275341

UNIVERSITY OF THE WESTERN CAPE
UNIVERSITEIT VAN WES-KAAPLAND

This book must be returned on or before the last date shown below.

Hierdie boek moet terugbesorg word voor of op die laaste datum hieronder aangegee.

NB: Telephonic renewals only between 17h00 – 22h00 at issue desk 959 2946

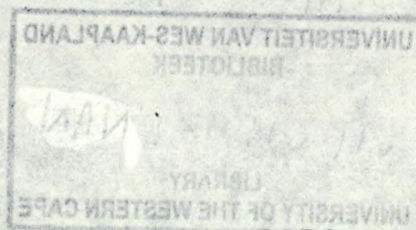
THE HANDLING OF UNDATED PIG EMBRYOS AND FOETUSES AS A PRELUDE TO HISTOLOGICAL STUDIES OF MORPHOGENESIS IN THE ORAL REGION

BAREND GABRIEL JANSEN VAN RENSBURG
Faculty of Dentistry
University of Stellenbosch

Thesis presented for the Degree of Master of Science in Dental Science at the University of Stellenbosch

UNIVERSITY of the
WESTERN CAPE

Promoter: Prof. C.W. van Wyk
Department of Oral Pathology
Faculty of Dentistry
University of Stellenbosch



December 1976

www.etd.ac.za

THE DEN
UNIVERSITEIT VAN WES-KAAPLAND
BIBLIOTEEK
611.018931 REN
LIBRARY
UNIVERSITY OF THE WESTERN CAPE

www.etd.ac.za

C O N T E N T S

		Page
ACKNOWLEDGEMENTS		i
SUMMARY		iii
I	INTRODUCTION AND AIMS OF THE STUDY	1
II	MATERIALS AND METHODS	7
	Summary of procedures carried out	7
	A Reception, initial handling and mass determination	7
	B Fixation	9
	C Measurement	10
	D Selection of specimens for histological investigation	15
	E Trimming	15
	F Decalcification	16
	G Processing for microscopy	16
III	RESULTS	18
	A Fixation and decalcification	18
	B General information on the sample	18
	C Instrument and measurement accuracy	19
	D Correlation between individual linear measurements	22
	E Correlation between linear measurements and mass	23
	F Variation within litters	24
	G Specimens selected for study	37
IV	DISCUSSION	39
V	CONCLUSIONS	53
VI	REFERENCES	56
VII	ILLUSTRATIONS	

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

ACKNOWLEDGEMENTS

A study of this nature is almost wholly dependant upon a ready supply of embryos and foetuses. In this regard the friendly co-operation and assistance of the Director of the Cape Town Municipal Abattoir, Dr. A.A.L. Albertyn and his staff is gratefully acknowledged.

I wish to record my appreciation to Dr. T.J. van W. Kotze and Dr. D.J. van Schalkwyk of the Institute for Biostatistics of the South African Medical Research Council for their guidance and assistance in the statistical evaluation of results.

I am thankful to Mr. W.F. Pretorius, senior technical officer in the Department of Anatomy of the University of Stellenbosch, for valuable advice and active participation in the designing of the measuring instruments. The apparatus to measure crown-tailroot length was constructed by Mr. C.A. Wijtenburg, instrument-maker on the staff of the Faculty of Medicine. His ability to produce a highly accurate instrument is much admired and appreciated.

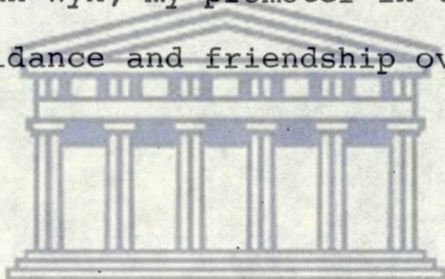
Mr. M.V. Jooste, photographer of the Faculty of Dentistry of the University of Stellenbosch and Mr. A.J. Louw, graphic artist of the Faculty of Dentistry, University of the Western Cape were responsible for all illustrations. Their contributions are acknowledged with gratitude.

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

My sincere thanks is due to Mrs. C.F.M.E. du Toit for typing the manuscript.

I am indebted to Mr. F.J. Greyling, medical technologist in the Department of Oral Biology of the Faculty of Dentistry, University of Stellenbosch for his excellent advice on technical matters and active assistance in this project.

In conclusion I would like to express sincere gratitude to Prof. C.W. van Wyk, my promoter in this investigation, for his wise guidance and friendship over a period of many years.



UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

SUMMARY

The author is interested in the morphogenesis of the oral region including the nasopalatine complex. With the intention of undertaking a study of the embryological development in this area, perusal of available literature failed to reveal a single comprehensive description of the reception and handling of embryonic and foetal material, mensuration and preparation for miscroscopy.

Human material for embryological study is relatively scarce in the Republic of South Africa. According to the literature there is, however, a distinct similarity between human and domestic pig development in certain regions, notably the palate. Furthermore, pig embryos and foetuses are available in comparative abundance from sows slaughtered at abattoirs. As a consequence of the above-mentioned factors it was decided to undertake a preparatory study in order to firstly evaluate existing methods of handling of embryonic and foetal material and secondly, to statistically evaluate data relating to mass and measurements. The aim was to draw a comparison with existing information and to select a sample for investigation.

Embryos and foetuses were removed from slaughtered sows in a fresh state and removed to the laboratory immersed in 10 per cent neutral buffered formol saline. In the laboratory foetal membranes were removed, umbilical cords cut and the

UNIVERSITY of the
WESTERN CAPE

specimens weighed. They were then placed in Bouin's solution for final fixation and decalcification.

Instruments were designed to measure crown-tailroot length, crown-rump length and dorsal profile length. After one day in Bouin's solution all specimens were measured.

In order to determine the accuracy of the weighing and measuring procedures ten fixed specimens were weighed and measured on seven consecutive days. Statistical analysis of this data indicated that crown-rump length was the most accurately determinable linear measurement, judged by both the coefficient of variation and the standard deviation. On this basis crown-rump length was chosen as the criterion for selecting the sample to be studied. Correlation between linear measurements and between linear measurements and mass for the entire series showed a very strong positive relationship between all the parameters indicating that a dimensional relationship was maintained during growth.

After measuring, the small specimens were embedded whole while larger embryos and foetuses were decapitated. A method was described for trimming and embedding these heads in such a way that subsequent sectioning would take place in a standardised transverse plane. In larger specimens this procedure had to be delayed until demineralization had taken place.

Conclusions based on a consideration of data for the entire

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

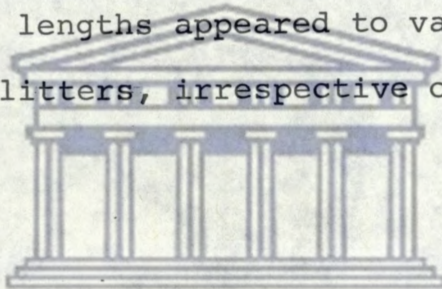
population included the following:

1. The mean number of specimens per litter was 6,475.

2. The number of pigs per litter stayed relatively constant throughout the period of gestation.

3. Mass showed a greater intra-litter variation than any of the three linear measurements recorded.

4. Relatively, lengths appeared to vary less in older than in younger litters, irrespective of litter size.

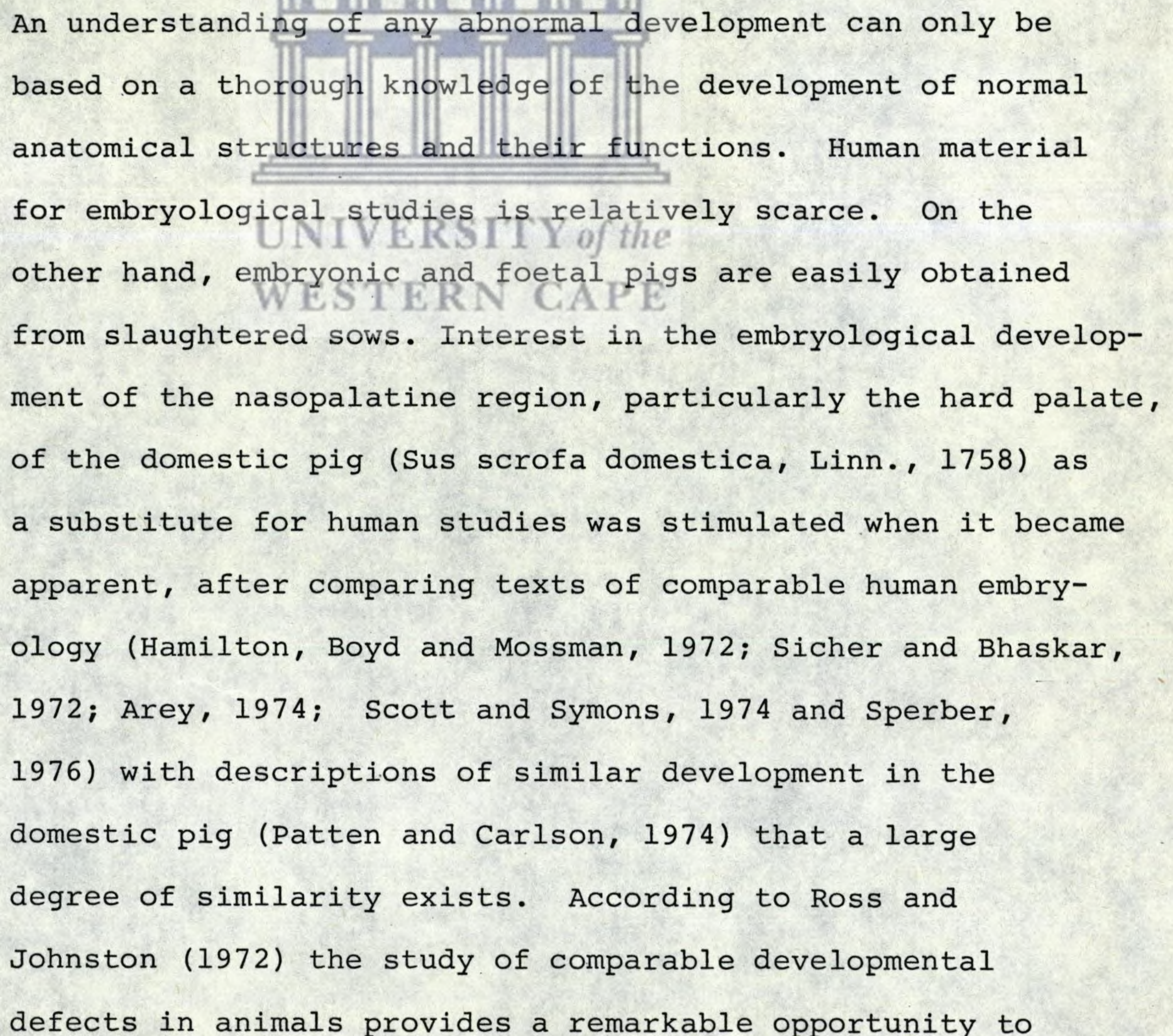


UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

I INTRODUCTION AND AIMS OF THE STUDY

Developmental abnormalities in the oral cavity and especially clefts of the palate present the clinician with an array of problems. Therapy in these cases usually includes surgery to repair the defect, orthodontics to restore or improve function, speech therapy and occasionally psychiatric aid. Treatment is usually of fairly long duration and involves specialists in many fields. Ross and Johnston (1972) state that many kinds of facial clefts occur but that most are rare. Clefts of the lips and palate occur, according to these authors, fairly frequently.



An understanding of any abnormal development can only be based on a thorough knowledge of the development of normal anatomical structures and their functions. Human material for embryological studies is relatively scarce. On the other hand, embryonic and foetal pigs are easily obtained from slaughtered sows. Interest in the embryological development of the nasopalatine region, particularly the hard palate, of the domestic pig (Sus scrofa domestica, Linn., 1758) as a substitute for human studies was stimulated when it became apparent, after comparing texts of comparable human embryology (Hamilton, Boyd and Mossman, 1972; Sicher and Bhaskar, 1972; Arey, 1974; Scott and Symons, 1974 and Sperber, 1976) with descriptions of similar development in the domestic pig (Patten and Carlson, 1974) that a large degree of similarity exists. According to Ross and Johnston (1972) the study of comparable developmental defects in animals provides a remarkable opportunity to

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

investigate the specific causes and development of the condition. It may be possible to apply these findings to human development. The foregoing considerations motivated the use of embryonic and foetal pigs in the present study.

Certain problems are encountered in the general handling of the specimens prior to histological studies of embryological development. The author was unable to find comprehensive descriptions dealing either singly or in combination with the initial receipt and handling of soft embryonic material, fixation and decalcification procedures, size determination and trimming of the heads before final processing and sectioning. It is furthermore desirable to analyse data relating to litters and individual specimens statistically for comparison with existing information.

UNIVERSITY of the
WESTERN CAPE

The initial receipt and handling of embryonic and foetal pig material is poorly described in the literature. Lowrey (1911), in a study of prenatal growth of pigs simply states that his pig litters were obtained from meat packing houses and were studied in a fresh condition where possible. In other instances they were preserved in a 5 per cent aqueous solution of formalin. In a similar study Warwick (1928) states that uteri were obtained as soon as carcasses were opened and all measurements and other records taken as soon as possible on the killing floor. The umbilical cords were cut from the bodies, foetal membranes were removed, excess moisture allowed to drain and specimens

www.etd.ac.za

were then weighed. Marrable and Ashdown (1967) simply state that membranes were removed and specimens measured and weighed before preservation. Umbilical cords were cut at the amnio-somatic junction.

Lowrey (1911), when considering comparative masses and measurements of different litters, states that a possible source of error may lie in the fact that litters were not subjected to a standard form of handling. In the study reported by Lowrey (1911) large foetuses were weighed to 0,1 g while smaller specimens were weighed to 1,0 mg. The smallest embryos (15 mm) were weighed to 0,1 mg. Warwick (1928) weighed his material to 0,1 g. Marrable and Ashdown (1967) based the fineness of their mass determinations on the age of the specimens, weighing embryos aged 26 to 27 days to 0,01 g, those aged 27 to 55 days to 0,1 g and older material to a gram. There appears to be a lack of uniformity in weighing accuracy accepted by different authors.

UNIVERSITY of the
WESTERN CAPE

For fixation of embryonic and foetal material Lowrey (1911) used a 5 per cent aqueous solution of formalin. He states that it is well known that specimens preserved in formalin show an increase in mass which sometimes amounts to 10 or 15 per cent of the total. Lowrey (1911) mentions this fact as a possible source of error in his calculations since not all his litters were fixed in this solution, some being studied fresh.

Von Bartheld (1956) employed Bouin's solution. He states

www.etd.ac.za

that the heads and especially the jaws of even his biggest specimens were adequately fixed by this method, the solution being able to enter through the mouth and the nose. An additional advantage of this solution, according to von Bartheld (1956), is that it simultaneously decalcifies the material.

Marrable and Ashdown (1967) simply state that the embryos were preserved but no further information on the method is supplied.

Pig embryos and foetuses have in the past been measured in various ways. Lowrey (1911) and Warwick (1928) measured crown-rump lengths but the exact method of measuring is not described although Warwick (1928) mentions the use of calipers.

UNIVERSITY of the
WESTERN CAPE

Ullrey et al (1965) used crown-rump lengths for 30-day foetuses. Fifty-one-day-old and older foetuses and newborn pigs were measured from the most anterior part of the frontal crest to the anus while lying in a normal unstretched position. The exact techniques are not specified nor are the reasons given for employing two methods.

According to von Bartheld (1956) who performed his histological study on pigs of unknown ages, the crown-rump length is not a sufficiently accurate method to distinguish specimens in different stages of development. He proposes a technique whereby the length from the top edge of the snout tip, along the dorsal mid-sagittal plane to the tail

www.etd.ac.za

root (dorsal profile length) is measured by means of a thin wire.

Marrable and Ashdown (1967) define the length of an embryo as the length in millimeters of a straight line passing from the root of the tail to the ventral border of the external auditory meatus and projected to cut the profile of the head (crown-tailroot length). Marrable (1971) adds that, in early embryos, the otic vesicle instead of the external auditory meatus is the central landmark.

Trimming of specimen heads prior to further processing for histological study of the oral cavity and associated structures is mentioned by only one author. Von Bartheld (1956) merely states that the entire heads were used. No further information is provided.

UNIVERSITY of the
WESTERN CAPE

The statistical evaluation of data relating to many aspects of prenatal growth has been fairly adequately dealt with in available literature. The mean number of specimens per litter was determined by various authors (Lowrey, 1911; Warwick, 1928 and Davies, 1976). Intra-litter size variations were demonstrated by Warwick (1928), Pomeroy (1960) and Marrable (1971). Warwick (1928) and Marrable and Ashdown (1967) are of the opinion that intra-litter size variation increases with embryonic age and that mass shows the greatest intra-litter variation. For this reason Marrable (1971) considers mass a less satisfactory estimator of prenatal age than length. There have been no attempts to statistically evaluate the accuracy of measuring

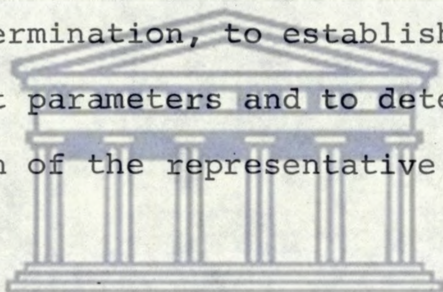
www.etd.ac.za

instruments and measurements nor have attempts been made to correlate size parameters.

The aims of the present investigation are the following:

(i) To describe a technique for the handling and processing of embryonic and foetal pig material prior to histological study of embryological development in the oral region.

(ii) To statistically evaluate data relating to mass and size determination, to establish a correlation between different parameters and to determine a basis for the selection of the representative specimens to be studied.



UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

II MATERIALS AND METHODS

Summary of procedures carried out

Embryos and foetuses were obtained from sows belonging to the Landrace breed and crosses between Landrace and Large White breeds slaughtered at the Cape Town Municipal Abattoir. Approximately 10 minutes after the animals were killed the uteri were opened and embryos and foetuses exposed. The entire uteri were then placed in 10 per cent neutral buffered formol saline. In the laboratory the membranes surrounding the specimens were removed and the umbilical cords cut. Specimens were then dried and weighed. Thereafter they were placed in Bouin's solution. One day later the specimens were measured, decapitated, the heads trimmed and returned to Bouin's solution for completion of fixation or for decalcification where indicated. After fixation and decalcification the specimens were processed for histological examination.

UNIVERSITY of the WESTERN CAPE

A. Reception, initial handling and mass determination

Through the courtesy of the Director of the Cape Town Municipal Abattoir it was possible to be present when pigs were slaughtered and carcasses opened. Pigs are rendered unconscious by means of an electric shock (150 volts) applied to the head through tong electrodes. The unconscious pigs are then hoisted into a hanging position by their hind legs and killed by means of a stab wound through the neck into the heart. They quickly bleed to death. Hereafter they are dehaired and the carcasses are

www.etd.ac.za

then opened and all organs removed.

The uteri of all sows were examined by the author and an inspector of the abattoir who indicated which uteri were pregnant (Fig. 1). These uteri were then immediately slit longitudinally, foetal membranes were nicked to enhance initial contact of the specimens with the fixing fluid and all the material was immersed in 10 per cent neutral buffered formol saline solution for transport to the laboratory.

The following is the formula for the solution employed:

Sodium dihydrogen phosphate (anhydrous)	3,50 g
Disodium hydrogen phosphate (anhydrous)	6,50 g
Sodium chloride	8,50 g
Formalin (40 per cent formaldehyde)	100,00 ml
Distilled water up to	1000,00 ml

(Solution adjusted to pH 7)

UNIVERSITY of the
WESTERN CAPE

At the laboratory foetal membranes were removed from each specimen (Fig. 2) and the umbilical cords cut as close to the body as possible (Fig. 3). In the case of smaller specimens this procedure was carried out under a Wild dissecting stereomicroscope (Fig. 4). As the specimens were limp and very difficult to handle at this stage, extreme care had to be exercised not to injure them. A particularly vulnerable area in very young embryos is the ventral body wall which is not entirely consolidated and is covered by a delicate skin which is easily stripped off. Specimens were quickly blotted with a soft cloth to remove excess surface moisture.

www.etd.ac.za

Most specimens were weighed on a Mettler H 10 W Analytical Balance (Fig. 5). The manufacturers specify a capacity of 160 g for this balance which is capable of weighing to 0,1 mg. Specimens considered too large were weighed to 1 g on a Model 750 S Ohaus Triple Beam Balance (Fig. 6) with a capacity of 2610 g.

It was considered desirable to determine the accuracy of the weighing procedure on the Analytical Balance. Ten randomly selected fixed specimens, ranging in mass from 0,4196 - 11,2717 grams, as determined by a single determination, were arranged in an ascending order of mass. On seven consecutive days mass was recorded. It was attempted to handle every specimen in exactly the same way as on the previous day. To prevent mass loss by dehydration weighing was performed in an air-conditioned laboratory maintained at 20°C. The recordings were performed after each specimen had been quickly blotted with a soft cloth to remove excess moisture. Results were statistically evaluated (Table 3).

UNIVERSITY of the
WESTERN CAPE

B. Fixation

Specimens were placed in Bouin's solution for final fixation. The following is the formula for Bouin's solution (Culling, 1974):

Picric acid (saturated aqueous solution)	75 ml
Formalin (40 per cent formaldehyde)	25 ml
Glacial acetic acid	5 ml

One day after the specimens were transferred to Bouin's

S.U. GENEESKUNDE BIBLIOTEEK

www.etd.ac.za

solution they were found to be firm enough to be measured.

C. Measurement

As mentioned in Chapter I of this report 4 different ways of measuring pig embryos and foetuses have been described in the literature, viz.: crown-tailroot length (CTL), crown-rump length (CRL), dorsal profile length (DPL), and the length from the most anterior part of the frontal crest to the anus.

Instruments were designed to measure CTL, CRL and DPL. The apparatus (Figs. 7 and 8) designed to measure CTL (Fig. 9) was constructed largely from perspex and consists of a heavy base. A fine wire passes through the base at one end and is drawn vertically through a raised platform 4 cm above the base. After this wire has passed through the platform it is connected to a spring attached to a platform at the same height at the other end of the base. The spring ensures that the wire is kept taut. Longitudinally, along the middle of the base, a slot is present in which a perspex bar slides and is so constructed that it can be fixed in any position by means of a perspex screw. The advancing edge of this slide has a wedge-shaped vertical attachment. When the bar is advanced the vertical edge of the wedge contacts the vertical wire at the other end of the base. On the lower surface of the base, vertically below and parallel to the horizontal wire, two fine parallel lines are present.

Embryos and foetuses were placed on their left sides on

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

different sizes of removable flat angular perspex sheets (Fig. 10). All measurements were performed with the specimens lying on their left sides. The sheets were manipulated in such a way that the root of the tail, between the embryonic body and the tail, was in contact with the wire. The horizontal wire and the indicating lines on the base of the platform were visually brought into line when viewing from above and the specimen so positioned that this now single line intersected the embryonic head in the plane described by Marrable and Ashdown (1967). The edge of the wedge on the sliding bar was gently approximated to the head (Figs. 11 and 12) and fixed by means of the screw.

The specimen was now in the position described by Marrable (1971) for measurement of the crown-tailroot length. The embryo was removed and the horizontal distance between the vertical edge of the wedge and the inner aspect of the wire recorded by means of a Vernier caliper (Fig. 13). This measurement was the crown-tailroot length.

UNIVERSITY of the
WESTERN CAPE

To measure the crown-rump length (Fig. 14) precision-made stainless steel blocks 6 cm high were constructed. A horizontal slot to receive the measuring arm of a Vernier caliper was made in each block. The arms were clamped in position by means of screws in each block.

The specimen was next placed on its left side on the lighted surface of a transparancy viewing box. Against this bright background the blocks of the Vernier scale were carefully manipulated to lightly contact the crown of the head and rump at the most protrusive points (Figs. 15 and 16). The

www.etd.ac.za

crown-rump length was recorded.

Dorsal profile length (Fig. 17) was measured on an apparatus consisting of a wooden platform with a cork surface. At the one end a vertical pillar was constructed of perspex while a vertical sheet of perspex carried a horizontal bar at the other end (Fig. 18). Through the base at one end a vertical wire passed to the horizontal bar and was strung horizontally across to the vertical pillar at a height of 8 cm. Gauge 000 black surgical silk was knotted around the vertical wire so that the knot and attached silk could slide up and down. The specimen was placed on its left side on the cork surface with the external aspect of the root of the tail against the vertical wire. The knot was manipulated to the mid-sagittal plane at the tail-root and was positioned along the back, neck, head and snout in the same plane. No tension was placed on the silk which adhered to the slightly moistened specimen. At the snout tip the silk was gripped with a small artery forceps (Figs. 19 and 20). The specimen was removed and the silk laid out along a straight line. No tension was placed on the silk. A pin was stuck into the cork surface at the point where the silk met the artery forceps. The forceps was removed and the distance between the vertical wire and the pin provided the dorsal profile length (Fig. 21).

It is clear that the instruments designed to measure the lengths of the specimens depend to a very large extent on the human eye. To ensure efficiency and accuracy only one

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

person operated the instruments.

To assess the accuracy of the measuring instruments, and consequently the type of measurement displaying the highest degree of consistent accuracy, all three measurements were repeated at daily intervals for seven days on the same test specimens used for the determination of weighing accuracy. To prevent possible distortion by dehydration every specimen was returned to its fixative for ½ hour between each measurement. Recordings were performed after each specimen had been quickly blotted with a soft cloth to remove excess moisture. Results were statistically evaluated (Table 3).

Following the experiment to determine accuracy of mensuration every specimen in the collection was measured and CTL, CRL and DPL determined.

UNIVERSITY of the
WESTERN CAPE

A statistical evaluation of data relating to the entire collection was carried out with reference to the following:

1. Litter size. The mean number of embryos or foetuses per litter was arithmetically determined.

2. Percentage relative frequencies of litter sizes. Litters containing the same number of embryos or foetuses were grouped together and the number of litters of the same size thus obtained expressed as a percentage of the total number of litters available for study (Table 1).

3. Relationship of gestational maturity to litter size.

www.etd.ac.za

Crown-rump length was considered a suitable indicator of gestational maturity. All the litters were arranged in ascending order of mean CRL. The list was arbitrarily divided into 10 sections each consisting of four litters. In this way the least mature specimens, having the smallest CRL, were grouped in section 1 and the most mature specimens in section 10. The mean number of embryos or foetuses in each section was established (Table 2).

4. <u>Correlation between individual linear measurements</u>.

Graphic correlations were established between individual linear measurements. CTL was correlated to DPL, CRL to DPL and CTL to CRL. Regression equations were calculated from the original data by the method of "least squares". Correlation coefficients were statistically determined.

5. <u>Correlation between linear measurements and mass</u>.

UNIVERSITY of the WESTERN CAPE

Correlations were established between each linear measurement on the one hand and mass. The two relevant observations for each embryo or foetus were plotted on a graph and the curve of "best fit" calculated from the original data by the method of "least squares". Third degree multipl correlation coefficients were statistically determined.

6. <u>Variation within litters</u>. Intra-litter variations were established with reference to mass, CTL, CRL and DPL (Table 4). Because the ages of the specimens are unknown data was firstly grouped according to litter size commencing with the smallest litters containing two pigs. Secondly, relevant data was grouped in

www.etd.ac.za

ascending order of mean magnitude for each parameter within the confines of the group of litters of the same size. The range of variation, standard deviation (SD) and coefficient of variation (CV) for each litter was established. Subsequently, mean coefficients of variation within each paramater were determined for the smallest and the largest litter in each group of equal-numbered litters (Table 6).

7. <u>Percentage relative frequencies of observations</u>

Individual observations for the entire population were listed in ascending order of magnitude for each parameter. The observations were divided into "cells", e.g. 0 - 60 grams, 60 - 120 grams; 0 - 20 millimeters, 20 - 40 millimeters and the number of observations in each "cell" expressed as a percentage of the total number of observations (Table 7).

UNIVERSITY of the
WESTERN CAPE

D. <u>Selection of specimens for histological investigation</u>

Specimens were selected for study on the basis of CRL (Table 8). In litters consisting of more than two members a single embryo or foetus whose CRL was closest to the mean CRL for the litter as a whole was chosen. In litters of only two members both specimens were used.

E. <u>Trimming</u>

Small specimens likely to be unnecessarily mutilated by the procedure were not trimmed but embedded as a whole

www.etd.ac.za

while in some larger specimens final trimming had to be delayed till demineralization had taken place.

Two primary landmarks were kept in mind at the initial trimming of the specimens, viz. the lower border of the mandible and the posterior border of the otic vesicle or external auditory meatus.

The head of each specimen was separated from the neck by a cut passing below the level of the lower border of the mandible and as nearly parallel to the lip slit as possible. After this had been accomplished a second cut passing at right angles to the first behind the external auditory meatus separated the posterior portion of the head from the anterior portion (Fig. 22).

F. Decalcification

UNIVERSITY of the
WESTERN CAPE

Specimens were decalcified in Bouin's solution and the initial presence of calcification as well as the end-point of decalcification were determined roentgenographically (Fig. 23) employing accepted methods (Brain, 1966).

G. Processing for microscopy

After a minimum fixation period of 4 days for smaller specimens and after decalcification had been completed in bigger heads specimens were initially dehydrated in 50 per cent alcohol for 4-6 hours, then in 60 per cent alcohol for 2 hours and finally in 70 per cent alcohol for 2 hours before further processing. The above procedures were carried out with the aid of mechanical agitation at

www.etd.ac.za

room temperature.

After the initial dehydration tissues were transferred to an automatic processor where they were immersed in 80 per cent alcohol for 2 hours, then taken through two changes of 96 per cent alcohol. The first period lasted 2 hours, the second 1 hour. Three changes of 1 hour each in 100 per cent alcohol followed.

Clearing of the tissue was accomplished according to the schedule described by Luna (1968) in which it is subjected to three changes of chloroform, the first two of 1 hour duration each and the final one of 2 hours duration. For impregnation two changes of paraffin wax (melting point $46^{\circ}C$ - $48^{\circ}C$) of 2 hours duration each were used. Final impregnation was achieved by means of Paraplast Plus (melting point $56^{\circ}C$) for 1½ hours.

UNIVERSITY of the
WESTERN CAPE

Embedding was performed in a mixture of four parts of Paraplast Plus and one part of paraffin wax with a melting point of $56^{\circ}C$. Specimens were embedded in such a way that transverse sections could be obtained, sectioning being commenced at the trimmed surface dorsal to the otic vesicle or external acoustic meatus and at right angles to the mouth slit (Fig. 24).

www.etd.ac.za

III RESULTS

A. Fixation and decalcification

It was found that formalin-fixed specimens were still limp and difficult to measure after one day. When Bouin's solution was employed as an alternative this problem was overcome and the specimens were firm and retained their shape during handling after one day in this fixative. Adequate penetration of fixative occurred in all instances and satisfactory decalcification was obtained (Fig. 23).

B. General information on the sample

Forty litters of pigs, consisting of 259 unsexed specimens, were collected in the way previously described. The mean number of pigs per litter was 6,475.

UNIVERSITY of the WESTERN CAPE

Percentage relative frequencies of litter sizes are shown in Table 1 and Fig. 25. As can be seen 47,5 per cent of the total of 40 litters had from five to seven piglets while 17,5 per cent had six.

TABLE 1

RELATIVE FREQUENCY OF LITTER SIZE

Number of specimens per litter (litter size)	Number of litters	Relative frequency of litter size (%)
2	4	10,0
3	4	10,0
4	2	5,0
5	6	15,0
6	7	17,5
7	6	15,0
8	3	7,5

www.etd.ac.za

9	4	10,0
12	1	2,5
14	2	5,0
17	1	2,5

When considering the relationship between gestational age (as indicated by CRL) and litter size in the population studied (Table 2) it is obvious that the youngest litters (Section 1) contain the most members. The small numbers of litters available preclude any definite general conclusions although it does seem that litter size stays relatively constant throughout gestation.

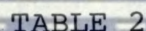


TABLE 2

RELATIONSHIP BETWEEN GESTATIONAL MATURITY AND LITTER SIZE

Section	Mean CRL range (mm)	Mean litter size
1	8,3100 - 14,0229	9,25
2	15,1867 - 17,2500	6,00
3	17,2500 - 20,0114	6,50
4	21,5900 - 30,9550	5,50
5	32,7867 - 34,9120	6,75
6	36,6867 - 42,7067	5,00
7	43,1733 - 49,2333	7,75
8	51,1840 - 56,7125	6,25
9	57,6200 - 83,0444	5,50
10	90,7600 - 185,8333	6,25

UNIVERSITY of the
WESTERN CAPE

C. Instrument and measurement accuracy

The author has previously mentioned the desirability of

www.etd.ac.za

evaluating instrument accuracy in the measurement of embryonic and foetal material. When considering the results of the investigation designed to determine the accuracy of the instruments it is clear (Table 3) that CRL is the most accurately determinable linear variable (SD 0,0959; CV 0,26%). When the initial mass or measurement is compared with the mean of the data obtained from repeated weighing or measuring it is furthermore apparent that the specimens did not increase or decrease in size over the seven days in any set pattern.

TABLE 3

ACCURACY OF TECHNIQUE

MASS

Specimen number	Initial Mass (g)	Mean (g)	Variance
1	0,4196	0,4201	0,0000
2	1,1790	1,1973	0,0003
3	2,5521	2,5613	0,0001
4	3,8033	3,7993	0,0002
5	4,5853	4,5477	0,0009
6	5,7816	5,7672	0,0005
7	6,5500	6,6261	0,0021
8	7,4710	7,4454	0,0006
9	9,5508	9,5649	0,0003
10	11,2717	11,2463	0,0004

Mean Standard Deviation (SD) = 0,0230

Mean Coefficient of Variation (CV) = 0,43%

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

C T L

Specimen number	Initial CTL (mm)	Mean (mm)	Variance
1	14,3000	14,0371	0,0194
2	19,9200	19,5543	0,0332
3	27,6400	26,9143	0,1648
4	35,1800	35,3600	0,0307
5	34,0800	33,9800	0,0607
6	39,2800	38,8743	0,4173
7	44,6400	44,5086	0,1189
8	41,8400	41,6171	0,0205
9	47,7800	47,5371	0,0797
10	50,4600	50,2457	0,1406

Mean SD = 0,3295

Mean CV = 0,93%

UNIVERSITY of the
WESTERN CAPE

C R L

Specimen number	Initial CRL (mm)	Mean (mm)	Variance
1	15,8000	15,7400	0,0136
2	21,9200	21,8600	0,0043
3	29,6600	29,7143	0,0078
4	37,8000	37,8057	0,0042
5	35,2000	35,2086	0,0090
6	40,9000	41,1057	0,0169
7	46,8600	46,7743	0,0058
8	43,9000	43,8086	0,0054
9	50,4200	50,6200	0,0145
10	53,3200	53,5286	0,0104

www.etd.ac.za

Mean SD = 0,0959

Mean CV = 0,26%

D P L

Specimen number	Initial DPL (mm)	Mean (mm)	Variance
1	33,0800	31,9429	0,3899
2	43,2000	43,1143	0,2553
3	57,8600	57,4743	0,4478
4	70,0600	70,2914	0,0753
5	74,8600	75,7886	0,2033
6	81,5800	80,8543	0,4265
7	84,9800	84,9029	0,1426
8	86,6000	85,9400	0,1835
9	90,6800	91,0143	0,1129
10	103,2200	103,0657	0,3422

UNIVERSITY of the
WESTERN CAPE

Mean SD = 0,5078

Mean CV = 0,70%

Standard Deviation and Coefficient of Variation of the obtained data may be summarised as follows:

Parameter	SD	CV(%)
Mass	0,0230	0,43
CTL	0,3295	0,93
CRL	0,0959	0,26
DPL	0,5078	0,70

D. Correlation between individual linear measurements

In order to correlate the abovementioned parameters the data for the entire series was used.

www.etd.ac.za

When CTL was plotted on the X-axis and DPL on the Y-axis the regression equation calculated from the original data was that of a straight regression line (Fig. 26). A Correlation Coefficient (r) of 0,99275 exists in this relationship.

Correlation between CRL and DPL was determined in the same way (Fig. 27) with CRL on the X-axis and DPL on the Y-axis. In this relationship r = 0,99375.

Correlation between CTL and CRL (Fig. 28) is very strong, almost perfect, due to the similarity of the dimensions measured. The regression equation calculated from the data by the method of "least squares" is again that of a straight line, r = 0,99947.

UNIVERSITY of the
WESTERN CAPE

E. Correlation between linear measurements and mass

Correlations were established between each of the linear measurements CTL, CRL, DPL and mass.

The third degree multiple correlation coefficients were 0,99196 (Fig. 29), 0,99315 (Fig. 30) and 0,99691 (Fig. 31) respectively.

All three indicate a "strong positive relationship". There appears, furthermore, to be an insignificantly higher correlation between DPL and mass than between either CRL (second highest correlation) or CTL and mass.

www.etd.ac.za

The scatter of the plotted points indicates that actual intra-litter variations in all parameters become of greater magnitude with an increase in individual size of the specimens. All the curves are third degree curves.

F. Variation within litters

In all parameters and in all the groups of litters studied there is no clear indication of a change in variation, judged by coefficients of variation, with an increase in mean specimen size. Litter size appears to have little influence on the coefficients of variation (Table 4). Mass shows the largest mean variation (CV 9,8167%) while DPL (CV 3,4384%) displays the smallest variation of the linear measurements (Table 5).

UNIVERSITY of the
WESTERN CAPE

TABLE 4

INTRA-LITTER VARIATIONS

MASS

Litter size	Mean (g)	Range (g)	SD	CV%
2	0,5221	0,4859 - 0,5582	0,0510	9,7682
2	3,2240	3,2156 - 3,2321	0,0100	0,3101
2	12,2053	11,7468 - 12,6638	0,6484	5,3124
2	33,2421	32,4823 - 34,0018	1,0744	3,2320
3	0,3354	0,2443 - 0,4186	0,0877	26,1478
3	1,8632	1,6400 - 2,2228	0,3145	16,8795
3	3,8602	3,7000 - 4,0773	0,1949	5,0489
3	8,3264	7,9000 - 8,7626	0,4314	5,1811

www.etd.ac.za

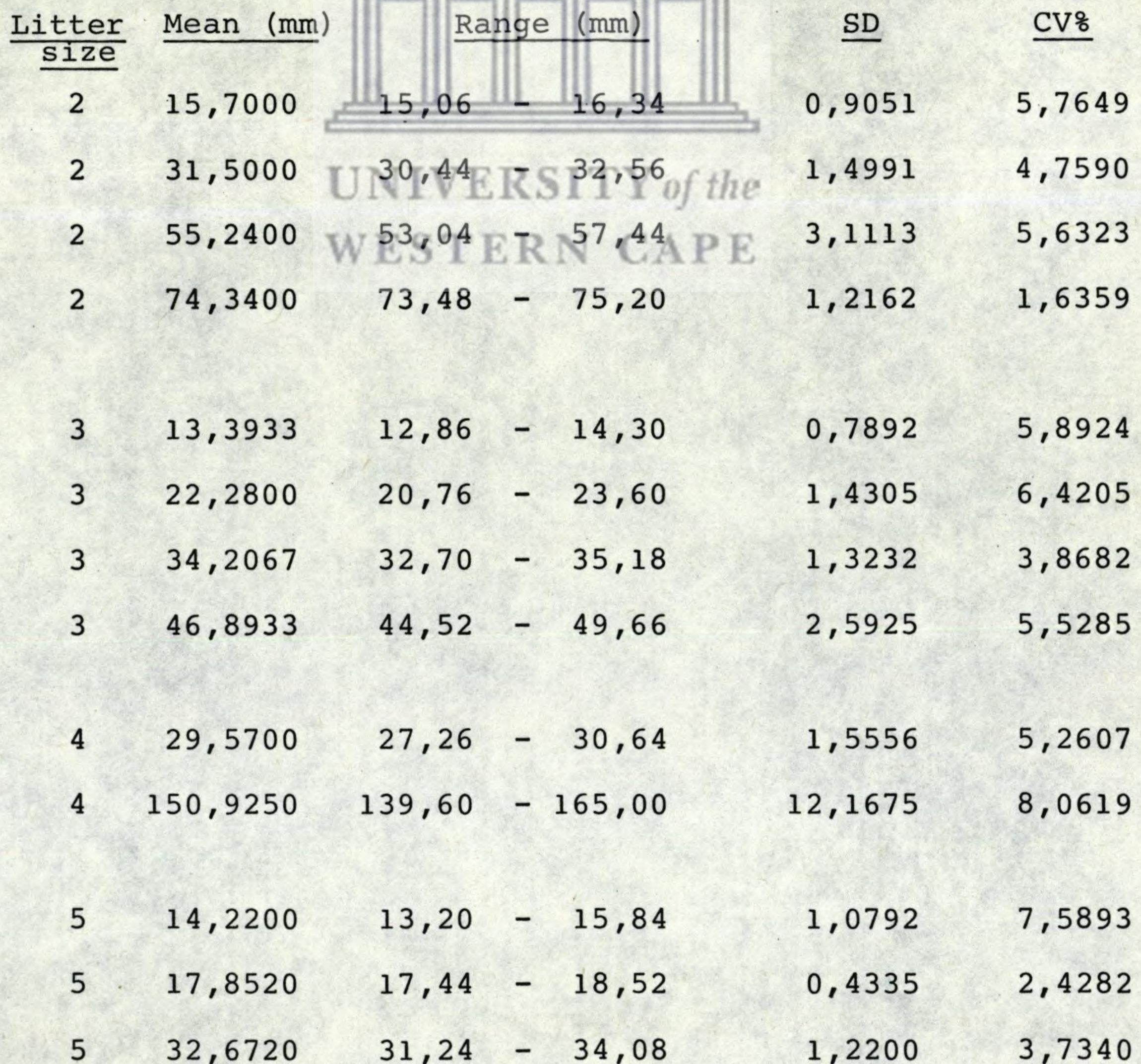
4	2,7494	2,5457 -	2,8764	0,1556	5,6594
4	251,5000	200	- 287	38,5616	15,3326
5	0,5018	0,4547 -	0,5500	0,0447	8,9079
5	0,8242	0,7452 -	0,8752	0,0500	6,0664
5	4,1159	3,5182 -	4,5853	0,3909	9,4973
5	4,1572	4,0293 -	4,2721	0,1221	2,9370
5	7,6078	7,1153 -	8,3300	0,4553	5,9846
5	10,1586	9,0746 -	11,2717	0,8593	8,4588
6	0,1341	0,1062 -	0,1646	0,0265	19,7613
6	1,1191	0,9878 -	1,2855	0,1025	9,1591
6	2,9264	2,4955 -	3,8048	0,4566	15,6027
6	7,0268	4,9435 -	8,2065	1,1030	15,6970
6	10,4928	9,7514 -	11,2664	0,5368	5,1158
6	11,5751	10,8177 -	12,1112	0,4725	4,0820
6	464,3333	422	- 502	35,1549	7,5710
7	0,2529	0,1976 -	0,3158	0,0374	14,7884
7	0,2696	0,1969 -	0,3255	0,0458	16,9881
7	0,4487	0,3040 -	0,5331	0,0894	19,9242
7	0,8485	0,7968 -	0,9090	0,0412	4,8556
7	5,5069	5,1145 -	5,9967	0,3035	5,5112
7	201,0000	182	- 215	11,1505	5,5475
8	2,1115	1,7650 -	2,5521	0,2532	11,9914
8	12,0533	9,7800 -	14,0168	1,3402	11,1189
8	64,0742	52,8400 -	74,7630	6,6122	10,3195
9	0,5487	0,3936 -	0,6722	0,0900	16,4024

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

9	6,5166	5,9258 - 7,4710	0,5237	8,0363
9	21,7917	18,9861 - 23,2305	1,5109	6,9333
9	35,2555	30,0753 - 38,6592	2,9866	8,4713
12	0,8688	0,7212 - 1,0648	0,0949	10,9231
14	3,6086	2,9169 - 4,2558	0,3122	8,6515
14	8,3190	7,0355 - 9,1630	0,7126	8,5659
17	0,1831	0,1038 - 0,2790	0,0600	32,7689

Mean SD 7,3982; CV 9,8167%


UNIVERSITY of the WESTERN CAPE

C T L

Litter size	Mean (mm)	Range (mm)	SD	CV%
2	15,7000	15,06 - 16,34	0,9051	5,7649
2	31,5000	30,44 - 32,56	1,4991	4,7590
2	55,2400	53,04 - 57,44	3,1113	5,6323
2	74,3400	73,48 - 75,20	1,2162	1,6359
3	13,3933	12,86 - 14,30	0,7892	5,8924
3	22,2800	20,76 - 23,60	1,4305	6,4205
3	34,2067	32,70 - 35,18	1,3232	3,8682
3	46,8933	44,52 - 49,66	2,5925	5,5285
4	29,5700	27,26 - 30,64	1,5556	5,2607
4	150,9250	139,60 - 165,00	12,1675	8,0619
5	14,2200	13,20 - 15,84	1,0792	7,5893
5	17,8520	17,44 - 18,52	0,4335	2,4282
5	32,6720	31,24 - 34,08	1,2200	3,7340

www.etd.ac.za

5	35,3733	34,30 - 36,38	1,0416	2,9445
5	41,9920	41,24 - 42,86	0,6149	1,4643
5	48,2200	43,00 - 51,18	3,2184	6,6744
6	6,2167	5,46 - 7,06	0,5867	9,4374
6	19,4967	18,74 - 20,66	0,7382	3,7862
6	30,7167	28,88 - 34,06	2,1975	7,1540
6	40,3700	36,74 - 42,72	2,3228	5,7537
6	49,7500	48,16 - 50,72	0,9933	1,9965
6	50,0500	47,68 - 51,72	1,5429	3,0827
6	181,9500	173,50 - 183,00	5,6617	3,1116
7	9,0857	8,26 - 10,80	0,8398	9,2430
7	12,3429	11,56 - 13,32	0,5795	4,6950
7	14,7857	13,56 - 15,76	0,9602	6,4941
7	18,7371	17,62 - 19,40	0,7489	3,9968
7	37,8257	36,82 - 38,86	0,8037	2,1247
7	141,9971	135,90 - 146,80	3,6147	2,5456
8	24,5850	22,00 - 27,64	2,2903	9,3158
8	53,6125	49,04 - 55,70	2,2187	4,6979
8	86,5375	82,88 - 88,86	1,8497	2,1374
9	14,0575	12,28 - 16,04	1,0396	7,3953
9	41,1400	38,88 - 44,64	1,8559	4,5111
9	59,8756	57,46 - 62,80	1,6530	2,7607
9	78,4000	72,56 - 82,08	3,9190	4,9987
12	14,1433	12,86 - 15,24	0,7949	5,6203

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

14	32,5214	28,88 - 36,04	2,4052	7,3957
14	43,8771	40,70 - 46,52	1,7312	3,9455
17	9,4988	7,82 - 11,82	1,1054	11,6370

Mean SD 2,4533; CV 5,0346%

C R L

Litter size	Mean (mm)	Range (mm)	SD	CV%
2	17,2500	16,80 - 17,70	0,6364	3,6892
2	33,9400	32,74 - 35,14	1,6971	5,0002
2	57,6200	54,86 - 60,38	3,9032	6,7740
2	80,0000	79,44 - 80,56	0,7290	0,9112
3	15,1867	14,38 - 15,80	0,7295	4,8035
3	24,8400	23,60 - 27,18	2,0277	8,1630
3	36,6867	35,96 - 37,80	2,6865	7,3228
3	49,2333	46,94 - 52,36	2,8044	5,6961
4	30,9550	30,74 - 31,26	0,2258	0,7294
4	155,8600	145,30 - 167,74	9,9616	6,3913
5	17,8120	17,00 - 19,50	1,0093	5,6664
5	19,6000	19,04 - 19,88	0,3265	1,6658
5	34,9120	32,82 - 37,26	1,6462	4,7152
5	36,9933	36,68 - 37,34	0,3312	0,8952
5	44,0680	43,18 - 45,00	0,7528	1,7082
5	51,1840	47,20 - 53,98	2,6865	5,2487
6	8,3100	7,06 - 9,28	0,8525	10,2587
6	21,5900	21,08 - 22,10	0,4005	1,8550

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

6	32,7867	30,74 - 36,36	2,2469	6,8530
6	42,7067	38,54 - 46,08	2,6269	6,1510
6	51,6667	50,76 - 52,76	0,8468	1,6389
6	52,3733	49,56 - 54,54	1,8273	3,4889
6	185,8333	178,00 - 189,00	4,0702	2,1902
7	11,8000	10,50 - 13,68	1,1465	9,7161
7	14,0229	13,12 - 14,56	0,4869	3,4721
7	16,4571	14,62 - 17,54	0,1375	0,8355
7	20,0114	19,24 - 20,50	0,4363	2,1802
7	40,1857	39,12 - 40,92	0,6066	1,5094
7	144,5429	135,98 - 149,50	4,5083	3,1190
8	27,0725	25,20 - 29,66	1,8777	6,9358
8	56,7125	53,14 - 59,26	2,2344	3,9398
8	90,7600	87,20 - 93,16	1,8014	1,9847
9	17,2500	15,34 - 19,12	1,1254	6,5240
9	43,1733	40,58 - 46,86	2,0942	4,8506
9	63,3444	61,22 - 66,82	1,8758	2,9612
9	83,0444	77,38 - 86,52	3,6564	4,4029
12	16,1633	14,88 - 16,94	0,6649	4,1136
14	33,5986	31,66 - 37,38	1,5000	4,4644
14	46,4057	42,88 - 48,84	1,8752	4,0408
17	11,9906	10,16 - 14,40	1,2844	10,7117

Mean SD 2,2218; CV 4,4258%

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

D P L

Litter size	Mean (mm)	Range (mm)	SD	CV%
2	32,9900	32,06 - 33,92	1,3152	3,9866
2	67,4300	66,54 - 68,32	1,2587	1,8666
2	103,8200	103,64 - 104,00	0,2546	0,2452
2	135,9300	135,56 - 136,30	0,5233	0,3849
3	29,6800	24,66 - 33,08	4,4376	14,9514
3	48,9733	47,80 - 51,12	1,8617	3,8014
3	72,0800	70,06 - 73,68	1,8462	2,5613
3	90,4933	90,00 - 91,32	0,7203	0,7959
4	64,6000	61,90 - 66,00	1,8344	2,8396
4	234,3500	219,60 - 241,80	10,0285	4,2792
5	35,6840	33,92 - 38,04	1,5985	4,4795
5	42,5800	41,68 - 44,00	0,9125	2,1430
5	63,7600	62,94 - 64,68	0,8743	1,3712
5	73,1840	67,70 - 75,86	3,2984	4,5069
5	78,6240	77,26 - 79,80	0,9832	1,2505
5	97,3320	90,34 - 103,22	6,2946	6,4671
6	22,4300	21,60 - 24,86	1,2252	5,4623
6	43,4733	43,00 - 44,40	0,5339	1,2281
6	65,1367	62,00 - 69,60	2,1937	3,3678
6	77,4600	71,00 - 82,24	3,6649	4,7313
6	91,1133	88,86 - 94,96	2,1091	2,3148
6	94,3067	87,84 - 97,46	3,5068	3,7185
6	282,3000	275,50 - 289,50	4,6230	1,6376

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

7	27,2600	24,58 - 29,46	1,5545	5,7024
7	29,5171	28,10 - 30,96	1,2270	4,1569
7	34,3914	31,50 - 36,88	1,9148	5,5676
7	39,9057	39,28 - 40,80	0,6053	1,5168
7	72,6486	70,94 - 75,40	1,6985	2,3379
7	219,6229	215,26 - 225,50	3,5680	1,6246
8	57,5675	53,94 - 59,66	1,9051	3,3093
8	102,6150	97,78 - 106,00	2,9044	2,8303
8	163,3650	156,06 - 175,38	5,8391	3,5742
9	35,0125	32,00 - 37,66	1,9956	5,6996
9	83,5667	79,80 - 86,60	2,2217	2,6585
9	118,2222	114,86 - 120,08	1,8719	1,5833
9	136,0311	128,36 - 139,42	3,5245	2,5909
12	33,0600	30,02 - 34,66	1,4039	4,2465
14	65,4271	63,00 - 70,90	1,7952	2,7438
14	81,0986	77,86 - 84,86	2,3126	2,8515
17	27,3176	23,16 - 32,62	2,8103	10,2875

Mean SD 2,8868; CV 3,4384%

TABLE 5

SUMMARY OF COEFFICIENTS OF VARIATION AND STANDARDS OF DEVIATION FOR THE ENTIRE SERIES

Parameter	SD	CV%
Mass	7,3982	9,8167

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

CTL	2,4533	5,0346
CRL	2,2218	4,4258
DPL	2,8868	3,4384

Comparison of coefficients of variation of the smallest and the largest litter, as determined by mean values, within each group of equal-numbered litters shows a consistently smaller value in the larger litters for each parameter with one exception, viz. the two litters with four members each (Table 6).

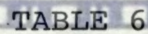


TABLE 6

DETAILED COMPARISON OF COEFFICIENTS OF VARIATION (CV%) FOR THE ENTIRE SERIES

MASS

UNIVERSITY of the WESTERN CAPE

Litter size	Number of litters	Smallest mean mass litter (CV%)	Largest mean mass litter (CV%)
2	4	9,7682	3,2320
3	4	26,1478	5,1811
4	2	5,6954	15,3326
5	6	8,9079	8,4588
6	7	19,7613	7,5710
7	6	14,7884	5,5475
8	3	11,9914	10,3159
9	4	16,4024	8,4713
12	1	10,9231	
14	2	8,6515	8,5659
17	1	32,7689	

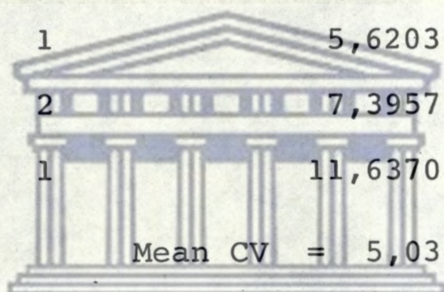
Mean CV = 9,8167%

www.etd.ac.za

C T L

Litter size	Number of litters	Smallest mean CTL litter (CV%)	Largest mean CTL litter (CV%)
2	4	5,7649	1,6359
3	4	5,8924	5,5285
4	2	5,2607	8,0619
5	6	7,5893	6,6744
6	7	9,4374	3,1116
7	6	9,2430	2,5456
8	3	9,3158	2,1374
9	4	7,3953	4,9987
12	1	5,6203	
14	2	7,3957	3,9455
17	1	11,6370	

Mean CV = 5,0346%



UNIVERSITY of the WESTERN CAPE

C R L

Litter size	Number of litters	Smallest mean CRL litter (CV%)	Largest mean CRL litter (CV%)
2	4	3,6892	0,9112
3	4	4,8035	5,6961
4	2	0,7294	6,3913
5	6	5,6664	5,2487
6	7	10,2587	2,1902
7	6	9,7161	3,1190
8	3	6,9358	1,9847
9	4	6,5240	4,4029
12	1	4,1136	

www.etd.ac.za

14	2	4,4644	4,0408
17	1	10,7117	

Mean CV = 4,4258%

D P L

Litter size	Number of litters	Smallest mean DPL litter (CV%)	Largest mean DPL litter (CV%)
2	4	3,9866	0,3849
3	4	14,9514	0,7959
4	2	2,8396	4,2792
5	6	4,4795	6,4671
6	7	5,4623	1,6376
7	6	5,7024	1,6246
8	3	3,3093	3,5742
9	4	5,6996	2,5909
12	1	4,2465	
14	2	2,7438	2,8515
17	1	10,2875	

Mean CV = 3,4384%

The nature of the population studied is illustrated by Table 7 and Figs. 32, 33 and 34.

When individual observations for the entire population were listed it was found that small specimens predominated (Table 7). It can be seen that 91 per cent of specimens had a mass of less than 60,00 g while approximately 80 per cent of specimens had a CRL of less than 60 mm.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

TABLE 7

PERCENTAGE RELATIVE FREQUENCIES OF OBSERVATIONS

MASS

Cell limits (g)	Number of observations	% Relative frequency
0 - 60	236	91,12
60 - 120	6	2,32
120 - 180	0	0,00
180 - 240	8	3,09
240 - 300	3	1,16
300 - 360	0	0,00
360 - 420	0	0,00
420 - 480	3	1,16
480 - 540	3	1,16

UNIVERSITY of the
WESTERN CAPE

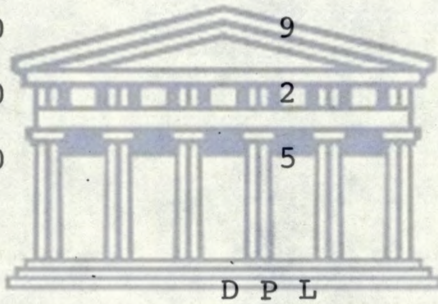
C T L

Cell limits (mm)	Number of observations	% Relative frequency
0 - 20	94	36,29
20 - 40	61	23,55
40 - 60	65	25,10
60 - 80	9	3,47
80 - 100	13	5,02
100 - 120	0	0,00
120 - 140	3	1,16
140 - 160	7	2,70
160 - 180	3	1,16
180 - 200	4	1,54

www.etd.ac.za

C R L

Cell limits (mm)	Number of observations	% Relative frequency
0 - 20	85	32,82
20 - 40	61	23,55
40 - 60	67	25,87
60 - 80	14	5,41
80 - 100	15	5,79
100 - 120	0	0,00
120 - 140	1	0,39
140 - 160	9	3,47
160 - 180	2	0,77
180 - 200	5	1,93



D P L

UNIVERSITY of the
WESTERN CAPE

Cell limits (mm)	Number of observations	% Relative frequency
0 - 30	32	12,36
30 - 60	74	28,57
60 - 90	81	31,27
90 - 120	35	13,51
120 - 150	12	4,63
150 - 180	8	3,09
180 - 210	0	0,00
210 - 240	10	3,86
240 - 270	1	0,39
270 - 300	6	2,32

www.etd.ac.za

G. Specimens selected for study

Using CRL as a basis for selecting the sample to be studied (Table 8) it is obvious that a fairly continuous series of specimens from 8,04 - 91,10 mm are available. An additional feature of the selected specimens is that their CRL is fairly close to the mean value for their litter.

TABLE 8

SELECTION OF SPECIMENS FOR STUDY

Litter number	Litter size	Mean litter CRL (mm)	Range (mm)	Specimen(s) selected (mm)
1	6	8,3100	7,06 - 9,28	8,04
2	7	11,8000	10,50 - 13,68	12,02
3	17	11,9906	10,16 - 14,40	12,06
4	7	14,0229	13,12 - 14,56	13,94
5	3	15,1867	14,38 - 15,80	15,38
6	12	16,1633	14,88 - 16,94	16,00
7	7	16,4571	14,62 - 17,54	16,50
8	2	17,2500	16,80 - 17,70	both
9	9	17,2500	15,34 - 19,12	17,32
10	5	17,8120	17,00 - 19,50	17,84
11	5	19,6000	19,04 - 19,88	19,62
12	7	20,0114	19,24 - 20,50	19,92
13	6	21,5900	21,08 - 22,10	21,60
14	3	24,8400	23,60 - 27,18	23,74
15	8	27,0725	25,20 - 29,66	26,66
16	4	30,9550	30,74 - 31,26	30,98
17	6	32,7867	30,74 - 36,36	31,98

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

18	14	33,5986	31,66	-	37,38	33,52
19	2	33,9400	32,74	-	35,14	both
20	5	34,9120	32,82	-	37,26	35,24
21	3	36,6867	35,96	-	37,80	36,30
22	5	36,9933	36,68	-	37,34	36,96
23	7	40,1857	39,12	-	40,92	40,10
24	6	42,7067	38,54	-	46,08	43,34
25	9	43,1733	40,58	-	46,86	43,90
26	5	44,0680	43,18	-	45,00	43,84
27	14	46,4057	42,88	-	48,84	47,10
28	3	49,2333	46,94	-	52,36	48,40
29	5	51,1840	47,20	-	53,98	51,00
30	6	51,6667	50,76	-	52,76	51,46
31	6	52,3733	49,56	-	54,54	52,86
32	8	56,7125	53,14	-	59,26	56,66
33	2	57,6200	54,86	-	60,38	both
34	9	63,3444	61,22	-	66,82	62,86
35	2	80,0000	79,44	-	80,56	both
36	9	83,0444	77,38	-	86,52	82,16
37	8	90,7600	87,20	-	93,16	91,10

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

IV DISCUSSION

Fixation and decalcification

It was decided to use formalin as the initial fixative for three main reasons. Firstly, formalin in various solutions is widely recognized as one of the best overall fixatives (Luna, 1968 and Culling, 1974). Secondly, the solution employed (10 per cent neutral buffered formol saline) is in general use in the laboratory and is consequently freely available and thirdly, it is a colourless solution and convenient to use during the initial stages when the material had to be frequently handled.

All material was however transferred to Bouin's solution after the mass was determined because firstly, the specimens had to be measured as quickly as possible. It was found that they tended to stay limp and were still not easy to handle after one day in formalin but in Bouin's solution they became firm and could be easily handled. Secondly, further fixation and decalcification was necessary in most of the larger specimens and Bouin's solution was considered suitable for this dual purpose.

The advantages of Bouin's fluid as a fixing solution are well known. According to Malleson and Bulleid (1924) it is a valuable fixative and has a definite place as a decalcifying agent. These authors state that it is especially useful for decalcifying foetal jaws, fixation and decalcification then taking place concurrently. Baker,

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

Silverton and Luckcock (1966) recommend Bouin's solution for the fixation of embryos and state that the required period of immersion is between 6 and 24 hours. They maintain that very little tissue distortion is produced by Bouin's solution but that the fixative penetrates poorly. No mention is made of the decalcifying properties of the solution. Luna (1968) appears cautious in his description of Bouin's solution as a fixative and describes adverse reactions which may occur following its use when the picric acid which it contains is allowed to remain in the tissue after embedding. Culling (1974), in contrast to the opinion of Baker, Silverton and Luckcock (1966), states that Bouin's fluid penetrates rapidly and evenly, causing very little tissue shrinkage. Culling (1974) is however in agreement with these authors when stating that fixation is usually complete in 24 hours. Culling (1974) also makes no mention of the decalcifying properties of Bouin's solution. In the present study Bouin's solution was found to be an entirely satisfactory fixative and decalcifying agent and the author is in agreement with von Bartheld (1956) who holds similar views.

UNIVERSITY of the
WESTERN CAPE

Trimming

Removal of the heads from the bodies is of course essential especially in the case of larger specimens. This procedure could only be carried out after decalcification had been accomplished. Decapitation and trimming of the heads not only permits correct and easy orientation during embedding but also eliminates unnecessary work

www.etd.ac.za

during sectioning.

Processing

An important procedure in the processing of all tissues fixed and/or decalcified in Bouin's solution is dehydration. According to Luna (1968) such tissues are subjected to deleterious effects when the picric acid has not been properly removed. He recommends that all tissues which have been fixed in Bouin's solution be washed in several changes of 50 per cent alcohol for 4-6 hours and agitated constantly to ensure proper removal of the acid. They may then, according to Luna (1968), be stored in 70 per cent alcohol. An interesting comment by Luna (1968) is that the harmful effects of picric acid, if not removed, may continue in the embedded specimen for a number of years, resulting in a well-stained section being obtained soon after embedding and a poorly stained section later from the same block.

UNIVERSITY of the
WESTERN CAPE

Culling (1974) is of the opinion that delicate or research material should be dehydrated slowly and recommends that a 50 per cent alcohol wash should be followed by dehydration in 60 per cent alcohol for 2 hours before being placed in 70 per cent alcohol. The reason given by Culling (1974) is that tissue immediately immersed in too great a concentration of alcohol after an aqueous fixative will usually show a high degree of shrinkage due to too rapid removal of water.

www.etd.ac.za

Final dehydration, according to Luna (1968), involves immersion of the specimen firstly in 80 per cent alcohol, then 95 per cent alcohol for 2 hours, followed by another bath of 95 per cent alcohol for 1 hour. Tissue is then subjected to 100 per cent alcohol for 1 hour followed by two further periods each of 1 hour duration in fresh 100 per cent alcohol. This procedure was only slightly modified in the present study (see p.17).

Clearing of tissue was accomplished by means of chloroform which, according to Culling (1974), is probably the most widely used clearing reagent because of its tolerance by delicate tissue.

For wax impregnation of delicate material such as foetal and areolar tissue Culling (1974) advises a melting point of 45°C. The temperature (46°C - 48°C) used in this study was slightly higher, being so specified by the manufacturers (Merck). Paraplast Plus, a material which, according to Culling (1974) penetrates tissue very rapidly, was used in the final stage of impregnation while a mixture of Paraplast Plus and paraffin wax at a melting point of 56°C was used for the final embedding.

UNIVERSITY of the WESTERN CAPE

Weighing procedure and mass

Differences exist in the weighing accuracy adopted by different authors (Lowrey, 1911; Warwick, 1928; and Marrable and Ashdown, 1967). It was the author's initial

www.etd.ac.za

experience that the analytical balance employed was not entirely reliable and showed fluctuation at the 0,1 mg level when repeatedly activated for the same specimen. It is not implied that the fault rests with the balance but that it can be attributed rather to factors beyond easy control viz. dehydration of the specimen while on the weighing platform and air currents. It was however, subsequently found possible to obtain consistently accurate recordings by employing a careful technique. Only one person operated the scale and recordings were carried out with a minimum of delay.

Mass, as an indicator of embryonic and foetal growth is apparently not favoured probably because of its tendency towards greater intra-litter variation in animals which habitually produce more than one offspring during a single gestation. Marrable (1971) summarises views on the unsuitability of mass as a comparative parameter when he states that masses of early embryos are much influenced by factors such as the amounts of cord included, varying amounts of retained blood and dehydration when unprotected by amniotic fluid. He does however state that mass has the advantage that it is independent of shape. Notwithstanding these views Marrable (1971) does take cognisance of mass to the extent that graphs showing the relationship between mass and gestational age and prediction equations for mass from gestational age are provided. Only mass, however, can be considered as an index of growth, according to Balinsky (1970) who furthermore states that

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

from a biological viewpoint growth is an increase of mass of living substance.

Litter size

The mean number of specimens per litter of 6,475 compares favourably with the findings of Lowrey (1911). This author used 22 litters comprising "about" 130 individuals, providing a mean number of approximately 5,9 per litter. Lowrey (1911) quotes Long (1906) who stated that pig litters average 7,8 to the litter. Warwick (1928) states that litter size diminishes from 11,4 at the 20th day of gestation to 6,8 at the 110-day stage (close to full term), probably as a result of intra-uterine degeneration and resorption of embryos and foetuses. The mean number (6,475) found in this study is however less than the 10,23 births per litter reported by Davies (1976). In the present collection three specimens were considered degenerate on visual examination and were not included in the study. Table 2, although not based on the same number of litters as used by Warwick (1928), tends to show that in the population studied litter size stays relatively constant in relation to gestational maturity.

UNIVERSITY of the
WESTERN CAPE

Measurement

No comparisons of accuracy between different ways of measuring embryonic and foetal material have in the past, with the possible exception of von Bartheld (1956), been carried out and no record has been found in available

www.etd.ac.za

literature of instruments designed to perform specific measurements. Furthermore, although different types of measurement have been used by various authors, as described, no clearly motivated reasons, again with the exception of von Bartheld (1956), are ever given for the use of a specific method.

Hamilton, Boyd and Mossman (1972) state that two or three measurements are usually employed in estimating age of specimens. These are crown-rump length, crown-heel length which is, in the author's opinion, applicable only to human and possibly primate material and neck-rump length in embryos which are markedly flexed. According to Hamilton, Boyd and Mossman (1972) measurements of very early embryos are unreliable due to the variable degree of flexion. Arey (1974), who agrees with Hamilton, Boyd and Mossman (1972), believes that human material can be measured in various ways, three of which can be applied to the majority of non-human mammalian specimens. He states that the commonest measurement is the crown-rump length which he describes as the distance from vertex to breech. For very young embryos in which body flexion has not yet occurred he advises the greatest length (GL) as the measurement of choice, while neck-rump length is recommended for embryos in which the head is greatly flexed.

Although certain figures are given in both the above-mentioned works no attempt at correlation of data is made and no clear developmental ages or stages are described

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

when specific measurements are applicable. Any worker wishing to correlate personal work with these figures would be at a loss to decide which measurement to employ.

Von Bartheld (1956), who performed his study on domestic pigs, is believed to be the first to critically compare measurements in order to distinguish different stages of development. He decided that crown-rump measurements were not sufficiently accurate and resorted to measuring the dorsal profile length. Reasons for selecting this measurement are not discussed and no statistical evaluation of his data is provided but the author does mention that the crown-rump lengths showed greater intra-litter variation than the dorsal profile lengths. In his series von Bartheld (1956) measured all the specimens from a litter in this way and, after determining the mode, used specimens corresponding to the mode in his histological study. Marrable (1971) is of the opinion that early embryos are difficult to measure because of their continuing change of shape and lack of sharply defined morphological landmarks. Only at about 20 days, when the embryo is in the region of 10 mm in length according to his method of measuring, has the wave of torsion nearly passed and permanent reference points become clear. He introduces yet another parameter viz. the crown-tailroot distance. One presumes that the appearance of three linearly arranged

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

landmarks which can be expected to enhance accuracy is the main criterion for selecting this measurement above other methods.

Measurement of the distance from the most anterior part of the frontal crest to the anus was not performed in this study. Ullrey et al (1965) create the impression that their measurements of crown-rump length and this measurement are perfectly correlated since no distinction is drawn in their article between specimens measured in these two ways. This is difficult to accept without supporting statistical data. No exact method for recording this measurement is described in available literature and the landmarks are difficult to determine. Although Ullrey et al (1965) state that the measurement was easily made and that variations between normal individuals of the same age were small no figures to confirm the merits of the measurement are provided.

UNIVERSITY of the WESTERN CAPE

Instrument accuracy

Results of the mensuration experiment lead one to the conclusion that the instruments designed show a high degree of accuracy. Furthermore, on the available evidence (Table 3) one must assume that crown-rump length is a more consistently accurate single measurement than either crown-tailroot length or dorsal profile length. Another major advantage of crown-rump length is that it is easy to measure. The excellent reliability displayed

www.etd.ac.za

by these methods, particularly CRL, indicate that a single measurement is adequate.

Correlation between linear measurements

Correlation between linear measurements are believed to have never previously been determined. Neither have correlations between linear measurements and mass been established. The establishment of these correlations for the entire series was motivated by a desire to know whether a dimensional relationship in different parameters exists. Results show that the correlation between crown-rump length and dorsal profile length (Fig. 27) is fractionally stronger than the correlation between crown-tailroot length and dorsal profile length (Fig. 26). The difference is however insignificant due to the similarity of mensuration of crown-rump length to crown-tailroot length. All the correlations are very strong.

UNIVERSITY of the
WESTERN CAPE

Correlation between linear measurements and mass

Correlation determinations between linear measurements and mass show that third degree multiple correlations are all very high and do not differ significantly from one another. The correlation between dorsal profile length and mass is marginally higher than that for the other two, indicating that dorsal profile length should be a more accurate reflection of embryonic size than the other measurements. Furthermore, it should not be

www.etd.ac.za

affected to the same extent by positional changes in the embryonic body. The measurement error for DPL is, however, larger than that for CRL (Table 3).

A general conclusion which may be drawn from the findings in these correlation experiments is that a positive dimensional relationship in all the measured parameters exists in an embryo and a foetus during all phases of growth.

Intra-litter variations in mass

Lowrey (1911), although not publishing supportive data, states that total mean body mass of males is consistently higher than in females. He does not, however, take this sex difference into consideration in any of his conclusions on relative growth of organs except in the case of the gonads. Warwick (1928) remarks that much variation exists in the size of foetuses of the same age especially during the last half of pregnancy. Pomeroy (1960) states that intra-litter variation in foetal mass increases with litter size. He further states that male foetuses were slightly heavier than female foetuses at all stages. Ullrey et al (1965) combine data on mass of males and females in their study of growth of the pig foetus except in the case of the gonads and the thyroid where they find that males have heavier gonads than females and that females have heavier thyroid glands. Marrable and Ashdown (1967) and Marrable (1971) state that wide variation in

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

the sizes of embryos from the same litter exist throughout pregnancy, masses being more variable than lengths judged by a comparison of their respective coefficients of variation. Their data also suggests that variation in lengths and masses is greater in the second half of gestation than in the first, thus agreeing with Warwick (1928). Marrable (1971) is consequently of the opinion that mass is a less satisfactory indicator of prenatal age than length because of its greater variability.

Mass shows the greatest intra-litter actual variation, standard deviation and coefficient of variation (Table 5). In this respect the author agrees with Marrable and Ashdown (1967) and Marrable (1971). With one exception the coefficient of variation of the heaviest litter is less than the coefficient of variation of the lightest litter in any equal-numbered group (Table 6). In two cases comparisons were impossible due to the fact that only one litter was available for study. It is significant that the largest CV values were found in litters of small mean mass (CV 26,1478%, mean litter mass 0,3354 g; CV 19,9242%, mean litter mass 0,4487 g). Low CV values were found in litters with a higher mean litter mass (CV 0,3101%, mean litter mass 3,2240 g; CV 3,2320%, mean litter mass 33,2421 g).

On the evidence of the above findings the author finds it difficult to agree with Warwick (1928), Marrable and Ashdown (1967) and Marrable (1971) that intra-litter

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

variation increases with embryonic age. On the contrary, the author suggests that relative intra-litter variation, as reflected by coefficients of variation, diminishes with an increase in the gestational age. Although the percentage of degenerate foetuses reported by Warwick (1928) is low (3,68%) he does show that the greatest percentage tends to degenerate during earlier stages of gestation and that the figure shows a gradual diminution with increasing age. A larger percentage of degenerate foetuses in young litters would tend to increase the coefficients of variation compared to older litters. In the present study there is no evidence to suggest an increased variation with increased litter size, judged by coefficients of variation.

Intra-litter variation in length

UNIVERSITY of the
WESTERN CAPE

In the analysis of intra-litter variations in length, dorsal profile length was found to have the smallest mean coefficient of variation followed by crown-rump length and crown-tailroot length (Table 5). In all these parameters there is, with some exceptions, a decreased coefficient of variation in larger specimens of the same litter group (Table 6). These findings again disagree with those of Pomeroy (1960), Marrable and Ashdown (1967) and Marrable (1971) who suggest that variation in length increases with gestational age. They, however, seem to consider the absolute variability as measured in terms of standard deviations. In the measurement of biological

www.etd.ac.za

material of this nature such an effect is to be expected. It is therefore, more appropriate to consider the relative variability as measured in terms of coefficients of variation as was done in this study. According to Marrable (1971) intra-litter size variation may be caused by various factors such as the initial size of the egg, its genetically controlled rate of development, time of arrival in the uterus, speed of implantation and differing times of fertilisation.

The sample selected for study

The material used in this study (Table 8) was selected on the basis of crown-rump length. It was decided not to include the three outstandingly large litters in the histological study. The specimens selected for study form a fairly continuous series up to approximately 90 mm which is well beyond the size at which completion of oral morphogenesis can be expected, according to descriptions by Marrable (1971) and Patten and Carlson (1974).

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

V CONCLUSIONS

In the present study conducted on 40 litters of pigs consisting of 259 specimens the following conclusions can be made:

1. The mean number of specimens per litter is 6,475.

2. Although the youngest litter group appears to consist of more members per litter than older litters there is no tendency for litter size (number of specimens per litter) to diminish with an increase in gestational age in the general population studied.

3. Instruments specially designed to measure embryos and foetuses in different parameters i.e. crown-tailroot length (CTL), crown-rump length (CRL) and dorsal profile length (DPL) functioned to a high degree of accuracy.

4. Crown-rump length appears to be the most accurately determinable linear measurement, followed by dorsal profile length and crown-tailroot length. Mass is, judged by its coefficient of variation, second to crown-rump length in accuracy, as determined by the accuracy study.

5. Correlation determinations between the linear measurements of individual specimens using the whole series of embryos and foetuses show a very strong correlation (maximum 0,99947, minimum 0,99275)

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

between all the dimensions. This proves that a definite dimensional relationship is maintained with increase in size of the specimens studied.

6. Third degree multiple correlations between individual linear measurements and mass (maximum 0,99691, minimum 0,99196) are very strong and add further to the concept of a dimensional relationship maintained by embryos and foetuses during growth.

7. In all the specimens mass shows a greater intra-litter variation, judged by the coefficient of variation, than any measurement.

8. There is no clear indication of increased variation in mass with an increase in litter size.

9. Litters of older gestational age (estimates based on size and mass) show a tendency towards a smaller relative variation in mass, judged by the coefficient of variation, than younger litters.

10. A determination of intra-litter variation in individual lengths shows that dorsal profile length displays the smallest variation, judged by the coefficient of variation, followed by crown-rump length and crown-tailroot length.

11. Relatively, lengths appear to vary less in older litters than in younger litters, irrespective of the litter size.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

12. Crown-rump length, by virtue of its reliability and ease of recording, forms a reliable basis for determining the developmental status of embryos and foetuses. In the author's opinion size is a correct alternative to, and perhaps a better indicator than, age in descriptions of embryological development. A population such as the present one in which size is carefully correlated to gestational age would however fulfil the descriptive embryologist's dream.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

VI REFERENCES

Arey, L.B. (1974) Developmental Anatomy, Revised 7th ed., Ch. 6, p. 104; Ch. 13, pp. 227-230. Philadelphia : W. B. Saunders Company.

Baker, F. J., Silverton, R.E. & Luckcock, E.D. (1966) An Introduction to Medical Laboratory Technology, 4th ed., Ch. 14, p. 278. London : Butterworths.

Balinsky, B.I. (1970) An Introduction to Embryology, 3d ed., Ch. 18, p. 591. Philadelphia : W.B. Saunders Company.

Brain, E.B. (1966) The preparation of Decalcified Sections, Ch. 3, pp. 128-132. Springfield : Charles C. Thomas.

Culling, C.F.A. (1974) Handbook of Histopathological and Histochemical Techniques, 3d ed., Ch. 3, p. 45; Ch. 3, p. 49; Ch. 5, p. 76; Ch. 5, p. 79; Ch. 5, p. 81; Ch. 5, p. 83. London : Butterworths.

Davies, P.V.A. (1976) Personal communication.

Hamilton, W.J., Boyd, J.D. & Mossman, H.W. (1972) Human Embryology, 4th ed., Revised by Hamilton, W.J. & Mossman, H.W., Ch. 8, pp. 176-177; Ch. 11, pp. 294-302. Cambridge: W. Heffer & Sons Ltd., Baltimore : The Williams and Wilkins Company.

UNIVERSITY of the WESTERN CAPE

www.etd.ac.za

Long, J. (1906) Quoted by Lowrey, L.G. (1911).

Lowrey, L.G. (1911) Prenatal growth of the pig. The American Journal of Anatomy, 12, 107-138.

Luna, L.G. (1968) Editor : Manual of Histological Staining Methods of the Armed Forces Institute of Pathology, 3d ed., Ch. 1, p.3; Ch. 1, p.5; Ch. 2, p.16. New York : The Blakiston Division, McGraw-Hill Book Company.

Malleson, H.C. & Bulleid, A. (1924) Practical Dental Histology and Bacteriology, Part 1, p. 10. London : John Bale, Sons & Danielsson, Ltd.

Marrable, A.W. (1971) The Embryonic Pig : A Chronological Account, Ch. 3, pp. 89-98. Tunbridge Wells, Kent: Pitman Medical Publishing Co. Ltd.

Marrable, A.W. & Ashdown, R.R. (1967) Quantitative observations on pig embryos of known ages. Journal of Agricultural Science, 69, 443-447.

Patten, B.M. & Carlson, B.M. (1974) Foundations of Embryology, 3d ed., Ch. 19, pp. 443-445. New York : McGraw-Hill Book Company.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

Pomeroy, R.W. (1960) Infertility and neonatal mortality in the sow. I : Lifetime performance and reasons for disposal of sows. Journal of Agricultural Science, 54, 1-17.

Ross, R.B. & Johnston, M.C. (1972) Cleft Lip and Palate, Ch. 1, p. 1. Baltimore : The Williams and Wilkins Company.

Scott, J.H. & Symons, N.B.B. (1974) Introduction to Dental Anatomy, Ch. 2, pp. 47-52. Edinburgh : Churchill Livingstone.

Sicher, H. & Bhaskar, S.N. (1972) Editors: Orban's Oral Histology and Embryology, 7th ed., Ch. 1, pp. 7-11. Saint Louis : The C.V. Mosby Company.

UNIVERSITY of the
WESTERN CAPE

Sperber, G.H. (1976) Craniofacial Embryology, 2nd ed., Ch. 3, pp. 35-38. Bristol: John Wright & Sons Ltd.

Ullrey, D.E., Sprague, J.I., Becker, D.E. & Miller, E.R. (1965) Growth of the swine fetus. Journal of Animal Science, 24, 711-717.

Von Bartheld, F. (1956) Ontwikkeling en Betekenis van Glazuurknoop en Glazuurstreng bij het Melkgebit van het Varken. Ch. 3, pp. 58-60. Thesis for the Degree of Doctor of Medicine, University of Groningen.

www.etd.ac.za

Warwick, B.L. (1928) Prenatal growth of swine. Journal of Morphology and Physiology, 46, 59-84.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

VII ILLUSTRATIONS

FIG. 1. Pregnant pig uterus after removal from carcass. Two uterine horns (A) can be seen leading from the vagina (B). Thickenings (C) in the horns indicate the position of embryos or foetuses.

UNIVERSITY of the
WESTERN CAPE

FIG. 2 Foetal membranes which envelope each specimen have been removed but the foetus still remains attached to its placenta by the umbilical cord.

www.etd.ac.za

FIG. 1

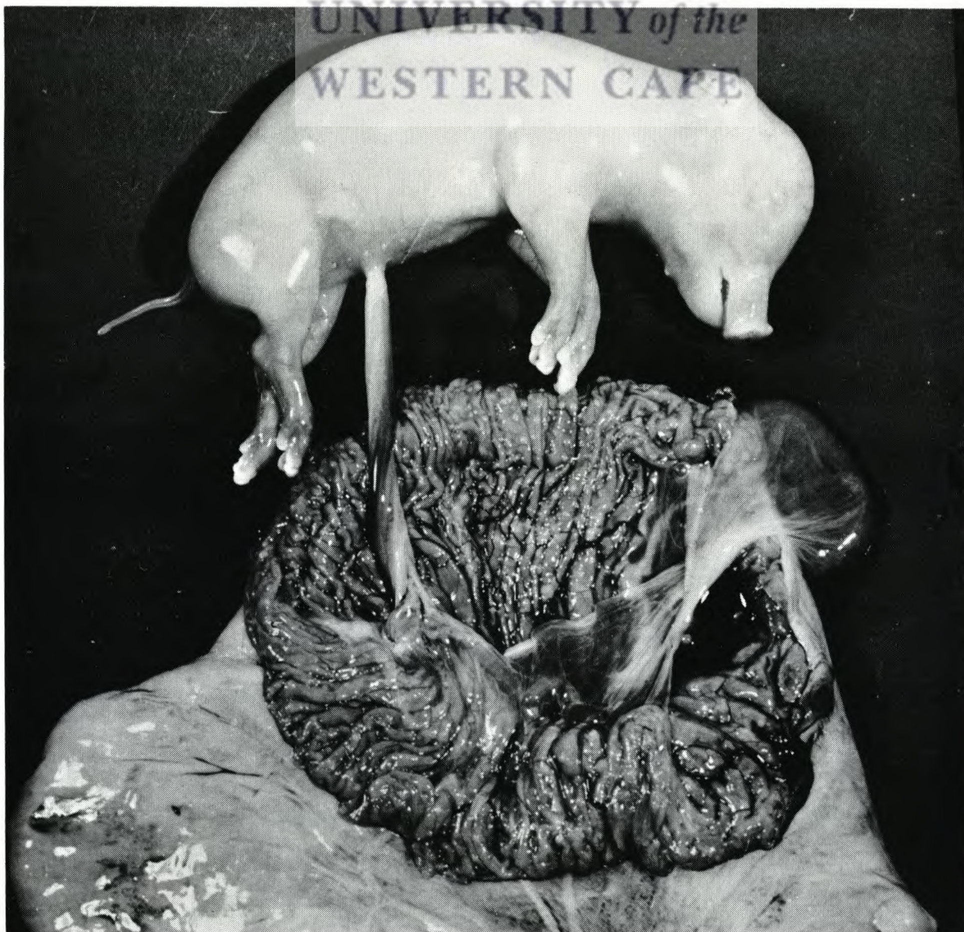


FIG. 2

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

FIG. 3. The umbilical cords are cut as close as possible to the body of the specimen. The arrow indicates the area where the umbilical cord was severed in this embryo of 20,50 mm. crown-rump length.

UNIVERSITY of the
WESTERN CAPE

FIG. 4. The Wild dissecting stereomicroscope which was used in the removal of foetal membranes and the severing of umbilical cords in small and delicate specimens.

www.etd.ac.za

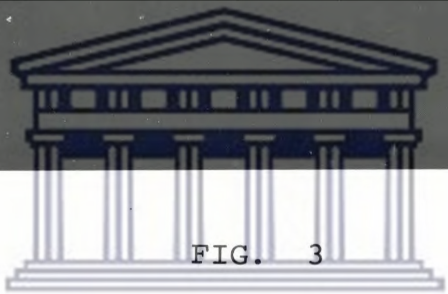


FIG. 3

UNIVERSITY of the
WESTERN CAPE

FIG. 4

www.etd.ac.za

FIG. 5. The Mettler H 10W analytical balance employed in mass determination of most of the specimens.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

UNIVERSITY of the
WESTERN CAPE

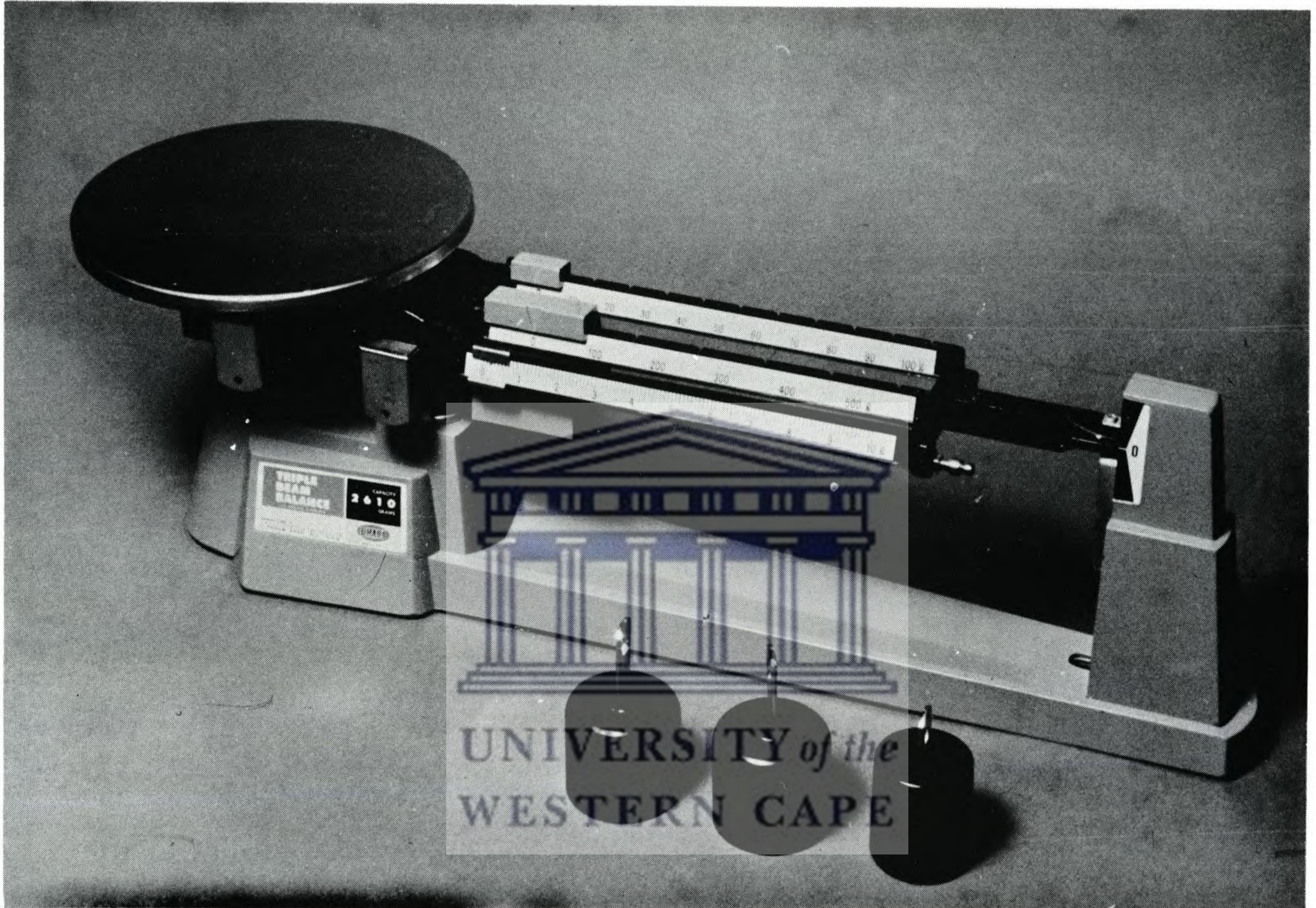
FIG. 5

www.etd.ac.za

FIG. 6. The model 750 Ohaus Triple Beam Balance which was used for the determination of mass in a few very large specimens.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za



UNIVERSITY of the
WESTERN CAPE

FIG. 6

www.etd.ac.za

FIG. 7. The apparatus designed to measure crown-tailroot length. A triangular perspex sheet on which small embryos were placed before manipulating them into the correct position for measuring is shown in the foreground. The larger sheet lying on the apparatus was used for bigger specimens.

FIG. 8. A drawing of the apparatus shown in fig. 7. The apparatus consists of a perspex base (A). A fine horizontal wire (B) is connected to a spring (C) attached to a raised platform at one end of the apparatus and is drawn vertically through a platform at the other end before being attached to the base. A perspex bar (D) which can be fixed in position by means of a screw (E) slides in a sunken slot (F) in the base. The advancing edge of the sliding bar has a chisel-edged vertical attachment. Two fine parallel lines (G) have been drawn in the base vertically below the horizontal wire.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

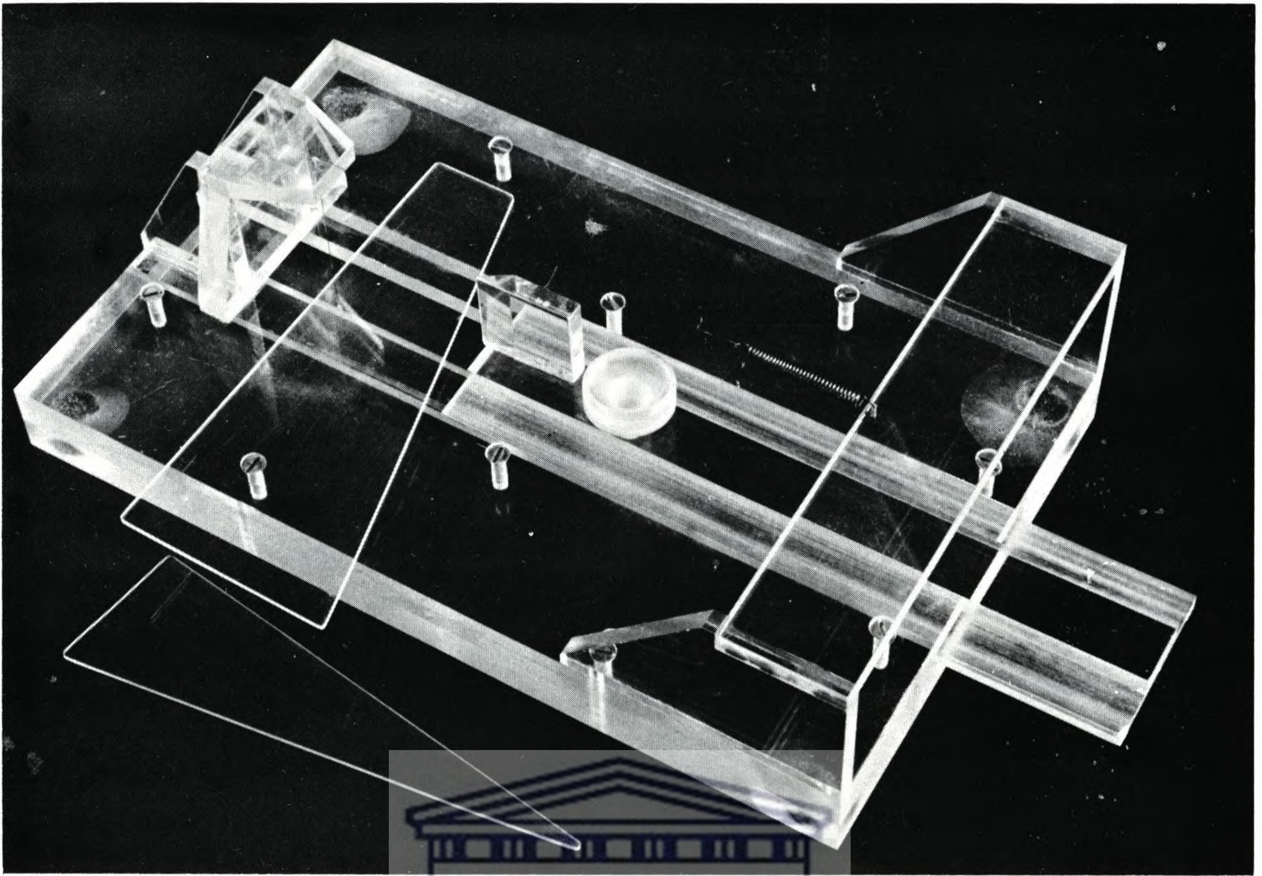


FIG. 7

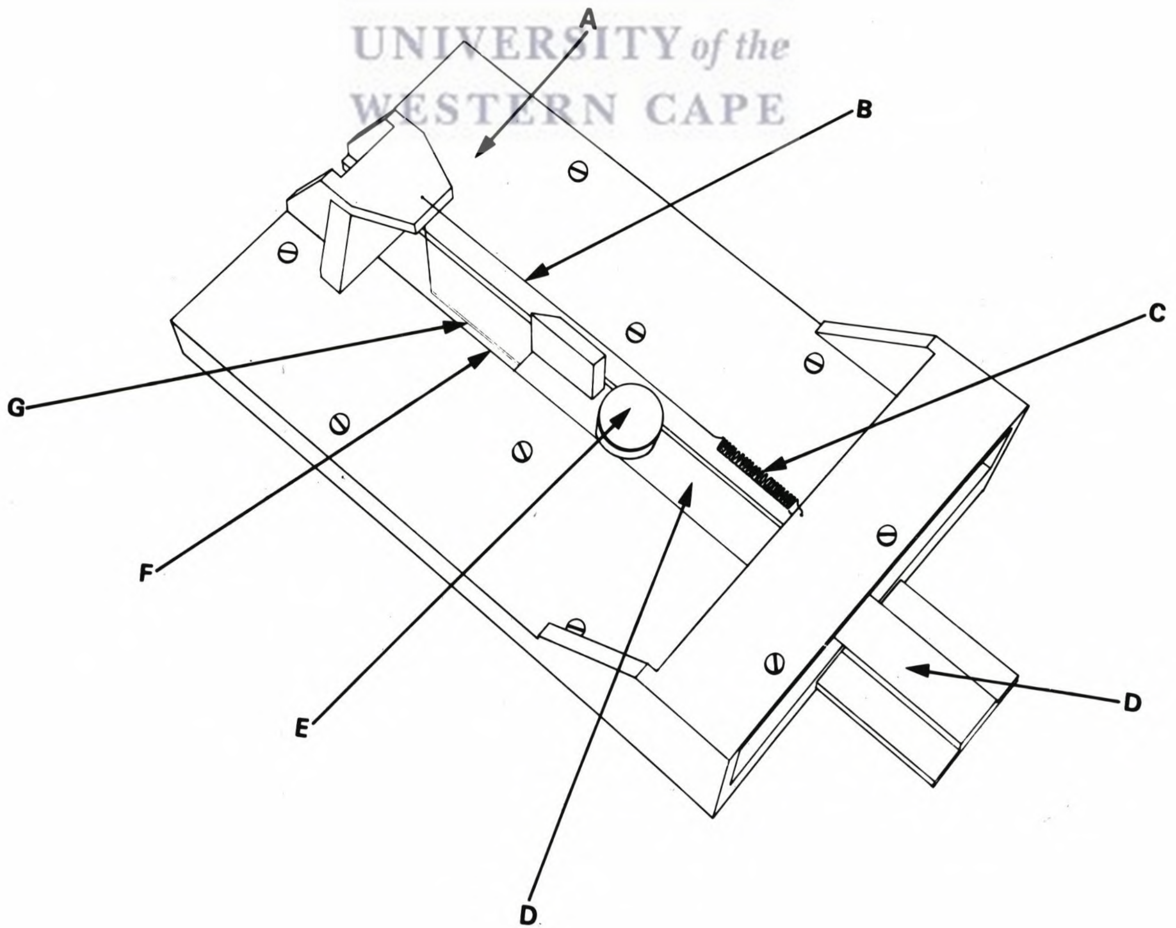


FIG. 8

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

FIG. 9. Crown-tailroot length. Crown-tailroot length is the length of a straight line passing from the root of the tail to the ventral border of the external auditory meatus and projected to cut the profile of the head.

FIG. 10. The specimen is placed on its left side on a perspex sheet (shown in fig. 7) with the vertical wire (arrowed) in contact with the root of the tail and is then manipulated into position.

www.etd.ac.za

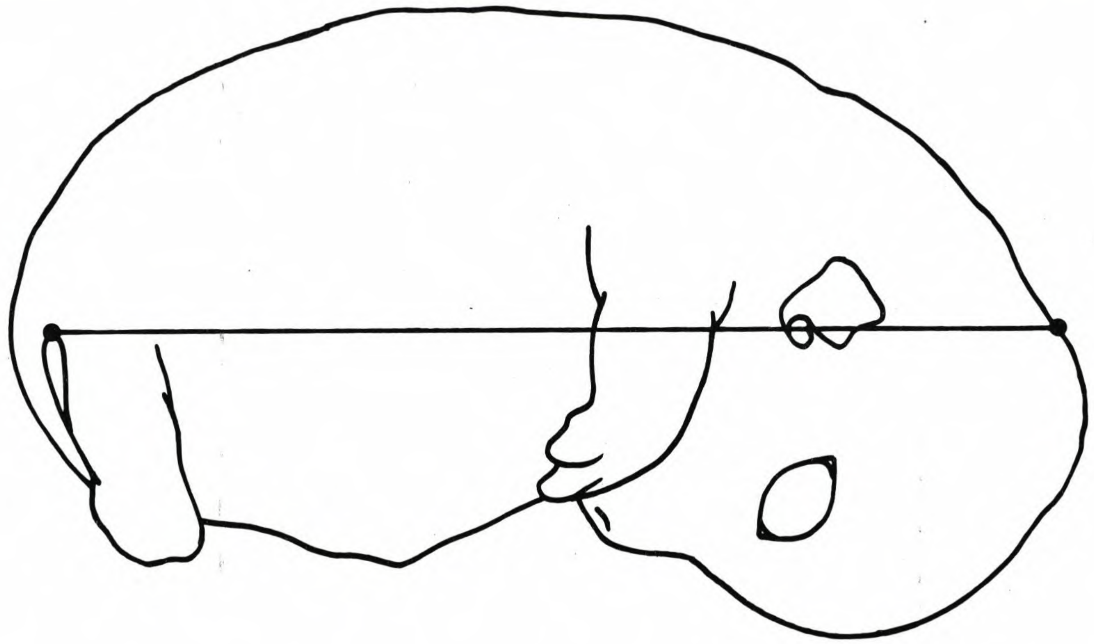


FIG. 9

UNIVERSITY of the
WESTERN CAPE

FIG. 10

www.etd.ac.za

FIG. 11. After the vertical wire is brought into contact with the root of the tail the specimen is manipulated into the correct position by visually aligning the horizontal wire with the two parallel horizontal lines (not demonstrated in this photograph) on the perspex base.

UNIVERSITY of the
WESTERN CAPE

FIG. 12. This drawing shows the perspex sheet (A) on which the specimen is lying, the vertical chisel-edged attachment (B) and the vertical wire (C). The specimen is now in a position where crown-tailroot length can be measured.

www.etd.ac.za

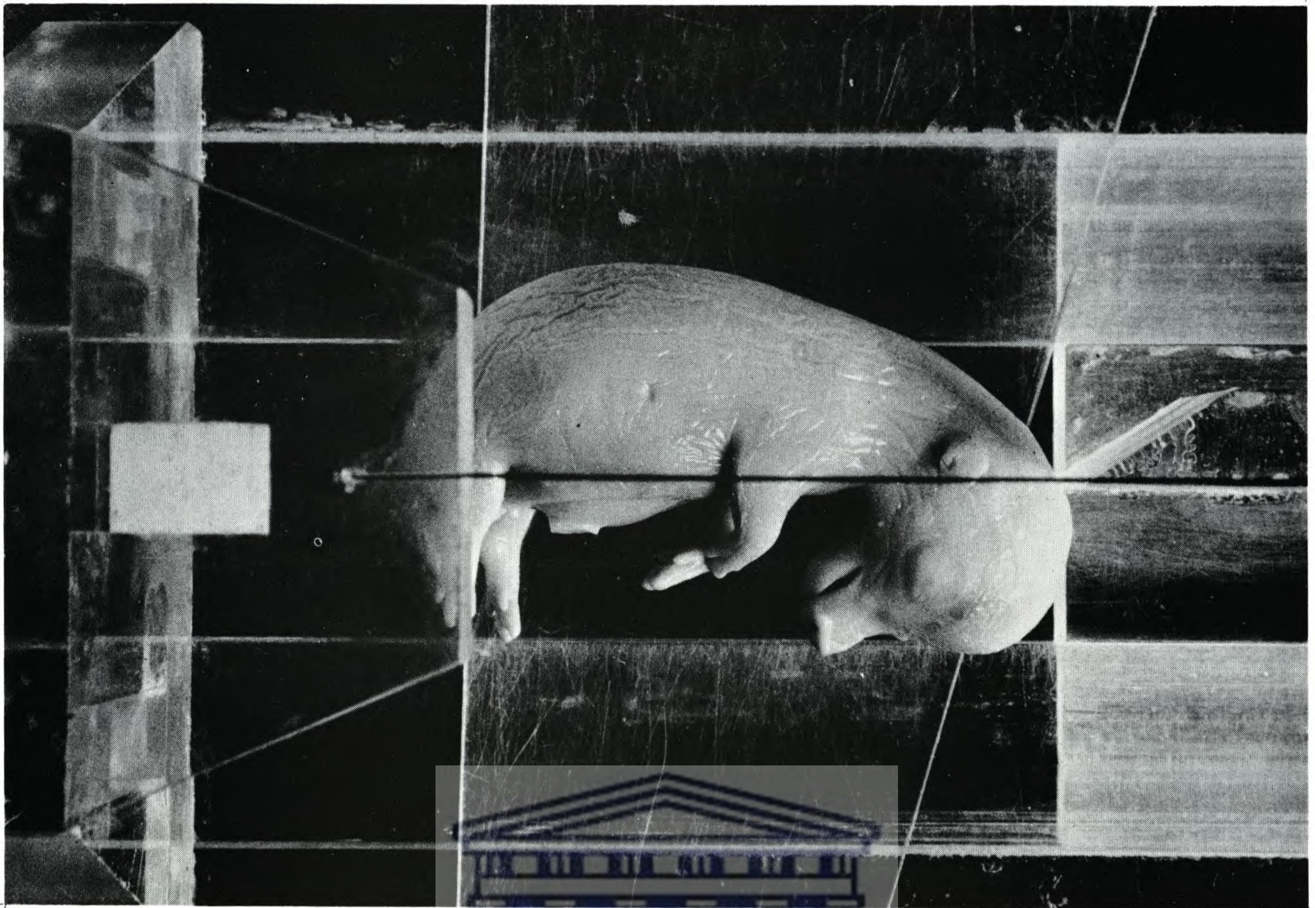


FIG. 11

UNIVERSITY of the
WESTERN CAPE

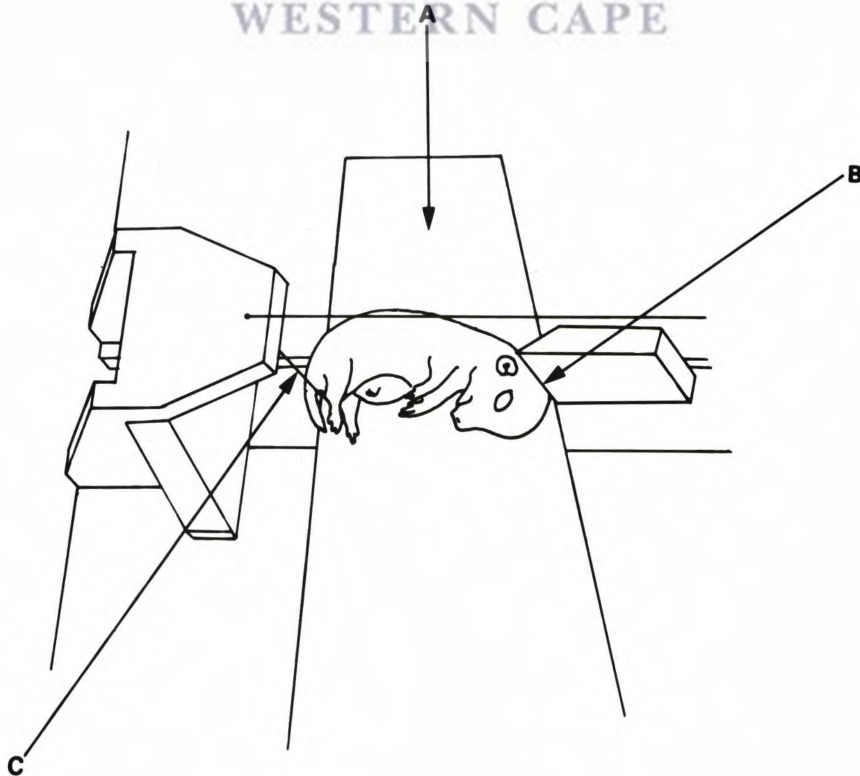


FIG. 12

www.etd.ac.za

FIG. 13. After the sliding bar has been fixed with the specimen in the correct position for crown-tailroot distance to be measured the specimen is removed and the distance between the vertical chisel-edged attachment and the vertical wire (figs. 10, 11 and 12) measured. This measurement is the crown-tailroot length.

FIG. 14. Crown-rump length is the greatest length of an embryo or foetus measured from the vertex to the external aspect of the root of the tail.

www.etd.ac.za

FIG. 13

UNIVERSITY of the
WESTERN CAPE

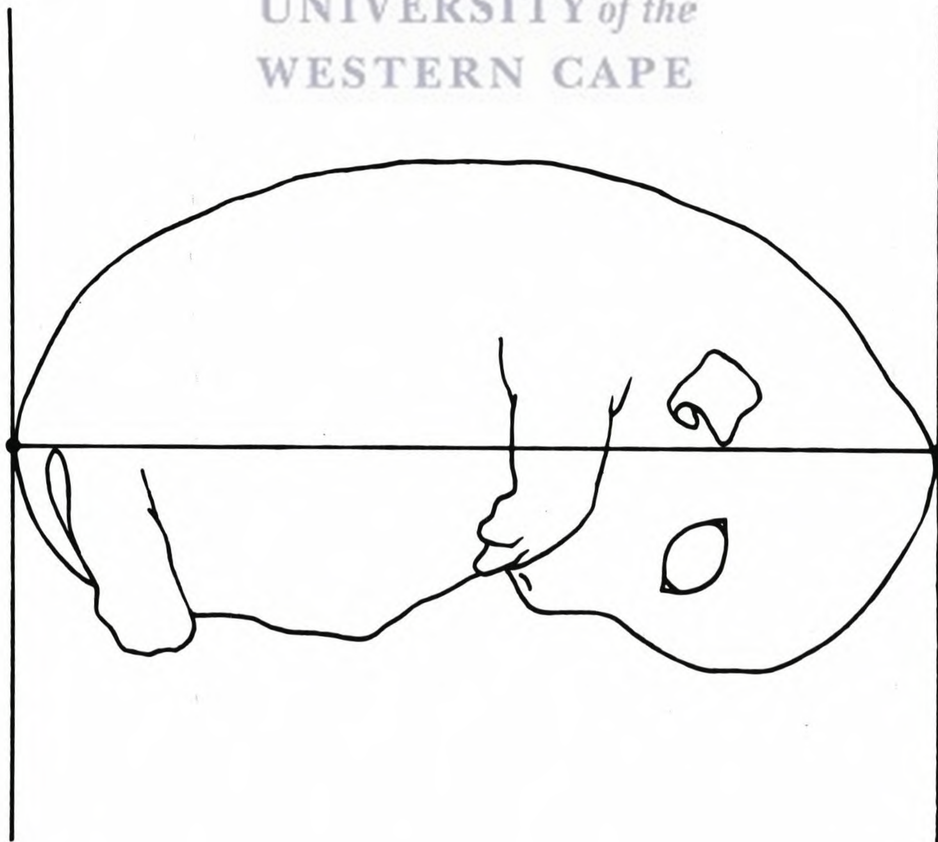


FIG. 14

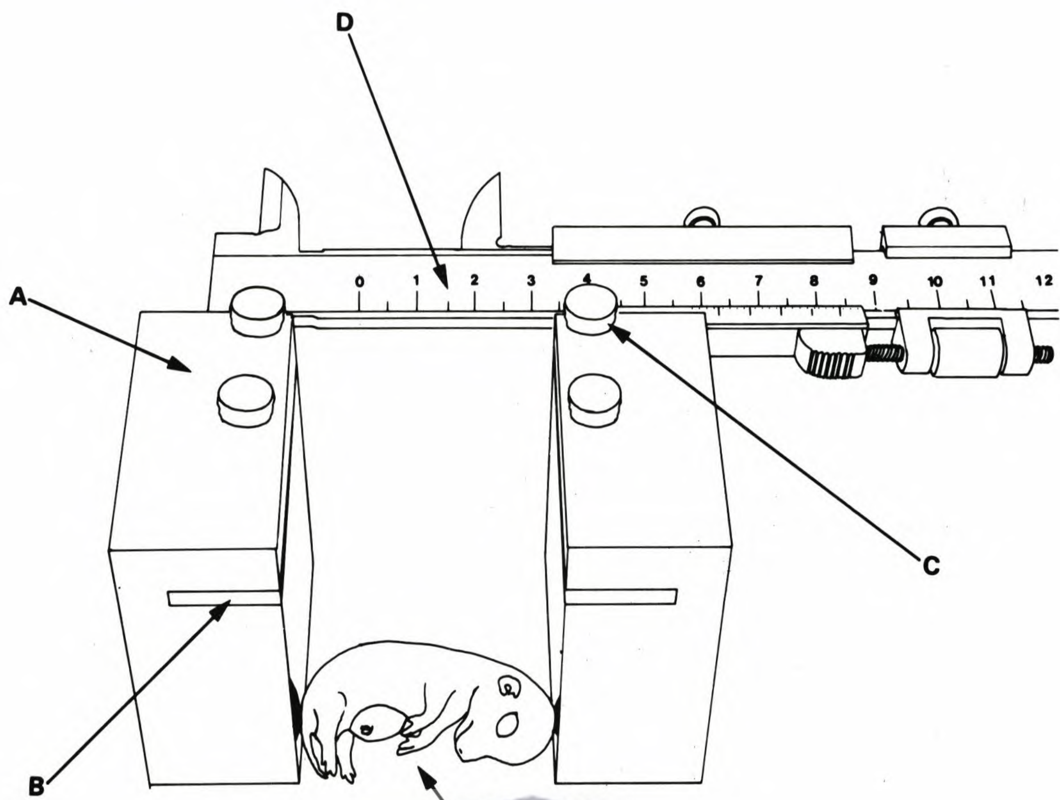
www.etd.ac.za

FIG. 15. The apparatus to measure crown-rump length consists of 2 precision-engineered stainless steel blocks (A). Slots (B) to receive the measuring arms of a Vernier caliper were made in the blocks. The arms were fixed by means of screws (C) and the distance between the blocks read off the scale (D). The specimen was measured lying on its left side against the lighted background (E) of an X-ray viewing box.

UNIVERSITY of the
WESTERN CAPE

FIG. 16. The specimen in position for the recording of crown-rump length. The lighted background and the polished inner surfaces of the blocks provided a reflection at both ends and compression of the specimen was avoided by observing the reflections.

www.etd.ac.za



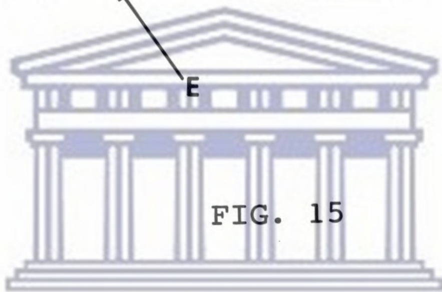


FIG. 15

UNIVERSITY of the
WESTERN CAPE

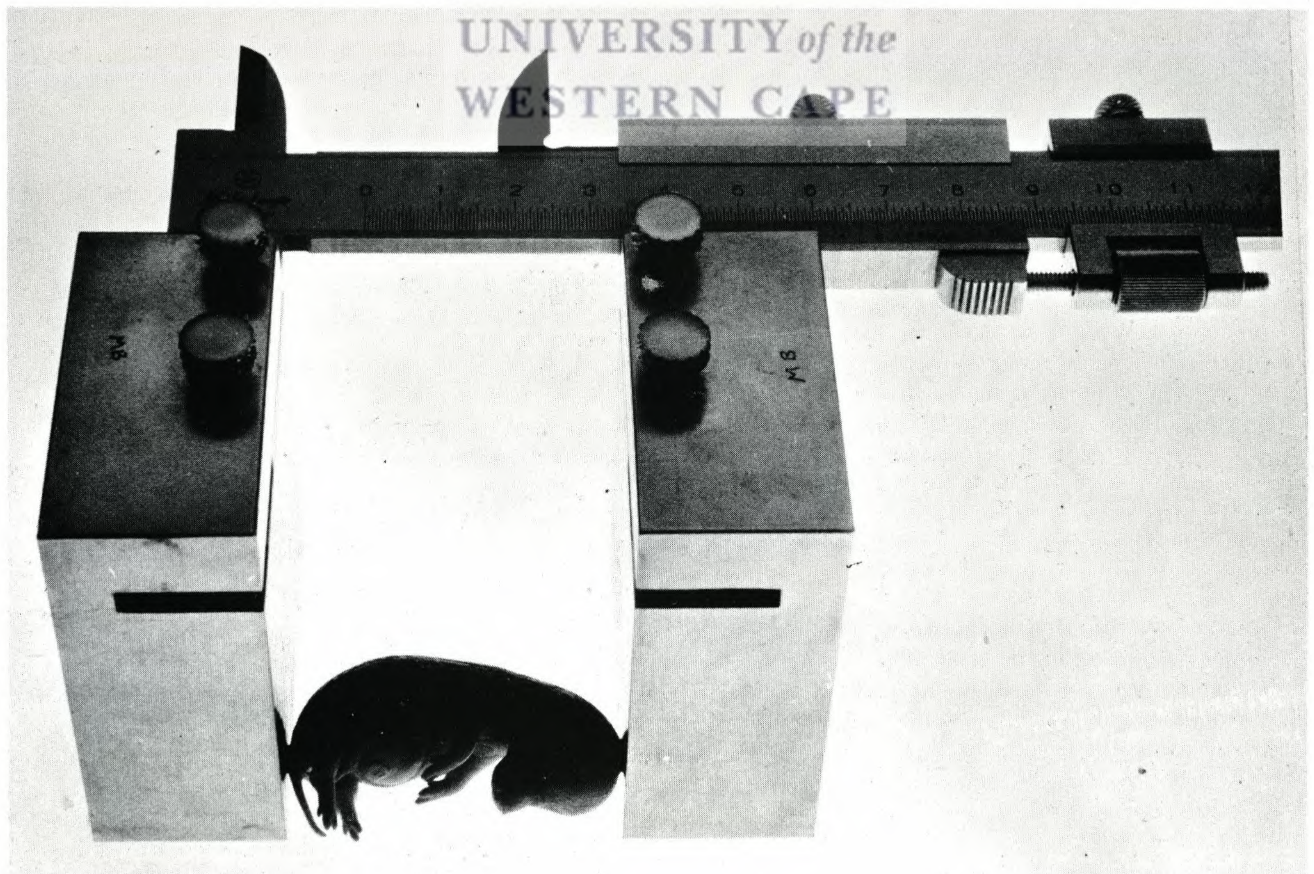


FIG. 16

www.etd.ac.za

FIG. 17. Dorsal profile length is the distance from the outer aspect of the root of the tail along the dorsal midsaggital plane to the snout tip.

UNIVERSITY of the
WESTERN CAPE

FIG. 18. The apparatus to measure dorsal profile length consists of a cork surface on a wooden platform. At one end a vertical pillar (A) receives the end of a horizontal wire (B) which passes through a horizontal bar (C) and is then strung vertically down (D) and is fixed to the base of the wooden platform. Silk (E) is knotted around the vertical wire.

www.etd.ac.za

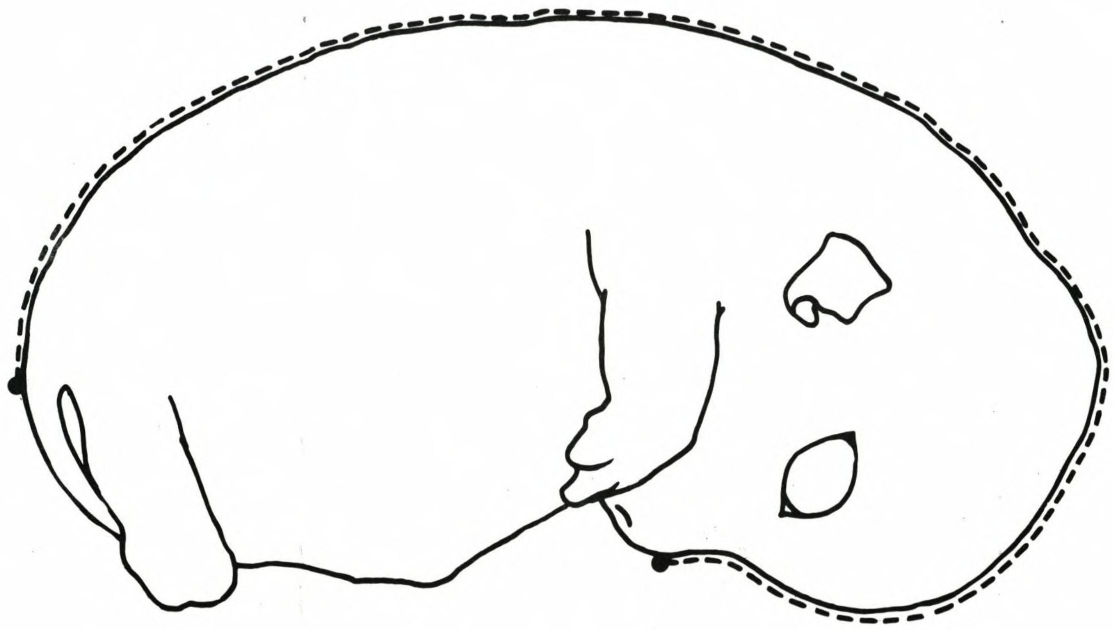


FIG. 17

UNIVERSITY of the
WESTERN CAPE

FIG. 18

www.etd.ac.za

FIG. 19. The specimen is lying on its left side on the cork surface with the outer aspect of the root of its tail against the knot (arrowed). The silk has been laid along its dorsal midsaggital plane and clamped at the snout tip.

UNIVERSITY of the
WESTERN CAPE

FIG. 20. Dorsal view of the same specimen shown in fig. 19.

www.etd.ac.za

FIG. 19

UNIVERSITY of the
WESTERN CAPE

FIG. 20

www.etd.ac.za

FIG. 21. After removal of the specimen the silk is laid out along a straight line and a pin (arrowed) pushed into the cork next to the inner edge of the artery clamp tip. The distance between the pin and the vertical wire is the dorsal profile length.

UNIVERSITY of the
WESTERN CAPE

FIG. 22. This drawing illustrates the cuts made during the trimming of the head. Cut 1 separates the head from the body while cut 2 removes the unwanted posterior portion of the head. The unshaded portion is further processed for microscopy (see fig. 24).

www.etd.ac.za

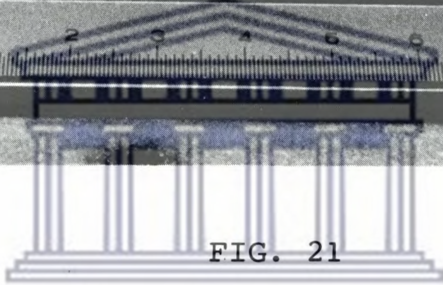


FIG. 21

UNIVERSITY of the
WESTERN CAPE

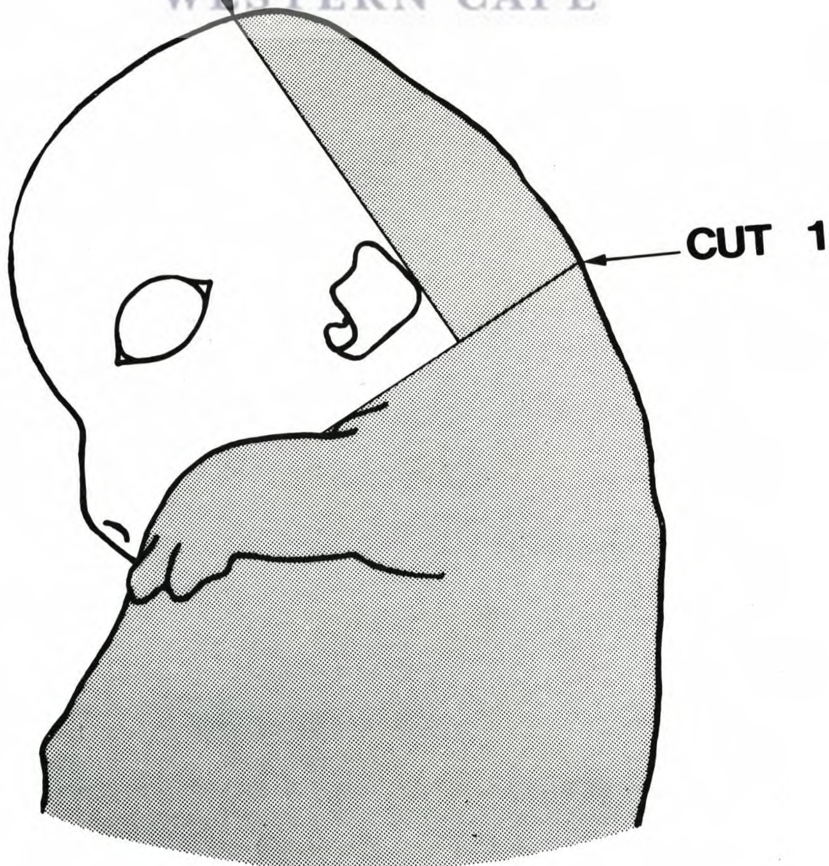


FIG. 22

www.etd.ac.za

FIG. 23A. Specimens were decalcified in Bouin's solution. The end-points of decalcification were determined roentgenographically as is shown in these photographs (figs. 23A, B, C, D and E) of a specimen of 91.80 mm. crown-rump length. Arrows indicate foci of mineralisation. Fig. 23A shows the specimen before decalcification was commenced.

FIG. 23B. Specimen after 1 day in Bouin's solution.

www.etd.ac.za

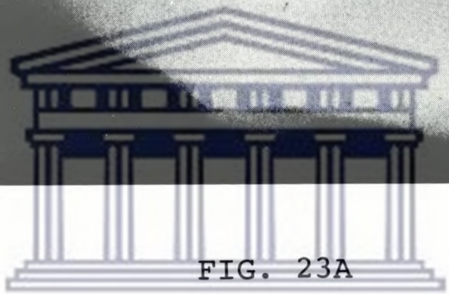


FIG. 23A

UNIVERSITY of the
WESTERN CAPE

FIG. 23B

www.etd.ac.za

FIG. 23C. Specimen after 2 days in Bouin's solution.

UNIVERSITY of the
WESTERN CAPE

FIG. 23D. Specimen after 3 days in Bouin's solution.

www.etd.ac.za

C

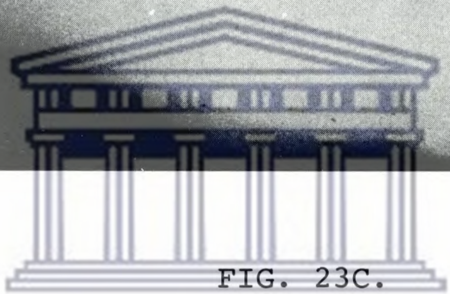


FIG. 23C.

UNIVERSITY of the
WESTERN CAPE

D

FIG. 23D.

www.etd.ac.za

FIG. 23E. Decalcification is complete in this specimen after 4 days in Bouin's solution.

UNIVERSITY of the
WESTERN CAPE

FIG. 24. The trimmed and processed head is embedded in a wax block (A) and is ready for sectioning in the transverse plane. The snout tip lies deep in the wax away from the surface shown. As sectioning proceeds the snout tip will be approached. A section obtained from the surface illustrated will pass through the brain (B), the eyes (C), dorsal recesses of the nose (D) and the mouth slit (E).

www.etd.ac.za

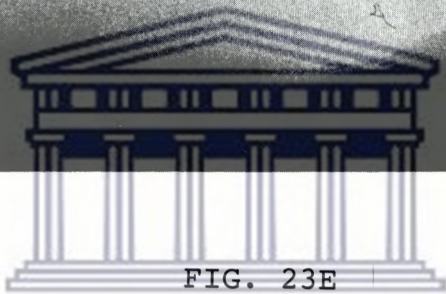


FIG. 23E

UNIVERSITY of the
WESTERN CAPE

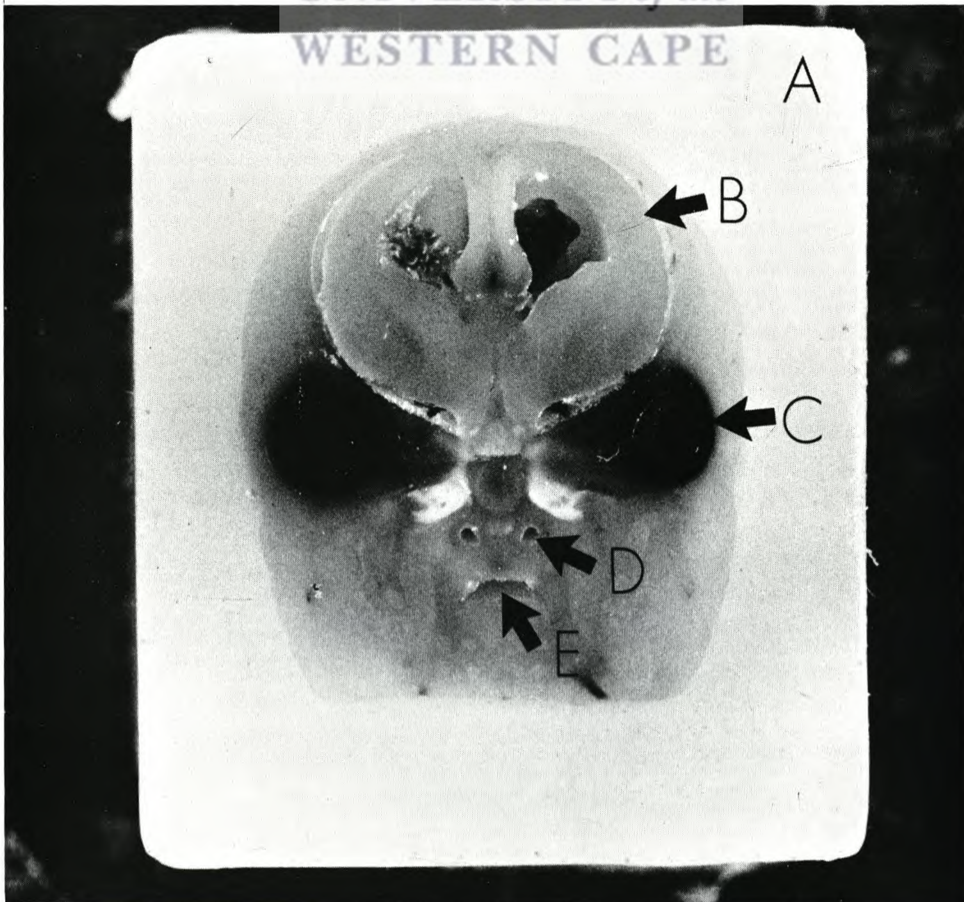


FIG. 24

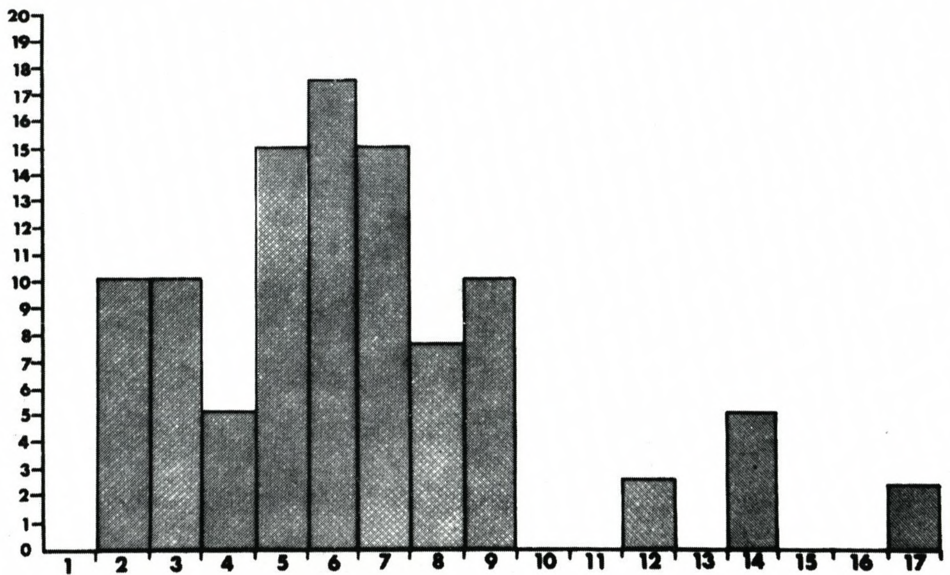
www.etd.ac.za

FIG. 25. This histogram illustrates very clearly that litters of 6 occur with the greatest relative frequency (17,5%) in the population studied. Litters of 5 and 7 members have an equal relative frequency (15%).

UNIVERSITY of the
WESTERN CAPE

FIG. 26. In this graph CTL has been plotted on the X-axis and DPL on the Y-axis. Data for the entire population was used. The regression equation calculated is that of a straight line demonstrating a fairly high coefficient of correlation (r = 0,99275).

www.etd.ac.za



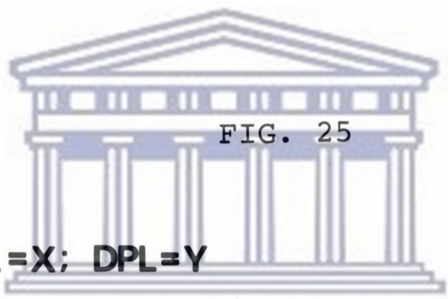


FIG. 25

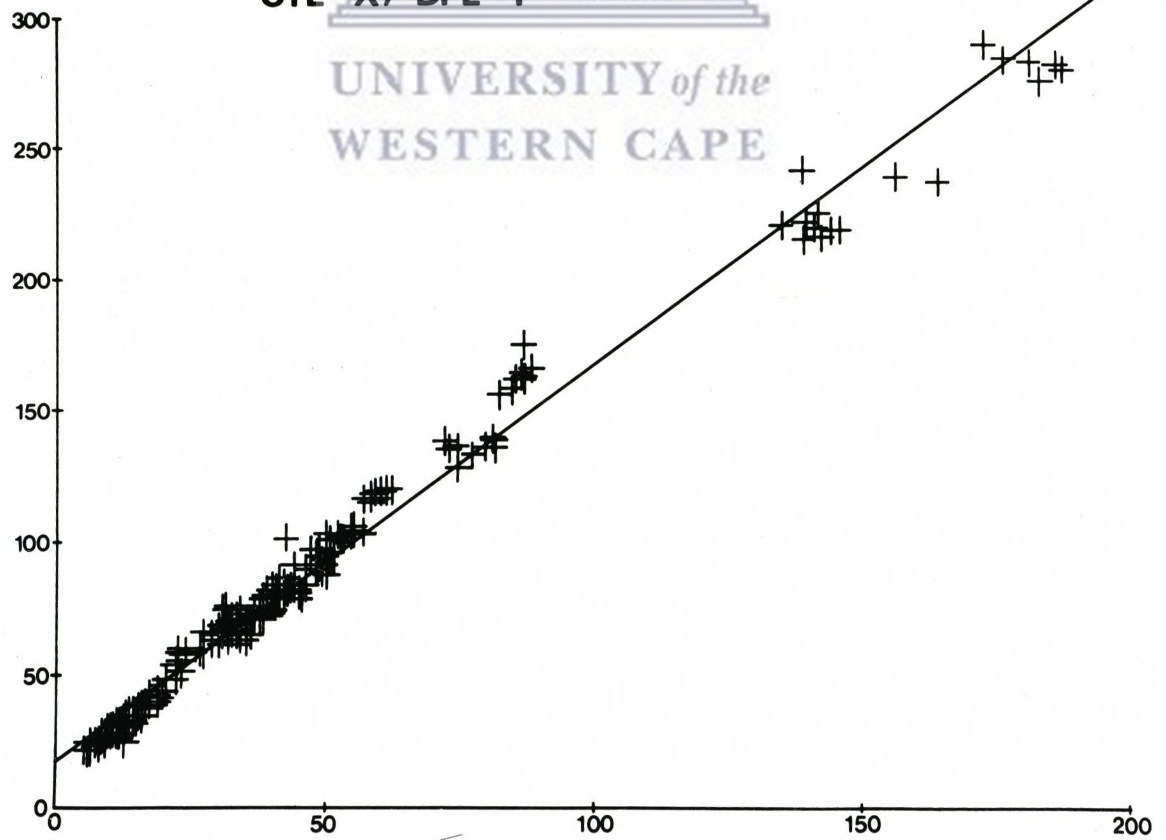


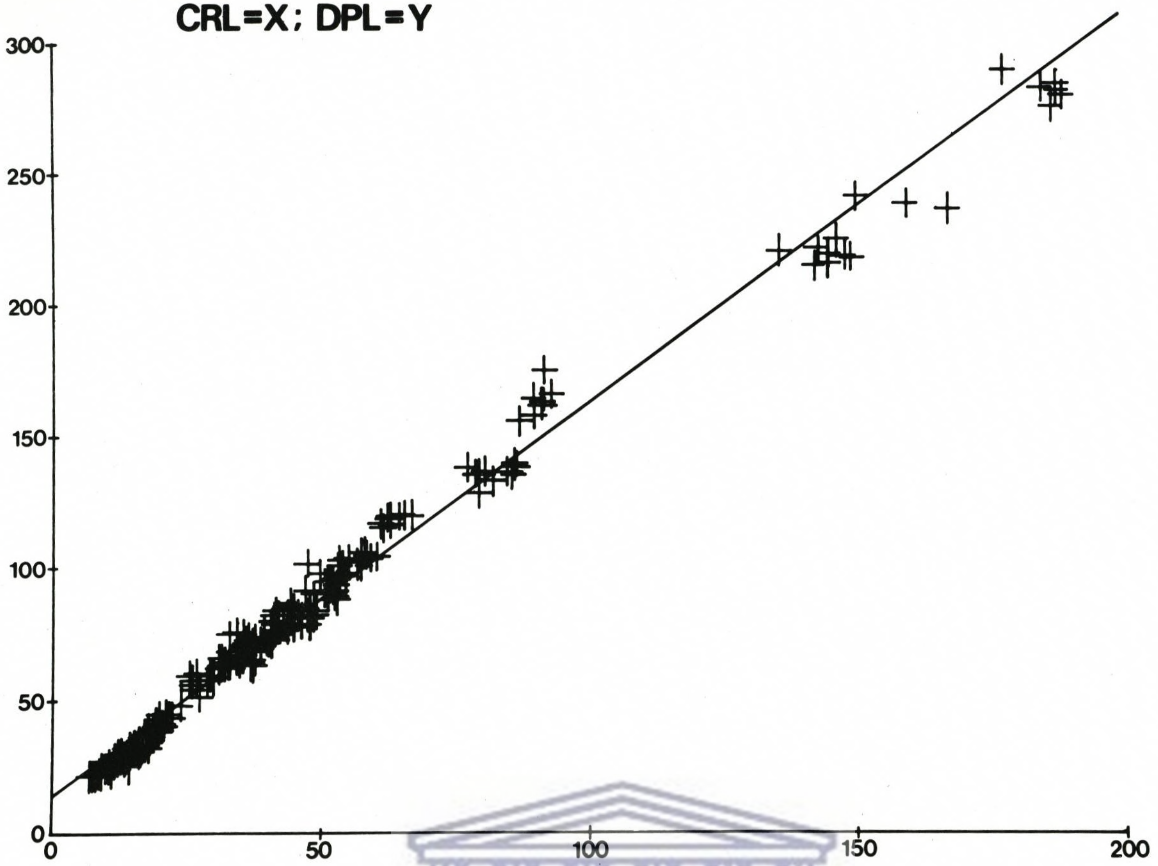
FIG. 26

www.etd.ac.za

FIG. 27. In this graph CRL is plotted on the X-axis and DPL on the Y-axis. In this relationship r = 0,99375.

FIG. 28. Correlation between CTL and CRL, due to the similarity in parameters measured, is almost perfect with r = 0,99947.

www.etd.ac.za



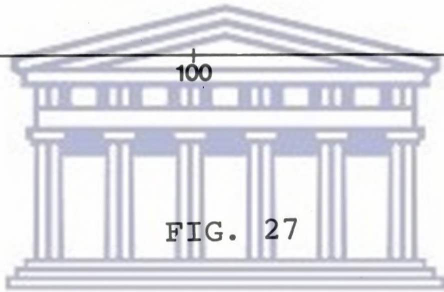


FIG. 27

UNIVERSITY of the
WESTERN CAPE

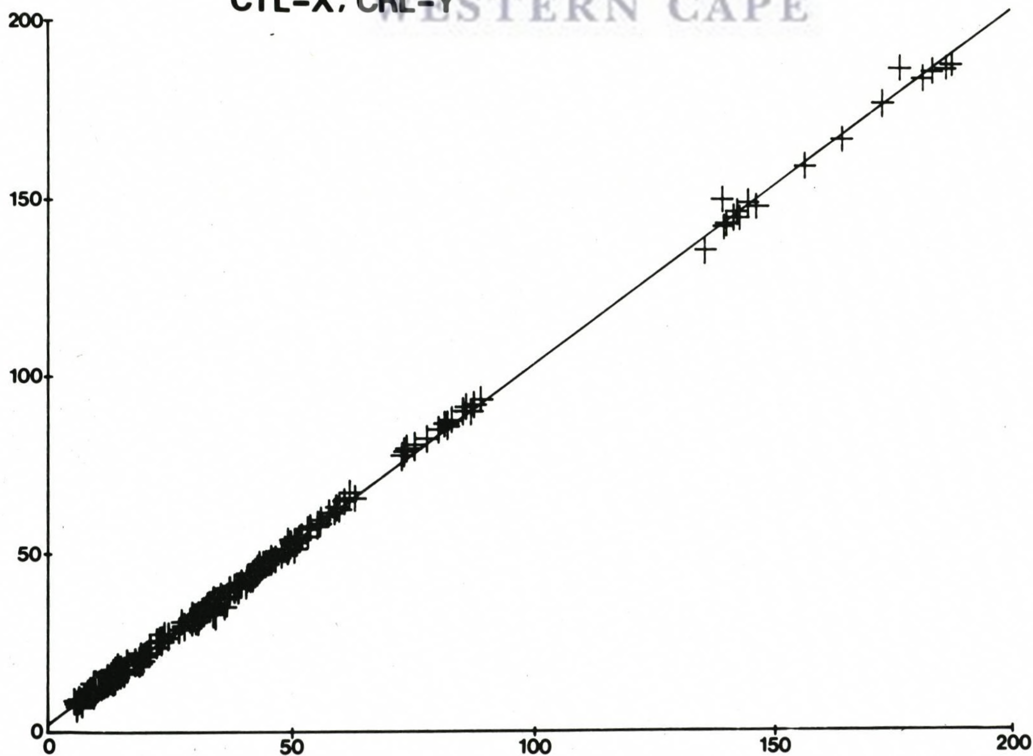


FIG. 28

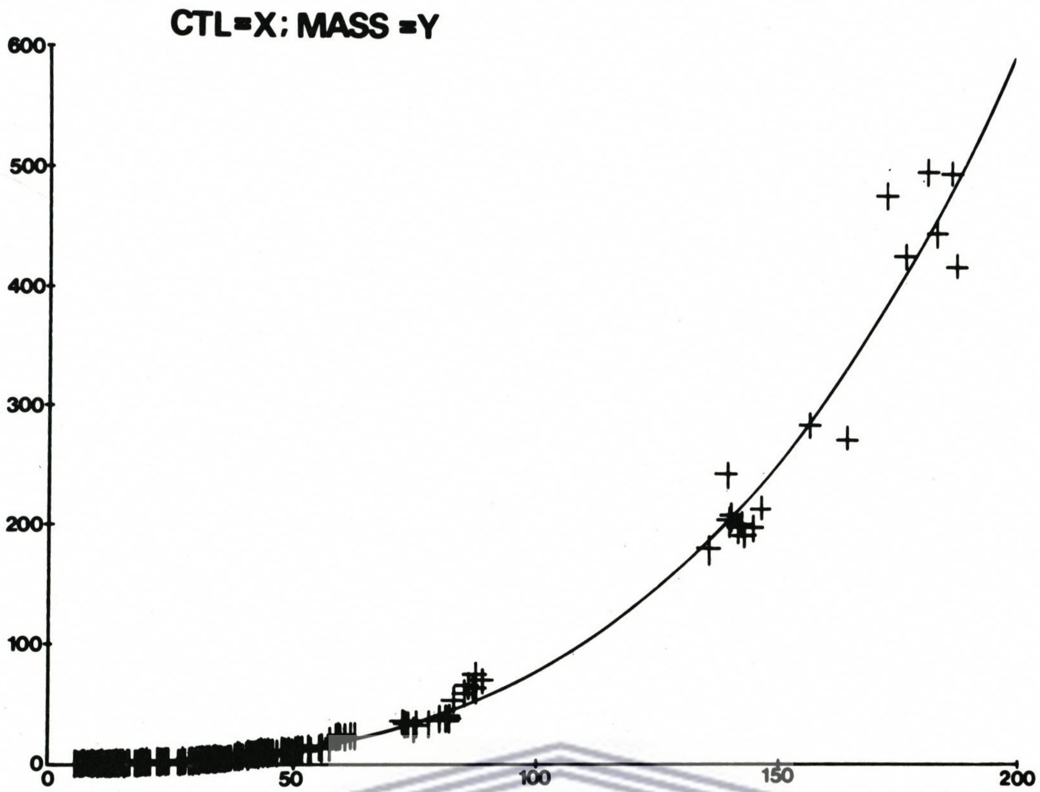
www.etd.ac.za

FIG. 29. A curve of "best fit" calculated from CTL and Mass data for the entire series. CTL is plotted on the X-axis and Mass on the Y-axis. In this relationship R = 0,99196.

UNIVERSITY of the
WESTERN CAPE

FIG. 30. A curve of "best fit" illustrating the relationship when CRL is plotted against mass, R = 0,99315.

www.etd.ac.za



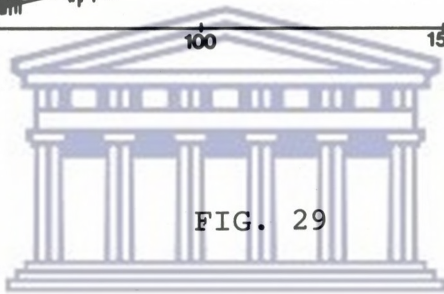


FIG. 29

UNIVERSITY of the
WESTERN CAPE

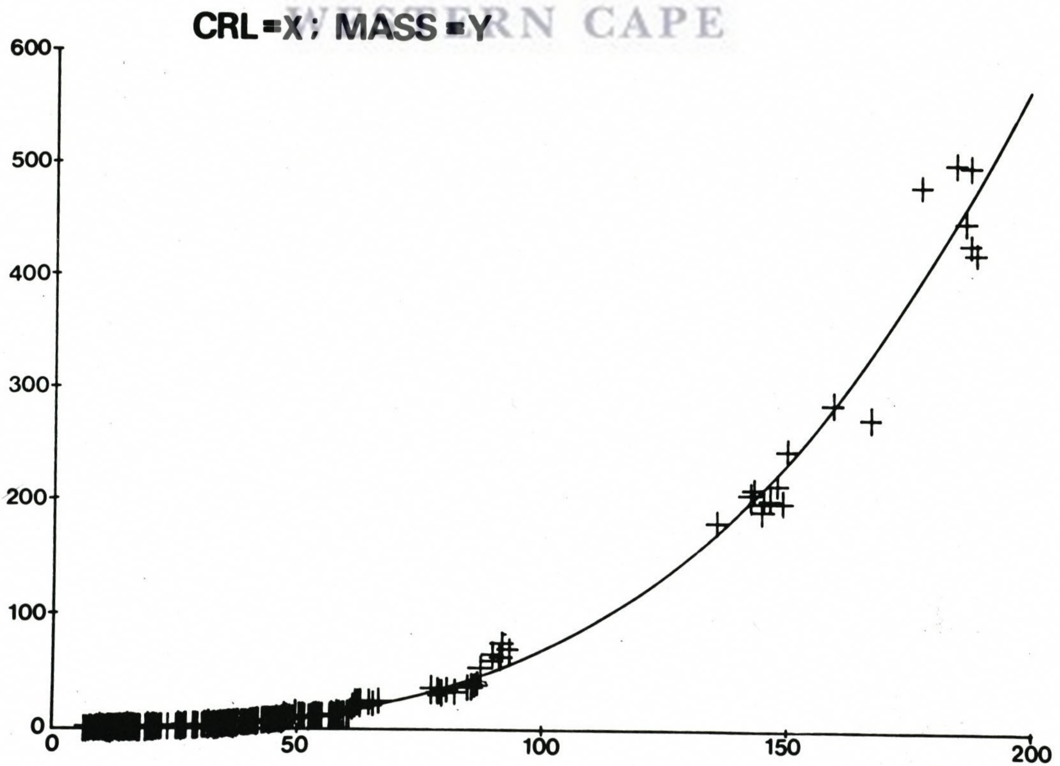
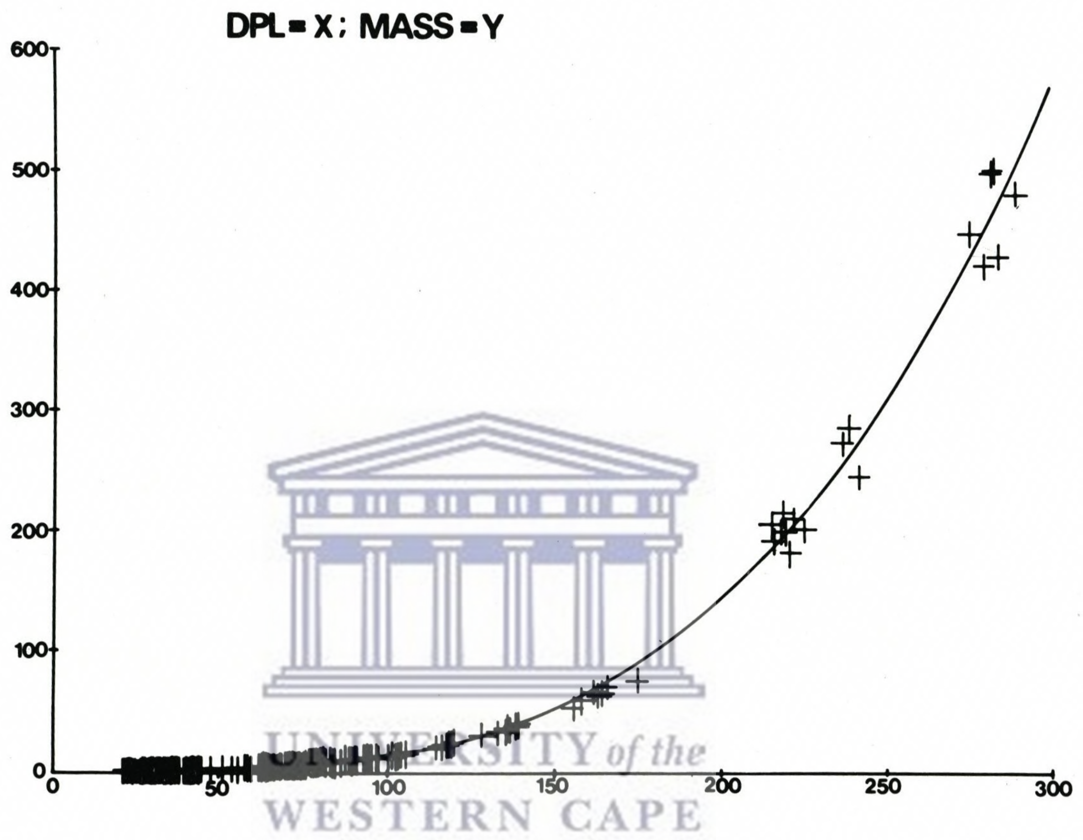


FIG. 30

www.etd.ac.za

FIG. 31. A curve of "best fit" showing the relationship of DPL to Mass, R = 0,99691.

www.etd.ac.za



UNIVERSITY of the
WESTERN CAPE

FIG. 31

www.etd.ac.za

FIG. 32. A histogram showing the predominance of specimens of less than 20 mm. CTL. Most specimens are below 60 mm.

UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

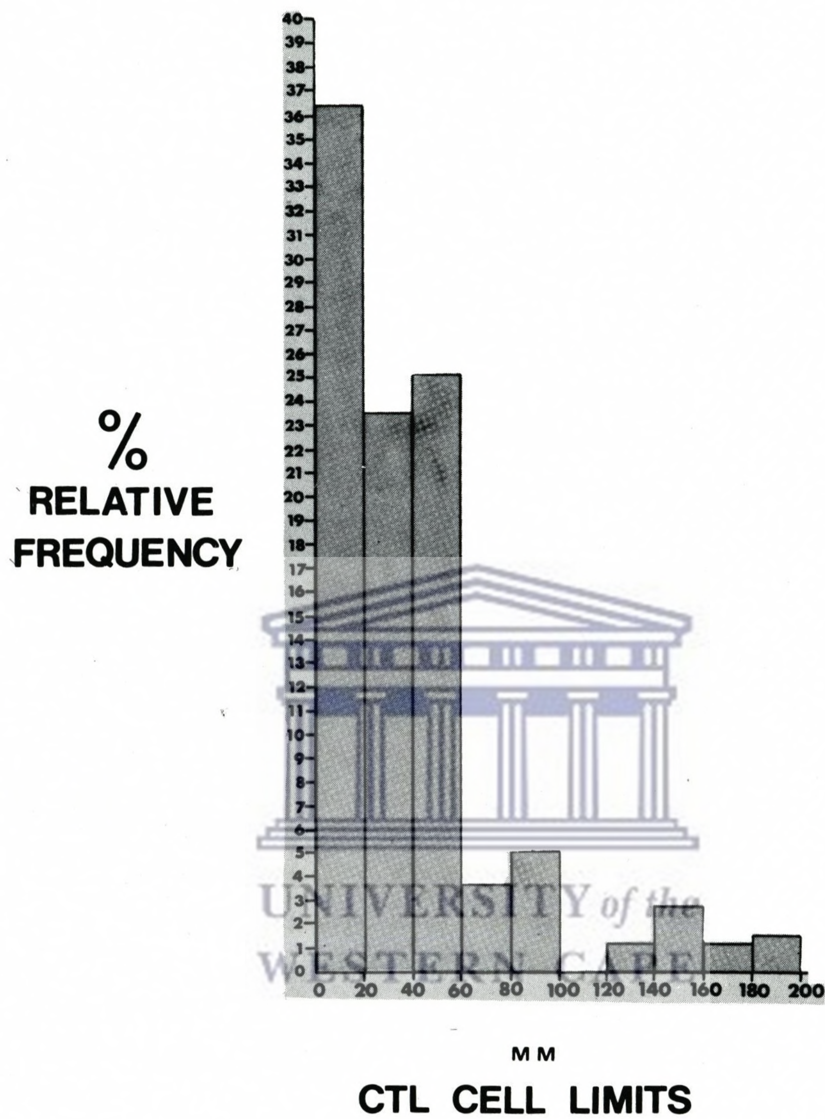
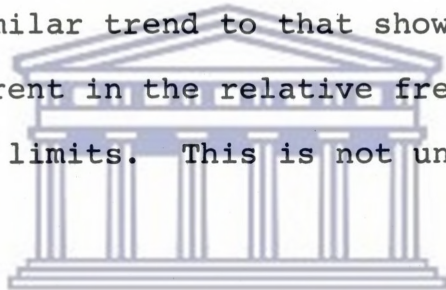


FIG. 32

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

FIG. 33. A similar trend to that shown in Fig. 32 is apparent in the relative frequencies of CRL cell limits. This is not unexpected.



UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

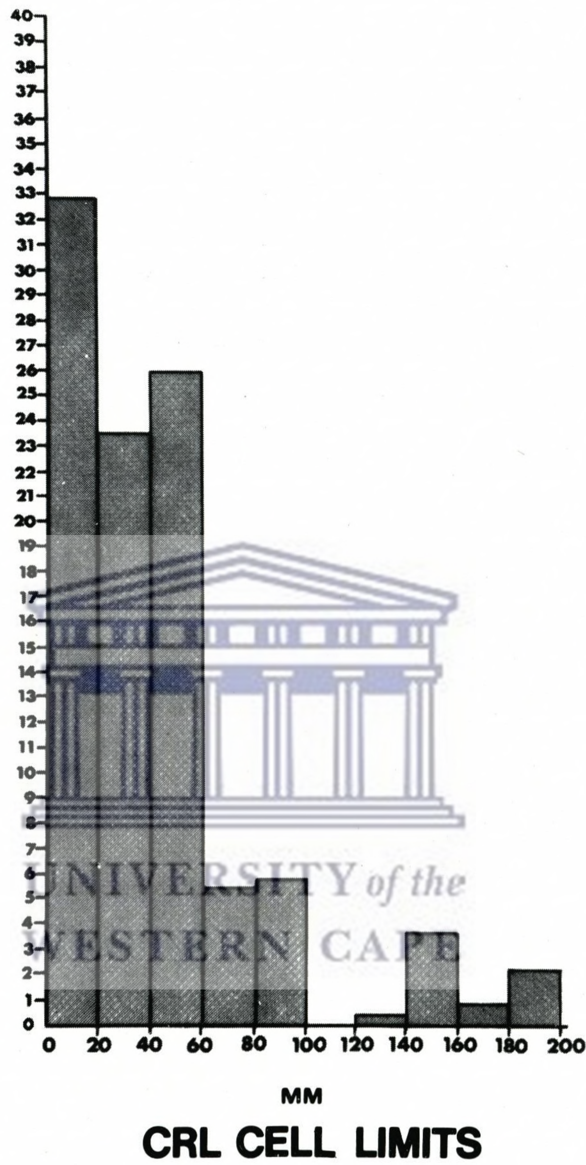
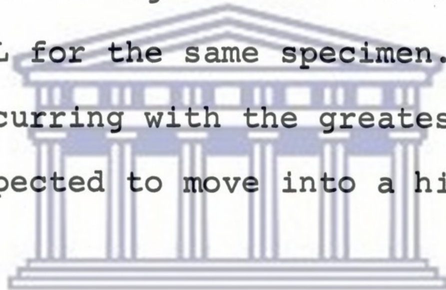


FIG. 33

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za

FIG. 34. An interesting deviation from the histograms shown in figs. 31 and 32. The "shift to the right" in the DPL cell limit with the greatest relative frequency is probably due to the greater magnitude of DPL relative to CTL and CRL for the same specimen. Thus the cell limit occurring with the greatest frequency can be expected to move into a higher category.



UNIVERSITY of the
WESTERN CAPE

www.etd.ac.za

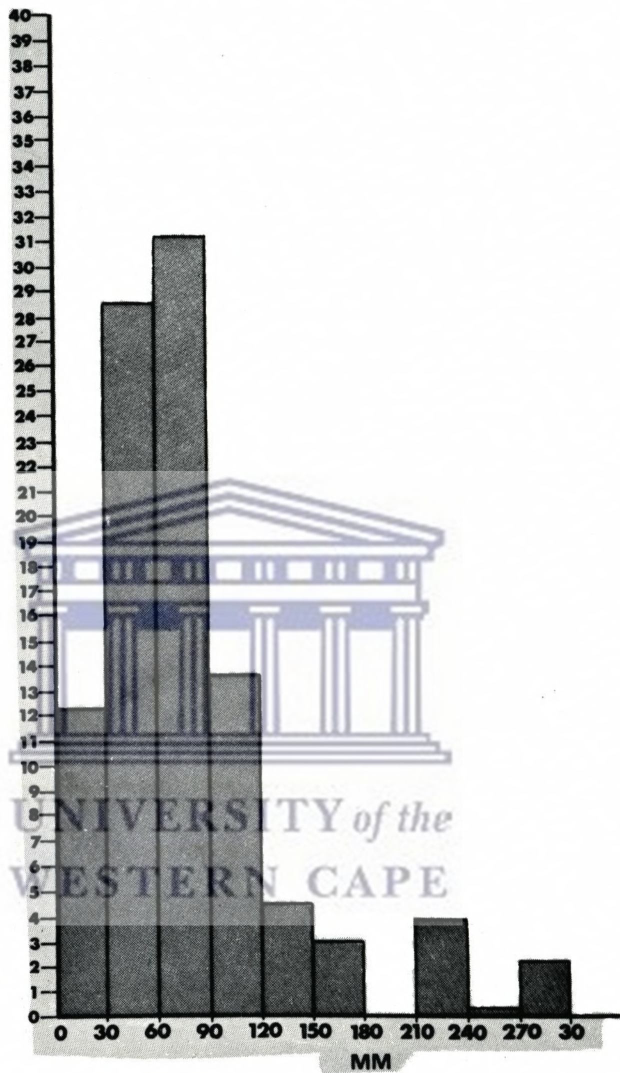


FIG. 34

UNIVERSITY of the
WESTERN CAPE
www.etd.ac.za