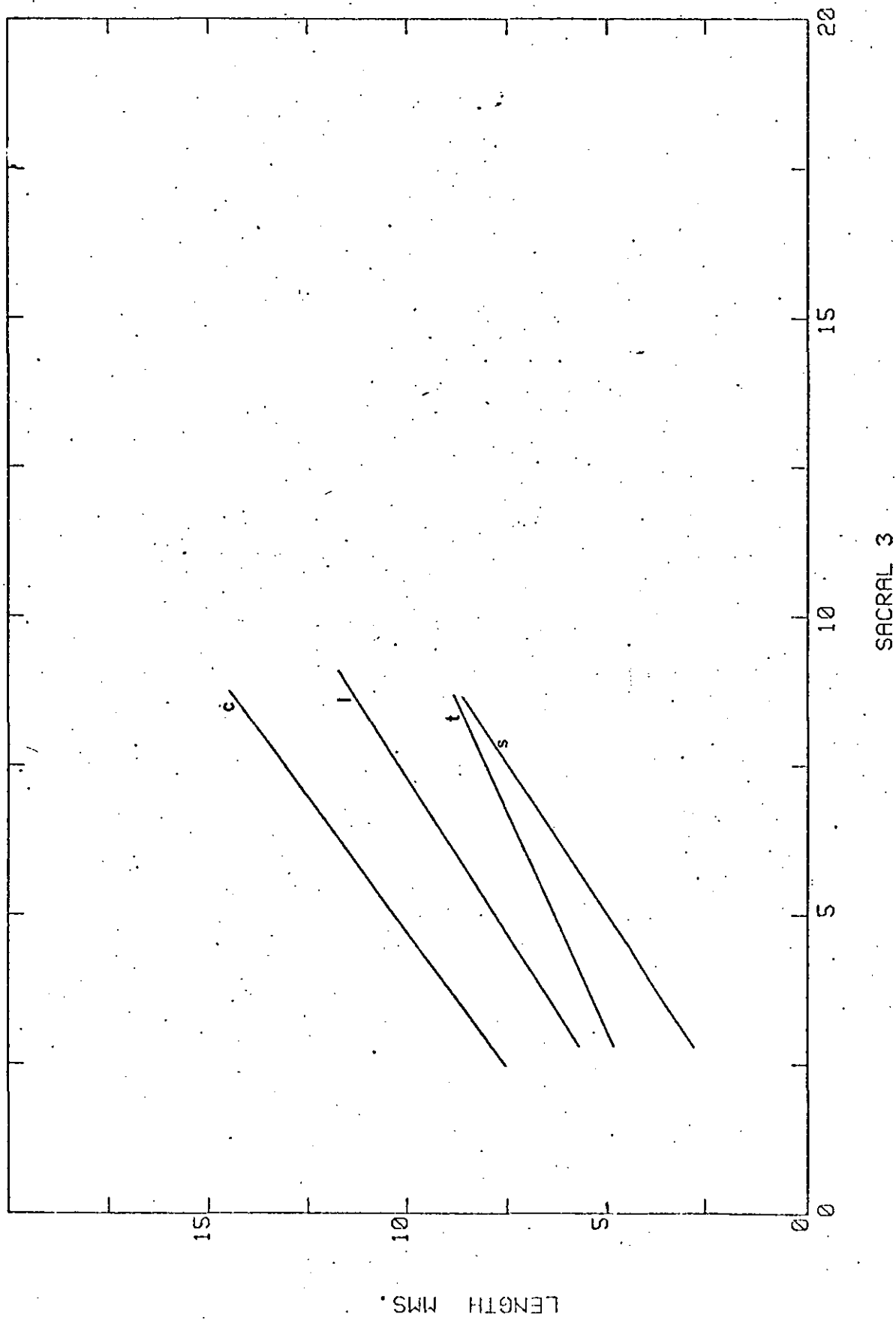


Figure 81. The width of the vertebral column compared to the width of S3.

width of vertebra T_6 , the width of vertebra L_4 fluctuates getting progressively larger and finally narrower, whilst that of vertebra S_3 remains constant. In comparison with the width of vertebra L_3 , the width of S_3 remains constant. Therefore the development of the cervical and lumbar enlargements of the vertebral column is more complicated than at first appeared. In relation to each other they appear to pass through different phases of enlargement at different times which results in changes in proportional growth. If this is related to growth of the spinal cord, such discrepancy might manifest itself in terms of fetal movements with possibly different landmarks in terms of motor development being able to be distinguished at different times related to phases of column and cord growth.

It is anticipated that the equations of Tables 43, 44, 45 and 46 and the graphs of Figures 78, 79, 80 and 81, will be used in further studies of the vertebral column particularly where the fetal age is not known.

The high multiple coefficients of correlation (R) for each equation shown in Tables 43, 44, 45 and 46 indicate that an accurate estimate of the width of any particular vertebra can be estimated from the width of any of the other three vertebrae used, and the S.E.E. attached to each equation will allow a first approximation to be made for normal growth. It is hoped that such facilities will have diagnostic use in further clinical studies of fetal abnormalities, with particular reference to spina bifida and scoliosis.

SUMMARY

1. Normal standards are presented for the growth in width of the individual vertebrae. It is hoped that such standards might be used for the detection and investigation of fetal abnormalities.
2. Early in fetal life the cervical and lumbar enlargements of the vertebral column are not as pronounced as later in fetal life.
3. In terms of patterns of growth in width, the vertebral column is perhaps more appropriately divided into 5 regions, namely $C_1 - C_5$, $C_6 - C_7$, $T_1 - T_{12}$, $L_1 - S_1$, $S_2 - S_5$.
4. In some vertebrae at least, growth in width follows a different pattern of development to growth in length.

ANOMALIES OF THE VERTEBRAL COLUMN

Congenital malformations of the vertebral column are common (Shanks and Kerley, 1971). Some of these malformations are gross and incompatible with life whilst others are trivial and no more than anatomical variants. The evolutionary development of the vertebral column can account for many of the anomalies that are to be found although in some cases it is impossible to define the limits between what is merely variation from the norm and pathological. In this study, the identification of the vertebrae, especially the 12th and the various ossification centres provided an opportunity to quantify the various anomalies that are to be found in vertebral formation including the ribs.

140 fetuses were found to possess ossification centres for the costal processes of vertebra C₇. Of these, 109 fetuses had centres on both sides, 10 fetuses had a centre on the left side only, and 21 fetuses had centres on the right side only.

However, from the cross-sectional data obtained from fetuses it is uncertain whether these centres would have remained separate and become true cervical ribs or whether they would have fused with the rest of C₇ if they had developed. These findings are supported by Noback and Robertson (1951) who found "cervical ribs" to be present in 34 specimens out of a total of 136, with 24 fetuses having bilateral centres, 7 fetuses having centres only on the right side, and 3 specimens having centres only on the left side. Similarly O'Rahilly and Meyer (1956) in a rather less-detailed study, found that 11 fetuses out of the 77 studied, possessed "cervical ribs" but gave no details of whether these were unilaterally or bilaterally placed. Therefore it is apparent that separate ossification centres for the costal processes of vertebra C₇ are common although it is not clear whether or not these would have developed into one of the many

-variations of cervical ribs described by Shanks and Kerley (1971).

Ossification centres for extra ribs were also found associated with the vertebra L_1 . 7 fetuses were found to have lumbar ribs, 4 of which had centres present on both sides, and 3 of which had centres present on the right side only. No fetuses were found in which centres were present on the left side only. Noback and Robertson (1951) report the presence of lumbar ribs in 3 specimens, all of which possessed only unilateral centres, 2 being on the right and 1 on the left, whilst O'Rahilly and Meyer (1956) do not report any findings of lumbar ribs. It appears therefore that lumbar ribs are a common occurrence although they are far less common than ossification centres for the costal processes of vertebra C_7 . It is conceivable that anomalies in both cervical and lumbar regions could lead to the presence of 14 or more ribs within a single fetus. In this study only one fetus was found in which this occurred. This particular fetus was found to possess lumbar ribs on both sides and ossification centres for the two costal processes of vertebra C_7 . Again, however, it could not be determined whether 14 separate ribs would have actually developed.

Prefixation of the thorax is another vertebral anomaly that appears to be an acceptable variation (Shanks and Kerley, 1971). In this study 14 fetuses were found to possess only 11 ribs and yet also possess bilateral ossification centres for vertebra C_7 , but it is difficult to be conclusive because it is unknown whether any further costal ossification centres would have appeared had the fetuses been allowed to develop.

Although this study was not concerned directly with the morphological appearance of the ossification centres, nevertheless certain anomalies were observed. The assumption was made that for each vertebral centrum only one centre of ossification would appear apart from that for the dens in which two paired bilateral centres would appear. In several instances "dumbbell" shaped centra ossification centres suggested that ossification had commenced from bilateral centres but a regular pattern both within and between

fetuses was not readily apparent. In one fetus two separate centres of ossification for the centrum of C₂ were present and in two fetuses the dens was found to possess four centres of ossification. These particular anomalies require further attention because they could well shed light upon the development of hemi-vertebrae which are so prevalent in scoliosis. Similarly two fetuses were found to possess an extra lumbar vertebra but the actual significance of this finding is difficult to assess.

GENERAL SUMMARY, CONCLUSION, AND FUTURE WORK.

One of the major aims of this study was to produce radiographic standards of normal growth for the vertebral column and ossified shafts of the long bones of the limbs in the human fetus. In addition to providing a description of the way in which a particular parameter grew in relation to another parameter, it was intended that these standards would also allow abnormal growth to be identified in other fetuses. Therefore, throughout the study, it has been emphasised that the graphs would be particularly suitable for further studies of fetal abnormalities such as anencephaly, spina bifida, and scoliosis. The collection of fetuses with these abnormalities has already begun with this idea in mind.

Fetuses with anencephaly, spina bifida, and scoliosis are being collected and investigation of their vertebral and limb development is planned in conjunction with investigation of the spinal cord. Figure 82 shows photographs of one of the fetuses with spina bifida. Figure 83 shows the corresponding lateral and A.P. radiographs of the same fetus. The exposed neural tissue of such fetuses is very susceptible to damage unless great care is taken and in the proposed joint study this must be taken into account. Therefore a new rig in which the fetuses can be held during radiography without damaging the neural tissue will have to be built. In Figure 83 only very slight traction was allowed to be applied to the fetus using the present rig in order that the exposed neural tissue was not damaged and consequently the resultant radiographs are "poor". Nevertheless they are only intended to act as an example of how the normal standards can be used and not as an exact representation of this study. Even so, several measurements have been taken from the radiographs and compared with maximum and minimum normal values expected for a fetus of that age (0.340 yr. from L.M.P. dates). (See Table 47). Very briefly, it can be

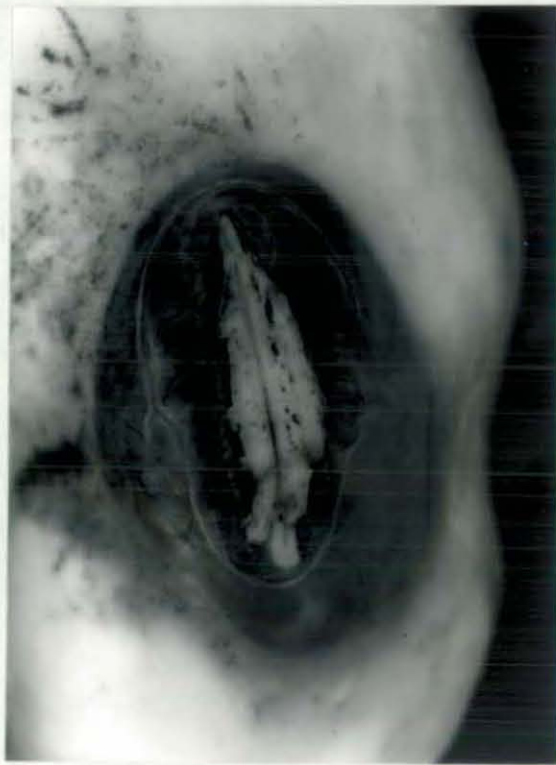


Figure 82. An 18 week old fetus with spina bifida.



Figure 83. Lateral and A.P. radiographs of the fetus in Figure 82.

Table 47 Measurements taken from Radiographs of a Fetus with Spina Bifida (Fig 82 & 83) and Compared with Normal Measurements

Vertebra	INTER NEURAL ARCH			Long Bone	LIMB LENGTHS			Region	COLUMN LENGTHS		
	Dist Meas mm	Min Normal Dist mm	Max Normal Dist mm		Dist Meas mm	Min Normal Dist mm	Max Normal Dist mm		Dist Meas mm.	Min Normal Dist mm	Max Normal Dist mm
C1	11.0	9.1	12.1	L Hum	32.2	26.3	33.2	Cerv Cen	15.4	15.3	22.1
C2	9.3	8.0	10.2	L Rad	26.3	21.4	27.5	Thor Cen	37.4	35.17	43.7
C3	10.5	8.6	10.9	L Uln	29.4	23.4	30.3	Lumb Cen	19.8	18.9	23.7
C4	10.3	8.8	11.0	L Fem	32.0	26.1	33.9	Sacr Cen	10.0	8.6	15.6
C5	10.1	8.9	11.1	L Tib	-	21.4	30.2	Cerv N.A.	16.8	13.8	20.0
C6	9.6	8.7	10.8	L Fib	-	21.2	28.6	Thor N.A.	40.9	38.6	46.8
C7	8.8	6.5	8.8	R Hum	30.5	26.0	33.2	Lumb N.A.	19.5	17.7	23.7
T1	8.3	7.1	9.2	R Rad	25.9	21.1	27.7	Sacr N.A.	10.0	9.2	14.4
T2	7.0	6.2	8.1	R Uln	29.2	23.2	30.6				
T3	5.9	5.8	7.7	R Fem	32.3	24.5	35.5				
T4	5.7	5.6	7.3	R Tib	26.0	21.6	29.8				
T5	5.9	5.4	7.1	R Fib	25.0	20.9	28.6				
T6	5.9	5.3	7.0								
T7	5.9	5.3	7.0								
T8	5.9	5.4	7.0	L = left							
T9	5.9	5.3	7.0	R = right							
T10	6.7	5.4	7.1								
T11	7.2	5.3	7.1								
T12	9.1	5.6	7.6								
L1	9.6	6.1	8.1								
L2	9.7	6.4	8.4								
L3	9.6	6.5	8.4								
L4	8.9	6.4	8.3								
L5	8.6	6.4	8.4								
S1	8.4	6.3	8.6								
S2	7.2	4.1	7.3								
S3	6.7	3.5	5.9								
S4	6.7	3.2	5.8								
S5	-	3.6	5.4								

seen that there is abnormal growth in the width of the vertebral column between vertebrae T₁₁ and L₅ and that all other measurements appear within the normal range. This immediately demonstrates that in this particular fetus at least, there is normal longitudinal growth in the lumbar region of the vertebral column although there is abnormal growth in its width in this region. This has not been able to be shown previously and serves as an example to illustrate the type of work that can now be undertaken. Obviously the application of the graphs is not restricted to any specific abnormality and all of the standards produced are equally applicable to any situation.

The standards would also be applicable to radiographs taken of fetuses in utero but this has a limited application because such films are taken only in rare instances. Of the 37 letters sent to Departments of Radiology throughout the U.K. 24 replies were received. It appears that radiography is used mainly to estimate the gestational age, particularly in the later weeks of pregnancy although ultrasound is now rapidly replacing radiography in this respect. Some radiologists prefer ultrasound both from a safety and ethical point of view as well as the convenience of the technique, but, even so, radiography complements ultrasound either by confirming a diagnosis or by revealing abnormalities which are not detectable by ultrasound. Bearing in mind that the radiation dose received by the fetus in utero during radiography has been reduced considerably by modern radiographic techniques (Russell and Pritchard, 1975) perhaps an argument can be developed to support an increase in fetal radiography particularly where ultrasound has suggested a particular fetal abnormality is present and the diagnosis requires confirmation, or even where ultrasound cannot detect an abnormality although one is strongly suspected. The argument is strengthened by the fact that ultrasound can now be used to determine precisely the fetal position in utero and such a procedure would lead to a very precise radiographic location, resulting in a reduction in radiation dose to the fetus and mother.

Experiments along these lines are being undertaken at present.

In order to determine the best combinations of exposure factors for viewing fetal ossification centres at varying depths in the maternal abdomen, pieces of bone are being placed in a "phantom" maternal abdomen and radiographed. These pieces of bone are at different stages of ossification and are being placed at varying depths within the phantom. From the results it is hoped that the best visualisation of a specific ossification centre can be achieved. Although these experiments are limited at present to the distal femoral epiphysis and proximal tibial epiphysis and consequently to the later stages of pregnancy, they could easily be extended to include other ossification centres in earlier fetal life.

The present Laws dealing with abortion material make it very difficult to obtain fetuses beyond the age of 24 weeks. A logical extension to the present study would be to include fetuses between the ages 24 weeks and 40 weeks and arrangements for this to be undertaken have been made with a local hospital. From this extension it is hoped that a continuous line of development can be found to connect early fetal life with postnatal work and also that some of the questions posed by the results of this study can be answered.

In addition to extending the work in terms of absolute fetal dimensions, however, it is also hoped to examine the morphological presentation of the ossification centres throughout fetal life. Such a project has already been started (Ward, 1976) and it is hoped that from this a fully comprehensive picture of the osseous development of the vertebral column and limbs can be achieved.

A further extension to the study could be made towards relating the development of the vertebral column to the development of the limbs. It would be interesting if the limbs could be related to specific parts of the vertebral column or, on a finer basis, if different parts of the limbs could be

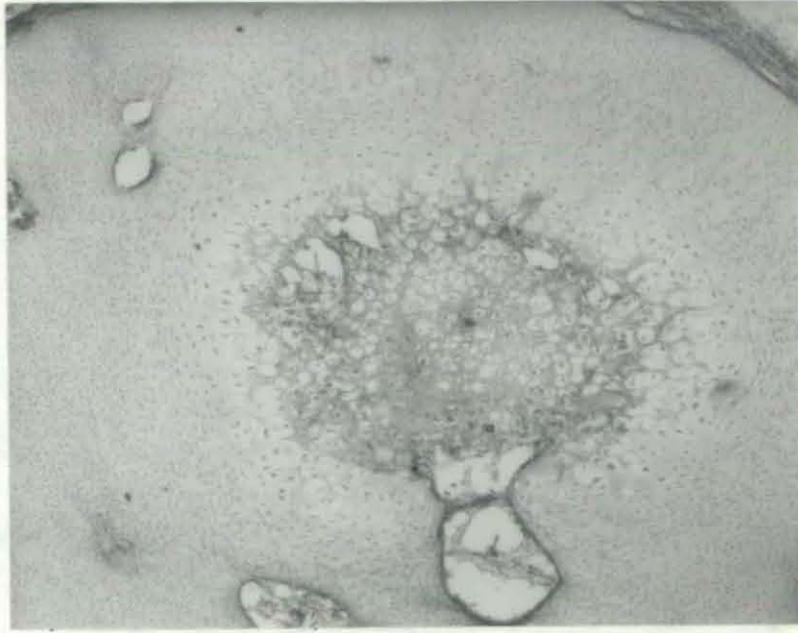
related to different parts of the vertebral column. It might then be possible to correlate abnormalities in these separate regions and in doing so achieve a better understanding of the specific abnormality. In this respect, the use of allophenic mice by Moore and Mintz (1972) appears to hold some potential. Although their method of distinguishing the different characteristics of the composite strains of mice within the individual vertebrae, was subjective they were nevertheless able to determine that individual vertebrae were the product of several sources of cells. If similar procedures can be applied to the long bones of the limbs, the results can be correlated with those from the vertebral column and, hopefully, the questions posed would be answered. It has been arranged that experiments along these lines, using allophenic mice, will be carried out. A more objective procedure for the assessment of the differences between morphological appearances is being developed and it is hoped to include the refinement of morphanalysis procedures (Rabey, 1968) into the views that are observed.

One of the most interesting aspects of this study centred upon the monitoring of fetal movements in utero for their development is, as yet, uncharted. Although the movements in this study were monitored for a specific purpose, namely to establish that the fetus moves all parts of its body from a very early age, the experiments to investigate this aspect of fetal life have continued. At present methods are being investigated by which fetal movements may be quantitated and at the same time experience is being gained in recognition of specific fetal movements in anticipation of further studies.

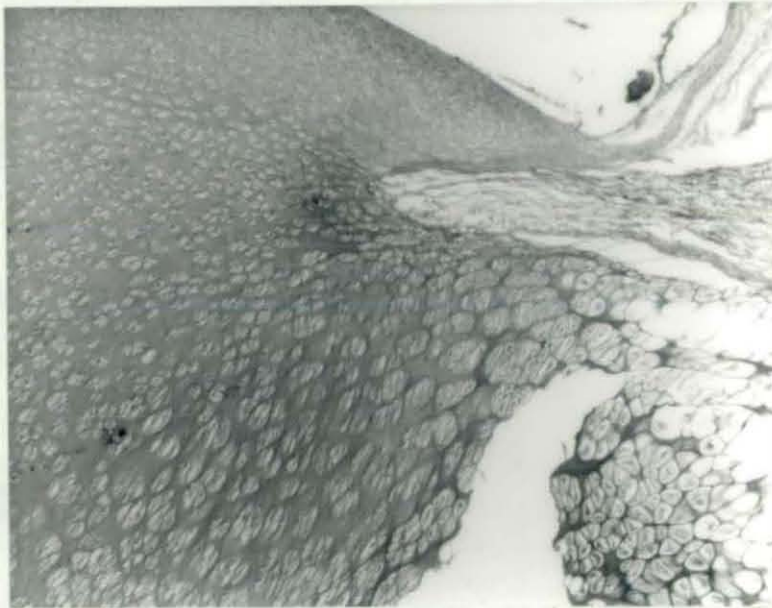
In conjunction with these studies of fetal movements, histological methods are being investigated by which cartilage, bone, muscle, tendon, and nerves can be recognised. It is hoped that the movements performed can then be correlated with the histological findings within a fetus and in this way a complete appraisal can be made of the locomotor system and its function within

the human fetus. It is also hoped to investigate in particular the piezo-electric effect in cartilage, the initial results of which have been very encouraging. (See Figure 84).

In a similar vein, comparative animal studies are also contemplated, the aim being to compare the development of the bipedal stance of man with all its associated problems especially in the lower back region, with the stance of other animals.



A



B

Figure 84. The effect of piezo-electricity on cartilage cells.

In Figure A the cartilage cells appear to be arranged in a circular manner around the developing ossification centre.

In Figure B the cartilage cells seem to be orientating themselves towards the insertion of the tendon.

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APPENDICES

APPENDIX A

A NIMBUS COMPUTER PROGRAMME

FOR POLYNOMIAL REGRESSION ANALYSIS - LOUGHBOROUGH

JOB KMB1,F,KMB1824
CARDLIST ,NIMBUS
SELECT APPLICATIONS
NIMBUS NO5R
JOB CORE 49K

DOCUMENT NIMBUS
PROBLM01264100100
(F5.1,F4.3)

—DATA IN FORM, XIY1—

FINISH

1

APPENDIX B

A PRG2 COMPUTER PROGRAMME

FOR POLYNOMIAL REGRESSION ANALYSIS - MANCHESTER

```
JOB KMB01, :MDPAS, CP76(P2000, T50)
ATTACH(BAGGY1, LIBSTATS, ID=LIBBAPPL)
LIBRARY(BAGGY1)
PRG2.
****S
  129
CRE. AGE
(26X, FS:1, 3X, F5:3)
DATA
  0 0.5 350
****S
****
```


APPENDIX C

GRAPH PLOTTING PROGRAMME, WITH OR WITHOUT

ORIGINAL DATA POINTS - LOUGHBOROUGH

JOB KMB5,F,KMB1824
JOB CORE 26000
LUFORTRAN
VOLUME 7500
RUN

LIBRARY (ED,SUBGROUPGRAF)
PROGRAM(KMB5)
COMPACT
COMPRESS INTEGR AND LOGICAL
INPUT 1=CRO
OUTPUT 2 = LPO
TRACE 2
END

MASTER BAGGY
DIMENSION XTITLE(3),YTITLE(3),X(1000),A(10),Y(1000),TEXT(6),FORM(5)
A),C(1000),D(1000),E(1000),F(1000)
READ(1,5)M,MM,LTEST,LPOINT
5 FORMAT(4I2)
IF(LTEST.EQ.0)CALL UTPOP
DO 6 JL=1,M
READ(1,1)XTITLE,YTITLE,XMIN,XMAX,YMIN,YMAX
1 FORMAT(3A8,3A8,4F0.0)
READ(1,8)TEXT
8 FORMAT(6A8)
IF(LTEST.EQ.0)CALL UTP4A (XMIN,XMAX,YMIN,YMAX,6.0,8.0,XTITLE,3,YTI
ATLE,3)
IF(LTEST.EQ.0)CALL UTP6V(TEXT,6,48,0.0,0.2,8.0,0.125)
DO 7 JK=1,MM
READ(1,2)N,I,ADD
2 FORMAT(13,I1,F0.0)
READ(1,4)(A(L),L=1,10)
4 FORMAT(10F0.0)
X(1)=MIN
DO 3 J=1,N
Y(J)=A(1)+A(2)*X(J)+A(3)*X(J)**2+A(4)*X(J)**3+A(4)*X(J)**4+A(6)*X(
AJ)**5+A(7)*X(J)**6+A(8)*X(J)**7+A(9)*X(J)**8+A(10)*X(J)**9
K=J+1
WRITE(2,20)X(J),Y(J)
20 FORMAT(2)6X,F12.6))
X(K)=X(J)+ADD
3 CONTINUE
IF(LTEST.EQ.0)CALL UTP4B(X,I,N,1)
IF(LPOINT.EQ.0)GO TO 13
READ(1,11)NO,FORM
11 FORMAT (13,5A8)
DO 12 LM=1,NO
READ(1,FORM)C(LM),D(LM)

APPENDIX C CONTINUED

$E(LM) = (C(LM) - XMIN) / ((XMAX - XMIN) / 6.0)$

$F(LM) = (D(LM) - YMIN) / ((YMAX - YMIN) / 8.0)$

XE=E(LM)

YF=F(LM)

IF(LTEST.EQ.0)CALL UPT3(1H,XE,YF,2)

WRITE(2,20)XE,YF

12 CONTINUE

13 CONTINUE

7 CONTINUE

6 CONTINUE

IF(LTEST.EQ.0)CALL UTPCL

STOP

END

FINISH

DOCUMENT DATA

—DATA—

APPENDIX D

GRAPH PLOTTING PROGRAMME. WITH OR WITHOUT

ORIGINAL DATA POINTS - MANCHESTER

```
PROGRAM GLOT(INPUT,OUTPUT,PFILE,TAPE1=INPUT,TAPE2=OUTPUT,TAPE7=PF
FILE)
DIMENSION ITITLE(27),X(1000),Y(1000),IFORM(8)
COMMON /SDTA/RV(400)
C READ IN NUMBER OF GRAPHS
READ(1,110)NUM
100 FORMAT(12)
DO 60 IND=1,NUM
READ(1,*)XMIN,XMAX,YMIN,YMAX
CALL FIXAXS(XMIN,XMAX,YMIN,YMAX)
C READ GRAPH TITLE
ITITLE(1)=80
READ(1,100)(ITITLE(1),1=2,9)
100 FORMAT(8A10)
C READ X-AXIS TITLE
ITITLE(10)=80
READ(1,100)(ITITLE(1),1=11,18)
C READ Y-AXIS TITLE
ITITLE(19)=80
READ(1,100)(ITITLE(1),1=20,27)
C SET A4 SIZE PLOT
CALL A4
C READ IN NUMBER OF POINTS
READ(1,120)N
120 FORMAT(14)
IF(N.GT.2)GO TO 80
ISYM=0
X(1)=XMIN
X(2)=XMAX
Y(1)=YNIM
Y(2)=YMIN
GO TO 90
80 CONTINUE
C READ IN DATA POINTS FORMAT
READ(1,130)IFORM
130 FORMAT(8A10)
C READ IN DATA POINTS
READ(1,IFORM)(X(I),Y(I),I=1,N)
C SET UP BASIC GRAPH AND AXES
READ(1,140)ISYM
140 FORMAT(I1)
90 CONTINUE
CALL FGPLT(X,Y,N,1,ISYM,1,0,ITITLE,2)
C READ IN NUMBER OF LINES
READ(1,150)NBEST
150 FORMAT(12)
DO 40 J=1,NBEST
C READ BEST FIT COEFFICIENTS
READ(1,*)A,B,C,D
```

APPENDIX D CONTINUED

```
C      READ IN NEW X-VALUES
      READ(1,*)AMIN,XINC
      READ(1,170)N
170  FORMAT(13)
      DO 70 M=1,N
      X(M)=AMIN
      AMIN=AMIN+XINC
      70  CONTINUE
C      DO BEST FIT COMPUTATIONS
      DO 20 K=1,N
      Y(K)=A+B*X(K)+C*(X(K)**2.0)+D*(X(K)**3.0)
      20  CONTINUE
C      CHANGE INK COLOUR
      READ(1,180)IPEN
180  FORMAT(11)
      CALL CHAPEN(IPEN)
C      PLOT SUPERIMPOSED LINE
C      READ PLOT TYPE AND SYMBOL TYPE
      READ (1,160)IPLLOT,ISYM
160  FORMAT(2I2)
      CALL FGPLT(X,Y,N,IPLLOT,ISYM,1,1,ITITLE,2)
      40  CONTINUE
      60  CONTINUE
      CALL DEVFIN
      STOP
      END
```

APPENDIX E

PROGRAMME TO ARRANGE

FETUSES IN ORDER OF SIZE AND TO AGE THEM

JOB KMB1,F,KMB1824

HPFORTRAN

C PROGRAMME TO ARRANGE THE FETUSES IN CHRONOLOGICAL ORDER

```
MASTER BAGGY
DIMENSION X(195),M(195),Y(195)
WRITE(2,7)
7 FORMAT(4X,'FETUS NUMBER',4X'CROWN RUMP EXTENDED',4X,'AGE DECIMAL'
A YEAR')
DO 2J=1,195
M(J)=J
2 CONTINUE
READ(1,1)X
1 FORMAT(F6,2)
DO 4 N=1,194
DO 4 I=N+1,195
IF(X(N).LE.X(I))GO TO 4
A=X(N)
X(N)=X(I)
X(I)=A
B=M(N)
M(N)=M(I)
M(I)=B
4 CONTINUE
DO 3 N=1,195
Y(N)=0.124671+0.00153X+0.000002X2
3 CONTINUE
WRITE(2,5) (M(I),X(I),Y(I),I=1,195)
5 FORMAT(7X,13,15X,F6.2,17X,F8.6)
STOP
END
FINISH
```

