TEETH AND JAWS OF XENOPUS LAEVIS

The time scales of tooth development and replacement in *Xenopus laevis* (Daudin), with observations on the growth of the jaws.

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Abstract

The prime concern of the Thesis was to establish absolute time scales for tooth development and tooth replacement, but the relationship between the dentition and growth of the jaws was also considered. The work was undertaken in three parts as follows:

(1) A longitudinal study was made of the first teeth to form and erupt (first generation teeth) in larval and newly metamorphosed animals. This was done by rearing 172 larvae in such a way that their rates of body growth (as measured by external criteria) were similar, and so the course of dental development could be followed histologically in a cross-sectional study. In this way the events in tooth development (amelogenesis, dentinogenesis, eruption, ankylosis and resorption) were observed and a time scale applied. On average each tooth took 26 days to develop and erupt, and then remained in its functional position for about 7 days. Individual tooth replacement was assessed to occur about every 16 days.

In a follow up longitudinal study 96 animals were used to assess the time taken for resorption of the first generation teeth. It was found that resorption could occur at any time after a tooth completed ankylosis, but that the process occurred in two phases - a phase of slow resorption lasting up to 8 days (called erosion), followed by a 48 hour phase of rapid resorption (called absorption). The histological differences between the phases are described.

(2) A longitudinal study of the dentitions of three large adult females was made by anaesthetising them at regular intervals and taking

wax impressions of their mouths. Charts were constructed, and the data analysed to obtain (a) the functional life span of the teeth, (b) the period of time tooth loci were unoccupied before eruption of the successional teeth, and (c) the replacement cycle time (defined as the period between eruption of a tooth and the eruption of its successor). Variation was found between animals, and the median functional life span ranged between about 24 and 29 days, and the median replacement cycle time between 38 and 42 days. These time scales were compared with the functional life span of the first generation teeth, and the pattern of tooth replacement discussed.

(3) In order to assess the relationship of the dentition to the jaws, metamorphosed animals of various sizes and ages were used to study proportional changes in the upper and lower jaws with increase in body size, and to study variations in the number and size of the teeth. Significant, but small, differences in jaw proportions were found between animals, and these are discussed in relation to local factors. An assessment was made of the importance of the dentition and jaws for feeding.

Since the first generation teeth began to form during the larval stages of the animal, and in view of the dramatic change in feeding patterns at metamorphosis, the larval lower jaw was examined from a functional viewpoint, and the rate at which it changed shape during metamorphosis assessed.

Part three of the work is presented first in the Thesis to enable the reader to appreciate the jaws and dentition as a unit.

Abbreviations used in the text

H + E	-	haematoxylin and eosin
L	-	left
NF	-	Nieuwkoop and Faber (1956)
R	-	right
UJ	-	upper jaw

CONTENTS

SECTION I - INTRODUCTION

A classification of the genus Xenopus can be given as follows:

Class Amphibia
Order Salientia (Anura)
Suborder Opisthocoela
Family Pipidae
Genus Xenopus (Wagler, 1827)
Species Xenopus laevis (Daudin, 1803)

Several species and subspecies are recognised in the genus Xenopus, which vary in body size, colouration, eye size, and egg colour and size. Karyotype analyses of the species have been made by Tymowska and Kobel (1972), and Tymowska and Fischberg (1973). The commonest subspecies is Xenopus laevis laevis, found in the temperate regions of South and West Africa, Malawi, Zimbabwe, Mozambique and Pretoriuskop (Deuchar, 1975). Animals from this subspecies have been used in this Thesis, and it will be referred to below as Xenopus. Comprehensive accounts of the life history and biology of Xenopus can be found in the following: (Beddard, 1894; Bles, 1905; Dreyer, 1913, 1914; Weisz, 1945a,b; Nieuwkoop and Faber, 1956, 1967; Brown, 1970).

Xenopus is a fully aquatic salientian with a stout body and long muscular hind limbs (Figure 1-1). It inhabits swamps, ponds and streams. The body is flattened dorso-ventrally and the limbs are splayed out at the sides, so that the animal normally lies flat against the river bed. In appearance and posture it is therefore quite different from the common European frogs and toads.

While the ventral skin colour is cream, the dorsal skin is a mottled

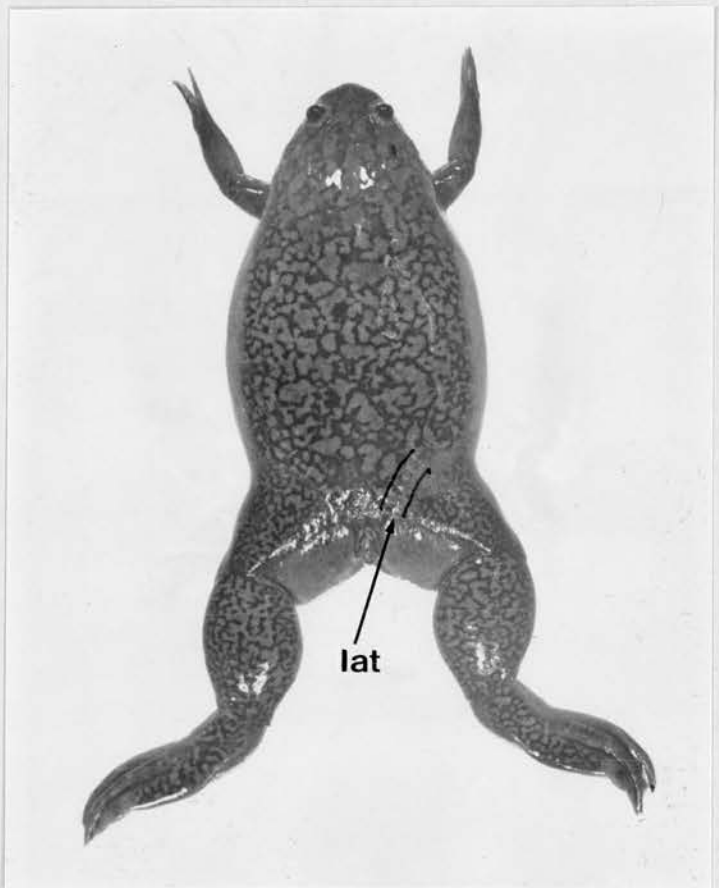


Figure 1-1

Dorsal view of female Xenopus in the resting position. Snout-vent length of this specimen — 107 mm.
Lat, right dorsal row of lateral line organs.

grey-green. However, the colour of the dorsal skin can change to almost black or pale buff depending on the overall colour of the background. The animal has a smooth skin, and no moveable eyelids.

The powerful rear limbs have extensive webbing between the toes to aid propulsive movements, and claws are present on the three inner toes of each hind foot. The claws have a number of functions, including the maintenance of a foothold on rocks in fast-flowing streams (Burton, 1975).

Like most other salientians, Xenopus develops first as a free-swimming larva which undergoes metamorphosis to the adult form. After metamorphosis the animal undergoes a period of rapid growth before it becomes sexually mature at about $1\frac{1}{2}$ - 2 years of age, when its snout-vent length may be 50 - 60mm.

Growth continues after sexual maturity, in common with many species of amphibians and reptiles, but while males are fully grown at 2 - 4 years (Brocas and Verzar, 1961; Deuchar, 1975), females continue to grow throughout life, although perhaps very slowly at extreme age. By 12 - 13 years of age laboratory bred females have been found to weigh 100 - 150g (Deuchar, 1975), and while specimens kept in captivity have survived to 15 years of age (Goin and Goin, 1971), the lifespan of Xenopus in its natural environment is difficult to determine (see Appendix 4).

Adult Xenopus is a predacious creature feeding on living and dead animals. The claws on the hind limbs are used for raking the mud on the bottom of pools, not only in the search for food, but also to make the water murky as camouflage against enemies (Cochrane, 1961). The food is torn up by the claws, pushed rapidly into the mouth with the

forelimbs, and swallowed whole.

Larval Xenopus, unlike the adult, feeds on detritus suspended in the water. This is filtered out by a mechanism located in the pharynx and supported by the branchial cartilages. The mouth is small in the larva, since its only function is the intake of water and suspended particles for filtration, and the mouthparts are devoid of horny beaks and keratinised pseudoteeth as are found in many other salientian genera e.g. Rana and Bufo (Goin and Goin, 1971; Bourges and Bachelerie, 1974).

The cartilaginous lower jaw of the larva is of a different shape to that of the adult jaw. The major change in lower jaw morphology occurs during metamorphosis, and is accompanied at this time by ossification of the lower and upper jaws. Previous work dealing with the jaws (Kotthaus, 1933; Paterson, 1939; Sedra and Michael, 1957) has been related to a description of the changes in morphology of individual cartilages and bones at metamorphosis, and has also been concerned with the homologies of the various parts of the chondrocranium.

However, since the overall change in lower jaw shape is so marked, it was decided to make a quantitative study of the growth of the larval lower jaw as a whole, and to assess the rate at which its dimensions changed at metamorphosis. This study is presented in Section IIIA.

Following from this it was decided to look at some aspects of the adult dentition, and to assess the changes in size and proportion of upper and lower jaws with increase in body size in metamorphosed and adult animals (see Figure 1-2). This study is presented in Section IIIB and C.

In Xenopus the teeth are monocuspid and found only on the upper jaw attached to the premaxillae and maxillae. The dentition is

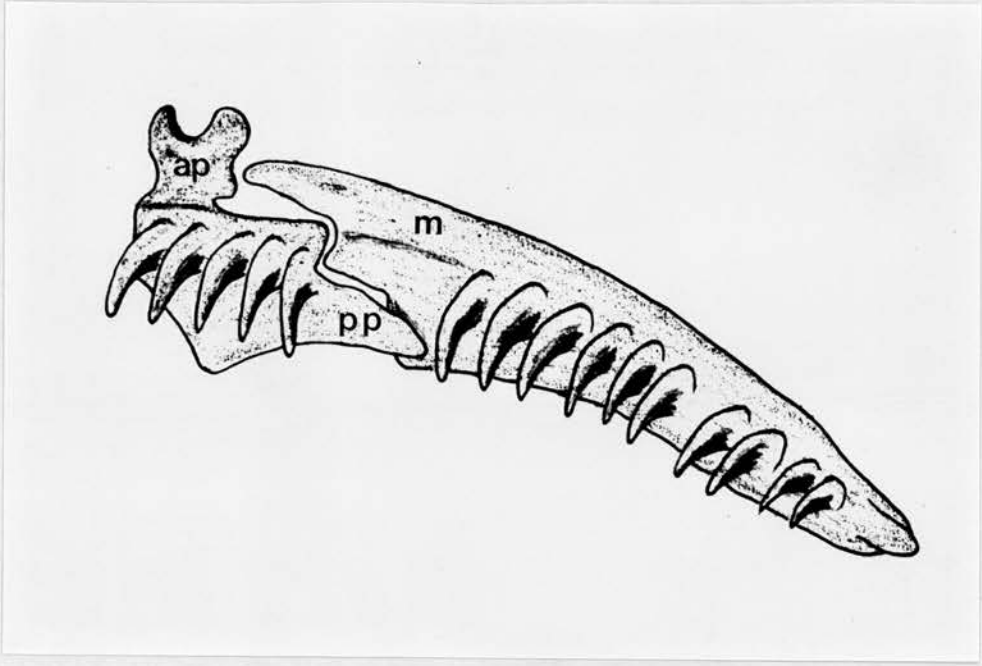


Figure 1-2

A view of the left half of the upper jaw from below, showing premaxilla and maxilla (m).

The premaxilla is tooth bearing and consists of the alary process (ap) and a palatal ledge called the pars palatina (pp). The alary process projects dorsally, lying approximately at right angles to the pars palatina, and is supported on the skull by a rod-like prenasal cartilage which articulates with the groove on its free upper edge.

The maxilla is also tooth bearing, and is supported anteriorly by a backward extension from the pars palatina of the premaxilla. Although the maxilla has no bony articulation posteriorly (the quadratojugal is absent), it is supported by a ligament, attached to its posterior end, which runs back to the pterygoid (the ligament is described in Appendix 3).

homodont, and the bulk of each tooth consists of orthodentine covered at the tip by a thin layer of highly calcified material (it is now accepted that this tissue is indeed a true enamel essentially of a similar nature to that of higher vertebrates (Soule, 1966; Chibon et al., 1971; Meredith Smith and Miles, 1971; Schmidt, 1970)). The dentine of each tooth surrounds a central pulp chamber, and here the odontoblasts are found only while the tooth is developing. When the tooth has completed its development it becomes ankylosed to a short bony pedicel which is continuous with the bone of the jaw. Examination of the teeth shows that only a small proportion of each tooth, that covered by enamel, actually protrudes through the epithelium (Figures 1-3, 1-4 and 1-5).

There is an extensive literature on the anatomy and histology of the dentitions of lower vertebrates and general accounts can be found in Owen (1840), Miles (1967) and Peyer (1968), while an appreciation of the general arrangement of amphibian dentitions can be gained from Hilton (1951), Goin (1958) and Parsons and Williams (1962). Although the events in the development of the teeth in many species are well documented, little work has been done on rates of tooth development.

A feature of lower vertebrate dentitions is that the teeth are replaced throughout the life of an animal, and consequently, when specimens are examined, either histologically or as whole mounts, the jaws show teeth in varying degrees of development. Previous workers have divided the sequence of events in the development of teeth of such polyphyodont dentitions into a number of stages (Gillette, 1955; Goin and Hester, 1961; Lawson, 1965a and 1965b; Lawson, Wake and Beck, 1971). By observing the relative incidence of the various stages, an

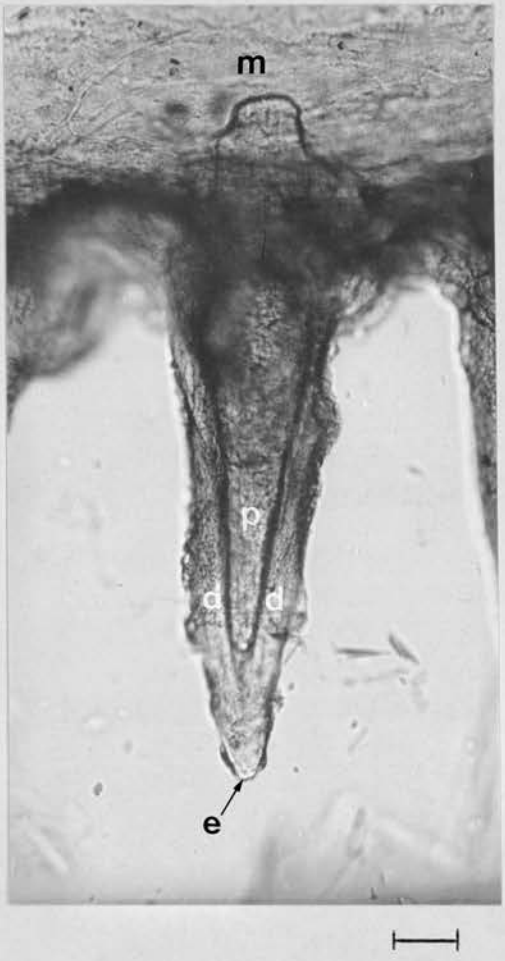


Figure 1-3

An ankylosed tooth of adult Xenopus. Cleared whole-mount preparation. The walls of dentine (d) surrounding the pulp chamber (p) are seen, while the enamel (e) is visible only as a narrow clear zone around the tooth tip. m, maxilla

Scale bar 100/um

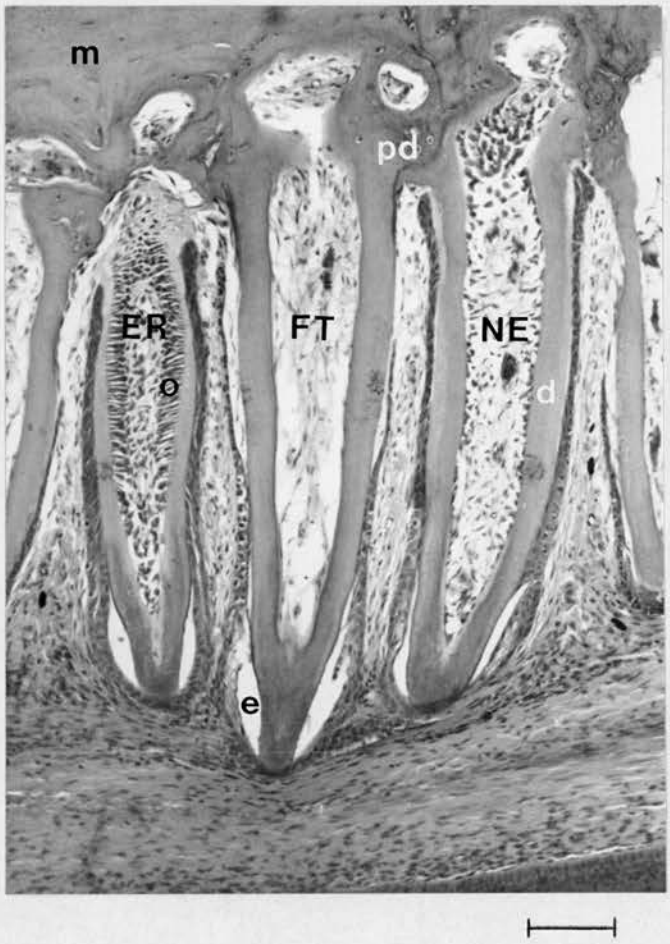


Figure 1-4

Three teeth of Xenopus in longitudinal section. Note the difference in cellularity of the pulp chambers. The pulp of the erupting tooth (ER) contains columnar odontoblasts (o), while no odontoblasts are recognisable in the pulp of the functional tooth (FT). The newly erupted tooth (NE) is just completing ankylosis to the pedicel (pd), and 'degenerate' odontoblasts are still recognisable on the pulpal aspect of the dentine (d).

e, space left by the enamel after decalcification;
m, maxilla

Decalcified, stained H+E

Scale bar 100 µm

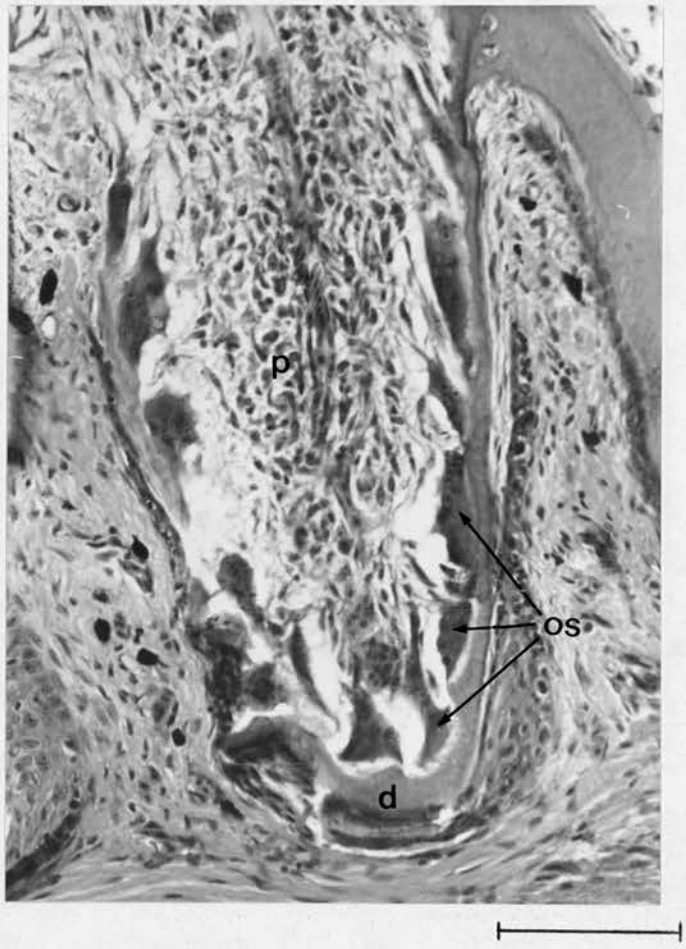


Figure 1-5

A longitudinal section of a tooth undergoing resorption in Xenopus. Note the cellularity of the pulp (p). The osteoclasts (os) appear to be actively resorbing the dentine (d).

Decalcified, stained H + E

Scale bar 100 um

estimate of the relative duration of each stage of tooth development can be made. Stages which occur frequently are assumed to be long-lasting, while a rare stage is assumed to be of short duration. In this way it has been deduced that the early stages of tooth development occupy a large proportion of the total developmental time, and therefore occur slowly. Eruption, ankylosis and resorption however, seem to occupy a small proportion of the developmental time, and are, therefore, assumed to occur rapidly.

However, it is not easy to measure the absolute time scale of tooth development in a polyphyodont dentition, because of the difficulty of fixing a base line from which the time taken for tooth development can be measured. Consequently, few estimates of absolute time scales have been made in lower vertebrates.

In Section IV an attempt was made to estimate the absolute time scale of tooth development in Xenopus. To circumvent the problem of establishing a base line in the dentition it was decided to follow the history of the first teeth to develop during the larval stages of the animal. Although in many amphibians, especially urodeles and caecilians, the larval teeth have a different morphology to the adult teeth (Parker and Dunn, 1964), in Xenopus the teeth of the larva are morphologically similar to those of the adult, the main difference being that of scale.

In order to follow the development of the larval teeth through the stages of eruption, ankylosis and resorption, a number of larvae were reared in such a way that their growth rates were similar, so that a longitudinal study could be made and the average time taken for the teeth to develop could be measured.

Only a few longitudinal studies have been made of the rates of tooth replacement in adult lower vertebrates. Edmund (1960, 1962) has measured the rates of tooth replacement in reptilian species using X-rays. More recently longitudinal studies of tooth replacement have been made in the slow-worm (Cooper 1966), the rainbow trout (Berkovitz & Moore, 1974, 1975; Berkovitz, 1977) and the piranha fish (Berkovitz & Shellis, 1978; Berkovitz, 1980).

It seemed pertinent, therefore, to make a study of the rate of tooth replacement in adult Xenopus. This was carried out using a dental impression technique and the work is presented in Section V.

SECTION II - MATERIALS AND METHODS

MATERIALS

Two adult males and two adult females were kept for breeding purposes, and, unless otherwise indicated, the specimens used for the Thesis were the progeny of these animals and were reared in the laboratory by the author (for breeding technique see Methods). The specimens used for each Section were as follows:

Section III

(A) 100 larvae from the same egg batch; details of the animals are given in Appendix 1.

(B),(C) 35 adult females and 7 adult males; details of the animals are given in Appendix 2.

(C) 5 adult females of unknown age, and 5 females 18 months old.

Section IV

(A) 148 larvae and 24 newly metamorphosed specimens reared from the same egg batch.

(B) 24 larvae and 72 newly metamorphosed specimens reared from the same egg batch.

Section V

3 large adult females obtained from Xenopus Ltd., Holmes Dale Nursery, South Nutfield, Redhill, Surrey.

METHODS

(1) The keeping of adult Xenopus

The conditions under which the animals were kept conformed with the guidelines for the care of amphibians laid down by the National Research Council (1974). Adults were kept in groups of two or three in glass aquaria containing 18 litres of dechlorinated tap water at 25°C. Stones were placed in the water as hiding places for the animals, which are nocturnal in their natural habitat, and each aquarium was covered with wire mesh to prevent the animals from escaping. The water temperature was controlled by heater/thermostat units supplied by UNO Products, Arnold Street, Nantwich, Cheshire, England. In the dark room in which the animals were kept the lights were controlled by a time clock, which turned the lights on at 0900 hrs, and off at 2100 hrs, each day.

Food was given on Monday and Thursday each week, and consisted of raw chopped liver or heart, which was spread around the bottom of the tanks in the late afternoon. On the day after feeding, the animals were netted and transferred to a clean tank containing preheated clean tap water which had been allowed to stand for 3-4 days so that any chlorine present in the water could evaporate.

(2) Breeding of Xenopus

Breeding could be induced artificially at any time of the year as and when eggs were required, and two males and two females were used for breeding purposes. When the animals were in condition to breed the females showed enlarged and reddened cloacal labia, while the males

possessed well-developed dark nuptial pads on the inner aspects of their forelimbs.

Although the females remained in breeding condition throughout the period of study, the males tended to lose condition after several months, with accompanying regression of their nuptial pads. To prevent this the males were injected every 5-6 months with a serum gonadotrophin preparation called Gestyl, supplied by Organon Laboratories Limited, Crown House, Morden, Surrey, England.

For this procedure 1000 i.u. of Gestyl were dissolved in 2ml of sterile water, and 0.2ml of this solution injected subcutaneously (for technique see later). The males returned to breeding condition about 7-10 days after injection.

Occasionally, 5-7 days after the administration of Gestyl, a male and female would display breeding behaviour and fertile eggs were obtained, but since this was an unpredictable event it was of little use as a standard method of breeding the animals. Consequently, for the routine induction of breeding a preparation of chorionic gonadotrophin, Pregnyl, was used (also supplied by Organon Laboratories), and the procedure adopted was as follows.

Prior to the administration of Pregnyl the breeding pair was isolated from the other animals and placed in an aquarium containing clean water at 25°C. Subsequently both male and female were injected with a solution of Pregnyl, 500 i.u. dissolved in 1ml of sterile water. The female was injected with 0.3ml of this solution at 1000 hrs, and with a further 0.4ml at 1600 hrs, while the male was given a single injection of 0.3ml at 1000 hrs only.

To give each injection the animal was netted and moved from its tank on to the bench. While still in the net, a damp towel was wrapped around net and animal in such a way that the right hindlimb and posterior end of the animal's body were exposed. The animal was firmly restrained and the needle point of the hypodermic syringe was then inserted through the skin of the dorsal surface of the right hindlimb, the point of injection being just lateral to the right dorsal rows of lateral line organs. From this position the needle point was advanced along the thigh and into the trunk towards the midline of the body. As this was done a slight resistance was felt indicating that the needle had entered the dorsal lymph sac, and it was here that the solution was deposited. After the injections the animals were returned to their tank. The above injection technique was used for the administration of Pregnyl and Gestyl.

When the injections of Pregnyl were completed the male and female were left undisturbed overnight and throughout the morning of the following day, during which time amplexus took place and eggs were deposited. When egg laying was completed the breeding pair were removed to another tank, to prevent them eating or physically damaging the eggs which were allowed to develop undisturbed.

The above procedure for the artificial induction of breeding could be repeated with the same breeding pair within six weeks.

(3) Care of the larvae

At 25°C the larvae hatched from the eggs after about 2 days, and attached themselves by mucous threads either to the water surface or to the glass sides of the aquarium. There they remained motionless

for a day or two, becoming active again when they commenced to feed about 3 days after hatching.

The larvae were fed each day on powdered nettles obtained from D. Napier and Sons, 17/18 Bristo Place, Edinburgh. On each feeding occasion, 3 teaspoonfuls of powder were made into a suspension with 100ml of water, and poured into the aquarium. Although this caused the water to become murky after a few days, the water was not changed during the first two weeks since the delicate larvae were very susceptible to physical damage during this period.

After the first two weeks, however, the water was changed weekly and, to do this without handling the larvae, the used water was siphoned off until only a depth of a few centimetres remained to cover the bottom of the tank. This allowed the tank sides to be wiped clean, and then preheated dechlorinated water was siphoned into the tank to return the water level to normal.

The development of the larvae was staged according to the Normal Table of Xenopus laevis (Nieuwkoop and Faber, 1956) in which there are 66 developmental stages between fertilisation and the completion of metamorphosis.

The larvae stopped feeding during metamorphic climax, but resumed feeding after metamorphosis, at which time they had changed their feeding habits and become predacious carnivores. Consequently newly metamorphosed specimens were fed on small Tubifex worms, supplied by Aquarama Ltd., 8 Middlefield, Pilrig, Leith Walk, Edinburgh.

(4) Administration of anaesthetics

The anaesthetic procedure was based on the technique of Kaplan (1969). The anaesthetic used for both larvae and adults was Tricaine methane sulphonate (MS222), supplied by Messrs. Sandoz Products Ltd., Sandoz House, 23 Great Castle Street, London W1.

Larvae were anaesthetised by placing them in a 1 litre solution of MS222 at a concentration of 0.2g per litre. Anaesthesia, as indicated by a failure to respond when touched, was achieved in 5-10 minutes.

Adult animals were immersed in a solution of MS222 at a concentration of 2g per litre and anaesthesia was obtained in about 30 minutes. The recovery period was of about equal duration and during part of this time the animal was kept in fresh shallow water with its head supported by hand, so that its nostrils were above the water surface to allow it to breath. When the animal recovered the ability to make spontaneous movements it was returned to its normal tank.

(5) Killing the animals

Before killing the animals they were anaesthetised and body measurements were taken as required. Larvae were then killed by immersion in fixative, but adult animals were first bled by cutting through the heart with a scalpel before being immersed in fixative.

Those animals which were to be prepared for histological examination were fixed in Bouin's solution while all other animals were fixed in 10% formalin.

(6) Histological preparations

The procedure adopted in the preparation of each specimen for histological examination was as follows:-

(i) The specimen was fixed for one week in Bouin's solution and then washed for 12 hours in gently trickling tap water, after which it was decalcified for 3 days in a solution of equal parts 50% formic acid and 20% sodium citrate.

(ii) After washing for 24 hours, the specimen was dehydrated by transferring it to absolute alcohol via a graded series of alcohols of increasing concentration.

(iii) The specimen was placed in a solution of equal parts absolute alcohol and methyl salicylate, which was changed twice with 30 minutes between changes, and then transferred to a solution of 500ml pure methyl salicylate and 500mg celloidin. It remained in the latter solution for at least 24 hours.

(iv) The specimen was then transferred to benzene prior to being embedded in molten wax. After embedding the specimen, the wax block was rapidly cooled under water and trimmed up. The block was then attached to the chuck of a Baird and Tatlock rotary microtome, serially sectioned at a thickness of 10/um or 5/um, and the sections mounted on glass slides.

(v) The 10/um sections were stained with haematoxylin and eosin and the 5/um sections stained with Heidenhain's iron haematoxylin using, in both cases, the technique of Culling (1957). The haematoxylin and

eosin stain was used on sections which were to be used for general visualisation of tissue structure and measurements, while Heidenhain's iron haematoxylin was used for those sections on which cell counts were to be made.

(7) Differential Staining of Cartilage and Bone in Whole Specimens

The procedure adopted here was as follows and was based on the technique of Wassersug (1976);

(i) The specimen was fixed in 10% formalin for 2 weeks, then skinned and all organs in the body cavity and pigmented fascia removed, including the eyes. The specimen was then washed for 24 hours in gently trickling tap water.

(ii) To stain the cartilage the specimen was placed in a solution of 90mg alcian blue, 600ml absolute alcohol and 400ml glacial acetic acid. The penetration of the alcian blue stain was monitored daily and depending on its size, the specimen was left in this solution for 48-96 hours. When adequately stained the specimen was dehydrated by transferring it to absolute alcohol.

(iii) The maceration of the soft tissues was accomplished by placing the specimen in a 1% potassium hydroxide solution, but for large adult specimens the potassium hydroxide concentration was increased to 5% in order to shorten the maceration time. In order to stain the bones and teeth, alizarin red S was added to the potassium hydroxide solution at a concentration of 20mg per litre, and the specimen was left in this solution for a variable time depending on its size. Maceration and bone staining in larvae were completed in 2-3 days, but with large

adults this time was increased to about 14 days despite the higher concentration of potassium hydroxide used.

(iv) When maceration and bone staining were completed the specimen was transferred to 100% glycerol via a graded series of glycerol-water solutions, and was kept in each intermediate fluid for 24 hours. The specimen was then stored in fresh 100% glycerol until the soft tissues became sufficiently transparent to allow the stained bone and cartilage to be examined. The clearing time of the soft tissues varied from 48 hours for larvae to several weeks for large adults.

SECTION III - THE JAWS AND DENTITION OF XENOPUS

In this Section a study was made of the jaws in *Xenopus* and it is presented in three parts as follows:

(A) growth of the lower jaw in the larva,

(B) dimensional changes of lower and upper jaws following growth in metamorphosed and adult animals,

(C) the dentition of metamorphosed and adult animals.

Section IIIA - Lower Jaw Growth in the Larva

Method

In 100 larvae between Nieuwkoop and Faber stages 47 to 66 inclusive, measurements of chondrocrania and lower jaws were made on 5 animals at each of the twenty developmental stages. (Details of the animals are given in Appendix 1.)

Eggs for the study were obtained by the standard method and the larvae allowed to develop to stage 47 by which time, although there were variations, a sufficient number of animals had developed at a similar rate to allow a study. Consequently, all larvae which reached stage 47 during the same day were chosen as the study group and subsequently the group was divided into batches of 50, with each batch in a separate tank for easier inspection of the animals as their development proceeded.

The aim while rearing the study group was to obtain for measurement animals which had developed at a similar rate. In so far as this could be achieved, it would then be possible to apply a time scale to the dimensional changes observed. To this end great attention was given to the animals' husbandry and to environmental control so that any variations affected them similarly.

Examination of the larvae was frequently carried out so as to eliminate the weak or slowly developing animals. Thus, as the animals developed beyond stage 47, any which did not reach subsequent developmental stages during the same day as the majority were discarded, so that only the fastest developing animals at each stage were used - 5 at

each stage.

By this method it was hoped to obtain for measurement animals which would have developed at a similar rate had they all been allowed to complete their development and thus apply a time scale to the results.

Each animal to be used for measurement was killed and the cartilage and bone (if present) of the skeleton were stained with alcian blue and alizarin red S respectively, while the soft tissues were rendered transparent, to allow examination of the skeleton, by immersing the specimen in glycerol (see Section II, Methods). When staining of the skeletal elements and clearing of the soft tissues were completed, the branchial and ceratohyal cartilages were removed. The chondrocranium was separated from the trunk so that the anterior and posterior ends of the chondrocranium and lower jaw could be clearly seen from the ventral aspect and the following measurements made:

(a) length of chondrocranium

(b) length of lower jaw

(c) width of lower jaw
(See Figures 3-1, 3-2, 3-3)

The measurements were made at X40 or X100 (depending on the size of the specimen) using an eyepiece graticule on a Nikon S microscope.

However, before making the measurements it was essential that the chondrocranium was orientated correctly (see below) and to make this easier it was mounted on the microscope stage in the following way: two glass slides were used, one 75mm x 25mm, the other 76mm x 37mm.

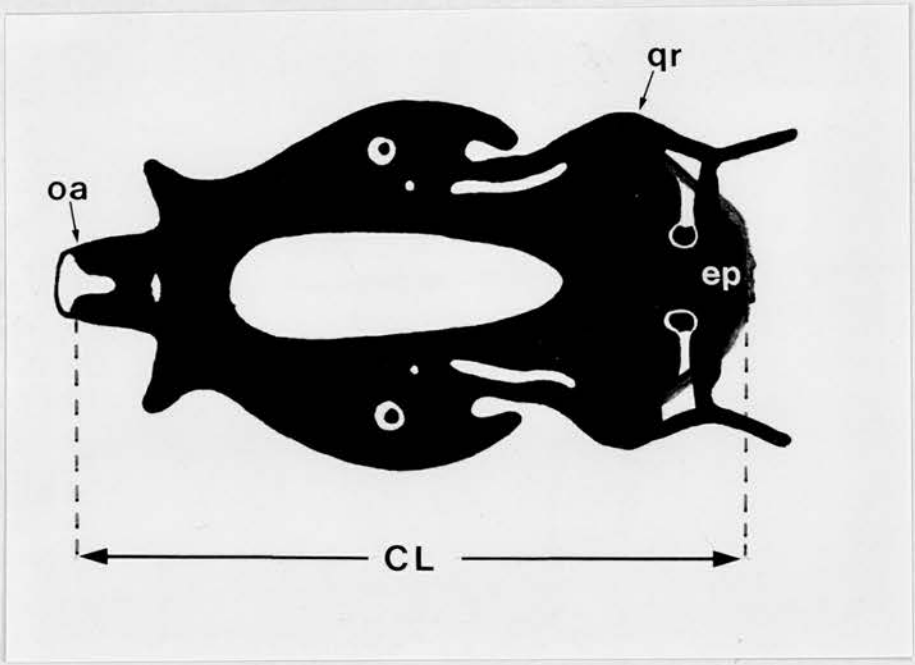


Figure 3-1

A dorsal view of the chondrocranium after removal of the branchial cartilages and ceratohyal cartilages. The lower jaw articulates ventrally with the quadrate region (qr) of the chondrocranium.

Chondrocranium length (CL) was measured from the anterior extremity of the ethmoid plate (ep) to the posterior extremity of the occipital arch (oa). Since the occipital arch is confluent with the atlas vertebra ventrally, measurements were made to the free dorsal aspect of the arch. (For lower jaw see also Figure 3-2).

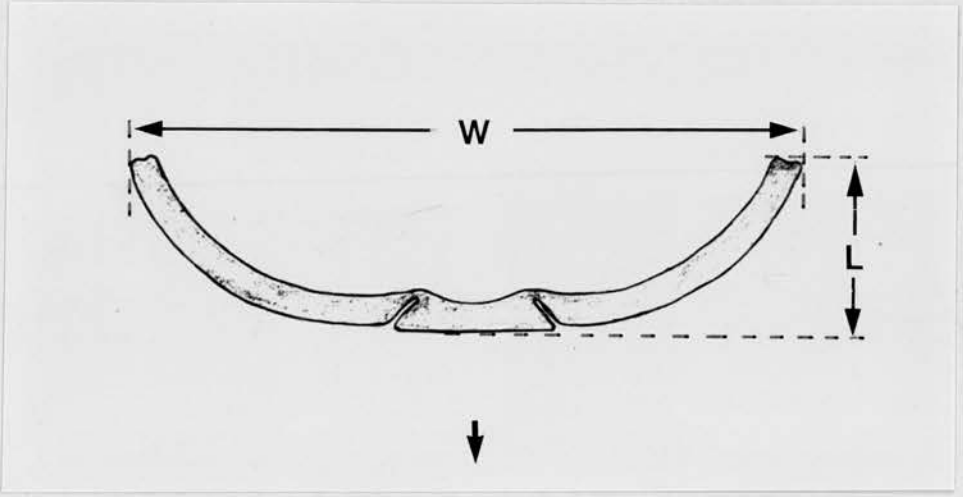


Figure 3-2

The appearance of the lower jaw (Meckel's cartilage) before metamorphosis showing the measurements width (W) and length (L). The posterior ends of the cartilage articulate with the quadrate regions of the chondrocranium. The central arrow points anteriorly.

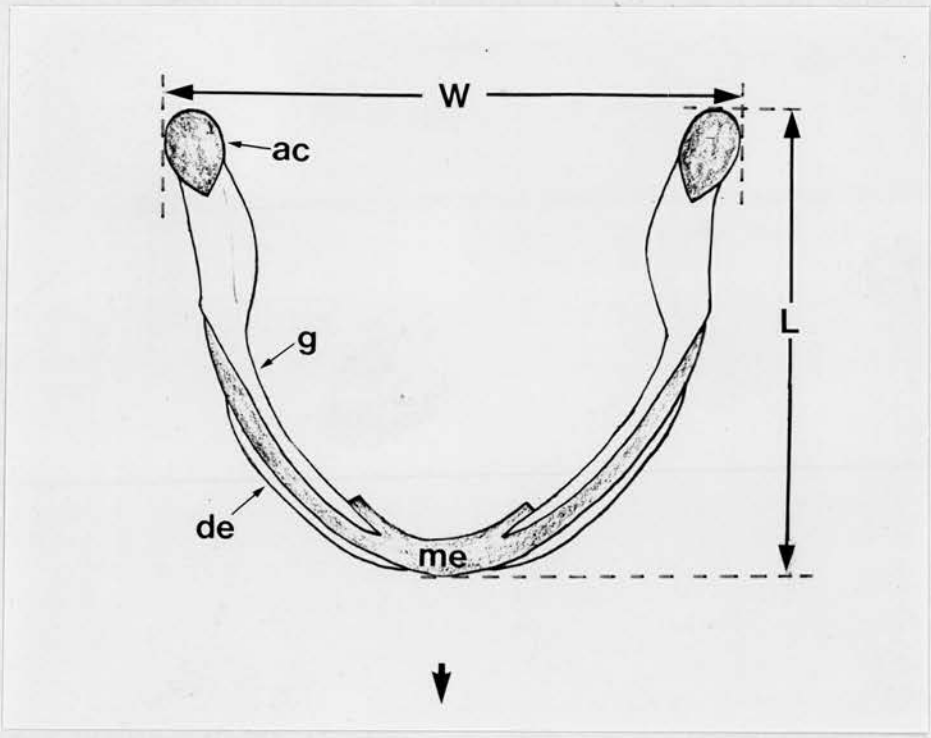


Figure 3-3

The appearance of the lower jaw during metamorphosis (cartilage-shaded, bone-unshaded), showing the measurements width (W) and length (L).
The bones, goniale (g) and dentale (de) are well ossified and surround Meckel's cartilage (me).
ac, articular cartilage.
The adult lower jaw is of similar shape, but the bones almost completely surround Meckel's cartilage.
The central arrow points anteriorly.

A shallow ring of wax, of sufficient diameter to contain the specimen, was heat-sealed to one surface of the smaller slide. The wax ring, which acted as a reservoir, was filled with glycerol and the specimen immersed in the glycerol ventral surface up. The smaller slide, with the wax ring holding the specimen facing upwards, was then placed on top of the larger slide. The larger slide, which now supported the smaller slide and the specimen,was fixed to the microscope stage.

Thus, although both slides could be moved in either the X or Y axis of the microscope stage, the smaller slide (and the specimen) could be moved independently since it was not fixed to the larger slide but could slide over its surface. By this method the specimen could be moved in one plane without the need for direct contact and this facility made easier the task of orientating the specimen.

Orientating the chondrocranium

Care was taken over the orientation of the chondrocranium and if necessary it was repositioned between measurements. The orientation was carried out in two stages to ensure that the specimen (a) was not tilted around its long axis and (b) lay with its antero-posterior (long) axis parallel to the X axis of the microscope stage.

To achieve (a) the specimen was viewed with the microscope and moved with a needle until the posterior ends of Meckel's cartilage were in the same plane of focus. The viscosity of the glycerol usually prevented displacement. In order to achieve (b) the assumption was made that the chondrocranium and Meckel's cartilage were symmetrical. Therefore an imaginary line joining the posterior ends of Meckel's cartilage would be at right angles to the long axis of the chondrocranium.

Thus if the imaginary line lay parallel to the Y axis of the microscope stage, then the long axis of the chondrocranium would be parallel to the X axis. Thus the specimen could be orientated using the posterior ends of Meckel's cartilage, which were readily identifiable.

The procedure was to move the specimen using the two-slide arrangement already described, since this dispensed with the need to touch it and so negate the efforts already made to achieve orientation (a) above. Accordingly the upper slide was moved relative to the lower slide until the specimen was orientated in such a way that when the microscope stage was moved along its Y axis both posterior ends of Meckel's cartilage passed through a predetermined point on the eyepiece graticule.

With the specimen correctly positioned the three measurements could be taken by moving the microscope stage along its X or Y axis as applicable and obtaining the values with the graticule.

Results (numbers in parentheses are standard deviations)

The changes in length of chondrocranium, width of lower jaw and length of lower jaw between stages 47 and 66 are shown in Table 3-1 and Figures 3-4, 3-5 and 3-6, which also indicate the time intervals over which these changes occurred.

The mean length of the chondrocranium increased from 2600 (185.4)/um at stage 47 to a maximum of 9450 (246.22)/um at stage 58. However, the rate of growth was not constant over this 33 day period. When the rate of growth per day is calculated from the mean lengths at various stages, it is seen to have been 347.33/um per day between stages 47 and 53, but to have dropped to 91.11/um per day between stages 53 and 58.

After stage 58 the mean length of the chondrocranium decreased, until at stage 62 the mean was 6074.24 (348.35)/um, and this reduction in length occurred at 675.15/um per day. There were small and insignificant fluctuations in the length of the chondrocranium between stages 62 and 66.

The width of the lower jaw increased between stages 47 and 58 from a mean of 1670 (135.09)/um to 5858.33 (142.89)/um, and this represented a mean growth rate of 126.92/um per day. After stage 58 the width decreased to a value of 4127.76 (457.85)/um at stage 62, a mean rate of 346.11/um per day. However, the greatest reduction in width occurred over the 1 day period between stages 61 and 62 when the lower jaw narrowed from 5548.7 (577.3)/um to 4127.76 (457.85)/um, a difference between the means of 1420.94/um ($P < .005$). There were further, but insignificant variations in the width between stages 62 and 66.

The length of the lower jaw increased constantly from 465 (45.41)/um at stage 47 to a maximum of 3455 (183.2)/um at stage 65, and thereafter there was an insignificant decrease at stage 66. The rate of growth increased from 45.34/um per day between stages 47 and 61, to 157.5/um per day between stages 61 and 66. However, over the 1 day period between stages 61 and 62 there was an increase in length of 700.37/um ($P < .01$), and therefore a rapid increase in the growth rate.

Table 3-1 and Figure 3-7 show the variations in the ratio of lower jaw width to lower jaw length during the study and it can be seen that the ratio dropped from 3.603 (0.268) at stage 47 to 1.272 (0.127) at stage 66. There were fluctuations in the ratio between stages 47 and 58, with a significant variation ($P < .001$) between stages 48 and 49. After stage 58 the ratio decreased, with the most significant change occurring between stages 61 and 62 ($P < .005$).

TABLE 3-1 CHANGES IN CHONDROCRANIUM AND LOWER JAW DURING LARVAL DEVELOPMENT

STAGE *	DAYS	MEAN LENGTH OF CHONDROCRANIUM (/um) (S.D.)	MEAN WIDTH OF LOWER JAW(/um) (S.D.)	MEAN LENGTH OF LOWER JAW(/um) (S.D.)	LOWER JAW WIDTH / LOWER JAW LENGTH (S.D.)
47	0	2600 (185.4)	1670 (135.09)	465 (45.41)	3.603 (0.268)
48	2	2845 (151.45)	1745 (89.09)	470 (27.39)	3.719 (0.203)
49	6	3595 (292.3)	2175 (96.82)	720 (41.08)	3.025 (0.136)
50	8	4890 (407.2)	3020 (312.95)	860 (118.06)	3.539 (0.388)
51	10	5900 (542.85)	3620 (300.73)	975 (58.63)	3.739 (0.546)
52	13	6780 (519.43)	4085 (424.12)	1110 (111.24)	3.699 (0.426)
53	15	7810 (634.58)	4685 (301.35)	1195 (92.53)	3.936 (0.353)
54	17	7840 (334.76)	4815 (177.31)	1335 (102.47)	3.622 (0.287)
55	22	8085 (298.22)	5025 (289.4)	1340 (158.71)	3.778 (0.348)
56	27	8560 (332.89)	4985 (350.27)	1520 (221.78)	3.351 (0.625)
57	29	9255 (363.75)	5506.8 (188.49)	1665.13 (182.59)	3.342 (0.412)
58	33	9450 (246.22)	5858.33 (142.89)	1700 (46.77)	3.444 (0.145)
59	34	8840 (376.91)	5800 (326.44)	1820 (147.27)	3.196 (0.199)
60	35	7765 (283.17)	5404.78 (186.05)	1937.08 (280.08)	2.834 (0.39)
61	37	6976.92(1169.47)	5548.7 (577.3)	2142.45 (248.94)	2.627 (0.492)
62	38	6074.24 (348.35)	4127.76 (457.85)	2842.82 (346.2)	1.476 (0.274
63	39	6210 (336.15)	4465 (285.92)	3065 (290.8)	1.461 (0.083)
64	41	6145 (447.7)	4180 (579.71)	3255 (320.35)	1.282 (0.106)
65	42	6320 (484.57)	4355 (412.08)	3455 (183.2)	1.259 (0.063)
66	45	6051.49 (277.13)	4314.55 (327.74)	3402.47 (172.47)	1.272 (0.127)

* n = 5 at each stage

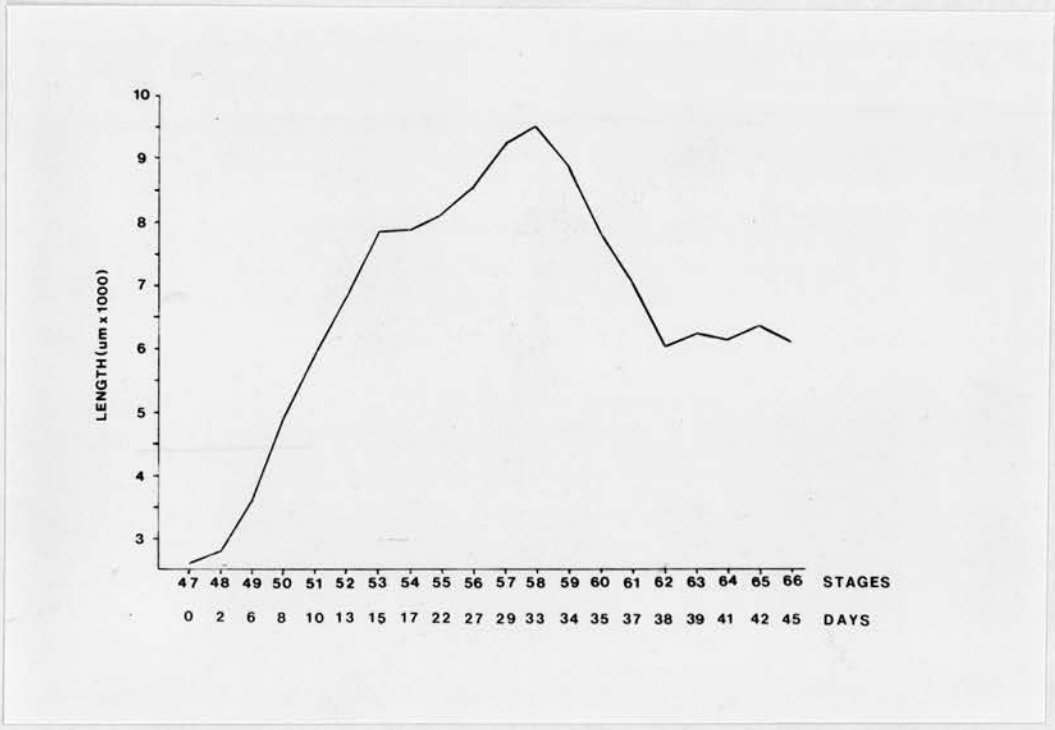


Figure 3-4

Changes in mean length of chondrocranium as larval development progressed from Nieuwkoop and Faber stage 47 to stage 66. The absolute time taken for development to each stage from stage 47 is shown in days.

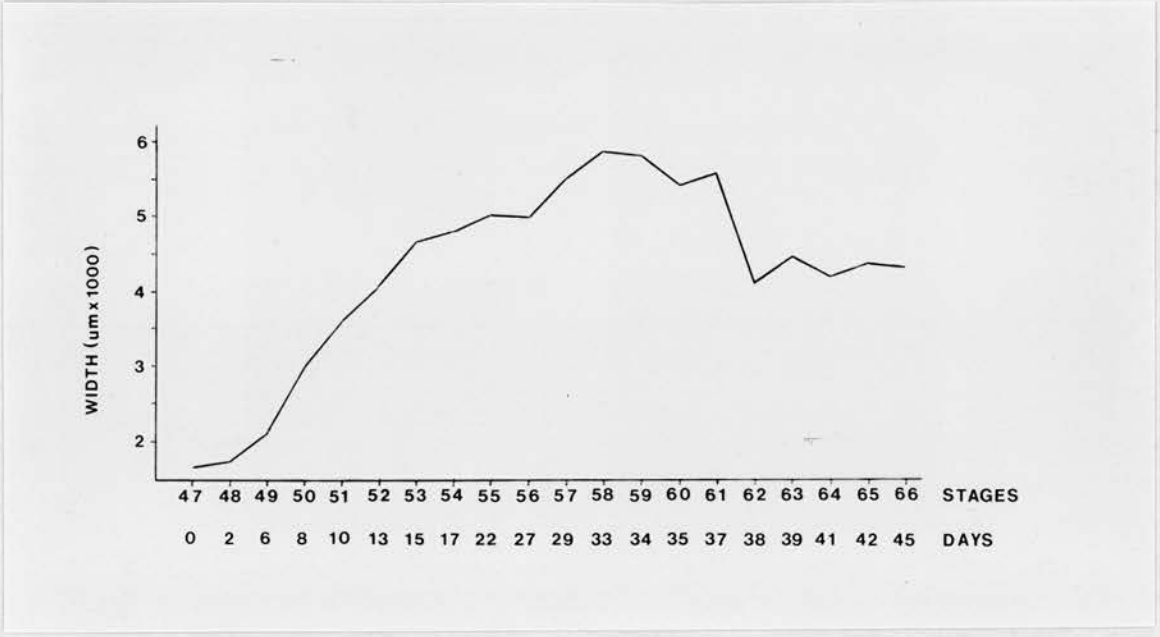


Figure 3-5

Changes in mean width of lower jaw as larval development progressed from Nieuwkoop and Faber stage 47 to stage 66, together with the time scale involved.

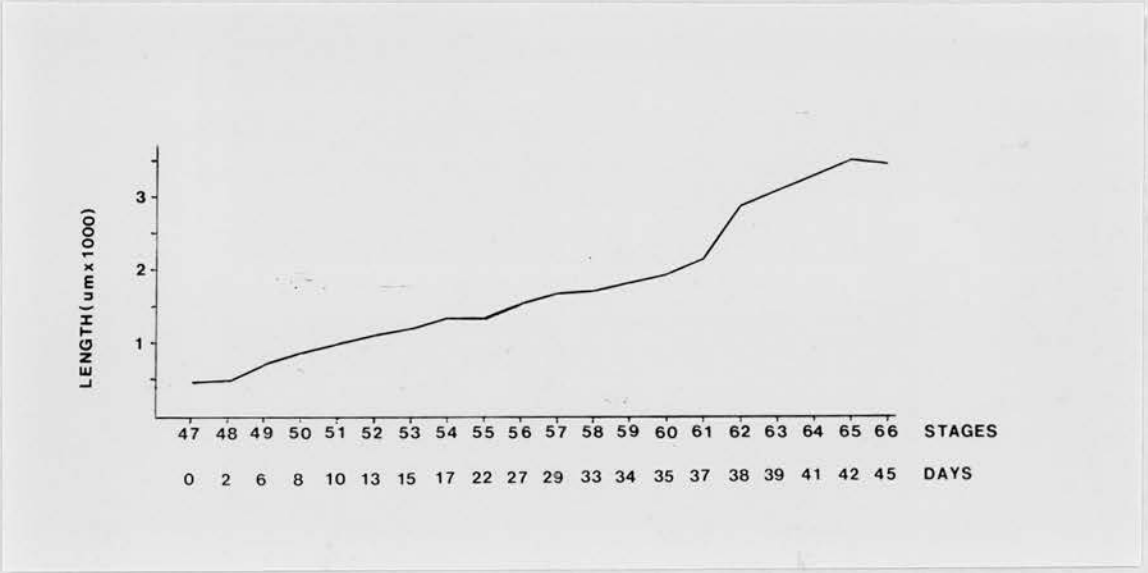


Figure 3-6

Changes in mean length of lower jaw as larval development progressed from Nieuwkoop and Faber stage 47 to 66, together with the time scale involved.

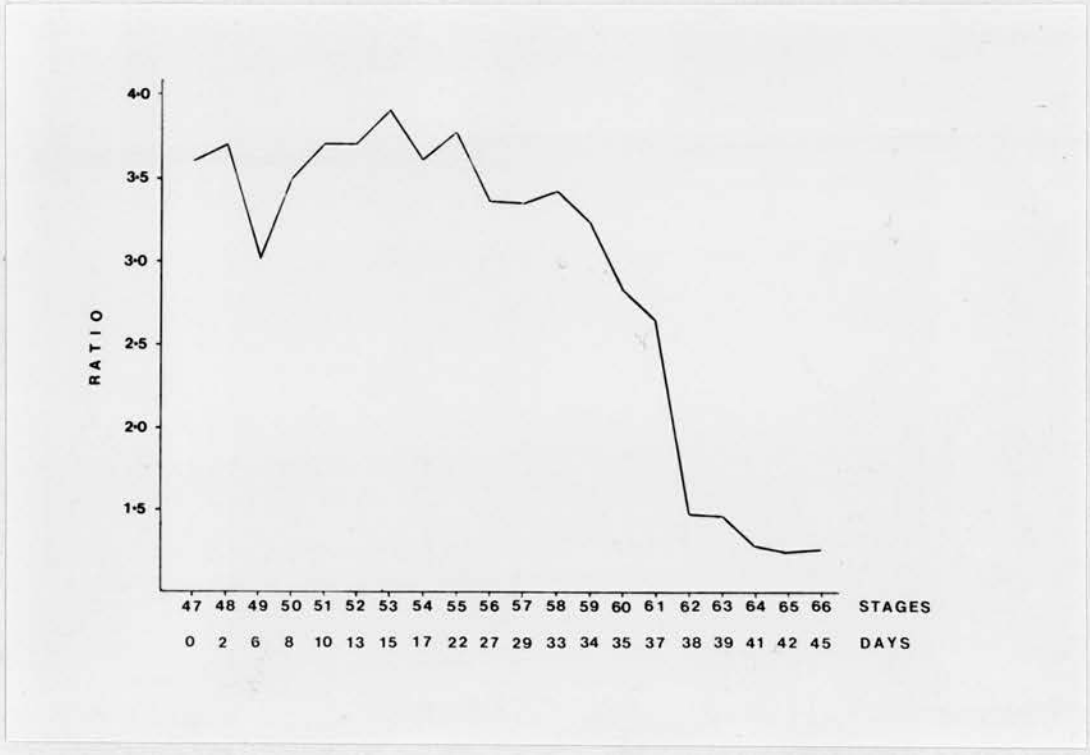


Figure 3-7

Changes in the mean lower jaw width to length ratio as larval development progressed from Nieuwkoop and Faber stage 47 to 66, together with the time scale involved.

Section IIIB - Dimensional Changes of Lower and Upper Jaws in Metamorphosed and Adult Animals

This study was an attempt to assess how the dimensions of the jaws varied with increasing body size, and while primarily concerned with female animals a small number of males was also examined. The animals varied in size and ranged from small newly metamorphosed specimens to very large adults. The animals were arranged as follows in 5 groups according to their snout-vent lengths:

Group A, 10 females with snout-vent lengths in the range 10.2 - 19.4mm

Group B, 10 females with snout-vent lengths in the range 20.8 - 27.4mm

Group C, 10 females with snout-vent lengths in the range 45.2 - 57.2mm

Group D, 5 females with snout-vent lengths in the range 99.5 - 115.0mm

Group M, 7 males with snout-vent lengths in the range 31.0 - 75.0mm.
(Full details of these specimens are given in Appendix 2)

Method

The animals were anaesthetised and their snout-vent lengths measured to the nearest 0.1mm. Before making the measurement each animal was laid on a glass slab, flat on its belly, with fore- and hindlimbs splayed out, an attitude which Xenopus normally adopts when resting (refer to Figure 1-1).

With the animal in this position the snout-vent length was measured using a travelling microscope. The tip of the snout and the cloacal labia were brought consecutively into coincidence with the eyepiece crossline and the amount of travel recorded.

The animal was then killed and the head removed from the trunk. The bones, teeth and cartilage of the skull were differentially stained and rendered visible by maceration and clearing as described under Methods, after which the hyoid apparatus and any soft tissue which hindered visibility were removed.

The sequence in which the measurements were made was dictated by technical factors. The sequence was as follows (see Figure 3-8):

(a) length of lower jaw
(b) width of lower jaw
(c) length of skull
(d) width of upper jaw
(e) length of upper jaw
(f) length of upper jaw tooth-free zone

The measurements were made with an eyepiece graticule at X40, and each skull was mounted on the microscope stage using two slides in the way described for the study of the larval chondrocranium, except that no glycerol reservoir was used.

The specimen was placed on the upper slide with its ventral surface facing upwards. Care was taken with the orientation of the specimen, and to ensure that it was not tilted around its long axis, but lay level on the stage, it was moved until the inferior borders of the posterior ends of the lower jaw were in the same plane of focus. It was then supported in that position with plasticine. From this point on the skull was not touched, but moved in relation to the axes of the microscope stage by sliding the upper slide on the lower.

The long axis of the skull was now orientated parallel to the X axis

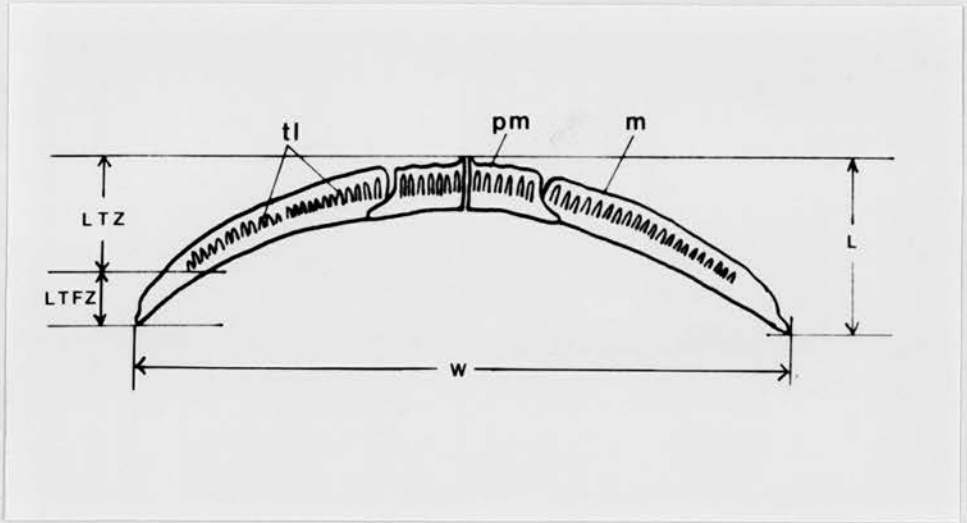


Figure 3-8

Drawing of the ventral aspect of the upper jaw showing premaxilla (pm), maxilla (m) and tooth loci (tl).

The parameters measured in Section IIIB are shown as follows; L, length of upper jaw; W, width of upper jaw; LTZ, length of tooth zone; LTFZ, length of tooth-free zone. Length of tooth-free zone was measured from the distal surface on the pedicel at the last tooth locus to the posterior end of the maxilla.

Length of skull was measured from the anterior extremities of the premaxillae to the posterior extremities of the occipital condyles.

The measurements of the lower jaw are the same as shown in Figure 3-3.

of the microscope stage, by squaring it along a line between the posterior ends of the lower jaw as described for the larval chondrocranium.

The length and width of the lower jaw were then measured, and when completed the lower jaw was removed, together with any soft tissue that obscured the upper jaw. The specimen was reorientated, this time using the posterior ends of the upper jaw.

The width and length of the upper jaw, the length of the tooth-free zone and the length of the skull were now measured.

The upper jaw was then dissected free and used later for tooth counts (see Section IIIC).

Results

The results are given in Tables 3-2, 3-3 and 3-4.

(a) Females. All parameters increased with increasing snout-vent length. Table 3-2 shows that the lower jaw width/length ratio varied between the groups, but the only statistically significant difference was between groups A and D ($P < .005$).

Table 3-3 indicates that there were variations in the upper jaw width/length ratio between groups, but there were no statistically significant differences except between groups A and B ($P < .001$).

The upper jaw tooth-free zone increased with increasing upper jaw length. However the ratio upper jaw length to length of tooth-free zone varied widely within and between groups. Accordingly, with the exception of groups A and B ($P < .01$), there were no statistically significant difference in the ratios.

Table 3-4 shows a fall in the skull length/upper jaw length ratio in group D as compared with the other groups and this was statistically significant ($P < .005$). The skull length/lower jaw length ratio decreased between groups with statistically significant differences between groups A and B ($P < .005$), and B and C ($P < .001$).

(b) Males. The males in group M were compared with the females in group C, since both groups had similar mean snout-vent lengths. However, no statistically significant differences were found between these two groups.

TABLE 3-2 MEAN WIDTH AND LENGTH OF LOWER JAW (/um)

GROUP (NUMBER IN GROUP)	MEAN SNOUT-VENT LENGTH OF GROUP(mm) (S.D.)	WIDTH (S.D.)	LENGTH (S.D.)	$\frac{\text{WIDTH}}{\text{LENGTH}}$ (S.D.)
A (10)	15.1 (2.65)	4427.8 (447.7)	3740.9 (426.65)	1.1863 (0.061)
B (10)	24.5 (2.55)	6287.5 (804.09)	5065.0 (406.07)	1.2382 (0.083)
C (10)	49.5 (4.06)	11510.0 (2244.6)	10005.0 (1209.96)	1.1478 (0.163)
D (5)	107.5 (7.17)	26090.0 (2038.12)	20065.0 (1316.20)	1.2997 (0.033)
M (7)	53.1 (19.51)	13185.7 (4762.74)	10453.6 (3276.79)	1.2495 (0.101)

TABLE 3-3 MEAN WIDTH AND LENGTH OF UPPER JAW (/um) AND MEAN LENGTH OF UPPER JAW TOOTH-FREE ZONE (/um)

GROUP (NUMBER IN GROUP)	MEAN SNOUT-VENT LENGTH OF GROUP(mm) (S.D.)	WIDTH (S.D.)	LENGTH (S.D.)	WIDTH / LENGTH (S.D.)	LENGTH OF TOOTH-FREE ZONE (S.D.)	LENGTH OF UJ / LENGTH OF TOOTH-FREE ZONE (S.D.)
A (10)	15.1 (2.65)	3753.9 (412.39)	1704.91 (168.64)	2.20 (0.10)	272.5 (50.62)	6.3884 (0.982)
B (10)	24.5 (2.55)	5262.5 (664.08)	2075.0 (199.65)	2.53 (0.187)	437.5 (132.94)	5.04 (1.149)
C (10)	49.5 (4.06)	9277.5 (967.42)	3515.0 (271.88)	2.65 (0.356)	895.0 (141.32)	4.0247 (0.786)
D (5)	107.5 (7.17)	22495.0 (1918.28)	8275.0 (1318.97)	2.74 (0.225)	1455.0 (168.08)	5.8409 (1.719)
M (7)	53.1 (19.51)	11307.1 (4521.49)	4010.7 (1349.99)	2.77 (0.233)	932.14 (271.84)	4.3045 (0.732)

TABLE 3-4 THE RELATIONSHIP BETWEEN LENGTH OF SKULL (/um) AND LENGTH OF UPPER JAW (/um)

GROUP (NUMBER IN GROUP)	MEAN SNOUT-VENT LENGTH OF GROUP (mm) (S.D.)	LENGTH OF SKULL (S.D.)	SKULL/UPPER JAW LENGTH RATIO (S.D.)	SKULL/LOWER JAW LENGTH RATIO (S.D.)
A (10)	15.1 (2.65)	6362.5 (576.66)	3.73 (0.182)	1.71 (0.071)
B (10)	24.5 (2.55)	8055.0 (704.23)	3.89 (0.215)	1.59 (0.064)
C (10)	49.5 (4.06)	13297.5 (942.55)	3.79 (0.234)	1.34 (0.082)
D (5)	107.5 (7.17)	26210.0 (920.19)	3.21 (0.376)	1.31 (0.056)
M (7)	53.1 (19.51)	14409.3 (3932.77)	3.66 (0.264)	1.40 (0.088)

Section IIIC - The Dentition of Metamorphosed and Adult Animals

There were three objectives in the study of the dentition:

(1) to assess the number of tooth loci with increase in snout-vent length,

(2) to compare the lengths of teeth in the anterior region with those in the posterior region of the adult jaw,

(3) to measure the parameters length of tooth, thickness of dentine and number of odontoblasts in adult teeth, for comparison with the teeth of newly metamorphosed animals measured in Section IV.

Method

(1) Number of tooth loci

A dissecting microscope was used to count the total number of tooth loci on each of the alizarin stained jaws dissected from the animals in groups A,B,C,D and M described in Section IIIB.

(2) Anterior-posterior tooth length comparison

Tooth length was measured at each locus along the right half of the jaw in five adult females of similar snout-vent length.

It had been observed that recently metamorphosed animals demonstrated about 20 teeth in each half-jaw, and so in this study teeth at loci 1-20 were classed as anterior teeth, and teeth at loci 21+ as posterior teeth.

After killing each animal the lower jaw was removed and the head

decalcified. Serial paraffin sections, cut at 10/um, were made of the right side of the head in the longitudinal plane and stained with H + E.

Measurements were made at X 32 using an eyepiece graticule (see Figure 3-9). The only teeth measured were those ankylosed in position and no measurements were recorded for erupting teeth, or for teeth in which extensive resorption had destroyed the continuity of the dentine between the base and the tip.

(3) Tooth Parameters in Adults

Five 18 months old adult females were used. They were prepared for study in the same way as the animals in group (2) above, except that the sections were cut at 5/um in the coronal plane and stained with Heidenhain's iron haematoxylin to facilitate nucleolar counts. Dentine measurements were made at X 32 using an eyepiece graticule, and nucleolar counts were at X 320.

As can be seen from Figure 3-9 fully ankylosed teeth do not demonstrate recognisable odontoblasts, so in this study the only teeth measured were those just commencing ankylosis and which displayed only a narrow bridge of hard tissue between the bone of the pedicel and the dentine of the tooth. Such teeth had reached maximum length, but still showed prominent odontoblasts. Only 20 teeth *in toto* met the above criterion.

The parameters recorded were; length of tooth, the thickness of the dentine at half the tooth length, and the number of odontoblasts in the pulp (see Figure 3-10).

An odontoblast was identified as a columnar cell apposed to the

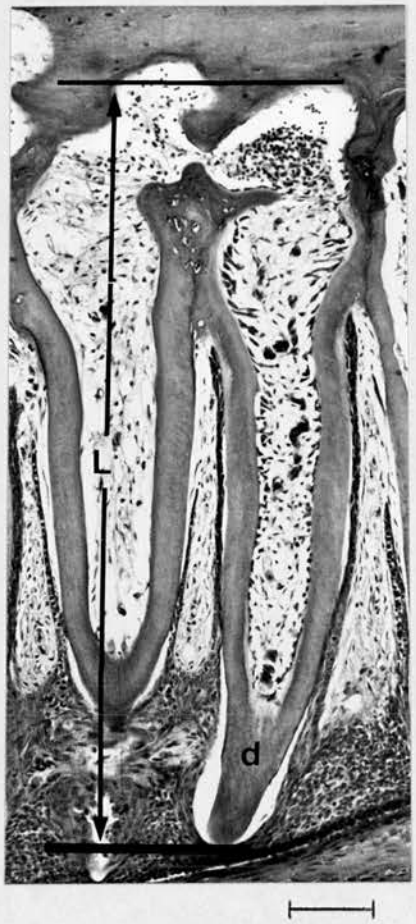


Figure 3-9

A longitudinal section of an ankylosed tooth showing the measurement made in Section IIIC(2). Tooth length (L) was measured from the oral surface of the maxilla to the tip of the dentine (d).

Since the enamel was only about 15/um thick, and was not present on the decalcified sections used, it was ignored in making the measurements.

Decalcified, stained H + E

Scale bar 100/um

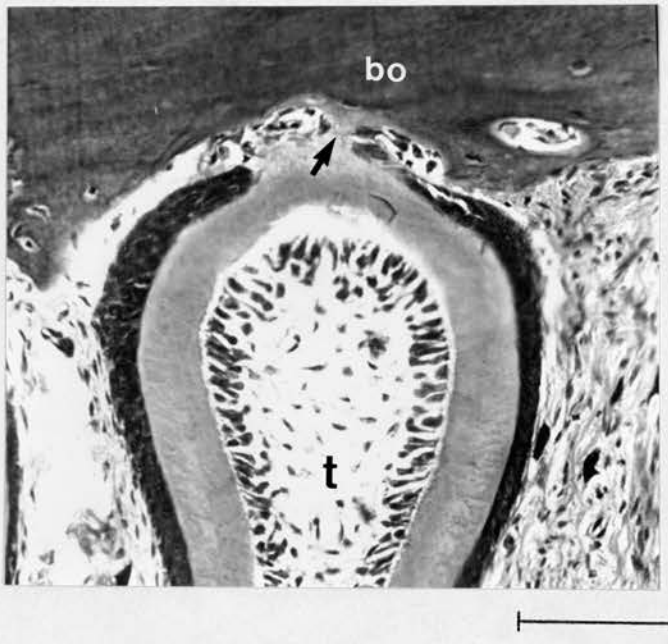


Figure 3-10

The photograph shows the features used in Section IIIC(3) to establish that an erupting tooth was on the point of commencing ankylosis to the bony pedicel. The photograph shows the base of a tooth (t) in longitudinal section connected to the bone (bo) by only a narrow bridge of dentine (arrowed); the bridge was present only on one section of the tooth.

By examining further sections of the same tooth, the parameter tooth length was taken as the greatest distance between the base of the unattached dentine to the tip of the dentine. Dentine thickness was measured midway between these two points.

Decalcified, stained H + E

Scale bar 100/um

pulpal surface of the dentine and possessing a prominent nucleus and nucleoli. Estimations of odontoblast numbers were made by counting nucleoli and the correction formulae of Konigsmark (1969) applied to those counts; after applying the correction the results were divided by two.*

In addition to the above an attempt was made to assess the height of the odontoblasts, again for comparison with those in the teeth of newly metamorphosed animals. No attempt was made to measure all the odontoblasts in each tooth, but instead a sample of about 40 from the basal $\frac{2}{3}$ of each tooth was measured. In the Results the height measurement is presented as the mean height of the odontoblasts in all 20 teeth.

Results

(1) Number of tooth loci

The results show (Table 3-5) that the number of tooth loci did not increase proportionately with snout-vent length. While there was a 227.8% increase in body length between groups A and C, the number of loci increased by only 39.3%. However between groups C and D body length increased by 117% and number of loci increased by a similar value of 105%.

This difference between the groups is emphasised by the changes in the snout-vent length/number of loci ratio. While significant differences occurred between groups A and B, and B and C ($P < .001$), there was no significant difference between C and D.

Similar results were obtained for the skull length/tooth loci ratio where the differences between groups A and B, and B and C were again

* The nucleus of each cell in *Xenopus* contains two nucleoli (Elsdale *et al*, 1958).

significant ($P < .005$), but not that between C and D. Thus the skull length and number of loci both increased by a factor of about 2 between groups C and D, whereas in the smaller animals there was a greater increase in skull length than number of loci.

The tooth zone length of the upper jaw (see Figure 3-8) increased with increase in snout-vent length, as also did the tooth zone length/tooth loci ratio. The increase in this ratio between the groups implies that there were fewer tooth loci per unit length of jaw in the larger animals, and there was a significant difference between groups A and D ($P < .001$).

There were no statistically significant differences between the males in group M and the females in group C.

(2) Anterior-posterior Tooth Length Comparison

The results are presented in Table 3-6, which shows that measurement of teeth was only possible at approximately two-thirds of the loci in each half-jaw. However, the differences between the means of anterior and posterior teeth was statistically significant in all animals ($P < .001$), the posterior teeth being about 25% shorter than the anterior.

(3) Tooth Parameters in Adults

The results are presented in Table 3-7, and are intended for comparison with similar parameters measured in the teeth of small newly metamorphosed animals studied in Section IV. For this purpose the data in Table 3-7 are presented again, together with the results of measurements on the smaller animals, in Table 4-1.

TABLE 3-5 THE RELATIONSHIP BETWEEN NUMBER OF TEETH, SNOUT-VENT LENGTH, SKULL LENGTH AND MAXILLARY TOOTH ZONE LENGTH. THE LAST TWO PARAMETERS WERE MEASURED IN SECTION IIIB, AND THE SAME ANIMAL GROUPINGS WERE USED.

GROUP (NUMBER IN GROUP)	MEAN SNOUT-VENT LENGTH (mm) (S.D.)	MEAN NUMBER OF TOOTH LOCI (S.D.)	SNOUT-VENT LENGTH / NUMBER OF TOOTH LOCI (S.D.)	MEAN LENGTH OF SKULL (μm)	LENGTH OF SKULL / NUMBER OF TOOTH LOCI (S.D.)	MEAN TOOTH ZONE LENGTH (μm) (S.D.)	TOOTH ZONE LENGTH / NUMBER OF TOOTH (S.D.) LOCI
A(10)	15.1 (2.65)	34.1 (1.97)	444.2 (87.21)	6362.5 (576.66)	187.3 (20.58)	1432.4 (148.11)	42.2 (5.09)
B(10)	24.5 (2.55)	36.2 (7.02)	691.2 (105.29)	8055.0 (704.23)	228.0 (34.63)	1637.5 (141.54)	46.6 (8.60)
C(10)	49.5 (4.06)	47.5 (5.93)	1049.9 (83.42)	13297.5 (942.55)	282.4 (27.90)	2620.0 (322.27)	55.8 (8.92)
D (5)	107.5 (7.17)	97.4 (9.56)	1107.4 (63.40)	26210.0 (920.19)	270.5 (19.19)	6820.0 (1479.06)	69.6 (9.57)
M (7)	53.1 (19.51)	53.1 (24.01)	1040.0 (170.04)	14409.3 (3932.77)	292.3 (60.31)	3078.57 (1114.71)	60.1 (6.62)

TABLE 3-6 A COMPARISON OF THE LENGTHS OF ANTERIOR AND POSTERIOR TEETH IN FIVE ADULT FEMALES

ANIMAL	SNOUT-VENT LENGTH (mm)	NUMBER OF TOOTH LOCI ON RIGHT SIDE	NUMBER OF TEETH MEASURED ON RIGHT SIDE	MEAN LENGTH OF TEETH AT LOCI 1-20, um (S.D.)	MEAN LENGTH OF TEETH AT LOCI 21+, um (S.D.)	MEAN LENGTH 21+ / MEAN LENGTH 1-20 X 100
1	63.0	37	26	815.0(86.25)	607.5(114.5)	74.54%
2	68.0	27	16	690.0(66.0)	512.5(43.25)	74.27%
3	65.5	34	25	760.0(90.5)	597.5(52.75)	78.62%
4	58.0	32	21	882.5(100.25)	642.5(48.25)	72.80%
5	67.0	31	20	780.0(90.0)	592.5(74.5)	75.96%

TABLE 3-7 THE PARAMETERS, MEAN LENGTH OF TOOTH, MEAN THICKNESS OF DENTINE AND MEAN NUMBER OF ODONTOBLASTS AS MEASURED IN FIVE ADULT FEMALES (20 TEETH IN TOTO)

SNOUT-VENT LENGTH OF ANIMALS n=5 mm (S.D.)	LENGTH OF TOOTH/um n = 20 (S.D.)	THICKNESS OF DENTINE/um n=20 (S.D.)	NUMBER OF ODONTOBLASTS PER TOOTH n=20 (S.D.)	MEAN HEIGHT OF ODONTOBLASTS IN BASAL $\frac{2}{3}$ OF TOOTH/um (S.D.)
73.2 (1.64)	841.1 (23.71)	36.2 (1.92)	2438.9 (135.62)	25.5 (3.24)

SECTION IV - THE TIME SCALE OF TOOTH DEVELOPMENT AND RESORPTION IN LARVAL AND NEWLY METAMORPHOSED XENOPUS

Section IVA - The Time Scale of Growth of the First Teeth to Develop

Introduction

In this study an attempt was made to estimate an absolute time scale for tooth development in Xenopus. As already stated it is difficult to measure the absolute time scale of tooth development in the polyphyodont dentitions of lower vertebrates, because of the impossibility of fixing a base line in the dentition. In an attempt to deal with this problem in Xenopus, it was decided to follow the history of the first teeth to form during the larval phase of the animal, and follow the development of these teeth through the stages of eruption, ankylosis and resorption, by examining histologically the teeth at even-numbered loci 6-14 in different animals reared at similar growth rates. In this way the events in the development of the teeth could be followed through time, and the time taken for the teeth to develop could then be measured.

Method

Eggs were obtained in the usual way, and the larvae allowed to develop to NF stage 53, at which time larvae which had reached this stage on the same day were segregated as the study group, and placed in tanks with about 50 animals per tank. From stage 53 onwards any larvae in the study group which did not reach subsequent developmental stages on the same day as the majority were discarded, and thereby it was hoped to obtain a population of animals developing at similar rates.

Since it was known from a pilot study that tooth development began during stage 55, the first specimens were killed when the larvae had reached this stage, and then every two days until stage 57, after which animals were killed as they reached each subsequent developmental stage, up to and including stage 66. The numbers of animals examined were as

TABLE 4-1 TOOTH PARAMETERS OF NEWLY METAMORPHOSED ANIMALS COMPARED WITH THOSE OF ADULTS

* Six teeth from each animal
\+ 20 teeth measured in toto (see text Section IIIC)

Animal group (number in group)	mean snout-vent length of group (mm) (S.D.)	mean length of dentine of erupted teeth (um) (S.D.)	mean thickness of dentine (um) (S.D.)	mean number of odontoblasts per tooth (S.D.)	mean height of odontoblasts in basal $\frac{2}{3}$ of tooth (um) (S.D.)
Newly metamorphosed* (15)	15.9 (0.8)	225 (2)	8.4 (0.4)	230 (24.3)	17.2 (2.8)
18 Months old adult female (5)	73.2 (1.64)	841.1+ (23.71)	36.2 (1.92)	2438.9 (135.62)	25.5 (3.24)

follows; 40 animals between stages 55-57, 12 animals for each stage between 58 and 66, and 24 newly metamorphosed animals. The staging of the animals was used only to judge their rates of overall development, and the time scale of tooth development given in the results is expressed in days.

All specimens were fixed in Bouin's solution, decalcified and double embedded. Serial sections were made of the head of each specimen. Half the specimens at each stage were cut at 10/um and stained with haematoxylin and eosin for measurement of the dentine, and half were cut at 5/um and stained with Heidenhain's iron haematoxylin to facilitate the estimation of cell numbers by making nucleolar counts. The correction formulae of Konigsmark (1969) were applied to these counts. The nucleus of each cell in Xenopus contains two nucleoli (Elsdale, Fischberg and Smith, 1958), so the results of the nucleolar counts were divided by two to obtain the number of cells.

All dentine measurements and cell counts were made on six teeth from each of the 12 animals at each stage. Additionally, 60 of the animals were examined in order to determine whether there was variation in the arrangement of the dentition as a whole.

Observations - The Arrangement of the Dentition

The teeth of the larvae and newly metamorphosed specimens were similar to those of the adult animals, the main difference being that of scale. The average length of the dentine of the fully formed larval teeth was 225/um and the dentine was about 8.4/um thick (Table 4-1). Each tooth was ankylosed to a short, ring-shaped bony pedicel, which, in the newly metamorphosed specimens, appeared to be continuous with the bone of the

maxillae or premaxillae.

Newly metamorphosed specimens demonstrated two series of teeth; a series in even-numbered tooth positions, and a series in odd-numbered tooth positions, starting from the mid-line. These teeth began to form during the larval stages of development, and did not erupt until the end of metamorphosis. Normally the even-numbered tooth series was the first to erupt. The first erupting odd and even series teeth will be called first generation teeth. In toto between 18 and 20 first generation even-numbered tooth germs were produced in each larva, while the 18–20 odd-numbered tooth germs developed between these, slightly later in time. This arrangement of the dentition will be called the symmetrical even-number type (SET) (See Figure 4-1).

However, out of a total number of 60 animals examined to see if this arrangement of the dentition was constant, 5 animals showed variations on this pattern. Two of the 5 animals showed a dentition which was the reverse of the common type, i.e. the teeth in the odd-numbered tooth positions erupted first. This arrangement of the dentition will be called the symmetrical odd-number type (SOT).

The three remaining animals which showed variations in the arrangement of their dentitions possessed dental arrangements which were asymmetrical. Two of these three animals showed the even-numbered teeth developing first on the right side, while the odd-numbered teeth were developing first on the left side; this is the asymmetrical type 1 (AT1) dentition. The remaining animal also possessed an asymmetrical dentition, but the reverse asymmetry of the atypical type 1 arrangement. This is the asymmetrical type 2 (AT2) dentition.

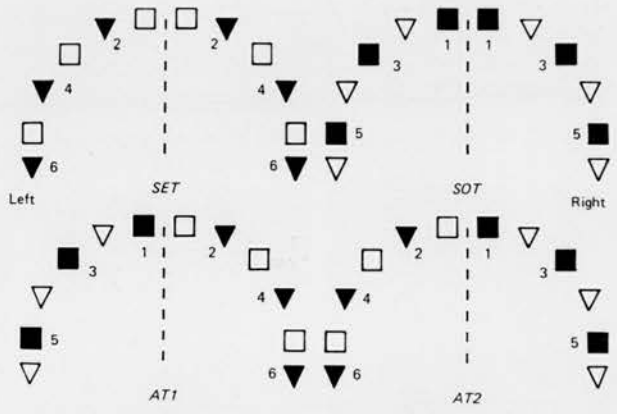


Figure 4-1

A diagram to illustrate the variations observed in the overall arrangement of the dentition of Xenopus. Four upper jaws are represented diagrammatically and only the 12 anterior tooth positions are shown in each jaw. The dotted lines represent the midlines.

SET, symmetrical even-number type dentition;

SOT, symmetrical odd-number type dentition;

AT1, atypical type 1 dentition; AT2, atypical type 2 dentition.

On each side tooth formation ceased abruptly at the back of the mouth. There were generally 20 tooth positions on each side of the mouth before metamorphosis. However, during metamorphic climax, two or three further tooth germs developed on each side posterior to the existing tooth germs. Each of these later tooth germs had a connexion with the oral epithelium, but not with any other tooth germ. They corresponded to tooth positions 21, 22 and 23, and did not appear to develop in sequence with the alternate arrangement of teeth 1-20.

Results - The development of individual tooth germs

This section is concerned with the time taken for the development, eruption, ankylosis and resorption of the sixth to fourteenth positioned teeth of the first generation even-numbered tooth series.

Odontogenesis began during the larval NF stage 55, and the day on which germ initiation occurred is counted as day zero of this study. Day zero was the base line from which the time scale of tooth development was measured.

Odontogenesis was characterised by a number of separate tooth germs which lay in relation to the lower aspect of the ethmoidal cartilage. The premaxillae and maxillae had not begun to ossify at this stage. The production of the tooth germs began posteriorly on each side and continued forward toward the mid-line. During the formation of each tooth germ, the basal cells of the oral epithelium in each tooth-bearing zone became columnar, and orientated with their long axes at right angles to the epithelial surface. The edges of each zone of basal cells invaginated to form a curved plate of cells, concave towards the dermis, resembling an inverted trough. Each trough was then roofed over by the epithelium to

form a short tube, closed at its anterior end and enclosing the mesodermal cells of the dental papilla. Each tube lay horizontally in the jaw and all had their long axes running anteroposteriorly (Fig. 4-2, 4-3, 4-4). This early period of tooth formation before hard tissue genesis had commenced was associated with frequent cell divisions in both the mesoderm and epithelium of each tooth germ. As yet no odontoblasts could be recognised.

Dentinogenesis commenced on the second day after germ initiation, and took the form of a short cone of dentine of average length 20 (1.8)/um; the dentine wall of this cone was about 2.3 (0.2)/um thick, and it was produced by about six odontoblasts. It is assumed that the odontoblasts differentiated from the pool of mesodermal cells, since the latter were no longer present in the papilla when all the odontoblasts had differentiated.

Figure 4-5 shows the average number of odontoblasts present in the dental papillae throughout the growth of the tooth germs. There was a clear division of dentinogenesis into a long period of slow growth, followed by a shorter period of rapid growth. This was reflected in a rapid increase in the length of the dentine and number of odontoblasts during the rapid phase.

From day 2 onwards there was a comparatively slow increase in the number of odontoblasts in the dental papilla until on day 17 there was an average of only 48 (4) odontoblasts present (Figure 4-5). They had produced a cone of dentine of average length 72 (4.5)/um, the walls of which were 3.5 (0.3)/um thick. Therefore, in 15 days only one third of the final length of the dentine had been produced. Day 19 marked the end of the slow phase of dentinogenesis.

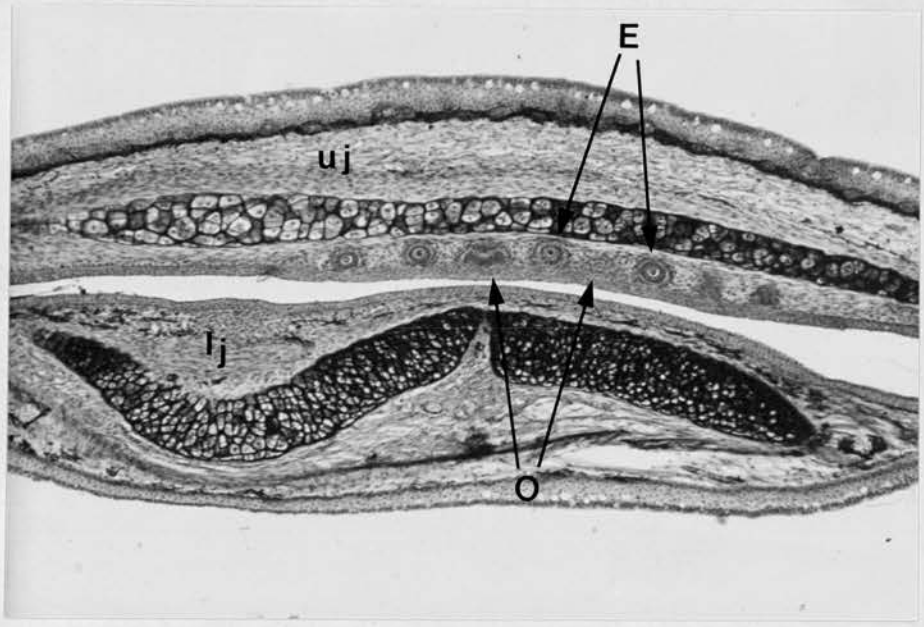


Figure 4-2

A coronal section of the head of a larva showing the upper (uj) and lower (lj) jaws, and the position of the tooth germs. The even-positioned tooth germs (E) have commenced dentinogenesis and enamel formation, while the odd-positioned tooth germs (O) are still at the 'trough' stage.

Decalcified, stained H +E

Scale bar 150/um

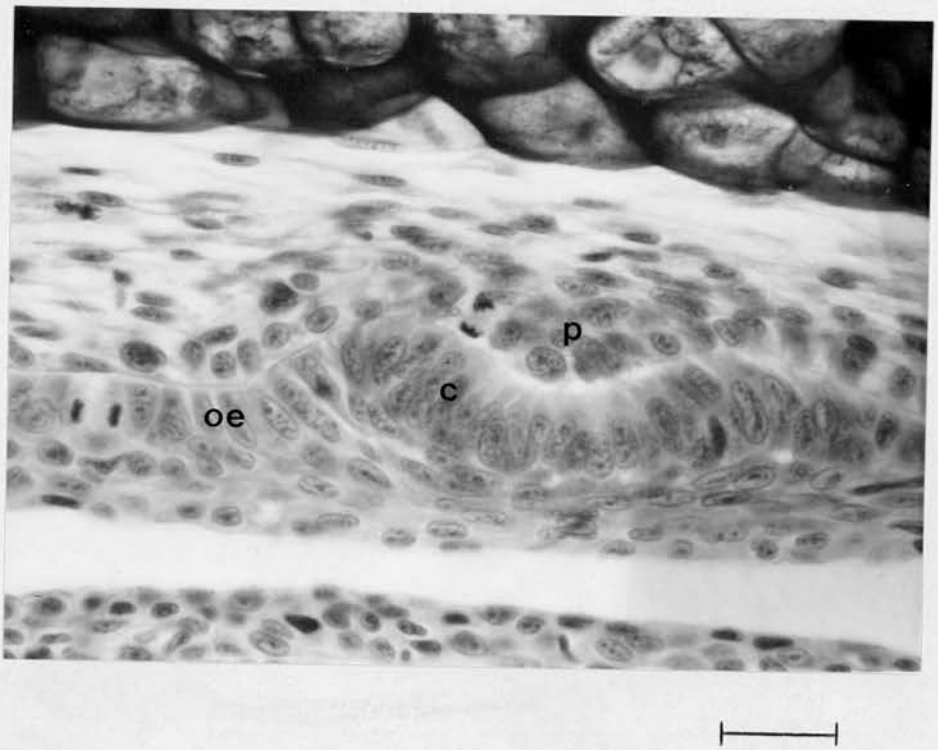


Figure 4-3

A coronal section of the head of a larva showing a tooth germ around day zero of its development. The basal cells of the oral epithelium (oe) have become columnar (c) and form a trough in which lie the future papillary cells (p). Mitoses were frequently visible at this time.

Decalcified, stained H + E

Scale bar 20 um

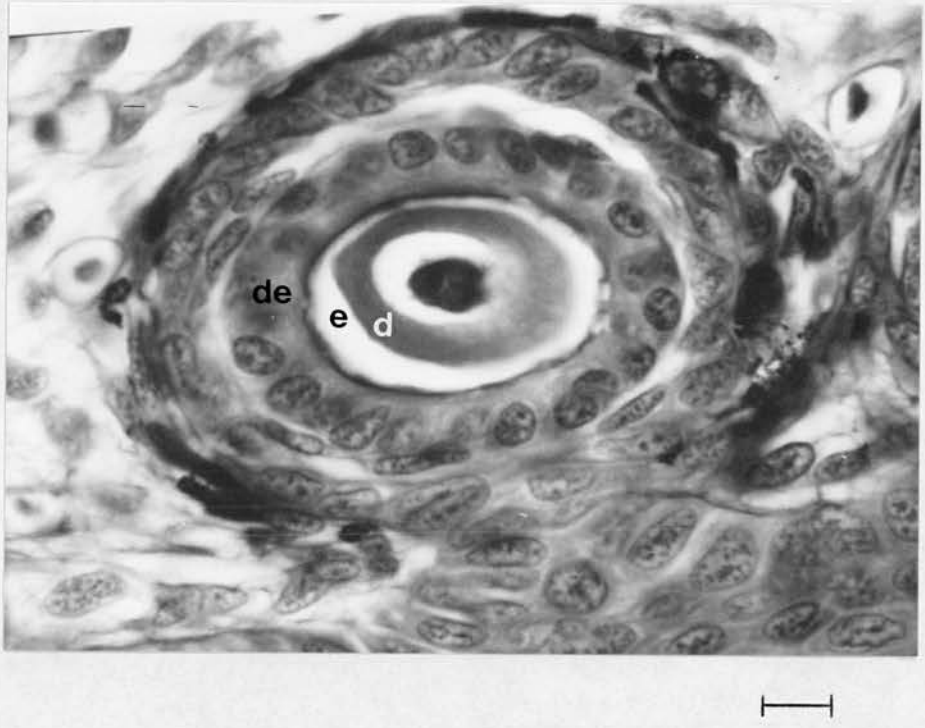


Figure 4-4

A coronal section of the head of a larva showing a tooth germ in transverse section on day 8. The dentine (d) is visible as a ring, and outside the dentine is the enamel space (e). A small amount of enamel matrix has collapsed onto the inner surface of the dental epithelium (de).

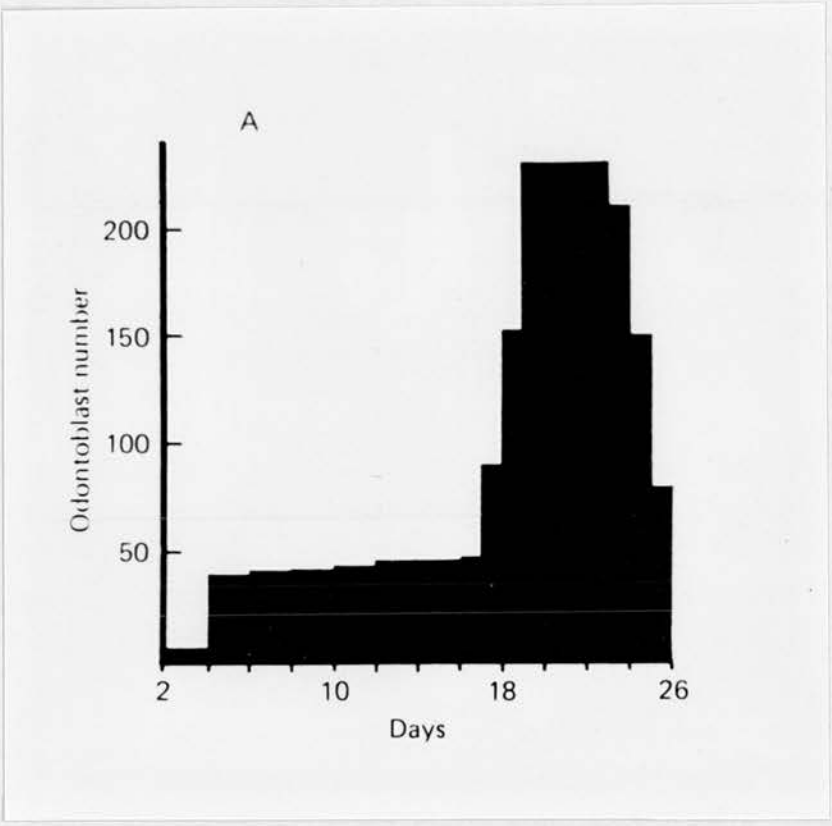
Decalcified, stained H + E

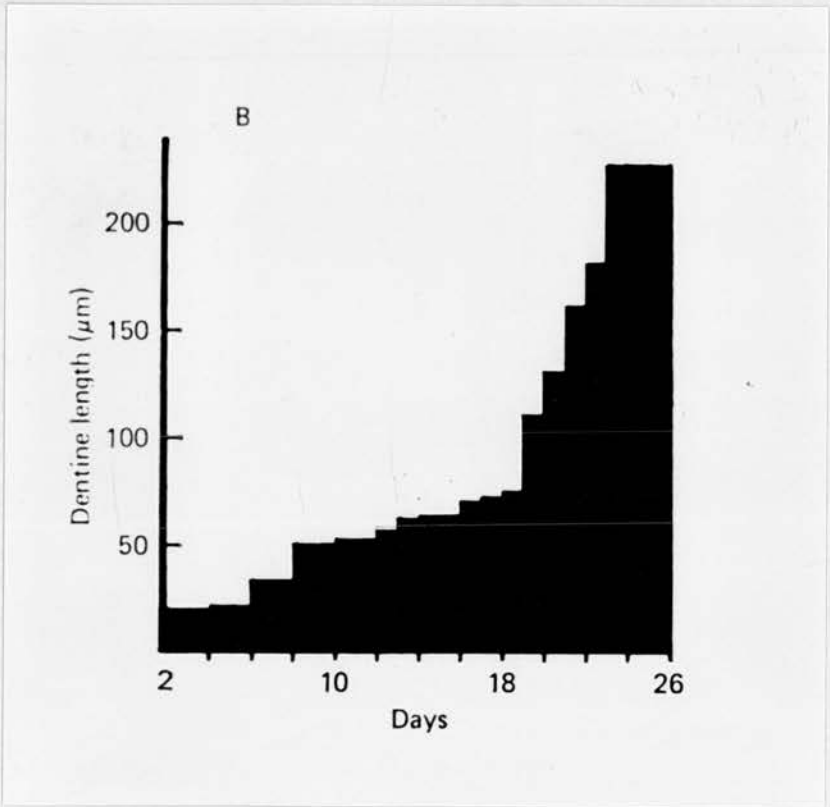
Scale bar 10/um

Figure 4-5 (following page)

(A) A histogram showing the mean number of odontoblasts present in the tooth germs over the first 26 days of tooth development; (B) a histogram showing the mean length of the dentine over the same period as A. The number of odontoblasts remained low during the slow growth phase (days 1-17). However, from day 18 onwards, the number of odontoblasts increased dramatically as the rapid phase began; this was accompanied by a concomitant increase in dentine length. After the maximum length of dentine was attained the number of odontoblasts dropped rapidly to zero at day 27. The two days lag period between the increase in odontoblast number and the increase in dentine length on days 17-19 may be an artefact caused by the method of sampling the specimens.

Figure 4-5


A
Odontoblast number
200
150
100
50
2
10
18
26
Days


B
Dentine length (μm)
200
150
100
50
2
10
18
26
Days

Formation of the enamel began shortly after dentinogenesis had begun and continued until about day 10. In decalcified sections the early enamel matrix stained a deep purple with haematoxylin. However, from about day 8 onwards the matrix was no longer present after decalcification, and an enamel space was left in tissue sections. Formation of the enamel matrix and its subsequent calcification appeared therefore to encompass a fairly short period in tooth formation.

During days 18, 19 and 20, there was a sudden and dramatic change in the appearance of the tooth germs, which heralded the beginning of a period of rapid dentinogenesis. First, the germs had begun their re-orientation to a more vertical position prior to eruption; secondly, each first generation even-positioned tooth germ had developed a dental lamina for its successional second generation tooth; and thirdly, there was a resumption of cell division in the mesodermal pool and a rapid differentiation of odontoblasts from that pool. The mitotic activity was such that by day 20 the total number of cells in the papilla averaged 230 (24.3), nearly all of which were active odontoblasts. This was approximately five times the number of odontoblasts present at day 17. The length of the dentine now averaged 189 (9)/um with walls 7.2 (0.2)/um thick. The base of the dentine wall showed for the first time a tapering at its basal edge, indicating a rapid deposition of material. In Figure 4-6 it can be seen that the odontoblasts forming the tip of each tooth presented a different appearance to those forming the base which had differentiated during the rapid phase. The odontoblasts at the tip were squatter than those at the base, and each possessed more than one odontoblast process. The basal odontoblasts were tall columnar cells with only one odontoblast process.

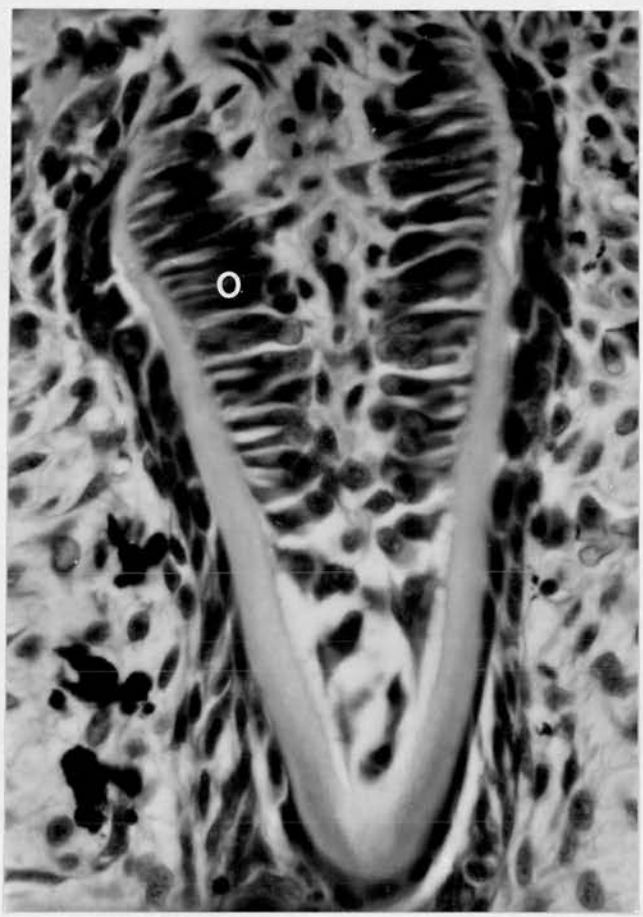


Figure 4-6

A longitudinal section of a tooth germ during the rapid growth phase (day 20), showing tall columnar odontoblasts (o) in the basal two-thirds of the pulp. Note thatthe odontoblasts at the tip of the tooth are squatter than those near the base.

Decalcified, stained H + E

Scale bar 20/um

The pulp cavity of each tooth now appeared to be filled only with odontoblasts and a blood vessel loop. The number of odontoblasts was maintained while the teeth erupted and the dentine attained its maximum length. The average length of the dentine was now 225 (2)/um with walls 8.4 (0.4)/um thick, and these values were reached on day 25. Between days 17 and 25 the dentine had more than tripled its length. Attachment to the pedicel commenced on day 23, and was completed by day 25. However, just before the completion of metamorphosis of the animals, the odontoblasts at the tips of the teeth were showing signs of degeneration. The cells had become flatter, and lay with their long axes orientated in the plane of the inner surface of the dentine. Their nuclei stained lightly, and some appeared pyknotic. This degeneration of the odontoblasts continued into the basal part of the teeth until day 27, when no active odontoblasts were seen. In their place was a small number of flattened cells, many of which lay along the inside of the dentine with their long axes parallel to its surface (Fig. 4-7). On day 27 the teeth in the odd-numbered tooth positions began their rapid growth phase.

The resorption of the even-numbered teeth occurred during days 32 and 33. Prior to the beginning of resorption, the connective tissue of the dental pulp was loosely woven and relatively acellular and avascular. At the beginning of resorption the connective tissue of the pulp became denser, more cellular and more vascular. Osteoclasts began to appear around the bony pedicel supporting the teeth. The soft tissue changes just described were seen in several of the post-metamorphic animals. However, the final phase of the resorptive process was so rapid that it was only observed in two specimens. In these two specimens large osteoclasts had invaded the pulp and were rapidly resorbing the dentine piecemeal from

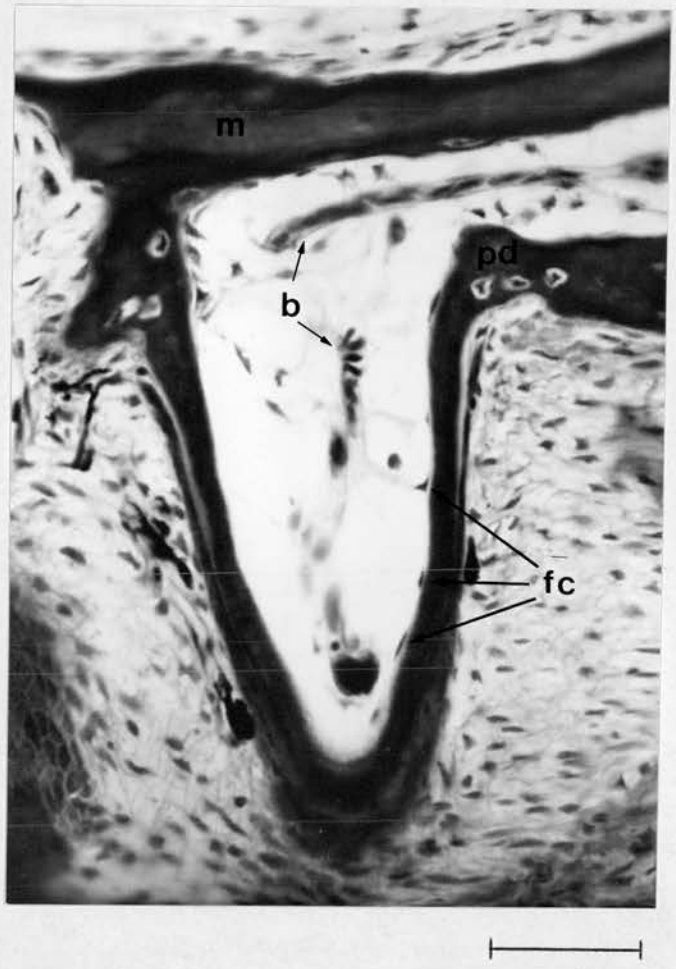


Figure 4-7

A longitudinal section of a tooth at the end of the rapid growth phase. Odontoblasts are no longer recognisable in the pulp, but a number of flattened cells (fc) are seen against the pulpal surface of the dentine.

m, maxilla; pd, bone of pedicel; b, blood vessel

Decalcified, stained Heidenhain's azan

Scale bar 50/um

its pulpal aspect (Figure 4-8). In those specimens in which the final phase of resorption was not seen, missing teeth indicated that it had occurred. Since developmentally missing teeth were not observed in any pre-metamorphic specimens, it is reasonable to assume that the teeth missing on days 33 and 34 had been resorbed.

The most striking aspect of the final resorptive process in the first generation teeth was the speed of the event. It is estimated that this phenomenon took only about 24 hours.

The timing of the events in tooth development are summarised in Figure 4-9.

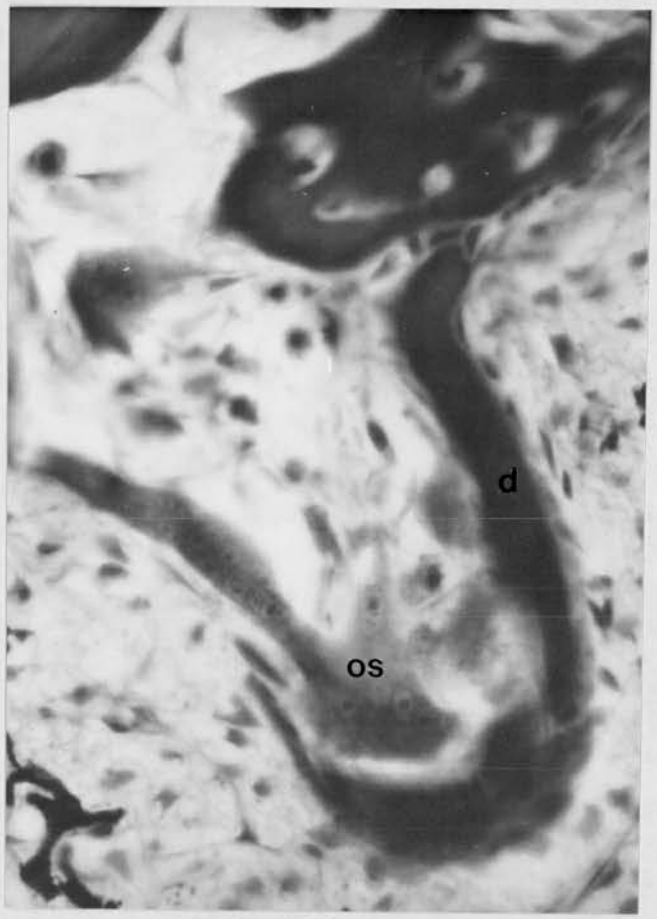


Figure 4-8

A longitudinal section of a tooth undergoing the final phase of resorption (day 32). Large osteoclasts are present in the pulp, and one wall of dentine has been completely resorbed.

os, osteoclast; d, dentine

Decalcified, stained H + E

Scale bar 50/um

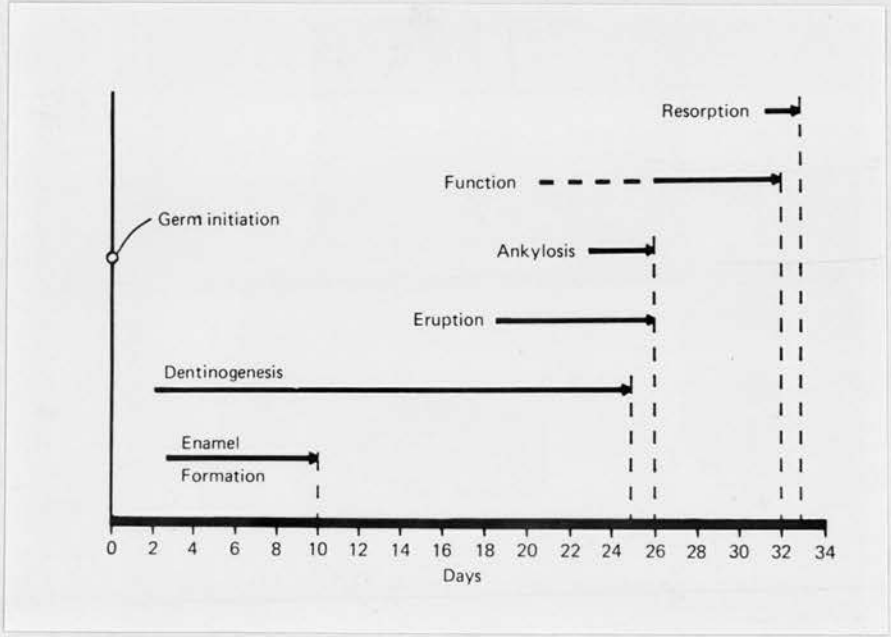


Figure 4-9

A summary of the events in the developmental cycle of the teeth relative to the absolute time scale involved. The length of each horizontal arrow indicates the duration of each process. Eruption is defined as commencing at the beginning of the rapid phase of dentinogenesis. Function is represented by a broken bar between days 20 and 26, since the teeth may have been functional between the time they pierced the oral epithelium and completed ankylosis.

Section IVB - Resorption of the First Teeth to Develop

Introduction

In this Section an attempt was made to assess more accurately the time scale of resorption of the first generation even-positioned teeth. Resorption was known to occur a few days after the completion of metamorphosis, and involved the removal of the first generation teeth prior to the eruption of the successional second generation teeth. The method used was similar to that in Section IVA, whereby larvae were reared at similar growth rates, so that the histological changes during the resorption of teeth at the same loci in different animals could be observed, and a mean time scale applied to these changes.

Method

For this study 96 larvae which had reached NF stage 65 on the same day were used. The animals were timed from stage 65 because this was an easy stage to identify using external criteria, and furthermore it was known from Section IVA that this was the stage at which the first generation even-positioned teeth were completing ankylosis. It was hoped that the animals in the group would continue to develop at similar rates over the short period of the study, a period which covered the functional life span of the teeth under examination.

Commencing three days after stage 65, 12 animals were killed at 1400hrs each day for eight days, and the period of study therefore encompassed the 4th - 11th days post stage 65.

The head of each specimen was serially sectioned in the coronal plane and the 10/um sections stained with haematoxylin and eosin, so that the resorption of teeth at loci 2, 4 and 6 on each side of the head could be examined.

In the study of resorption osteoclasts were recognised on the sections as large cells with more than one nucleus and well-developed eosinophilic cytoplasm, which were frequently associated with Howship's lacunae in dentine and bone. They presented an appearance distinct from the other cells in the connective tissues and tooth pulps, each of which had scanty cytoplasm and a single, small, darkly staining nucleus. No confusion arose between osteoclasts and odontoblasts, since the latter were not present in the ankylosed teeth under examination, while the dental epithelial cells were closely packed and possessed basophilic cytoplasm.

One animal died and was excluded from the results, and later one animal was found to possess an atypical type 2 dentition and another to possess an abnormal dentition (to be discussed later), and these animals were also excluded from the results.

Observations

4th - 6th days post stage 65

During this time each first generation tooth remained ankylosed to its pedicel, with the second generation tooth germ lying above it on the palatal side. Small isolated osteoclasts were observed in relation to the first generation teeth, but these were confined to the outer aspect of the palatal wall of dentine which was undergoing resorption (Figure 4-10). Where osteoclasts were not seen any resorption bays on the outer surface of the dentine were assumed to indicate previous tissue destruction.

Over this period the pulp of each first generation tooth remained comparatively avascular and acellular, being sparsely populated by cells having the appearance of fibroblasts, supported by a loosely arranged meshwork of fibres. A single layer of flattened cells lay against the

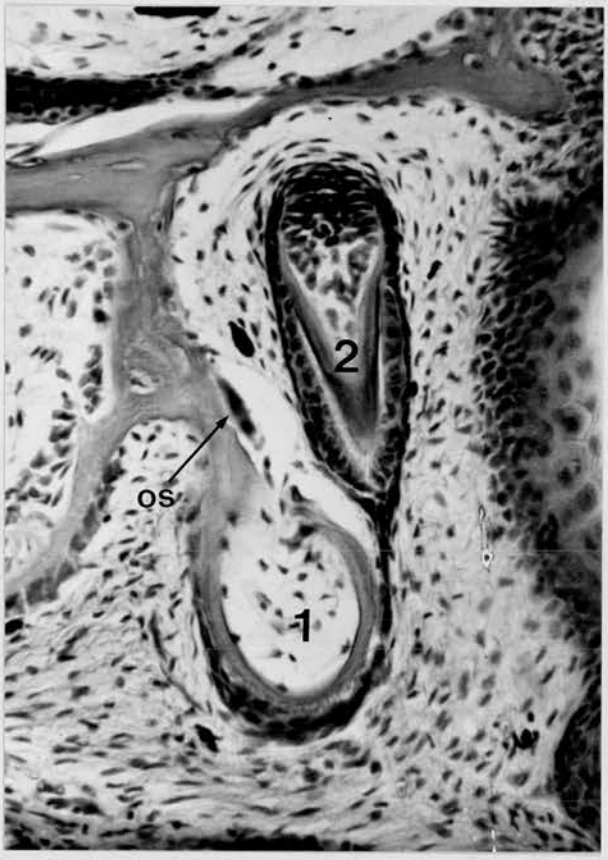


Figure 4-10

4th - 6th days post stage 65; a longitudinal section of a first generation tooth (1) and its successional second generation tooth germ (2) lying above it on the palatal side. During this time osteoclasts (os) were observed only on the outer aspect of the dentine of the first generation teeth.

Decalcified, stained H + E

Scale bar 50/um

dentine, with the long axes of the cells parallel to the dentine surface (Figure 4-11). Osteoclasts were not seen within the pulp until the 6th day, and then only in 8.33% of teeth examined (Table 4-2). Where osteoclasts were found in this situation they were not apposed to the internal surface of the dentine but were congregated in the basal part of the pulp and did not appear to be actively resorbing the dentine.

Throughout this 3 day period the successional second generation tooth germs remained small, their mean length being 72.5 (8.24)/um. This length corresponded well with that found for first generation tooth germs at the end of their slow growth phase in Section IVA.

7th - 8th days post stage 65

The histological picture presented during the 7th and 8th days was very different from that observed earlier (Figure 4-12). Most striking was the presence of osteoclasts around the periphery of the pulps of many teeth lying against the internal surface of the dentine, and these cells now seemed to be engaged in actively resorbing the dentine from its internal aspect. Dentine destruction was now more extensive than that observed during days 4-6. In two specimens an osteoclast was seen lying in the lingual foramen of the pedicel, as if in the process of migrating into the pulp.

The central regions of each pulp showed increased cellularity, and red blood corpuscles were more evident in the blood vessels, perhaps indicating an increased vascularity. However, measurements indicated that there was no significant difference in the mean diameter of the blood vessels when compared with those of teeth during days 4 - 6.

On the 8th day 74% of teeth examined showed the histological changes

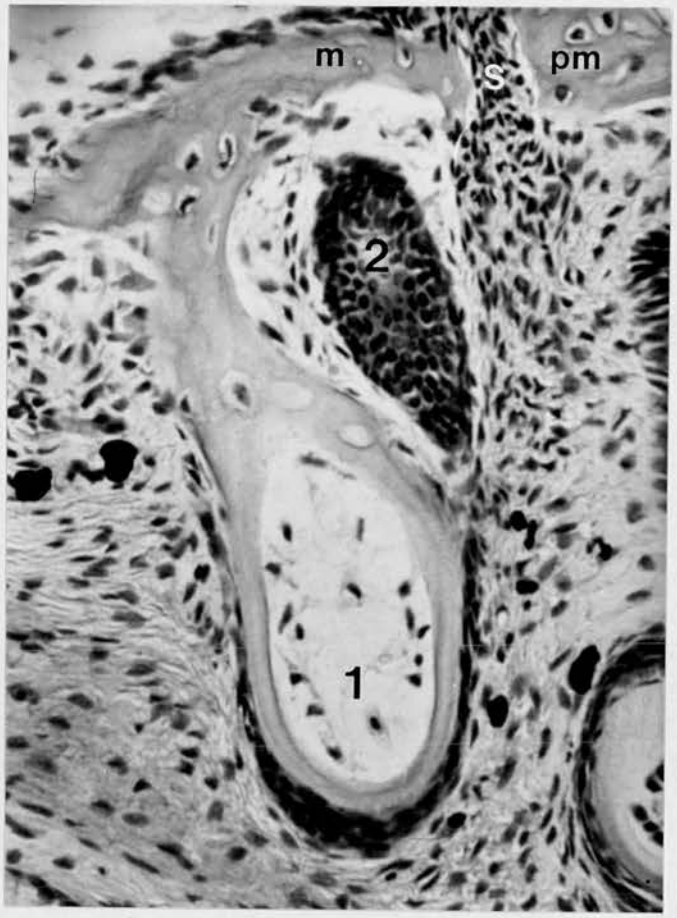


Figure 4-11

4th - 6th days post stage 65 — a longitudinal section of part of a first generation tooth (1) and its successional second generation tooth germ (2). The degree of cellularity of the pulp of the first generation tooth remains similar to that seen at the completion of dentinogenesis in the tooth shown in Figure 4-7.

From the premaxillary-maxillary suture(s) a band of fibrous tissue extends inferiorly, and note that both tooth (1) and successional tooth (2) lie on the same side of this band. (Compare with Figure 4-15, and see text page 51).

pm, premaxilla; m, maxilla

Decalcified, stained H + E

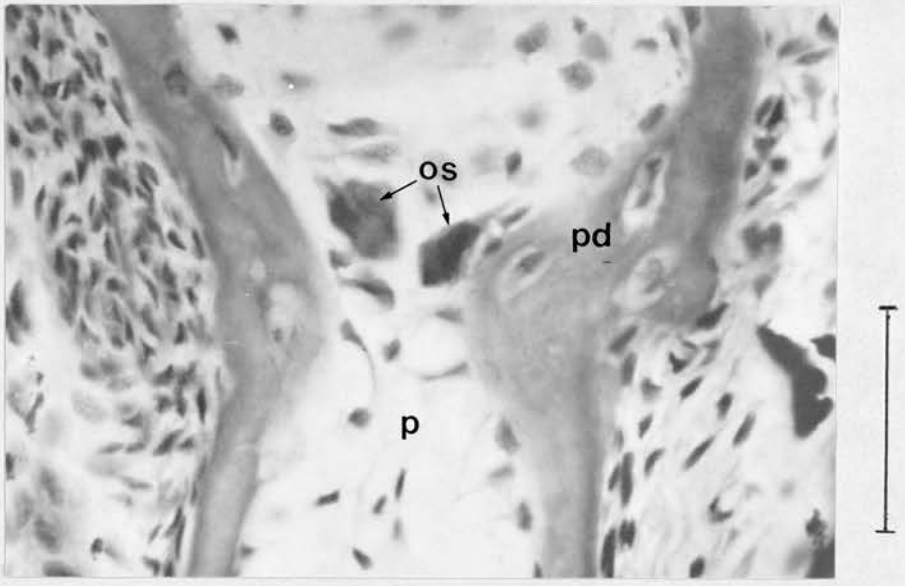
Scale bar 100/um

TABLE 4-2 THE RESULTS OF THE STUDY ON TOOTH RESORPTION

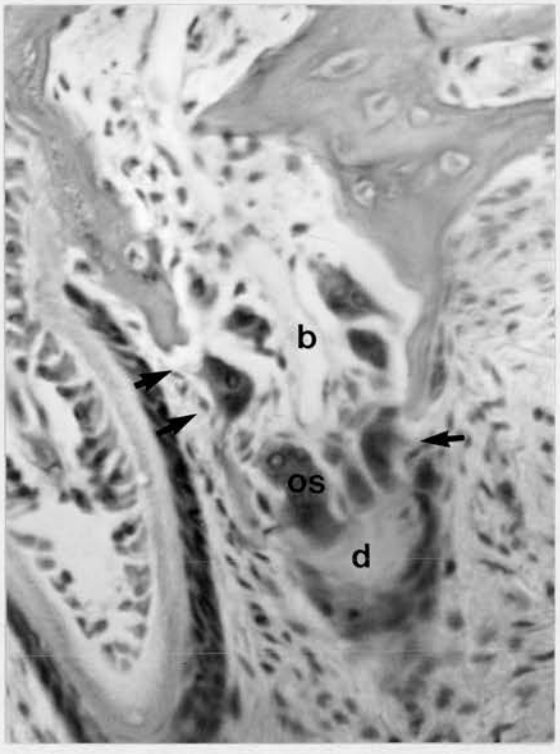
Days post stage 65	Number of specimens examined	Number of teeth examined	Number of specimens showing osteoclasts in pulp	% of specimens showing osteoclasts in pulp	Number of teeth showing osteoclasts in pulp	% of teeth showing osteoclasts in pulp	Number of specimens in which tooth tips present
4	11	66	-	-	-	-	-
5	12	72	-	-	-	-	-
6	12	72	2	16.67	6	8.33	-
7	12	72	8	66.67	37	51.39	3
8	11	66	10	90.91	49	74.24	7
9	12	72	2	16.67	12	16.67	2
10	12	72	2	16.67	4	5.56	1
11	11	66	-	-	-	-	-

Figure 4-12A and B

7th - 8th days post stage 65 — the appearance of the first generation teeth.



(A) Osteoclasts (os) lying in the lingual foramen of the pedicel (pd) near the base of the pulp (p).



(B) Osteoclasts (os) are present in the pulp, and areas of dentine have been resorbed (arrows).
d, dentine; b, blood vessel.

Both decalcified, stained H + E

Scale bars 50/um

described above, but also teeth were frequently observed of which only the tips remained unresorbed (Figure 4-13). Furthermore, in several cases such tooth tips, which would have projected through the oral epithelium when the teeth were functional, were found deep to the epithelium embedded in the connective tissue of the upper jaw. Osteoclasts were still present in what remained of the pulps of these teeth, but it was no longer possible to identify blood vessels with certainty in the pulps.

During this period (days 7-8) the mean length of the second generation tooth germs had increased to 110.78 (27.14)/um, and this would indicate that they were in their rapid growth phase and in the process of erupting (vide Section IVA).

9th - 11th day post stage 65

Only four specimens during this period showed teeth which were undergoing internal resorption by osteoclasts. In the remaining 31 specimens the first generation teeth were absent, and the second generation teeth were erupting. It seemed, therefore, that resorption had been completed in these specimens.

Further observations

One specimen, killed on the 4th day, possessed a dentition which appeared to be overcrowded, so that there was insufficient space for the first generation odd-positioned teeth to erupt in the normal way between the first generation even-positioned teeth. When the specimen was killed the odd-positioned teeth were erupting, and the dentine of the even-positioned teeth adjacent to the erupting odd-positioned teeth

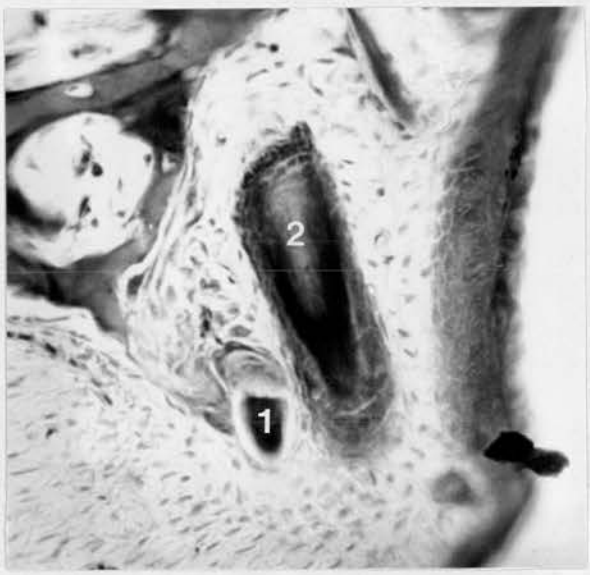


Figure 4-13

7th - 8th days post stage 65 — in this specimen only the tip of the dentine of the first generation tooth (1) remains.
2, successional second generation tooth.

Decalcified, stained H + E

Scale bar 50/um

had been completely resorbed, in one case 74% of the total length of the dentine had been destroyed (Figure 4-14).

However, the pulps of the even-positioned teeth retained their integrity, and appeared comparatively avascular and acellular. There were no osteoclasts within the pulps, and no indication that any internal resorption of dentine had occurred.

Two further specimens, killed on the 8th day, showed an interesting feature in relation to the tooth at the 6th locus, in the region of the premaxillary-maxillary suture. In both specimens the first generation tooth was ankylosed to the maxilla, while its successional tooth germ was developing beneath the premaxilla. Thus the teeth were on opposite sides of the suture, and furthermore were separated by a band of fibrous tissue which extended inferiorly between them. The width of this fibrous band was 66% of the width of the successional tooth germ. The pulp of the first generation tooth showed osteoclasts resorbing the dentine from its internal aspect (Figure 4-15).

Results

Resorption of dentine and bone extended from day 4 to day 10, but the process occurred in two phases. During days 4-6 resorption was seen predominantly on the outer aspect of the dentine, and involved minimal tissue destruction. But during days 7-8 osteoclasts were present in the pulp destroying the dentine from within, and the rate of resorption appeared to have accelerated, so that the entire thickness of the dentine was being destroyed from the base to the tip of each tooth.

Table 4-2 shows that the number of specimens and teeth demonstrating internal resorption rose dramatically on day 7, and reached a peak on

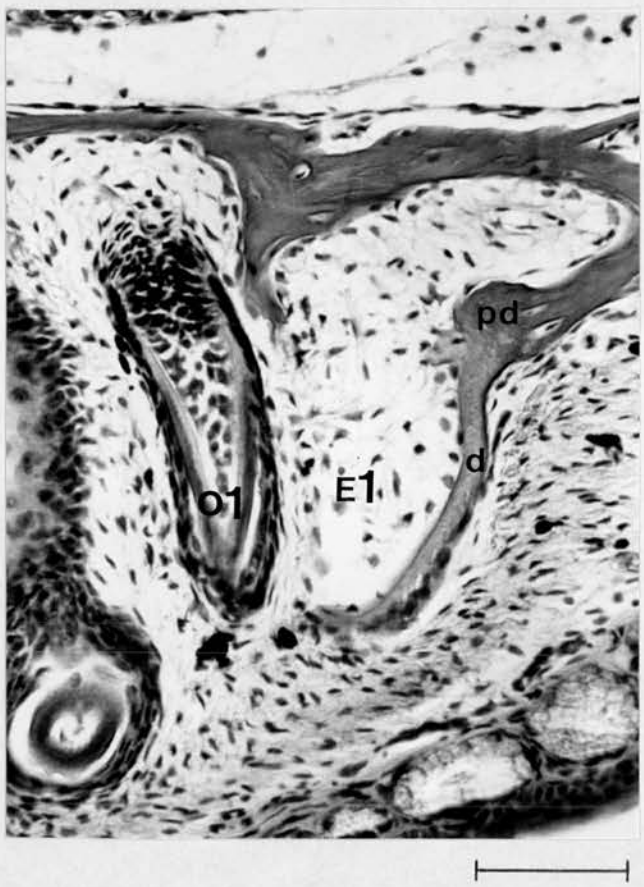


Figure 4-14

Longitudinal section of teeth from a specimen with a crowded dentition. The first generation odd-positioned tooth (O1) is erupting very near the first generation even-positioned tooth (E1), and the adjacent wall of dentine of E1 has been resorbed. Osteoclasts were not visible within the pulp of E1, and the degree of cellularity of the pulp remains similar to that of the teeth shown in Figures 4-7 and 4-11.
pd, pedicel of E1; d, dentine of E1.

Decalcified, stained H + E

Scale bar 50/um

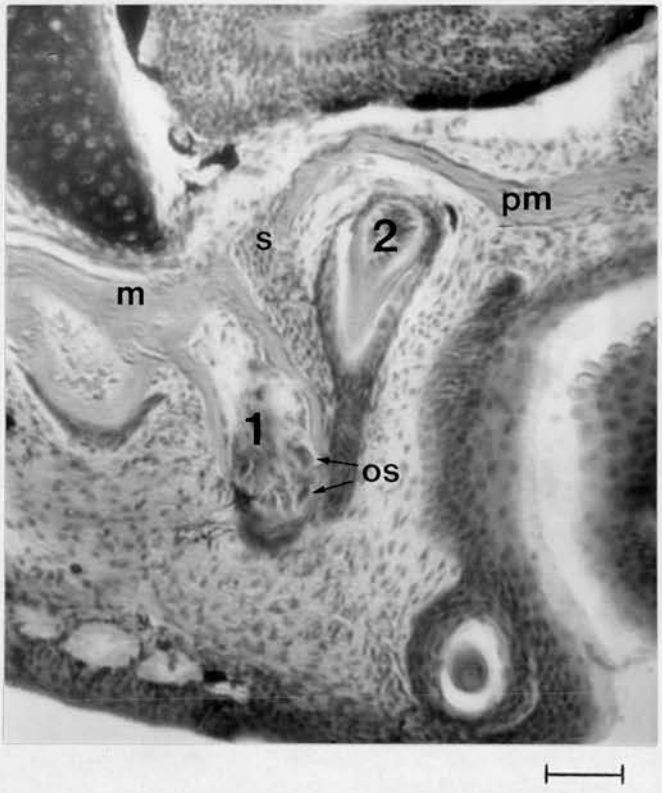


Figure 4-15

A specimen in which the first generation even-positioned tooth (1) and its successional second generation tooth germ (2), are situated on opposite sides of the band of fibrous tissue extending inferiorly from the premaxillary-maxillary suture(s). (Compare with Figure 4-11 which shows the normal arrangement of teeth at this locus (the sixth)).

Osteoclasts (os) are present in the pulp of the first generation tooth.

pm, premaxilla; m, maxilla

Decalcified, stained H + E

Scale bar 50/um

day 8. On day 8, seven of the eleven specimens examined showed teeth of which only the tips remained. On the assumption that the first generation teeth completed ankylosis at stage 65, then the majority of these teeth underwent internal resorption 7-8 days later and had been completely resorbed by the 9th day.

Internal resorption of individual teeth did not seem to occur in the same order in different animals, but in many specimens the order could not be discerned.

SECTION V - THE RATE OF TOOTH REPLACEMENT IN ADULT XENOPUS

Introduction

In this Section an attempt was made to estimate an absolute time scale for tooth replacement in adult *Xenopus*, by using a wax impression technique to record, *in vivo*, the state of the dentition over predetermined intervals of time.

However, it was clear before embarking on this work that there would be problems with the impression technique. Trials with a rubber base impression material showed that while this may have produced accurate impressions, the difficulties involved in their interpretation gave conflicting results. Consequently the rubber base technique was rejected, and sheets of gold-casting wax used as the impression material.

Unfortunately the wax was not capable of recording the presence or absence of teeth at each locus with complete accuracy, although a pilot study had shown individual impressions to be about 85% accurate. Consequently, it was decided for the study to take three wax impressions of each animal's mouth on each occasion, and to use a summation of the information on these impressions as being the nearest approximation to the real situation.

The difficulties involved with both recording techniques were due principally to the extremely small size of the teeth; only about 100 µm of each tooth actually protruded through the oral mucosa into the mouth. Furthermore, teeth undergoing ankylosis or resorption might be only loosely attached to the bone and be displaced by the impression material in such a way that they were not recorded.

It was also possible that inaccuracies were caused by the mobility of the maxilla. This mobility was demonstrable in living animals, and

appeared to be due to the absence of the quadratojugal bone.

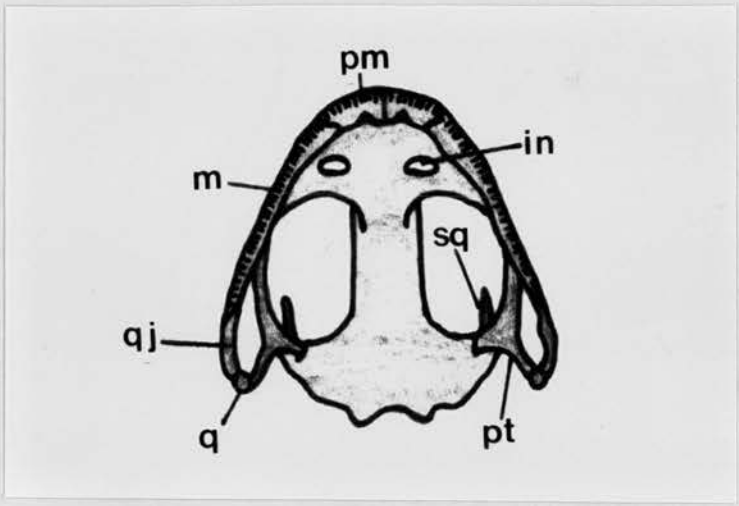
In the upper jaw of most salientians a quadratojugal is present on each side of the skull and articulates with the maxilla anteriorly and the quadrate posteriorly, so that the upper jaw, while composed of several bones, is a continuous bony arch and therefore a comparatively rigid structure (see Figure 5-1). However in Xenopus there is no quadratojugal, and consequently the posterior ends of the maxillae lie in soft tissues free of bony attachments, so that the upper jaw is a flexible structure which can be displaced by the lower jaw when the mouth is closed (see Figure 5-2 and 5-3). Movement of the maxillae caused by the pressure applied during the impression procedure might have resulted in teeth being pushed away from the wax and thus not recorded.

Method

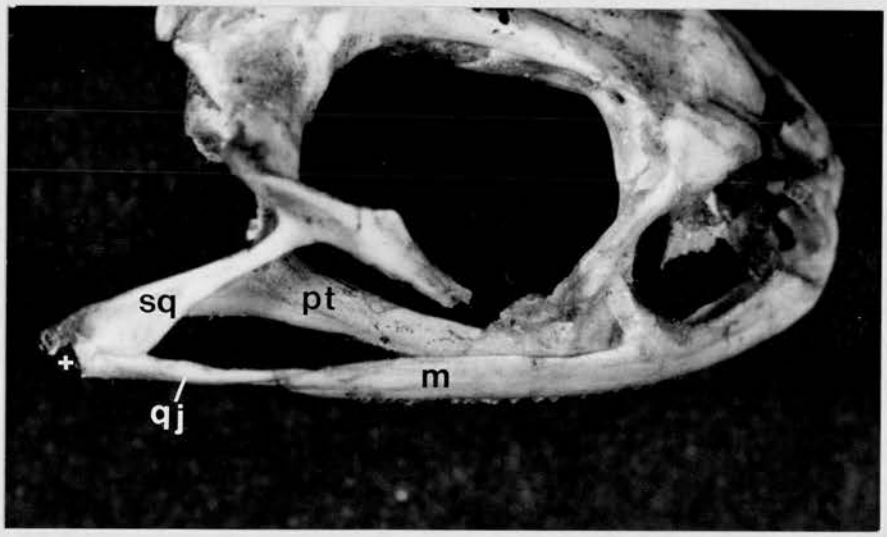
Three very large females of similar size were used (Table 5-1) and impressions were taken of each animal's mouth on Tuesday and Friday of each week for about 10 weeks. However, to preclude the possibility of a very rapid tooth replacement rate, impressions were taken daily during week seven. To take the impressions each animal was anaesthetised, and moved from its tank onto the bench where it was wrapped in a damp towel. A shaped sheet of Kemdent* gold-casting wax was then inserted into the mouth, and the jaws squeezed gently but firmly together for 10 seconds. The wax was then removed and the animal allowed to recover from the anaesthetic. After taking each impression the time of day was recorded to the nearest 10 minutes.

* Supplied by Associated Dental Products Ltd, Purton, Swindon, Wilts.

Figure 5-1A and B



A. Ventral view of Rana skull (simplified). The upper jaw extends as a continuous bony arch between the quadrates. q, quadrate; qj, quadratojugal; m, maxilla; pm, premaxilla; in, internal naris; pt, pterygoid; sq, squamosal.



B. View of Rana skull from right side to show the articulation between the posterior end of the maxilla and the quadratojugal. The quadrate is composed principally of cartilage and is not present on this dry skull, but its former position is indicated by "+". Lettering the same as in Figure 5-1A.

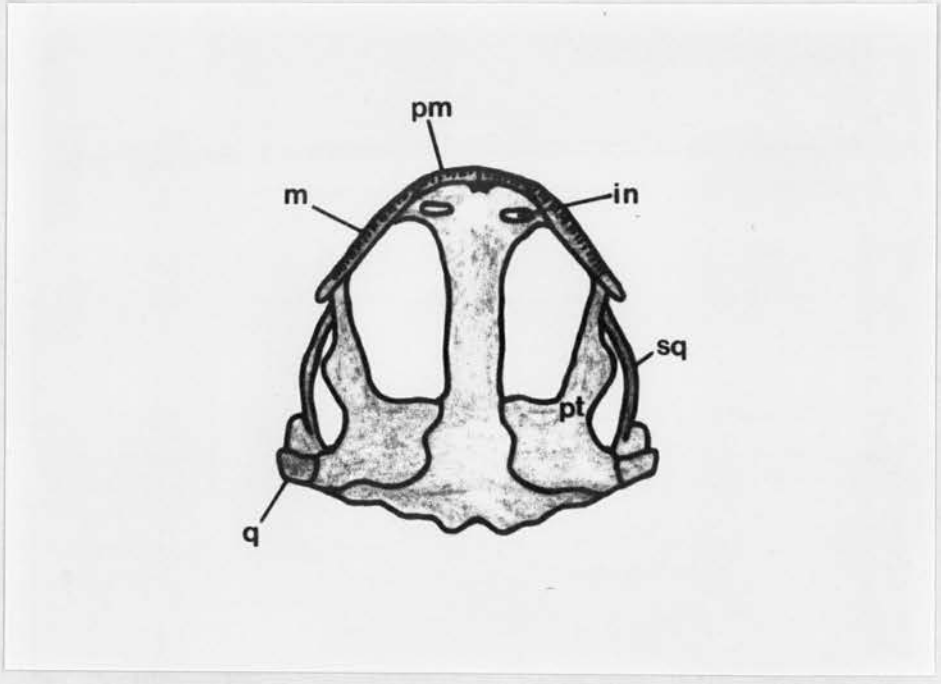
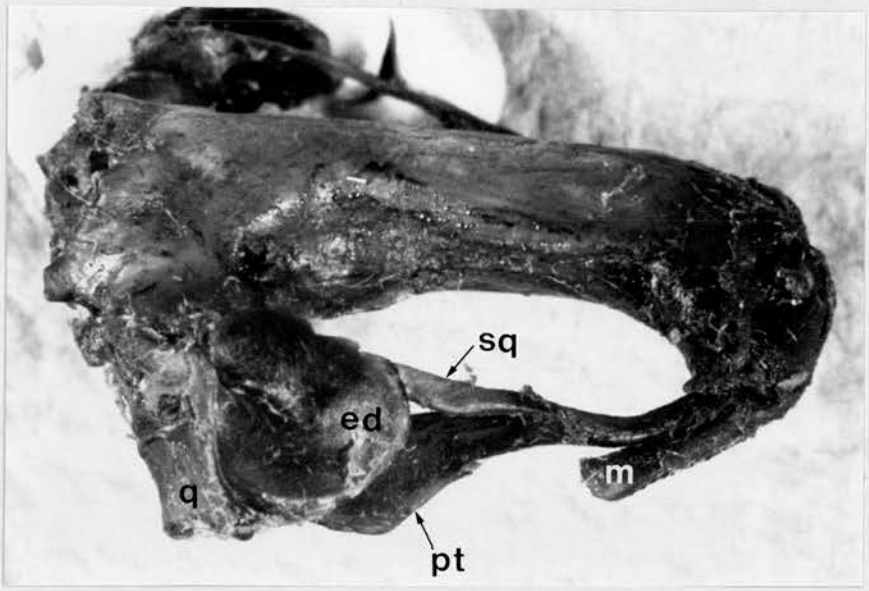


Figure 5-2

Ventral view of Xenopus skull (simplified). In the absence of the quadratojugal there is no bony link between the maxilla and the quadrate. Lettering the same as Figure 5-1A (on preceding page). Please also refer again to Figure 1-2, which follows text page 3.

Figure 5-3A and B



A. Skull of Xenopus from right side (macerated specimen), showing the posterior end of the maxilla ending freely at some distance from the quadrate. Lettering the same as Figure 5-1A; ed, cartilage of the eardrum.

B. Skull of Xenopus from right side (partially macerated specimen), showing a ligament (l) attached between the maxilla and the pterygoid. Compare with A above. The ligament is described in Appendix 3. Lettering the same as Figure 5-1A.

TABLE 5-1 A COMPARISON OF THE ANIMALS USED IN SECTION V

	Snout-Vent Length (mm)	Upper Jaw Width (mm)	Number of recorded tooth loci
ANIMAL 1	112	19.5	84
ANIMAL 2	107	17	77
ANIMAL 3	105	18	83

At the end of the study period the animals were killed and their bones and teeth stained with alizarin red S, so that the number and position of tooth loci recorded by the impression technique could be confirmed.

The impressions of the teeth in the wax were too small to be examined by direct observation, and so to interpret each impression it was mounted in a 35mm slide holder, and enlarged by projection at a standard lens-screen distance of one metre, so that a drawing of the image could be made on the screen. Any doubtful markings of the image were checked by examining the wax impression with a binocular microscope.

A series of drawings was thus obtained for each animal's dentition and these were compared with others in the series using tracings. In this way charts were constructed so that a longitudinal study of the changes in the dentition over the 10 week period could be made.

Results. (Figures 5-4 to 5-8 follow page 58)

The following terminology was used in the interpretation of the charts compiled from the impressions (Figure 5-4). When a given tooth A erupted it would appear in the impression record and be recorded by subsequent impressions until its resorption; this period was called the Functional Life. When tooth A was resorbed, no tooth would appear in the impression record at that tooth position until the eruption of its successional tooth B; this time period was called the Gap Length. The period from the eruption of tooth A to the eruption of tooth B was called the Replacement Cycle. The values obtained for these three variables were expressed in hours.

Over the period of study one or two replacement cycles were observed at each tooth position in each animal (Figure 5-5), but due to the difficulties with the recording technique there appeared to be variation in the functional life, gap length and replacement cycle times between tooth positions. This necessitated the use of a statistical method to analyse the data.

For this analysis it was necessary to make the assumption that the duration of the developmental cycle of all teeth in each animal should be similar. Each half of each animal's jaw was analysed separately, and for every tooth locus in each half-jaw the first replacement cycle was measured from the chart, together with the gap length encompassed by that cycle, and the functional life obtained by subtraction. The median values for the three variables were then calculated separately for each half of each animal's jaw.

Post-mortem examination of the alizarin stained jaws showed that, at a very small number of loci, no teeth had been recorded over the 10 week period. Two loci were unrepresented in the impression record in animal 1, three in animal 2, and four in animal 3. Although the unrecorded loci were in the anterior regions of the jaws, there was no correlation in their positions between the three animals. However, 95% of loci counted post-mortem were represented in the impression record, thus vindicating the technique employed.

Histograms showing the frequency of occurrence of replacement cycle times at different loci along the jaws, indicated that although there were variations, there was a clear peak in all animals at around 1100 hours duration (Figure 5-6). Similarly the frequency of occurrence

of gap lengths varied, but showed a definitive peak at 300 - 400 hours in animals 1 and 3, and 500 hours in animal 2 (Figure 5-7).

However, unlike the other two parameters, there was no clear peak in the frequency of the functional life times in any of the half-jaws, but there was a trend in all animals towards a value of 600 - 800 hours (Figure 5-8).

The median values for the replacement cycle, gap length and functional life of the teeth are shown in Table 5-2, and it can be seen that the values obtained for each half of each animal's jaw (calculated separately) were indeed very similar.

TABLE 5-2 THE REPLACEMENT CYCLE TIME, GAP LENGTH AND FUNCTIONAL LIFE OF TEETH IN THE ADULT

		Median replacement cycle time (hours) ± 10	Median replacement cycle time (days)	Median gap length (hours) ± 10	Median gap length (days)	Median functional life (hours) ± 10	Median functional life (days)
Animal 1	right	930	38.75	270	11.25	660	27.5
	left	930	38.75	230	9.58	700	29.17
Animal 2	right	1010	42.08	420	17.5	590	24.58
	left	990	41.25	410	17.08	580	24.17
Animal 3	right	910	37.92	330	13.75	580	24.17
	left	980	40.83	340	14.17	640	26.67

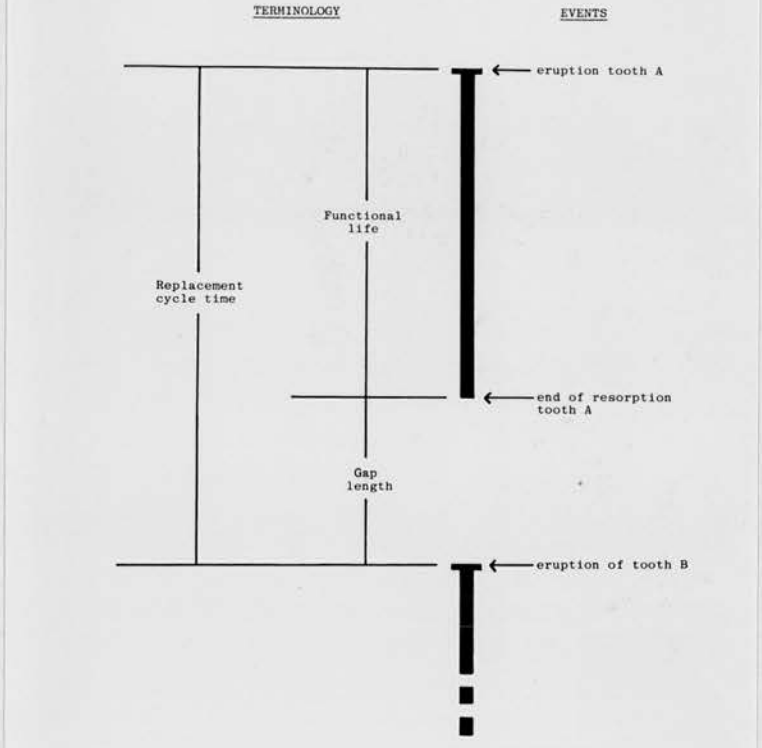


Figure 5-4

The terminology used in the charts compiled from the impressions - for explanation see text page 55. In the charts on the following pages (Figure 5-5A, B and C) the presence of a tooth at a locus is represented by a vertical bar as shown here under "EVENTS", and the absence of a tooth at that locus by a break in the bar.

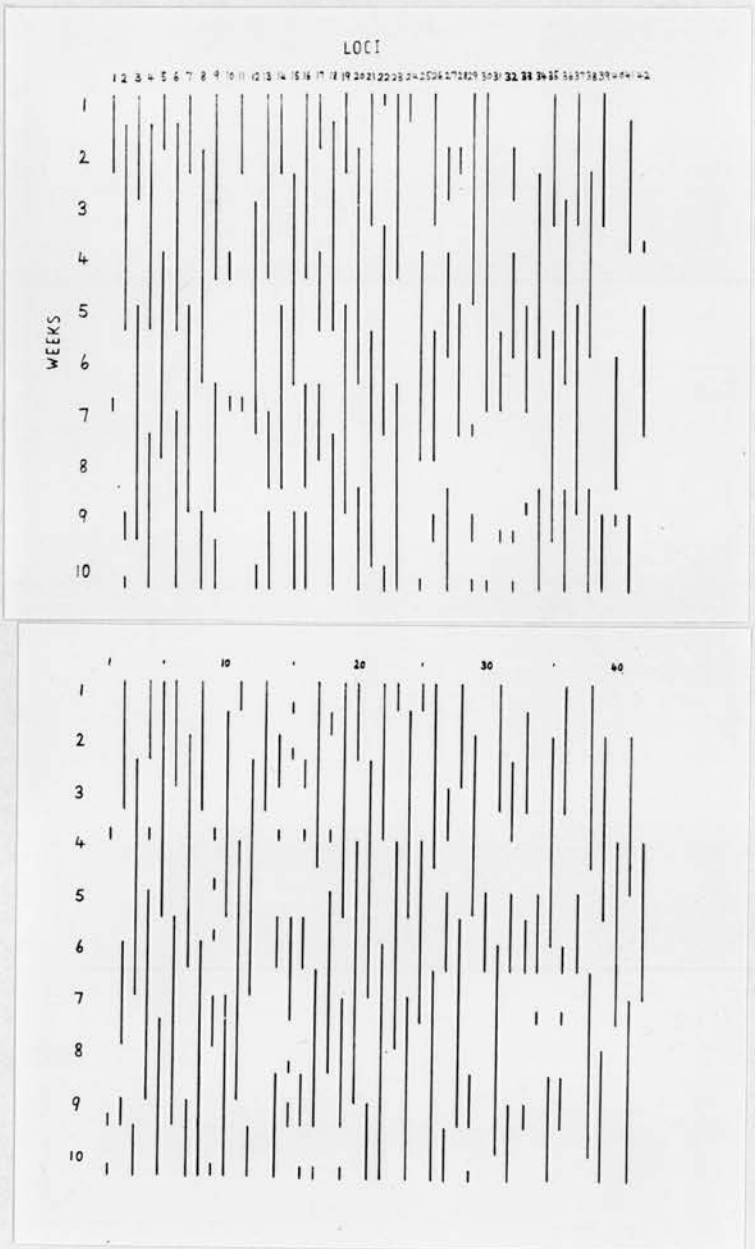
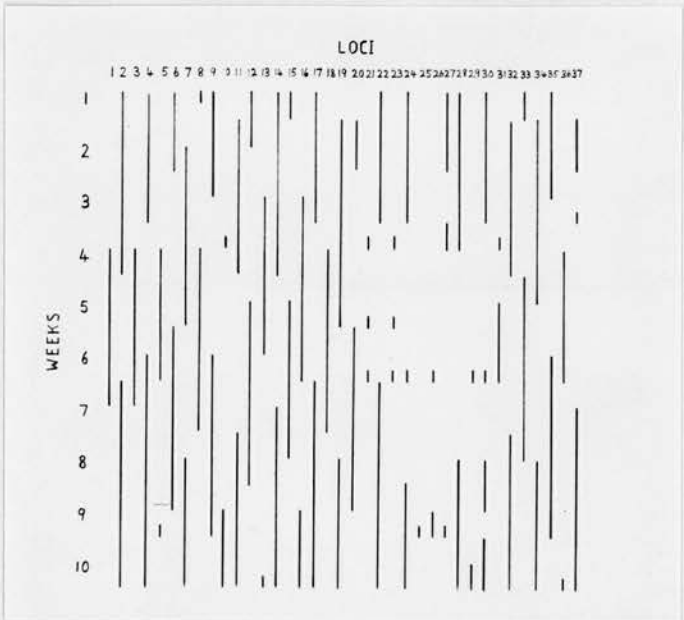
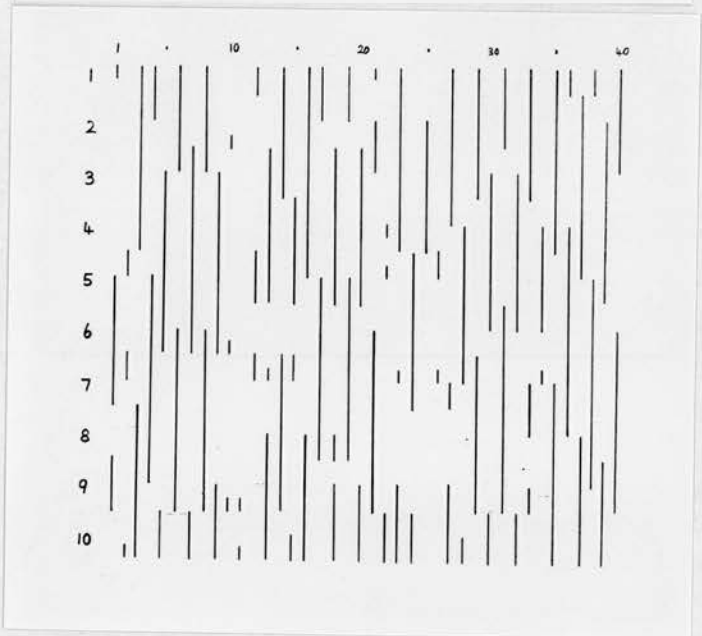


Figure 5-5A

Charts constructed for animal 1, showing the presence or absence of a tooth at each locus (numbered across the top) over the 10 week impression period. Upper chart - right side, lower chart - left side. The midline is to the left on both charts.



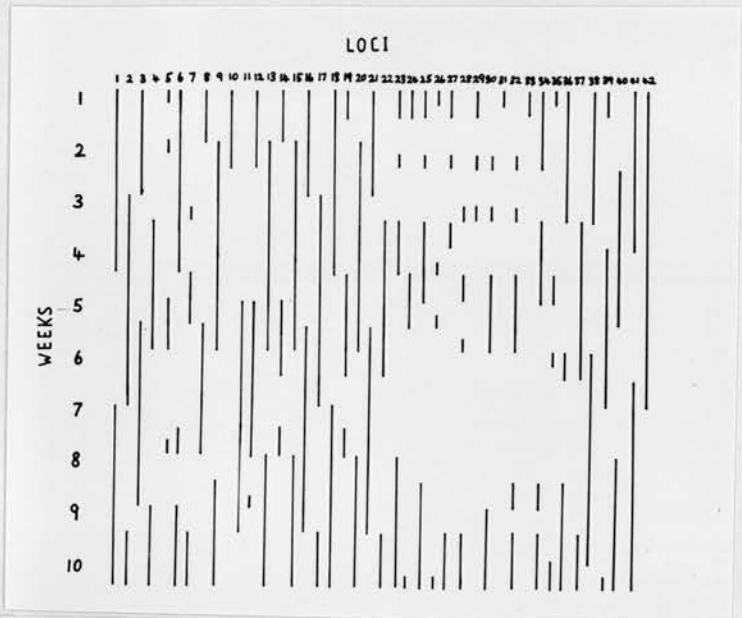
right



left

Figure 5-5B

Charts constructed for animal 2.



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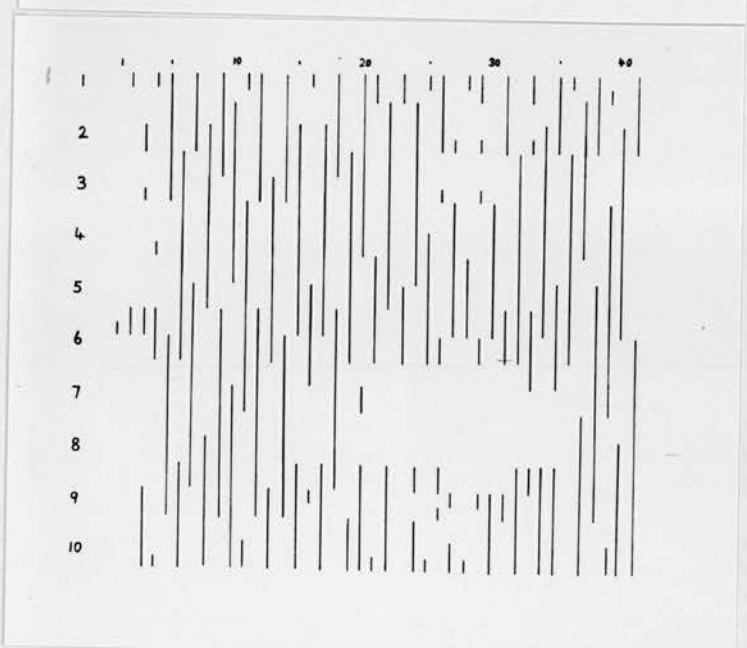


left

Figure 5-5C

Charts constructed for animal 3.

Figure 5-6A, B and C

(on the following three pages)

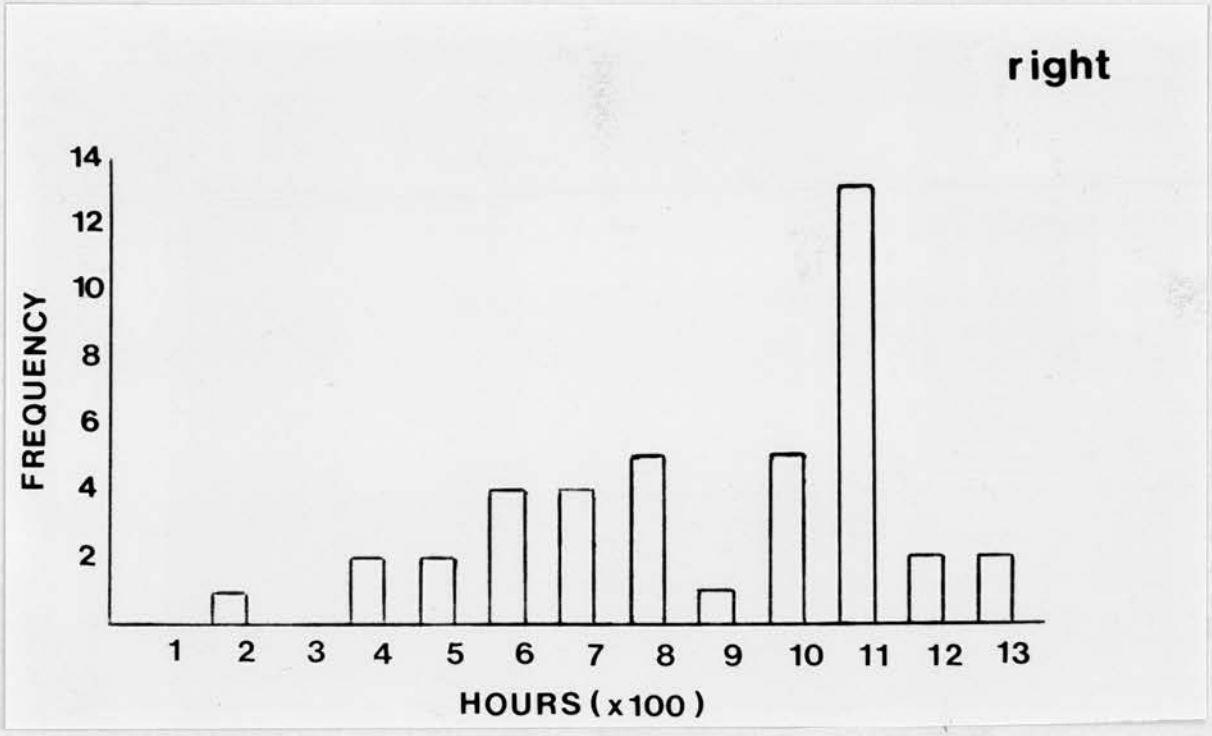
Histograms showing the frequency of occurrence of replacement cycle times (in hours) for each of the three animals. Each half of each animal's upper jaw was analysed separately, and the duration of the first replacement cycle at all loci in each half-jaw measured from the charts.

The histograms for each animal are presented on a separate page.

Figure 5-6A - Animal 1
Figure 5-6B - Animal 2
Figure 5-6C - Animal 3

On each page the upper histogram is an analysis of the right half of the jaw, the lower histogram of the left half. (This method of presentation is also applicable to Figures 5-7 and 5-8).

Figure 5-6A — Animal 1, replacement cycle



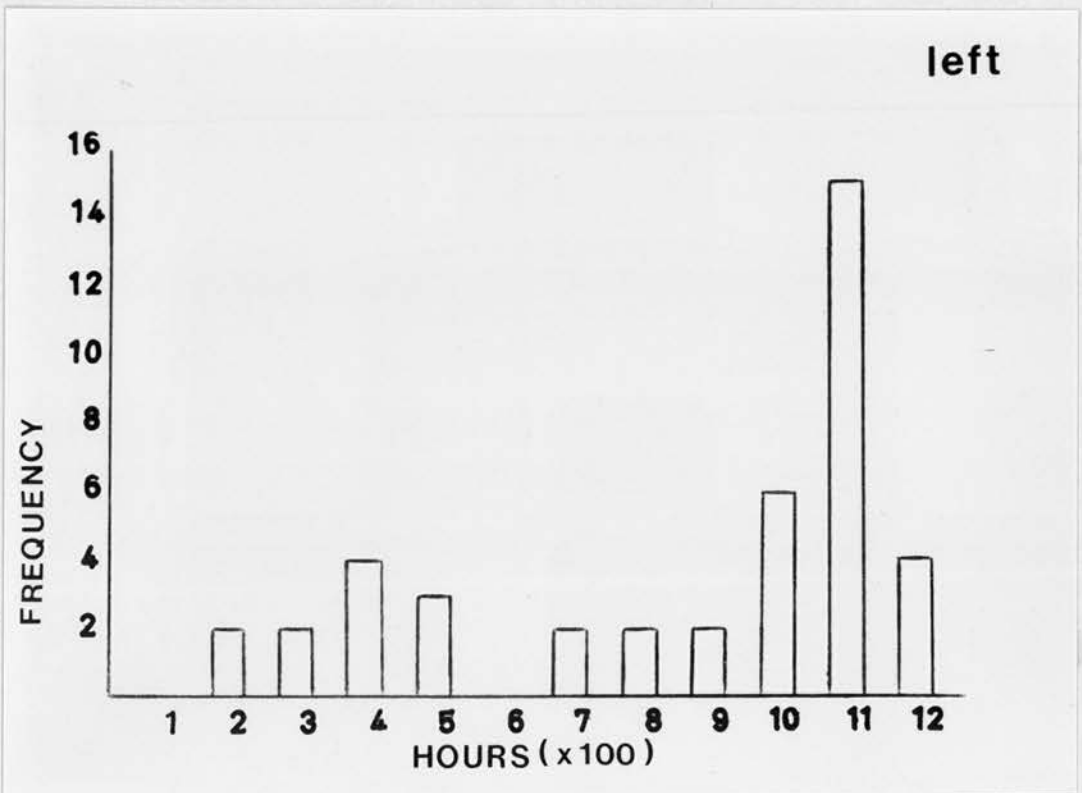
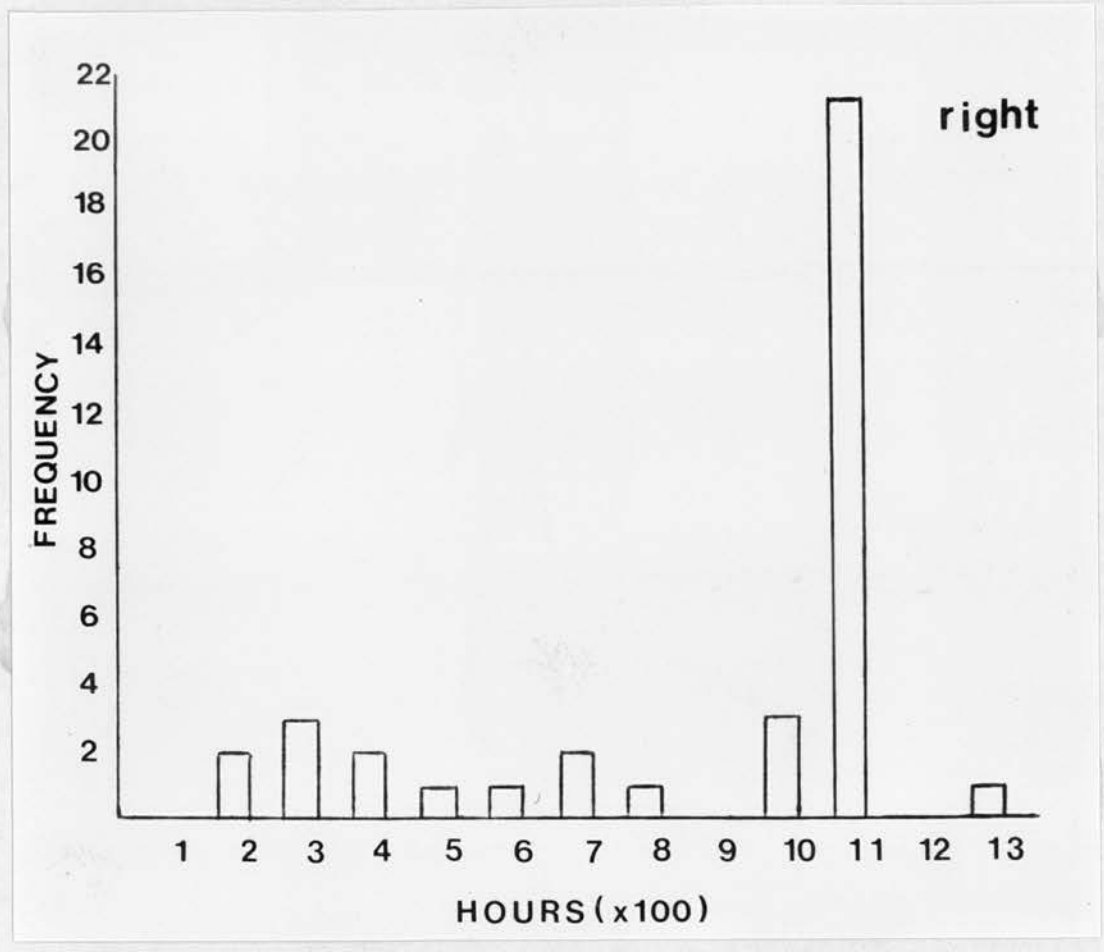


Figure 5-6B — Animal 2, replacement cycle



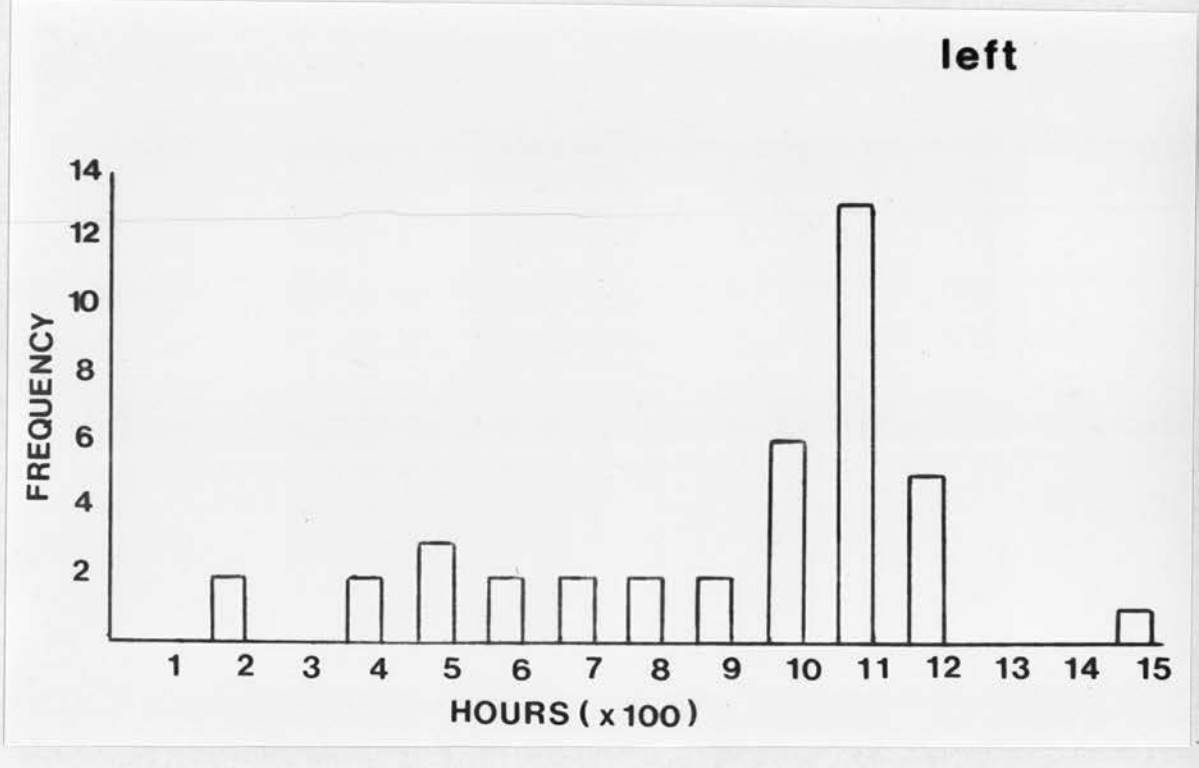
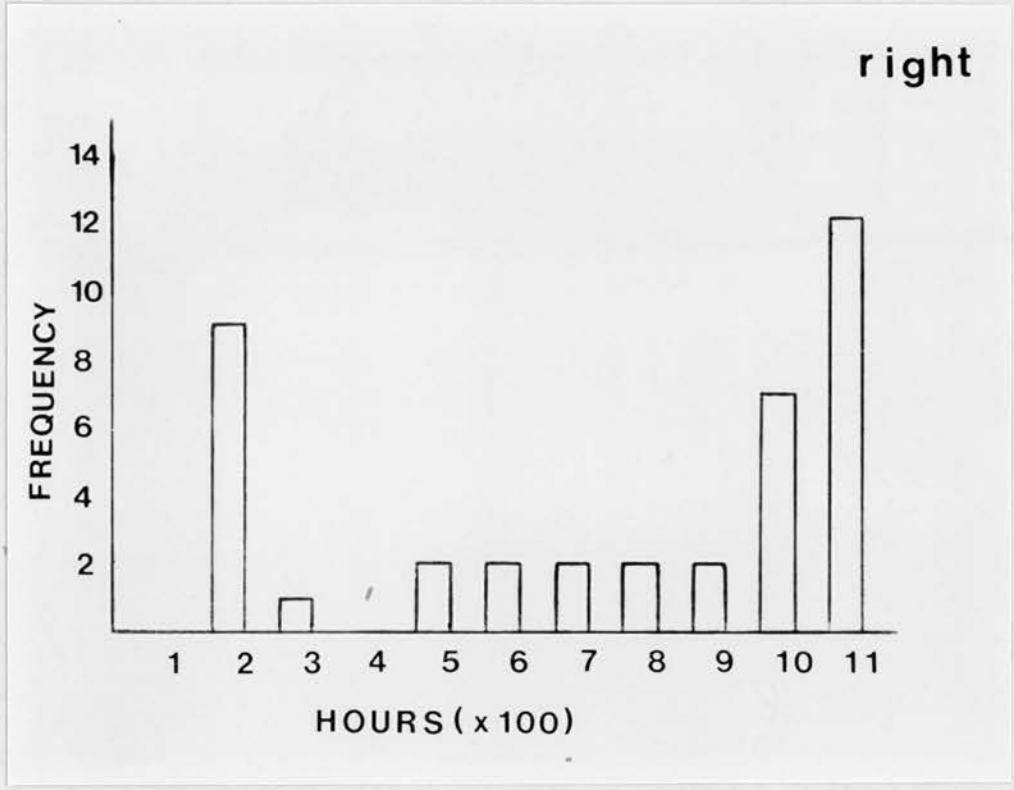


Figure 5-6C – Animal 3, replacement cycle



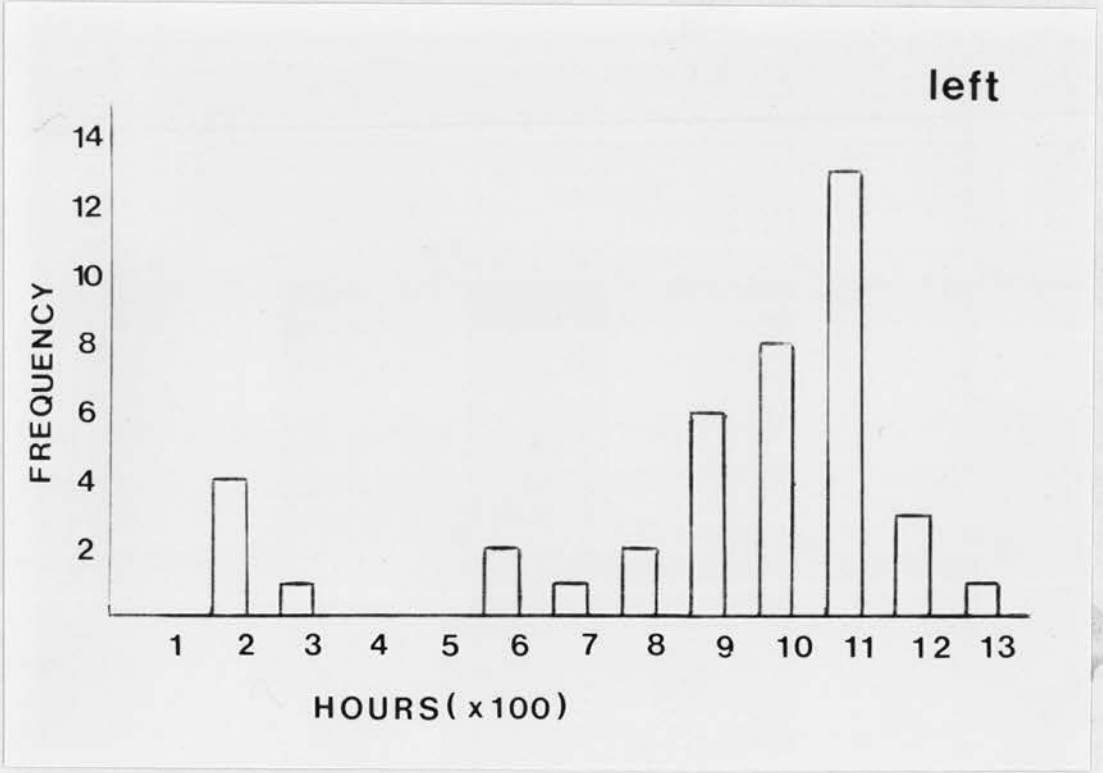


Figure 5-7A, B and C

(on following three pages)

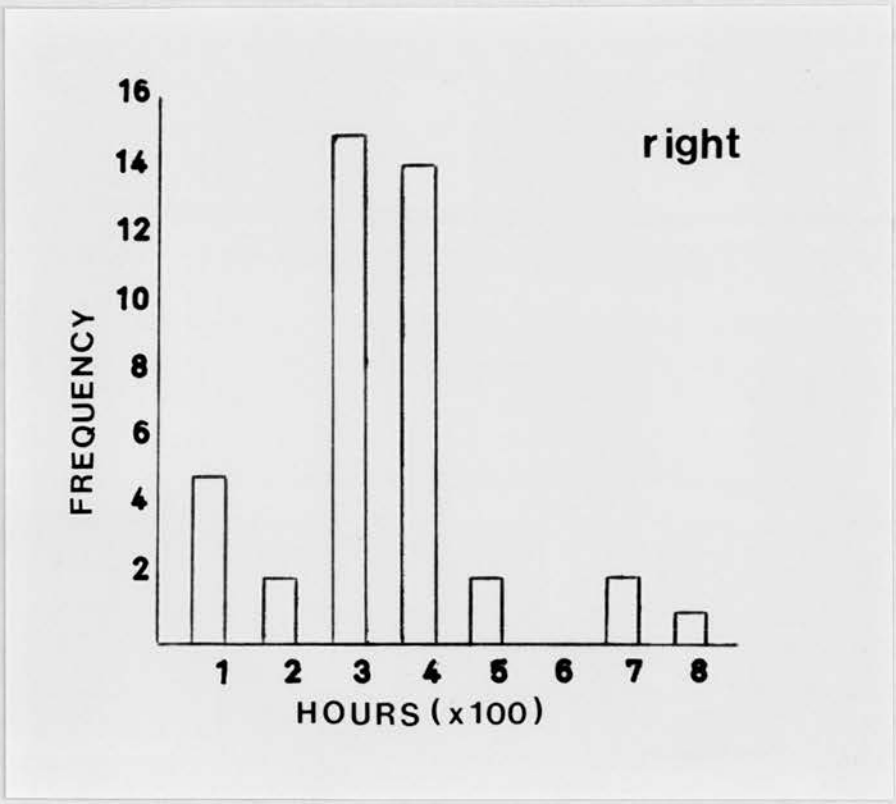
Histograms showing the frequency of occurrence of gap lengths (in hours) for each of the three animals. The method of analysis was the same as for the replacement cycle (Figure 5-6), but here the duration of the first gap length at all loci in each half-jaw was measured.

Figure 5-7A — Animal 1

Figure 5-7B — Animal 2

Figure 5-7C — Animal 3

Figure 5-7A — Animal 1, gap length



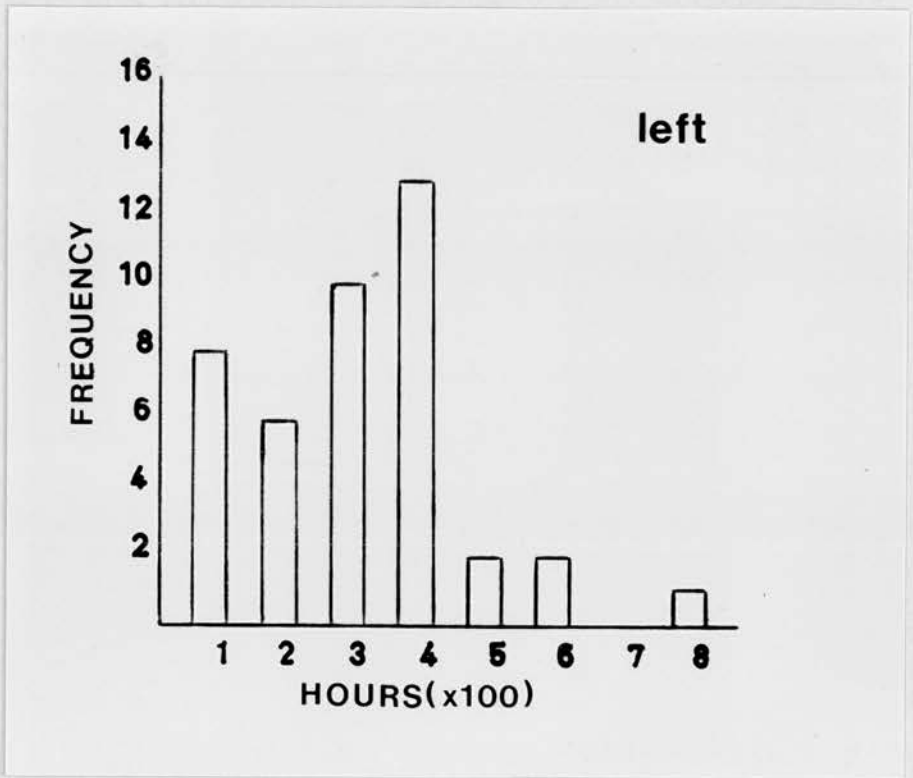
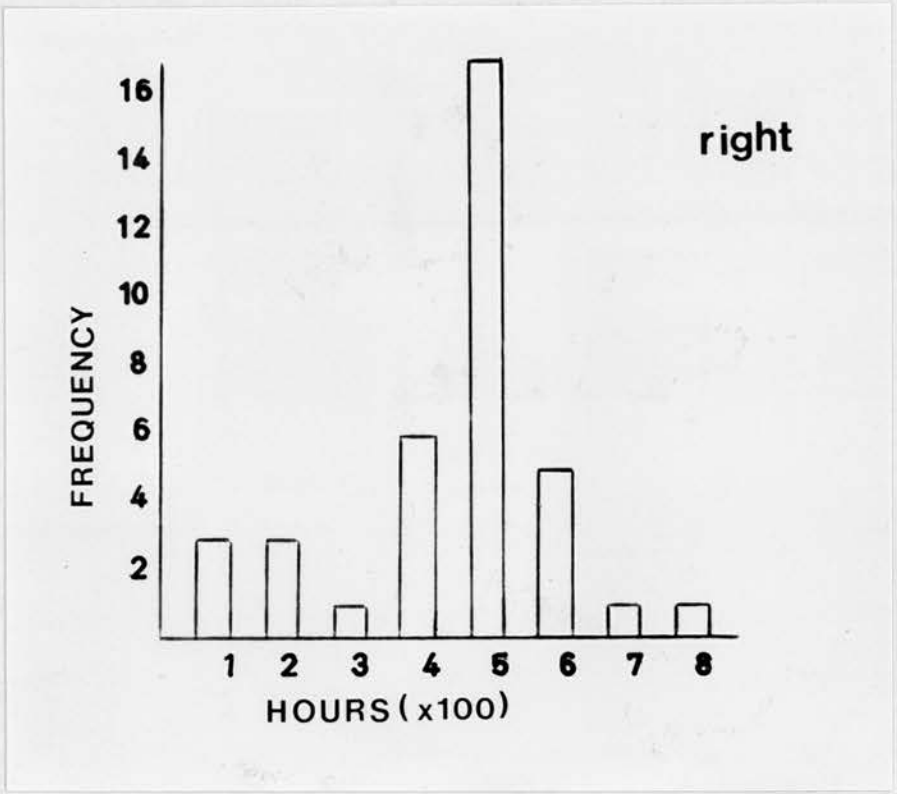


Figure 5-7B — Animal 2, gap length



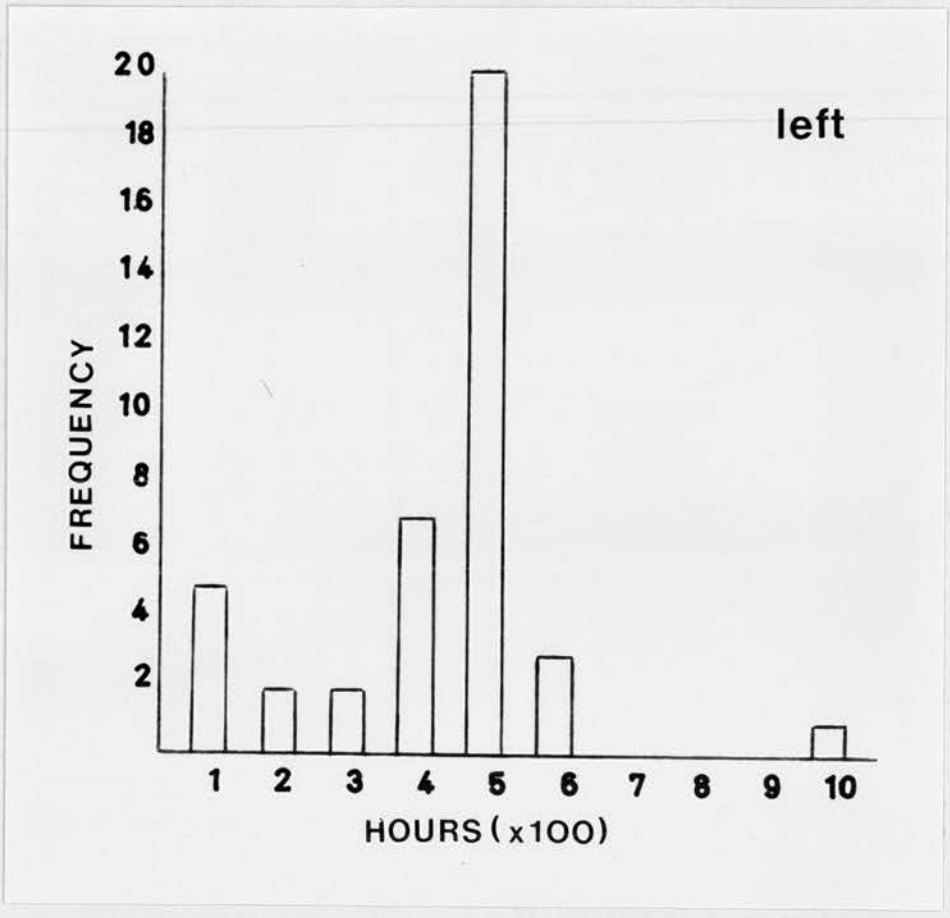
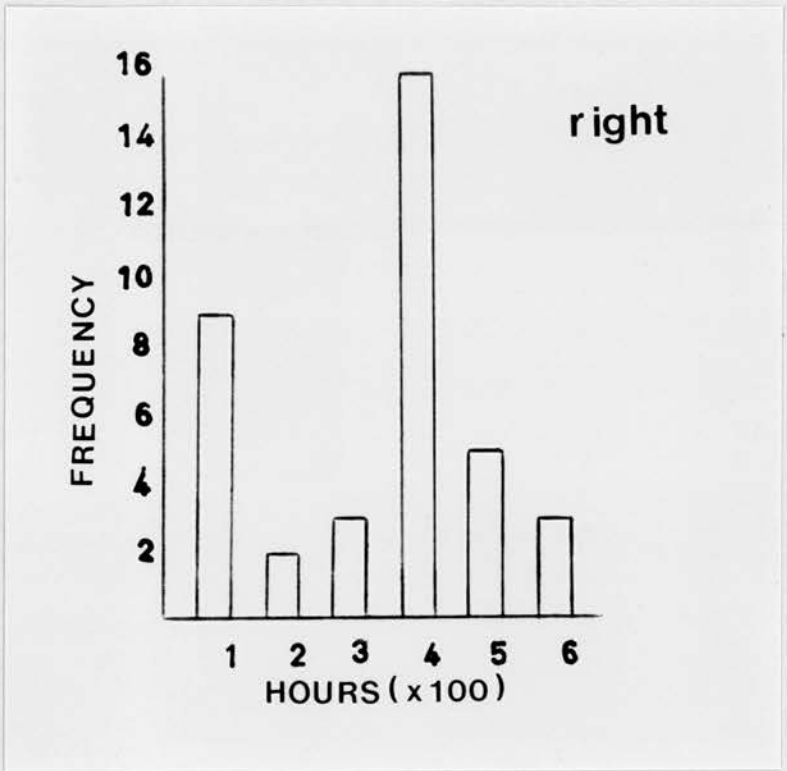


Figure 5-7C — Animal 3, gap length



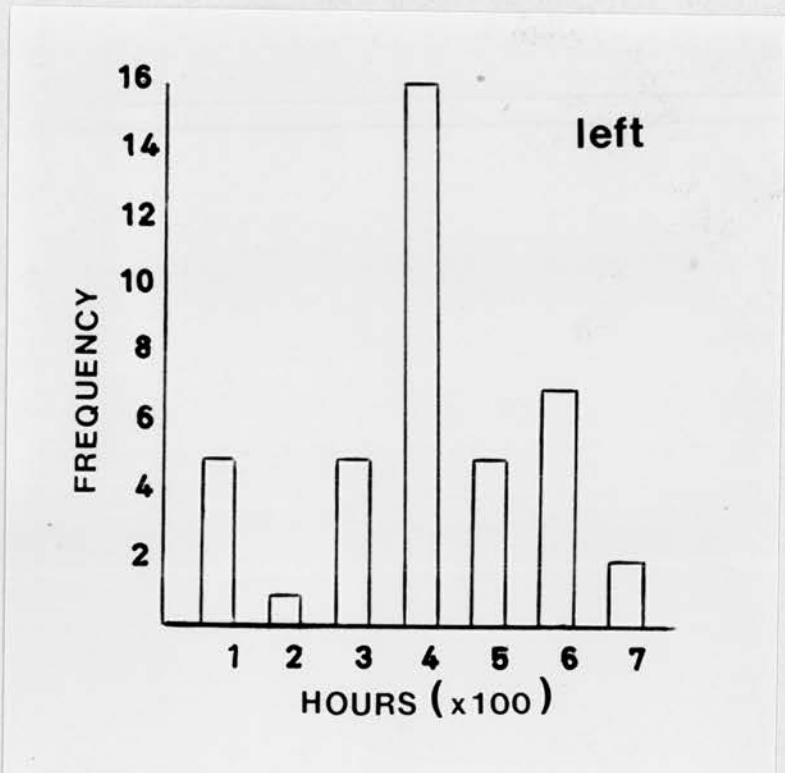


Figure 5-8A, B and C

(on following three pages)

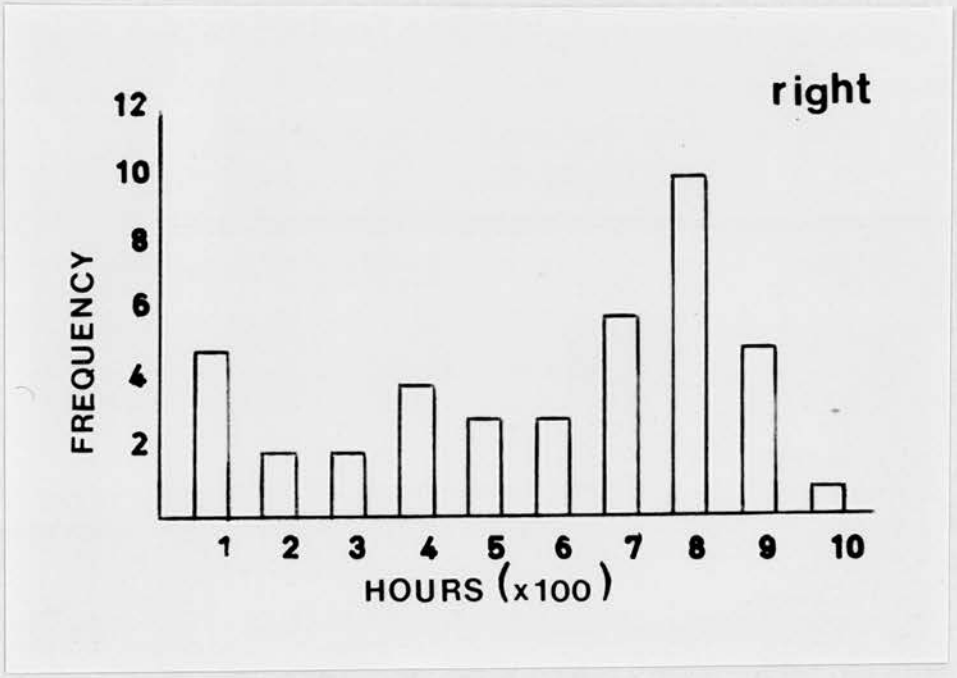
Histograms showing the frequency of occurrence of functional life times (in hours) of the teeth in each of the three animals. At each locus in each half-jaw the functional life was obtained by subtraction of the first gap length from the first replacement cycle.

Figure 5-8A — Animal 1

Figure 5-8B — Animal 2

Figure 5-8C — Animal 3

Figure 5-8A — Animal 1, functional life



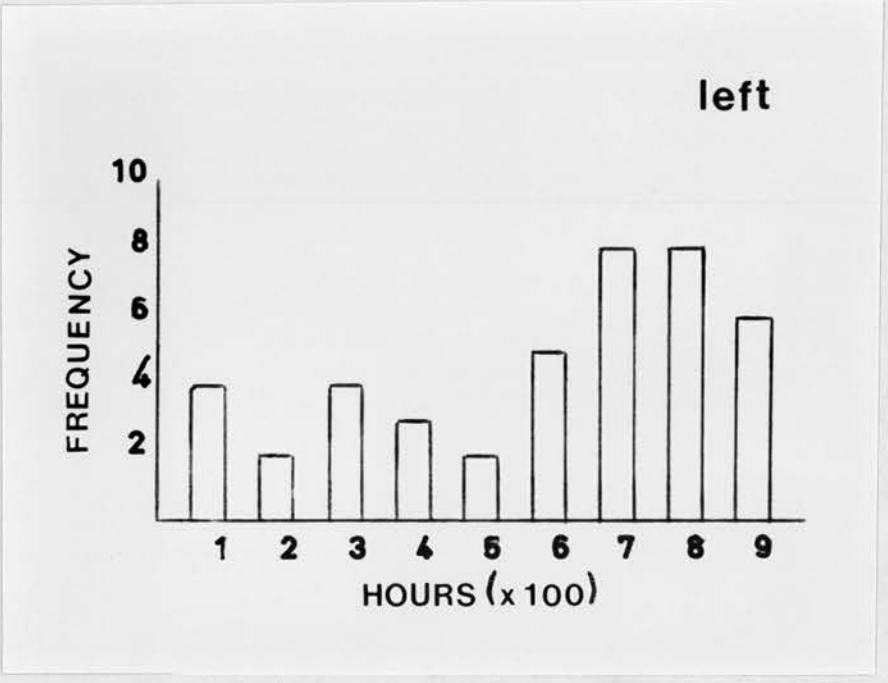
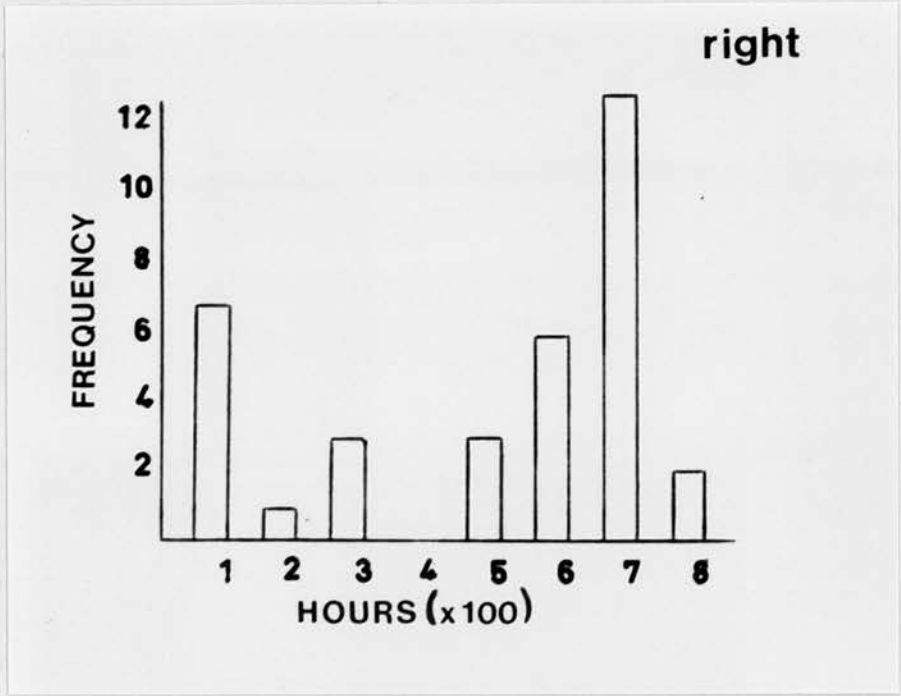


Figure 5-8B — Animal 2, functional life



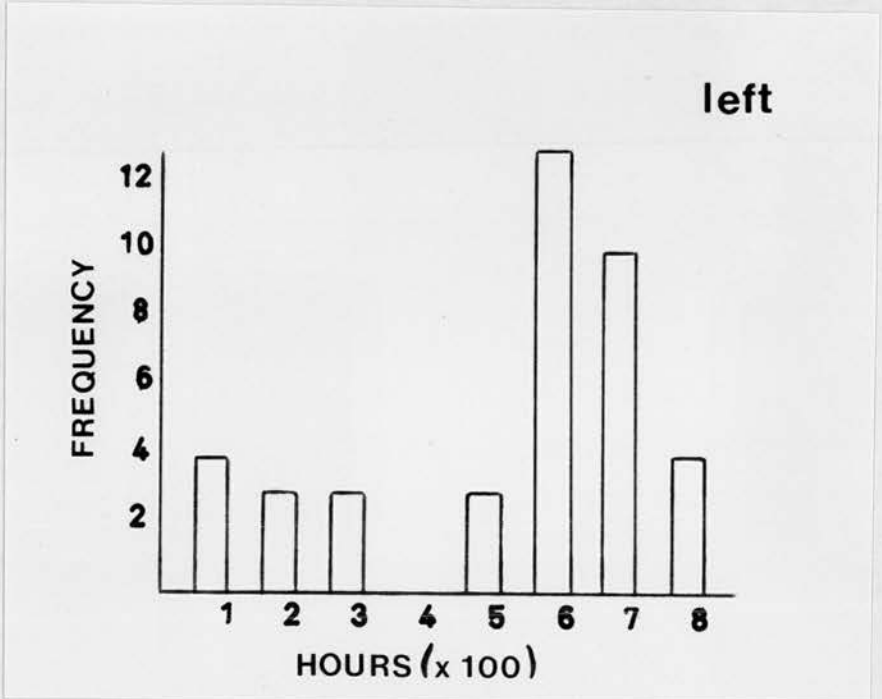
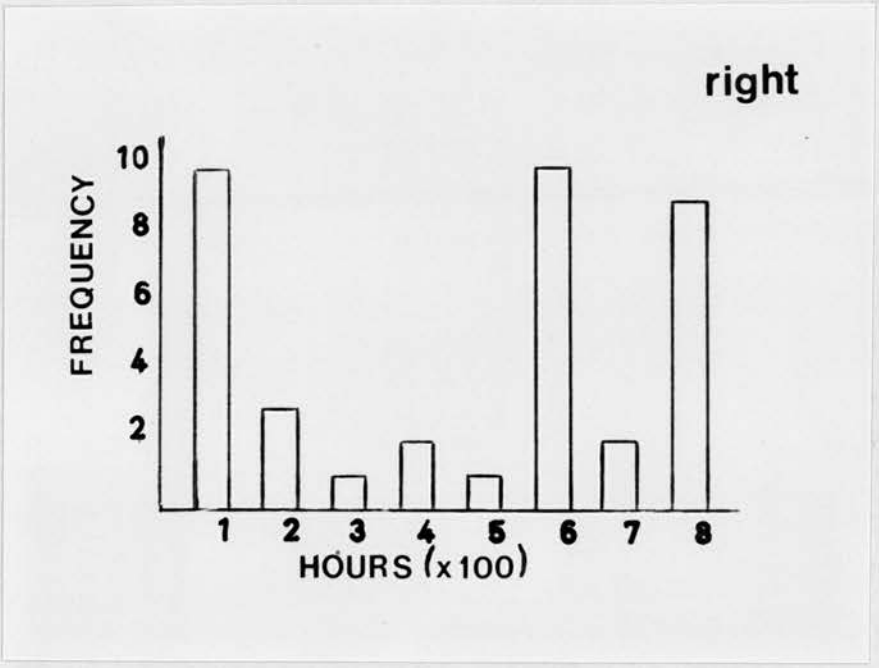
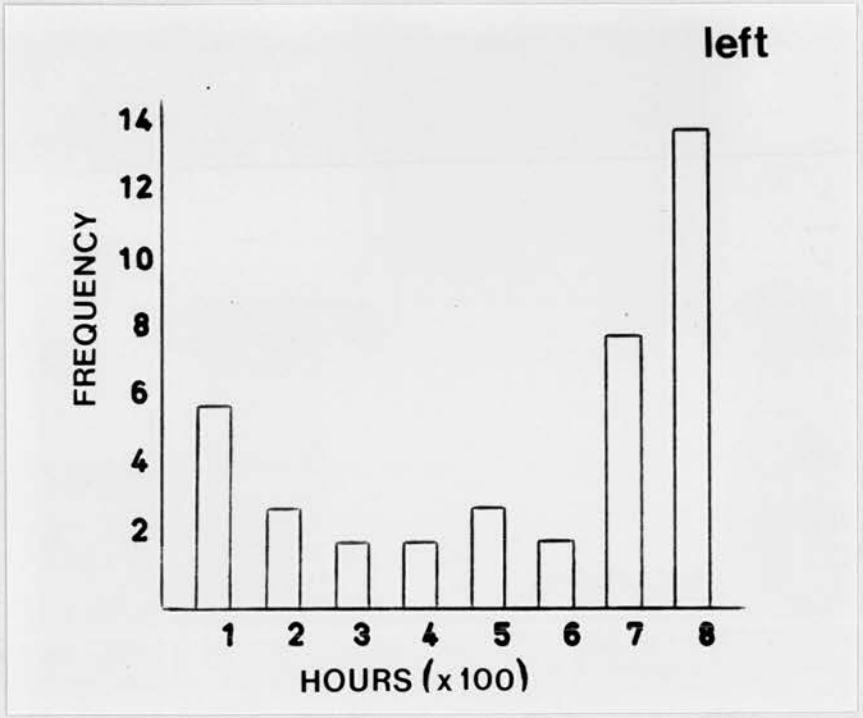


Figure 5-8C — Animal 3, functional life





SECTION VI - DISCUSSION

The jaws and dentition

The larva of Xenopus reaches its maximum length at stage 58 (Nieuwkoop and Faber, 1956), and correspondingly it can be seen from the results of Section IIIA that the chondrocranium increased in length from stage 47 to its maximum at stage 58, though the rate of increase was not constant. Subsequent to stage 58, during prometamorphosis and metamorphic climax, the chondrocranium decreased in length and ossifications occurred around it.

The width of thelower jaw also increased until stage 58, but thereafter decreased, while the length of the lower jaw continued to increase until stage 66. The rate of increase in the length of the lower jaw showed variation, and while it was 45.34/um/day between stages 47 and 61, it accelerated to 157.5/um/day during metamorphic climax (stages 61-66).

This latter period coincides with the reorganisation of the larval alimentary canal in preparation for the change from a vegetarian to a carnivorous diet, and during this time the larva does not feed (Nieuwkoop and Faber, 1956; Brown, 1970).

However, the greatest increase in lower jaw length and decrease in width occurred over one day between stages 61 and 62, when the length increased dramatically by 700.37/um, and the width decreased by 1420.94/um. As a result the lower jaw width/length ratio fell from 2.627 at stage 61 to 1.476 at stage 62, and continued to fall until stage 65, by which time the ratio had dropped to 1.259, and the proportions of the lower jaw were very different from those seen at the beginning of prometamorphosis.

As a result of the increase in length, the articulation of the lower jaw, which was previously at the front of the chondrocranium on the anterior ends of the palatopterygoquadrate bars, came to be further back at a level near the auditory capsules. This translation in position of the articulation with the quadrate increased the gape of the jaws considerably, and was accompanied by a caudal migration of the corner of the mouth, which before metamorphosis lay below and in front of the eyes, but which after metamorphosis came to lie behind the eyes.

De Beer (1937), Nieuwkoop and Faber (1956) and Paterson (1939) attribute the more caudal position of the lower jaw articulation and the increased length of the mouth slit, to a backward migration of the quadrate region of the palatopterygoquadrate bar. However, as emphasised by Pusey (1938) in a study of the jaws at metamorphosis in Rana temporaria, it is very difficult to assess relative movement of parts in such a dynamic situation because it is impossible to establish fixed points. In Xenopus while the lower jaw is lengthening the premaxillae, maxillae and septomaxillae are ossifying and there is development of additional cartilages and chambers in the olfactory region, which implies some forward growth in the anterior part of the chondrocranium. The more caudal position of the quadrate after metamorphosis is likely therefore, to involve both cranial and caudal growth components.

Nevertheless, whereas the mouth before metamorphosis was a wide but short slit, used only to allow the ingress of water and particles for the filter apparatus, after metamorphosis the mouth became proportionately

narrower and longer, with a greatly increased gape, to facilitate the now predacious habits of the animal.

In Section IIIB the proportional changes in the lower and upper jaws after metamorphosis were studied in females; a small group of males was also examined. In males the jaw parameters and proportions were similar to, and did not differ significantly from, the parameters of the medium sized females in the 50mm snout-vent range (group C). It should be remembered that males are sexually mature at 50-60mm snout-vent length and do not grow much above this size and it seems that for the parameters measured here there is no sexual dimorphism between adult males and females of comparable size.

In females however, to which all further comments apply, jaw parameters and skull length increased with increasing snout-vent length, and the increasing jaw size is related to the need for the capture of larger prey to meet the extra nutritional requirements of the larger body. There was remarkable uniformity in the proportions of the jaws between groups, although small and sometimes significant differences were found.

Up to about 50mm snout-vent length, the increase in the length of the lower jaw was greater than the increase in the length of the skull, whereas above this body size the length of the lower jaw and skull increased almost isometrically. This may reflect the need of young animals, which when small are vulnerable to a wider range of predators than adults, to increase the gape of the jaws for efficient and effective prey capture. Thus body growth can occur quickly until a size has been attained which makes these animals less attractive to smaller predators.

There was a significant increase in the lower jaw width/length ratio between the smallest and largest animals, so that in the largest females the lower jaw was proportionally wider. However, despite its significance the difference in the ratios was small, and it is suggested that it may not be due to a generalised growth phenomenon, but to functionally related growth at the posterior ends only. In particular there seemed to be a disproportionate increase in the size of the coronoid processes at the back of the jaw in large females, related to the larger attachment area required for the well developed adductor mandibulae muscles.

With reference to the upper jaw there was a greater increase in width than length in small animals below about 30mm snout-vent length, possibly again related to the need for efficient capture of prey. Above this body size the width/length ratio did not significantly change even in large females.

The length of the upper jaw had increased faster than the length of the skull in large females when compared with other groups. However, the upper jaw consists of tooth-bearing and tooth-free zones. The former, composed on each side of the premaxilla and part of the maxilla, supports the teeth, while the tooth-free zone at the posterior end of the maxilla gives attachment to a ligament running forwards from the pterygoid (more information on the ligament is given in Appendix 3). The zones increased in length with increase in body and skull length, with the tooth-bearing zone supporting more tooth loci in larger animals.

As described in Appendix 3 it is thought that the function of the ligaments attached to the tooth-free zones is to prevent the upper jaw splaying outwards when the mouth is closed. In large animals the

ligaments are thicker and are attached to a longer tooth-free zone than in small animals, and these two factors can be related to the increasing size and power of the adductor mandibulae muscles as the animal grows. More powerful muscles would permit more forceful elevation of the lower jaw against the upper, and the increase in size of the tooth-free zones and ligaments is probably a response to the greater forces the upper jaw is required to absorb as a result of this.

The number of tooth loci increased with increasing body length and skull length. However, whereas in smaller females body length increased faster than the addition of tooth loci, in large females the increase in the number of loci was greater than the increase in body length. Consequently the number of loci in larger, older females was greatly and disproportionately increased. It would appear that after the animal has reached its maximum body size tooth loci are still added to the dentition, and this indicates that the addition of loci is independent of general body growth. The evidence suggests that there is no cessation in the addition of loci or replacement of teeth in older animals.

The tooth-zone length/number of tooth loci ratio increased with increasing body size, and indicates that there are fewer loci per unit length of jaw in larger animals. Since there were no gaps in the dentition this can be attributed to an increase in width of the teeth (which was not measured in this study). As was to be expected the teeth of larger animals were longer than those of small animals, but within each jaw the posterior teeth were significantly shorter than the teeth at anterior loci.

Although the width of teeth was not measured, because of the practical difficulties of establishing points of reference, a few measurements were attempted and it was found that the posterior teeth, particularly those at the last six or seven loci in large females, were not as wide as more anteriorly placed teeth. It was also observed that teeth at the very back of the tooth zone were often eccentrically orientated, and the general impression gained was of overcrowding in this region of the maxilla, despite the presence of a long tooth-free zone behind the most posterior teeth. A similar situation was found in animals of all sizes (and ages) and it therefore does not appear to be an age-related phenomenon. It is tempting to suggest that the tooth zone and tooth-free zone are so distinct that, while the zones grow proportionally, tooth loci cannot be added on the tooth-free zone.

However that may be, the teeth of the adult were similar to those which began their development in the larva, the main difference being that of scale. This is unlike the situation in many urodeles where a definitive larval dentition is found in which the teeth are morphologically different from those of the adult (Kerr, 1960). It should also be pointed out that the teeth of Xenopus are not divided into proximal and distal parts separated by a zone of weakness, as described in many other amphibian species (Parsons and Williams, 1962; Casey and Lawson, 1981).

The functional life span of the teeth in relation to the feeding habits of the animal

While the complete developmental cycle of the first generation even-positioned teeth was about 33 days, each tooth spent about seven days in its functional position — a mere quarter of the time taken for its production. Thereafter each tooth was rapidly replaced by its successor. While the first generation teeth were being resorbed, the second generation even-positioned teeth were beginning their rapid growth phase, and were therefore about 16 days later in development. The members of the odd-positioned tooth series probably had a developmental cycle of similar duration, but were 8-9 days out of phase with the even-positioned series.

The teeth of very large adults spent about 24-29 days in their functional positions (vide impression study), i.e. about four times the functional life span of the first generation teeth. This does not seem a very great increase when it is considered that, judged by their body weights, the animals used for the impression study may have been more than 13 years old (Deuchar, 1975). Despite their age, tooth production was still occurring vigorously, and the teeth spent only a short time in their functional positions.

However the speed of tooth replacement may be linked to a fundamental embryological process involved in the development of the successional teeth, rather than to functional requirements.

Nevertheless it is possible that the possession of a dentition whose members are rapidly replaced may be of advantage to an animal, in that damaged teeth would be quickly replaced.

But in both young and adult animals only a few microns of each tooth actually protrudes through the epithelium, while the bulk of the tooth remains deep to it, and thus it is difficult to ascribe any feeding function to the teeth (see Figure 6-1).

When Xenopus is feeding, the prey is taken by a rapid forward thrust of the animal, with its mouth wide open. As the open jaws envelope the prey, the forelimbs are used to push it back into the mouth, which is then forcibly closed. It would seem that the arrangement of the soft tissue covered ridges in upper and lower jaws not only act as an effective seal, but are probably more effective in gripping the food than the teeth. In this respect bufonids, Rhinophrynus, some dendrobatids and microhylids, which do not possess teeth (Trueb, 1973), are able to capture and devour prey.

This study has not been able to ascribe any important function to the teeth for feeding, and yet throughout the life of the animal there is rapid production and resorption of the hard dental tissues. If the dentition is not of prime importance for the prehension of prey then some other function could account for its existence, and, based on the observed phenomena described in this study, the following speculation is advanced: the bony skeleton of Xenopus is not robustly constructed, and the bones consist, with very few exceptions, of thin trabeculae without Haversian systems, an arrangement which does not appear to provide a substantial store of calcium. Also, except in very old animals, large parts of the skull, limb girdles and epiphyses of the long bones consist of cartilage, much of it calcified. Since cartilage is avascular it would seem that calcium deposited there could not be easily recovered for metabolic purposes.

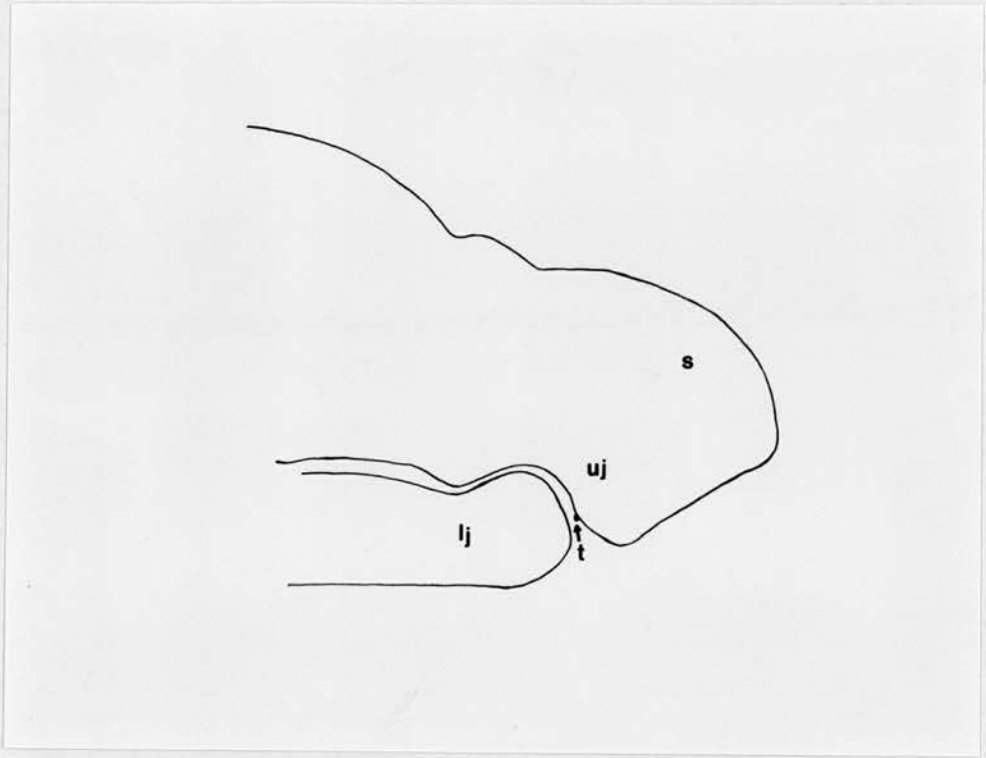


Figure 6-1

A drawing of the head of Xenopus in sagittal section to show the small size of the erupted part of a tooth (t) in proportion to the size of the jaws and mouth. Note that the position of the tooth is such that it is barely in the mouth cavity at all. With the jaws closed the margin of the lower jaw is seen to be of such a shape and size that it fits into a corresponding groove in the oral surface of the upper jaw. This arrangement may be effective in holding prey.

lj, lower jaw; uj, upper jaw; s, snout

While the repeated production at short intervals of large numbers of teeth must represent a considerable drain on the animal's calcium reserves, the calcium used is recycled, since the teeth are almost completely resorbed on replacement. The calcium is therefore released for general body requirements, or for re-use in further tooth production. It is speculated, therefore, that the teeth act as a calcium store which is readily and rapidly accessible. The degree of calcification of replacement teeth may vary inversely with the general calcium requirements of the animal.

Whatever the truth of that may be, the larger teeth of older animals spent a longer time in their functional positions than the smaller teeth of newly metamorphosed animals. Also, measurements in young adults indicate that the posterior teeth are significantly smaller than the anterior teeth, which suggests that the former have a shorter functional life span.

Cooper, Poole and Lawson (1970) have found differences between anterior and posterior teeth in Agamid lizards, where the row of teeth present at hatching is extended by addition of teeth posteriorly, the size of the additional teeth increasing as the jaw grows. In Uromastyx (where there is no replacement of teeth), Cooper and Poole (1973) have found that the distinction between teeth present at hatching and those added later posteriorly, remains clear even in older specimens, there being a particularly abrupt increase in size of the posterior teeth.

In comparing antero-posterior tooth length in Xenopus, teeth at loci 1-20 were classified as anterior teeth since these loci correspond approximately with those present before metamorphosis. The smaller posterior teeth had therefore developed at loci formed after metamorphosis,

and may have had a different functional life span and development cycle time.

It may not be prudent, however, to attach great significance to the differences between anterior and posterior teeth, since Cooper (1966) states that in Anguis fragilis the functional life of all the teeth appeared to be similar irrespective of their size or position. Thus in this study, in calculating the results, all teeth were assumed to have similar life spans, and if differences in functional life exist between teeth in Xenopus they could not be elucidated by the impression technique.

It was noted from the results of the impression study, that although the teeth in all three animals showed similar replacement cycle and functional life times, in animal number 2 the time interval between tooth loss and subsequent replacement (the gap length) was a larger proportion of the replacement cycle time than in the other two animals. Since the animals were of similar size with almost identical numbers of tooth loci, the significance of this difference is not clear, although Berkovitz and Moore (1974) using larger numbers of rainbow trout have related variations in the ratio of functional to non-functional time to the number of functioning tooth positions.

Since Xenopus is poikilothermic the functional life span of the teeth, and indeed the whole tooth development cycle, is probably temperature dependent, and in the wild seasonal variations are likely to occur as reported by Miller and Rowe (1973) for Necturus maculosus.

It is also possible that tooth development and replacement may slow down or cease in very old Xenopus, although no evidence for this was found in the present study. Nevertheless the possibility of this

happening in extreme old age cannot be entirely discounted since it seems that such a phenomenon may occur in Nile crocodiles (Poole, 1961) and monitor lizards (Bellairs and Miles, 1961).

The developmental cycle of the first generation teeth

As indicated in the Introduction, previous work on the development of teeth in amphibians has supplied information only on the relative durations of the events in the developmental cycle, whereas in the present study an absolute time scale was applied to these events. The study confirms the deductions of previous workers that the early stages of tooth development occur slowly, but that eruption, ankylosis and resorption occur rapidly (see Figure 4-9).

In Xenopus the complete developmental cycle of the first generation even-numbered teeth lasted about 33 days, from the initiation of the tooth germs until complete resorption. Tooth development began during prometamorphosis and continued until the end of metamorphic climax, therefore encompassing a similar period in the animal's life cycle as hind limb development. The development of the teeth during this period is presumably associated with the change in feeding habits of the animal.

During tooth development the formation of the dentine preceeded the formation of the enamel by about one day. Amelogenesis continued for seven days, and during the early stages an acid insoluble enamel matrix was formed. An enamel matrix stage has been identified in the frog Rana pipiens by Gillette (1955) and Zaki and Weber (1979), in urodeles by Kerr (1960) and Meredith Smith and Miles (1971), and in caecilians by Casey and Lawson (1981). In this respect it would appear that early enamel formation in amphibians is similar to mammals.

In Xenopus dentinogenesis lasted 23 days, but over this period

formation of the dentine did not occur at a constant rate. The process was distinctly biphasic, a long phase of slow production being followed by a shorter phase of rapid production, and it is difficult to understand the cause of, or need for, this change in rate.

However, dentine production depends on the activity of odontoblasts, and the numbers of these cells increased dramatically at the beginning of the rapid phase. It is possible to view the variations in the rate of odontoblast differentiation and activity as part of a monophasic control process, if it is speculated that the odontoblasts control their differentiation and activity autonomously by means of a "humoral factor"which they themselves produce. Such "humoral" mechanisms of control of cell differentiation have been discussed by Konigsberg (1971), de la Haba and Amundsen (1972), and Lash and Whittaker (1974).

With respect to dentinogenesis it is speculated that the concentration of the "humoral factor" at the end of the slow phase would be such as to cause large numbers of mesodermal cells to differentiate into odontoblasts. The "humoral factor" produced by these extra odontoblasts would cause the cells to become highly active in dentine production, giving rise to a rapid phase of dentinogenesis.

However that may be, during the rapid phase the teeth erupted, and, while it was not within the scope of this study to investigate the causes of eruption, it seemed that the phenomenon was caused primarily by growth of the tooth germs. Dentinogenesis ceased before ankylosis was completed and the teeth were carried to their final positions by the formation of the bony pedicel which, unlike many other salientians, is not a well developed structure in Xenopus.

Following the completion of dentinogenesis and ankylosis, odontoblasts were no longer recognisable in the pulps of the teeth, which thereafter appeared comparatively acellular and avascular. There was therefore a sudden disappearance of the odontoblasts after eruption. This rapid change is intriguing, and further investigations will be undertaken to elucidate the fate of the odontoblasts.

Nevertheless, in the absence of odontoblasts, it would seem that repair of dentine, or the formation of secondary dentine as found in mammals, would not be possible in ankylosed teeth, unless odontoblasts can be differentiated from pulpal cells after eruption, but no evidence for this was found.

Tooth resorption

Resorption of an ankylosed tooth involved osteoclasts, and was associated with the growth and eruption of its successional tooth. Yaeger and Kraucunas (1969), in an ultrastructural study of osteoclasts involved in tooth resorption in Rana pipiens, stated that these cells in amphibians were essentially similar to resorptive cells in mammals.

The longitudinal studies undertaken in Sections IVA and IVB showed that osteoclastic resorption of a tooth began soon after ankylosis, and continued until the tooth tissue had been removed to allow its successor to erupt. Resorption may be dependent upon the presence of a successional tooth, but this can only be speculation since no specimen was found in which successional teeth were missing.

The process did not occur at a constant rate, and the majority of tooth tissue was not resorbed until the successional tooth began to erupt. The study in Section IVA showed that during the eruption of a successional tooth, the destruction of its predecessor occurred with great speed. It was hoped to apply a more precise time scale to this rapid tissue destruction in Section IVB, but, while the study confirmed the rapidity of the process, the method used was not sufficiently refined to enable a more accurate assessment than before.

Nevertheless it is clear that the majority of the tooth tissue was destroyed in about 24-48 hours in a first generation tooth, a period representing only about 6% of the total developmental cycle.

Observations on the material in Sections IVA and IVB suggest that

not only does the speed of resorption increase when tooth replacement is about to occur, but also that the character of the process changes at this time. It seemed that resorption occurred in two phases, described here as erosion and absorption. Erosion was a slow, intermittent process lasting about seven or eight days. It involved resorption of the bony pedicel and dentine from their external aspects only, and could extend over the entire period that a first generation tooth spent in its functional position. The evidence suggests that erosion may be intermittent, since concavities, apparently unoccupied by osteoclasts, were seen on the external aspects of dentine and bone. It would appear that repair of these tissues does not occur, unlike the situation in deciduous teeth in many mammalian species.

Although as much as a quarter of the lingual side of a tooth could be destroyed by erosion, the pulp remained comparatively avascular and acellular. This was a vastly different appearance to the pulps of teeth undergoing absorption (see below).

Erosion was assessed to be a slow process which gradually created space to accommodate the growing successional tooth, which was in its slow growth phase. The process might depend upon (a) the proximity of the successional tooth, (b) the direction of growth of this tooth, and (c) pressure on the standing tooth caused by (a) and (b). No specimens were seen in which successional teeth were missing, so their role in erosion could not be assessed.

Absorption, however, was a rapid, continuous process lasting up to 48 hours, which involved gross histological changes in the pulp.

During absorption the pulp of the tooth was infiltrated by

osteoclasts which appeared to be destroying the dentine piecemeal from within, along its full length. Osteoclasts apart, the pulp became more cellular, and there was an impression of increased vascularity although there was no significant increase in blood vessel diameter.

This last observation was intriguing, and so the sections of the head of adult Xenopus used in Section III were re-examined. Here again it was found that there seemed to be an increased number of red blood corpuscles in the pulps of teeth undergoing absorption, yet there was no significant increase in blood vessel diameter. Indeed, in many cases where absorption was nearing completion, it was impossible to identify blood vessels, and isolated clumps of red blood corpuscles were seen, apparently lying free, in the pulp. An electron-microscopic study of the blood vessels in such teeth might clarify this situation. Nevertheless, these observations suggest that absorption might not be associated with increased vascularity, but with a reduction of blood flow prior to removal of the vessels.

However that may be, absorption appeared to be a period of intense osteoclastic activity during which the majority of the tooth constituents were rapidly destroyed. It was always associated with the rapid growth phase and eruption of the successional teeth.

It seemed that absorption involved the entire length and width of the dentine and that very little, if any, of each tooth was shed. Toward the end of absorption tooth tips were found deep to the epithelium embedded in the connective tissues of the jaw. In some cases, collections of osteoclasts surrounding spicules of dentine were found in a similar situation, which suggests that complete destruction of teeth may occur. However, since decalcified sections were used,

the fate of the enamel remains uncertain.

It is generally assumed that loss of teeth in amphibians is brought about by resorption of their lingual aspects to the extent that they are loosened and shed into the mouth (Gillette, 1955; Lawson, Wake and Beck, 1971). However, Gillette (1955), working on Rana pipiens, reported occasionally finding teeth which had been resorbed right down to their enamel tips. Gillette's study was not a longitudinal one, and so he could not assess if this was a normal occurrence for every tooth.

In Xenopus it appears that extensive absorption is the norm, and interestingly, complete internal resorption of dentine in the labyrinthodont teeth of the Lower Carboniferous rhipidistian fish, Rhizodus hibberti, has been reported by Cruickshank (1968).

When such complete absorption occurs it follows that the majority of tooth constituents could be made available for recycling, and this would be of clear advantage to an animal with a high rate of tooth turnover.

In the preceding discussion, histological observations have been interpreted as indicating that tooth resorption occurred as two processes, erosion and absorption. It has been postulated that erosion may be caused by a mechanical stimulus, namely the slowly growing successional tooth germ, and this suggestion is strengthened by the observations made on the animal with a crowded dentition. In this case there was insufficient space for the odd-positioned teeth to erupt in the normal way between the even-positioned teeth.

Consequently there was extensive destruction of adjacent teeth, and the amount of tissue destroyed was similar to that previously seen only during absorption. However, none of the pulpal changes associated with absorption was seen in the pulps of the teeth undergoing destruction. On the contrary the pulps retained their integrity, and remained relatively avascular and acellular, while the dentine seemed to have been destroyed from the outside only. This suggests that erosion was the process involved here, caused by mechanical factors involved in creating space for the crowded teeth. Furthermore it suggests that extensive tooth destruction only involves internal resorption of dentine (i.e. absorption) when a successional tooth is erupting.

Some evidence was found to suggest that absorption may not be caused by the same mechanical factors as erosion. Two specimens were examined in which the tooth at the sixth locus was separated from its successional tooth by a thick band of fibrous tissue. Here any pressure effects on the standing tooth by the growth of its successor would appear to be limited, yet the pulp of the standing tooth displayed the histological features associated with absorption.

In view of these observations on animals with abnormal dentitions, it is suggested that absorption may depend on a specific, intrinsic timing mechanism.

The duration of the developmental cycle in the teeth of adults

It was not possible in the impression study to measure directly the time scale of the complete developmental cycle of the adult teeth, from tooth germ initiation to complete tooth resorption, since the study only measured the functional life of the teeth. However by extrapolating from the results obtained in Section IV for first generation teeth, a tentative time scale can be obtained.

Although the first generation teeth were ankylosed in their functional positions for about eight days, the tips of the teeth erupted through the mucosa three days before the completion of ankylosis. Consequently the teeth were erupted through the mucosa for 11 days and this is equivalent to the functional life of the adult impression study. Therefore by substituting the functional life times obtained from the impression study for the 11 day value obtained for the first generation teeth, approximate values for the complete developmental cycle in the adults can be obtained (see Table 6-1). These values are obtained on the assumption that the proportions of the developmental cycle do not change with age, and are only accurate to $\pm$ 200 hours.

It can be seen that the developmental cycle in the adult is of about 60 - 70 days duration, and this agrees well with Gillette (1955), who showed by an indirect method (since his study was not longitudinal) that the developmental cycle in adult Rana pipiens was about 90 days.

In Section IIIC the number and height of the odontoblasts in young adult teeth were obtained, and these measurements, alongside similar measurements made on first generation teeth are presented in Table 4-1.

TABLE 6-1 THE DURATION OF THE COMPLETE TOOTH DEVELOPMENTAL CYCLE IN THE ADULT

		Duration of the complete developmental cycle	
		hours	days
Animal 1	R	1613.03	67.21
	L	1711.07	71.29
Animal 2	R	1442.19	60.09
	L	1417.75	59.07
Animal 3	R	1417.75	59.07
	L	1564.41	65.18

In comparing young adult teeth with first generation teeth it can be seen that the dentine is about 4.5 times thicker and 4 times longer, but that this dentine was produced by 10 times the number of odontoblasts, which were also of greater height, and perhaps therefore more active, than those of first generation teeth.

In view of the disproportionate increase in number and height of the odontoblasts, it could be concluded that the developmental cycle of teeth in young adults would be of similar duration to that in the teeth of newly metamorphosed animals. However, the results of the impression study indicate that this is not the case, and that the developmental cycle increases in length in the larger teeth of older animals.

The pattern of tooth replacement

This study was primarily concerned with the time scales of tooth development and replacement; however there are some observations which have relevance to the pattern of tooth replacement.

In the larvae and young animals studied the dentition was organised into two series of teeth, a series at even-numbered loci and a series at odd-numbered loci. The teeth in each series appeared to be developing synchronously, but the odd-positioned teeth were 8-9 days out of phase with the even-positioned teeth. It would seem, therefore, that the dentition was organised *de novo* in such a way that a regular alternating pattern of tooth replacement would occur as successive generations of teeth from each series erupted.

Alternating patterns of tooth replacement in lower vertebrates are described in the literature (Owen, 1840; Rose, 1893; Loomis, 1900; Harrison, 1901; Bolk, 1912; Woerdeman, 1921), and various theories have been proposed to account for this and other replacement patterns.

Hertwig (1874) considered tooth replacement as a functional adaptation, suggesting that resorption of standing teeth and the growth of their successors were a response to increased use of the dentition. However, Gillette (1955) recognised that the replacement of teeth in *Rana pipiens* occurred in a regular manner, and that this implied a mechanism to correlate the development of the individual teeth in the dentition.

Gillette suggested that the presence of a tooth germ inhibited the development of other tooth germs near to it, and that the inhibiting

effects of two tooth germs would be least at a point midway between them. A further tooth germ could therefore develop at this point and would be later in its development than the two existing germs. Thus, along the jaw, germs at alternate loci would be at similar stages of development, and this would result in an alternating tooth replacement pattern.

In amphibians regular (Lawson et al, 1971) and irregular (Miller and Rowe, 1973) replacement patterns have been recorded, whereas in reptiles, alternating and frequently precise replacement patterns have been described (Edmund, 1962; Cooper, 1963, 1965, 1966; Miller and Radnor, 1970; Cooper et al, 1970).

An explanation given by many authors to account for this regularity is embodied in the Zahnreihen Theory proposed in 1960 by Edmund (although the term Zahnreihe, meaning tooth row, was first coined by Woerdeman, 1921). As originally proposed this theory was applied to the dentition of reptiles, but has since been extended to explain replacement patterns in fish and amphibians.

The Zahnreihen Theory attributes control of tooth formation to a stimulus which moves posteriorly along the dental lamina initiating the development of tooth germs at pre-determined loci. Thus tooth germs initiated by the stimulus at the front of the jaw would be more advanced in their development than those at the back, and the teeth would consequently erupt along the jaw as a wave running antero-posteriorly.

The term Zahnreihe is used to describe all the teeth produced by a single stimulus, and a number of stimuli will produce a number of

Zahnreihen, causing a series of replacement waves of teeth. In terms of the Zahnreihen Theory, an alternate replacement pattern is a "special case" in which the Zahnreihen are two tooth spaces apart.

Apart from its developmental significance, the theory is used in an attempt to explain how, in a situation of repeated loss and replacement of teeth, a constantly functioning dentition is maintained. Demar (1972 , 1973) has constructed mathematical models of the permissible spacings between Zahnreihen which would be compatible with the maintenance of a functional dentition at all times.

However, it is questionable whether many amphibians require a fully functional dentition since they are poikilothermic animals with a comparatively low metabolic rate and do not require a constant food intake. Indeed it is difficult to ascribe any feeding function at all to the dentition of Xenopus, since only a few microns of each tooth tip actually protrudes through the epithelium.

The Zahnreihen Theory proposes that tooth formation is controlled by a series of stimuli passing along the jaw, but no physical basis for such stimuli has ever been found. In larval Xenopus tooth germs developed in sequence from the back to the front of the jaw, a phenomenon also observed by Osborn (1971) in Lacerta vivipara. During metamorphic climax two or three additional tooth loci appeared in Xenopus, and their production occurred from front to back, posterior to the existing loci. Such a change in the direction of sequential tooth development as the animals grew seems incompatible with a concept of stimuli moving unidirectionally anteriorly or posteriorly. However, wave reversal has been observed in Caiman sclerops by Miller and Radnor (1970), and age-related wave reversal was observed by Edmund (1962) in

Alligator mississippiensis.

Goin and Hester (1961) attempted to identify the Zahnreihen in adult Xenopus and admitted that this was difficult. Like Goin and Hester many workers describing Zahnreihen utilise whole-mount, cleared, alizarin stained specimens, and assign teeth to their respective Zahnreihe according to their size and degree of development.

In alizarin preparations soft tissue changes are not visible. However, in the histological investigations undertaken here it has been shown that the early stages of resorption involved soft tissue changes. Thus in the examination of alizarin preparations the commencement of tooth resorption in a standing tooth might not be recognised, and such a tooth assigned to the wrong Zahnreihe.

In this respect Lawson (1966) describing Rana temporaria, and Goin and Hester (1961) describing Hyla cinerea, state that there were large numbers of erupted teeth along the jaws which did not fit with the wave patterns they were attempting to recognise. Such "retained" standing teeth are generally accounted for by suggesting that their presence is due to breaks in the replacement waves, or by fluctuations in the spacing between Zahnreihen. Lawson, Wake and Beck (1971) state that variations in the replacement pattern were common in the red-back salamander, and were produced by fluctuations in the spacing of the Zahnreihen around a mean of 2.0.

However, in mathematical terms, if a regular replacement wave pattern is to exist in a dentition, then the spacings between Zahnreihen must be precise. Variations in the spacings would result in a disintegration of the regularity of the dentition, although this

condition might be reversible.

One consequence of the Zahnreihen Theory is that the presence of any apparently alternating sequence of tooth replacement is produced as a secondary effect of successive Zahnreihen. However, in Xenopus the teeth developed ab initio as an alternating series. Osborn (1970) suggested that once a row of alternating teeth was developed in sequence from the back to the front of the jaw, autonomous control of tooth development at each tooth position would maintain a wave-like replacement of the teeth. He related the order of tooth development to the migration of neural crest tissue into the jaws, and to the developing competence of the oral epithelium to respond to this. (The importance of neural crest tissue in amphibian tooth development has been demonstrated by, among others, de Beer, 1947, and Chibon, 1967). Osborn realised that it was often possible to construct many wave patterns in a dentition, but that their significance might be doubtful.

As an alternative to the concept of Zahnreihen, Osborn (1971, 1973) suggested that control of tooth replacement is mediated by a local mechanism acting at each locus. The local control mechanism has received support from Lawson, Wake and Beck (1971) and Miller and Radnor (1973). Osborn's concept is that growth of a tooth germ would be repressed by the presence of adjacent teeth and only when the adjacent teeth had erupted away from the tooth germ could its growth proceed. The pattern of tooth succession would, therefore, be established at the first appearance of the teeth.

In this respect Berkovitz and Shellis (1978) noted that in Serrasalmus (piranha fish) the teeth in each quadrant developed and

were replaced almost synchronously and that this pattern appeared to be established in the larva. They also suggested a local mechanism for the control of tooth replacement.

As already indicated, most work on tooth replacement patterns in lower vertebrates has been done on fixed specimens, and only a few longitudinal studies have been carried out in living animals. Such studies involve recording the presence or absence of teeth in a dentition over a period of time. They have been carried out in alligators using radiographs (Edmund, 1962), in Lacerta and Anguis fragilis using dental impressions (Cooper, 1963, 1966), in Salmo gairdneri using dental impressions (Berkovitz and Moore, 1974, 1975), and in Serrasalmus by visual inspection (Berkovitz and Shellis, 1978; Berkovitz, 1980).

Serrasalmus has a specialised dentition and the teeth in each quadrant of the jaw appear to be replaced almost in synchrony (Berkovitz and Shellis, 1978). In the other longitudinal studies alternating replacement waves have been recognised. However, Berkovitz and Moore (1974) were able to construct further wave patterns on their charts compiled from impressions in Salmo gairdneri, but state that the significance or causation of these was not known.

In the present study of Xenopus, observations made on fixed specimens suggest an alternating pattern of tooth replacement, since (a) the first generation teeth developed as an alternating sequence and (b) an alternating sequence could be recognised in some regions of the jaws of young adults examined histologically.

Unfortunately this could not be confirmed by the impression study,

because of the incomplete nature of the records. Nevertheless it was possible to construct wave patterns on the charts along parts of the jaws as shown in Figure 6-2, and these strongly suggest that the pattern of replacement remains predominantly alternate in very large, old, adults. However, caution is required in this interpretation, since Berkovitz (1980) has shown that significant changes in tooth replacement patterns occurred with age in the specialised dentition of Serrasalmus.

A high degree of symmetry was found in the dentition of young and old Xenopus. In the newly established dentition of the larva the teeth at even-numbered loci on both sides of the jaw normally erupted before those at odd-numbered loci (SET dentition; see Section IVA). Also in the young and adult animals examined in Section III the number of tooth loci on right and left halves of the jaw was identical in nearly every case.

However, with the alizarin technique used in Section III, it was impossible to identify tooth germs which had not commenced hard tissue genesis, and therefore it was impossible to establish if the adult dentitions displayed symmetry with respect to the eruption of teeth at even and odd-numbered loci as seen in the larvae. Nevertheless, since 1.8% of metamorphosing larvae possessed asymmetrical dentitions (AT1, AT2 type), and if the pattern of tooth replacement in a dentition remains the same as when first established, then it seems likely that asymmetrical replacement patterns will occur in a small proportion of adults.

In conclusion it seems that enough instances of an alternating pattern of tooth replacement have been recorded in lower vertebrates to

Figure 6-2A and B

(on following two pages)

Wave patterns (dotted lines) drawn on the charts of animal 1, right side, and animal 2, left side. These were the only charts on which all tooth loci identified post-mortem had been recorded by the impressions, and which could therefore be used for wave analysis.

The dotted lines were drawn by linking the times of eruption of successively younger teeth in both adjacent and alternate tooth loci, and represent waves of tooth replacement. On both the charts waves can be drawn with either a cephalad slope or a caudad slope. Waves with a cephalad slope indicate that the teeth in the wave would be progressively younger toward the front of the jaw, whereas waves with a caudad slope indicate that the teeth in the wave would be progressively younger toward the back of the jaw.

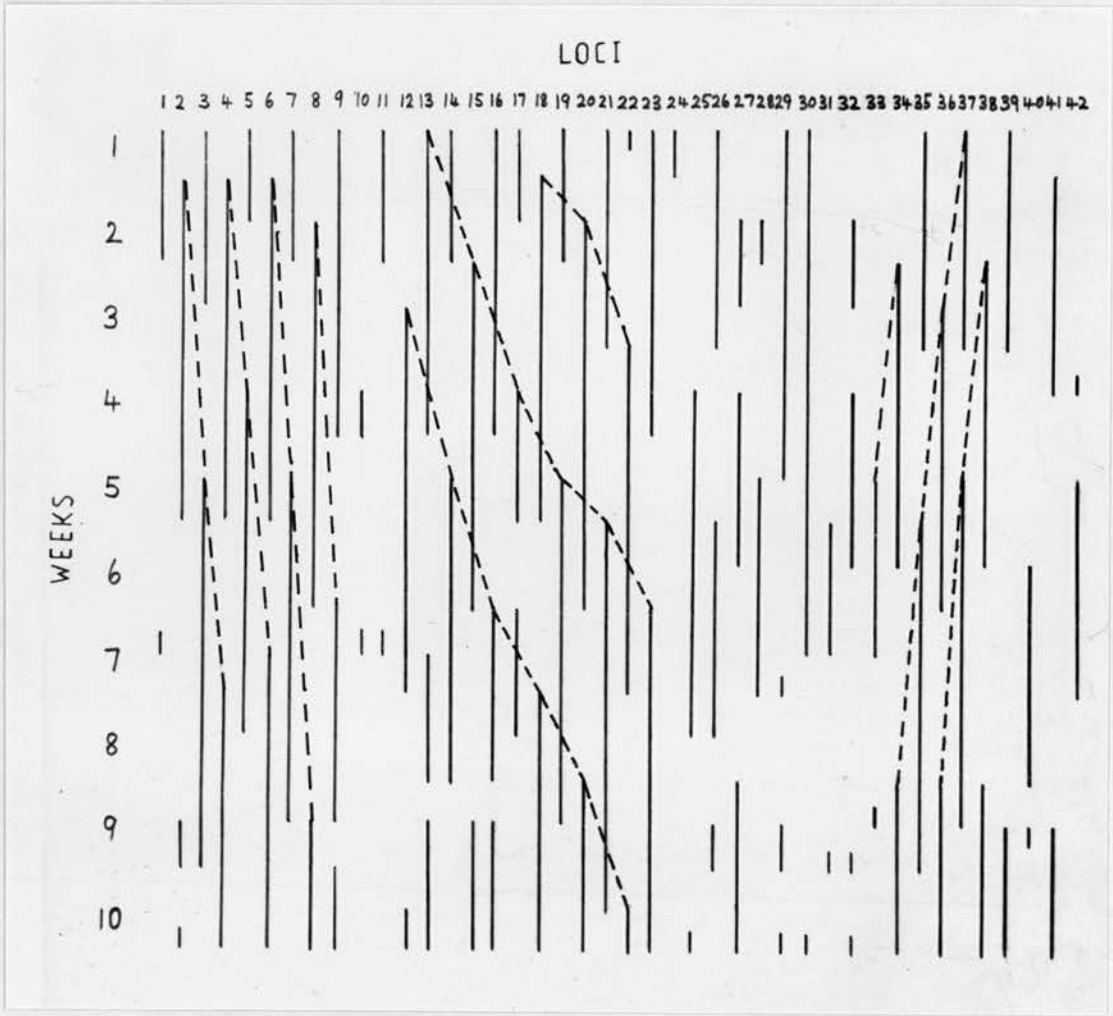


Figure 6-2A

Wave patterns drawn on the chart of animal 1, right side. Waves linking adjacent loci show both a cephalad and a caudad slope; those linking alternate loci show a caudad slope.

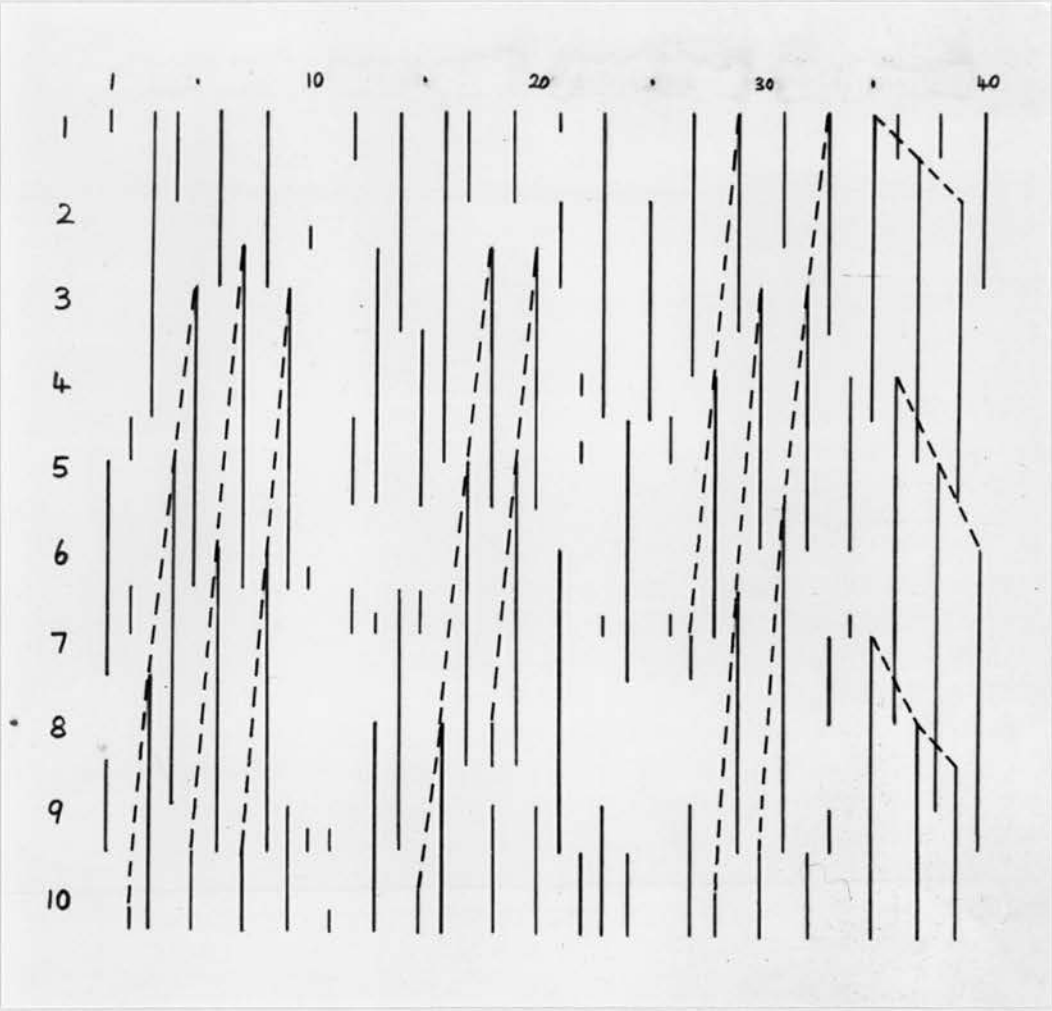


Figure 6-2B

Wave patterns drawn on the chart of animal 2, left side. Waves linking adjacent loci show cephalad slopes, those linking alternate loci a caudad slope.

suggest that it occurs fairly generally. Clearly the orderly pattern of replacement in many species implies a mechanism which correlates the developmental cycles of the teeth in a dentition, but the mechanism of control has yet to be elucidated.

APPENDIX 1

DATA FOR LARVAE IN SECTION IIIA

A letter key is used to represent the measurements in the Tables on the following pages.

The letter key is given below.

Letter	Measurement
A	length of chondrocranium (/um)
B	width of lower jaw (/um)
C	length of lower jaw (/um)

The stages are those of Nieuwkoop and Faber (1956).

STAGE	SPECIMEN NUMBER	A	B	C	$\frac{B}{C}$
47	1	2800	1800	525	3.429
	2	2375	1525	425	3.588
	3	2575	1725	425	4.059
	4	2475	1525	450	3.389
	5	2775	1775	500	3.55
48	1	2975	1875	475	3.947
	2	2625	1650	425	3.882
	3	2750	1675	475	3.526
	4	2925	1775	475	3.737
	5	2950	1750	500	3.50
49	1	3275	2050	650	3.154
	2	3900	2200	725	3.034
	3	3525	2250	725	3.103
	4	3375	2100	750	2.80
	5	3900	2275	750	3.033
50	1	4700	2875	775	3.71
	2	5425	3450	1050	3.286
	3	4400	2750	900	3.056
	4	5175	3250	800	4.063
	5	4750	2775	775	3.581
51	1	5500	3400	1025	3.317
	2	6775	4050	900	4.50
	3	6075	3825	925	4.135
	4	5500	3400	1025	3.317
	5	5650	3425	1000	3.425
52	1	6650	4050	1275	3.176
	2	6300	3550	1075	3.302
	3	6450	3825	975	3.923
	4	6875	4400	1075	4.093
	5	7625	4600	1150	4.0
53	1	8000	4725	1225	3.857
	2	8225	4675	1325	3.528
	3	7250	4675	1075	4.349
	4	7050	4250	1150	3.696
	5	8525	5100	1200	4.25

STAGE	SPECIMEN NUMBER	A	B	C	$\frac{B}{C}$
54	1	7850	4825	1400	3.446
	2	7775	4875	1200	4.063
	3	8400	5075	1425	3.561
	4	7550	4650	1250	3.72
	5	7625	4650	1400	3.321
55	1	7925	4750	1375	3.455
	2	8125	5150	1275	4.039
	3	7675	4700	1200	3.917
	4	8450	5375	1600	3.359
	5	8250	5150	1250	4.12
56	1	8175	5350	1375	3.891
	2	8950	5250	1500	3.50
	3	8325	4825	1550	3.113
	4	8850	5025	1300	3.865
	5	8500	4475	1875	2.387
57	1	9450	5575	1400	3.982
	2	8925	5375	1725	3.116
	3	8825	5400	1875	2.88
	4	9675	5809	1750.7	3.318
	5	9400	5375	1575	3.413
58	1	9325	5750	1725	3.333
	2	9425	6100	1700	3.588
	3	9625	5800	1625	3.569
	4	9125	5700	1750	3.257
	5	9750	5900	1700	3.471
59	1	8425	5875	2025	2.901
	2	8850	5525	1700	3.25
	3	9250	6150	1925	3.195
	4	8500	5400	1700	3.176
	5	9175	6050	1750	3.457
60	1	7775	5275	1925	2.74
	2	7375	5350	2025	2.642
	3	7750	5600	2000	2.80
	4	7750	5198.9	1485.4	3.50
	5	8175	5600	2250	2.489

STAGE	SPECIMEN NUMBER	A	B	C	$\frac{B}{C}$
61	1	8625	6075	1750	3.471
	2	7250	5225	2325	2.247
	3	7025	5025	2125	2.365
	4	6600	5150	2125	2.424
	5	5384.6	6268.5	2387.3	2.626
62	1	6200	4550	2725	1.67
	2	6600	4550	3050	1.492
	3	5850	4200	2375	1.768
	4	5700	3837.5	2775	1.383
	5	6021.2	3501.3	3289.1	1.065
63	1	6775	4850	3525	1.376
	2	5875	4300	2750	1.564
	3	6175	4600	3000	1.533
	4	6100	4100	2925	1.402
	5	6125	4475	3125	1.432
64	1	6025	4400	3150	1.397
	2	6325	4350	3200	1.359
	3	6650	4525	3575	1.266
	4	6275	4475	3550	1.261
	5	5450	3150	2800	1.125
65	1	6325	4300	3575	1.203
	2	7050	5025	3700	1.358
	3	6175	4375	3425	1.277
	4	5700	3925	3250	1.208
	5	6350	4150	3325	1.248
66	1	6225	4375	3425	1.277
	2	6450	4550	3625	1.255
	3	5900	4482.7	3236.1	1.385
	4	5782.5	3740	3501.3	1.068
	5	5900	4425	3225	1.372

APPENDIX 2 - DATA FOR ANIMALS IN SECTIONS IIIB AND IIIC GROUPS A, B, C, D AND M

A letter key is used to represent the measurements/ratios in the Tables on the following pages.

The letter key is given below.

Letter	Measurement/Ratio
A	Snout-vent length (/um)
B	length of skull (/um)
C	width of lower jaw (/um)
D	length of lower jaw (/um)
E	$\frac{\text{width of lower jaw}}{\text{length of lower jaw}}$
F	width of upper jaw (/um)
G	length of upper jaw (/um)
H	$\frac{\text{width of upper jaw}}{\text{length of upper jaw}}$
I	length of upper jaw tooth zone (/um)
J	length of upper jaw tooth-free zone (/um)
K	$\frac{\text{length of upper jaw}}{\text{length of upper jaw tooth-free zone}}$
L	number of tooth loci
M	$\frac{\text{snout-vent length}}{\text{number of tooth loci}}$
N	$\frac{\text{length of skull}}{\text{number of tooth loci}}$
O	$\frac{\text{length of upper jaw tooth zone}}{\text{number of tooth loci}}$
P	$\frac{\text{length of skull}}{\text{length of upper jaw}}$
Q	$\frac{\text{length of skull}}{\text{length of lower jaw}}$

KEY	SPECIMEN							
	A1	A2	A3	A4	A5	A6	A7	A8
A	10200	13600	13700	13800	14400	14500	15900	16600
B	5400	6650	5975	5925	6000	6075	6575	6725
C	3725	4525	4275	4150	4050	4125	4827.6	4525
D	3125	3750	3575	3550	3525	3375	3633.9	4100
E	1.192	1.207	1.196	1.169	1.149	1.222	1.328	1.104
F	3000	3800	3750	3550	3400	3675	3713.5	3925
G	1425	1775	1825	1575	1525	1575	1724.1	1825
H	2.105	2.141	2.055	2.254	2.23	2.333	2.154	2.151
I	1200	1550	1500	1275	1250	1350	1499.1	1550
J	225	225	325	300	275	225	225	275
K	6.333	7.889	5.615	5.25	5.546	7.0	7.663	6.636
L	38	36	32	35	32	32	34	35
M	268.42	377.78	428.13	394.29	450.0	453.13	467.65	474.29
N	142.11	184.72	186.72	169.29	187.5	189.84	193.38	192.14
O	31.579	43.056	46.875	36.429	39.063	42.188	44.091	44.286
P	3.79	3.747	3.274	3.762	3.934	3.857	3.814	3.685
Q	1.728	1.773	1.671	1.669	1.702	1.80	1.809	1.64

KEY	SPECIMEN							
	A 9	A-10	B1	B2	B3	B4	B5	B6
A	18400	19400	20800	21000	21600	23500	24600	26200
B	7075	7225	7325	7200	7125	7600	8300	8475
C	4875	5200	5425	5225	5400	5550	6450	7050
D	4250	4525	4375	4525	4700	4950	5225	5225
E	1.147	1.149	1.24	1.155	1.149	1.121	1.234	1.349
F	4275	4450	4700	4325	4400	4900	5275	5750
G	1925	1875	2000	1975	1750	1975	2125	2150
H	2.221	2.373	2.35	2.19	2.514	2.481	2.482	2.674
I	1550	1600	1500	1650	1450	1550	1725	1475
J	375	275	500	325	300	425	400	675
K	5.133	6.818	4.0	6.077	5.833	4.647	5.313	3.185
L	33	34	36	28	24	36	31	41
M	557.58	570.59	577.78	750.0	900.0	652.78	793.55	639.02
N	214.39	212.5	203.47	257.14	296.88	211.11	267.74	206.71
O	46.97	47.059	41.667	58.929	60.417	43.056	55.645	35.976
P	3.675	3.853	3.663	3.646	4.071	3.848	3.906	3.942
Q	1.665	1.597	1.674	1.591	1.516	1.535	1.589	1.622

KEY	SPECIMEN							
	B7	B8	B9	B10	C1	C2	C3	C4
A	26200	26500	26700	27400	45200	45400	46700	47200
B	8375	8500	9275	8375	11850	13025	12675	12250
C	6900	6975	7325	6575	9600	12425	7925	8800
D	5475	5250	5450	5475	8800	9050	9400	8950
E	1.26	1.329	1.344	1.201	1.091	1.373	0.843	0.983
F	5675	5775	6350	5475	9000	8250	9500	9550
G	2050	2325	2450	1950	3375	3475	3225	3225
H	2.768	2.484	2.592	2.808	2.667	2.374	2.946	2.961
I	1750	1800	1850	1625	2425	2450	2325	2475
J	300	525	600	325	950	1025	900	750
K	6.833	4.427	4.083	6.0	3.553	3.39	3.583	4.3
L	38	45	46	37	40	47	45	44
M	689.47	588.89	580.43	740.54	1130.0	965.96	1037.8	1072.7
N	220.39	188.89	201.63	226.35	296.25	277.13	281.67	278.41
O	46.053	40.0	40.217	43.919	60.625	52.128	51.667	56.25
P	4.085	3.656	3.786	4.295	3.511	3.748	3.93	3.798
Q	1.53	1.619	1.702	1.53	1.347	1.439	1.348	1.369

KEY	SPECIMEN							
	C5	C6	C7	C8	C9	C10	D1	D2
A	47600	49000	49900	52300	54900	57200	99500	102500
B	12750	13625	14025	14800	13675	14300	24850	26750
C	11250	11700	13725	13450	11275	14950	23175	26250
D	9125	10000	12325	11600	10000	10800	18450	20000
E	1.233	1.17	1.114	1.159	1.128	1.384	1.256	1.313
F	8025	9775	9925	9900	7925	10925	21775	23125
G	3600	3375	3825	4075	3625	3350	7400	8125
H	2.229	2.896	2.595	2.429	2.186	3.261	2.943	2.846
I	2550	2575	3125	3300	2500	2475	5875	6725
J	1050	800	700	775	1125	875	1525	1400
K	3.429	4.219	5.464	5.258	3.222	3.829	4.853	5.804
L	44	44	54	44	57	56	89	97
M	1081.8	1113.6	924.07	1188.6	963.16	1021.4	1118.0	1056.7
N	289.77	309.66	259.72	336.36	239.91	255.36	279.21	275.77
O	57.955	58.523	57.87	75.0	43.86	44.196	66.011	69.33
P	3.542	4.037	3.667	3.632	3.772	4.269	3.358	3.292
Q	1.397	1.363	1.138	1.276	1.368	1.324	1.347	1.338

KEY	SPECIMEN							
	D3	D4	D5	M1	M2	M3	M4	M5
A	105500	115000	115000	31000	35600	40800	43800	75000
B	25700	26650	27100	9900	11175	11675	12525	18640
C	25750	26375	28900	7925	9750	9925	10200	19300
D	20125	19650	22100	6450	7625	8025	9650	14050
E	1.28	1.342	1.308	1.229	1.279	1.237	1.057	1.374
F	19650	23125	24800	6450	7725	8000	8750	16550
G	7175	8175	10500	2575	3050	2925	3225	5200
H	2.739	2.829	2.362	2.505	2.533	2.735	2.713	3.183
I	5525	6675	9300	1850	2475	2150	2325	4150
J	1650	1500	1200	725	575	775	900	1050
K	4.349	5.45	8.75	3.552	5.304	3.774	3.583	4.952
L	87	106	108	31	39	33	33	78
M	1212.6	1084.9	1064.8	1000.0	912.82	1236.4	1327.3	961.54
N	295.4	251.42	250.93	319.35	286.54	353.79	379.55	238.97
O	63.506	62.972	86.111	59.677	63.462	65.152	70.455	53.205
P	3.582	3.26	2.581	3.845	3.664	3.992	3.884	3.585
Q	1.277	1.356	1.226	1.535	1.466	1.459	1.298	1.327

KEY	SPECIMEN	
	M6	M7
A	72500	73000
B	18375	18575
C	18200	17000
D	13675	13700
E	1.331	1.241
F	16175	15500
G	5525	5575
H	2.928	2.78
I	4400	4200
J	1125	1375
K	4.911	4.055
L	78	80
M	929.49	912.5
N	235.58	232.19
O	56.41	52.5
P	3.326	3.332
Q	1.344	1.356

APPENDIX 3 - THE LIGAMENTOUS ATTACHMENT OF THE MAXILLA TO THE PTERYGOID

As mentioned in the text, the maxilla has no posterior bony articulation in Xenopus, and therefore the posterior ends of the upper jaw are supported only by soft tissue. However, on each side of the upper jaw, a fibrous tissue ligament runs postero-inferiorly from the posterior end of the maxilla to the ipsilateral pterygoid (the pterygo-maxillary ligament). At its attachment to the maxilla the pterygo-maxillary ligament of each side becomes continuous with a band of fibrous tissue which extends forwards along the lingual aspect of the upper jaw. The fibrous band follows the curve of the jaw and continues across the midline at the inter-premaxillary suture. Thus each pterygo-maxillary ligament is connected indirectly to its fellow on the contralateral side.

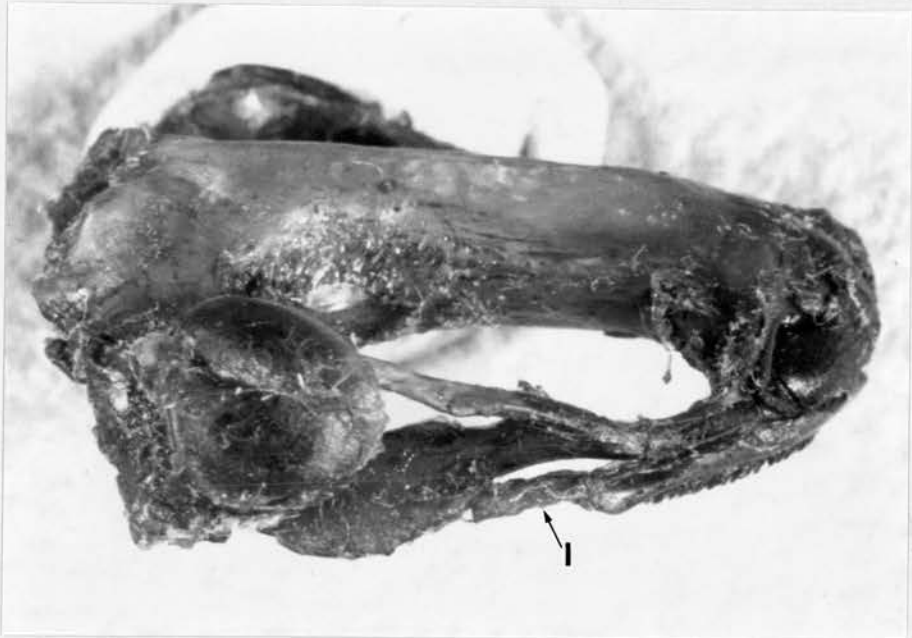
The presence of these ligaments would appear to limit displacement of the upper jaw by the lower when the mouth is closed. Furthermore, the attachment of the ligaments to the pterygoids, and their connection via the fibrous band, would seem to be effective in preventing the upper jaw from splaying outwards, as would occur when pressure is applied upwards by the lower jaw during rapid, forceful closure.

Specimens stained with alizarin red S, and only partially macerated, showed the pterygo-maxillary ligament clearly (Appendix Figure 1). In a large female of snout-vent length 115mm, the ligaments were about 3.5mm in length and 1.1mm in diameter; in the same animal the posterior end of each maxilla could move over a range of about 5mm in a postero-inferior direction before further movement was prevented by the ligament.

The histological appearance of the pterygo-maxillary ligament is shown in Appendix Figures 2, 3 and 4, which are coronal sections from a

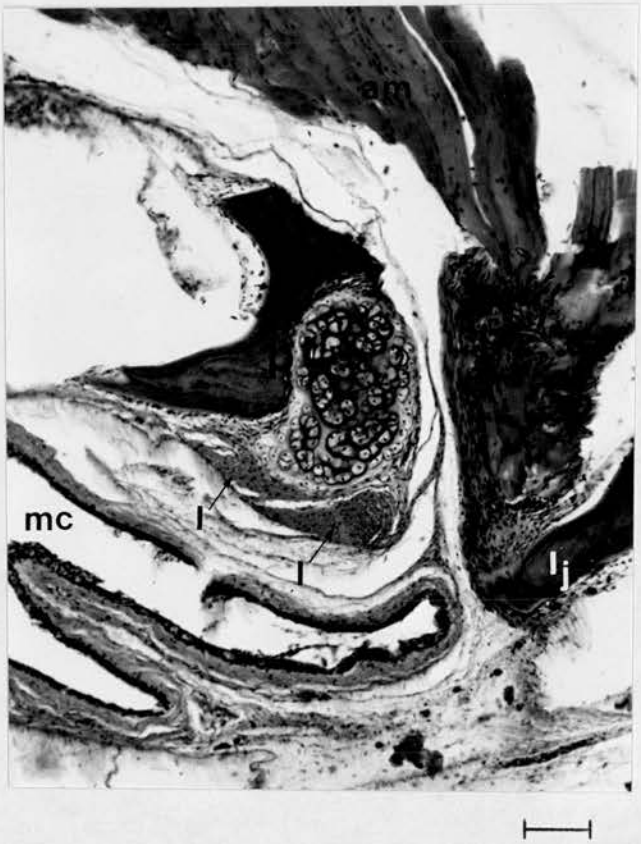
young female (snout-vent length 31.5mm). In this specimen the ligament shown was approximately 1680/um in length, and 202/um in diameter at its midpoint.

The pterygo-maxillary ligament does not seem to have been described elsewhere in the literature.



Appendix Figure 1

Skull of Xenopus from right side (partially macerated specimen) showing the pterygo-maxillary ligament (1) attached between maxilla and pterygoid. Compare with Figure 5-3A.



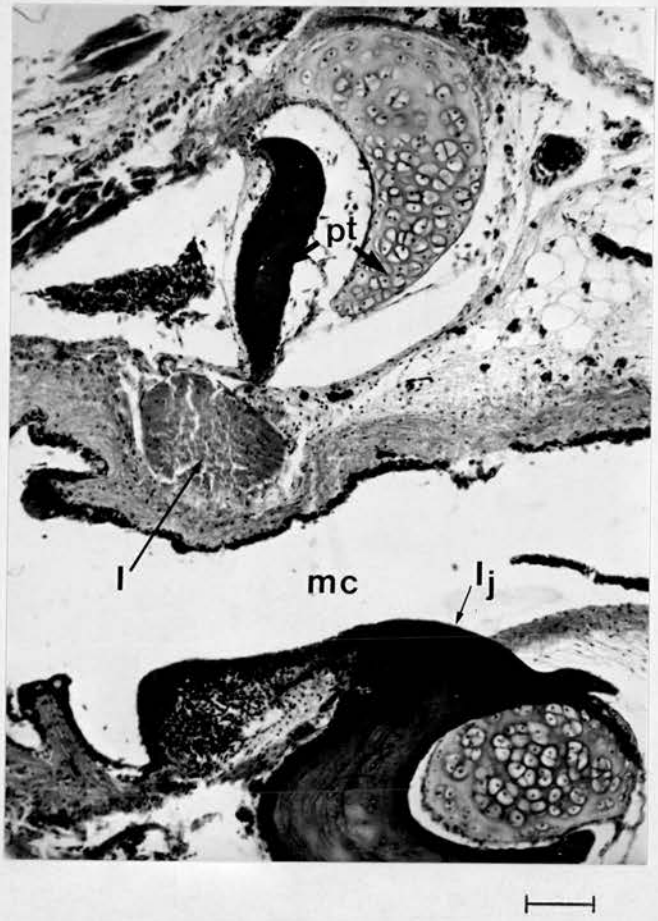
Appendix Figure 2

A coronal section of the left side of the head of Xenopus showing the posterior attachment of the pterygo-maxillary ligament (l) to the pterygoid (pt).

mc, mouth cavity; lj, lower jaw; am, adductor muscles of lower jaw.

Decalcified, Stained H + E

Scale bar 100/um

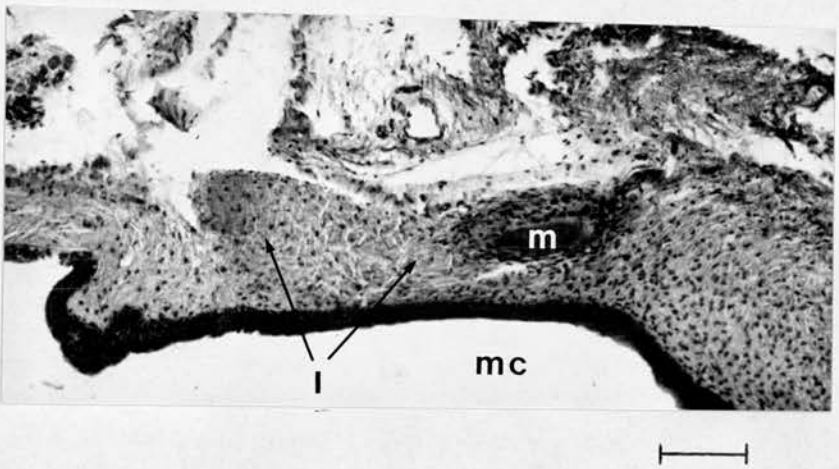


Appendix Figure 3

A coronal section of the left side of the head of Xenopus showing the pterygo-maxillary ligament (l) midway between its posterior attachment to the pterygoid and anterior attachment to the maxilla.
pt, pterygoid; mc, mouth cavity; lj, lower jaw

Decalcified, stained H + E

Scale bar 100/um



Appendix Figure 4

A coronal section of the left side of the head of Xenopus showing the pterygo-maxillary ligament (l) at its anterior attachment to the maxilla.
m, maxilla; mc, mouth cavity

Decalcified, stained H + E

Scale bar 100/um

APPENDIX 4 - AGE ASSESSMENT IN XENOPUS

Data are presented in this Appendix on 120 metamorphosed animals collected by the author. Most of the animals were not required for the main study, but 42 were used in Sections IIIB and IIIC. The ages of 98 of the animals were known, and were within the range 13-365 days.

It was decided to measure some body parameters in the living animals the aim being to ascertain whether or not some conveniently measured external features could be used for age assessment. It is difficult to obtain data for the rate of growth of Xenopus in the wild, but the animal has been reared in many laboratories, and its rate of growth is known under these conditions (see Section I - Introduction).

For most amphibians and reptiles information on growth has been obtained from captive animals, but this information is open to criticism on the grounds that there is no indication of the relationship between captive growth rates and growth under natural conditions. However, tag-recapture data as used in turtles by Harrisson (1956) and Bustard (1972), does provide information on the growth of animals in their natural environment.

If observations cannot be made directly on wild animals, an indirect method of assessing growth and age must be used. Thus age in vertebrates has been assessed in a number of ways, such as -

(a) incremental lines on the epidermal scales of fish (Bagenal, 1978).

(b) growth rings on the epidermal plates of the chelonian carapace (Bellairs, 1969),

(c) overall body length in Alligator mississippiensis (Oliver, 1955).

(d) incremental lines in dental cement e.g. the seal (Hewer, 1960),

(e) the state of development and degree of wear of the dentition in many mammals e.g. the hippopotamus (Laws, 1968),

(f) lens weight e.g. the elephant (Laws, 1952, 1967),

(g) closure of bony epiphyses and skull sutures in Man (Warwick and Williams, 1973).

Unfortunately it was clear that the majority of the above methods could not be used for Xenopus, since the animal has no large incrementally growing epithelial structures, the dentition is polyphyodont, and epiphyses are still present in the long bones of very large females. Accordingly, it was decided that the following external parameters would be measured to assess their value in age determination in Xenopus; (a) wet body weight, (b) snout-vent length, and (c) inter-narial width. The study was primarily concerned with female animals, since males stop growing at 2-4 years of age.

It was also decided to measure the diameter of the lens (although of no value for age assessment in vivo), since in the elephant (see above) lens weight has been used for age estimation, and it was hoped that lens diameter might prove useful in Xenopus.

After killing each animal both lenses were dissected out and stored on plasticine for 24 hours (tests had shown that drying and shrinkage of the lenses ceased after 18 hours). The dry lens diameter was then measured with an eyepiece graticule at X40 or X100. The values for lens

diameter presented in Table 1 are the mean of right and left lens for each animal.

The results are presented in Tables 1 and 2, and Table 3 shows the coefficients of correlation between the parameters.

The animals used in Sections IIIB and IIIC of the Thesis are cross-coded in Tables 1 and 2; the Section III code is given in parenthesis after the specimen number. Reference may thus be made to Appendix 2 where further details of these animals are presented.

TABLE 1 - RESULTS

Females

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
1(A2)	13	-	617.79	13600	1856.8
2(A1)	21	83	557.78	10200	1538.5
3	"	233	600.01	13400	1856.8
4(A5)	22	365	586.68	14400	1817.0
5(A3)	23	280	635.56	13700	1909.8
6	31	-	653.34	18900	2281.2
7(A4)	35	323	586.68	13800	1883.3
8(A7)	"	509	613.34	15900	2042.4
9	"	859	662.24	18200	2334.2
10	"	937	657.79	18900	2387.3
11	"	-	697.79	24000	2679.0
12	"	-	706.68	23500	2546.4
13	37	-	702.23	23200	2679.0
14	39	670	680.34	17400	2200.0
15	"	1127	709.92	20600	2375.0
16	"	1363	690.2	21800	2500.0
17	"	1505	729.64	22600	2625.0
18	"	1720	709.92	23400	2575.0
19	"	1837	759.22	24400	2725.0
20	"	1996	744.43	24700	2675.0
21	"	2355	729.64	25300	2725.0
22(B6)	"	2496	764.15	26200	2925.0

Females

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
23(B8)	39	2573	778.94	26500	2850.0
24(A6)	43	427	617.79	14500	1883.3
25(A9)	"	824	644.46	18400	2201.6
26	"	889	680.01	18700	2360.7
27	"	1037	688.9	20050	2307.7
28	"	1188	680.01	20700	2413.8
29	"	1402	680.01	21700	2599.5
30	43	1405	702.23	21850	2626.0
31	"	1906	702.23	23500	2679.0
32	"	-	648.9	18900	2334.2
33	"	-	653.35	18400	2440.3
34	47	564	608.9	15900	2095.5
35(A8)	"	568	648.9	16600	2148.5
36	"	811	657.79	18700	2201.6
37	"	813	666.68	18400	2281.5
38(A10)	"	963	675.57	19400	2533.1
39	"	1000	675.56	20800	2440.3
40(B1)	"	1110	684.45	20800	2387.3
41(B3)	"	1295	680.01	21600	2413.8
42	"	1391	706.68	21800	2519.9
43	"	1538	693.34	21900	2519.9
44	"	1691	702.23	22800	2466.8
45	"	1996	711.12	23650	2705.6
46	"	2219	760.01	25600	2944.3

Females

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
47(B10)	47	2736	724.46	27400	2758.6
48	"	-	644.46	16600	2148.53
49	"	-	728.9	23100	2679.0
50	53	514	675.57	16450	2069.0
51	"	823	666.68	17700	2254.6
52	"	921	684.45	19600	2228.1
53	"	1177	702.23	20100	2413.8
54	"	1289	671.12	20400	2413.8
55	"	1371	720.0	22000	2546.4
56	"	1472	688.9	21200	2466.9
57	"	1908	711.12	24100	2758.6
58	57	1113	677.79	20000	2466.8
59	57	1163	668.9	19850	2413.4
60(B2)	"	1229	684.45	21000	2466.8
61	"	1628	733.34	22250	2586.19
62(B4)	"	1719	733.35	23500	2652.5
63	"	1764	733.35	23550	2705.0
64	"	1771	711.12	23200	2599.5
65(B5)	"	2225	748.9	24600	2679.0
66(B7)	"	2711	768.9	26200	2970.8
67	"	-	711.12	22450	2652.5
68	63	1285	702.23	20450	2413.8
69	288	2613	857.82	28300	2900.0
70	"	3727	916.98	31500	3125.0

Females

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
71	288	8581	990.93	40600	3675.0
72	329	-	-	38500	3607.4
73	365	4467	936.5	32600	3300.0
74	"	7876	987.5	37600	3800.0
75	"	11846	1075.0	43600	4075.0
76(C2)	"	12962	1037.5	45400	4225.0
77(C6)	"	14784	1062.5	49000	4525.0
78(C7)	"	15613	1075.0	49900	4525.0
79(C8)	"	18857	1118.75	52300	4650.0
80	"	22978	1112.5	52100	4700.0
81	"	25136	1212.5	58700	5275.0
82(C10)	"	25422	1175.0	57200	5000.0
83	"	26665	1125.0	58900	4825.0
84	"	30513	1200.0	62100	5325.0
85	"	32119	1175.0	66300	5125.0
86(B9)	Not Known	2628	778.94	26700	2970.8
87(C1)	"	11476	1200.0	45200	4000.0
88	"	12808	1225.0	46300	3500.0
89(C4)	"	13663	1200.0	47200	4125.0
90(C3)	"	15048	1250.0	46700	4050.0
91(C5)	"	16332	1212.5	47600	4500.0
92	"	17021	1275.0	47100	4450.0
93	"	22666	1325.0	54500	4875.0
94(C9)	"	24834	1337.5	54900	4875.0

Females

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
95(D2)	Not Known	119767	1475.0	102500	8350.0
96(D1)	"	123970	1562.5	99500	7650.0
97(D3)	"	126493	1312.5	105500	7100.0
98(D4)	"	174022	1750.0	115000	8700.0
99(D5)	"	191244	1775.0	115000	9050.0

TABLE 2 - RESULTS

Males

SPECIMEN NUMBER	AGE (DAYS)	WEIGHT (MGM)	MEAN DRY LENS DIAMETER (/um)	SNOUT-VENT LENGTH(/um)	INTER-NARIAL WIDTH (/um)
1	31	-	595.56	15900	2095.5
2	33	-	635.56	17800	2334.2
3	40	-	646.67	15800	2122.0
4	47	-	751.12	26000	2214.8
5	53	1545	706.68	21950	2546.4
6	57	696	631.12	17400	2108.7
7	"	1718	737.79	22700	2679.0
8	"	2103	742.24	23900	2705.6
9	77	3362	826.68	27200	2997.3
10(M1)	365	3977	925.0	31000	3175.0
11(M2)	"	7752	1035.3	35600	3650.0
12(M3)	"	8410	1000.0	40800	3875.0
13(M4)	"	12792	1125.0	43800	3875.0
14	Not Known	4070	916.98	31500	3103.4
15	"	4700	852.89	33300	3156.5
16	"	9786	1200.0	41300	3875.0
17	"	13054	1225.0	44500	3825.0
18	"	15715	1125.0	49700	4675.0
19(M7)	"	50279	1375.0	73000	7100.0
20(M6)	"	51760	1362.5	72500	6200.0
21(M5)	"	59991	1362.5	75000	5800.0

TABLE 3 - THE COEFFICIENTS OF CORRELATION BETWEEN THE PARAMETERS MEASURED ($P < .001$ IN ALL CASES)

PARAMETERS	NUMBER OF FEMALES IN GROUP	COEFFICIENT OF CORRELATION
wet weight/snout-vent length	83	+ 0.9103
snout-vent length/inter-narial width	99	+ 0.9932
wet weight/age	74	+ 0.8494
dry lens diameter/age	82	+ 0.9030

In the group of animals with a comprehensive range of body size in which the measurements were made, high and significant correlations were found between all parameters in the females. However, since there were no animals in the study with known ages between 63 and 288 days, linear regression with the available data gave conflicting results in age determination.

The females reared to one year old showed large variations in their final weights, the lightest being only 14.63% of the weight of the heaviest. These variations were reflected in concomitant variations in snout-vent length and inter-narial width. Therefore, even in young females large variations in growth rates occurred.

Males are fully grown at 2-4 years, so that size is not an indication of age after this. However, a wide variation was found in the sizes of

males only one year old, and therefore even before they are fully grown the determination of age by the parameters described here may be impossible.

The wide range in size exhibited by the lenses taken from the one year old animals suggests that the lens is probably not a useful tool in age analysis (the smallest lens pair was only 78.06% of the diameter of the largest pair). However, the profile of the male in this respect remained similar to immature females of about 50mm snout-vent length. Since, in females weight increased faster than snout-vent length after sexual maturity, it may be that in animals above 50mm snout-vent length the ratio of weight - dry lens diameter has a place in sex determination.

These observations on the young of both sexes, indicate that the value of the criteria in age assessment is low. It is likely that a large female is old, but a small animal of either sex need not necessarily be young.

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APPENDIX 5

PUBLISHED WORK

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The time scale of tooth development and replacement in *Xenopus laevis* (Daudin)

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INTRODUCTION

There is an extensive literature on the anatomy and histology of the dentitions of lower vertebrates, and the events in the development of the teeth in many species are well documented. A characteristic feature of lower vertebrate dentitions is that the teeth are replaced throughout the life of the individual and the jaws at any given time show teeth in all stages of development.

Previous workers have divided the sequence of events during the development of individual teeth of such dentitions into a number of stages (Gillette, 1955; Goin & Hester, 1961; Lawson, 1965; Lawson, Wake & Beck, 1971). By observing the relative incidence of the various stages in fixed specimens, an estimate of the relative duration of each stage of tooth development can be made. Stages which occur frequently are assumed to be long-lasting, while a rare stage is assumed to be a rapid one. In this way it has been deduced that the early stages of tooth germ development occupy a large proportion of the total developmental time, and therefore occur slowly; while eruption, ankylosis and resorption occupy a small proportion of the developmental time and therefore occur rapidly.

However, it is difficult to measure the absolute time scale of tooth development in the polyphyodont dentitions of lower vertebrates. This is because in an animal examined at an arbitrary time it is impossible to fix a base line in the dentition. Consequently, few estimates of the absolute time scale involved in tooth development and replacement in lower vertebrates have been made.

Edmund (1960) has, however, measured the rates of tooth replacement in many reptilian species using long term, periodic X-ray analyses of live animals. More recently a direct measurement of the absolute time scale of the tooth cycle in the rainbow trout was made by Berkovitz (1977).

The present study is an attempt to estimate the absolute time scale of tooth development in the fully aquatic amphibian *Xenopus laevis*. To circumvent the problem of fixing a base line in the dentition it was decided to follow the history of the *first* teeth to develop during the larval stages of the animal. In order to follow the development of the teeth through the stages of eruption, ankylosis and resorption, it was necessary to rear a number of *Xenopus* larvae in such a way that their growth rates were similar and so a cross sectional study could be made. Having achieved this, animals were killed at various growth stages so that the events in the development of their teeth could be followed through time. The average time taken for the teeth to develop could then be measured.

An explanation given by many authors for the sequence of tooth replacement and organisation of lower vertebrate dentitions is embodied in the Zahnreihen Theory

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proposed by Edmund (1960). This theory attributes control of tooth formation to a 'stimulus' which moves caudad along the dental lamina initiating the development of tooth germs at pre-determined loci; the term Zahnreihe is used to describe all the teeth produced by a single 'stimulus'. A number of 'stimuli' will produce a number of Zahnreihen, and this will cause a series of replacement waves of teeth. The Zahnreihen Theory has been challenged by Osborn (1970, 1971) and De Mar (1972, 1973).

The present paper had the following objectives:

(*a*) to describe the arrangement of the dentition in *Xenopus laevis*;

(*b*) to obtain an absolute time scale for the events in the development of the first teeth to develop and erupt in larval and newly metamorphosed specimens, and to assess their rate of replacement;

(*c*) to estimate indirectly the rate of tooth replacement in the adult dentition;

(*d*) to assess the relevance of the Zahnreihen Theory for the dentition of *Xenopus laevis*.

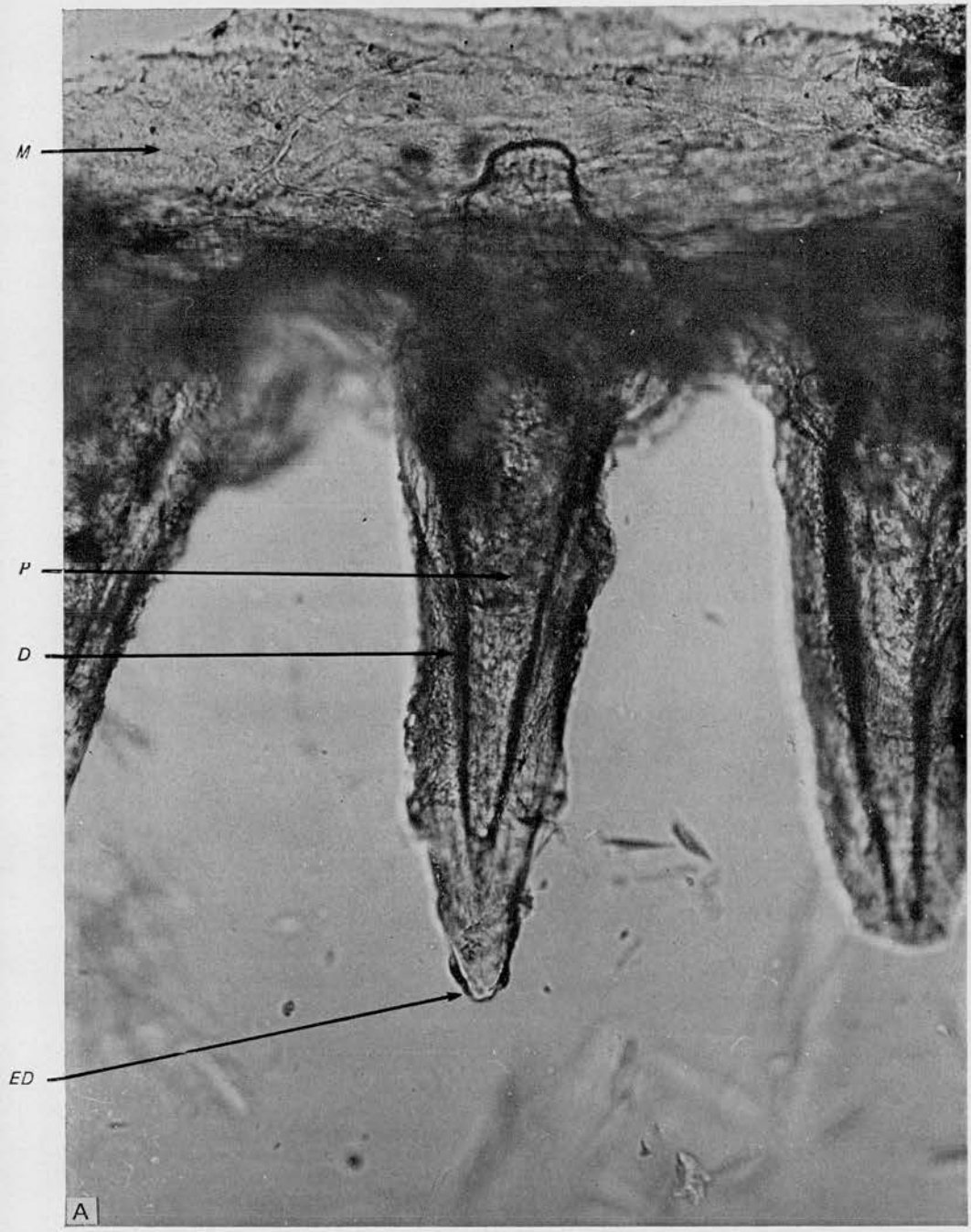
MATERIALS AND METHOD

Larval teeth

In order to study the first erupting teeth in the larvae, eggs were obtained by injecting a pair of adult *Xenopus laevis* with Gestyl, and the larvae obtained were reared in dechlorinated tap water at 25 °C and fed on nettle powder. The larvae were staged in their development using the external criteria in the Normal Table of *Xenopus laevis* (Nieukoop & Faber, 1956). This Table describes 66 stages between fertilisation and metamorphosis; this study was concerned only with NF stages 53–66, and some post-metamorphic juvenile stages. The staging of the animals was used only to judge their rates of development before they were killed; the time scale of tooth development given in the results is expressed in days.

In order to obtain a final sample of animals which had developed as nearly as possible at a similar rate, all very small, slowly developing, or abnormal animals were eliminated before the larvae had reached NF stage 53. The larvae were then divided into batches of approximately 50, and each batch was kept in a separate tank at a temperature of 25 °C. The fertility level of the eggs was exceptionally high, and so, from an initial number of several hundred larvae, a sufficient number was deemed to have developed at a similar rate to make analysis of the results possible.

Fig. 1. (A) An ankylosed tooth of 18 months old adult *Xenopus laevis*. Cleared whole-mount preparation. The walls of dentine (*D*) surrounding the pulp chamber (*P*) are clearly seen, while the enameloid (*ED*) is visible only as a narrow clear zone around the tooth tip. *M*, maxilla. ×25. (B) A longitudinal section of three teeth from an 18 months old specimen of *Xenopus laevis*. Note the difference in cellularity of the pulp chambers. The pulp chamber of the erupting tooth (*ER*) contains tall columnar odontoblasts (*OD*), which are visible on the pulpal aspect of the dentine (*D*). The newly erupted tooth (*NE*) is just completing ankylosis to the pedestal (*P*); 'degenerate' odontoblasts are still visible on the dentine surface. The 'functional' tooth (*FT*) possesses no odontoblasts in the pulp chamber, which is relatively acellular. *ES*, enameloid space; *M*, maxilla. Decalcified, stained haematoxylin and eosin. ×32. (C) A longitudinal section through the basal two thirds of a tooth in the functional position, from a newly metamorphosed specimen of *Xenopus laevis* (NF stage 66+). This tooth began its development during the larval NF stage 55. Odontoblasts are no longer present in the pulp chamber, but in their place are a number of flattened cells lying at right angles to the dentine surface (compare with Fig. 6). *D*, dentine; *B*, blood vessels; *P*, bone of pedestal; *M*, maxilla. Decalcified, stained Heidenhain's azan. ×100.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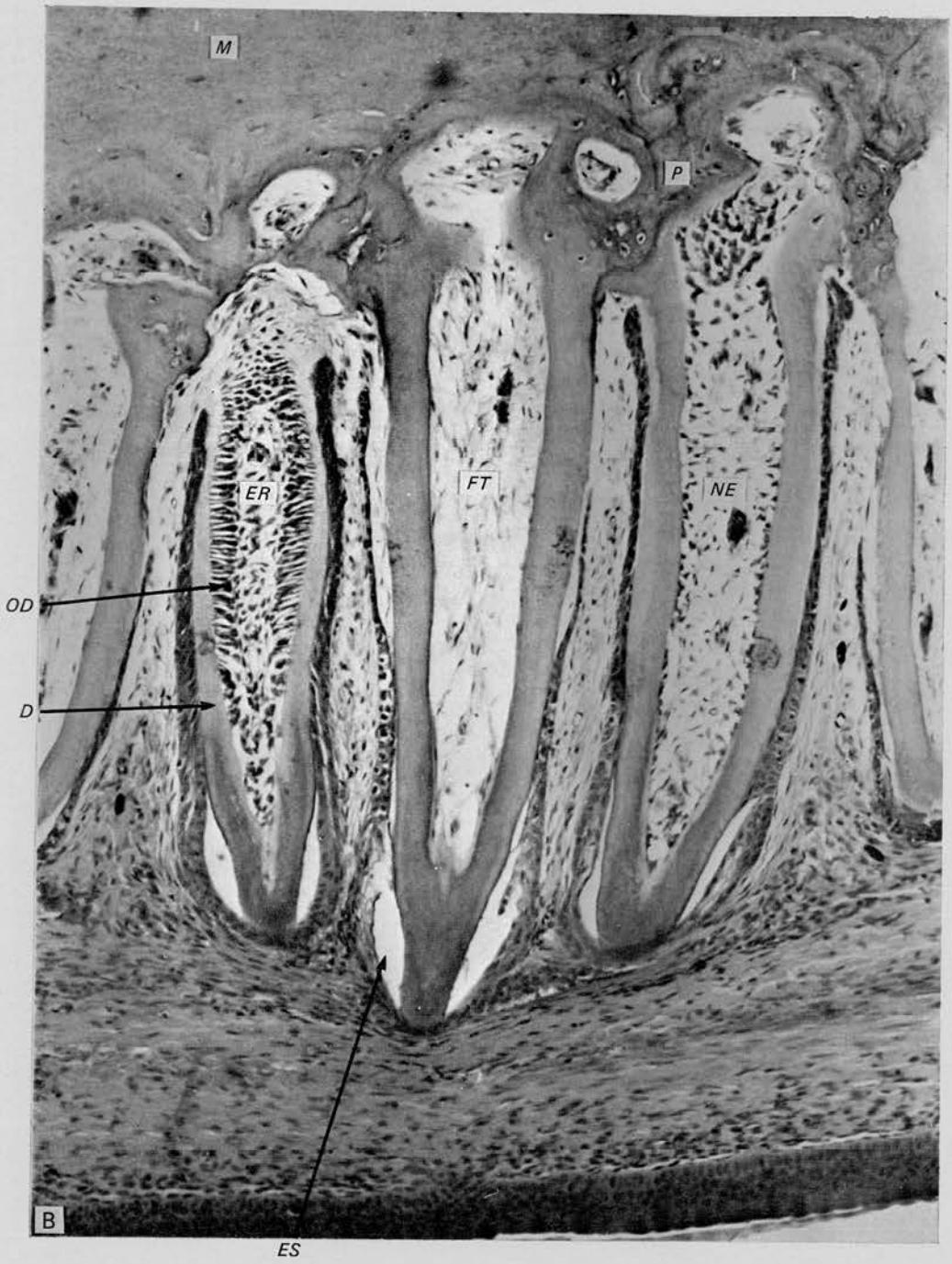
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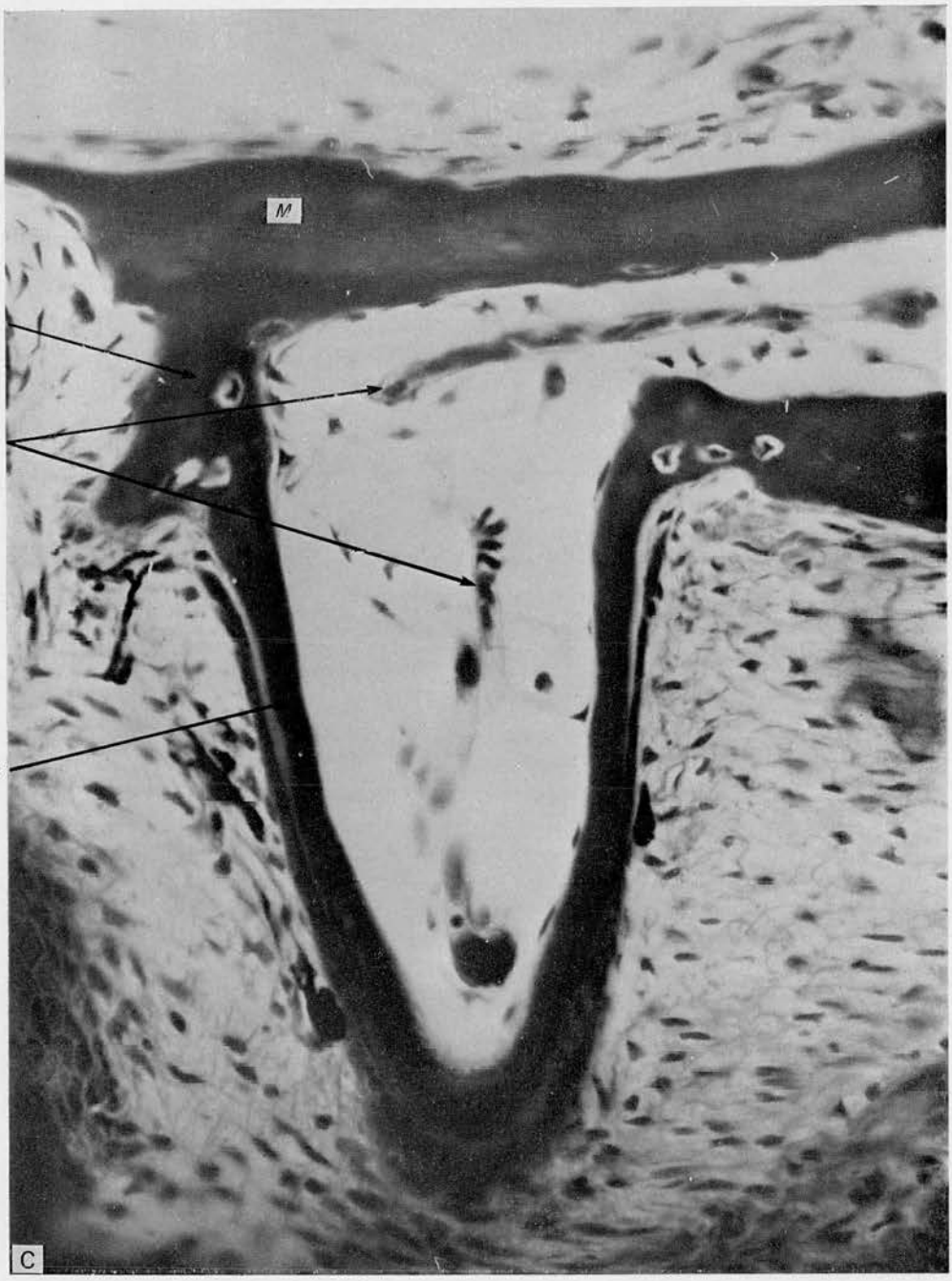
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Since it had been observed that the size and shape of the teeth of larvae from different egg batches can vary, in this study all the animals were from one batch of eggs. By the nature of the method of selection the animals eventually examined were the strongest and fastest developing.

During the more prolonged early stages, i.e. NF stages 55, 56 and 57, animals were killed every 2 days, but during the more rapid subsequent stages animals were killed as they reached each stage. The numbers of animals examined were as follows: 40 animals between NF stage 55–57; 12 animals for each subsequent stage between 58 and 66; and 24 animals at post-metamorphic stages. The total number of animals was therefore 172.

All specimens were fixed in Bouin's solution, decalcified and double embedded. Serial paraffin sections were made of each specimen. Half the specimens at each stage were cut at 10 μm and stained with haematoxylin and eosin for measurement of the dentine; half were cut at 5 μm and stained with Heidenhain's azan to facilitate cell counts.

Each cell of *Xenopus laevis* possesses two nucleoli, and so cell numbers were estimated by making nucleolar counts. The correction formulae of Konigsmark (1969) were applied to these counts. A number of nuclear counts was also made as a check, and these were always approximately 10 % lower than the nucleolar counts.

Sixty of the animals, which had developed beyond NF at 64, were examined in order to determine whether there was variation in the arrangement of the dentition as a whole.

Adult teeth

In order to study the dentition of the young adult, a breeding pair of *Xenopus* was injected with Gestyl and the eggs laid were reared to metamorphosis in dechlorinated tap water at a constant temperature of 25 °C. The larvae were fed on nettle powder. Ten of the metamorphosed juveniles were then reared under similar conditions to 18 months of age. They were fed on tubifex worms and liver. Two adult females of unknown age, but known to be over 3 years old, were also examined.

OBSERVATIONS

The arrangement of the dentition

The teeth of young and mature *Xenopus laevis* were found as a single row attached to the maxillae and premaxillae. The dentition was essentially homodont, and each tooth consisted of a comparatively simple cone of orthodentine, capped by a thin layer of enameloid (Fig. 1A). Unlike many other amphibian species examined by Parsons & Williams (1962), there was no division of the teeth into distal and proximal parts separated by a line of weakness.

The teeth of the larvae and newly metamorphosed specimens were similar to those of the adult animals, the main difference being that of scale. The average length of the dentine of the fully formed larval teeth was 225 μm, and the dentine was about 8·4 μm thick. Each tooth was ankylosed to a short, ring-shaped bony pedestal, which, in the newly metamorphosed specimens, appeared to be continuous with the bone of the maxillae or premaxillae (Fig. 1C). However, in the adults the bone of the pedestal was clearly distinguishable from the underlying bone of the jaw, and demonstrated many incremental and reversal lines (Fig. 1B).

Newly metamorphosed specimens of *Xenopus laevis* demonstrated two series of teeth; a series in even-numbered tooth positions, and a series in odd-numbered tooth positions, starting from the mid-line. These teeth began to form during the larval stages of development, and did not erupt until the end of metamorphosis. Normally the even-numbered tooth series was the first to erupt. The first erupting odd and even series teeth will be called first generation teeth in this paper. *In toto* between 18 and 20 first generation even-numbered tooth germs were produced in each larva, while the 18–20 odd-numbered tooth germs developed between these, slightly later in time. This arrangement of the dentition will be called the symmetrical even-number type (SET) (see Fig. 2).

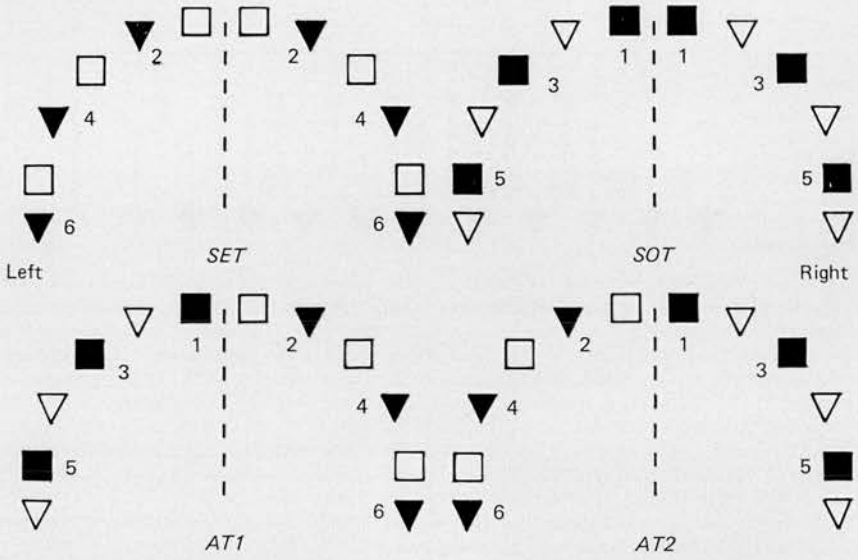


Fig. 2. A diagram to illustrate the variations observed in the overall arrangement of the dentition of *Xenopus laevis*. Four upper jaws are represented diagrammatically, and only the 12 anterior tooth positions are shown in each jaw. The dotted lines represent the mid-lines. *SET*, symmetrical even-number type dentition; *AT1*, atypical type 1 dentition; *AT2*, atypical type 2 dentition. For explanation see text.

However, out of a total number of 60 animals examined to see if this arrangement of the dentition was constant, 5 animals showed variations on this pattern. Two of the 5 animals showed a dentition which was the reverse of the common type, i.e. the teeth in the odd-numbered tooth positions erupted first. This arrangement of the dentition will be called the symmetrical odd-number type (SOT).

The three remaining animals which showed variations in the arrangement of their dentitions possessed dental arrangements which were asymmetrical. Two of these three animals showed the even-numbered teeth developing first on the right side, while the odd-numbered teeth were developing first on the left side; this is the asymmetrical type 1 (AT1) dentition. The remaining animal also possessed an asymmetrical dentition, but the reverse asymmetry of the atypical type 1 arrangement. This is the asymmetrical type 2 (AT2) dentition.

One animal, with a dentition of the SET type, demonstrated 'twinning' of one tooth germ. At the fifth tooth position on the left side there were two teeth, lying close together in a medio-lateral relationship; these were separated only by a mutual

zone of dental epithelium. The single successional tooth germ lay in relation to the lingual edge of the dental epithelium of the lingually placed abnormal tooth. All the other teeth in this specimen's dentition were present in their normal positions.

On each side tooth formation ceased abruptly at the back of the mouth. There were generally 20 tooth positions on each side of the mouth before metamorphosis. However, during metamorphic climax, two or three further tooth germs developed on each side distal to the existing tooth germs. Each of these later tooth germs had a connexion with the oral epithelium, but not with any other tooth germ. They corresponded to tooth positions 21, 22 and 23. It will be noticed from Fig. 3 that

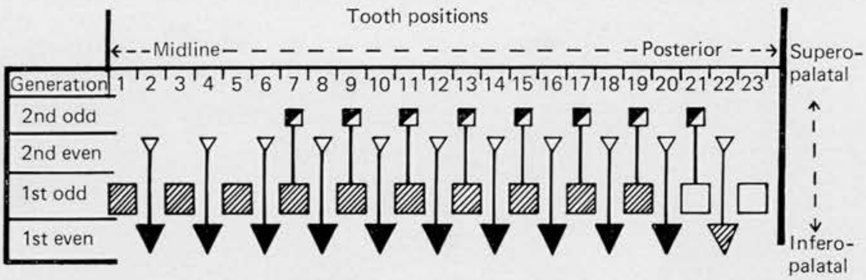


Fig. 3. A schematic representation of one side of the upper jaw of a typical specimen of *Xenopus laevis* just after the end of metamorphic climax (NF state 66+). The dentition is of the symmetrical even-number type, and the teeth are represented by triangular and square symbols, which are shown diagrammatically in a linear arrangement. Four generations of teeth are shown. Triangles represent even-positioned teeth; squares odd-positioned teeth. Solid triangles, teeth ankylosed in their functional positions: cross-hatched symbols, erupting teeth: clear symbols, tooth germs showing hard tissue genesis: semi-solid squares, tooth germs composed only of soft tissue. The first generation teeth in positions 1–20 began their development during the larval NF stage 55–56, whereas the second generation teeth in these positions began development later at about NF stage 60–62. The first and second generation teeth in positions 21–23 appeared during metamorphic climax; notice that their development is out of phase with the more anterior teeth. This may only be a transitory phenomenon.

these tooth germs did not appear to develop in sequence with the alternate arrangement of teeth 1–20. However, this was probably only a transitory phase in their development, since the teeth of the adults examined appear histologically to continue to develop alternately along most of the upper jaw.

The number of erupted teeth present in the upper jaws of the 18 months old adult animals was 30, compared with 20 in the newly metamorphosed animals. This is not a very great increase in such a comparatively long period of the animal's life span, during which the average width of the dental arch (measured between its posterior ends) had increased from an average of 4 mm to an average of 13·5 mm.

In the two adult females examined, whose exact age was unknown, but was certainly in excess of 3 years, there were 60 erupted teeth in one animal and 62 in the other (see Table 1).

The teeth of the 18 months old adult animals were larger than those in the newly metamorphosed juveniles. The average length of the dentine of an adult tooth was 840 μm, and the thickness of the dentine of each tooth averaged 36 μm. The histological picture presented by such a tooth was virtually identical with that of a larval tooth, except that blood vessels were more evident, presumably because of the higher nutritional requirement of the larger number of odontoblasts present in the pulp.

Table 1. *Variations of tooth numbers in Xenopus laevis with size and age*

Specimens	No. of animals	Snout–vent length of specimen (mm)	Maximum width of dental arch (mm)	No. of erupted teeth in upper jaw
Newly metamorphosed	22	16±1	4±0·5	20±2
18 months adult males	4	66±2	14±1	28±3
18 months adult females	6	73±1	13±2	34±6
3 years+adult females	2	110±2	20±1	61±1

The development of individual tooth germs in the larva

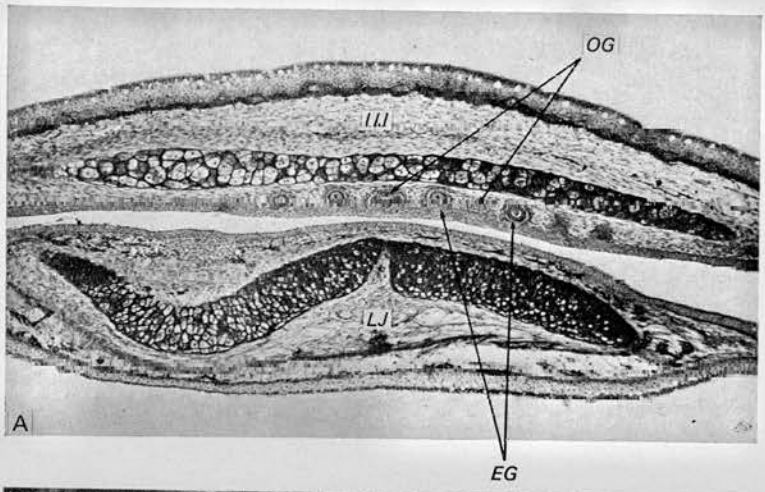
This section of the paper is concerned with the time taken for the development, eruption, ankylosis and resorption of the sixth to fourteenth positioned teeth of the first generation even-numbered tooth series. The sixth tooth was normally the last tooth on the premaxilla before the premaxillary–maxillary suture, and was therefore readily identifiable.

Odontogenesis began during the larval NF stage 55, and the day on which germ initiation occurred is counted as day zero of this study. Day zero was the base line from which the time scale of tooth development was measured.

Odontogenesis was characterised by a number of separate tooth germs which lay in relation to the lower aspect of the ethmoidal cartilage. The premaxillae and maxillae had not begun to ossify at this stage. The production of the tooth germs began posteriorly on each side and continued forward toward the mid-line. During the formation of each tooth germ, the basal cells of the oral epithelium in each tooth-bearing zone became columnar, and orientated with their long axes at right angles to the epithelial surface. The edges of each zone of basal cells invaginated to form a curved plate of cells, concave towards the dermis, resembling an inverted trough. Each trough was then roofed over by the epithelium to form a short tube, closed at its medial end and enclosing the mesodermal cells of the dental papilla. Each tube lay horizontally in the jaw and all had their long axes running antero-posteriorly (Fig. 4). This early period of tooth formation before hard tissue genesis had commenced was associated with frequent cell divisions in both the mesoderm and epithelium of each tooth germ. As yet no odontoblasts were present.

Dentinogenesis commenced on the second day after germ initiation, and took the form of a short cone of dentine of average length 20 (1·8) μm; the dentine wall of this cone was about 2·3 (0·2) μm thick, and it was produced by about six odontoblasts. (In this section of the paper all measurements and counts of cells were estimated using six teeth from each of 12 animals representative of the stage being described. The number in parentheses after each measurement is the standard deviation.) It is assumed that the odontoblasts differentiated from the pool of mesodermal cells, since the latter were no longer present in the papilla when all the odontoblasts had differentiated.

Figure 5 shows the average number of odontoblasts present in the dental papillae throughout the growth of the tooth germs. There was a clear division of dentinogenesis into a long period of slow growth, followed by a shorter period of rapid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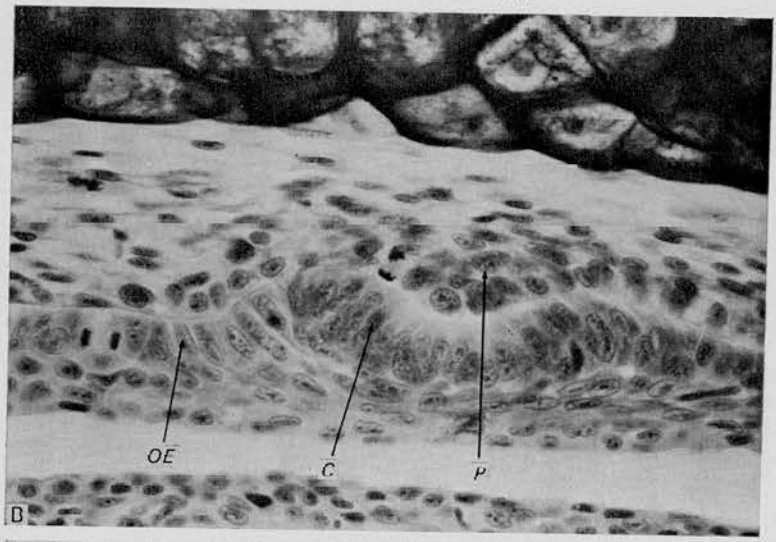
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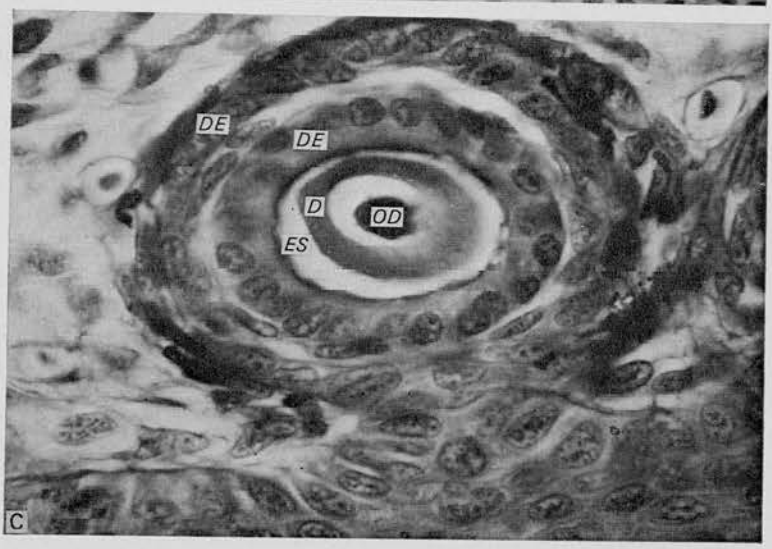
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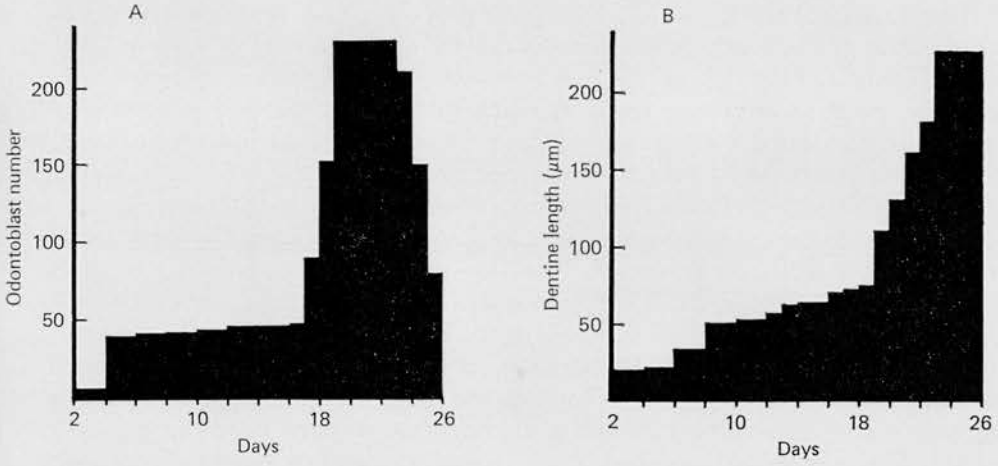


Fig. 5. (A) A histogram showing the average number of odontoblasts present in the tooth germs over the first 26 days of tooth development. (B) A histogram showing the average length of the dentine of the tooth germs throughout the same period as A. The number of odontoblasts remained low during the slow growth phase (days 1–17). However, from day 18 onwards, the number of odontoblasts increased dramatically as the rapid growth phase began; this was accompanied by a concomitant increase in dentine length. The 2 days lag period between the increase in odontoblast number and the increase in dentine length on days 17–19 may be an artefact produced by the method of sampling the specimens. After the maximum length of dentine was attained the number of odontoblasts dropped rapidly to zero. On day 27 there were no odontoblasts present.

growth. This was reflected in a rapid increase in the length of the dentine and number of odontoblasts during the rapid phase.

From day 2 onwards there was a comparatively slow increase in the number of odontoblasts in the dental papilla until on day 17 there was an average of only 48 (4) odontoblasts present (Fig. 5). They had produced a cone of dentine of average length 72 (4·5) μm, the walls of which were 3·5 (0·3) μm thick. Therefore, in 15 days only one third of the final length of the dentine had been produced. Day 19 marked the end of the slow phase of dentinogenesis.

Formation of the enameloid began shortly after dentinogenesis had begun and continued until about day 10. In decalcified sections the early enameloid matrix stained a deep purple with haematoxylin. However, from about day 8 onwards the matrix was no longer present after decalcification, and an enameloid space was left in tissue sections. Formation of the enameloid matrix and its subsequent calcification appeared therefore to encompass a fairly short period in tooth formation.

Fig. 4. (A) A low power coronal section of the head of a larva showing the upper (*UJ*) and lower jaws (*LJ*), and the general position of the tooth germs. The even-numbered tooth germs (EG) have commenced dentinogenesis and enameloid formation, while the odd-numbered tooth germs (*OG*) are still at the 'trough' stage. Decalcified, stained haematoxylin and eosin. ×16. (B) A transverse section of a tooth germ around day zero of its development. The basal cells of the oral epithelium (*OE*) have become columnar (*C*) and form a trough in which lie the future papillary cells (*P*). Frequent mitoses are visible at this stage. Decalcified, stained haematoxylin and eosin. ×160. (C) A transverse section of a tooth germ on day 8. The dentine (*D*) is visible as a ring surrounding the odontoblasts (*OD*). Outside the dentine is the space (*ES*) left by the enameloid after decalcification; a small amount of enameloid matrix is still present and has collapsed on to the inner surface of the dental epithelium (*DE*). Decalcified, stained haematoxylin and eosin. ×256.

During days 18, 19 and 20, there was a sudden and dramatic change in the appearance of the tooth germs, which heralded the beginning of a period of rapid dentinogenesis. First, the germs had begun their re-orientation to a more vertical position prior to eruption; secondly, each first generation even-positioned tooth germ had developed a dental lamina for its successional second generation tooth; and thirdly, there was a resumption of cell division in the mesodermal pool and a rapid differentiation of odontoblasts from that pool. The mitotic activity was such that by day 20 the total number of cells in the papilla averaged 230 (24.3), nearly all of which were active odontoblasts. This was approximately five times the number of odontoblasts present at day 17. The length of the dentine now averaged 189 (9) μm with walls 7·2 (0·2) μm thick. The base of the dentine wall showed for the first time, tapering at its basal edge, indicating a rapid deposition of material. In Figure 6 it can be seen that the odontoblasts forming the tip of each tooth presented a different appearance to those forming the base which had differentiated during the rapid phase. The odontoblasts at the tip were squatter than those at the base, and each possessed more than one odontoblast process. The basal odontoblasts were tall columnar cells with only one odontoblast process.

The pulp cavity of each tooth now appeared to be filled only with odontoblasts and a blood vessel loop. The number of odontoblasts was maintained while the teeth erupted and the dentine attained its maximum length. The average length of the dentine was now 225 (2) μm with walls 8·4 (0·4) μm thick, and these values were reached on day 25. Between days 17 and 25 the dentine had more than tripled its length. Attachment to the pedestal commenced on day 23, and was completed by day 25. However, just before the completion of metamorphosis of the animals, the odontoblasts at the tips of the teeth were showing signs of degeneration. The cells had become flatter, and lay with their long axes orientated in the plane of the inner surface of the dentine. Their nuclei stained lightly, but some appeared pyknotic. This degeneration of the odontoblasts continued into the basal part of the teeth until day 27, when no active odontoblasts were seen. In their place was a small number of flattened cells, many of which lay along the inside of the dentine with their long axes parallel to its surface (Fig. 1C). On day 27 the teeth in the odd-numbered tooth positions began their rapid growth phase.

The resorption of the even-numbered teeth occurred during days 32 and 33. Prior to the beginning of resorption, the connective tissue of the dental pulp was loosely woven and relatively acellular and avascular. At the beginning of resorption the connective tissue of the pulp became denser, more cellular and more vascular. Osteoclasts began to appear around the bony pedestal supporting the teeth. The soft tissue changes just described were seen in several of the post-metamorphic animals. However, the final phase of the resorptive process was so rapid that it was only observed in two specimens. In these two specimens large osteoclasts had invaded the pulp and were rapidly resorbing the dentine piecemeal from its pulpal aspect (Fig. 7). In those specimens in which the final phase of resorption was not seen, missing teeth indicated that it had occurred. Since developmentally missing teeth were not observed in any pre-metamorphic specimens, it is reasonable to assume that the teeth missing on days 33 and 34 had been resorbed.

The most striking aspect of the final resorptive process in the first generation teeth was the speed of the event. It is estimated that this phenomenon took only 12–24 hours.

The timing of the events in tooth development are summarised in Figure 8.

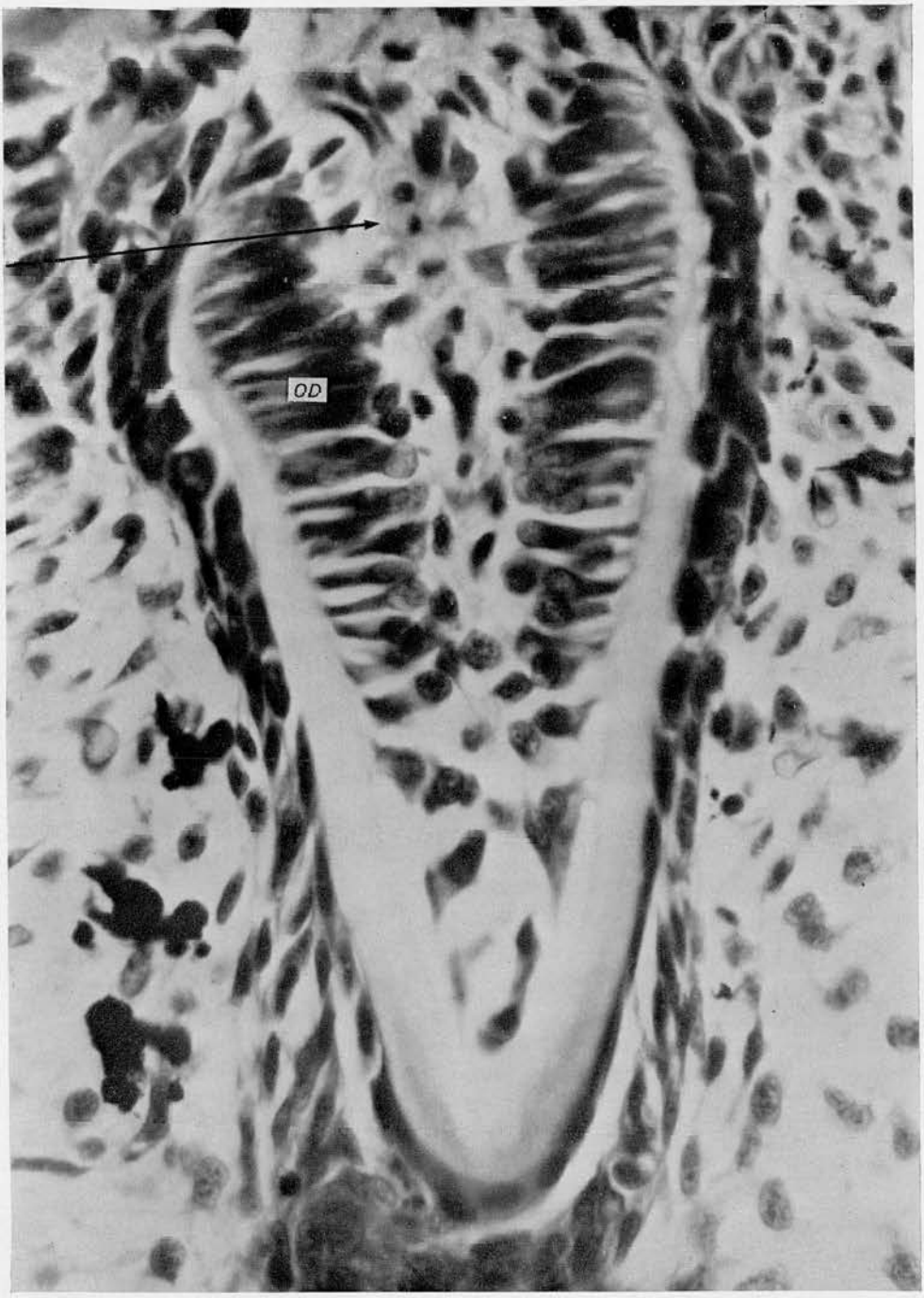
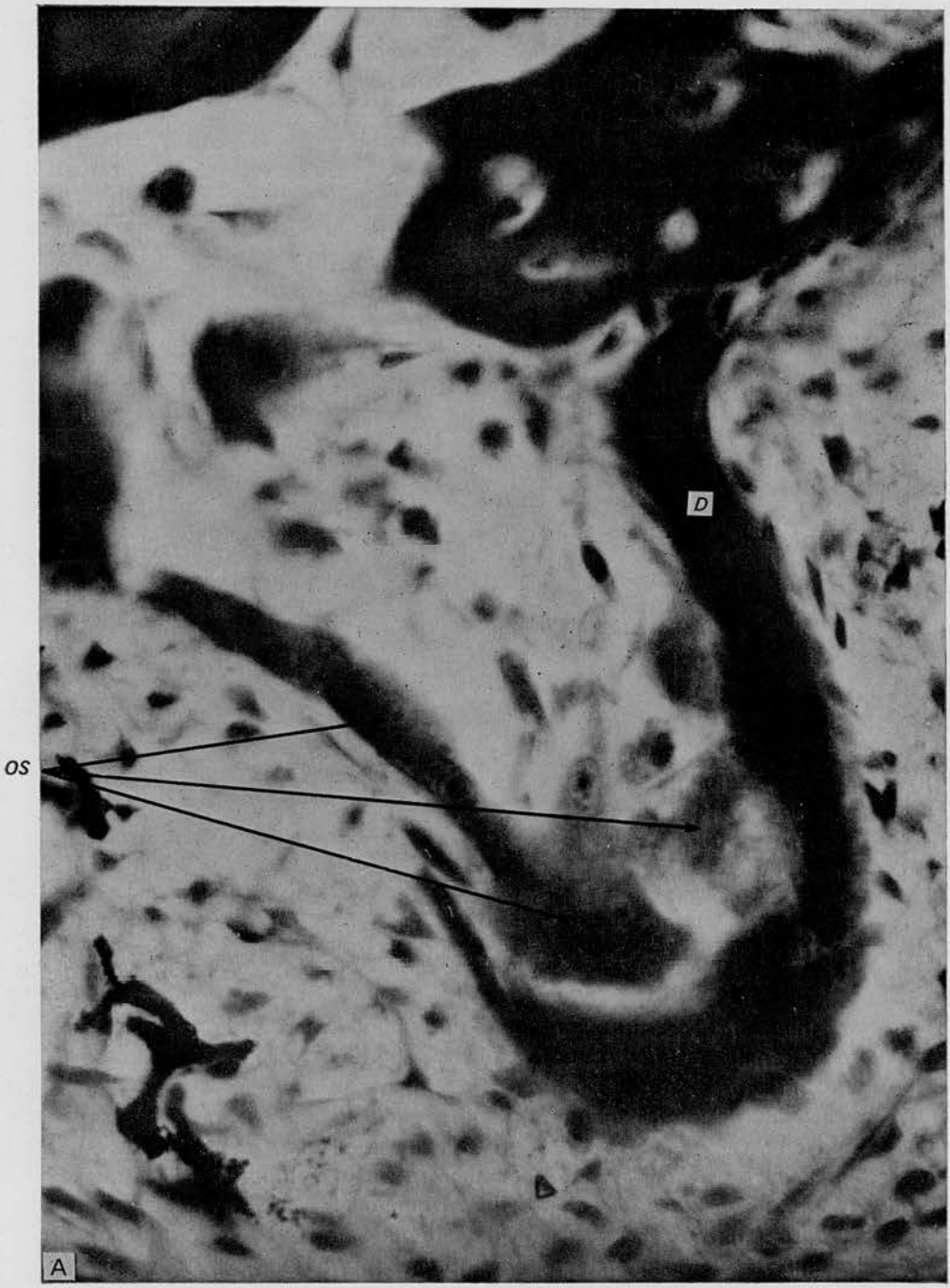


Fig. 6. A longitudinal section of a tooth germ during the rapid growth phase (day 20). The basal region of the pulp is filled with tall columnar odontoblasts (*OD*) and blood vessels (*BV*). Note that the odontoblasts at the tip of the tooth are squatter than those at the base (see text). Decalcified, stained haematoxylin and eosin. × 160.


D
OS
A

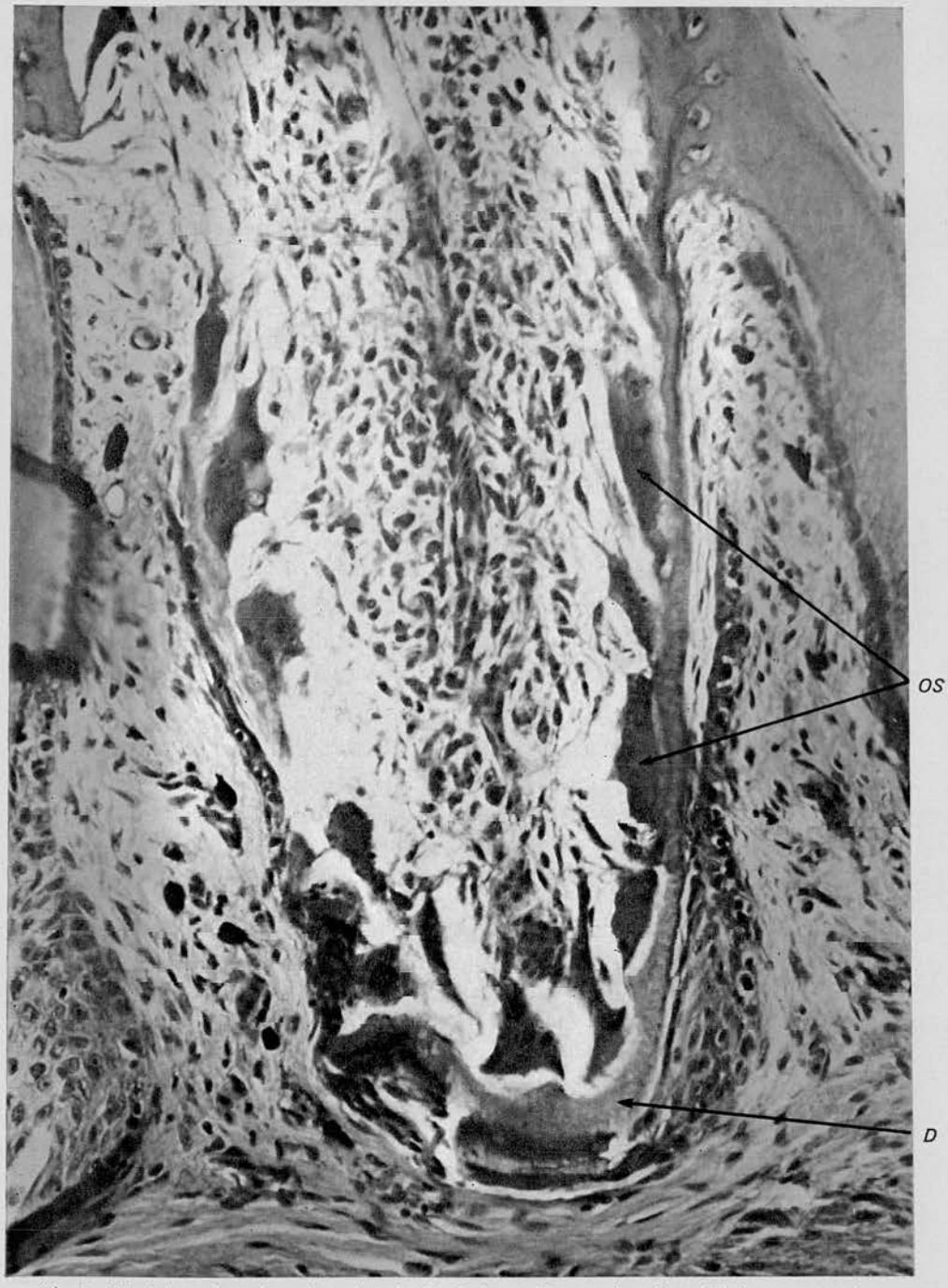


Fig. 7. (A) A larval tooth undergoing the final phase of resorption (day 32). Large osteoclasts (*OS*) are present in the pulp and one wall of dentine has been completely resorbed. *D*, dentine. Decalcified, stained haematoxylin and eosin. × 160. (B) A tooth from an 18 months old adult specimen undergoing the final phase of resorption. Note the cellularity of the pulp. (Compare with Fig. 1B.) The osteoclasts (*OS*) appear to be actively resorbing the dentine (*D*). Decalcified, stained haematoxylin and eosin. × 64.

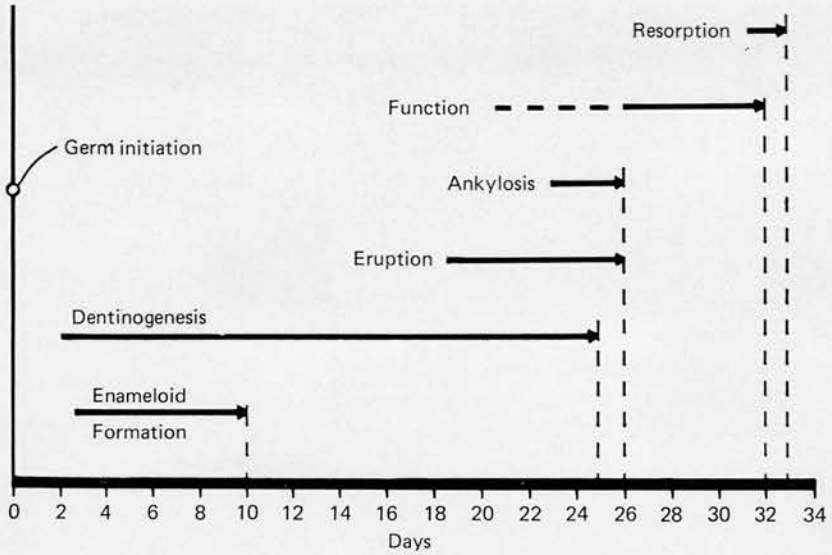


Fig. 8. A diagram summarising the various events in the developmental cycle of the individual teeth relative to the absolute time scale involved. The length of each horizontal arrow indicates the duration of the process written alongside it. Eruption is defined here as starting at the beginning of the rapid phase of dentinogenesis. During the final stages of eruption, dentinogenesis ceased and the teeth were carried to their final positions by the formation of the bony pedestal. Function is represented by a broken bar between days 20 and 26, since the teeth may have been functional between the time they pierced the oral epithelium and became ankylosed.

A comparison of larval and adult teeth

In order to assess indirectly the rate of tooth replacement in the adult animals, the number and height of the odontoblasts associated with dentinogenesis in the fully developed adult teeth were estimated. The count was done by the same method as was used for the larvae (see Materials and Method). By counting all the odontoblasts in adult teeth which were undergoing ankylosis, an estimate of the maximum number of odontoblasts required to produce each adult tooth was obtained. This allowed a comparison to be made with the maximum number of odontoblasts associated with dentinogenesis in the larval teeth. Table 2 shows this comparison, and the length and thickness of the dentine of larval and adult teeth.

Table 2. *Variation of cell numbers and quantities of dentine with age in Xenopus laevis*

Age of animal	Average thickness of dentine of erupted teeth (μm)	Average length of dentine of erupted teeth (μm)	Average number of odontoblasts producing this length of dentine	Average height of odontoblasts in basal $\frac{2}{3}$ of tooth (μm)
Newly metamorphosed*	8·4 (0·4)	225 (2)	230 (24·3)	17·2 (2·8)
18 months†	36 (2·1)	840 (20·6)	2427 (139)	25·3 (3·5)

*, Fifteen animals, six teeth from each animal.
†, Four animals, four teeth from each animal.
The numbers in parentheses are the standard deviations.

DISCUSSION

The Zahnreihen Theory

There are many features of the dentition of *Xenopus laevis* which throw doubt upon the relevance of the Zahnreihen Theory in this animal.

Although the Zahnreihen Theory may be a useful descriptive tool, it has little heuristic value: its principal *raison d'etre* is to explain how, in a situation of repeated loss and replacement of teeth, a constantly functioning dentition is maintained. De Mar (1973) has constructed models of the permissible spacings between Zahnreihen which would be compatible with the maintenance of a fully functional dentition. In terms of the Zahnreihen Theory, alternate replacement of teeth is a 'special case' in which the Zahnreihen are two tooth spaces apart.

However, it is questionable whether many amphibians require a fully functional dentition at all times, since they are poikilothermic animals with a comparatively low metabolic rate and do not require a constant food intake. Indeed it is hard to ascribe any function at all to the teeth of *Xenopus laevis*, since only a few microns of each tooth tip actually protrudes through the oral epithelium into the mouth. The prey is taken by a rapid forward thrust of the animal, with its mouth wide open. As the open jaws envelop the prey, the forelimbs are used to push it back into the mouth, which is then closed. The arrangement of the soft tissue ridges in the upper and lower jaws not only acts as an effective seal, but these are probably more effective in gripping the food than the teeth. Bufonids manage to capture and devour food without teeth. A rapid turnover of the teeth in a dentition cannot be taken as sure evidence that such a dentition is of great functional value, as is suggested by Peyer (1968) for *Rana pipiens*. Nor can function be accepted as the prime impetus for tooth development.

The Zahnreihen Theory proposes that tooth formation is controlled by a series of 'stimuli' passing backwards along the jaw and initiating a series of tooth germs at successive loci. No physical basis for such stimuli has ever been found. In larval *Xenopus laevis*, tooth formation was observed to begin at the back of the mouth and progress forwards on each side to the mid-line. Osborn (1971) has observed a similar phenomenon in *Lacerta vivipara*. He suggested (Osborn, 1970) that once a row of alternating teeth was developed in sequence from the back to the front of the jaw, autonomous control of tooth development at each tooth position would maintain a wave-like replacement of the teeth.

One consequence of the Zahnreihen Theory is that the presence of any apparently alternating sequence of tooth replacement is produced as a secondary effect of successive Zahnreihen. However, in larval *Xenopus* the teeth developed *ab initio* as an alternating series, and a similar alternation appeared to be borne out histologically in the adult.

In larval *Xenopus*, tooth germs developed in sequence from back to front at about 40 loci in the upper jaw. More tooth loci were present in adult *Xenopus*. Two or three of the additional loci appeared in the larvae during metamorphic climax, and their production has been observed to occur from front to back posterior to the existing teeth. This difference in the direction of tooth production as the animal grows seems incompatible with any concept of 'stimuli' moving undirectionally mesially or distally.

Goin & Hester (1961) attempted to trace the Zahnreihen in adult *Xenopus* and

admitted that they were difficult to determine. Many workers describing Zahnreihen utilised whole mount, cleared, alizarin-stained preparations for their investigations. However, the histological investigation undertaken here indicated that not only was the final phase of resorption extremely rapid, but also that the early stages of resorption involved only soft tissue changes, and could not be visible in whole mount specimens. The soft tissue changes involved alterations in connective tissue density in the pulp, and the accumulation of comparatively inactive osteoclasts at the tooth apex. These changes would remain undetected in whole amount preparations and would obscure the temporal status of standing teeth, not only in *Xenopus*, but possibly in other amphibian species also. Standing teeth which were about to be resorbed might thus be assigned to the wrong Zahnreihe. In this respect Lawson (1966), describing the Zahnreihen in *Rana temporaria*, states that there was a large proportion of apparently retained standing teeth. A similar phenomenon has been noted by Goin & Hester (1961) in *Hyla cinerea*.

The rate of tooth development and replacement

Although the first generation of even-positioned teeth of *Xenopus laevis* erupted during metamorphic climax, it was not easy to assess the manner in which tooth development was related to the hormonal changes associated with metamorphosis. Tooth development began during prometamorphosis and continued until the end of metamorphic climax. Development of the first generation teeth therefore encompassed a similar period in the animal's life cycle as hind limb development. However, unlike the hind limbs, development of succeeding generations of teeth continued after metamorphosis and followed the same stages as the first generation teeth over a similar time scale.

Dentinogenesis was a biphasic process, a long slow phase being followed by a shorter rapid phase. This did not seem to be the most direct method of producing the dentine composing the comparatively simple conical teeth of *Xenopus laevis*. However, dentine production depends on the presence and activity of odontoblasts, and it is possible to view the variations in the rate of odontoblast differentiation observed in this study as being part of a monophasic control process if it is speculated that the odontoblasts control their differentiation and activity autonomously by means of a hypothetical humoral factor which they themselves produce. Such humoral mechanisms of control of cell differentiation and activity are not unknown biologically (Konigsberg, 1971; de la Haba & Amundsen, 1972).

However that may be, the complete tooth cycle of the even-numbered first generation teeth was about 33 days. In the frog *Rana pipiens* Gillette (1955) calculated indirectly that the duration of the individual tooth cycle was about 90 days. Gillette's estimate was based on a constant rate of dentine deposition, but as demonstrated here in *Xenopus laevis*, the rate of dentine deposition may vary.

In *Xenopus* each first generation tooth spent only about 7 days in its functional position – a mere quarter of the time taken for its production. Thereafter each tooth was rapidly replaced by its successor. While the first generation even-numbered teeth were being resorbed, the second generation even-numbered teeth were just beginning their rapid growth phase, and were therefore about 16 days later in development. The members of the odd-positioned tooth series probably had a replacement cycle of similar duration, but 8–9 days out of phase with the even-positioned tooth series.

It is possible that the possession of a dentition whose members are rapidly replaced may be of advantage to an animal, in that damaged teeth would be quickly replaced. However, it seems more likely that the short period spent by each tooth in its erupted position indicates that the teeth may not subserve any important function in *Xenopus laevis* at all, and that the speed of tooth replacement may be linked to a fundamental embryological process involved in the development of the successional teeth.

A direct measurement of the rate of tooth replacement could not be made in the adult animals because, from the nature of the material, a precise time scale was not available for either longitudinal or cross sectional studies. However, the measurements (Table 2) indicated that the adult teeth were formed by greater numbers of odontoblasts than the juvenile teeth, and that the odontoblasts in the adult teeth were also of greater height than those in the juvenile teeth. These facts may indicate that they were more active as dentine producers. If this is so, it seems probable that in both juvenile and adult *Xenopus laevis* the time taken for tooth development may be of a similar order. This view is supported by the finding in all the adult specimens, of whatever age, of several teeth undergoing resorption.

It is more than possible, however, that in the wild there would be seasonal variations in the rate of tooth replacement, as reported by Miller & Rowe (1973) for *Necturus maculosus*. Also, tooth replacement may be prolonged in senescent animals, and, further, it is questionable whether tooth succession is kept up indefinitely.

SUMMARY

One hundred and seventy two larval specimens of *Xenopus laevis* were reared in such a way that their rates of development (as measured by external criteria) were similar, and so the course of dental development could be examined histologically in a cross sectional study. In this way the events of tooth development were observed, and a time scale constructed for these events. The teeth took an average time of 26 days to develop, erupt and become ankylosed to the bony pedestal, after which each tooth was in a functional position for only about 7 days. Individual tooth replacement was assessed to occur about every 16 days.

By comparing the number and size of the odontoblasts responsible for dentinogenesis in 18 months old adult *Xenopus laevis* with the odontoblasts in the larval specimens, the conclusion was drawn that, despite the larger size of the adult teeth, the time involved in their development and replacement may well be of similar duration to the smaller larval teeth.

The significance of the findings for the Zahnreihen Theory is discussed.

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Tooth resorption in Xenopus laevis

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Ninety five specimens of newly metamorphosed Xenopus laevis were examined to analyse the events of tooth resorption, and to obtain a time scale for these events. The results suggested that tooth resorption may be separated into two processes (1) erosion and (2) resorption proper.

Erosion was a slow process extending over several days, and involved the removal of a small, variable quantity of tissue from the bony pedestal and base of the standing tooth; the degree of this erosion depended on the proximity of the successional tooth to the standing tooth. During this period no osteoclasts were observed within the pulp, but appeared to be confined to the outer aspect of the bone and dentine, where they were removing the hard tissues from without. Two specimens showed abnormal erosion and despite the removal of a large proportion of dentine in these cases, resulting in contact between the pulp and the surrounding connective tissues over a large area, no internal resorption was observed. Although erosion was followed by resorption proper, these two processes did not seem to be interdependent. *Resorption proper was a rapid process lasting less than 24 hours. It was characterised by the infiltration of the pulp by osteoclasts, which resorbed the dentine piecemeal from within along its full length from the base to the tip of the tooth. The vast majority (in probability all) the dental tissue was resorbed, and its constituents were therefore made available to the animal for recycling. It is suggested that while erosion may be due to local mechanical factors associated with the growth of an underlying successional tooth, resorption proper may depend on a more specific, intrinsic timing mechanism.

* For resorption proper read absorption.

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Time Scale of Tooth Development and Replacement in Adult Xenopus laevis.

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Shaw (1979, J. Anat. 129 (2), 323-342) has estimated an absolute time scale for tooth development and replacement in larval and newly metamorphosed Xenopus laevis. The present study was an attempt to determine an absolute time scale for tooth development in adult Xenopus laevis. Three large females (snout-vent lengths 112 mms, 107 mms, and 105 mms) were maintained for about 10 weeks at 21°C.* Twice weekly each animal was anaesthetised with MS 222 and impressions taken of its teeth using gold casting wax. The very small size of the teeth necessitated the making of camera lucida drawings of each impression. Tracings of such drawings laid over drawings of subsequent impressions enabled a longitudinal study of changes in the dentition to be made. A statistical analysis of the resulting data yielded the following results:-

Animal 1: erupted life of individual teeth, 660 ± 10 hours; teeth replaced every 930 ± 10 hours; total tooth development cycle time 67.22 ± 8.7 days.

Animal 2: erupted life of individual teeth, 590 ± 10 hours; teeth replaced every 1010 ± 10 hours; total tooth development cycle time 60.09 ± 7.8 days.

Animal 3: erupted life of individual teeth, 580 ± 10 hours; teeth replaced every 910 ± 10 hours; total tooth development cycle time 59.07 ± 7.6 days.

The total tooth development cycle time is obtained by extrapolation from the results of Shaw (1979, see figure 8 page 338).

* For 21°C read 25°C.