THE EFFECT OF RADIATION ON THE DEVELOPMENT AND GROWTH OF TEETH: A CRITICAL REVIEW OF THE LITERATURE.

This review is submitted as part of the requirements for the candidature of the Author for the degree of Master of Dental Surgery, University of Sydney.

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A C K N O W L E D G E M E N T S

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I N T R O D U C T I O N

This critical review of the literature has been undertaken as a result of an enquiry by a dermatologist (Poyzer, 1971) as to the radiosensitivity of developing teeth.

He pointed out that many such specialists are irradiating certain types of "birth marks" (haemangiomas) on the faces of infants and young children with as much as 800r (Worth, 1963), to induce these disfiguring and often psychologically traumatic lesions to disappear. Despite the fact that many of these lesions disappear with time anyway, tremendous parental pressure is put on dermatologists to "do something" immediately. Radiation appears to be the treatment of choice in these situations, as other forms of treatment, e.g. cryotherapy, surgery and sclerosing solutions, are likely to produce unsightly scarring.

Compared with many other topics, there is scant literature on the subject. I have attempted to survey all the English language reports available up to and including those listed in the second quarter of the publication "Dental Index" (1973) and to present this survey in the form of a critical review. This has not been an easy task. The earlier authors were severely hampered by a lack of fundamental knowledge of radiotherapy theory.

Many of the later authors either failed to give sufficient details of physical constants used, and thus their experiments cannot be repeated or directly compared with others, or else, although obviously expert radiotherapists or radiologists, they had little knowledge of dentistry.

The literature is readily divisible into two types of reports:

1. The case histories of treated human subjects and the effects produced.
2. The reports of experiments involving laboratory animals.

The basic format for the second group, the experimental literature, has been to give a detailed review of the well documented reports, there being rarely more than two or three such reports on any particular aspect of odontogenic radiobiology, and, at the appropriate places, to add the observations of other authors.

Finally, I have attempted to draw some meaningful conclusions.

A SUMMARY OF TOOTH DEVELOPMENT

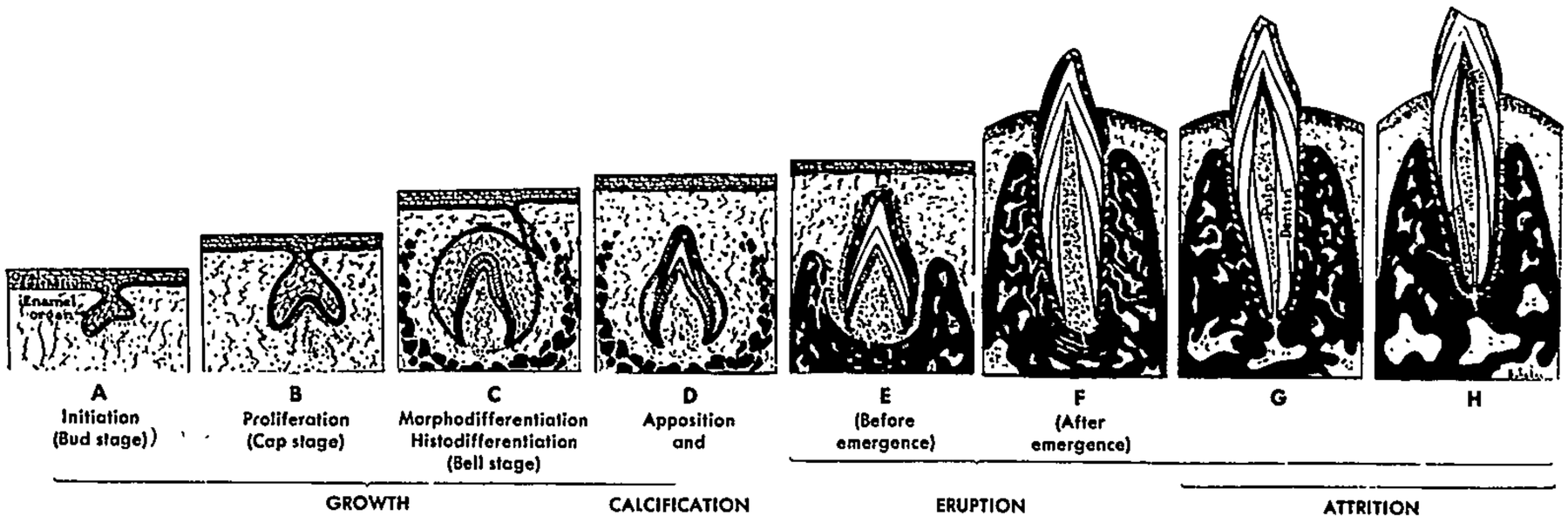
The dental lamina arises in the primitive stomodeum from the 34th to the 38th day (11 mm. C.R.). So predictable is the time at which the teeth develop that odontogenesis has been recommended as a means of determining foetal age (Garn et al, 1970). Tonge (1969) noted that at the 21 mm. C.R. (36-38 days) the incisor areas of the human foetus exhibit "disturbed areas of odontogenic epithelium but that invagination had not occurred".

The dental lamina consists of an epithelial thickening near the free margin of each jaw, and is the anlage of the enamel organ. By a process of rapid and varying proliferation the basal cells of the dental lamina invade the underlying mesenchyme and successively form the Bud, Cap and Bell stages of tooth development. (See Fig. 1, Page 4).

In response to this epithelial proliferation, and not merely a condensation of mesenchyme, the underlying mesenchymal tissue becomes highly organised and can be readily differentiated from the adjacent connective tissue. Blood vessels become more numerous and the cells adjoining the inner enamel epithelium become larger, columnar and polarised. These cells are the odontoblasts.

Fig. 1.

Successive stages of development of a tooth derived from a non-persistent pulp.
(From Orban, 7th ed.)



The Tomes' processes of the ameloblasts become surrounded by matrix material and separated from the cells proper by terminal bars. They then become transformed into matrix substance secreted by the ameloblasts. This process of formation, separation and transformation of the Tomes' processes is repeated until the full width of the enamel matrix is formed.

(b) Mineralization and maturation.

These occur as two separate but closely related events. Initial mineralization (25-30% of total) occurs as the rods and interprismatic substance are laid down. Maturation then follows as a gradual mineralization starting at the dentino-enamel junction and at the crown tip, and proceeding towards the surface and the cemento-enamel junction.

2. DENTINOGENESIS.

The outer spindle shaped cells of the dental papilla adjacent to the stimulating inner enamel epithelium undergo differentiation, becoming aligned along the basement membrane and seemingly attached to it by several short processes. The nuclei are basally placed and the cells become markedly elongated. They begin to take on the appearance of actively secreting cells by containing many organelles and a pronounced endoplasmic reticulum.

The mature, nondividing odontoblasts then begin to move away from the dentino-enamel junction, laying down dentin. As they recede they leave the odontoblastic processes embedded in the dentin matrix. Initial dentin formation occurs at the cusp tips and progresses in a conical fashion towards the pulp and the cemento-enamel junction.

Predentin, the uncalcified organic matrix, precedes the formation of the mineralised substance.

Korff's fibres appear as fibrillar bundles between the odontoblastic cells. They are a major constituent of the dentin matrix, and the collagen content is vital for the proper mineralization of the new dentin. The periodicity of collagen has been found vital to the process of mineralization.

Mineralization.

This begins at the dentino-enamel junction following the laying down of sufficient predentin. Beginning in small discrete islands it advances towards the pulp and becomes a confluent and almost homogeneous material.

3. ROOT FORMATION.

Following completion of the crown portion of the dentin, future dentin formation of the root is influenced by Hertwig's epithelial root sheath derived from the epithelial dental organ.

This double layer of epithelial cells stimulates the differentiation of odontoblasts from the mesenchyme.

Disintegration of the epithelial root sheath (to form the Cell Rests of Malassez) allows direct contact of mesenchyme with the outer dentinal surface. Cuboidal cells, cementoblasts, derived from this mesenchyme, lay down a comparatively thin layer of cementoid tissue which then matures to cementum.

RADIOBIOLOGY: SOME RELEVANT FACTS

It is not the purpose of this review to explain the physics of ionizing radiation or the rationale of radiotherapy. However it is necessary to preface a discussion of radiobiology with some relevant facts, based upon Upton (1963) and the British Medical Bulletin (Jan. 1973).

Radiotherapy began approximately 60 years ago, and has had problems in its development similar to those of any other developing medical speciality. From a complete ignorance of dose measurement, dose-time relationship and even the radiosensitivity of the disease in question, radiotherapy has matured to a highly sophisticated and reasonably rational form of treatment.

The effect of irradiation is one of transfer of energy to the diseased cells and this is usually achieved by the use of X-ray, electron or neutron beam generators, or radioactive isotopes emitting β-particles. However the effects of radiation from different sources are similar (Warren, 1944).

This energy transfer is to all irradiated cells, i.e. both host and diseased tissue alike within the path of the beam, and is only of value when its effect on the unwanted cells is significantly different to that on the host cells.

Bergonie and Trebondeau, near the turn of the century, stated that the radiosensitivity of a cell is proportional to its functional and morphological differentiation. This is a good general rule, but there are some quite graphic exceptions.

1. RADIATION CHEMISTRY.

Biological molecules may be affected in two ways:

(i) direct "hit", or energy transfer,

(ii) formation of excited ionised atoms and molecules which promptly react with biological macromolecules. Water molecules are believed to play a large part in the process with low energy transfer (LET) irradiation. Formation of these active radicals is enhanced by a high partial pressure of oxygen.

The effect of both (i) and (ii) is to transfer the energy to biological molecules. This absorbed energy tends to become concentrated in particular molecular bonds.

As irradiation is a "hit or miss" phenomenon its effect may be of little or no consequence, or lethal to that cell. However, whatever the actual mechanism of injury it is apparent that only small amounts of energy are involved.

2. THE CHROMOSOMES.

Radiation is known to cause breakage of the chromosomes, presumably in a random manner at or near the point of energy absorption.

Following such a break a number of things may follow:

(i) reunion of the broken ends,

(ii) union with the broken ends of adjacent chromosomes,

(iii) remain unjoined, leading to the loss of genetic information at anaphase.

Other induced changes include surface "stickiness" and inactivation of the centromeres. The full significance of these effects of radiation induced changes in the chromosomes is as yet little understood. However, the radiosensitivity of individual cells is variable, generally being high at or following chromosome replication.

3. DAMAGE AND REPAIR TO NUCLEIC ACIDS.

As the reproductivity of a cell is one of its first activities to be affected by irradiation, an obvious target for sublethal damage is the desoxyribonucleic acid (DNA) of the nucleus.

Irradiation has been found to produce cross linking either within the DNA strand or between paired strands. However, the nature of such links is unknown.

Damaged portions of the DNA strand appear to be excised with the aid of enzymes and the exposed area subject to "repair synthesis" or "unscheduled synthesis".

The capacity for repair to DNA exists throughout the cell cycle and a significant increase in the molecular weight of DNA, indicating rejoining of broken strands following irradiation, has been found to occur within 20 minutes of irradiation.

Rejoining of broken strands may also be accomplished by the filling in of the gap with new nucleotide sequences. Although it is obvious that the change of the sequence of nucleotide bases may or may not have an effect on the cell chemistry and that these changes occur in a random manner, the importance of this type of repair is unknown.

Cell death will occur for many different reasons as it is unlikely that more than a small percentage of cells should be injured in identical ways by a direct "hit".

4. MITOSIS.

The effects of non lethal irradiation doses on cellular mitotic division is characteristic and includes:

(i) An initial decline in numbers of dividing cells,

(ii) A following period in which the numbers of dividing cells ~~are~~ remain reduced, the length of this period being dose dependent,

(iii) An increase of mitotic activity to a level exceeding that of the pre-irradiated tissue,

(iv) A gradual settling back to a level approximately equal to that of the pre-irradiated tissue.

5. CELLULAR MORPHOLOGY.

Changes occur within the cells similar to those seen for most non-specific toxic stimulants. These changes include:

(i) Nuclear swelling, pyknosis and vacuolation,

(ii) Nuclear budding and lobulation,

(iii) Karyolysis,

(iv) Chromatin clumping,

(v) Extrusion of chromatin,

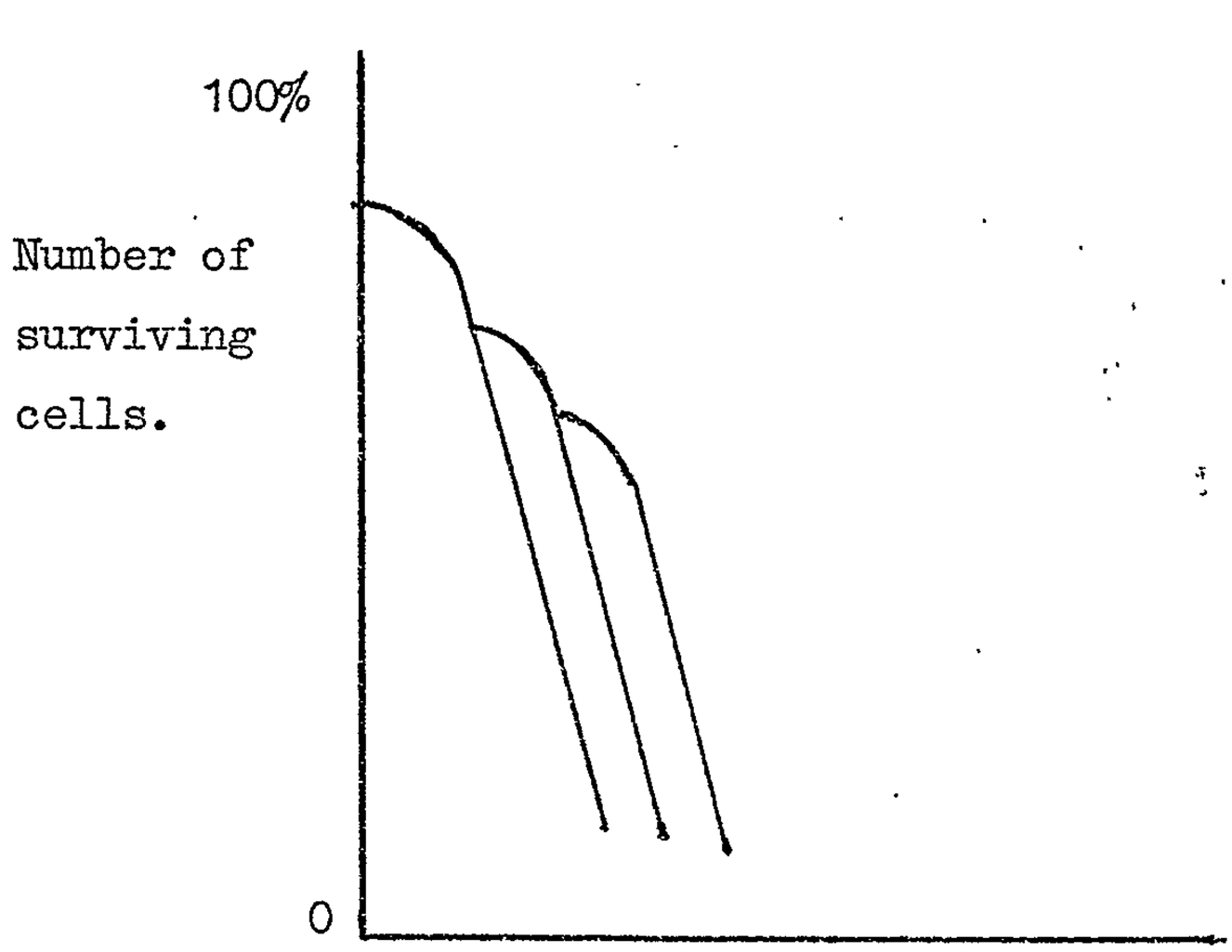
(vi) Loss of mitochondria.

These changes can often be detected within minutes of the irradiation.

6. CELL SURVIVAL CURVES.

The relationship between cell survival and radiation dose is often demonstrated by a graph plotting the surviving fraction of cells, i.e. those which have not lost their capacity to proliferate, on a logarithmic scale, against dose on a linear scale.

When plotted for LET radiation, ~~the type used for all reviewed experiments,~~ with the exception of neutrons which is a form of HET radiation, mammalian cells show a characteristic initial region of low sensitivity. This initial low sensitivity produces a shoulder on the graph. The shoulder suggests that diploid and polyploid cells can receive some degree of nonlethal injury before being subject to death by additional energy transfer. Fractional dosages produce a series of shoulders and this indicates that cells are able to repair some of their radiation injuries between doses.



Such cell survival curves have been found to predict, quite accurately, the response of haemopoietic death and intestinal death in mice. The curves were derived from studies of tissue cultures of the respective stem cells. However they do not take into account such factors as variation of oxygen tension in a given tissue mass and so can only indicate cell sensitivity and are therefore not necessarily accurate for tissues or tumours.

The extent of the shoulder, i.e. the size of the nonlethal dose, varies between the different types of mammalian cells and there are many other factors which affect this property (Dq) in a given cell colony. It has been found that for a given cell line the value of Dq can range between almost zero for cells in mitosis and 700 rads for those in late synthetic phase.

Consequently the ability of a cell to divide is usually lost before its ability to survive. This factor of sublethal damage, however, may also produce characteristically poor response of irradiated tissue to injury. Much of this response can be attributed to poor blood supply, but something more than ischaemia[a] is necessary to explain the onset and the characteristics of many of the "so-called" delayed effects observed in the following reports.

7. DEFINITIONS AND ABBREVIATIONS.

HVL	-	half value layer
Kv, KVP	-	Kilovoltage potential
m.a.	-	milliampere
r, Roentgen	-	production of 1.610×10^{12} ion pairs/gm air
R, Rad.	-	absorption of 100 ergs/gm (190R = 200r very approximately)
TSD	-	target (X-ray tube) skin distance.

HUMAN CASE HISTORIES

"As a result of continuing advances in the techniques and modalities used in radiation therapy, a larger percentage of head and neck tumours are treated by radiation or by a combination of radiation and surgery. These advances have resulted in increased survival rates and decreased morbidity". (Blozis et al, 1968).

Evidence of the effects of radiation on developing teeth has come largely from case histories of children who have received radiotherapy. The results of such early therapy often produced deformities and problems, such as that of osteoradio-necrosis, greater than those of the initial lesion.

The first report of radiation damage to developing teeth was made by Leist in 1926 (as quoted by Kimeldorf et al, 1963). He found that three children of a group of six showed a "marked reduction in the eruption of deciduous teeth". The abdomens of the mothers had been irradiated with X-rays or β- emitting radium. No details of methods of application, dose, apparatus used, or even the rationale behind such treatment appears to have been given. This is true for all the earlier reports, and even as late as 1961 (Binford et al), full details were not given of experimental work, such reports concerning themselves merely with

recording the clinical results rather than the details of the therapy that produced those results. Consequently, other than noting the various deformities produced and the age at which irradiation took place, little value can be placed on such reports.

As described in Chapter 2, radiation damage is instantaneous, but the actual results of that damage may not be revealed for some time.

Partial anodontia resulting from X-radiation was reported by Blozis et al (1968), Brown (1949), Gorlin et al (1963), Rushton (1947), Stafne et al (1947), and Philip (1957). (See Figs. 2, 3 and 4, Pages 20 and 21).

Stafne et al (1947) logically stated that the observed effects of irradiation depended on:

"(i) Intensity of irradiation,

(ii) Age of the patient,

(iii) Length of interval between exposure and observations,

(iv) Susceptibility of patient",

and the degree of anodontia and other defects appear to correspond with these factors.

Point (iii) is very important, as delayed eruption is almost a constant feature of reports of irradiation of children. No other author has raised the point of patient susceptibility.

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Fig. 2.

A. Five year old patient with eosinophilic granuloma.
B. Radiograph following 6000r. The radiograph on the left was taken 18 months post irradiation and the one on the right 21 months later. Note that tooth formation has stopped and that the apex is closing.
C. Same patient at 11 years of age. Note small lower third of face.
D. Intra oral view of same patient as in C. Note partial anodontia.
(From Blozis et al, 1968).

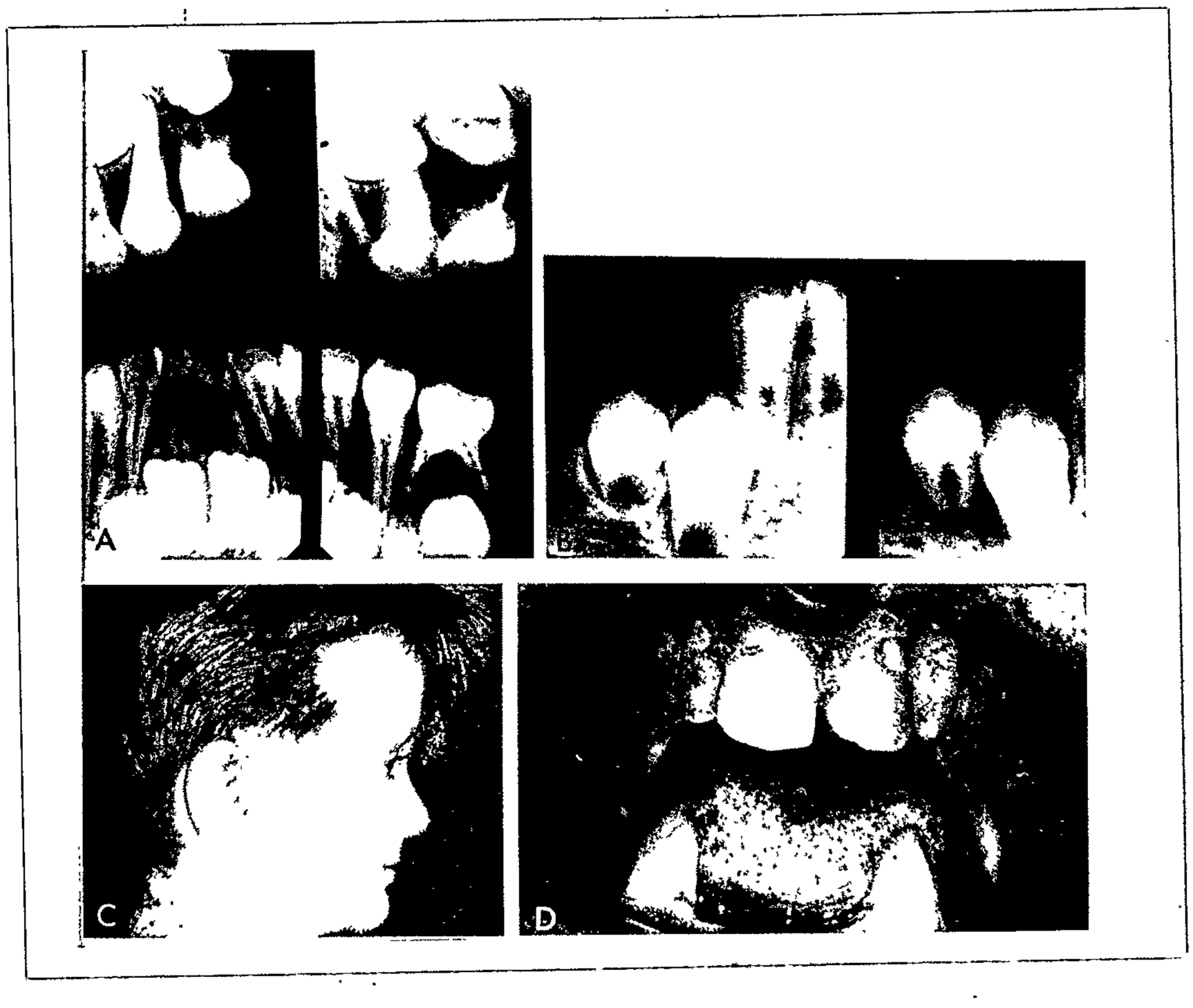


Fig. 3.

Female at age 3 months irradiated by 2 radon implants. The first gave 2000r, the second, implanted 11 days later, gave 6600r over a period of six days. Total dose of 8600r for a rapidly growing, malignant, very anaplastic, possibly angeoblastic lesion.

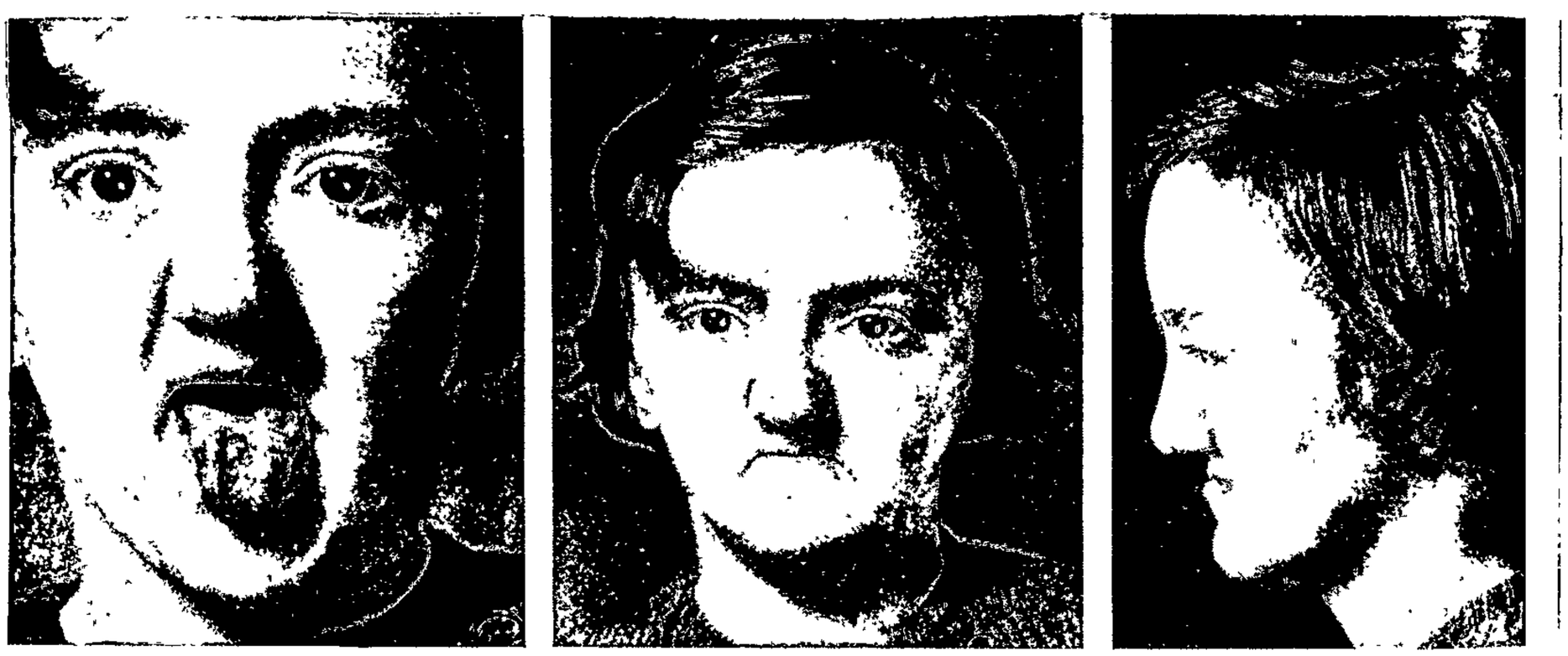


Fig. 4.

X-rays of patient in Fig. 3. Note micrognathia of maxillae (mandibular growth area, i.e. the condylar region, well removed from field), partial anodontia.
(From Philip et al, 1957).

Blozis et al (1968) stated that "3000r will stop tooth development at its current level of formation. However these teeth will usually erupt". This statement is rather misleading as he gave no indication of the stage of development at which a tooth will fail to erupt, except by saying that "teeth which have not begun to develop will be inhibited and never develop". It appears that once crown formation is complete the tooth may erupt, even without root formation. Thus the degree of anodontia is determined by the developmental stage of the teeth, and this has been confirmed by the experimental work of Gowgiel (1961) on monkeys.

Microdontia.

Dawson (1968) quoted the case of a male (case 18), irradiated for an haemangioma of the face and side of tongue at 11 weeks of age over a single 8 x 8 cm. field. The physical factors were 230 Kv. skin dose 1000r 5 cm. depth dose 580r. At 12 weeks the same field was irradiated, skin dose 580r, 5 cm. depth 290r. At 6 years of age he showed left side micrognathia and left side microdontia compared with the right side. No specific teeth were mentioned, nor the degree of micrognathia or microdontia.

He gave a further example of a male (case 24) aged 9 years (20 x 10 cm. field bridge technique from zygoma to

nipple 230 kv., maximum dose 3250r minimum dose 2900r), whose teeth were well formed but small. However, as most of the crowns of the permanent teeth were almost certainly calcified by this age, his observations were most probably incorrect.

Further reports of microdontia by Gorlin et al (1963) (total dose 7470r, right side 2650r, left side 4820r), Rushton (1947) (no therapeutic details), Philip et al (1957), Kimeldorf et al (1963) and Stafne et al (1947) (no therapeutic details), confirm the possibility of microdontia following irradiation of developing teeth. However, apart from Lyon et al (1964), who stated that at a total body dose of approximately 170r there is no effect on tooth morphology, none of the above authors attempted to give a threshold level above which microdontia occurs, nor attempted to explain the mechanics of this effect.

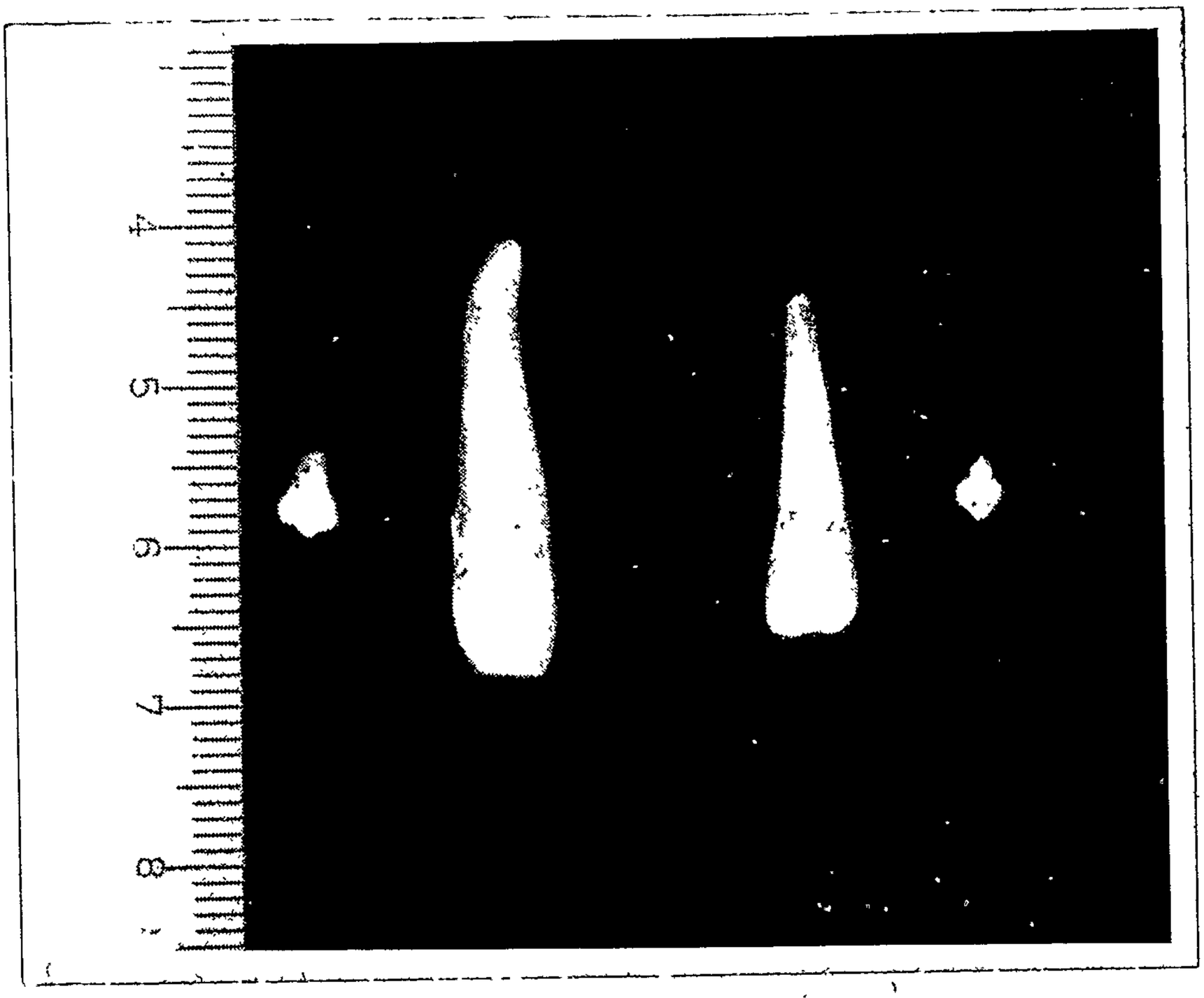
Poyton (1968) suggested that damage due to irradiation can occur as early as the tooth germ stage. Retardation of amelogenesis and impaired activity of odontoblasts, together with a decrease of mitotic activity of both ectodermal and mesodermal elements, may be responsible for the clinical findings of microdontia and anodontia. Poyton further stated that the specific damage is at a cellular level on either the chromosomes or an impairment of DNA activity, and that "... interference with the vascular supply undoubtedly has some bearing." (See Figs. 5 and 6, Page 24 and 25.)

Fig. 5.

Comparison of the models of the irradiated mandibular and maxillary arch of a 12 year old female with those of a child of comparable age.
(From Adachi et al, 1957).

Fig. 6.

Comparison of normally sized teeth with those removed from a similar position in an irradiated patient.
(From Murai et al, 1954).



Root Deformities.

Consistent with the non specific nature of radiation damage, root abnormalities are found to be quite varied in nature. Varieties of deformations have been reported ranging from those indistinguishable from dilaceration produced by trauma (Gorlin et al, 1963), to the eruption of crowns with a complete absence of root formation (Rushton, 1947).

Between these two extremes lies considerable variation in the range of root deformity. The most common finding is short conical diminutive roots which appear to complete formation earlier than expected. Bruce et al (1950), Gorlin et al (1963), Rushton (1947), Stafne et al (1947) (who also mention incomplete root calcification), Poyton (1968), Weyman (1968), Philip et al (1957), Kimeldorf et al (1963) and Worth (1963), all cited examples of such teeth.

There is no way of predicting exactly what type of irradiation or what dose will produce a specific root deformity. The influential factors appear to be:

(i) developmental age of tooth,

(ii) dose absorbed by tooth structure,

(iii) particular susceptibility of patient (Stafne, 1947).

No particular tooth appears to be more likely than any other to sustain damage, and different types of root deformity will be

found in the same patient, e.g. Gorlin et al (1963) noted partial anodontia, microdontia, dwarfing and abnormal curvature of roots and enamel hypoplasia in the same patient.

Calcification.

The effects of radiation on calcification appear unpredictable and most bizarre. Gorlin et al (1963) mentioned premature completion of tooth formation, as did Bruce et al (1950). Stafne et al (1947) reported a case of lower incisors, canines and first molars where completion occurred 3 - 6 years earlier than normal.

Poyton (1968) stated that enamel may show hypoplasia with other tooth structures barely being affected, or the converse, i.e. enamel caps may be formed but the rest of the tooth markedly affected.

Some authors have compared the teeth in the irradiated area with those in the non-irradiated area, e.g. Dawson (1968), case 18. However, no author has mentioned any history of other physical damage to the teeth or appears to have attempted to rule out familial tendencies.

Kimeldorf et al (1963) drew attention to the fact that teeth have a multiembryonic tissue source together with an excellent vascular and nerve supply. These authors also pointed out that developing teeth have their own source of progenitor cells and undergo proliferation, differentiation, growth and

secretion in a highly organised manner. Alterations in this predictable pattern of activity can readily be detected as teeth do not undergo the normal process of anabolism and catabolism which is usual for other hard and soft tissues of the body, and consequently show little capacity for repair. Thus the tooth provides a permanent record of the instantaneous damage of radiation exposure.

No attempt, other than that of Poyton (1968), has been made in the literature to explain the sporadic non-specific changes found, such as the range of root deformity and the different types of tooth damage that have been reported in the one mouth.

Poyton commented that the developmental damage started with the tooth germ and that primitive pulp cells may be so damaged that differentiation of odontoblasts does not take place.

He found that the rate of DNA synthesis was markedly depressed following irradiation of the tooth germ. This was indicated by a decrease of the uptake of radioactive thymidine when compared with the control. This finding indicates that the rate of mitosis is decreased in irradiated cells. He postulated that the radiation produced a change in the cells of the proliferation stage of tooth development.

Except in severe cases, it is difficult to diagnose radiation damage without the help of a case history. In fact,

too much weight has been placed on the evidence given by case history, and no statistical analysis has been attempted with the exception of Stasek et al (1965).

Stasek et al (1965) examined the delayed changes in a group of children (2/12 - 10 years old) irradiated with 125-300r for cervical lymphadenitis (72 cases) and hyperplasia of the thymus (5 cases). The physical constants of the irradiation were 6 x 8 cm. and 10 x 30 cm. fields, HVL 0.2 to 0.8 mm. Cu. 10 m.a. 120-160 Kv., 40-44 r/min., fractionated dosages of 10-50r each.

Although their report did not mention damaged developing teeth, it did mention three cases of facial asymmetry and unilateral mandibular hypoplasia. The hypoplasia took the form of a decreased volume and flattening, and they regarded such hypoplasia as "... manifestations of retardation of bone growth ...". They commented further that the mandible exhibited "... more marked ..." changes than did the bone of the cervical spine.

The almost casual and empirical manner in which the earlier reports, and also some of the later ones, were presented is unfortunately similar to the design of the earlier treatment plans. Dawson (1968) bore this out when he said: "Unhappily lessons learnt in the laboratory are not always remembered in the clinic. The pages of the literature and Annals of the Law Courts record many instances where irradiation of a childhood blemish has

resulted in a degree of disability that could never have been matched by the untreated disease."

Finally, it appears that no author except for Leyon et al (1964), who reported that 170r total body radiation had no effect on tooth morphology, has been in a position to give an indication of threshold dose values, and one has to rely on experimental work and its subsequent problems of extrapolation.

MONKEY

Gowgiel (1961), studying the mechanisms of eruption, irradiated 18 Macacus rhesus monkeys (14-28 months) with 4500, 5500 and 7500r, and kept them for up to $3\frac{1}{4}$ years post-irradiation. Physical factors were 250 KVP 15 m.a. TSD 50 cm. half value layer 1.5 mm. Cu. dose rate 44 r/min.

He found that at all dose levels the permanent teeth were completely inhibited from further development, and that the uncalcified anlage of the third molar had disappeared (the deciduous teeth were fully formed and erupted). Over a period of $5\frac{1}{2}$ weeks to $3\frac{1}{4}$ years he found profound changes.

The pulps of the teeth rapidly became obliterated, and the solid pulpless teeth rested on a layer of dense bone. (See Figs. 7 and 8, Page 32 and 33.)

If the teeth failed to erupt, he found that the pulpal tissue was still obliterated, but more slowly. These teeth occurred in the higher dosage levels, and ankylosis was not observed in the animals receiving lower dosage (4000, 5500r). (See Fig. 9, Page 34).

The partially formed teeth were found to calcify normally and slowly erupt into position, even though some - mainly mandibular teeth - had no roots. The deciduous teeth were exfoliated normally following normal tooth resorption and the aberrant size

Fig. 7.

Control non-irradiated animal. Compare with Fig. 8. (From Gowgiel, 1961).

Fig. 8.

Irradiated animal showing rootless teeth (arrowed), obliteration of pulps and partial anodontia.
(From Gowgiel, 1961).

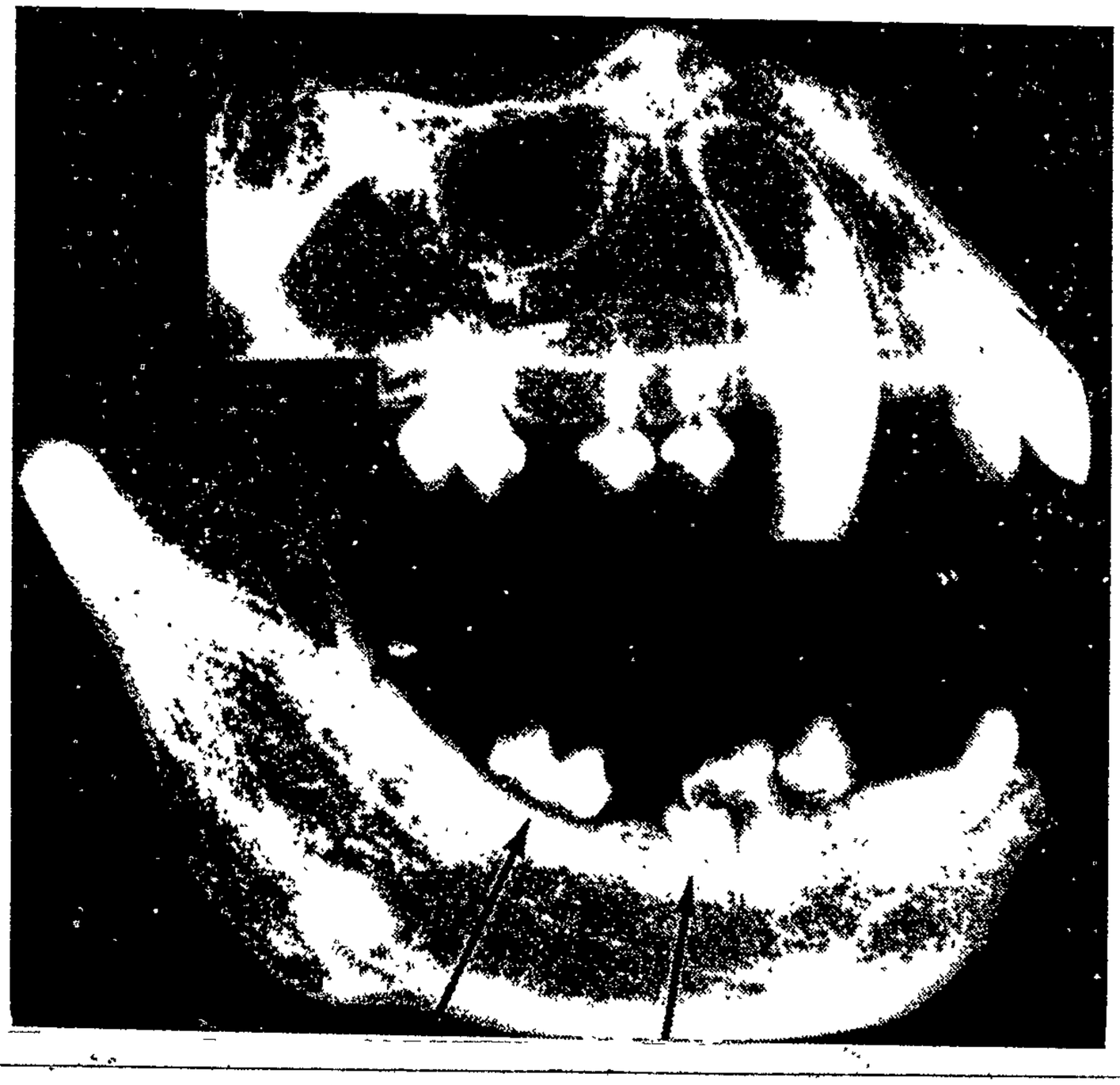
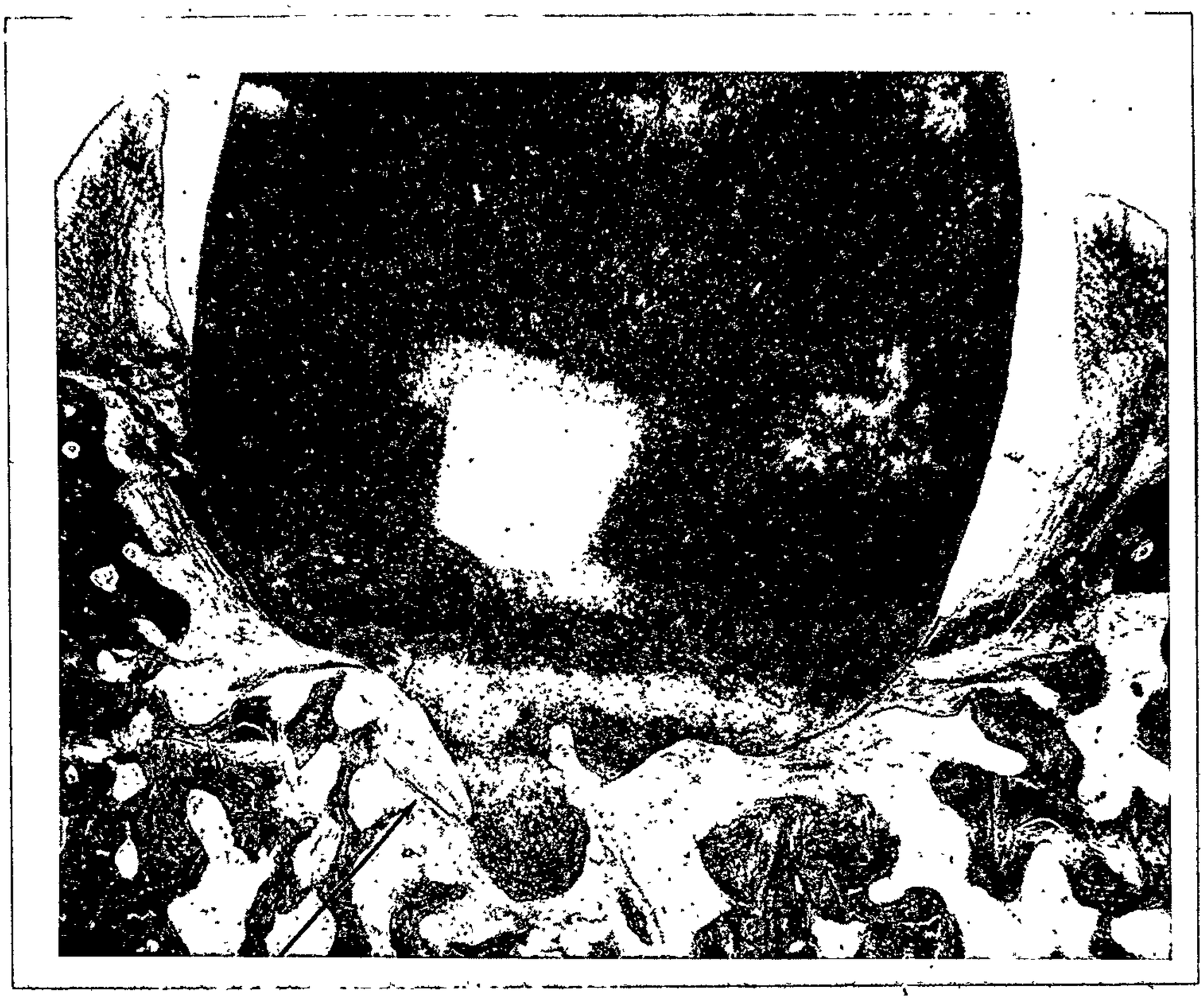


Fig. 9.

Ankylosis of a rootless tooth.
(From Gowgiel, 1961).



of the following teeth had no effect on this process. However he did not state if they were lost sooner or later than normal.

Following eruption the rootless crowns were attached to the gingiva in full occlusion, inexplicably "without any looseness", for 18 months.

Histologically the changes were very marked, although Gowgiel made little effort to differentiate between the changes at different dose levels.

There was an abrupt change from normal odontoblasts to low cuboidal cells associated with widely spaced dentinal tubules. This appearance was "maintained for 30-50 cells ..." - presumably towards the root - "... and then gave way to polygonal-shaped cells and osteodentin formation." Adkins (1967), in rats, found evidence of mitosis of pulpal connective tissue cells and their subsequent production of osteodentin. Gowgiel gave no such explanation, and appears to have assumed that it was produced by these altered cells. However, he described macroscopically the complete obliteration of the pulpal chamber. This pulpal obliteration by osteodentin has not been noted by most other authors following experiments on different animals. However Gowgiel (1967) obtained similar results with rats.

The epithelial root sheath was found to terminate on osteodentin near the junction of this substance with normal dentin.

Enamel formation was not found beyond the normal dentin, and as the sections were decalcified no comment could be made on the normality of the calcification of either the dentin or enamel.

The outer surface of the osteodentin was covered with cementum and the follicular sac was attached to cementum. The follicular sac only developed into periodontal ligament when attached to cementum. Elsewhere it did not develop any further.

From these findings Gowgiel concluded that neither pulpal proliferation nor root formation was a factor in tooth eruption.

MOUSE

Mice are similar to rats in that they have one set of teeth with continuously erupting incisors. Their response to irradiation is also similar, as the following reports indicate.

Burstone (1950) studied the effect of irradiation on the teeth and supporting structures of mice 2-28 days old at the time of irradiation. Using 50 Kv. 2 m.a. at 5000 r/min. he irradiated three lots of mice with 1500, 3000 and 5000r. He noted in the introduction of his report the lack of standardisation of previous experiments.

Seven days later, in the 5000r group, he observed distortion of the developing structure of the third molars, the absence of normal ameloblasts, and the complete absence of enamel matrix formation.

At seven days the 3-day old group who received 1500r produced some osteodentin and abnormal odontoblasts. The ameloblasts appeared normal. (See Figs. 9, 10 and 11, Page 38).

At 12 days, the 3000 and 5000r groups of animals showed gross changes in the root structure of the first and second molars. This consisted of abnormal apices, composed of a mass of dentinoid and globular material. Doses of 1500r and 3000r retarded the molar root formation to a lesser degree than doses

Fig. 9.

Normal third molar of a 9 day old non-irradiated mouse.

Fig. 10.

Third molar of a 9 day old mouse 7 days post irradiation.

Fig. 11.

High power view of Fig. 10. Note abnormal odontoblasts and osteodentin. From Burstone (1950).

of 5000r.

The long term effect of irradiation on older mice consisted of atrophied or fibrotic pulps (60 days) and root ankylosis of the molars. Burstone concluded that: "The degree of radiation damage to the teeth and jaws of mice is dependent upon the age at which the animal is irradiated and the stage of histogenesis of the individual tooth."

At 5000r there was a cessation of histodifferentiation of ameloblasts, and less marked changes at 1500 and 3000r. However, these last two dosages "severely" damaged the odontoblasts which subsequently produced osteodentin.

"There is a retardation or cessation of root formation (first and second molars) following exposures of 1500 to 5000r ..." - presumably due to interrupted mesodermal differentiation - "... while the development of the coronal dentin is only slightly altered ..." (Burstone, 1950) - presumably because the mature odontoblasts are relatively insensitive.

Brunst (1954) compared the effects of local irradiation on the jaw and tooth development of young axolotls and mice. (See Fig. 12, Page 40). Of 60 1-3 day old mice irradiated with 2000-3000r (55 Kv. 3 m.a. no filter 10 cm. TSD) locally to the upper or lower jaw, only three survived the two month observation period. (Two additional mice also survived, but showed no signs of irradiation effects; an obvious experimental method defect).

Fig. 12.

Suppression of development of jaws and teeth in mouse treated by irradiation.

A. Sagital section through basal portion of incisor of the upper (not treated) control jaw. Original X52.

B. Sagital section through irradiated lower jaw of same animal as A. Original X52.

C. Sagital section through the basal portion of the incisor of the upper jaw of control animal. Original X52.

B-bone, BM-bone marrow, E-epithelium, M-muscle, D-dentin, DP-dental pulp.

(From Brunst, 1954).

One survivor was from a group of four irradiated animals (2000r), and showed a great suppression of lower jaw growth and a complete lack of lower teeth. The upper incisor grew to the characteristic sickle shape due to its lack of attrition.

The second animal was from a group of five 3-day old animals irradiated with 3000r (upper jaw). There was partial suppression of growth of the upper jaw and absence of teeth. The incisors of the lower jaw were abnormally large.

The third animal was from a litter of seven, five of which were irradiated with 2000r when 2-days old (upper jaw). This mouse also showed partial suppression of upper jaw growth and a complete lack of teeth. The incisors of the lower jaw were approximately normal size.

Histological examination of the irradiated experimental jaws showed that the tooth primordia and anlagen were absent, despite the fact that at the age of irradiation they were present in the control group.

60 days following irradiation the histological picture was normal, indicating that the "stable state" had been achieved.

This finding may be questioned as many authors have reported, at this dose level, obvious changes such as cyst formation.

Brunst concluded that: "... when the developing jaws of two widely separated animals such as the axolotl and the mouse

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were treated with roentgen irradiation, the biological reaction of both was essentially the same. Both exhibited inhibition with respect to the development of the treated jaw and disappearance of the teeth and teeth primordia". He further concluded that there was always a more marked effect on the lower jaw than the upper, but this observation may be suspect due to his low number of surviving animals.

However his observation of not only the interruption of tooth development but gross anodontia is most interesting. This is the first report in the literature of anodontia of this magnitude.

The incisors have been further studied by Upton et al (1956). They found that a single dose of 1350r to the head, given to 9-10 week old RF and LAF mice, caused defects of the incisors that led to death from starvation.

Using a dose range of 500-1800r (250 Kv. at 100-137 r/min. RF mice 30 m.a. TSD 93.7 cm. 3mm. Al filter HVL 0.44 mm. Cu., LAF mice 15 m.a. TSD 50 cm. no filtration), they studied the protective effects of intraperitoneal injections of 9 mg. 0.01% solution 2-mercaptoethyl- guanidine H Br. (MEG).

They found that "grossly detectable dental defects" in the 113 LAF mice exposed to 1350r became evident within 60 days. These gross defects consisted of varying degrees of loss, erosion

administered. There was little difference between the respo to the β-emitter of either the phosporous or colloidal gold, indicating once again the local nature of the response rathe than the effect on some distant organ.

He remarked on the apparently higher sensitivity of odontoblasts compared with ameloblasts for equivalent doses, as the odontoblasts appeared more histologically affected.

Mathews (1963) studied the effect of 300r on foetal mi (15 days I.U.). He reported an overall normal developmental pattern for the dentin and for the cervical enamel (which wa formed post-irradiation). However the enamel of the molar c exhibited "... gross structural changes and defects ...", including anucleated ameloblasts. He concluded that the pri differentiation area of the ameloblasts was markedly radio-sensitive, and that the cervical enamel was formed from new ameloblasts which had differentiated from the stellate retic post-irradiation.

or tipping of one or more incisors, and that the time of onset (presumably the time at which these defects erupted above the gingiva) varied with the dose of radiation. "However administration of MEG before irradiation greatly lessened the damage from a given dose. After exposure to 1800r, dental defects were evident in 50% of the 30-day survivors within 40 days, and the median time required for development of gross defects was 45 days, and the median survival time 40 weeks". (Upton et al, 1956). They concluded that the effect of MEG in this experiment was a 30% reduction in radiosensitivity.

The authors in this report based their results on macroscopic evidence only. Consequently, characteristic changes in the dentin were missed and they were able to report that no defects were noted in mice exposed to less than 1000r.

In attempts to irradiate only those structures concerned with odontogenesis, Burstone (1950, J. Exp. Zool.) injected radioactive phosporous (H_3PO_4, p^{32} 5-60 μc/gm. body unit) and colloidal gold (both of which remain localised, the phosphate being incorporated into the tooth structure). He found the response of the developing structures similar to that provoked by external irradiation anodontia, root deformities and general disturbances of odontogenesis. The severity of the effects were dependent on the age of the animal and on the actual dose

RAT

Introduction.

The rat is a very suitable animal for this type of experimental investigation. It has two types of teeth, continuously growing incisors and non-regenerating molars whose cusps are bare of enamel.

It was first used by Leist (1926, as quoted by Kimeldorf et al, 1963), who described the characteristic "dentin niche". Following the introduction of dental radiography, Smith (1938) confirmed the work of Leist. He irradiated six rats with clinical diagnostic dosages (TSD 7", 10 m.a. spark gap 3"), and could find no effect. At "well above clinical dosage level" he noted that:

(i) Odontoblasts and dentin were affected, and

(ii) The effect was temporary.

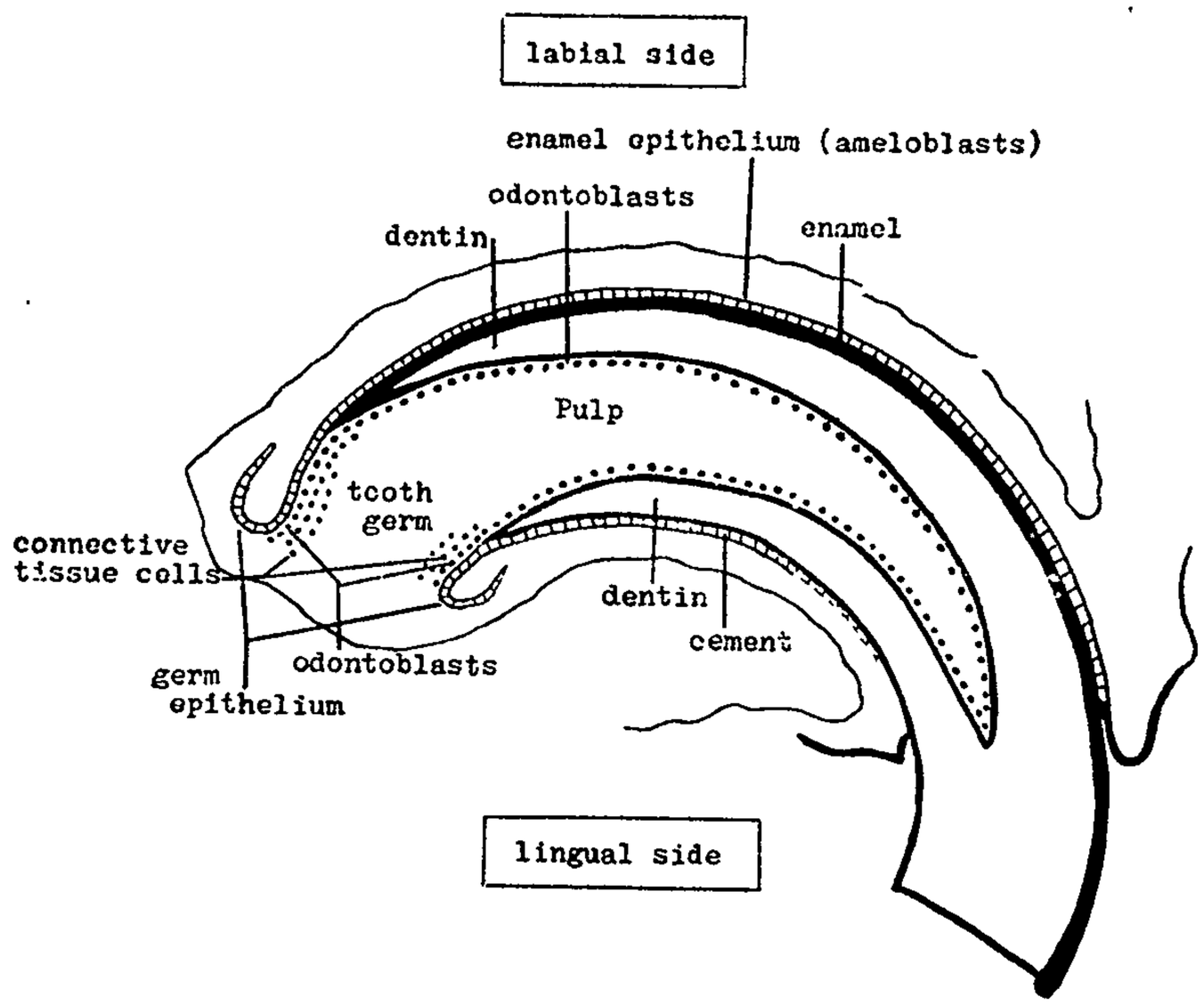
However, he made no mention of any latent damage to the rat molars.

The Rat Incisor. (See Fig. 13, Page 46).

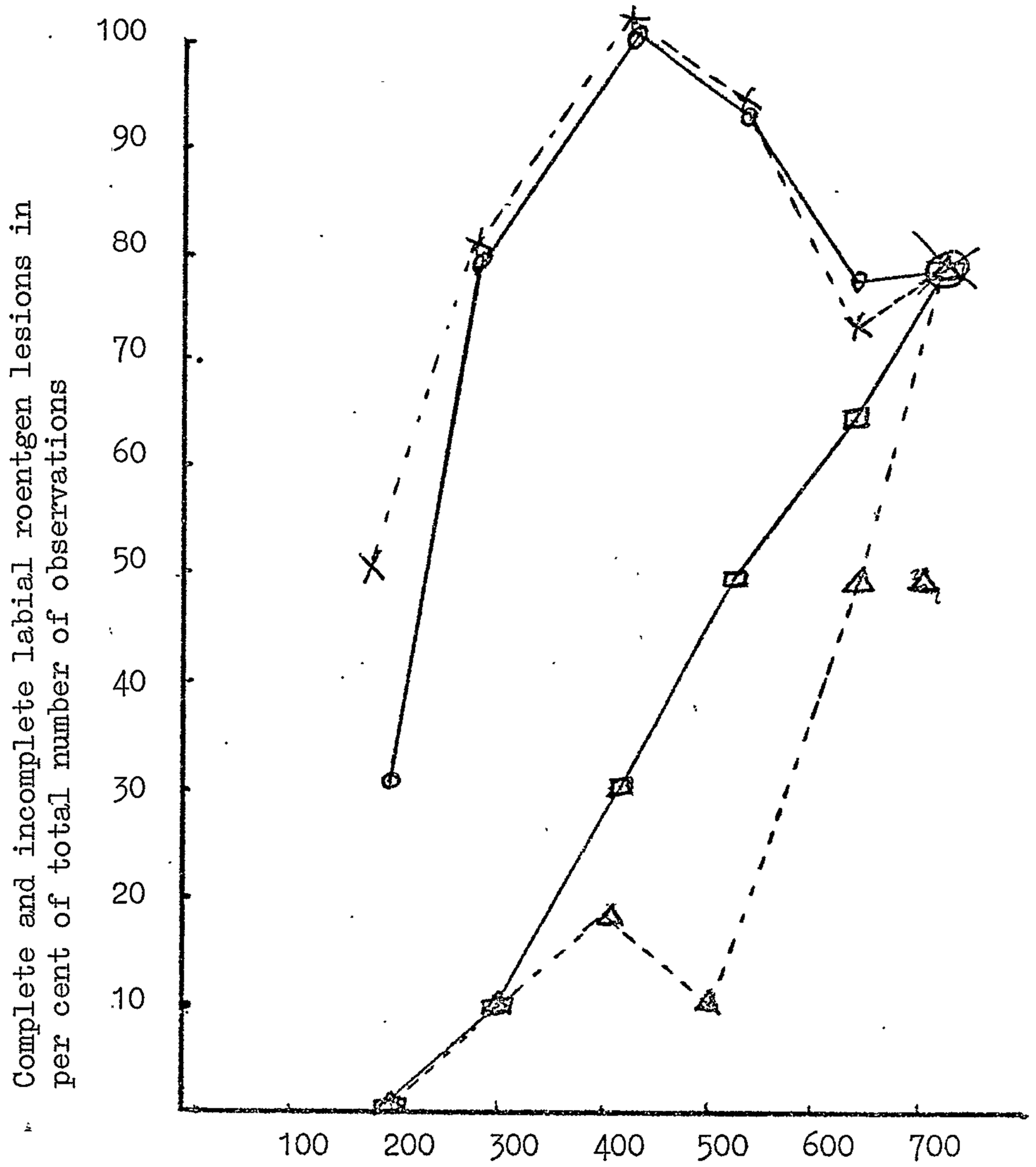
Koppang (1967), in one of the best articles on this subject, stated that "... in the continuously growing incisors of certain species the effect of irradiation on different cells at various stages of development can be observed simultaneously".

Fig. 13.

Diagram of the continuously growing rat incisor.
(From Adachi et al, 1957).



Not only is a permanent record of irradiation damage of the cells produced by the growing tooth, but we may also observe their attempts at recovery following sublethal dosages. The degree of damage varies approximately in proportion to the absorbed radiation dose (See Table on Page 48 and Graph below).



Microscopical Diagnosis	% changes in				% changes in				Radiation dose in r
	Left superior incisor		Right superior incisor		Left inferior incisor		Right inferior incisor		
	Lab.	Ling.	Lab.	Ling.	Lab.	Ling.	Lab.	Ling.	
Complete lesions	80	20	80	10	80	50	80	10	
Incomplete lesions	0	20	0	0	0	10	0	10	700
No observable lesions	20	60	20	90	20	40	20	80	
Complete lesions	67	0	70	0	56	0	44.5	0	
Incomplete lesions	11	0	0	0	11	0	11	0	600
No observable lesions	22	100	30	100	33	100	44.5	100	
Complete lesions	67	0	90	0	33	0	0	0	
Incomplete lesions	22	0	0	0	22	0	10	0	500
No observable lesions	11	100	10	100	45	100	90	100	
Complete lesions	89	0	100	0	30	0	20	0	
Incomplete lesions	11	11	0	0	0	0	0	0	400
No observable lesions	0	89	0	100	70	100	80	100	
Complete lesions	22	0	40	0	0	0	0	0	
Incomplete lesions	56	0	40	0	10	0	10	0	300
No observable lesions	22	100	20	100	90	100	90	100	
Complete lesions	10	0	20	0	0	0	0	0	
Incomplete lesions	20	0	30	0	0	0	0	0	200
No observable lesions	70	100	50	100	100	100	100	100	

From Koppang (1969).

Binford et al (1961) reported that changes occurred in the cemento-enamel junction at 400r and became well defined at 500r. (This compares interestingly with a report by Dawson (1968) that damage to the epiphyseal growth centre of the long bones will occur at 400r).

At 500-1000r Medak et al (1950) reported a slowing down of eruption, and at 600r Binford et al (1961) reported that osteo-dentin begins to form.

At 1500r English et al (1954) reported more varied signs of damage. The dental niche becomes so large that the tooth is actually amputated, and for a time the animal has four incisors in the lower jaw rather than the normal number of two. This is due to a complete amputation of the tooth by irradiation-damaged tissue. The old teeth eventually become loose and fall out. The new, or "extra" incisors lie to the mesial of the exfoliated ones, and indicate a general phase of recovery of tooth-forming elements.

They also reported that the maxillary teeth are not as affected as those of the mandible and that the new tooth structure is atypical. (Willhoit et al, 1965, and Koppang, 1969, disputed this finding of increased mandibular radio-sensitivity when compared with the maxillary teeth, stating that the reverse is true).

Occasional missing ameloblasts allow invagination of osteoid material into the resulting spaces and there is inclusion of "cellular and indiscriminate matter" in the new tooth substance. The pulp chambers of the new teeth are enlarged, indicating a slowing down of dentino-genesis.

Medak et al (1952) reported that the primary changes following doses of 1000r to 2000r are well localised to the labial zone of proliferation at the basal end of the incisors. (This comment also is disputed by Koppang, 1969, together with Adkins, 1967).

Sublethal doses of radiation, above a threshold level, produce characteristic changes. Koppang (1967) has defined the two most common findings as follows:

(i) "dental niche formation"; narrow dentinal zone, narrow predentin border, degenerated depolarised odontoblasts, osteodentin (depolarised cementoid) in the pulp,

(ii) "intradental lesion of classical character"; interdental pulp tissue with narrow predentin border, degenerated depolarised odontoblasts, osteodentin.

"An incomplete roentgen lesion does not refer to any niche formation but to a certain reduction in the thickness of the

predentin and/or dentin zone combined with degenerated depolarized odontoblasts and osteodentin in the pulp". (Koppang, 1967).

Using four groups of animals and a dose range of 400-700r 12 m.a. 175 KVP TSD of 24 cm. 0.5 mm. Cu. and 87.94 r/min., she administered the selected dosage to all members of the groups. The groups were then sacrificed at intervals of one week, the results classified in tables and the position of the distinctive elements noted according to Pindborg's classification (1950). She also investigated the protective effect of cyestaemine.

The cyestaemine (10 mg. hydrochloride intraperitoneal 5 minutes prior to radiation) was found to have little effect, apart from a tendency for an increase of the radiation effect at lower dosages and a decrease of this effect at high dosages. She commented that the relationships should be investigated further.

The main effect of irradiation at this level (400-700r) was found in the dentin. No changes were demonstrated in either the ameloblasts or in enamel matrix formation. However young odontoblasts were damaged when irradiation occurred in the active metabolic stage.

This finding of the relative radio-resistance of ameloblasts when compared with odontoblasts is contrary to evidence given by English et col (1951), studying swine, who found

hypoplasia, alteration of enamel structure and a response above 400r much the same as to other damaging stimuli. However they agreed with Koppang that the most affected cells were those most metabolically active.

Collett et col (1965) stated that the degree of osteodentin production is dependent upon the odontoblastic metabolic rate. However the extent of the reaction is determined by the stage of differentiation.

Dale (1953) stated that the ameloblasts (rat) were markedly radiosensitive (250 KVP at 15 m.a. Al parabolic filter and $\frac{1}{2}$ mm. Cu., 375, 500, 625 and 750r groups, single whole body dose; animals sacrificed 30 days post-irradiation). At 375r he found that enamel was more affected than dentin. Small irregular amorphic discoloured enamel rods with dark inclusion bodies in the interprismatic substance were noted, but "... no apparent effect on calcification of either enamel or dentin". Thus he concluded that the disturbance is probably due to an interference or inhibition of the laying down of enamel and dentin matrix, rather than one of calcification.

Hansen et col (1957) stated that the "latent period" characteristic of radiation injury precludes the determination of which is the more radiosensitive. This is supported by Koppang (1967) and also by Pliess et col (1960). These authors

point out that the primary cellular damage may not be directly demonstrable, the effect being shown up later by matrix faults.

Adachi et al (1957) agreed with this last comment, and stated that not only are the germ cells sensitive but that their predecessors, the connective tissue cells, are as well. This could explain anodontia in animals who have been irradiated before actual tooth development has started to occur. However the latter authors then stated that they believe that odontoblasts are the more sensitive of the two types of cells. (See Fig. 14, Page 54).

1. THE PATTERN OF INJURY TO INCISORS FOLLOWING IRRADIATION.
- 24 Hours Post-Irradiation.*

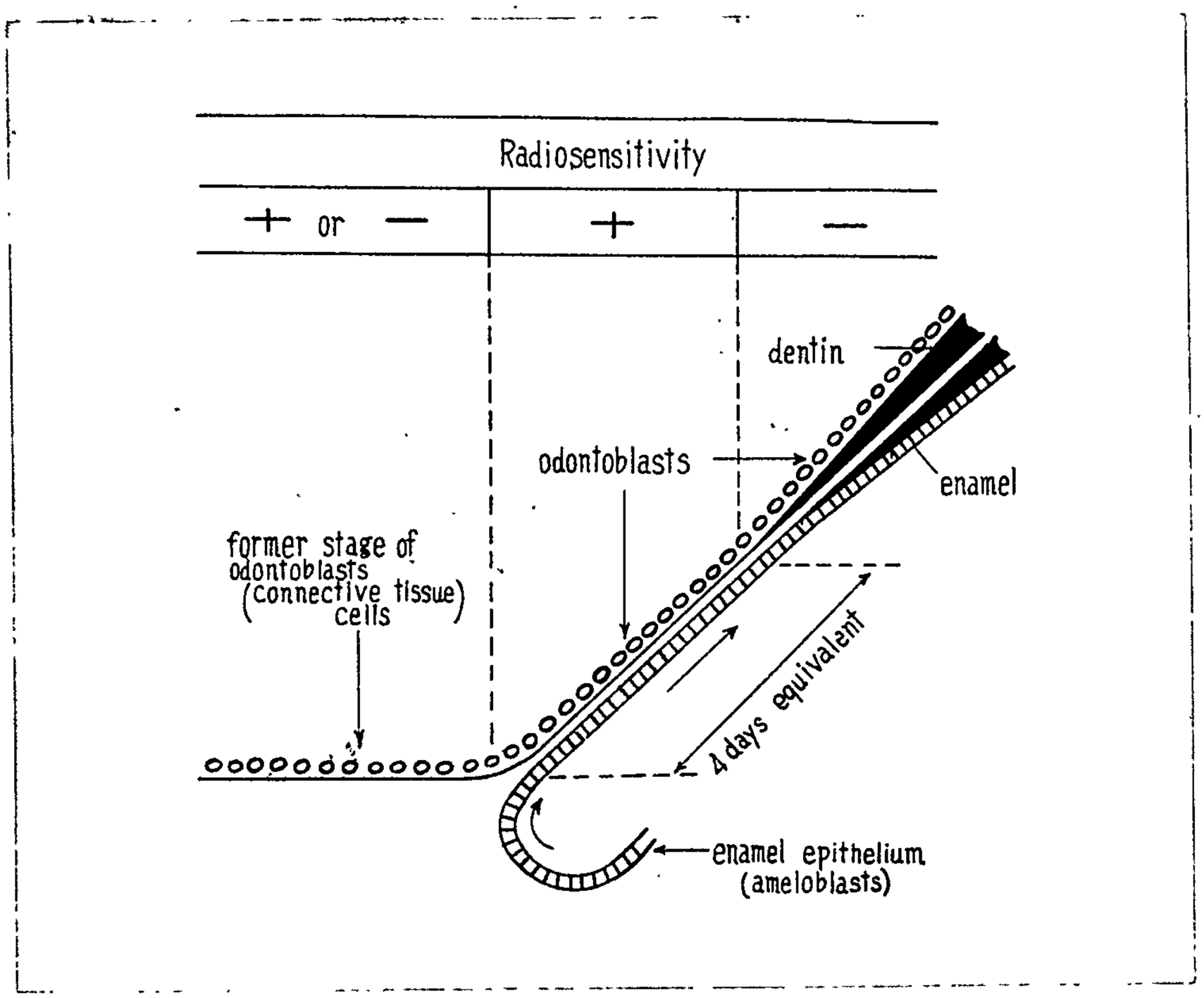
Adkins (1967) reported that necrosis of connective tissue cells near the apex of the incisors (presumably the odontoblastic precursors) was apparent as karyorrhexis. However, there was no necrosis of the germinal epithelium (ameloblast precursors) nor of ameloblasts or odontoblasts.

However, Willhoit et col (1971) reported that the amelogenic cell migration from the beginning of the enamel matrix is slowed down from 466 $\pm$ 66 mm. per day to almost zero following irradiation (single exposure X-ray 600r 280 KVP 1.4 mm. Cu. HVL 31 cm. TSD 305 r/min.). Their experiment indicates that the

* Unfortunately copies of Koppang's excellent illustrations were not available for reproduction. The reader is referred to both 1967 and 1969 articles.

Fig. 14.

Schematic diagram of radiosensitivity of the developing tooth. Note that the author indicates that the radiosensitivity of both ameloblasts and odontoblasts occurs in the same region, i.e. that of proliferation.
(From Adachi et al, 1957).



maturation-differentiation compartment is not decidedly sensitive at this dose rate and level, and that subsequent tooth deformity is due to the blockage of the proliferative compartment.

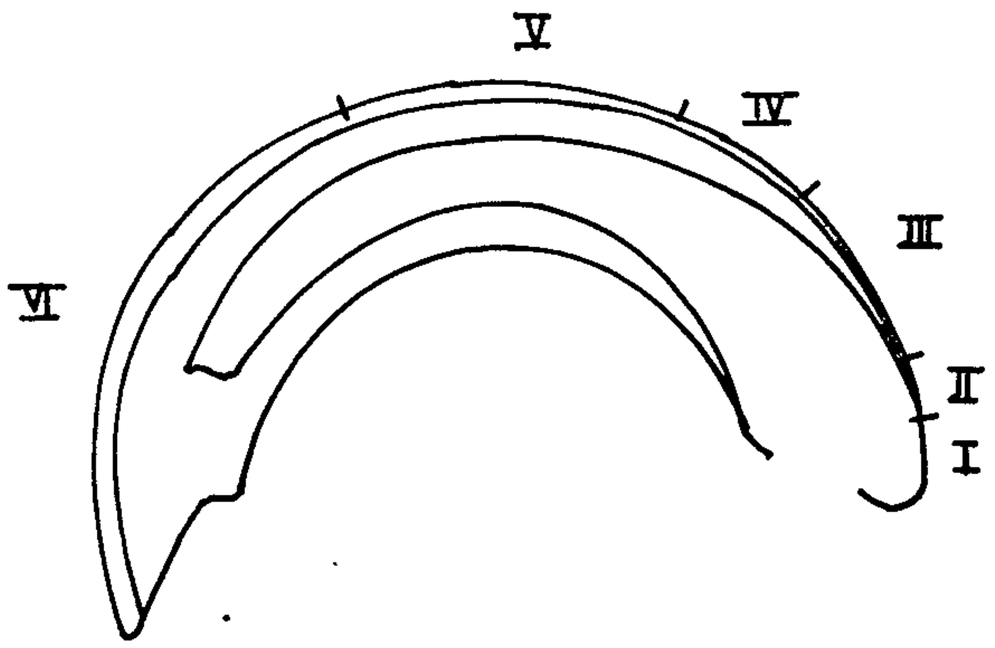
The number of necrotic cells found by Adkins (1967) was closely related to the amount of radiation per dose, i.e. 1x1200R produced many necrotic cells, 2x600R less and 4x300R least. However, the areas of oedema produced at the apical region appeared more severe in the 4x300R animals than in the 1x1200R. No explanation was given for this finding.

Hansen et al (1957) could find no significant variation from normal after 16 and 24 hours.

- 48 Hours Post-Irradiation.

Adkins (1967) reported that osteodentin is present on both the labial and lingual aspects of the pulpal chamber. This tissue was first seen in the 4x300R group and occurred initially in the sub-odontoblastic tissues. The osteodentin always rested on a 16 μ thickness of dentin labially separated from it by vital but now cuboidal odontoblasts.

Koppang (1967) reported similar osteodentin occurrence, but at a lower X-ray dose level (maximum 700r as against Adkins' 1200R). She further found the lingual osteodentin to be of different distribution than the labial, being more diffuse and not strictly confined to the niche area. This variation was not mentioned by Adkins.



Schematic illustration of the sectors of the labial side of the rat incisor according to Pindborg.

In 1952 Medak et al found that the primary changes were well localised to the labial zone of proliferation of the basal end of the incisor (1000-2000r), and liquefaction necrosis was present in this area, disappearing in a further two days. Adkins (1967), however, noted "large oval intracellular spaces adjacent to the lingual epithelial diaphragm".

- 3 Days Post-Irradiation.

The odontoblasts on the labial aspect become less intensely staining and more cuboidal, i.e. they change shape and cease secretion (Adkins, 1967).

- 4 Days Post-Irradiation.

Medak et al (1952) reported that the oedema had subsided and secondary histological changes could be noted, including degeneration of developing odontoblasts, the formation of osteo-dentin and irregularities of tissue at the dentino-enamel junction. These changes in days 2, 3 and 4 are the forerunners of the formation of the dentin niche.

More severe dosages (3000r-4000r) produced fluid-filled large cyst-like cavities which resulted in further odontoblastic damage by mechanical compression.

- 1 Week Post-Irradiation.

The lesion following moderate exposure (300-700r) is located between sectors III and IV (after Pindborg) in the

maxillary incisors and more especially in the mandibular incisors (Koppang, 1967). The labial lesions are more common than the lingual and superior incisor lesions more common than inferior. The lesion now appears as one of defective dentin formation.

- 2 Weeks Post-Irradiation.

Koppang (1967) reported that the lesion has now progressed to lie between sectors IV and V in the maxillary incisors and in the coronal portion of sector IV of the mandible. Complete lesions were never seen lingually and the complete labial lesions had not changed their characteristics. Depolarized odontoblasts were associated with osteodentin which was separated from the regular dentin. She did not discuss this interesting production of odontoblastic-like cells whose origin, presumably from pulpal mesenchyme, occurred remote from the influence of the enamel organ.

However Adkins (1967) describes this osteodentin more precisely:

"The osteodentin was an eosinophilic organic matrix in which only a few cells became entrapped in lacunae. It was bounded by numerous plump deeply stained low columnar cells orientated with their long axis at right angles to the surface of the osteodentin. The nuclei, located in the basal third of the cells,

were vesicular and contained prominent nucleoli. In some of these cells a pale area was apparent within the cytoplasm similar to the negative image of the prominent Golgi net seen in active osteoblasts.

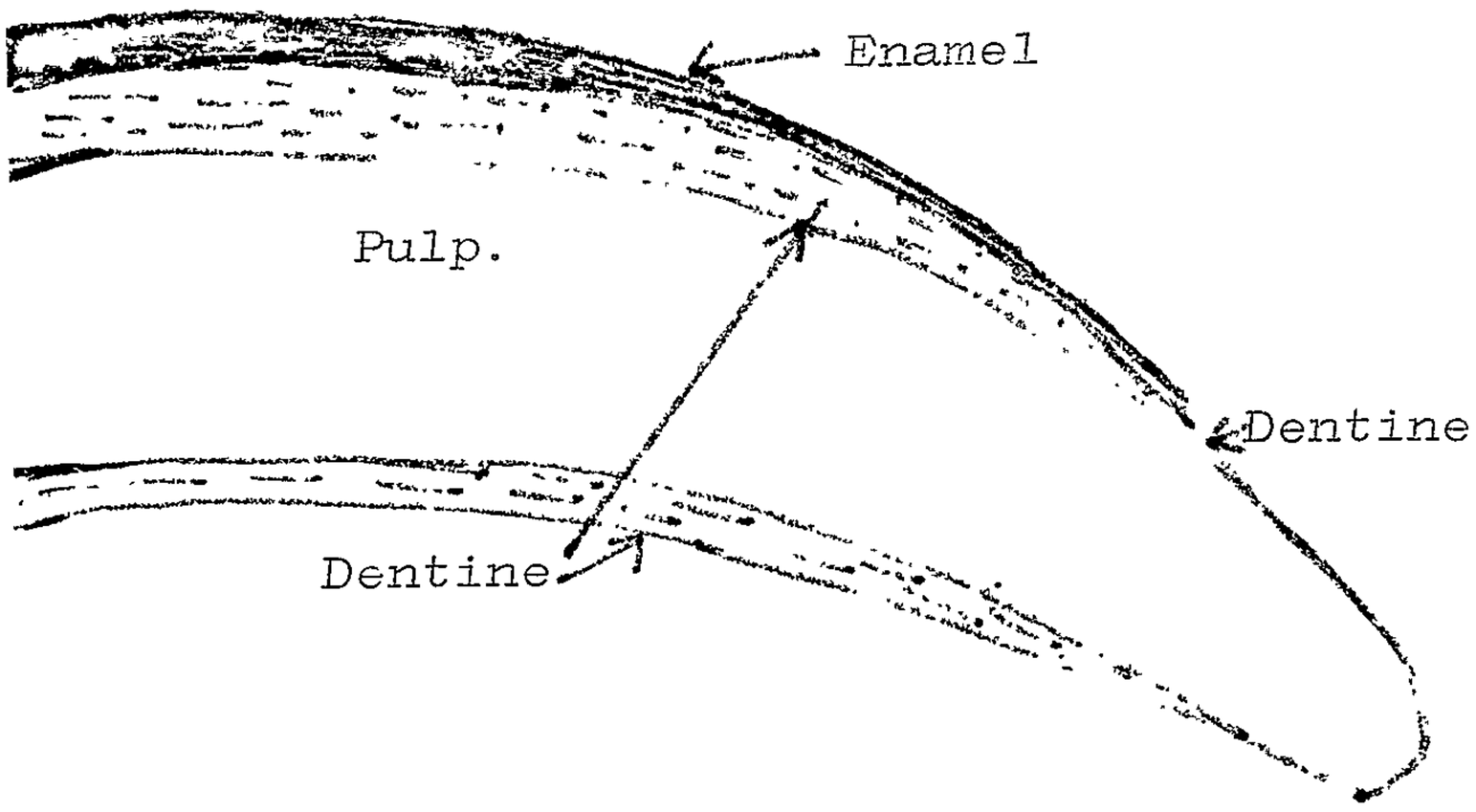
The cytoplasm of these cells was basophilic and was stained intensely by pyronin Y. Following ribonuclease digestion this affinity for pyronin Y was lost".

He stated, however, that they only occur in areas adjacent to dentinal damage and that there was no evidence to suggest that they were derived from the damaged odontoblast. Further, the pulpal cells were seen to gradually alter their staining characteristics to those just described, indicating differentiation of odontoblasts from pulpal mesenchyme.

The lesion is now well defined, being sharply limited both longitudinally and transversely, and of characteristic mushroom shape with a stork of pulpal tissue. There is an increase in thickness of the dentin compared with that of one week, and the mushroom shape of the defect, found in the thickest part of the dentin, is probably due to attempted repair. (See Fig. 15, Page 59).

Fig. 15.

Rat Incisors.
Marked changes produced at 19 days by 600r x 3.
Note large labial disruption[A] and complete lingual disruption [B] producing a potential severence of the tooth.
(From Adachi et al, 1957).



Schematic diagram of normal rat incisor of same stage of development as that in the opposite diagrame.

- 3 Weeks Post-Irradiation.

The lesion is located in the apical third of section V in maxillary incisors, and more apically in mandibular incisors. The lesion now lies in considerably thicker dentin and the "stork of the mushroom" is much smaller, indicating further repair. The contents of the niche consist of a strand of globulous, eosinophilic, homogeneous substance, rather acellular, the longitudinal axis of which is parallel to the dentino-enamel junction in the longitudinal axis of the tooth. "Labial, and to a minor extent lingual globules of osteodentin comprised as in earlier post irradiation stages the complete roentgen lesion". (Koppang, 1967).

- 4 Weeks Post-Irradiation.

The lesion now lies near the junction of sectors V and VI, i.e. near the zone of ameloblastic atrophy. In mandibular teeth it lies in the central part of V. (Koppang, 1967).

- 5 Weeks Post-Irradiation.

Castanera et al (1963) reported the appearance of gingival lesions at approximately 35 days post irradiation. This lesion was visible as a chalk white zone on the labial aspect of the teeth. They stated that the degree of visibility and the size of the zone was directly related to the size of the dose, being least at 430 rads X-ray and most at 360 rads neutrons. They reported that in every case the zone was the full width of the

tooth, but that the size of the lesion varied from tooth to tooth by as much as a factor of two.

- Later Stages and Recovery.

The dentinal niche is gradually filled in with osteodentin and the whole defect then covered, on the pulpal side, by normal dentin. Due to the continuous normal growth of the epithelial enamel organ and the depressed rate of pulpal proliferation, the dentino-enamel junction takes on a wavy appearance (Medak et al, 1952).

There appears to be a marked difference between labial and lingual response of the odontoblasts (Koppang, 1967). Murai et al (1958), citing their earlier work (1936, 1943, 1944), equated the development of lingual tooth structure of rat incisors to human root structure formation, and stated that irradiation damage to the labial side of rat incisors is analogous to that of the human tooth in the stage of crown formation.

- The more severe dental niches so weaken the teeth that breakage, both sub- and supra-gingival, is common. Kimeldorf et al (1963) noted that this breakage has a profound effect on the metabolism of the animal, and may even be responsible for its death from starvation, and that the age of the animal has been found to be directly related to the incidence of damage and subsequent breakage. This weakness of hard tissue was found

to be responsible for the breakage of the incisors which occurred in 68% of juvenile rats, 11% of young adult rats, and 12% of old rats, following 215 rads of neutrons.

2. RADIO-SENSITIVITY: ODONTOBLASTS AND AMELOBLASTS.

There is a pronounced variation of odontoblastic susceptibility from tooth to tooth in the same animal. Collett et col (1965) reported that the minimum osteodentinogenic dose at the 50% level of occurrence was 600r for incisors, and no effect was noted in the third molars (physical factors 220 KVP 64.4 r/min. TSD 40 cm. 12 x 12 cm. field, 9-13 day old rats) at almost three times (1500r) this dose. No reason for this apparent variation was given.

Koppang, in her initial study (1967), reported that the superior incisors, despite their lower metabolic rate, were more susceptible than the lower incisors, but observed that this result could have been due to experimental error. She also noted that in both superior and inferior incisors the labial, enamel-covered odontoblasts appeared more sensitive than the lingual, cementum-covered odontoblasts, but that this also could be due to experimental error.

This apparent difference in odontoblastic sensitivity has been noted by Murai et al (1958). However many reports, e.g.

Castanera et al (1963), dealt with live animals, and mentioned only the labial aspects of the teeth. Other reports, e.g. Adachi et al (1957), dealt with high dose ranges (3 x 600r), and made no comment on this apparent difference, except that the lingual lesion is less defined and the osteodentin less demarcated when compared with the labial.

The apparent difference in sensitivity between the upper and lower incisors and labial and lingual odontoblasts was further investigated by Koppang (1969).

In her initial study (1967) she found that single head doses of X-ray as low as 300r were capable of inducing niche formation in the superior incisors, but that 400r of similar quality was necessary to produce niche formation in the lower incisors. (See tables of her results for the 1969 investigation for both tooth sensitivity discrepancies and labial and lingual niche formation on Page 48).

In the 1969 investigation, using similar quality X-rays, animals and experimental method, she found that when the X-ray beam was directed laterally, instead of from above, the incidence of lingual lesions was much higher. However, these lesions were still very different from those of the labial surface. They were not as distinctly limited, not as niche shaped and were associated with larger amounts of osteodentin. The lingual

lesion was found to lie more towards the clinical crown than the labial lesion, but this was probably due to the curved nature of the tooth resulting in the shorter crown tip-root length.

She reported that at 700r there was no difference in the numbers of superior and inferior lesions. However there was a difference at 600r, which became statistically significant at 500r for the right incisors (see table and graph, Pages 48 and 47), and for both sides at 400r. Experimental method faults and the small number of animals in her sample may have been responsible for this obvious anomaly of left and right variation.

The apparent difference between labial and lingual odontoblastic elements was also noted in this experiment, and appears to prove conclusively that labial odontoblasts are more radio-sensitive than lingual odontoblasts (see table, Page 48). However it must be realised that all mention of the radio-sensitivity of the odontoblasts refers to the production of the classical "niche", We are therefore considering the odontoblast precursor. Adkins (1968) has shown that the mature odontoblast, laying down dentin, is remarkably radio-resistant and his study indicated that at 1200R the rate of dentin deposition is hardly affected.

Murai et al (1958) attempted to equate rat labial and lingual incisor odontoblast sensitivity with that of human crown and root formation, contending that the two, respectively, are analogous. However, there have been no clinical reports of dwarfed or damaged crowns associated with normal roots and, unlike rat incisors, post irradiated developed human teeth show little apparent resumption of normal odontogenesis.

Adachi et al (1957) studied the maturation pattern of odontoblasts in relation to their radio-sensitivity. They found that, by using a lead acetate injection technique, the niche is produced by cells in the tooth germ area. Fractionated dosage indicates that the connective tissue cell precursors of odontoblasts and the fully secreting mature odontoblasts are relatively insensitive and that the germ areas in a region of four days' growth equivalent are very sensitive. Further, as successive niches become larger following repeated doses the initial area of differentiation may also exhibit sensitivity. (See Fig. 14, Page 54). They considered that ameloblasts have a much lower sensitivity, but make no further comment on this.

Gowgiel (1967) noted that the rat molars appear to be more sensitive than the incisors, as after 2000r the molars ceased development, while the development of the incisors continued after a period. However he also noted that, microscopically,

there appeared to be less cellular damage in the molars. He gave no reason for this anomaly.

The very different types of experimental design result in conflicting reports. Dale (1953) reported that ameloblasts have a marked radio-sensitivity, whilst Adachi et al (1957) claimed that it was less marked than that of odontoblasts.

However, no author appears to have commented on the fact that although new mesodermal activity in the form of osteodentin occurs following sublethal doses of irradiation, no ectodermal activity occurs producing new or malformed enamel to the same extent. The current ameloblasts appear to continue forming enamel, but there has been no report of new ameloblasts trying to lay down enamel on the osteodentin. The lack of such reports appears to support the view of Dale (1953).

The recurring bands of hypo- and hyper-calcification in incisors reported by Willhoit et al (1965) and Dale (1950) are two of the few references to post irradiation enamel production. This lack of post irradiation enamel formation could be explained in two ways. Either ameloblast differentiation and subsequent enamel formation is dependent on a prior normal dentin formation (as we saw in the introduction to this review), or ameloblasts are very sensitive to irradiation prior to their differentiation from the enamel organ. Graham (1963), in a similar study to that

of Mathews (1963) with mice, irradiated foetal rats at 11 days I.U. with 250r. He reported that those survivors, who were subsequently born and who showed no retardation of growth, exhibited alteration to the maxillary incisor tooth bud in the form of lack of differentiation of the odontoblasts when compared with ameloblasts. More deformed foetuses showed less pulpal differentiation with vacuolated cells at the dentino-enamel junction and some dentin formation. There was no enamel.

Unfortunately most of the apparently reliable reports have dealt with decalcified sections and consequently most of the enamel histology is destroyed, e.g. Koppang and Adkins. Even where apparently well conducted experiments, such as those just cited, cover similar ground it is difficult to form conclusions as to the fate of the ameloblasts. This problem is well illustrated by a comparison of three apparently well written reports of good experimental method. In 1957 Hansen et col. reported that they could find no significant deviation from normal in the incisor teeth following 1500r of 200 Kv. X-ray at 16 and 24 hours, while both Koppang (1967) and Adkins (1967) reported similar and very marked changes at lower dose levels (300-700r, 3 x 400r, and 4 x 300r). At later post irradiation stages all three reports agreed on the type of changes occurring.

Eruption.

The effects of irradiation on eruption rate have been well documented by Medak et al (1950), using a straight line regression technique utilizing data of least squares for each group as the dose increased and an established control rate of 2.6 $\pm$ 0.6 mm. per day. Their findings were as follows:

500-1000r - a slight increase.

2000r - decrease for four weeks, followed by a return to normal.

3000r - this was found to be the minimum dose to stop eruption.

They postulated that this may be produced either by local effects or by hypophysis damage. The alternative reason, however, appears to have been ruled out by Schour and Van Dyke (1932). English (1956) came to the same conclusion that radiation damage is a local effect. However, this report dealt with the effect of 1500r on odontoblasts, and their subsequent replacement by newly differentiated cells.

Gowgiel (1967) irradiated the maxillae and mandibles of 10-day old Holtzman albino rats with a single dose of 2000r. Physical factors were 1.5 mm. Cu. TSD 21.5 cm. field 10 x 7 mm. 180 r/min. He found that this dose caused a permanent cessation of further root formation in the molars and only temporary cessation in the incisors. However, rootless teeth, and those whose

pulps were obliterated by osteodentin, continued to erupt. He concluded that eruption was a result of follicular activity.

Weinreb et al (1949) reported that with a single dose of 400r (100 Kv. 5 m.a. TSD 12.5 cm. 320 r/min.), not only did the irradiated upper incisors show a decrease and finally a cessation of eruption, but the mature odontoblasts continued to lay down dentin, thus increasing the thickness of incisor dentin post irradiation, whilst there was no further differentiation of new odontoblasts. We may, therefore, conclude that the cessation of eruption of the incisors is due to a cessation of odontoblastic differentiation.

This is in marked contrast to those teeth which do not normally grow persistently. Gowgiel (1961) with monkeys, and Murai (1958) with cats, have reported the eruption of rootless teeth; teeth whose crowns were formed prior to eruption, whose roots were prevented from forming, and yet the crowns erupted.

3. RADIOMIMETIC AGENTS.

Hebborn et al (1965) investigated the response of rat incisors to radiomimetic agents. Using various strains of albino rats, they investigated the effects of alkylating agents, including triethylene melamine (TEM) 4 *p* [bis(2-chloroethyl)amino] phenylbutyric acid (Chlorambucil) and 1,4-dimethanesulfonoxy-butane busalfan (Myrlan). The agents were given in either a single dose or divided over a period of from three to five days.

They found that some substances (nitrogen mustards) produced defects, including tooth fractures, loss, regrowth or no regeneration, or atypical regrowth, pulpal oedema and vacuolation at the basal end, degeneration of young odontoblasts which had begun secreting predentin matrix (the last two observations being made 48 hours following treatment), osteodentin formation, (See Fig. 16, Page 71), development of normal dentin both proximal and distal to the affected region producing a niche, (See Fig. 17, Page 72), and marked degeneration of tooth bud elements (See Fig. 18, Page 73) with subsequent tooth socket invasion by alveolar bone. This led them to comment that the lesions produced by X-rays and some alkylating agents (nitrogen mustards) are similar.

They pointed out that previous studies, e.g. Medak (1950), Adachi et al (1957), and Pliess et al (1960) had indicated that odontoblast sensitivity occurred during the phase of differentiation and that the extent of the lesion depended on the dose as a time/intensity relationship.

Hebborn et al stated that their results can be compared with those of Schour et al (1941), Walbach et al (1933) and Schour (1942), who demonstrated similar lesions in rat incisors following Vitamin A and D deficiency, parathryoidectomy, or treatment with colchicin. They also concluded that the

Fig. 16.

Effect of Radiomimetic Agents.
Structure of dentin niche showing:
A. Reduced dentin layer.
B. Degenerated odontoblasts.
C. Osteodentin layer.
(From Hebborn et al, 1965).

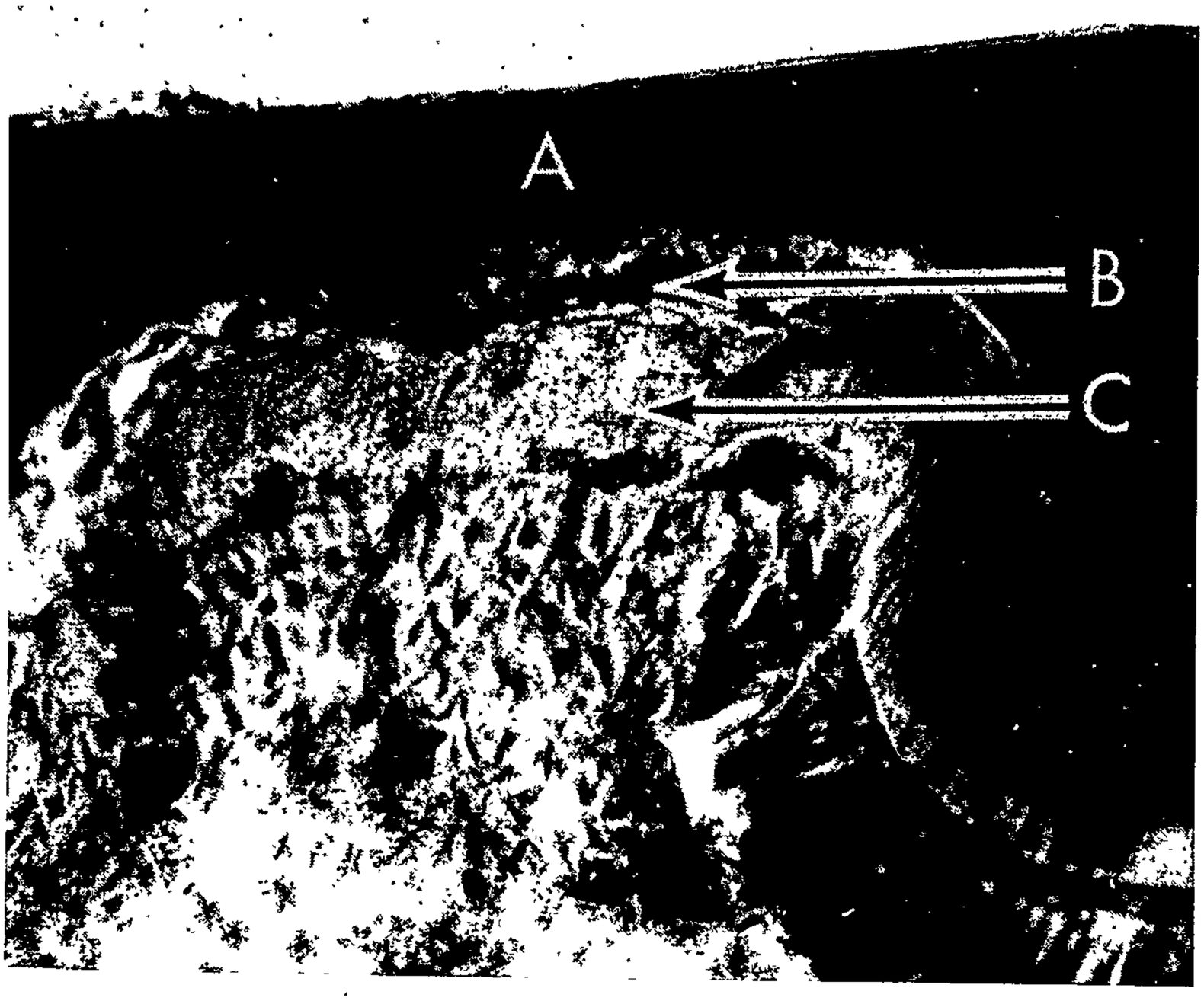


Fig. 17.

Effect of Radiomimetic Agents.
Section of maxillary incisor 15 days after treatment with an alkylating agent. Note dentin niche[A] and waviness of dentino-enamel junction. B
(From Hebborn et al, 1965).

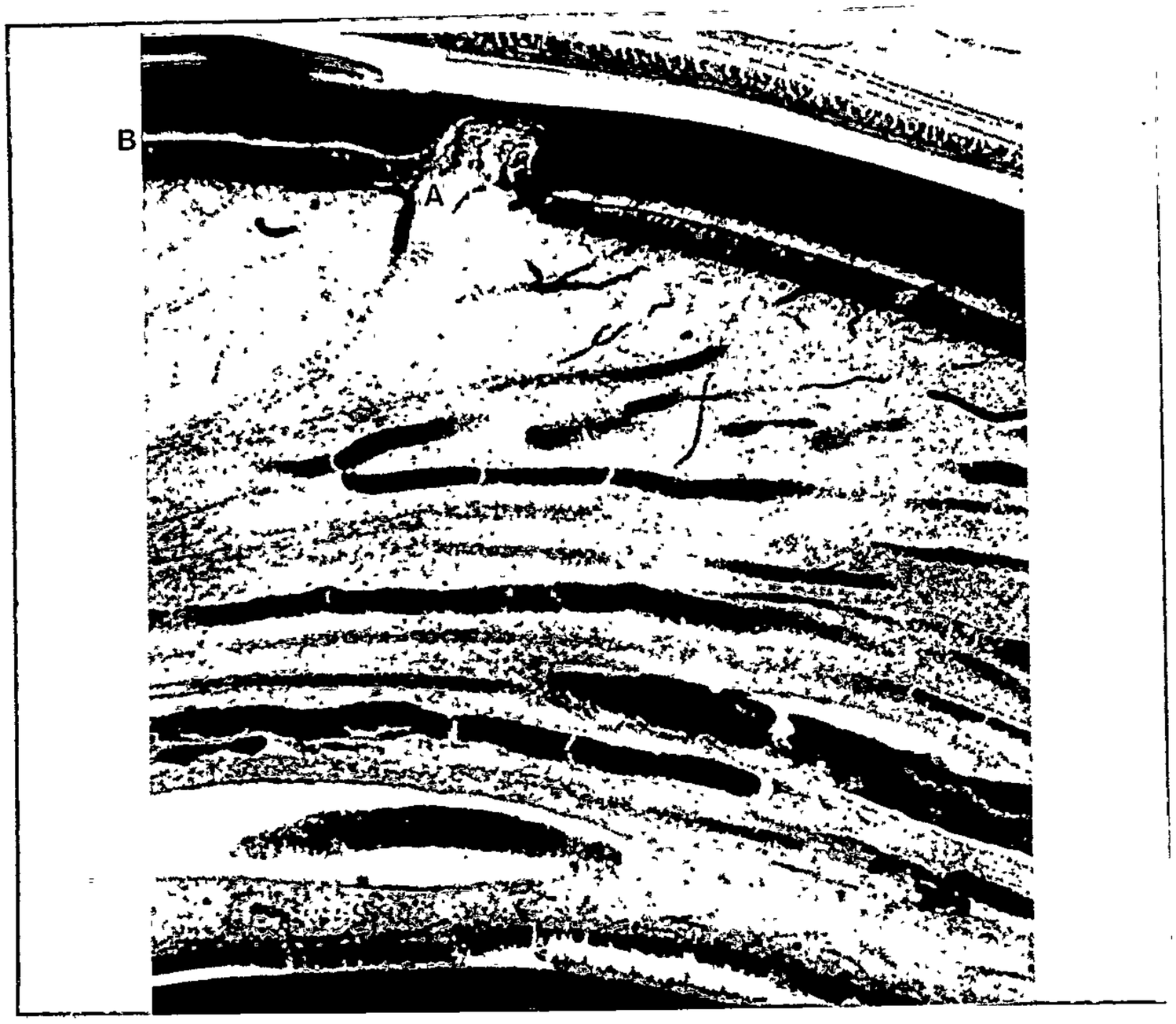


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Fig. 18.

Effect of Radiomimetic Agents.
Section of maxillary incisor 36 hours after treatment with an alkylating agent. Note liquefaction.
(From Hebborn et al, 1965).

reaction to irradiation is of a non specific nature. It is probably due to the rapid rate of cellular differentiation and to the subsequent inability of the damaged cells to either repair themselves or be replaced by normal cells. Tooth structure is an almost unique tissue by virtue of its lack of catabolism.

Consequently, Koppang's (1967) definition of the "complete roentgen lesion" and the "intradental lesion" cannot be accepted as one defining a specific response to irradiation.

4. THE EFFECT OF NEUTRONS.

Willhoit et al (1965) reported the effect of neutron irradiation on adult Sprague Dawley rats. Five groups, each of 10 animals, were exposed to neutron doses of 45, 125, 170, 220 and 240 rads. They found that the severity of the effects were dose dependent, increasing with increasing doses; the effects were essentially the same as those produced by X-rays; and that the relative potency of neutron exposure appeared to be about x4. *that of X-rays.*

They reported that at 80 rads and above all animals showed constriction of the incisor shaft beginning at 36-38 days post irradiation, i.e. it took a little over five weeks

for the defect to erupt. Exposure below this level produced no defects.

Their report appears to indicate that the first response, a "waviness" or "dip" in the enamel, (see Fig. 19, Page 76), is seen at 80 rads, and they commented that enamel hypoplasia was observed before any changes in dentinogenesis and that this finding supports the view that ameloblasts direct the odontoblastic activity. Why this conclusion should be drawn from such results is not clear. However it is clear that, in their experiments, ameloblasts were more sensitive than odontoblasts.

Castanera et al (1963), in a jumbled and not very contributory report, have also studied the effect of X-rays (430 or 680 rads) and neutrons (230 or 360 rads) on rat incisors. (See Figs. 20 and 21, Pages 77 and 78). Their report dealt only with macroscopic observations of unsectioned and non-dissected animals.

They reported that the eruption of the incisors is slowed progressively as the dose increases. This compares interestingly with the results of Medak et al (1950) who found that at between 500-1000r the eruption rate was increased slightly. However Castanera et al were dealing with whole body irradiation, while those animals of Medak et al had only their heads irradiated.

It is difficult to sort out the results obtained with those rats irradiated with neutrons from those irradiated with X-rays.

Fig. 19.

Radiograph of maxillary incisor after exposure to 170 rads neutrons.
A - enamel dip, B - dentin niche.
(From Willhoit et al, 1965).

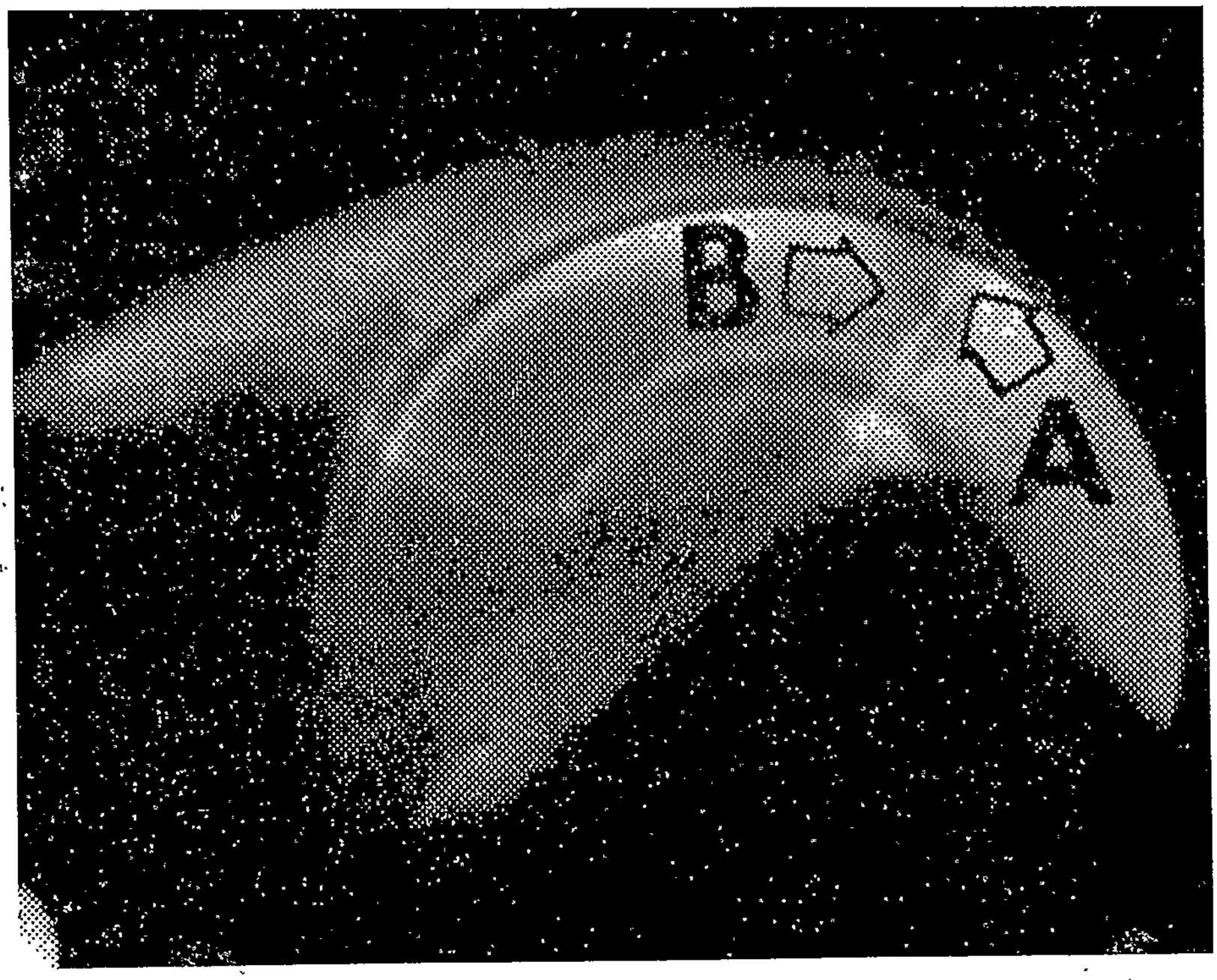


Fig. 20.

Incisor teeth of rat irradiated with 320 rads neutrons (HET) 57 days previously. Note:

(i) white defects and constriction of lower incisor,
(ii) upper defects [redacted] (not yet erupted).

(From Castanera et al, 1963).

Fig. 21.

Incisor teeth of rat irradiated with 560 rads neutrons 7-8 days previously. Note:

(i) broken lower incisor at constricted region,
(ii) maxillary incisors show lesions similar to those of mandibular incisors at 57 days.

(From Castanera et al, 1963).

However from their tables we may conclude that the neutrons produced more severe lesions than X-rays. (98% of animals had lesions of all four incisors when exposed to 320 rads of neutrons against 94% of animals with lesions of all four incisors exposed to 680 rads of X-rays). (Physical factors were 250 KVP 15 m.a. HVL 1.5 mm. Cu. TSD 40" 27 r/min.). They gave the neutron/X-ray potency ratio as approximately 2:3, which contrasts with the level of 1:4 given by Willhoit et al (1965).

HAMSTER

The hamster has a typical rodent dentition except that, unlike rats, the molar cusps are covered with enamel.

Medak et al (1954) found that the reactions of hamsters' incisors to irradiation (4800r 250 Kv. 30 m.a. 50 cm. TSD, 300r alternate sides each day until full dose given) were similar to those of the rat, with the exception of the odontogenic epithelium.

The basal end of the incisors became oedematous and by the end of the fourth week underwent necrosis and liquefaction. This pulpal destruction resulted in the death of associated odontoblasts and these cells were not replaced. However, according to the authors, the fully formed odontoblasts continued to lay down dentin until the pulp was completely obliterated. Osteodentin formed adjacent to this material. Presumably this osteodentin was laid down by newly differentiated pulpal connective tissue cells, although the authors did not mention this form of pulpal reactionary proliferation.

The odontogenic epithelium was compressed by the expanding, so-called, cyst. The epithelium was distorted and appeared as a strand of low cuboidal or squamous cells displaced by the expanding basal cavity. These displaced remnants proliferated, producing

several "knoblike" areas. The proliferated areas were apparently organised to some extent but the authors did not label these areas as abortive efforts by the distorted odontogenic epithelium to form tooth structure.

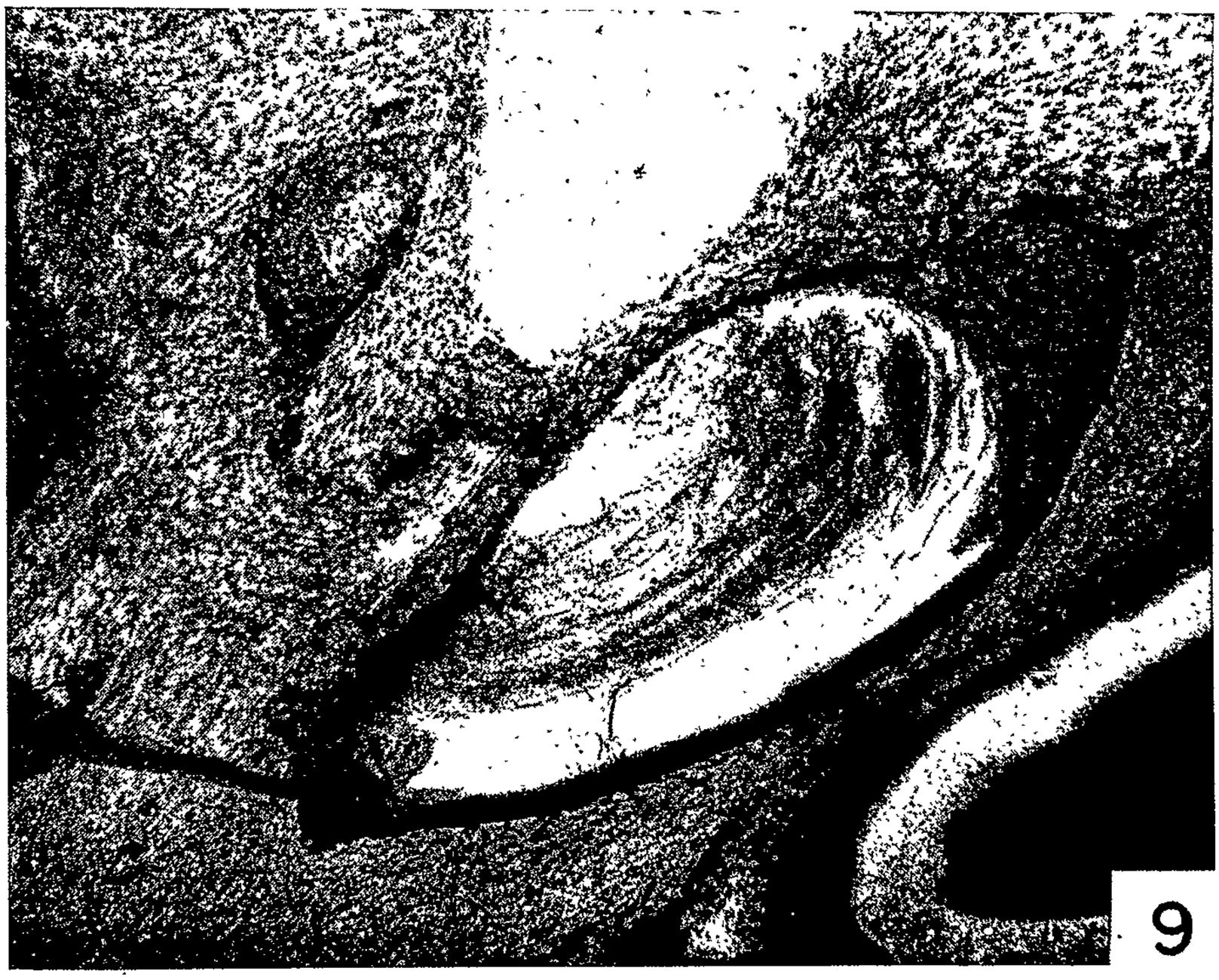
Those teeth which were partially exfoliated by the expanding cyst-like cavity were eventually supported by connective tissue and bone in the old socket.

Animals sacrificed later (7 weeks post irradiation) showed a large cyst "... along the lingual border of the tooth socket". (This probably was not the cavity described previously as the cyst at the basal end of the tooth, as the authors had described it as having been filled in with connective tissue). The wall of this second cyst was irregular in thickness and apparently composed of epithelial cells which contributed to the lumen content of keratin. (See Fig. 22, Page 82). The authors pointed out that this activity replaced any attempt to form normal tooth tissue, whereas in rats the reverse is true, i.e. the new epithelium makes abortive attempts to form tooth structure. Brunst (1954), in his article on axolotls, disputed this odontogenic potential of epithelium which has differentiated following irradiation.

Bruce (1950), using radon (dose approximately 1000-2000r), reported dwarfed roots and missing third molars following irradiation

Fig. 22.

Late stage of localised epithelial proliferation showing a cyst filled with keratin.
(Medak et al, 1954).



of 5-70 day old hamsters. Gross changes were limited to animals less than 10 days old.

In a further study with Kaplan (1953), the authors again reported undeveloped roots, anodontia and dwarfed teeth using single doses of 250, 500, 1000, 2000 and 4000r at 130 Kv. and 160 r/min. At 250r there was no effect, at 500r some effect, and at 1000r a pronounced effect.

Generally the first molars were found to be less sensitive then the third molars, not because of any inherent difference in radiosensitivity, but because the first molars were further developed and hence more differentiated than the third molars. However this finding of decreased radiosensitivity with increasing developmental age of the tooth is directly opposed to the results obtained by Murai et al (1958), which are discussed in the section on the Cat.

Kaplan and Bruce divided the response into two basic categories, labelling the resultant anodontia as a follicular lethal response, and the dwarfing as a sublethal response. Generally they found that the molar response was related to the dose and increased as the dose increased. The response was gradated from mild to severe as follows:

(i) incomplete formation of third molar roots,

(ii) dwarfing of third molars,

(iii) incomplete formation of second molar roots,

(iv) incomplete formation of first molar roots,

(v) anodontia of third molars.

PIG

In a well set up study, English et col (1951) studied the effect of single doses 250-800r of X-rays at the rather high KVP of 2000 (1.5 m.a. HVL=4.3 mm. lead. 14.7 r/min. TSD 2 mm.). They too commented on the excellence of developing teeth as objects of study for radiobiologists, pointing out the multi-embryonic origin of teeth and the complicated and highly organised pattern of differentiation the various components undergo. They gave ameloblasts as an example of a group of cells which undergo "... several stages of proliferation, growth and differentiation". They continued: "With these changes one naturally expects an alteration in its susceptibility to irradiation ...".

Most of the structural changes were found to occur in the enamel, the pulp being mostly unaffected apart from subdentinal haemorrhage and insignificant alterations to the odontoblasts. However, haemorrhage into the alveolar crypt was found to occur above 400r. This finding is in common with other studies, e.g. that of Dale (1953), dealing with whole body irradiation.

The dentin itself showed minimal alteration except for some spherical areas of dense acido-phylic staining material, which was presumably osteodentin, lying below a pronounced incremental line.

The enamel showed a characteristic response to non-specific toxic factors, the response above 400r consisting of a break up of the normally regular laying down of enamel with the formation of enamel pearls (see Figs. 24 and 25, Page 87), cavities and hypocalcification. Where irradiation of 400r or more occurred at the time of a cusp tip formation, faulty amelogenesis (as outlined above) occurred. (See Fig. 23, Page 87). However, in those animals surviving long enough, e.g. 71 days post irradiation, the subsequently formed cervical enamel was reportedly normal. (See Fig. 26, Page 87). This is a most interesting result for two reasons. Firstly, it is the first report of complete recovery of amelogenesis in non continuously growing teeth, and secondly, it appears to indicate that:

(i) the orderly differentiation of ameloblasts is not determined by the more advanced preceding cell higher up in the "cap" stage of development, as the later differentiated and more cervical ameloblasts were normal, and

(ii) the functional ameloblast is more radiosensitive than its precursor.

The authors suggested that the change of polarity of the ameloblast is the point at which this increase of radiosensitivity occurs. The ameloblasts of the enamel organ do not change their polarity together but rather in a progressive manner, starting

Fig. 23. Second molars, 2 pigs aged 5 mths. 16 dys. at irradiation (800r). Sections 12 dys. post irradiation. (a) Normal enamel; (b) gnarled enamel external to (a). Both formed by same ameloblasts. Normal post irradiation enamel (c) formed by new ameloblasts.

Fig. 24. Second molar of pig which received 450r. Thin layer of normal enamel over occlusal third. No normal ameloblasts either along calcified band (o) or enamel. (e) enamel pearls. (h) haemorrhagic effects into crypt and in pulp. (i) pronounced imbrication line in dentin.

Fig. 25. Resorption of enamel (r). Complete second enamel layer, formed architecturally different from that of pre-irradiated tooth.

Fig. 26. Normal enamel and dentin formed post irradiation in cervical area.
(From English et col 1951).

23

24

25

26

at the tip of the cusp and progressing towards the cervical area. At any one time during crown development ameloblasts ranging from low cuboidal to fully mature cells may be found. Thus the procedures of English et col appear to be selective, as only the more mature cells were damaged.

These irregularities (viz. enamel pearls, cavities and hypocalcification) were found to occur in areas where the ameloblasts were most metabolically active. The authors reported that ameloblastic sensitivity is proportional to ameloblast metabolic activity, finding that those ameloblasts whose polarity had not changed at the time of irradiation were unaffected.

They concluded that the damaged enamel resulted from damaged ameloblasts and that the resultant poor enamel matrix was normally calcified. From this we may deduce that the protein synthesis function of the ameloblasts is more readily disturbed by irradiation than the calcification function. In fact no author has reported an investigation, with any animal, of the effects of irradiation on calcification, largely concerning themselves instead with decalcified sections.

English et col (1951) and Willhoit et al (1965) are the only authors to report enamel changes producing deformed enamel overlying normal dentin. Both these studies involved whole body irradiation (X-rays and neutrons) and both studied ground sections.

CAT

The cat was one of the first experimental animals used for radiobiological studies. Racamier et col (1905) (as quoted by Kimeldorf et al, 1963) reported markedly altered dentition of a kitten following irradiation, and that the teeth showed a greater degree of retardation than did the facial bones.

Gorvey (1951) repeated the experiment using a black female four day old animal. The dosage was 1500r delivered as a single dose in a period of five minutes. No other physical factors of the apparatus were given, and as only one animal was used his results must be appraised with this severe limitation of experimental design in mind.

He reported dwarfed incisors, with the right side smaller than the left, and loss of the deciduous teeth long before untreated animals of similar size. The permanent dentition was markedly affected, only two permanent anterior teeth erupting, one of these being "... severely affected."

Murai et al (1958) irradiated the heads of 13 young cats, marking the stage of dentinogenesis at the time of irradiation by means of lead acetate injections. Physical qualities of the apparatus were 160 KVP, 3 m.a. 0.5 mm. Cu. and 1.0 mm. Al. HVL 0.8 mm. Cu. TSD 23 cm., doses of 1800-3360r. The animals were

then kept for periods ranging from 13 to 375 days.

It is difficult to decide from the authors' writing exactly what dosages the animals received. Although the text has doubtless suffered in translation from the original Japanese, there can be little excuse for reporting, under the physical factors heading, a dose range of 1800-2000r, and then under the results heading, an applied dose of 1120r x 2 (animals 15, 19, 20), and 1120r x 3 (animal 14).

Thirteen days following a dose of 2240r one animal's radiograph showed no abnormalities of the jaws. However, histological examination indicated a decrease in the number of pulpal cells near Hertwig's epithelial root sheath and a "... disturbance of histo-differentiation ...".

Following 1800r (600r x 3) a total of three animals were sacrificed at periods of 20, 37 and 88 days (see Fig. 27, Page 91), following irradiation. From an initial response at 20 days consisting of a decreased number of connective tissue and odontoblastic cells and a disturbance of odontoblastic differentiation, they found that a wavy or zig-zag layer appeared in the basal portion of the mandibular premolar. This can be identified from the accompanying photographs as the dentino-enamel junction, although the authors did not name this structure. (See Fig. 28, Page 92). This waviness was probably caused by unequal

Fig. 27.

Cat, 88 days post irradiation (600r x 3). Note cessation of root formation and dwarfing in permanent teeth.
(From Murai et al 1958).

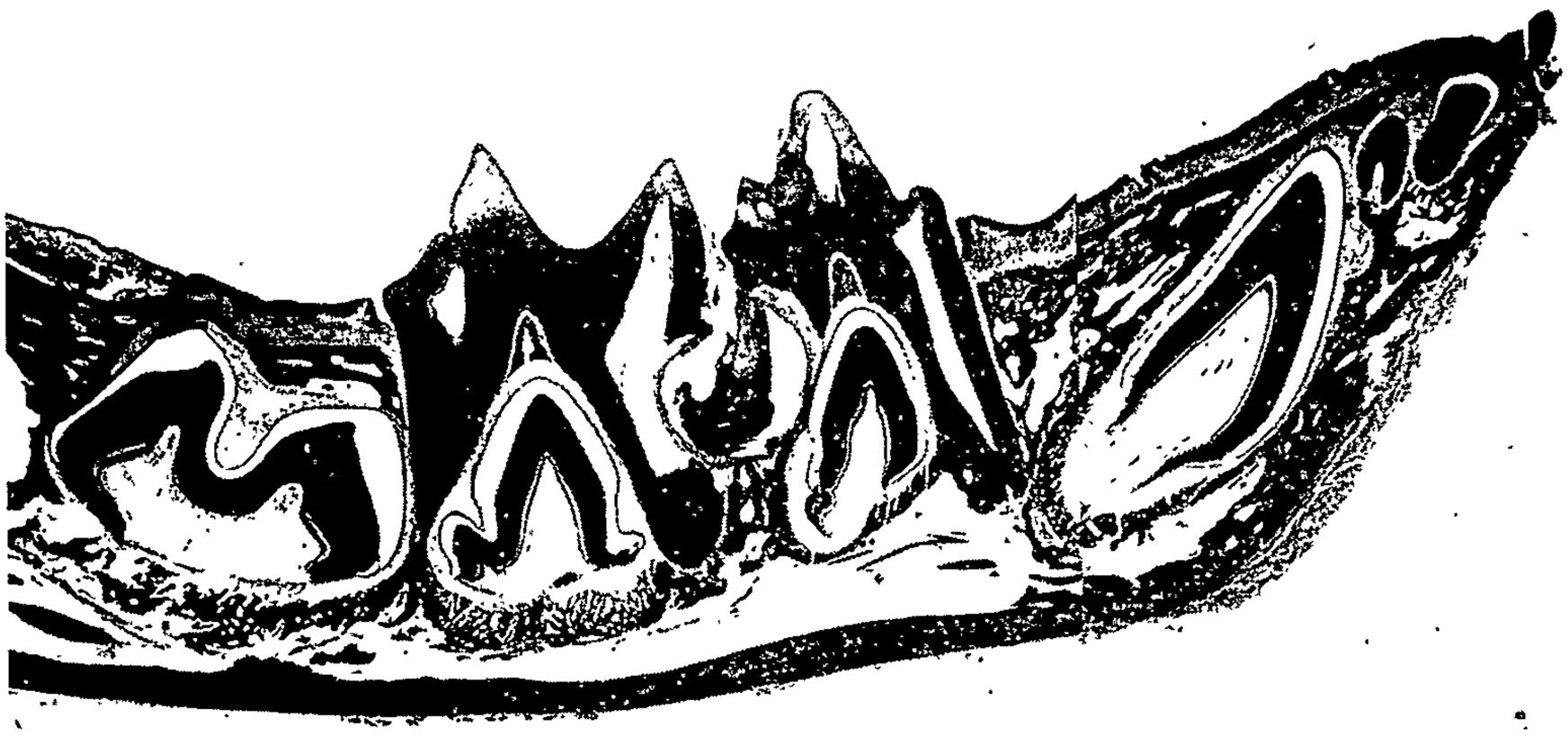
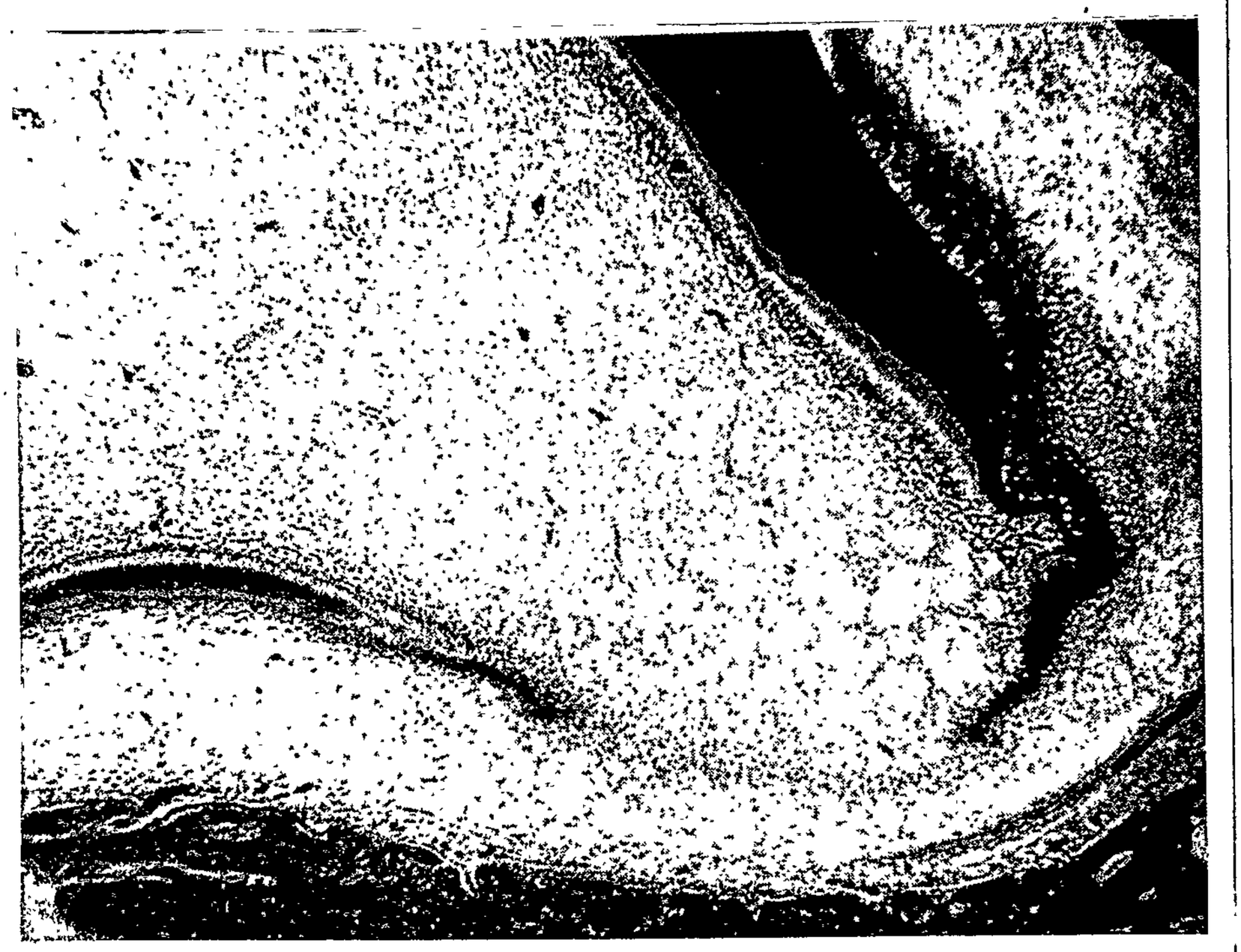


Fig. 28.

Developing Premolar (Cat).
Note wavy dentino-enamel junction and abrupt cessation of normal odontoblasts.
(From Murai et al, 1958).



growth of the disturbed ameloblasts and odontoblasts. This is borne out by their illustration (see Fig. 28). No recovery was seen at 37 days. However, at 88 days histo-differentiation was increased in some places. The roots of the premolars and molars ended abruptly and showed no further formation, but that of a canine was completed though more slender than normal. Other signs of irradiation damage included root ankylosis, abscess formation and partial obliteration of pulpal chambers by the osteodentin.

Animals exposed to 2240-3360r and sacrificed following periods of 186 to 375 days showed similar responses; namely, a gradual increase in the rate of apposition of dentin (the authors did not give the pre-irradiation apposition rate for all animals), dwarfing of teeth, loss of permanent teeth following eruption due to their rootless form, impaction of some third molars and slender canine roots. The decrease in root width of canines was reported in the result section of the article. However under the later discussion heading, similar effects to premolars were also mentioned, as well as resorption of dentin and tooth buds, and some recovery of the odontoblasts.

One animal was kept until the teeth were more advanced in their development before being subject to irradiation (no age given). Presumably all the permanent crowns were formed at the

time of irradiation, as they showed no defects. However, the roots were slender in shape and the eruption of the permanent teeth retarded.

The very interesting result of a completely different response by the molar and premolar roots is pointed out by the authors. They found that the developing premolars reacted to irradiation by producing a slender root. However, odontogenesis of the adjacent molar was more severely affected, and no root would form. They concluded that the molar and pre-molar teeth have different patterns of response to irradiation damage, but offered no explanation for this phenomenon. However, it would probably be more correct to conclude that the response of the two teeth is identical, the molars being the more sensitive and thus the more acutely affected of the two teeth.

By comparing primordia, each at different stages of development at the time of irradiation, the authors found that recovery was more likely in the earlier stages of crown development than in the later stages.

The molar normally erupts before the premolar in this species. However the irradiated premolars, which produced roots, erupted before the irradiated rootless molars. This result is most interesting from two points of view. Firstly, we have seen that Gowgiel (1961), following experiments with monkeys, concluded that

root formation was not a factor in tooth eruption. Secondly, as the premolars of the cats at the time of irradiation were less advanced in their development than the molars, the authors' conclusion that recovery is decreased with increasing developmental age appears to be verified - a conclusion that may well be disputed.

The lead acetate marking methods used by the authors indicated a response by the mature odontoblasts not previously reported in the literature. They found that the mature dentin producing odontoblasts, which have been reported as highly radio-resistant cells, were affected by moderate doses of irradiation, and that this effect took the form of an initial decrease in the apposition rate of new material followed by a gradual return to normal.

The results of these authors are very similar to the pattern of irradiation damage reported in humans. They found partial anodontia - dwarfed roots, ankylosis of deciduous teeth and a variety of responses in the same animal. They commented that the cat possesses a heterodont and dyphodont type dentition similar to that of man, and suggested that the radiosensitivity of teeth is determined not only by the develop-mental stage at which the tooth is irradiated but by the type

of tooth as well. This is a most interesting conclusion.

Donohue et col (1964), in a similar study to that of Murai et al (1958), used 25 domestic kittens and observed the changes in dentin deposition following exposure to 2000r (2 x 1000r, 7 days apart, 110 Kv. 10 m.a. TSD 20cm. 3mm. Al. filter). Although their results were very similar to those of Murai et al, their conclusions conflicted. They found that the mature odontoblasts were "minimally" affected and less radiosensitive than the immature cells, and that the apposition rate of dentin changed. This indicates that the mature odontoblast is less radiosensitive than the immature odontoblast, as the dentin continued to grow in width due to the activity of the mature radio-resistant odontoblast, but not in length because of the radiosensitivity of the immature odontoblasts. They also reported a decrease of eruption rate.

They further concluded that eruption is not due to "periodontal pressure", but this conclusion is probably not as accurate that of Murai et al.

No author who has dealt with the cat has attempted to report on the effects of irradiation on enamel. From the

illustrations of Murai et al it would appear that the amelo-
blastic response is very similar to that which we have seen in
other animals.

AXOLOTL

Brunst (1954) made a comparison of the effects of irradiation on tooth development in young axolotls and mice. Using 36 Siredon Mexecanum axolotls he irradiated 22 animals with 3000r at 60 days post hatching, and 14 with 2000-4000r at 20-22 days post hatching. Physical factors of the apparatus were 55 KVP 3 m.a., no filter, 10 cm. TSD, through a 2 cm. slit in lead to either the dorsal aspect of the maxillae or ventral aspect of the mandible, the opposing jaw being protected by a lead shield.

13 days post irradiation the animals treated with 4000r at the age of 22 days showed a complete suppression of mitotic activity and a cessation of the differentiation and development of teeth in the exposed tissue.

23 days post irradiation degenerative giant cells were seen in the epithelium, and the author concluded that it "... is a clear sign of the primary acute reaction to irradiation".

The author found that the enamel epithelium had disappeared and that cells, probably macrophages, were invading the residual tooth structure. This is the only account of actual destruction of unerupted tooth structure in the literature surveyed.

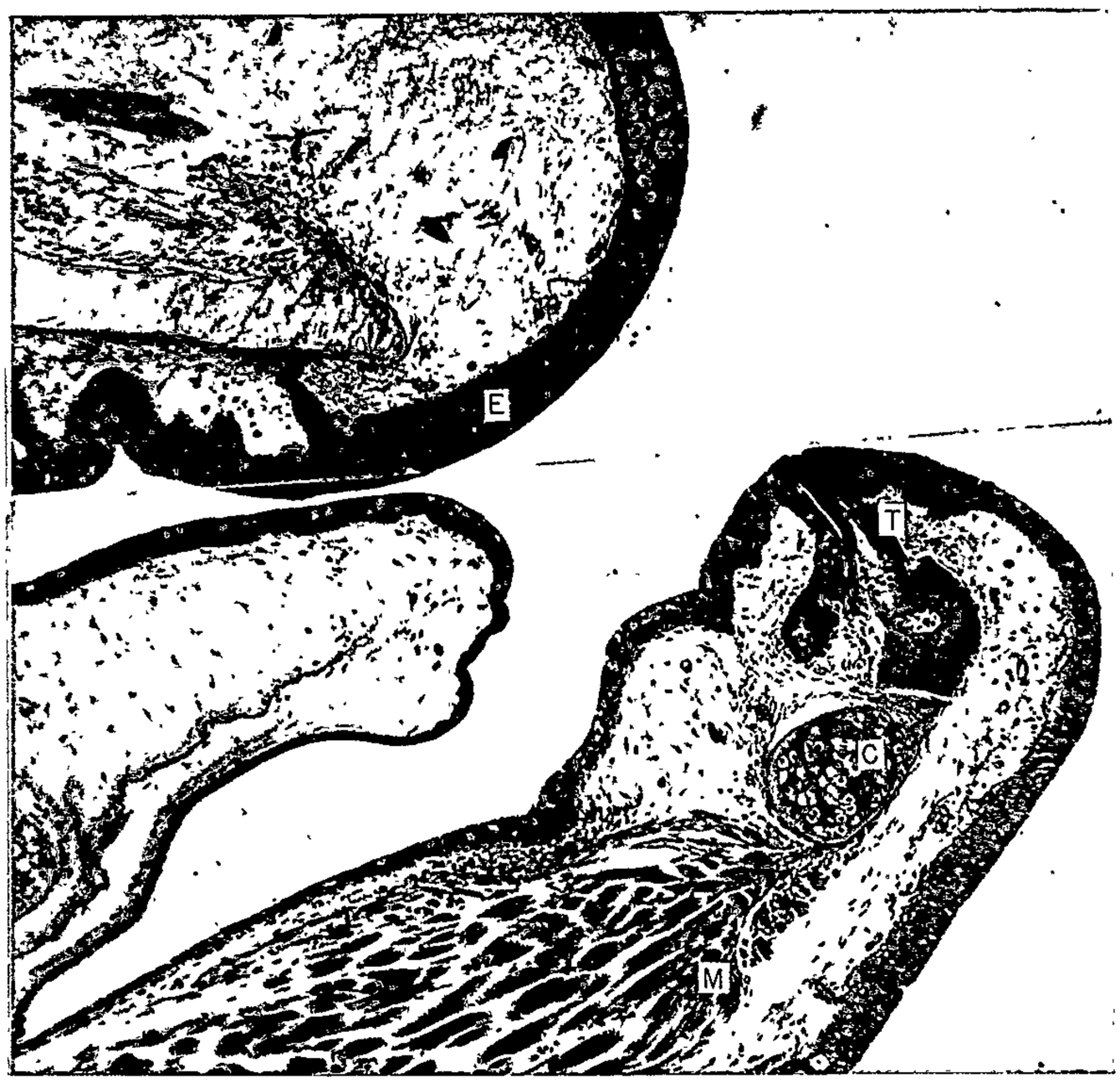
This process of tooth resorption was almost completed 34 days post irradiation (4000r). The degenerative epithelium previously described had been replaced with normal epithelium from, the author presumed, the oral cavity. However no sign of new tooth development was seen and Brunst concluded that only a very narrow region of the oral mucosa is capable of producing tooth structure. This conclusion may be unsound, as it has been shown embryologically that structural development is not only a property of certain cells, as Brunst pointed out, but that those cells must be in the correct position at the correct developmental time for the required structure to develop. It may well be that any epithelium may have odontogenic potential, and this view is supported by such occurrences as dermoid cysts, in which teeth may form completely removed from the oral cavity. Further, as we have seen previously in the section on the Hamster, Medak et al (1954) pointed out that varying attempts of newly differentiated epithelium at odontogenesis in rats were made following irradiation.

At 58 days the tissue of the treated jaws appeared normal. However the tooth buds had completely disappeared and the treated jaws were edentulous, (See Fig. 29, Page 100).

In the discussion section of his article, Brunst reported a greater response from the lower jaw than the upper, indicating

Fig. 29.

Suppression of development of upper jaw and teeth of axolotl by irradiation. Sagital section through upper irradiated and lower control jaw of an animal 110 days after treatment with 2000r of 20 day old animal.
C - cartilege, E - epithelium, M - muscle, T - tooth.
(From Brunst, 1954).



a higher radiosensitivity of the lower jaw. However, he concluded that this may have been due to the physical factors of his unfiltered radiation.

Unfiltered radiation produced at 55 KVP would have contained a high proportion of soft, poorly penetrating rays. The result would have been to expose the mandibular teeth to higher dosages of irradiation than the maxillary teeth, as the maxillary teeth would have been partially protected by the intervening cranium.

C O N C L U S I O N

The following table is a summary of some of the reported effects of irradiation occurring at different dose levels. The dosages were achieved with a multitude of different types of radiation sources and are the total dose given, whether a single dose or fractionated dosages.

Dose	Animal	Reported Effects
80r	Rat	Constriction of incisor shaft, enamel hypoplasia seen before any change in dentin, HET radiation (Willhoit et col, 1965).
170r	Human	Long term study as a result of radio-active fallout. No observed effect (lyon et al, 1964).
200r	Rat	No effect (macroscopic), (Castanera et al, 1963).
250r	Hamster	No gross changes observed (Kaplan et col, 1953).
	Rat	Enamel malformation in foetal rats (Graham, 1963).
300r	Human	Histological changes in the epiphysis of growing bone (Dawson, 1968).
	Rat	Labial niche formation in upper incisor Koppang, 1967).
360r	Rat	360r neutrons will produce more damage than 430 rads X-rays (Castanera et al, 1963).
375r	Rat	"Marked ameloblastic radiosensitivity", dentin less affected (Dale, 1948).
400r	Rat	Labial niche formation lower incisor (Koppang, 1969).

Dose	Animal	Reported Effects
		Significant effect on the dentino-enamel junction. Well developed by 500r (Binford et col, 1961).
	Pig	Hypoplasia, alteration of enamel structure. Haemorrhage into alveolar crypt, formation of enamel pearls, enamel cavities and hypo-calcification of enamel (English et col, 1951).
	Human	The epiphysis of long bones may be damaged producing stunting of growth (Dawson, 1968).
500r	Hamster	Evidence of root dwarfing of third molars (Kaplan et col, 1953).
	Rat	500-1000r produced a slight increase in the rate of eruption of upper incisors (Medak et al, 1950).
600	Rat	Amelogenic cell migration slowed to almost zero (Willhoit et al, 1971).
600r	Rat	Formation of osteodentin (Binford et col, 1961, and Koppang, 1967).
700r	Rat	(400-700r) No change demonstrated in enamel or ameloblasts. Young odontoblasts damaged (Koppang, 1967). (See 400r and 375r).
		Minimum dose needed to produce lingual lesions in incisors (Koppang, 1969). (See 400r).
1000r	Rat	Unerupted non-calcified third molars unaltered (Binford et col, 1961).
		500-1000r produced a slight increase in the eruption rate of incisors (Medak et al, 1950).
1200r	Rat	Number of necrotic cells closely related to dose (Adkins, 1967).
	Hamster	Incomplete root formation and dwarfing of first and second molars, anodontia of third molars (Kaplan et col, 1953).

Dose	Animal	Reported Effects
1350	Mouse	Incisor defects leading to death from starvation (Upton et al, 1950).
1500r	Rat	A general recovery of tooth forming elements (English et al, 1954; English, 1956).
		No effect at 16 and 24 hours post irradiation (Hansen et col, 1957).
	Mouse	Some osteodentin and abnormal odontoblasts. Ameloblasts normal (see 375r) (Burstone, 1950).
1580	Human	Micrognathia and microdontia (Dawson, 1968).
1800r	Rat	600x3 produced a more marked effect than 150x12 (Adachi et al, 1959).
	Cat	Reduction of pulpal and odontoblastic cell numbers, mature odontoblasts continued to function, no sign of recovery of odontogenic epithelium at 37 days post irradiation. Ankylosis of root of deciduous molar (Murai et al, 1958).
2000	Mouse	(1-3 days old) Complete lack of lower teeth (Brunst, 1954).
2000r	Hamster	Incomplete root formation, anodontia of third molars or dwarfed third molars (Kaplan et col, 1953; Bruce, 1950).
	Rat	(1000-2000r) Changes well localised to the labial part of the odontogenic zone. Waviness of dentino-enamel junction. Slight injury to ameloblasts causing enamel hypoplasia (Medak et al, 1952). (See 375r, 400r and 1500r).
		Permanent cessation of root development, crowns still erupted. Only temporary cessation of incisor growth (Gowgiel, 1967).
3000r	Rat	Minimum effective dose to produce a complete cessation of eruption (Medak et al, 1950).

Dose	Animal	Reported Effects
		Production of large cyst-like cavities (Medak et al, 1952).
	Human	Will stop tooth development at present level of formation. Teeth will usually erupt (Blozis et col, 1968).
4000	Axolotls	Resorption of tooth structure (Brunst, 1954).
	Rat	Incisor growth in length stopped immediately; growth in width undisturbed (Weinreb et al, 1949).
4500	Monkey	Permanent tooth development stopped (Gowgiel, 1961).
4800	Hamster	No dentin formation from differentiating odontoblasts. Mature odontoblasts continued to function but abnormal pulp obliterated by dentin, cyst formation, and no normal tooth structure formed post irradiation (Medak et al, 1954).
5000r	Mouse	Cells of ameloblastic layer appeared as low cuboidal or squamous types. 1500-5000r severely damaged the odontoblastic layer. Surviving odontoblasts resembled osteoblasts, osteodentin formed. Odontoblasts were more radiosensitive than ameloblasts (See 375r, 1500r, and 2000r). Atrophy and degeneration of pulp (Burstone, 1950).
7470r	Human	Microdontia (Gorlin et al, 1963).

The effects of ionizing irradiation on developing human teeth appears to depend on:

(i) the developmental age of the teeth,

(ii) the dose absorbed by the teeth,

(iii) the method of administration of the dose,

i.e. time and number of divided doses,

(iv) susceptibility of the patient (e.g. age),

and these factors have been demonstrated in experiments dealing with animals.

The pattern of damage appears to vary according to the type of dentition that the subject possesses. We have seen that the human response can be varied and quite bizarre, exhibiting differing degrees of anodontia, microdontia, root deformations, delayed eruption, hypocalcification and premature loss of teeth. Many of the responses can be demonstrated in the same individual. However the reports of the case histories of the human studies indicate that until very recently there has been no strict standardisation of the physical factors of exposure. Consequently we must accept these reported aberrations of tooth development as indicators of likely consequences of irradiation damage. In contrast to this, the reports of the better work done on experimental animals indicate that we can predict very accurately ($p < 0.05$ and $p < 0.01$) the response that will be obtained (Koppang, 1969).

There appears to be a basic similarity in the radiosensitivity of similar types of teeth (e.g. those with persistent pulps) developed by different animals and a marked contrast of radiosensitivity between different types of teeth in the

same animal, and even a difference between similar odontogenic elements within the same developing tooth. For example, from the table and the review of the literature, it is apparent that hamster and rat incisor responses are very similar. However the rat molar at 2000r exhibits a permanent cessation of root development, whilst the incisors are only temporarily affected (Gowgiel, 1967). Further, the labial odontoblasts of the rat incisors are affected at 400r, whilst the lingual odontoblasts of the same tooth require a minimum dose of 700r to show signs of damage (Koppang, 1967).

The comparative radiosensitivity of ameloblasts and odontoblasts obviously requires a more thorough investigation. Various reports, e.g. those of Willhoit et al (1965), who reported a marked ameloblastic sensitivity, and Koppang (1967), who reported a comparative radio-resistance when compared with odontoblasts, are diametrically opposed, and obviously one must be wrong.

However, it appears that ameloblasts are the more sensitive cells (see table for 80r, 375r and 400r). Many of the reports were based on evidence obtained from decalcified sections, and so the conclusions drawn as to the state of the enamel could well be erroneous. No author has attempted to correlate the changes exhibited in ground sections, decalcified

sections, and intra-cellular changes occurring in any one group of experimental animals.

In the chapter on the development of teeth we saw that there are basically two distinct but closely related phases in odontogenesis. Firstly, the differentiation of the ameloblasts, odontoblasts and cementoblasts together with the subsequent production of the enamel matrix predentin or precementum. Secondly, the mineralization of the various structures.

There has been no investigation in the reviewed literature of the effects of radiation on this vital second phase of odontogenesis. Even in the exhaustive reports featuring extensive lesions the authors have merely referred to "hypo-calcification", "normal calcification", or "hypercalcification". It would be a most interesting study to follow through the maturation of irradiation-damaged ameloblasts or odontoblasts, their subsequent production of deformed (?) or abnormal (?) or normal (?) matrix, etc., and the degree of resultant mineral-ization. It may well be that changes in the pattern of mineralization of enamel could be the most radiosensitive phase of odontogenesis, as hypoplastic enamel is found in the human dentition far more frequently than hypoplastic dentin.

In the chapter on radiobiology we saw that there is a marked variation in the radiosensitivity of cells, ranging

from almost zero for cells in mitosis to 700 rads for cells in late synthetic phase, and yet the bulk of the reviewed articles have been concerned with tissue doses far in excess of 700r. No author has attempted to correlate the tissue changes, or lack of, in response to significant dosages of irradiation with intracellular changes which apparently occur at very much lower dosage levels. Further, there has been a marked scarcity of observations of intracellular changes provoked by ionizing radiation.

One very interesting point which has not been made in the literature except by Worth (1963) and Stasek et al (1965), is that not only is the radiosensitivity of the ameloblast and odontoblast determined by their degree of differentiation and, in the case of ameloblasts their polarity, but also it appears by the age of the animal concerned. For example, we have seen that Mathews (1963) found with foetal mice that enamel was affected at 300r, and that Graham (1963) observed similar effects in foetal rats at 250r. These changes appear to be far more marked than those reported by even the most observant studies dealing with older animals. Koppang (1967) and Adkins (1967), using rats, reported seemingly far less marked cellular changes.

This finding of decreasing radiosensitivity with increasing odontogenic maturation is contrary to that of

Murai et al (1958) who found, with cats, that recovery was more likely in the earlier stages of crown development than in the later stages.

The observation of variable radiosensitivity has been borne out by the odontoblasts, the mature cells continuing to lay down dentin (albeit deformed dentin) following irradiation, long after the immature cells have disintegrated. However the reverse appears to be true for the ameloblasts. These cells, following a change in polarity, appear to be more radiosensitive (English et al, 1951) than their less differentiated precursors.

By comparing the reports of Stasek et al (1965) on human response and Adkins (1967) on rats, we may conclude that the human developing dentition is very radiosensitive, the threshold for damage lying below 300r, and possibly 125r (at 120-160 Kv.). Stasek et al reported a mandibular hypoplasia when children's mandibles were irradiated at this (300r) dosage level, and Adkins (1967, 1968) reported similarly severe mandibular hypoplasia and defects to the developing teeth or rats at 1200r. It is likely that the radiosensitivity of developing human teeth is at least equal to, or greater than, that of the mandible.

Finally, it appears that there is a definite risk of

damage to developing teeth with most forms of ionizing radiation of sufficient quantity to induce haemangiomas to disappear. However, Yttrium 90, a β-emitter, is capable of producing a useful level of soft ionizing radiation that will not penetrate to a sufficient depth at a sufficient dosage to produce damage to the underlying developing teeth. This may be an alternative source of irradiation to the more penetrating X-rays.

However, with the demise of the widely held belief that there is a "safe" level of radiation and a realisation that all levels of radiation are potentially hazardous (Kenward, 1973), it would appear to be unwise to treat infantile benign lesions with any form of irradiation.

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