ACTA ORTHOPAEDICA SCANDINAVICA
SUPPLEMENTUM NO. 116

From the Department of Orthopaedic Surgery (Head: Prof. G. Wiberg) and the Department of Oral Roentgenology (Head: Prof. K.-Å. Omnell), University of Lund, Sweden

RATE OF APPOSITION OF DENTINE IN UPPER INCISORS IN NORMAL AND HORMONE-TREATED RATS

WITH A TENTATIVE COMPARISON OF THE APPOSITION OF DENTINE AND LAMELLAR BONE

BY

SVEN-ARNE AHLGREN

MUNKSGAARD
Copenhagen 1968

Statistical adviser: Peter Vorwerk
Translator: L. J. Brown

LUND 1968
CARL BLOMS BOKTRYCKERI A.-B.

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EXPLANATIONS

Throughout the investigation apposition of dentine is measured in arbitrary units — measuring units (1 unit=10 μm).

The various technical terms used such as section, interval, point, etc. are explained on pages 35—36 and 52. The abbreviations used in Tables in the Appendix are explained on pages 46—47.

Tables with Roman numbers are collected in the Appendix.

INTRODUCTION AND PURPOSE OF THE INVESTIGATION

In the course of personal preliminary experimental investigations on the effect of the growth hormone on the rate of bone repair and lamellar bone formation it was realised that accurate quantitative determinations would offer considerable technical difficulties. Because of the constant remodelling of bone tissue the interpretation of the results could be difficult.

Bone repair has been studied by manual testing of the stability of the fractured bone (Koskinen 1959, 1965), by roentgenography with or without planimetric measurement of the callus as seen in the roentgenogram (Koskinen 1959, 1963, 1965), by examination of histologic sections (Koskinen 1959, 1965, Hulth & Olerud 1964, Pritchard 1964), by autoradiography (Koskinen 1959, 1965), by measurement of the mechanical strength of the fractured bone during healing (Lindsay & Howes 1931, McKeown, Lindsay, Harvey & Howes 1932, Falkenberg 1961, Wray & Goldstein 1966), by studying the uptake and disappearance of radioactive isotopes (Marshak & Byron, Jr. 1945, Bauer 1954, Bohr 1955, Wendeberg 1961), by angiography (Göthman 1961, Hulth & Olerud 1964), by tetracycline labelling (Hulth & Olerud 1964) and by microradiography (Nilsonne 1959, Hulth & Olerud 1964).

Lamellar as well as cancellous bone has been widely studied also with the aid of radioactive isotopes (Bauer, Carlsson & Lindquist 1961, Dymling 1964, Nordin, Smith & Glass 1964, Bauer 1966), microradiography (Jowsey 1966) and vital dyes, such as alizarine (Harris 1960) and the tetracyclines (Harris 1960, Lee 1963, 1964, Frost, Villanueva & Roth 1960, Tapp 1966).

During the above-mentioned personal investigations interest was focused on dentine. This tissue is similar to bone, but its physiology is

less complicated because no resorption occurs in normal dentine. It was thought that its rate of formation could be studied with comparatively simple laboratory methods. It had been realised by the Swedish anatomist Retzius as early as 1838 that "similarity between dentine and bone is closer than that suggested by the first microscopic examinations". Also other investigators have recognised such similarities (Weidenreich 1925, Orban 1957, Ørvig 1958, 1967, Bernick & Ershoff 1964, Zussman & Ioachim 1964).

According to Sognnaes (1955), the microstructural organisation of bone, enamel, dentine and cementum "is comparable insofar as they all contain (a) a fibrous framework, (b) an amorphous ground-substance, and (c) inorganic crystals, deposited in that order, whereas the vascular and cellular elements of bone, cementum, dentine, and enamel decrease in significance in that sequence".

Bone and dentine resemble one another also in chemical composition and ultrastructure. In both tissues the bulk of the organic matrix consists of collagen, and the collagen in dentine is very similar to that in bone (Piez & Likins 1960, Eastoe 1964, 1967). The mineral phase in both tissues is an apatite (De Jong 1926, Gross 1926, Hodge 1955 Carlström & Engström 1956, Posner 1960).

Bone and dentine differ from one another in one essential respect: in bone, formation and resorption occur simultaneously; in normal dentine, only formation (Schour 1938, Schour & Massler 1943 b, Sognnaes 1955), but, as in bone, exchange of ions takes place (Armstrong 1955, Sognnaes 1955).

In the incisors of rodents, dentine continues to form throughout the animal's life. In his studies on the parathyroid glands and calcium metabolism Erdheim (1911) considered the incisors of the rat to be an extremely sensitive indicator. He also stressed what may be called their kymographic properties: what was registered remained until the incisor was worn away. He compared the incisor with bone, where the continuous formation and resorption make the interpretation of a microscopic section very difficult, and recommended the use of the incisor in investigations of calcium metabolism. Also in fairly recent literature incisor dentine formation in the rat has been described as continuous, very regular and very sensitive to experimental interference (Schour & Massler 1962).

Preliminary personal investigations showed that the rate of apposition of dentine in the upper incisors of the white rat varied in a more complicated way than previously supposed. Therefore, before pro-

ceeding to the investigation of dentine as a test tissue for hormonal influence it was considered necessary to re-investigate the normal rate of apposition of incisor dentine in the rat. Several experimental investigations of the action of the growth hormone have been performed on female rats. It was therefore primarily decided to confine the investigation to rats of this sex. The study was limited to the upper incisors for two reasons. First, owing to their shape, it is easier to obtain acceptable histologic sections from these teeth than from the lower incisors, and second, to keep the number of histologic sections and measurements within reasonable limits.

Since it was desired to compare dentine and bone formation, it was decided to study endosteal and periosteal bone formation, because bones in the rat have no Haversian systems (Enlow & Brown 1958).

The purposes of the present investigation were:

to chart the normal pattern of apposition of dentine of the upper incisors in the female white rat,

to find out whether the rate of apposition of dentine could be influenced by the administration of a hormone, and

to compare the rate of apposition of dentine and of lamellar bone.

EARLIER INVESTIGATIONS

I.

MORPHOLOGY OF THE UPPER INCISORS, PARTICULARLY OF THE DENTINE AND A SHORT NOTE ON BONE FORMATION

INCISOR

Gross morphology

According to Mummery (1924), "the distinguishing features of the rodent dentition are the presence in each jaw of a pair of long curved incisors with chisel-shaped edges and growing from persistent pulps" (Figs. 1—2).

Eruption of the incisors and formation of dentine occur simultaneously and continuously throughout the animal's life. This growth is normally counterbalanced by mechanical attrition at the incisal edges (Weinreb, Assif & Michaeli 1967).

The upper incisor has been described as a 210° segment of a logarithmic spiral with a lateral shear (Schour & Massler 1962), but also as a segment of a circle, about 210°, at 5 months of age (Addison & Appleton 1915). The length of the upper incisor, along the outer, convex side at 5 months of age (Addison & Appleton 1915) is 23.3 mm; the extra-alveolar length, 8.7 mm.

The main part of the rat incisor consists of dentine. The labial aspect and part of the lateral and medial aspects are covered with a layer of enamel; the rest with a thin layer of cement (Figs. 2—3).

The longitudinal or "extrusive" growth of the incisor can be measured either as eruption rate or as attrition rate (Sessle 1966, Weinreb et al. 1967). Sessle found these values to be close to each other in the lower incisors. In the upper incisors the situation is similar. Thus, according to Addison & Appleton (1915), the rate of attrition is 2.2 mm/week. Similar figures have been given by Schour & Massler (1962) and Stüben & Knothe (1965) for the rate of eruption.

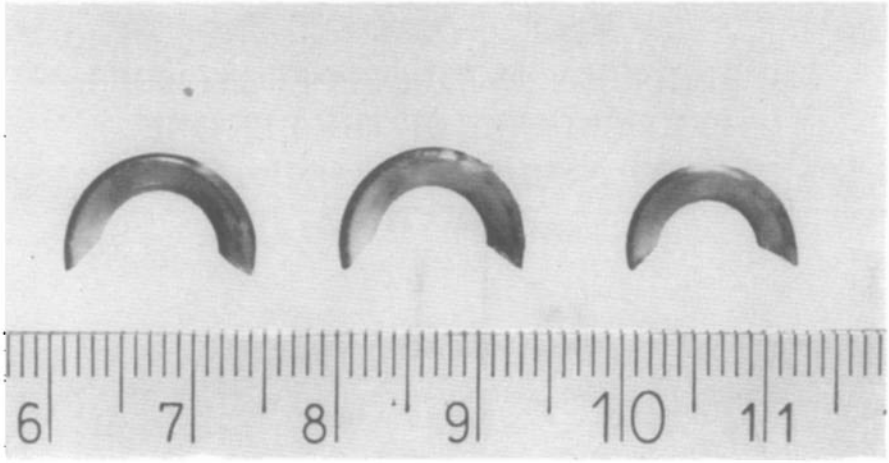


Fig. 1. Incisors from rats of different ages. Ages (left to right) approx. 10, 4 and 2 months. Incisal edge to left.

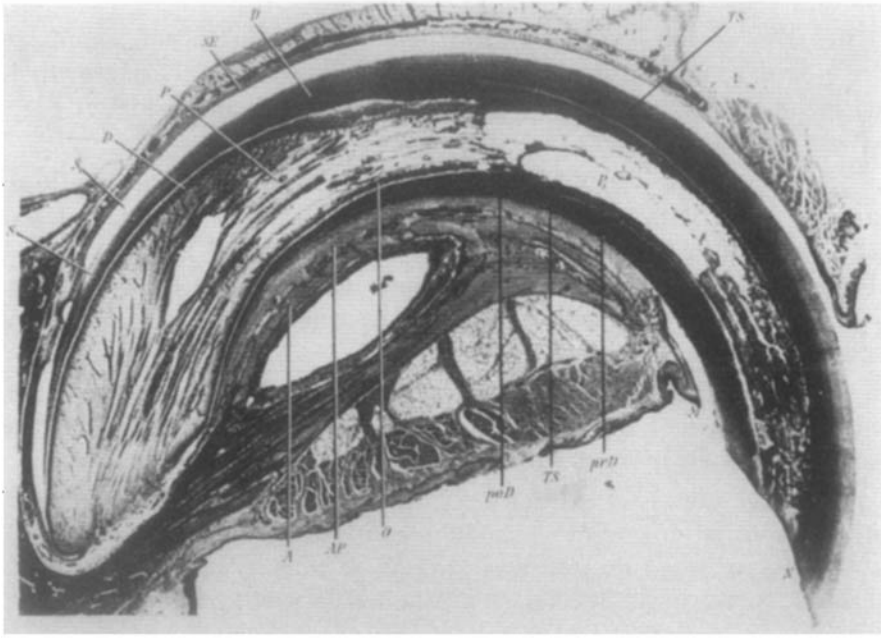


Fig. 2. Photomicrograph of a section of an incisor (After Erdheim, 1911). Symbols: P=pulp, S=enamel, D=dentine, SE=enamel epithelium, O=odontoblasts, AP=alveolar periosteum, A=the bony alveolus of the tooth, M=oral mucous membrane, N=incisal end of the tooth. The other symbols used are not essential to the understanding in this connection.

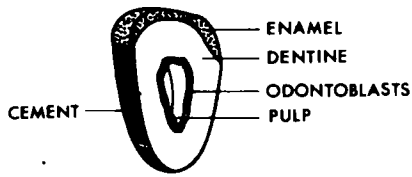


Fig. 3. Schematic drawing of transverse section of an incisor.

Dentine

Dentine is formed by odontoblasts lining the pulp cavity (Figs. 2—3) and giving off cell processes extending through the dentine tubules. Owing to the continuous eruption of the tooth the odontoblasts move towards the incisal edge at the same time as dentine formation continues (Schour & Massler 1962). During this process the layer of dentine grows thicker and the pulp space consequently narrower.

The first stage in dentine formation consists of the formation of an organic matrix, which later becomes calcified (Erdheim 1911, Schour & Massler 1962, Bevelander & Nakahara 1966, Symons 1967). As in the osteone, the mineralisation does not follow immediately after the formation of the matrix but a zone of uncalcified tissue, the predentine, normally exists like the osteoid (Frost & Villanueva 1960 a).

Besides collagen, the main organic constituent (Stack 1955, Battistone & Burnett 1956, Piez & Likins 1960, Eastoe 1964, 1967), and small amounts of other proteins, the matrix contains mucopolysaccharides, lipids, citrate and lactate (Stack 1955, Eastoe 1967).

The mineral of dentine — like that of other mammalian hard tissues — consists mainly of an apatite. In normal dentine there is probably only one crystalline phase with a structure resembling that of hydroxyapatite (Carlström 1955, Jenkins 1966, Trautz 1955, 1967).

Earlier studies on the rate of apposition of rat incisor dentine

In 1921 Marshall stated: "The interstitial growth of the dentine of the persistent teeth of the rat, as shown by azo dyes under the microscope, amounts approximately to 0.01 mm per day."

Schour and co-workers have performed several studies on the rate of dentine apposition, using sodium fluoride or alizarine red S as intravital markers or calculating the rate during known time periods of experimental interferences. They claim the daily rate of apposition of dentine in the white rat's incisors, from 40 days of age onwards, to be very close to 16 μm (Schour & Smith 1934, 1935, Schour & Steadman

1935, Schour 1936, Schour & Hoffman 1935, 1937, 1939 a and b, Schour & Massler 1962). During the early life of the animals the rate is somewhat higher (Herzberg & Schour 1941).

Weinmann (1942) measured the rate of apposition of dentine in the upper incisors of the white rat after injection of alizarine red S and strontium chloride. The values given for the daily appositional rate deviated only moderately from the 16 μm given by Schour and co-workers, except one value, where the rate is given as 9.5 μm. Differences in the rate after injections of alizarine red S and strontium chloride were found and were attributed to a difference in toxicity of the two substances, the former substance being the more toxic one.

In an extensive survey of dentinogenesis Symons (1967) refers to Schour and co-workers and states: "In the rodent incisor the 16 μm increments represents the amount of dentine laid down and calcified daily."

BONE

The periosteal and endosteal formation of new bone in the shafts of long bones occurs by appositional growth on existing surfaces and is a comparatively simple process. Concentric layers of new bone form, and the process can be compared to the formation of annual rings in a tree (Frost, Roth, Villanueva & Stanisavljevic 1961, Sissons 1961, Vanderhoeft, Kelly & Peterson 1962).

II.

INTRAVITAL MARKERS IN THE STUDY OF RATE OF APPOSITIONAL GROWTH OF DENTINE AND BONE

Vital dyes and radioactive isotopes have been widely used as markers in calcified tissue research. John Belchier (1736), who found that the bones of pigs, fed madder root, stained red, also noticed that the teeth were stained.

Administration of an appropriate marker at suitable intervals results in the development of bands visible microscopically in histologic sections. If the time intervals are known and the distances between the bands measured, the rate of formation of tissue can be expressed as a linear measure per unit of time.

For a marker to be acceptable for the present investigation it should produce bands not only in growing dentine, but also simultaneously in growing lamellar bone. Perusal of the literature in the search for a suitable marker revealed that in the rat the appositional growth of dentine had been studied with the aid of different markers. Marshall (1921) tried several vital dyes, including alizarine. Pflüger (1931) used uroporfyrin. Alizarine red S (Schour & Hoffman 1939 b, Weinmann 1942, Johannessen 1961), strontium chloride (Weinmann 1942), sodium fluoride (Schour & Hoffman 1939 b) and chlorazol fast pink B (Johannessen 1964) have also been used.

Several of the dyes used by Marshall (1921) did not stain satisfactorily. The staining properties of alizarine were satisfactory, but the use of this dye affected the weight gain of the animals. Also Weinmann (1942) found that alizarine had toxic effects. Studying bone formation Harris (1960) and Harris, Travis, Friberg & Radin (1964) observed a toxic effect of alizarine red S. Of the other markers mentioned, sodium fluoride and strontium chloride are known to interfere with mineralisation (Schour & Smith 1935, Weinmann 1942, Pindborg

1950, Yaeger, Hinrichsen & Cohen 1964, Yaeger 1966) and chlorazol fast pink B with the weight gain of the animals (Johannessen 1964). Uroporfyrin seemed to be less readily available.

It was soon realised that it was among the tetracyclines that the marking agent was to be sought. The staining properties of the tetracyclines were initially observed simultaneously in the skeleton and in the teeth (André 1956, Rall, Loo, Lane & Kelly 1957, Milch, Rall & Tobie 1957, 1958), where they are deposited in the enamel and dentine undergoing calcification at the time of administration of the marker and form a compound with yellowish fluorescence in UV-light (Zipkin & Larson 1960, Bevelander, Rolle & Cohlan 1961, Boyne & Miller 1961, Owen 1961, Owen, Stevenson & Keilin 1962, Hulth & Olerud 1962, Harcourt 1963, Storey 1963, Yaeger, Hinrichsen & Cohen 1964, Bevelander 1964, Bennett & Law 1965, Bevelander & Nakahara 1965, Löfgren, Nylen & Omnell 1965, Zástava, Málek, Zák & Kole 1966, Zussman 1966, Bennett 1967).

They accumulate also in bone where mineralisation is active (Ghosez 1959, Harris, Jackson & Jowsey 1962, Urist & Ibsen 1963) and form a compound fluorescing in UV-light.

They are bound to the mineral and possibly also to the matrix (Steendijk 1964, Bevelander & Nakahara 1965). It has been suggested that calcium salt reacts more readily in the initial stage of formation of apatite than later (Urist & Ibsen 1963).

The potential value of the tetracyclines in studies of the rate of dentine formation has been realised, but not exploited, by Grøn & Johannessen (1961) and Hulth & Olerud (1962).

In skeletal tissues the tetracyclines have been used in studies of: bone grafts (Puranen 1966), lamellar bone formation (Harris 1960, Vanderhoeft, Kelly & Peterson 1962, Frost 1960, 1962, Frost, Roth, Villanueva & Stanisavljevic 1961, Frost & Villanueva 1960, Frost, Villanueva & Roth 1960, Frost, Villanueva, Roth & Stanisavljevic 1961, Hulth & Olerud 1962, Stanisavljevic, Roth, Villanueva & Frost 1962, Holmes 1963, Lee 1963, 1964, Landeros & Frost 1964, Landry & Fleisch 1964, Tapp 1966), endochondral calcification and bone formation (Hulth & Olerud 1962, Hansson 1964, 1967, Tapp 1966, Sundén 1967), and callus formation (Milch et al. 1958, Urist & Ibsen 1963, Hulth & Olerud 1964).

Tetracyclines may have certain side-effects such as: discoloration of the teeth in children and enamel hypoplasia (Schwachman & Schuster 1956, Schwachman, Fekete, Kulczycki & Foley 1958, Wallman & Hilton 1962, Hamp 1967), inhibition of calcification in enamel and dentine in

the rat incisor (Bevelander et al. 1961), retardation in development of the skeletal system in the chick embryo (Bevelander, Nakahara & Rolle 1960), disturbed mineralisation in tissue cultures of embryonic mice bone (Saxén 1965, 1966), retardation of skeletal growth in the dog (Owen 1963), and disturbed endochondral bone growth in the rabbit (Hansson 1967).

The disturbances of tooth development vary with the type of tetracycline used and the dose given, and probably also with the route of administration. For children Wallman & Hilton (1962) believed oxytetracycline to be less toxic than tetracycline. This opinion is shared by Kienitz (1964). Studying the effect on enamel of rat incisors Omnell, Löfgren & Nylen (1966) and Löfgren, Omnell & Nylen (1968) found oxytetracycline to produce less severe disturbances than tetracycline hydrochloride.

In the incisors of the rat Antalovská & Králové (1966) found a hypomineralised band in the dentine after having fed the animals a diet containing tetracycline hydrochloride. The daily dose was about 13 mg/kg bodyweight. Grøn & Johannessen (1961) found that a dose of oxytetracycline of 10 mg/kg bodyweight did not produce any microradiographically demonstrable hypomineralisation in dentine in the molars of the white rat. In dogs even considerably larger doses (Owen 1963) had no effect on the degree of mineralisation of the dentine, investigated with microradiography.

Even if the degree of mineralisation may be affected, it does not necessarily follow that also the rate of matrix formation will be impaired. Information on this point is rather scanty. A search of available literature failed to reveal a comparative study of the rate of appositional growth of bone or teeth measured by tetracycline marking and by some other method. Neither could any study be traced of the rate of such appositional growth after different doses of tetracycline.

Cohlan, Bevelander & Tiamsic (1963) found the growth in length of the fibula in premature infants to be decreased during the administration of tetracycline. Chu, O'Hara & Keitel (1964) could not find any disturbances of the growth process in the fibula, but they used oxytetracycline and in smaller doses. The mode of administration was also different. Owen (1963) gave large doses of tetracycline, chlortetracycline and oxytetracycline daily to young dogs for 4 weeks. He found retardation of bodyweight increase and longitudinal growth of radius and femur only after the administration of chlortetracycline. This retardation ceased on withdrawal of the agent and was followed by

rapid growth. Hansson (1967) considered oxytetracycline to be the least toxic of the tetracyclines and used it in his study of endochondral bone growth. Oxytetracycline was also used by Sundén (1967) in a similar investigation. These investigators used rabbits as experimental animals and gave 1 mg oxytetracycline/kg bodyweight. In an investigation of the growth in length of the tibia in young rats Tapp (1966) used tetracycline in a dose of 75 mg/kg bodyweight. He compared the results obtained with the tetracycline method with those obtained with a radiological method and found a disparity. The tetracycline method was considered more accurate. The possibility of a toxic effect of tetracycline was not discussed.

When the tetracyclines have been used as markers in lamellar bone fairly large doses have been given. In a survey, "Tetracycline bone labeling", Frost et al. (1961) say: "About 75 milligrams per kilo in animals is adequate." The dosage of chlortetracycline to dogs employed by Lee (1964) varied between 20 and 50 mg/kg bodyweight intravenously. Tapp (1966) gave 75 mg tetracycline/kg bodyweight to rats.

In a survey of "The effect of tetracycline on mineralization and growth" Bevelander (1964) concludes: "absence of adverse side effects when trace amounts are administered." However, he did not state what could be considered as traces.

Summarising, the use of oxytetracycline in small doses carries at most a small risk of interfering with mineralisation and/or appositional growth of dentine and lamellar bone. It was therefore decided to use this marker in the present investigation.

III.

BRIEF SURVEY OF EFFECTS OF PITUITARY GROWTH HORMONE OR HYDROCORTISONE ON DENTINE AND BONE

INTRODUCTION

In the investigation of the possibility to influence the rate of apposition of dentine it was decided to use 2 agents: one expected to accelerate dentine formation and one expected to retard it. The growth hormone and hydrocortisone were selected (see below).

The following survey of the effects of the hormones is extended to comprise the effects observed in dental and skeletal tissues not only in some experimental animals but, for comparison, also in man.

PITUITARY GROWTH HORMONE

Pituitary growth hormone (GH) is a protein and has been extracted from the hypophysis of various animals. Extensive surveys of the chemistry and physiology of growth hormone have been given by Knobil & Hotchkiss (1964), Matsuzaki & Raben (1965) and Evans, Briggs & Dixon (1966). A species specificity exists: in man, only GH from man and monkeys is said to have any effect, while the rat responds to GH from several species (Papkoff & Li 1962, Raben 1962 a).

It is an anabolic agent and promotes the growth of most tissues. In man and experimental animals GH has been found to promote nitrogen retention (Bartlett 1955, Russell 1955, Shorr, Carter, Smith, Jr., Kennedy, Havel, Roberts, Sonkin & Livingstone 1955, Beck, McGarry, Dyrenfurth & Venning 1958, Bergenstal & Lipsett 1960, Henneman, Forbes, Moldawer, Dempsey & Carrol 1960). In mineral metabolism retention of calcium, phosphorus, sodium and potassium has been reported (Shorr et al. 1955, Beck et al. 1958, Raben 1962 a). However, also the opposite effect on the calcium balance has been observed

(Knobil & Hotchkiss 1964). GH also influences fat and carbohydrate metabolism (Campbell 1955, Greenbaum 1955, Shorr et al. 1955, Henneman & Henneman 1960, Raben 1962 a).

In hypopituitarism in man dental development is retarded, while in acromegaly and gigantism the excessive production of the growth hormone can lead to overgrowth of the mandible, supra-eruption of teeth and increased size of dental arch (Schour & Massler 1943 a, Garn, Lewis & Blizzard 1965, Jenkins 1966).

The effects of GH on rat incisors seem to have received comparatively little attention. Schour & van Dyke (1932 a) and Schour (1934 a) found severe changes in the incisors of the hypophysectomised white rat. The eruption was poor, the teeth were distorted and abnormally small. After the administration of the growth-promoting hormone of the pituitary the eruption rate of the incisor increased, but the histological alterations were not significantly influenced (Schour & van Dyke 1932 b, Schour 1934 b). Baume, Becks, Ray & Evans (1954) found hypophysectomy to cause a rapid decrease in the eruption rate of the upper incisors in the rat. Dentinogenesis was affected, but less severely than amelogenesis. They also described folding of the incisors and diminished pulp space, findings which have also been made by other investigators (Becks, Collins, Simpson & Evans 1946, Christensen & Pindborg 1950).

In normal animals the administration of growth-promoting hormone did not alter the eruption rate but increased bodyweight (Schour & van Dyke 1932 b, Schour 1934 b). Becks, Collins, Asling, Simpson, Li & Evans (1948) treated normal female rats for several months with growth hormone. They found increased activity of the odonto- and ameloblasts in the incisors. No change of eruption rate was found. Baume, Becks & Evans (1954) found administration of growth hormone to normal adult female rats to have no effect on the rate of incisor eruption, but the teeth increased in size. None of the above-mentioned investigators carried out any studies on the rate of apposition of dentine.

Asling & Evans (1961) have surveyed the regulation of skeletal development by the anterior pituitary. Their account is based chiefly on data from the rat. Pituitary dwarfism and short stature in man have been successfully treated with GH (Raben 1962 b, Tanner & Whitehouse 1967). The effect of GH on skeletal tissue in human acromegaly is well known. Growth hormone normalises skeletal development in hypophysectomised rats and causes overgrowth in normal rats (Evans, Becks, Asling, Simpson & Li 1948, Asling, Simpson, Moon, Li & Evans 1955, Asling, Simpson & Evans 1965). The tibia test is a bioassay for

GH, which increases the epiphyseal width in hypophysectomised female rats (Evans, Simpson, Marx & Kibrick 1943, Greenspan, Li, Simpson & Evans 1949, Geschwind & Li 1955).

Koskinen (1959, 1963) claimed GH to accelerate fracture healing in man and in the rat.

HYDROCORTISONE

Cortisone and hydrocortisone (cortisol) are glucocorticoids and have a catabolic effect (Ashmore & Morgan 1967). The effects they produce on systemic administration are similar (Asboe-Hansen 1958).

In the rat Schour & Rogoff (1936) found the calcification of dentine to be disturbed after adrenalectomy.

Goldsmith & Ross (1953) gave cortisone to pregnant rats and found "accelerated differentiation and organization" of the odontoblastic layer in the lower incisors of the offspring. They also discovered signs of increased deposition of dentine. Administration of cortisone retards dentinogenesis in the molars of white rats (Johannessen 1964).

In children with Cushing's syndrome (Liddle 1967) and after the administration of corticosteroids (Friedman & Strang 1966) growth is decreased. The osteoporosis occurring in Cushing's syndrome and after therapeutic administration of the hormones is known (Isaksson & Lindholm 1965, Klein, Villanueva & Frost 1965, Collins 1966). The healing of fractures is said to be retarded in patients with Cushing's syndrome (Asboe-Hansen 1958).

Similar findings have been made in experimental animals after the administration of glucocorticoids, *viz.* retarded growth in young rats (Sissons & Hadfield 1955, Storey 1960, Ailabouni, Dikstein, Zor & Sulman 1966), retarded formation of lamellar bone in the rat (Stanisavljevic, Roth, Villanueva & Frost 1962), and impaired bone repair in the rat (Duthie & Baker 1955, Koskinen 1959, Storey 1960, Hulth & Olerud 1964) and the rabbit (Ragan, Howes, Plotz, Meyer, Blunt & Lattes 1950, Göthman 1961).

AUTHOR'S INVESTIGATION

IV.

MATERIAL AND METHODS

ANIMALS

Sprague-Dawley rats were used. It was initially intended to breed all the animals at the experimental unit in order to know their ages with a high degree of accuracy. However, it was discovered that the weights of such animals at 10 months of age were lower than expected from the normal weight curve for animals from a breeding centre. From then on adult animals were purchased from a breeding centre. When young animals were required, pregnant rats were bought, and their young were used when they had reached desired age.

After arrival the animals were observed for at least a week before being used in the experiments. The same room was used for all experimental categories. Cycling of light and darkness was achieved by artificial light. The mean temperature ranged from about 24°C, as measured during 5 days in February, to about 26°C, as measured during 5 days in July. The relative humidity was slightly below 40 per cent. Only minor variation of the temperature and humidity was found during an uninterrupted 48-hour control period. The animals were kept in the same type of cages and fed the same diet (pellets and water *ad libitum*) as in the breeding centre. The pregnant and lactating animals were given a special type of food. During the experiments the animals were weighed at regular intervals.

Of the 271 animals used in the experiments proper, only one died.

VITAL MARKING

Oxytetracycline (OTC) was used (Terramycin®, Pfizer). The stock solution contained 50 mg OTC per ml and was diluted in physiologic saline before use.

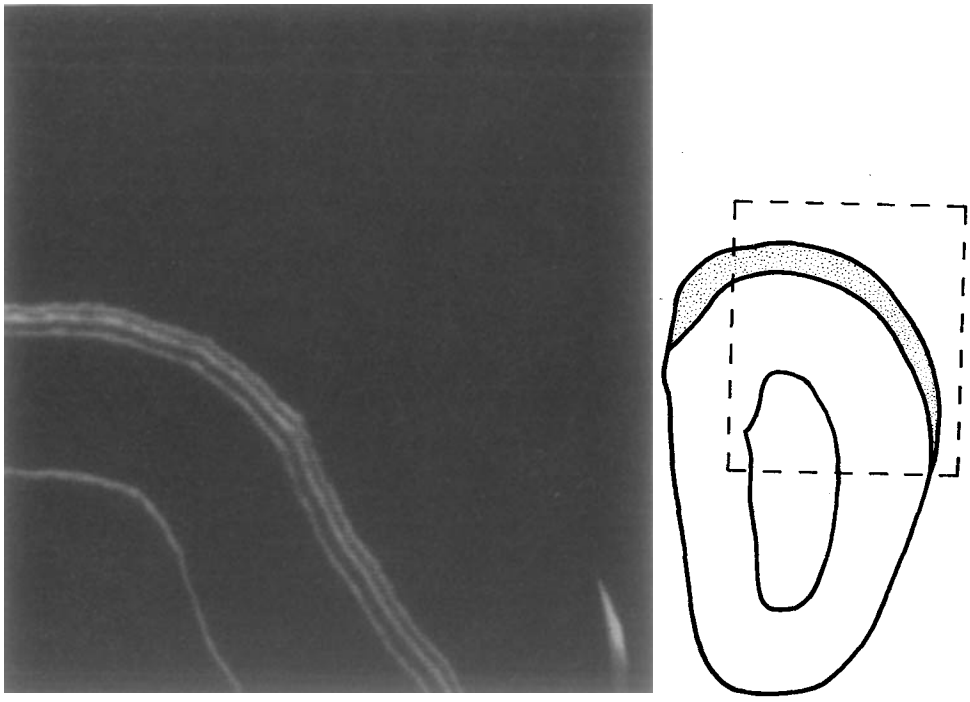


Fig. 4. Part of transverse section showing 4 fluorescent bands. The 3 located close to each other produced by intraperitoneal injections of OTC and the single more central one by an intravenous injection. The drawing shows position of photographed part in the transverse section. About 10 mg OTC/kg bodyweight. Adult female rat. 86×.

Before deciding upon the route of administration the fluorescent bands produced by intraperitoneal and intravenous (into a tail vein) injections were compared in respect of clearness. Five adult female rats were given 3 consecutive intraperitoneal injections of OTC (about 10 mg/kg bodyweight) at intervals of approximately 24 hours. Eight days after the last of these injections the animals were etherised, the femoral vein was surgically exposed and the same dose of OTC was injected into it. No appreciable difference was found between the clearness of the first 3 fluorescent bands and that of the fourth (Fig. 4). Being technically simpler the intraperitoneal route was therefore chosen for the administration of OTC.

Oxytetracycline was given at intervals of 8, 4, 8 and 4 days, *i.e.* the

injections were given on days No. 0, 8, 12, 20 and 24. These intervals were chosen because it was initially thought advisable to use periods that were multiples of each other. Preliminary experiments had also shown that when the experimental period was about 3—4 weeks, the pattern of growth of the dentine during this period could be traced in about half of the number of histologic sections to be taken from the tooth. (In some experiments [page 76] OTC was given at 6 day intervals.)

Young animals received 0.5 mg (vol. 0.5 ml) and adult animals 2 mg (vol. 1 ml) OTC at each injection. This means that the dose given to normal animals never exceeded 10 mg OTC/kg bodyweight. In each experimental category the OTC was injected at the same time of day.

Four days after the last injection of OTC the animals were sacrificed (on day 28) by ether. The upper incisors and femur and tibia on both sides were removed, either immediately or after storage of the cadavers in the deep-freeze. The bones were afterwards placed or replaced in the deep-freeze for later investigation. The choice of a 4-day interval between the last injection of OTC and sacrifice was based on the assumption that apposition of dentine during this interval could be measured and included in the investigation, and on the fact that measurement to the innermost fluorescent band was easier when it was not situated too close to the dentine border.

HISTOLOGIC TECHNIQUE

After fixation in 70 per cent ethyl alcohol for at least 24 hours the teeth were embedded in Ward's Bioplastic.

In preliminary experiments both longitudinal and transverse sections were prepared. However, as a rule, longitudinal sections permitted only measurement of distances between fluorescent bands in the middle part of the tooth. The fluorescent bands in the apical and incisal ends were not always well-defined, which made it impossible to measure the distances between them with acceptable precision. This haziness of the bands was thought to be due to technical difficulties in the preparation of the sections, which were in turn, at least partly, ascribable to the spiral shape of the tooth. Nor did they allow examination around the whole periphery of the tooth. Transverse sections proved easier to prepare and to be more informative.

The plastic blocks were ground until the contours of the tooth, viewed from the side, were clearly visible through a thin layer of the embedding material. The planes in which the transverse sections were to be

placed were determined in the following way: the mid-point of an imaginary line connecting the apex and the incisal edge was determined and a line was drawn at right angle to it. The 2 right angles thus formed were divided into 2 equal parts. Each of these 4 parts was then likewise divided into 2 equal parts. These radial lines were marked on the block (Fig. 5 a). The marking was facilitated by the use of a glass plate with engraved lines.

Segments of the tooth (Fig. 5 b) were cut with a circular saw and ground manually to a thickness of approximately 70—100 μm. The grinding technique was essentially that described by Frost (1958) for bone. Each section was placed dry on slide and covered with a cover-glass.

MEASURING TECHNIQUE

A conventional binocular microscope (Zeiss, Oberkochen) equipped with an accessory for fluorescence microscopy was used. The source

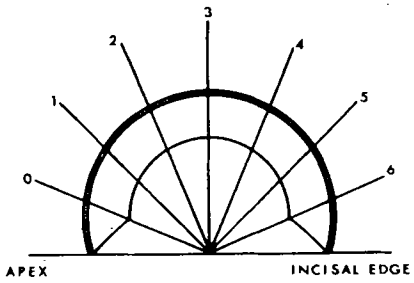


Fig. 5 a.

Figs. 5 a and 5 b. Schematic drawing showing location and number of histologic sections. Fig. 5 a shows lines for intended segmentation of tooth. Fig. 5 b: segments cut with the saw. These are later ground to histologic sections of suitable thickness.

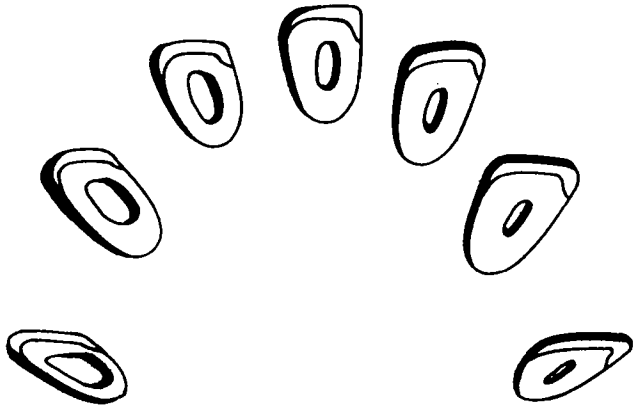


Fig. 5 b.

of radiation was an Osram super pressure mercury lamp, HBO 200 W. The primary filters were BG 38/2.5 and BG 12/4, allowing transmittance of light with a wavelength between 3300 and 5000 Å. As a secondary filter a barrier filter 50 (Schott) was used. An ocular (Kpl 12.5 ×) was equipped with a 10 mm micrometer, divided into 100 parts. The objective was planachromatic, 10 × (NA 0.22) magnifica-

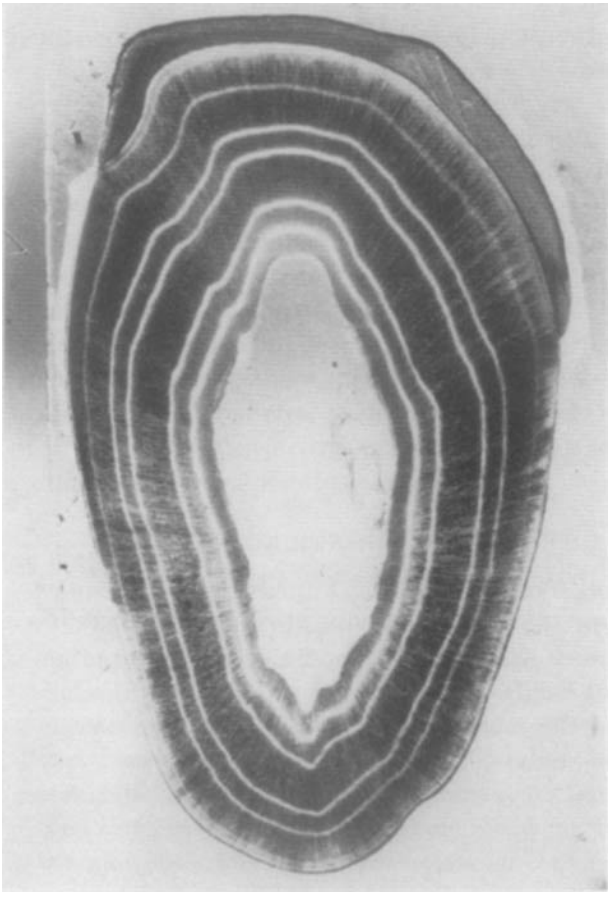


Fig. 6.

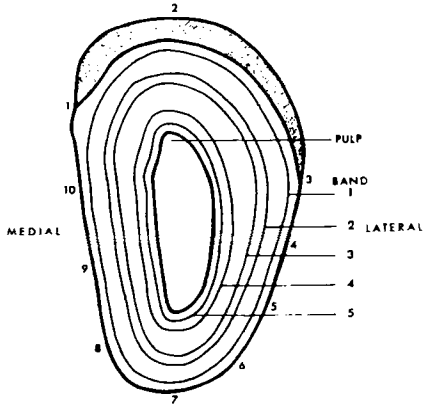


Fig. 7.

Fig. 6. Photomicrograph of a transverse section from an incisor. Five fluorescent bands can be seen. The radial striations reflect dentine tubules. 37×

Fig. 7. Schematic drawing of a transverse section showing location and numbers of the measuring points and the fluorescent bands.

tion. The calibration was made so that each part (unit) in the ocular micrometer corresponded to 10 μm.

Distances between consecutive fluorescent bands, as measured in the direction of the dentinal tubules (Fig. 6), were determined at points numbered 1—10 (Fig. 7). The bands were numbered 1 to 5 (Fig. 7).

Measuring was started at point 1 and continued around the section. Measuring points 1, 2, 3 and 7 were fairly easy to identify. Points 4, 5, 6, and 8, 9, 10 were distributed evenly along the lateral and medial aspects. At point 2 the thickness of the entire dentine layer, from the dento-enamel junction to the pulp (=DE-pulp distance=height of the dentine layer), was measured.

The fluorescent bands were usually well defined. Attempts were always made to measure the distance between the peripheral borders of consecutive fluorescent bands, this part of the bands forming first. As a rule, the positioning of the eyepiece was not difficult except in some areas where the tubules were slightly curved and an approximation had to be made (Fig. 6). In the sections situated nearest the incisal edge, bands 4 and 5 were sometimes very close to each other. At points 1 and 3 they could sometimes not be distinguished from one another, and then the distance between them was said to be 0. The distances found between bands at various points were entered in separate record sheets, one for each section.

PRELIMINARY ANALYSIS OF THE MEASURING METHOD

It soon became obvious that the technical measuring difficulties varied with the location of the section. It was also more difficult to measure the distance between a fluorescent band and the pulp outline than between 2 fluorescent bands.

To test the precision of the measuring technique a number of sections from animals used only for preliminary investigation were measured by the author and by a specially trained assistant. Distances between 2 consecutive fluorescent bands and between a band and the outline of the pulp chamber were measured. The sections used allowed measurement at every point from 1 to 10. Each distance was measured twice. The author performed 3520 and the technical assistant 3920 measurements.

Owing to the construction of the measuring device, over- or under-measurement of the distance by one major grade (5 measuring units) occasionally occurred. Therefore, as a precaution, when a difference

of at least 4 units was found between double determinations a third measurement was made, and then those 2 of 3 measurements that appeared most correct were used.

Analysis showed that the distance between a fluorescent band and the pulp chamber was more difficult to measure. This was examplified by the residual standard deviation, which was approximately 50 per cent higher in spite of the above-mentioned procedure with a third measurement. This distance was therefore not used separately in the subsequent investigations.

Concerning the distance between 2 consecutive fluorescent bands, on the basis of 38 sections measured by the author and 39 sections measured by the technical assistant it was found that the reproducibility of double determinations in sections 1—4 was similar and for these taken together. For the author it was 0.56 ± 0.18 and for the technical assistant 0.69 ± 0.17. The corresponding figures for section 5 were 0.73 ± 0.10 and 0.72 ± 0.21, respectively. Tabulation and a rough comparison showed that the measurements could be made with approximately the same precision at all points. No significant difference was found between the absolute values noted by the 2 examiners.

In the actual experiments measurements were made by the technical assistant and by the author, and each distance was measured on 2 days. The measurements made by the assistant were controlled by the author in randomly selected sections.

PREPARATION FOR STATISTICAL ANALYSIS

The continuous eruption of the incisors prevented the occurrence of 5 fluorescent bands in all sections 0 to 6. A loss of material occurred because of "missing bands". Some injections of OTC resulted in the appearance of only faint bands or no bands at all. Finally, some sections were torn or damaged during preparation. Thus, the vast material available was not complete in every respect but had to be surveyed and arranged to provide maximal information. To facilitate the understanding of what follows, it might be convenient here to give a list of certain definitions.

S e c t i o n designates each of the transverse regularly spaced histological sections of the incisor and are numbered 0 to 6 inclusive according to their distance from the apex (Figs. 5 a and b).

B a n d. A fluorescent band resulting from administration of OTC. The band produced by the last administration of OTC is given the

number 5 and is situated nearest the pulp, the one produced by the first injection being situated nearest the periphery and is given the number 1. The bands in between are numbered 2, 3 and 4 (Fig. 7).

Interval refers to time. Interval 1 is the period between the 2 first injections of OTC, *i.e.* those injections resulting in bands 1 and 2. Interval 2 is the period between the second and third injections of OTC, resulting in fluorescent bands 2 and 3. Accordingly, interval 3 is the period between the third and fourth injections of OTC (bands 3 and 4) and interval 4 the period between the fourth and fifth injections of OTC (bands 4 and 5).

Distances are to be understood as the distances along the dentinal tubules between fluorescent bands at different points. These distances are the linear measures of the amount of dentine formed during a given period. This measure is given in measuring units (1 unit= 10 μm) throughout the investigation and called measured value, value measured or observation.

Measuring points are the places on the periphery of the tooth (Fig. 7), which were selected for measurement of distances between fluorescent bands along the dentine tubules.

Set. To obtain a picture of the pattern of dentine apposition from different points of view the observations were arranged in certain combinations and such a combination is called a set.

The purpose of the arrangement of observations into sets was to find out whether and, if so, how the rate of apposition of dentine varied within a certain period between the sections and — in a single section — how the rate of apposition varied during different intervals.

The distances between bands 1—2 and 2—3 were regularly found only in sections 3, 4 and 5. It was thought that this number of sections was not large enough to allow satisfactory statistical analysis. The following sets were found suitable.

The distances between bands 3 and 4 were found to occur rather regularly from section 2 to 5 inclusive. It seemed suitable to study apposition during this 8-day interval through 4 sections.

The distances between bands 4 and 5 (a 4-day interval) were, as a rule, represented in sections 1 to 5 inclusive and provided a set.

The distances between all the 5 bands were rather regularly found in sections 3, 4 and 5, and each of these constituted a set.

Thus, the rate of apposition of dentine during an 8-day and a 4-day interval could be investigated in different sections. The rate during different intervals of the entire experimental period could be studied

in each of 3 sections from the middle of the tooth towards the incisal edge.

As to the young rats, the distances between bands 3—4 and 4—5 were investigated in sections 3 to 6 inclusive and from 2 to 6, respectively. Only in sections 5 and 6 were all 4 distances regularly represented in these young animals.

Hereinafter these sets will be referred to as 3—4, 4—5, 3, 4, 5 and 6 with above-mentioned meanings for adult and young animals. These sets were used whether the statistical calculations were made on the basis of observations at all 10 measuring points taken together or at a single measuring point. In this system section 0 is of no use. Section 6 is useful only when investigating young animals. It was studied in a few adult animals, but at this level apposition of dentine seemed to have stopped almost completely, and measurement of distances between the inner bands was very difficult or impossible. When section 5 was located too far incisally, the same difficulties were encountered.

When double determinations of the distances had been made the material was surveyed and divided into the above-mentioned sets, right and left side separately. Then the 2 observations in each set were compared. If the difference between the 2 determinations was 4 (measuring) units or more, the distance was measured a third time and those 2 that appeared to be most correct were used. In an experimental series 5 sets were made for the right tooth and 5 for the left in adult animals and 4 in young animals. When the analysis was made on the basis of all 10 measuring points taken together, 50 measured values (100 with the second determination) were necessary, when set 4—5 was to be investigated; it was 40 (80) for the other sets from right and left tooth in each animal. If one or more values for a given tooth was missing the tooth could not be included in this analysis. When the individual measuring points were investigated, the demands were not so high and consequently more material could be used.

STATISTICAL METHODS

Owing to the large number of data to be handled the statistical analysis was made with a computer. This required the transfer of the values to punched cards. In one of the computer programs selected, only one value could be registered on each card. In each card entries were made not only of the value measured but also of the total height of the dentine layer at point 2 and an identification code com-

prising the number of the measuring point, the number of the animal, right or left side, interval and section. As the intervals were not of equal length, distances found for the 4-day intervals in sets 3, 4, 5 and 6 had to be doubled. This was made by a specially designed program. A list of the cards was made.

To study the variations in dentine apposition between animals, sections and measuring points and their interactions, analysis of covariance was carried out. Program BMD 03V (IBM), analysis of covariance, version of Feb. 19, 1964, University of Utah Computer Center, was selected. The thickness of the dentine layer was chosen as a covariable to correct for the variation in the locations of the sections and for the possible differences in size of the teeth.

Differences found were said to be

highly significant (***) when $P < 0.001$
significant (**) when $0.001 < P < 0.01$
almost significant (*) when $0.01 < P < 0.05$

The variation in the rate of apposition of dentine was investigated further and described with the aid of program BMD 05R (IBM), polynomial regression, version of April 13, 1964, Health Sciences Computing Facility, UCLA. Program BMD 05R can dispose of only 500 measured values. This meant that when the 10 measuring points together were the subject of the investigation only 10 (set 4—5) or 12 (the other sets) teeth could be investigated simultaneously. Arbitrarily the first of the 2 available observations was selected for this analysis. For the statistical analyses, not performed with the aid of a computer, conventional methods were used.

SOURCES OF ERROR

Material

Using rats from an inbred stock, standardising their nutritional and environmental conditions and maintaining these as close as possible to earlier conditions helps to keep the animal material uniform. Before and during the experiments weighing and inspection of the animals served as a kind of "health control".

The adverse effects of OTC on growth were discussed earlier, and it was concluded that in view of the smallness of the doses used in the present investigation such effects were surely minimal. To investigate

the possible influence of OTC on the rate of dentine apposition, the following experiments were made. Twenty-five young animals were divided into 3 groups of 8, 8 and 9 animals and given intraperitoneal injections of OTC; 1.0, 10.0 and 20.0 mg/kg bodyweight, respectively. The mean weight of the animals at the beginning of experiment was 140.8 g; at the end, 168.3 g with negligible differences between the 3 groups. The rate of apposition at measuring point 2 was studied during an 8-day and a 4-day interval following the injections of OTC. No significant difference in appositional rate was found between the 3 groups. A corresponding experiment was made on 24 adult animals divided into 3 equal groups. Mean weight at beginning of the experiment 265.3 g and at the end 272.4 g with negligible differences between the groups. Neither in this experiment were any significant differences found in appositional rate. The results are based upon 141 observations from 10 animals in the 2 groups given 1.0 mg OTC/kg bodyweight, 293 observations from 15 animals in the 2 groups given 10.0 OTC/kg bodyweight and 259 observations from 14 animals in the 2 groups given 20.0 mg OTC/kg bodyweight.

It was also noted that when the lowest dosage (1.0 mg/kg bodyweight) was employed the bands were fainter and more difficult to recognise and the frequency of missing bands was higher. The dosage of OTC employed for the experiments proper was thought convenient and not to have any appreciable effect on the rate of apposition of dentine.

Technique

In each category the injections were given at the same time of the day, with a few minutes' deviation.

An intraperitoneal injection of a tetracycline compound will produce a fluorescent mark in calcified tissues within 30 minutes (Milch et al. 1957, Löfgren, Nylen & Omnell 1965). Storey (1963) found tetracycline-induced fluorescence in enamel 15 minutes after an intraperitoneal injection, and Johnson (1966) found fluorescence at the dentine-predentine junction within minutes after the injection, but did not say how the injections were given. It may be assumed that the absorption starts very soon after an intraperitoneal deposition, that the accumulation in tissues undergoing calcification follows in close succession and consequently that the marking in the tissue corresponds well to the time of the administration.

Not every injection of OTC resulted in a fluorescent band. One explanation may be that OTC was deposited in such a place (possibly in the bowel) that very little or no absorption occurred. If only little absorption occurred, the fluorescence could be below the visible limit. If the fluorescence is primarily weak but discernible, the small amount of fluorophore might be washed away during the fixation or during the preparation of sections and then the bands will not be visible. A possible source of error was to mistake one missing band for another, for example, to believe that band 4 was missing when in reality it was band 5. The experimental period was then read as 12 days instead of 8. To avoid this, every section was controlled by both observers. Otherwise missing bands were not a source of error.

To investigate whether deep-freezing might influence the distances measured, a group of animals comprising 6 adult and 4 young rats was investigated. The adult rats were given 2 injections of OTC at a 4-day interval; the young ones, according to the usual scheme. The upper incisors were removed immediately after sacrifice. The right one was placed directly into alcohol for fixation and the left one was deep-frozen. After an appropriate period of fixation the right incisor was embedded in plastic. The left one was thawed and frozen again 3 times before it was fixed and embedded. Corresponding distances were measured in ground sections of the right and left tooth. The material comprised 190 pairs (right-left) of observations from the adult animals and 100 pairs from the young ones. Sections from 1 to 5 inclusive and all 10 measuring points were represented. Only the first of the double determinations on the right side were compared with those on the left side. The differences between the right and the left teeth were calculated. No significant difference was found between right and left teeth. Normally, no significant difference exists between right and left teeth (see page 50). Deep-freezing of bone tissue before the preparation of sections for fluorescence microscopy does not diminish the intensity of the fluorescence (Milch et al. 1957, Frost, Roth, Villanueva & Stańisavljevic 1961, Hulth & Olerud 1964). In the present material visual inspection did not reveal any difference in intensity of fluorescence between the left and right teeth.

It is quite clear that the relative crudeness of the method used for determining the positions at which the sections were to be taken implies a fairly large source of error. It is also to be observed that the tooth or a segment of it is not repeatedly divided into 2 equal parts, except at the site of section 3, even if the angles are so divided. As the tooth

is not a perfect semi-circle but a 210° segment of a spiral, the planes of the sections deviate from the radius, except section 3. This deviation was estimated in the following way. It was thought justifiable to disregard the slight spiral form of the upper incisor. Assuming the adult tooth to occupy 210° of a circle the following calculations were made. From the point P a line was drawn in the way illustrated in Fig. 8, where d is the distance between P and the true centre of the circle. For the angle marked 15°, this holds, sin 15°=d:r. According to the sine theorem, for the triangle containing the angle α d : sin α=r : sin 45°. The following deduction is made: sin α=sin 45° · sin 15°. α=10.55°. Thus, the section cuts the tooth at an angle deviating by 10.55° from the radius and the measured distance will be l=t : cos 10.55°=1.017 t. (Fig. 9). At section 5, where the total height of the dentine layer is roughly 90 units, this distance is measured 1.5 units too long. The distances between bands rarely exceed 20 units and then the error (0.34 measuring units) can be ignored.

The sections may be placed apically or incisally to the intended place on the curvature of the tooth. It was thought necessary to compensate for this methodological error. It is obvious that the height of the dentine layer increases towards the incisal edge. For every section the total height of the dentine layer from the dento-enamel junction to the pulp was determined at measuring point 2. The distance was measured twice, if necessary, 3 times. It may seem illogical to use the outline of the pulp chamber as the limit of this distance and not band 5, it having been shown that the distance between a band and

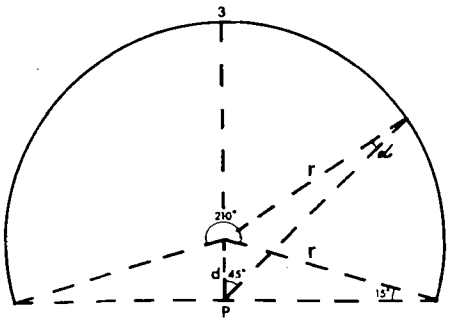


Fig. 8.

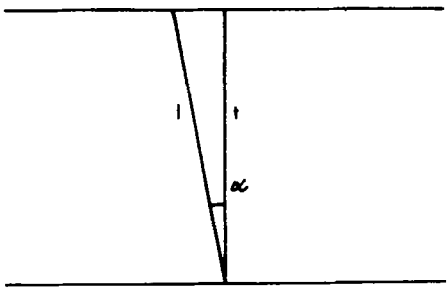


Fig. 9.

Figs. 8 and 9. Drawing showing how deviation of section 5 from radius is estimated.

the pulp was more difficult to measure than that between 2 fluorescent bands. It was nevertheless used because it was desired to measure the height of the whole dentine layer. The values were not used directly in the estimation of the rate of apposition of dentine but as a more accurate description of the location of the section than its number. First an attempt was made to define the section with the aid of the DE-pulp distance at point 2. As a trial, it was simply assumed that section 1 were those whose dentine height at point 2 varied between 25 and 35 μm. The other sections were assigned to appropriately spaced groups. The method worked fairly well for sections from the apical parts of the teeth but for those from the incisal parts the overlapping between the DE-pulp values was pronounced, and using this method would have meant an unnecessary loss of material. Instead it was decided to have the DE-pulp value as covariable in the statistical analysis, this way making a correction.

When these DE-pulp observations were taken together and scrutinised it was also possible to check that the sections had not been confused; for example section 3 mistaken for section 4. This control was possible because it was known that the DE-pulp values should be increasing from section 0 or 1 to 5 and 6 for each tooth.

If one section of a tooth had a high (or low) DE-pulp value the other values for the same tooth were, as a rule, also high (or low). It seemed that if a section, *e.g.* the one first cut, was placed incisally to the position intended, all the others were also placed too near the incisal end and then, of course, the DE-pulp values were higher than they should have been. However, it appeared that the spaces between the sections were regular. If the sections were located too far apically, the DE-pulp values were lower than the average but — as it seemed — still fairly regularly spaced.

Measuring method

Measuring along the dentinal tubules seems convenient as the tubule is described as "the path of cellular activity" (Herzberg & Schour 1941). It is, of course, not possible to place the measuring device along a single dentinal tubule. It must, however, be placed more or less parallel to the striation visible in the histologic sections and produced by dentinal tubules (Fig. 6). Once the measuring device had been placed in position on a section, it was not moved until all distances at the point had been measured.

In what follows apposition of dentine is given in measuring units. If these units were converted to μm it would, perhaps, give a false impression of accuracy. The precision of the measured values is known, but the true value for apposition of dentine is not. When apposition of dentine is later expressed as μm per day, this reservation should be borne in mind.

It is possible that the measuring points do not correspond strictly to each other in the different sections. The enamel may perhaps cover a varying part of the circumference of different sections of one and the same tooth and the reference points consequently have a different location. Judging from inspection under the microscope, this difference, if any, was small. It was therefore not considered necessary to investigate this matter separately. It was decided to use the method developed and then find out what the variation could be. It was considered probable that at most a comparatively slight shift could occur between the odontoblasts and the dentine they had formed and into which their cell processes passed.

The third measurement was made because the construction of the measuring device favoured faulty readings of about 5 units. These errors are to be regarded as gross errors. As such, an attempt to eliminate them was justifiable (Hallert 1967). To estimate the effect of this attempt on the calculations, an analysis was made during the course of the investigation. In the category comprising 16 adult normal animals 1400 measurements were made on teeth from the right side and 1420 on teeth from the left side. The third measurement was made in 16 and 20 cases, respectively. In young animals treated with growth hormone 2530 measurements were made on teeth from the right side, and the third control made in 24 cases. In the analysis of covariance only the variation within replicates was influenced. Investigation showed that it was about 5 per cent "too low".

Statistical analysis

Every calculation and step in the preparation for statistical analysis was made at least twice and the punching of cards was controlled. The computer programs must be regarded as adequate and the manual calculations made do not argue against this assumption. The covariable proved to be a small source of error in some of the calculations. When investigating the differences between intervals in one section it served the intended purpose. When investigating how a distance varied be-

tween sections the covariable slightly strengthened the variation. However, analysis showed that when this small error was corrected for, there was still a highly significant variation.

The sources of error, some of which were discussed above, contribute to the variation of the observations. Estimates of such variations will be given in subsequent chapters.

V.

NORMAL APPOSITION OF DENTINE

MATERIAL

Four categories of animals were used.

1) Adult rats, age about 5 months at sacrifice.
2) Adult rats, age exactly 10 months at sacrifice. These animals were reared at the experimental unit and their weights at sacrifice were lower than normal.
3) Adult rats, age about 11 months at sacrifice. They were breeders bought from a breeding centre and their weights at sacrifice were considered normal.
4) Young rats, age 58 days at sacrifice.

RESULTS

Adult animals

Age 5 months

Computer analysis. Since the most extensive analysis was performed on data about the 5-month old animals, this category will be dealt with first. It comprised 25 animals. Three could not be used in the computer analysis because fluorescent band 4 was missing. The number of animals used in the analyses of the different sets are given in the tables or can be obtained by adding 1 to the number of degrees of freedom for factor T (tooth). The mean weights and weight ranges are given in Table 1. As the animals were not numbered until they were sacrificed the weights of the individual animals were not known until then. When the observations had been grouped in the 5 sets, covering the 10 measuring points together, it was possible to calculate the mean weight for each set, right and left side separately. The range of the

Table 1. *Mean weights and weight ranges in grams at beginning and end of experiment in different categories of adult female rats.*

Day \ Age at end of experiment	Bodyweight (mean and range) adult animals		
	5 mths.	10 mths. (low weight)	11 mths. (normal weight)
0	272.4 250—295	262.2 240—290	335.6 314—360
24	296.0 270—320	276.1 240—310	334.6 294—368

mean weights in these 10 sets was from 286.1 to 297.1 grams on day No. 28. For teeth from the right side the mean weights of the animals contributing observations to the analysis of each of the 10 points in each of the 5 sets were calculated. The resulting 50 mean weights clustered around the value of 290 grams on day No. 28.

Analysis of covariance was made for the 5 sets, right and left tooth separately. The height of the dentine layer at point 2 was used as covariable. The results are given in detail in Tables I—II in the Appendix. Variations between sections (S), between measuring points (P) and their interaction (SP) were regarded as systematic. Variation between animals (=teeth, T) was regarded as random variation as were the interactions tooth-section (TS), tooth-measuring point (TP), tooth-section-measuring point (TSP) and within replicates (R).

That the different variations can be neglected (null hypothesis: no variation) can be investigated with F-test. The analysis was performed by forming the following quotients: for T, T : TS; for S, S : TS; for

Tables 2—3. *Summary of the result of analysis of covariance. 5 month old normal female rats.*

Table 2.

Source of variation	Set 3—4		4—5		
	Right	Left	Right	Left	Right and Left
T	—	—	*	—	—
S	***	***	***	***	***
P	***	***	***	***	***
TS	***	***	***	***	***
TP	—	—	—	—	—
SP	***	***	***	***	***
TSP	***	***	***	***	***

Table 3.

Source of variation	Set 3		4		5	
	Right	Left	Right	Left	Right	Left
T	—	—	—	—	—	*
I	***	***	***	***	***	***
P	***	***	***	***	***	***
TI	***	***	***	***	***	***
TP	—	***	***	***	***	**
IP	***	***	***	***	***	***
TIP	***	***	***	***	***	***

P, P : TP; for TS, TS : TSP; for TP, TP : TSP; for SP, SP : TSP and for TSP, TSP : R. In the analyses of sets 3, 4 and 5 the factor section (S) was replaced by factor interval (I). When forming the quotients T : TS, T : TI; TS and TI were chosen instead of TP because they were higher than TP. Thus, a model with variance components was used.

For sets 3—4 and 4—5 the results are given in Table 2. All the systematic variations S, P, SP were considerable. A high value for SP meant that the measuring points behaved differently in the different sections. Of the variance components, T and TP could be ignored because they did not differ significantly from 0. The others differed significantly from 0 and of them TS is to be noted. This value meant that although the teeth were similar, there was variation between sections. This may be explained by the previously mentioned observation that all the sections of a given tooth may be placed too near the apex or the incisal edge. The same explanation may apply to TSP. If the values found for a given tooth are high in section 2 those for section 5 will be low and vice versa.

In sets 3, 4 and 5 the systematic variations I, P and IP were large (Table 3). T could be ignored, but the others differed significantly from 0. That TI, TP and TIP could not be ignored may be due to differences between the positions of the sections in different teeth.

To increase the number of animals available for analysis set 4—5 was analysed in right and left teeth taken together. Each animal was represented by only one tooth. The findings (Tables I and 2) were the same as earlier.

Having shown the existence of a significant variation, the next step was to describe the variation more closely.

For the 5 sets and the 10 measuring points taken together polynomial regression was performed, right and left side separately. The purpose

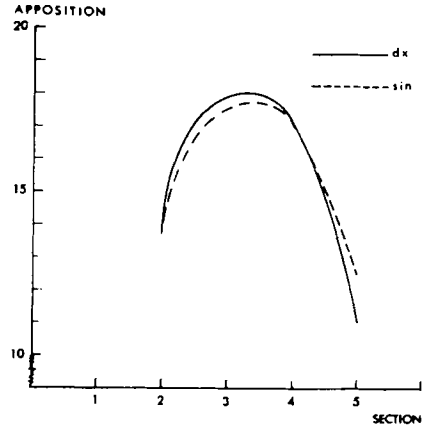


Fig. 10.

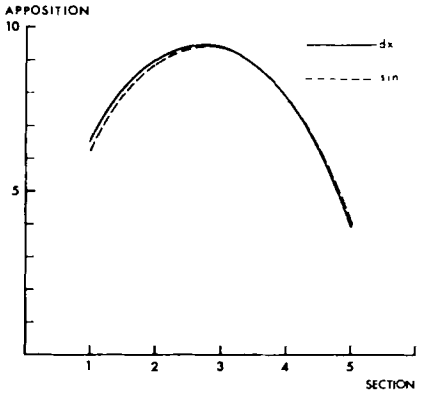


Fig. 11.

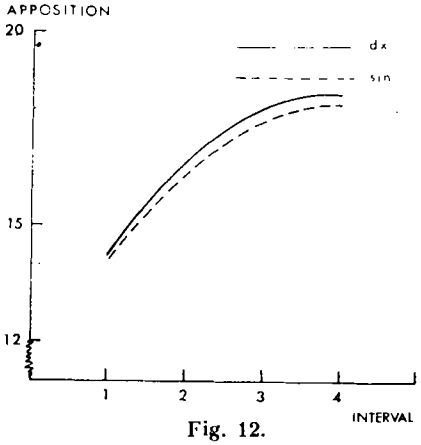


Fig. 12.

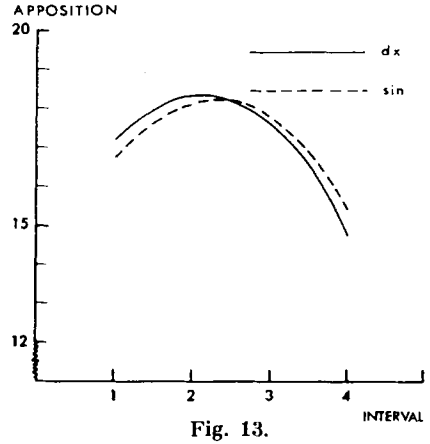


Fig. 13.

was to describe the variation between sections or intervals with the aid of a polynomial of the lowest possible degree. A second degree polynomial was found suitable. With the thickness of the dentine layer as the dependent variable (Y) and interval or section as the independent variable (X) Y was calculated according to the formula:

$\acute{Y}$ = intercept + (1st) regression coefficient · X + (2nd) regression coefficient · X^2

The values for Y for 10 measuring points taken together and the number of animals used for calculations in each set are given in Table III and the standard deviations in Table IV. The corresponding graphs are found in Figs. 10—14.

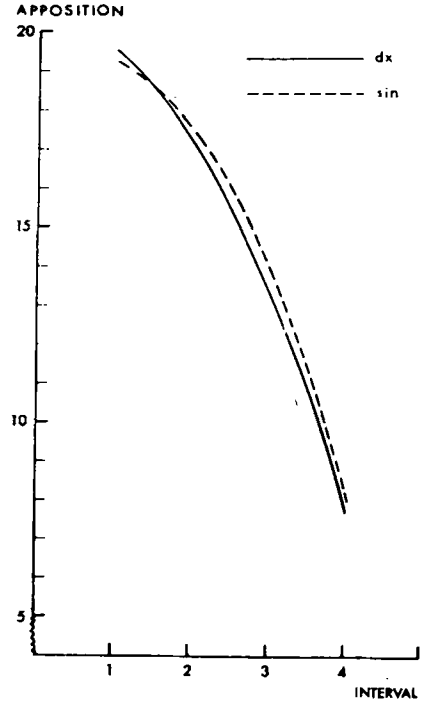


Fig. 14.

Figs. 10—14. The calculated variation of dentine apposition for 10 measuring points taken together. 5-month old normal rats. Fig. 10, set 3—4. Fig. 11, set 4—5. Fig. 12, set 3. Fig. 13, set 4. Fig. 14, set 5.

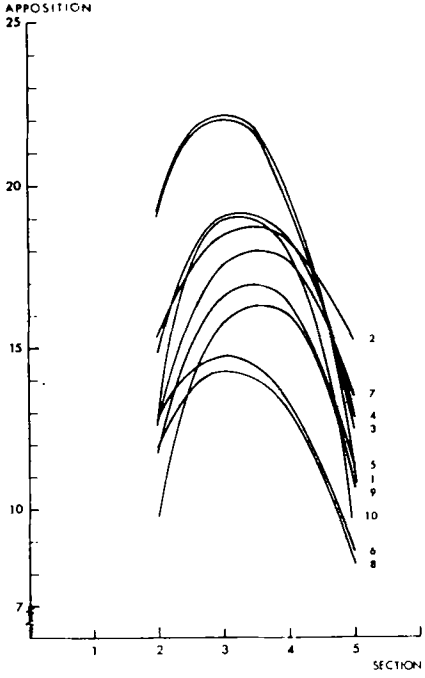


Fig. 15.

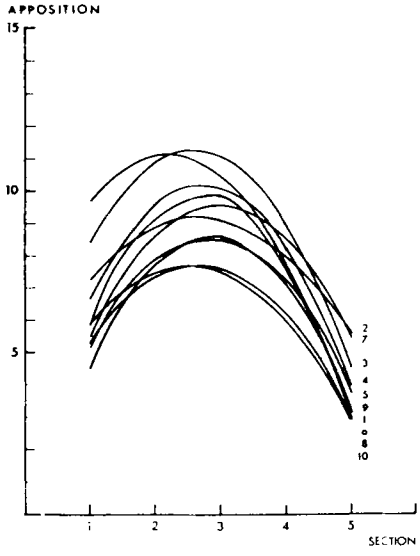


Fig. 16.

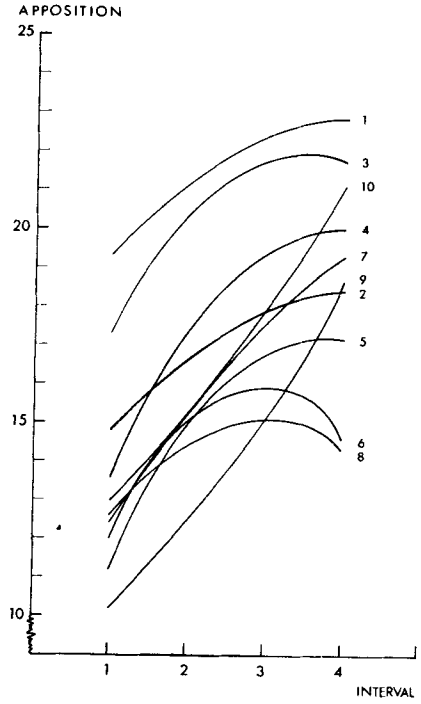


Fig. 17.

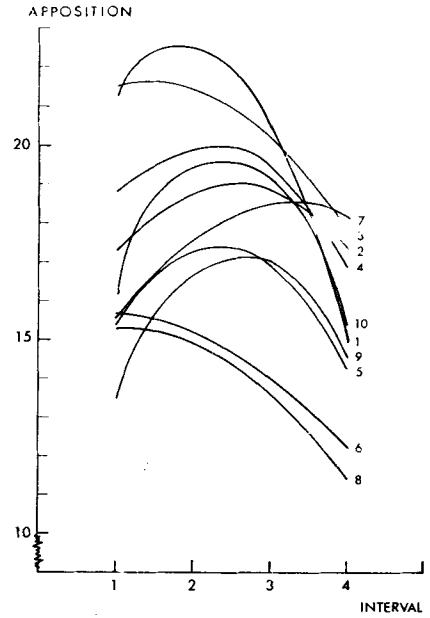
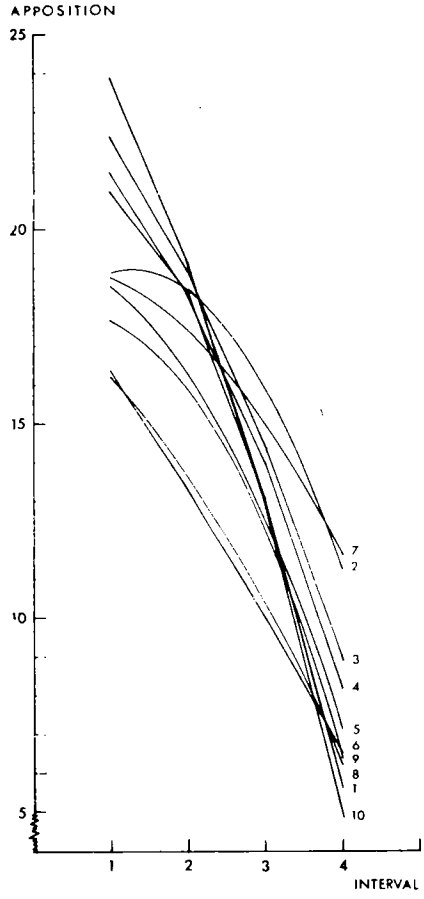


Fig. 18.

Polynomial regression was then performed for each of the 10 measuring points in the 5 sets. Because the right and left teeth were found to be similar the calculations were arbitrarily performed for the right side only. The calculated values for apposition of dentine and the number of animals on which they were based are given in Table V, the standard deviations in Table VI, and the graphical illustrations in Figs. 15—19.

Extended and simplified analysis. The curves hitherto obtained for the apposition of dentine supported the natural assumption that apposition in the different sets reflected only part of the apposition in the whole tooth. It was thought desirable to try to make full use of all the values measured in order to describe the pattern of apposition of dentine as completely as possible. Moreover, the way in which the analysis had hitherto been performed was very time-consuming and a simplified method would be of value.

The measured values and the polynomials showed the right and the left teeth to be similar. To confirm this, analysis of variance was made.



Figs. 15—19. The calculated variation of dentine apposition for each of the 10 measuring points in the different sets, right side. 5-month old normal rats. Figures to the right of the curves denote measuring points. Fig. 15, set 3—4. Fig. 16, set 4—5. Fig. 17, set 3. Fig. 18, set 4. Fig. 19, set 5.

Fig. 19.

Distances used for the analysis were DE-pulp or DE-band 5. These were chosen because they seemed to show the greatest variation. A mean value was calculated from the 2 replicates. Differences were found between animals and between right and left tooth from the same animal, too. The variation between the right and left teeth was not smaller than that between animals. However, no systematic difference was found between the right and the left teeth. The analysis was also performed for other categories of normal and hormone-treated animals with the same results. It was therefore considered justified to use the data obtained from both the right and left side in the same animal in the analysis.

As it had became clear that the positions of the sections varied somewhat from tooth to tooth, another basis for classification was sought. Since the thickness of dentine increases towards the incisal edge it is obvious that the distance from the dento-enamel junction to a fluorescent band increases towards the incisal edge. If, for example, interval 4 is chosen, the values measured for this interval can be arranged according to the distance DE-band 4. The higher the latter value the closer the section will be to the incisal edge. For a given value for DE-band 4 the apposition of dentine for the following 4 days was known. The DE-band values were divided into classes: DE-band=1 (measuring unit) was the first class, DE-band=2 was the second class, etc. These classes were taken into groups of 5, 1—5, 6—10, and so on.

Data on all the 25 animals were used in this analysis. The calculations were made without the aid of a computer and could not easily be performed for every one of the 10 measuring points. It was thought suitable not to make them for the 10 points together. As the variation at point 2 was low, this point was selected. This analysis was performed for time periods 8 and 4 days long, *i.e.,* interval 3 and intervals 2, 4 and 5-pulp, respectively. The animals were not of the same age during the different intervals. The intervals were compared in pairs. The differences between corresponding groups of classes were given different weights depending upon the number of observations, added and compared to the standard deviation. A comparison between intervals 2 and 4 revealed no significant difference in appositional rate between them, but between intervals 1 and 3 a highly significant difference existed. Intervals 2 and 4 were treated together and interval 3 was selected as representative of an 8-day interval. For the 4-day intervals it was decided to include the observations in interval band 5-pulp for DE-band values higher than 50. Otherwise the values corresponding to high DE-band values would have been rather few. The use of values measured between a fluorescent band and the pulp chamber may introduce an error, as the inner border of dentine is difficult to identify. A comparison between the observations for interval 4 and band 5-pulp chamber revealed a wider variation among the latter, but no significant difference. It was thought that the disadvantage of a somewhat wider variation was outweighed by the larger number of values on which to base the curve.

Tables VII—VIII give the number of values in each group of classes, the mean values and the standard deviation for individual values. The resulting graphs are in Figs. 20—21. On the X-axis the thickness of the

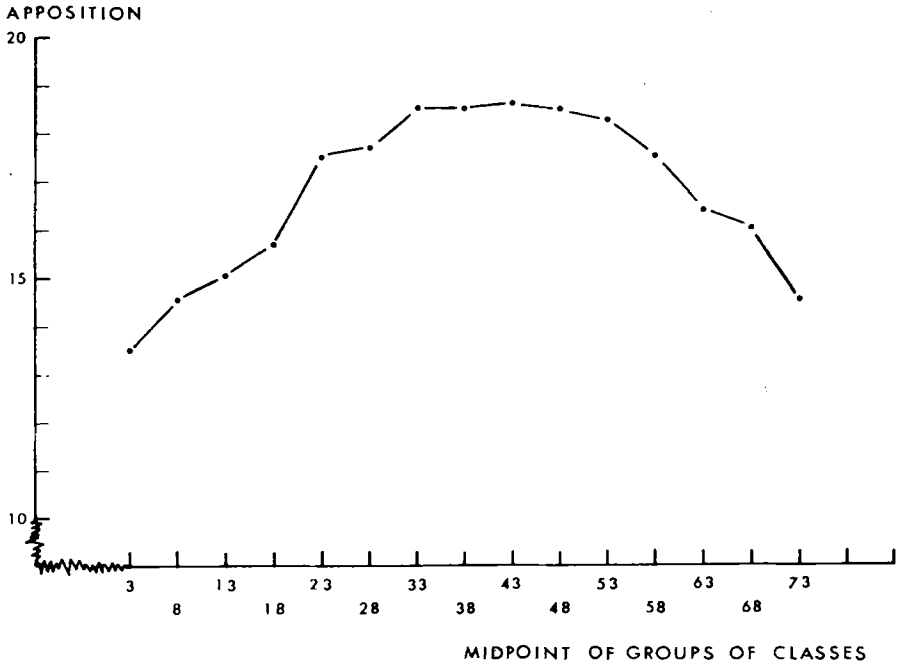


Fig. 20. The observed variation of dentine apposition at point 2 during an 8-day interval. 5-month old normal rats.

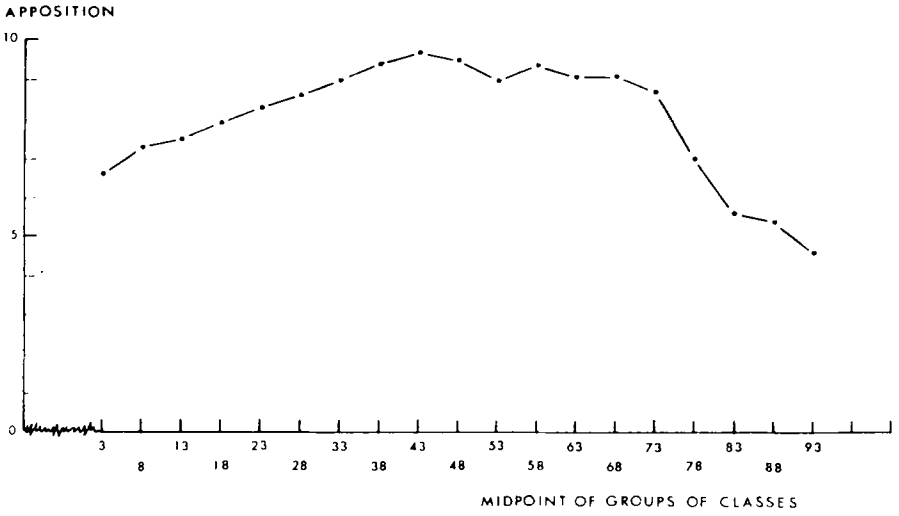


Fig. 21. The observed variation of dentine apposition at point 2 during 4-day intervals. 5-month old normal rats.

dentine layer from the dento-enamel junction to the actual band is given as values for the midpoints of the groups of classes. The corresponding measured values for apposition of dentine during the following 8 or 4 days are given along the Y-axis. The curve for the 8-day interval is based on 183 observations and that for the 4-day intervals on 499 measured values.

During the calculations it was also found that the standard deviations were larger when the values for DE-band were high. The standard deviations are not given for each group of classes but a border was chosen between the 2 groups with midpoints 48 and 53 (Tables VII—VIII) because the increase of the standard deviations began there.

Age 10 months, low weight

This category comprised 9 animals. Mean weights and weight ranges are given in Table 1. All the animals are included in the analysis.

Analysis of covariance was performed and the details are given in Tables IX—X. The same model with variance components was applied

Tables 4—5. *Summary of the result of analysis of covariance. 10-month old female rats, low weight.*

Table 4.

Source of variation	Set 3—4		4—5	
	Right	Left	Right	Left
T	—	—	—	—
S	***	***	***	***
P	***	***	***	***
TS	***	***	***	***
TP	**	—	—	—
SP	***	***	**	***
TSP	***	***	***	***

Table 5.

Source of variation	Set 3		4		5	
	Right	Left	Right	Left	Right	Left
T	—	—	—	—	***	—
I	***	***	***	***	***	***
P	***	***	***	***	***	***
TI	***	***	***	***	***	***
TP	***	***	***	***	***	***
IP	***	***	***	***	***	***
TIP	***	***	***	***	***	***

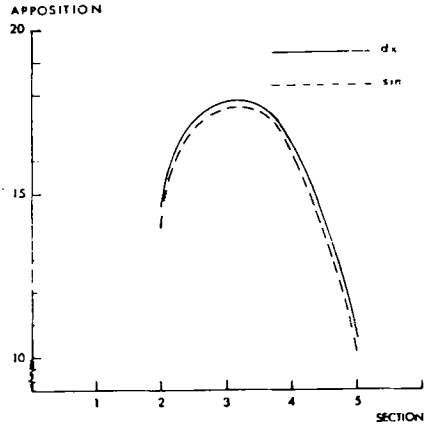


Fig. 22.

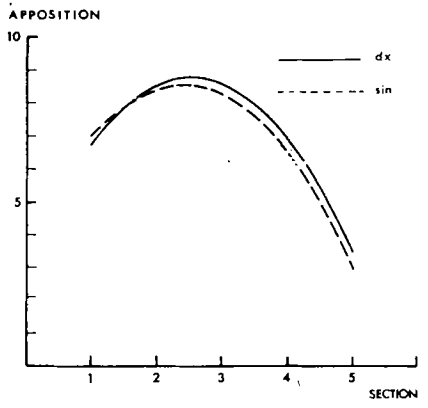


Fig. 23.

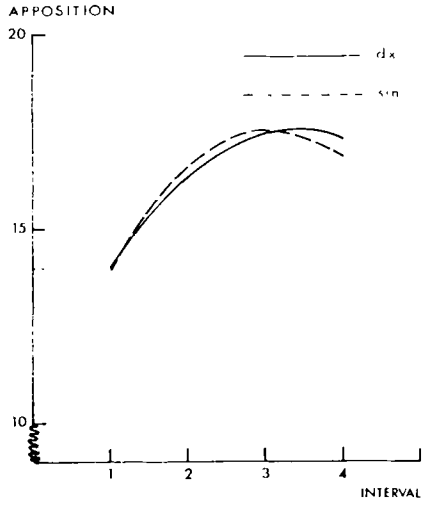


Fig. 24.

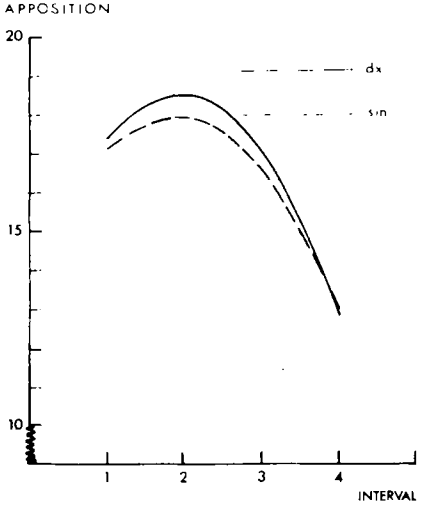
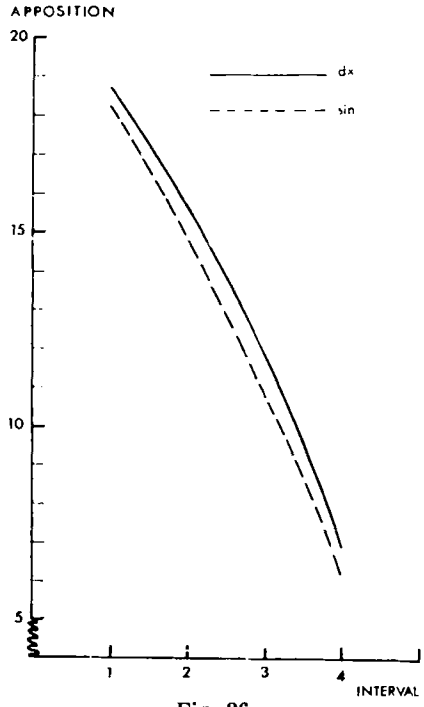


Fig. 25.

and the results are in conformity with those obtained for the 5-month old animals (Tables 4—5).

Polynomial regression was performed for the 10 points taken together and the results are given in Tables XI—XII and Figs. 22—26. The general picture of apposition of dentine was the same as that obtained earlier. At this stage of the investigation it was not considered necessary to perform "extended and simplified analysis" for this category.



Figs. 22—26. The calculated variation of dentine apposition for 10 measuring points taken together. 10-month old rats. Low weight. No treatment. Fig. 22, set 3—4, Fig. 23, set 4—5. Fig. 24, set 3. Fig. 25, set 4. Fig. 26, set 5.

Fig. 26.

Age 11 months

This category comprised 16 animals. Mean weights and weight ranges are given in Table 1. Data on 13 were used in the computer analysis.

The details of analysis of covariance are given in Tables XIII—XIV and in summary in Tables 6—7.

Polynomial regression was performed for the 10 measuring points taken together. The values for dentine apposition calculated from the polynomial are given in Table XV and the standard deviations in Table XVI. The corresponding graphs are Figs. 27—31. The pattern of dentine apposition was essentially the same as in the categories investigated earlier.

Young animals

This category comprised 27 animals, and the mean weights and weight ranges for males *and* females together during the experiment, and for females only on day 28, are given in Table 8. On day 28 the mean weight for males was 235.9 grams and the range 208—278. The analysis

Tables 6—7. *Summary of the result of analysis of covariance. 11-month old normal female rats.*

Table 6.

Source of variation	Set 3—4		4—5	
	Right	Left	Right	Left
T	*	—	—	—
S	***	***	***	***
P	***	***	***	***
TS	***	***	***	***
TP	*	***	—	*
SP	***	***	***	***
TSP	***	***	***	***

Table 7.

Source of variation	Set 3		4		5	
	Right	Left	Right	Left	Right	Left
T	***	—	—	—	**	***
I	***	***	***	***	***	***
P	***	***	***	***	***	***
TI	***	***	***	***	***	***
TP	***	***	***	***	—	***
IP	***	***	***	***	***	***
TIP	***	***	***	***	***	***

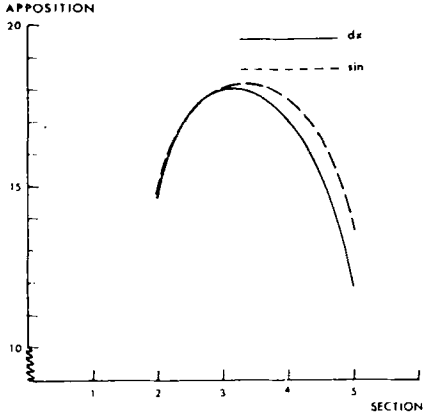


Fig. 27.

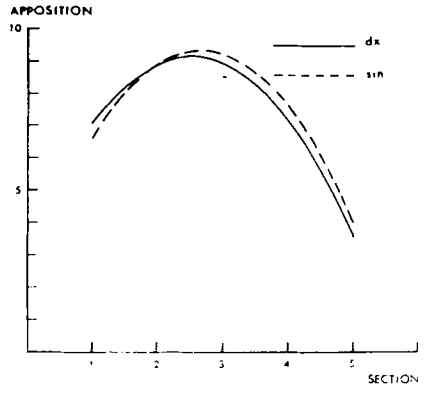


Fig. 28.

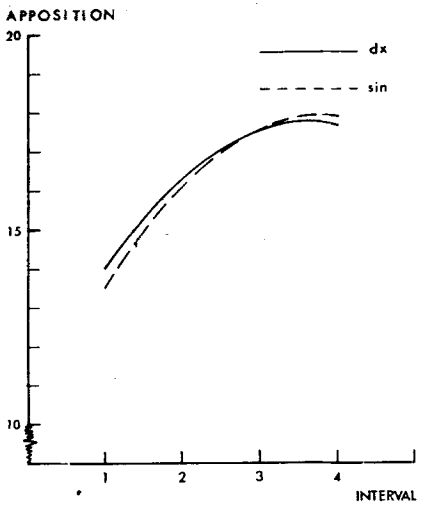


Fig. 29.

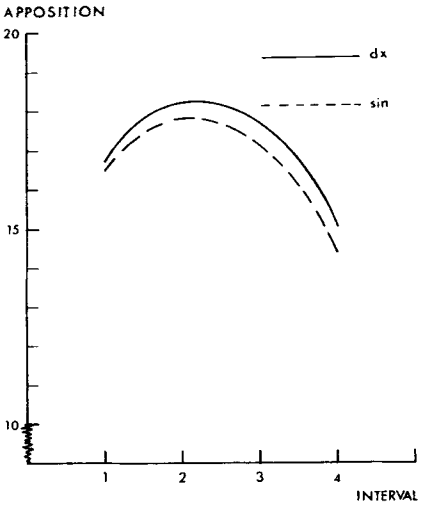


Fig. 30.

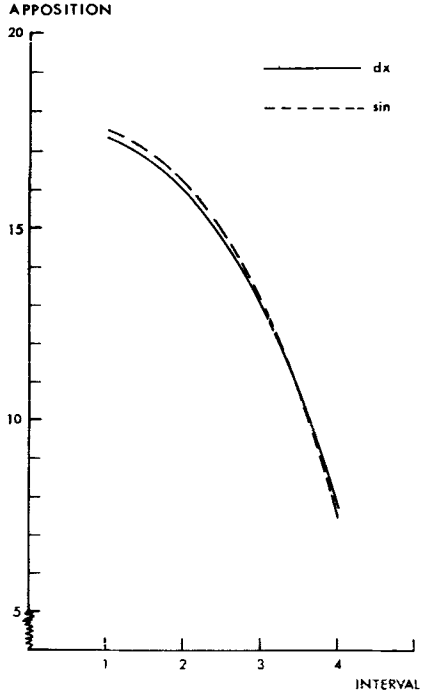


Fig. 31.

Figs. 27—31. The calculated variation of dentine apposition for 10 measuring points taken together. 11-month old normal rats. Fig. 27, set 3—4. Fig. 28, set 4—5. Fig. 29, set 3. Fig. 30, set 4. Fig. 31, set 5.

made with the aid of the computer is based on values obtained for 19 animals; in the following analysis, data on all 27 animals.

Table 8. *Mean weights and weight ranges in grams during experiment in different categories of young animals. Treatment from Day 13 through 24. The figures for Day 28 (sacrifice) apply to females only, otherwise they are given for males and females together. Age at beginning of experiment: 30 days.*

Day	Bodyweight (mean and range) young animals		
	No treatment	GH	Solvent for GH
0	78.1	78.6	83.9
	58— 99	50—106	55—119
12	131.0	140.7	144.6
	103—200	85—194	104—215
24	184.3	193.5	207.1
	140—242	121—264	137—276
28 (females only)	175.7	179.7	174.4
	148—188	133—217	147—196

The results of covariance analysis are given in Tables XVII—XVIII. The same model with variance components as for the adult animals was applied and the result is given in Tables 9—10. The findings are the same as for the adult animals. The factors section, interval and measuring point and their interactions are the important ones and their influences are highly significant. That factor tooth in one case is significant does not invalidate this statement. Concerning the significant interactions the same explanations may apply as to the adult animals.

The analysis was carried further and, for the 10 points taken together, the values measured were fitted to a second degree polynomial. The values calculated from the polynomial are given in Table XIX and the standard deviations in Table XX. The graphs are in Figs. 32—35.

The analysis of individual measuring points was performed only for set 4—5 right side. The calculated values and the standard deviations are given in Tables XXI—XXII and the graph in Fig. 36.

In the "extended and simplified analysis" it was found necessary to treat the intervals of equal length separately because it was found that apposition of dentine during intervals 1 and 2 differed systematically and significantly from respectively 3 and 4. The mean values for

Tables 9—10. *Summary of the result of analysis of covariance. Young normal female rats. Age at beginning of experiment: 30 days.*

Table 9.

Source of variation	Set 3—4		4—5	
	Right	Left	Right	Left
T	**	*	—	—
S	***	***	***	***
P	***	***	***	***
TS	***	***	***	***
TP	—	—	*	*
SP	***	***	***	***
TSP	***	***	***	***

Table 10.

Source of variation	Set 5		6	
	Right	Left	Right	Left
T	—	—	—	—
I	***	***	***	***
P	***	***	***	***
TI	***	*	***	***
TP	—	—	—	*
IP	***	**	***	***
TIP	***	***	***	***

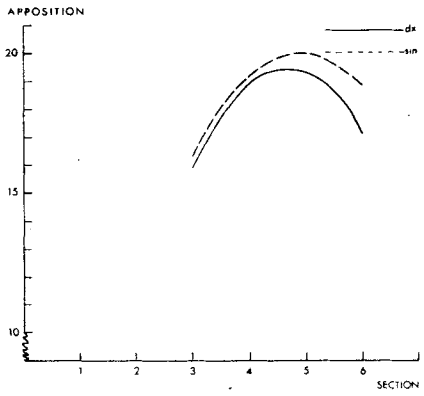


Fig. 32.

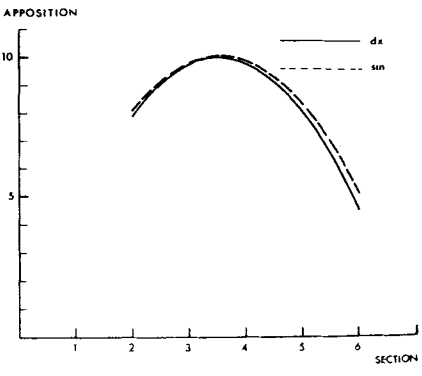


Fig. 33.

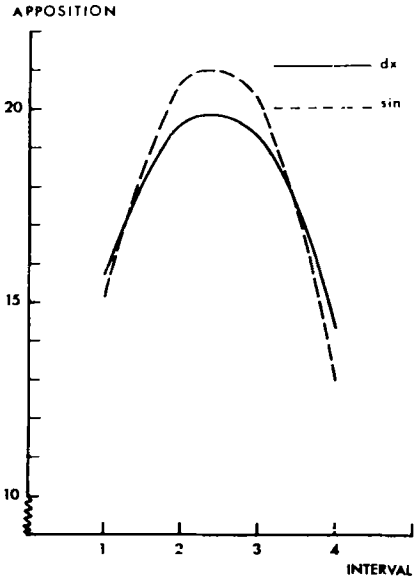


Fig. 34.

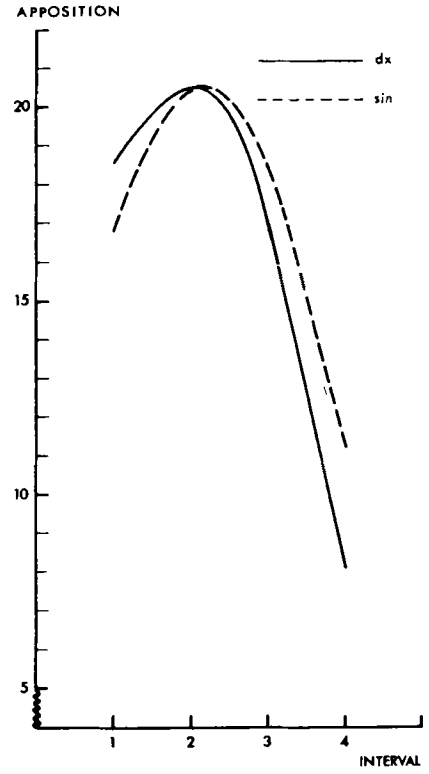


Fig. 35.

Figs. 32—35. The calculated variation of dentine apposition for 10 measuring points taken together. Young normal rats. Fig. 32, set 3—4. Fig. 33, set 4—5. Fig. 34, set 5. Fig. 35, set 6.

the different groups of classes, the number of observations and the standard deviations for an 8-day and a 4-day interval are given in Tables XXIII and XXIV respectively. The corresponding graphs are Figs. 37—38.

The pattern for dentine apposition in young animals is essentially the same as in adult animals. The important exception seems to be that in young animals the course of events is faster. This can be seen, for example, in Fig. 34 which should be compared with Fig. 14, which illustrates the apposition of dentine in an adult animal. In section 5 both describe the events during the 24 day experimental period. In adults, only the decrease in apposition can be seen, while in young

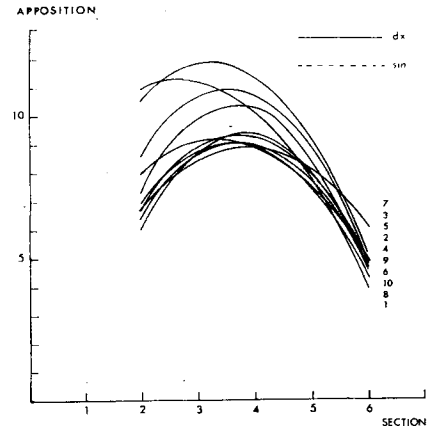


Fig. 36. The calculated variation of dentine apposition for each of the 10 measuring points in set 4—5, right side. Young normal rats. Figures to the right denote measuring points.

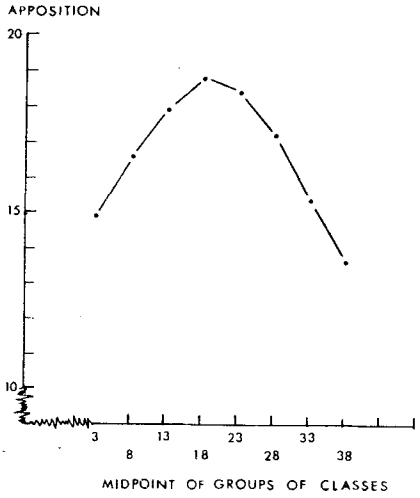


Fig. 37.

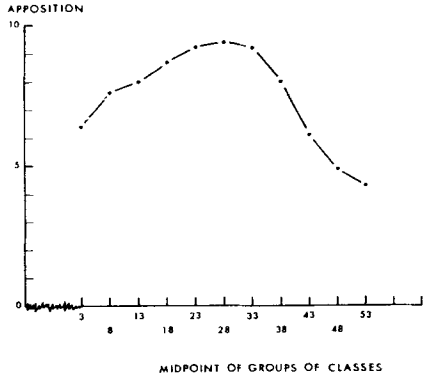


Fig. 38.

Fig. 37. The observed variation of dentine apposition at point 2 during and 8-day interval. Young normal rats.

Fig. 38. The observed variation of dentine apposition at point 2 during a 4-day interval. Young normal rats.

animals the increase, the maximum and the decrease occur during the same period.

Determination of the age of the incisor at different levels

It has been shown that the sites of the sections varied somewhat from tooth to tooth and that the effect of this variation was overcome by the use of a different classification. It would be of even more value if an age determination could be made.

The following calculations were made with the aid of the earlier calculated means of values measured for apposition at point 2 for the 4-day intervals in the adult 5-month old animals (Table VIII). These values had earlier been arranged in groups of classes according to the distance from the dento-enamel junction to the peripheral band of these intervals. The mean values for the groups of classes were known. If the mean value for a group of classes is added to the value for the midpoint of the group the sum denotes the apposition during an unknown time plus 4 days. To simplify the analysis distance in measuring units with even figures was chosen. With the rule of three the corresponding time in days can be calculated. The problem was, however, that the start of apposition of dentine had to be determined by extrapolation.

The reasoning is easier to follow if reference be made to Table 11. The midpoint of the first group of classes was 3 and the mean value for apposition during the following 4 days was 6.57. Then the mean thickness of the dentine layer at the end of these 4 days was 9.57. We selected distance 3—9. When the thickness of dentine increased from 3 to 9, six measuring units were added. Since we knew that the apposition of 6.57 units took 4 days, it was calculated that apposition of 6 measuring units took 3.65 days. For the distance 0—3 it was assumed that the apposition of 6 measuring units took 4 days. Then 3 units took 2 days. These 2 days are added to the 3.65 days. Now we knew that the apposition of 9 units, starting from 0 took 5.65 days. This procedure was used for the following groups of classes and the result is given in Table 11. The standard deviations of these age determinations were estimated (Table 11).

From the abovementioned it follows that the X-axis in Figs. 20—21 (page 53) and 37—38 (page 62) may also denote time because the height of the dentine layer is related to time.

However, it should be remembered that these age determinations

Table 11. *Age determination (in days) of different parts of the incisor. 5-month old normal rats. Value in italics is extrapolated.*

Midpoint of groups of classes	Mean apposition	Groups selected (in measuring units)	Duration in days for groups selected	Distance from dento-enamel junction in measuring units (left column) and corresponding age in days (right column)		SD (age)
3	6.57	3— 9	3.65	3	*2.0*	—
8	7.33	9—14	2.73	9	5.7	0.1
13	7.46	14—19	2.68	14	8.4	0.1
18	7.87	19—24	2.54	19	11.1	0.1
23	8.30	24—30	2.89	24	13.6	0.1
28	8.61	30—35	2.32	30	16.5	0.1
33	8.96	35—40	2.23	35	18.8	0.1
38	9.38	40—45	2.13	40	21.0	0.1
43	9.67	45—50	2.07	45	23.2	0.1
48	9.47	50—55	2.11	50	25.2	0.1
53	9.00	55—60	2.22	55	27.4	0.1
58	9.16	60—65	2.18	60	29.6	0.1
63	9.12	65—70	2.19	65	31.8	0.1
68	9.05	70—75	2.21	70	33.9	0.1
73	8.67	75—80	2.31	75	36.2	0.2
78	6.96	80—84	2.30	80	38.5	0.2
83	5.61	84—88	2.85	84	40.8	0.2
88	5.39	88—93	3.71	88	43.6	0.2
93	4.57	93—97	3.50	93	47.3	0.3
				97	50.8	0.5

are derived from observations of apposition at point 2 and are only valid for this point. Another limitation is that the beginning of apposition of dentine was determined by extrapolation.

SOURCES OF ERROR

The choice of systematic and random variations in the model for analysis of covariance may be regarded as well founded. The variations between sections, intervals, measuring points and their interactions are primarily the topic of the investigation. The F-test showed that in a selected group of animals the variations between animals (T) were negligible compared with other variations studied. This is in accordance with general biological principles in experimental investigations.

Concerning the choice of a suitable degree for the polynomial, it was thought that the second degree would fit most cases. To check this, the F-test was carried out and for different categories of normal animals it was found that a second degree polynomial was satisfactory.

This can also be shown by comparing the values measured for dentine apposition with those calculated with the aid of the polynomial. Table XXV gives the means of measured values at each of the points in the different sets for the 5-month old normal adult animals, right side. On comparison of these values with the corresponding *calculated* values in Table V, good agreement will be found. A curve of the third and fourth degree can always be adapted to 4 and 5 measured values, respectively. From a biological point of view, a function of the second degree seems to be natural and logical. It was then decided to describe the apposition of dentine as a second degree polynomial.

As it had been proved that highly significant differences existed between measuring points, it was natural that the variations of the adapted curves were less for the individual points than for the points taken together, Tables IV and VI. Among the individual points, 2 was the one with the least variation, *i.e.* standard deviations for this point generally had the lowest values. This was shown for the different sets in the category of adult 5-month old animals. For the young ones it was calculated only for set 4—5, right side. Experience showed that measuring at point 2 was comparatively easy, regardless of the age of the animals. It was therefore not considered necessary to make a complete computer analysis for all sets in young animals. Nor was it considered necessary to make this analysis for the older adult animals at this stage of the investigation.

The limitations of the age determinations of the incisor were mentioned in the description of the method. The standard deviations given describe the uncertainty of the mean values for apposition. The variation depending upon the systematic errors such as: assumption of linear apposition of dentine and the selection of groups for the age determination, are not included. This latter variation is hard to estimate but is probably not greater than the variation given.

DISCUSSION

Three phases could be recognised in the curve for the rate of apposition of dentine: a rising one, a maximum one and a falling one. The result was the same whether it was based on measurements made during a given time interval in different sections or during different time intervals in sections. The general pattern was the same whether the curves were based on the values noted at a single measuring point or the 10 measuring points were combined to a "mean" measuring

point. In the former case there were obvious differences between the different points with regard to the magnitude of the measured values and also with regard to the occurrence of the maxima of apposition (Figs. 15—19, 36, pages 49—51 and 62). When all the measured values from point 2 were exploited for the investigation of the appositional rate, as described in the simplified analysis, again the same pattern emerged.

Thus, when 3 different methods were used for the analysis the same picture of dentine apposition was obtained in 4 experimental categories of animals.

Though it appeared improbable that a fundamental methodological error was the cause of the variation found, the method for taking histologic sections may again be touched upon. Sections with number 3 were those closest to the radius of the tooth and the values for dentine apposition in these sections ought to be closest to the true values. Sections from the apical and incisal parts deviated from the radius and the values found for apposition of dentine were "too large". The values measured and accounted for previously, however, showed the opposite pattern. The conclusion can be drawn that the deviation of the sections from the radius counteracts rather than contributes to the variation found in apposition of dentine. In the statistical investigation of the variation between intervals in one and the same histologic section deviation of the section from the radius has no effect on the proportions between the intervals. That observations at point 2 were selected for the simplified analysis may to some extent have reduced the error connected with the cutting of segments. In one plane a possible rotation of the section may occur round an axis roughly corresponding to the place where measurements at point 2 are performed and if this occurs, the measured values are affected little or not at all by this error.

The conclusion may be drawn that there are good reasons to assume that the variation in the rate of apposition of dentine found and described is true and not simply apparent. Even visual inspection of the histologic sections shows a variation (Figs. 39—43).

Schour and co-workers (page 17), who investigated this subject, did not describe this variation in apposition of dentine and this may, at least partly, be attributed to their technique. A survey of their reports shows that they used both transverse and sagittal sections from the incisors for measurements, but — as far as can be seen — they used only 1 transverse section per tooth. The technical difficulties in making longitudinal sections may explain why the variation was not discovered in these. It is also impossible to know with certainty from

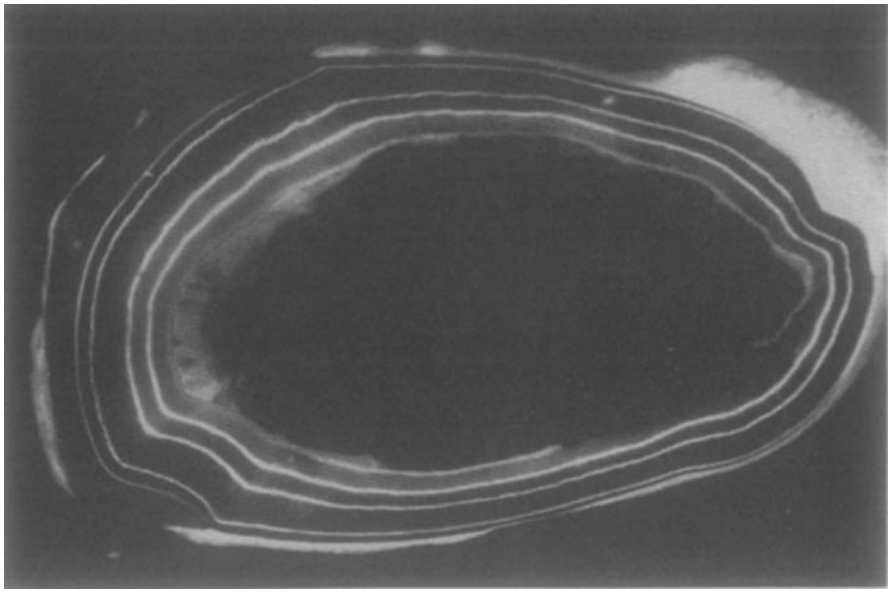


Fig. 39.

Figs. 39—43. Microphotographs of sections 1—5 from an 11-month old normal animal. Fig. 39: section 1, 4 fluorescent bands (2—5) visible. Fig. 40: section 2, 4 fluorescent bands (2—5) clearly visible, band No. 1 is discernible under the enamel (top of the picture). Figs. 41—43: sections 3, 4 and 5, respectively. Five fluorescent bands visible. 37×.

their reports where on the circumference of the tooth the measurements were made. Weinmann (1942) mentioned differences similar to those reported above, but he thought them to be due to the toxicity of his marking substances.

A search of the literature revealed no reports indicating that the variation of dentine apposition in the white rat's upper incisors has ever been so closely described as in the present investigation.

When the pattern of apposition of dentine was compared in the 3 categories of animals of different ages and normal weights it was found that young animals differed considerably from adult animals. The course of events was much faster in young animals (Figs. 19 and 34, pages 51 and 61). In young animals apposition during intervals 1 and 2 differed highly significantly from that during intervals 3 and 4, respectively. The difference may perhaps be explained by the quicker development of the animals at this age. Consequently the different ages

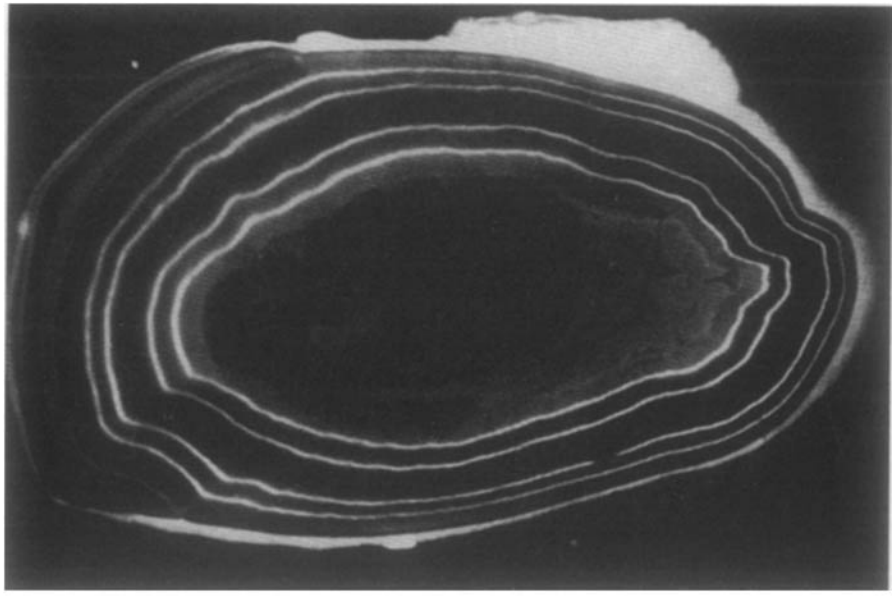


Fig. 40.

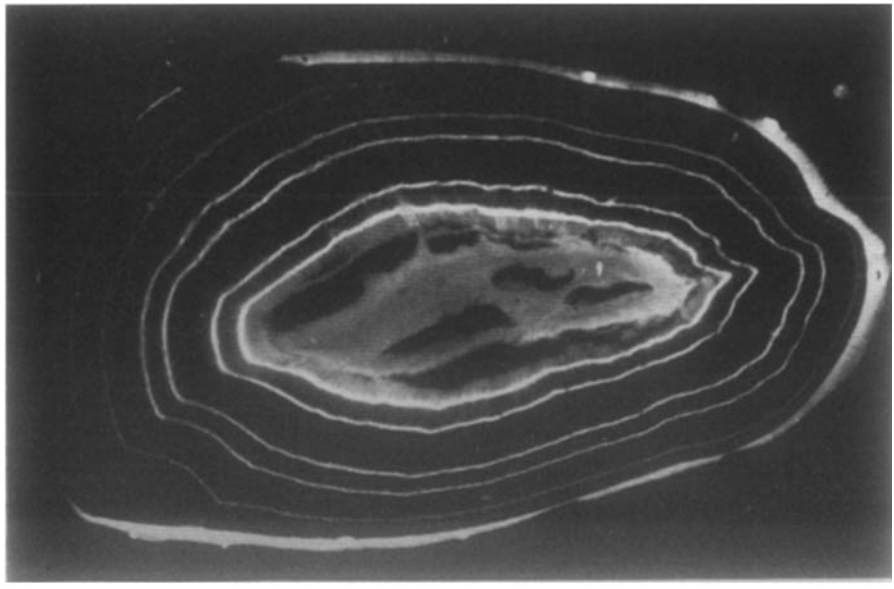


Fig. 41.

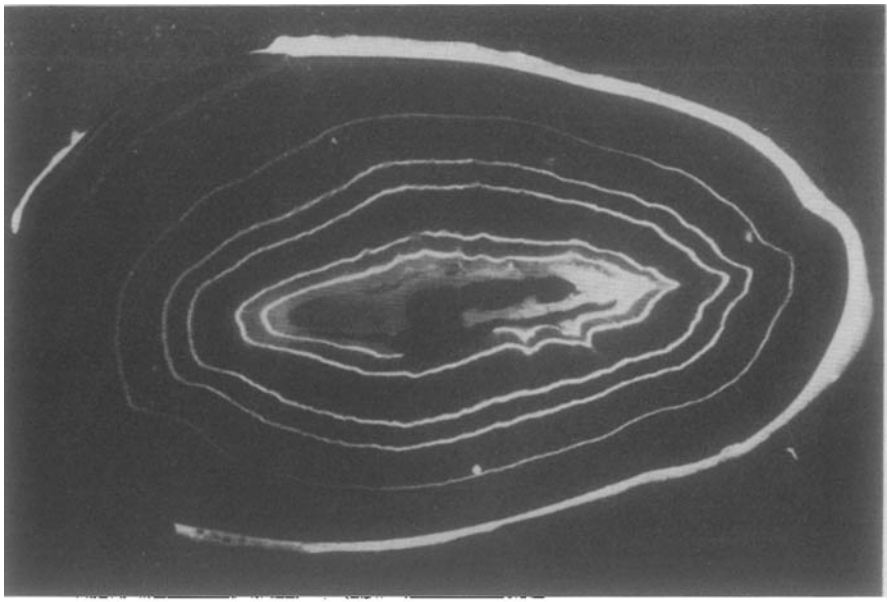


Fig. 42.

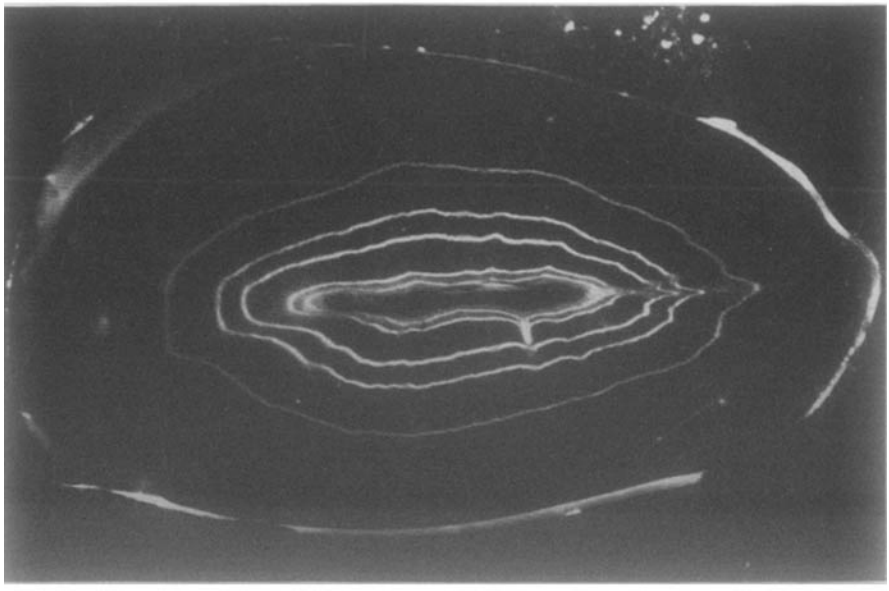


Fig. 43.

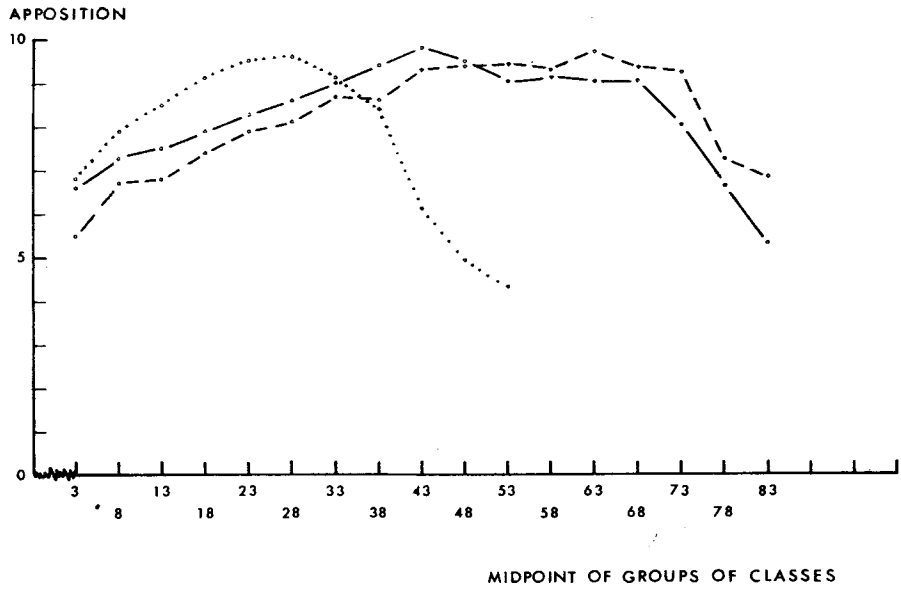


Fig. 44. Dentine apposition in normal animals of different ages. Y-axis: mean value from 4-day intervals 2 and 4. X-axis: midpoints of groups of classes (=age of the formative cells). o — o — o 5-month old animals. o - - o - - o 11-month old animals. o ···· o ··· o young animals.

of the animals during different intervals affects dentine apposition more in young animals than in adults.

In the 2 categories of adult animals of normal weight, age about 5 and 11 months, respectively, the differences in pattern of apposition were much smaller despite a comparatively larger difference in age (Fig. 44). The 10-month old animals of low weight differed somewhat from those with normal weight. During the increase in rate of apposition of dentine the difference was small, but the maximum occurred somewhat earlier, and was not of the same magnitude as in the animals with normal weight, and the decrease was somewhat more rapid.

The age determinations were founded on observations made at point 2 and are valid only for this point. They are based upon the formation of dentine. They cannot forthwith be used to describe the chronological age of the odontoblasts corresponding to point 2 but denote the "functional age" of these odontoblasts. It is true that the difference may be small, but the distinction ought to be made. With the method described, age determinations can be made for any point, if the laws of growth

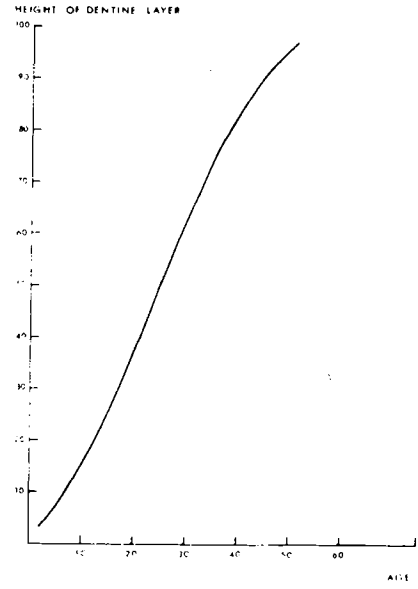


Fig. 45. Height of the dentine layer (Y-axis) versus incisor age in days (X-axis). 5-month old normal animals (*cf.* Table 11).

of the incisor, are to be analysed further. For this investigation no such further analysis was necessary.

If desired, the appositional rate in μm per day can be calculated by multiplication of the figures given by 10 and division by the actual interval in days (4, 6 or 8). If it is made for sections 3, point 2 in 5-month old animals with the aid of values calculated from the polynomial (Table V), it will be found that the mean rate increases from 18.5 μm during interval 1 to 23.0 μm during interval 4. Much greater differences can be found for other measuring points and other sections.

If the curve for height of the dentine layer versus time (Fig. 45) is studied, a slight but discernible S-form can be found. This S-form, though more marked, is found in curves describing other types of growth such as weight in relation to age of the rat (Zucker, Hall, Young & Zucker 1941, Zucker & Zucker 1942), the number of individuals in relation to age in a population and weight in relation to age of individuals (Brody 1945). Thus, the growth of incisor dentine is in agreement with some other types of growth.

The comparison between the formation of 2 types of calcified tissues: dentine in the rat incisor and bone in an osteone, may be of interest. Jee & Arnold (1954), using Ca^{45} as a tracer, found that the rate

of bone apposition in a Haversian system was highest during the first half of the formation process. Marshall, Jowsey & Rowland (1959) made similar findings. Lee (1964) studied the bone formation rate in osteones with the aid of tetracycline markings and found a linear relationship between appositional growth rate and diameter of the marker. However, he admitted that at the lower rates of growth the correlation was not so good. Frost (1964) described the rate of bone formation in a Haversian system by means of an S-shaped curve, based on a histogram.

It is possible that different forms of growth can be described by S-shaped curves. As measurement at low rates of growth may be difficult the values forming the basis of the 2 ends of the curve may be difficult to obtain. If a method does not allow measurement of low rates of growth, it will record only the fairly linear part of the curve. This may explain the discrepancies between the findings by different authors regarding Haversian systems.

VI.

APPOSITION OF DENTINE UNDER INFLUENCE OF PITUITARY GROWTH HORMONE AND OF HYDROCORTISONE

INTRODUCTION

The purpose of this study was to find out whether administration of either of the hormones had any effect on the rate of growth of dentine. The growth hormone was studied for its effect on both adult and young animals; hydrocortisone, only on adult animals.

CHOICE OF HORMONE PREPARATIONS

Growth hormone can be obtained from different sources. The porcine growth hormone preparation Somacton has previously been used in investigations in man and experimental animals.

Carstensen, Paulsen & Rudberg-Roos (1955) gave Somacton and insulin to patients with advanced pulmonary tuberculosis and in 8 cases they found "definite improvement of the general condition in all but one patient and a weight increase in all but two cases". Saikku (1956) found Somacton to have a maturing effect on granulation tissue and to increase the tensile strength of healing experimental wounds. Brummer (1966) studied the formation of adhesions around a traumatised tendon and found Somacton to accelerate the formation of collagen.

Somacton has been reported to accelerate healing of fractures of the tibia in the rat (Koskinen 1959). Given in combination with thyrotropin it is said to promote fracture healing in humans (Koskinen 1963).

It was decided to use this preparation of growth hormone [1] because it was fairly readily available and, second, because it has been reported to have positive effect also on the formation of mineralised tissue.

[1] Generously supplied by AB Ferring, Malmö.

The hormone was delivered in vials containing 50 I.U. of GH (in dry substance). Before use it was dissolved in 5 ml of solvent. The solvent consisted of distilled water containing 5 per cent glucose and 0.5 per cent phenol. With the aid of hydrochloric acid the pH was adjusted to 3—4.

Stanisavljevic et al. (1962) reported that hydrocortisone acetate retarded lamellar bone formation more than cortisone acetate. In their study of callus formation Hulth & Olerud (1964) used cortisone acetate (Cortodrin®, Astra), which was found to retard bone repair. The same manufacturer's preparation of hydrocortisone acetate (Hydrocortodrin®, Astra)[2] was used in the present investigation.

It is a suspension containing 25 mg of hydrocortisone acetate (17-hydroxycorticosterone-21-acetate) per millilitre. The suspension medium used in the control experiments was supplied by the manufacturer.[2] It contains Tween 80, phenylcarbinol, carboximethylcellulose, sodium chloride and distilled water.

The descriptions of the hormones, the solvent and the suspension medium have been obtained from the manufacturers.

DURATION OF HORMONE TREATMENT

It was intended to study dentine formation before and during hormone treatment in one and the same animal. The duration of hormone treatment was selected so that the earlier used investigation period (24 days) was divided into 2 parts. In the beginning and without knowledge of the effect of the above hormones on dentine formation it seemed natural to use a control period of the same length as the period of treatment. It was considered self-evident that the control period should precede the hormone period.

DOSAGE

It was decided to use such large doses that a lack of response could not reasonably be ascribed to too small a dose.

When deciding upon the dose of GH it had to be taken into account that the supply was not unlimited and that the protein solution might cause local irritation. If such a reaction occurred, it might interfere with the result. When choosing between 1/2 and 1 millilitre of solution,

[2] Generously supplied by AB Astra, Södertälje.

i.e., 5 or 10 I.U. GH, it was decided to give 10 I.U. of GH daily to adult animals and 2 I.U. daily to young animals, which doses are well above those used by Koskinen (1959).

The supply of hydrocortisone could be regarded as unlimited. In young rats Johannessen (1964) found oral administration of cortisone to retard dentinogenesis in the molars. The dose is not expressed in mg per kg bodyweight but, judging from the figures given, it varied between 17 and 40 mg/kg bodyweight. Follis, Jr. (1951) found 20 mg cortisone acetate per kg bodyweight to have no effect on the epiphyseal cartilage in the rat. Profound disturbances occurred when the dose was 40 or 50 mg. Stanisavljevic et al. (1962) found a daily dose of 1 mg hydrocortisone acetate to retard lamellar bone formation. In the present investigation it was decided to give a daily dose of 25 mg (vol. 1 ml) hydrocortisone, *i.e.* a very large dose.

The doses of GH and hydrocortisone were not intended to be comparable.

ROUTE OF ADMINISTRATION

According to Greenspan et al. (1949), the effect of GH, investigated by the tibia test, is the same whether the hormone is given subcutaneously, intraperitoneally or intravenously. It was decided to give GH subcutaneously to the adult animals and to reserve the intraperitoneal route for the suspension of hydrocortisone acetate, in case it should be desired later to give the two hormones simultaneously. Because of the smallness of the young animals it was easier to inject GH intraperitoneally. Hydrocortisone has been reported to be effective when given intraperitoneally (Stanisavljevic et al. 1962).

MATERIAL AND METHODS

Adult animals

Two series of experiments, primary experiments and control experiments were performed.

Primary experiments

Thirty-two female rats were divided into 2 equal groups. One group was given a daily intraperitoneal injection of 25 mg (1 ml) hydrocortisone acetate and the other a daily subcutaneous injection of 10 I.U. porcine GH (1 ml) on the last 12 days of the earlier used experimental period. corresponding to intervals 3 and 4. At end of the experiment the

animals were a little more than 4 months old. The different techniques used were the same as those described in Chapter IV.

Control experiments

One hundred female rats were divided into 5 equal groups of practically the same mean bodyweight. The cages were randomly distributed in the stable. Five injections of OTC were given at *6 day intervals*; otherwise the animals were treated in the way described earlier. Intervals of equal length were used to facilitate the simplified analysis. At the end of experiment the animals were slightly above 4 months.

Of those 100 animals, group 1 served as untreated controls, while the other 4 groups received various injections.

1) No treatment.
2) Daily subcutaneous injection of 10 I.U. (1 ml) of porcine GH during the last 12 days of the experimental period.
3) Daily subcutaneous injection of 1 ml solvent for GH during the last 12 days of the experimental period.
4) Daily intraperitoneal injection of 25 mg (1 ml) hydrocortisone acetate during the last 12 days of the experimental period.
5) Daily intraperitoneal injection of 1 ml of the suspension medium for hydrocortisone acetate during the last 12 days of the experimental period.

The previously described histologic and measuring techniques were used with the exception that measurements were made only at measuring point 2 and always by the author personally. When making the measurements the author did not know to which group of animals a given histologic section belonged. Some of the observations were checked by the technical assistant in randomly selected sections.

Young animals

Forty-five animals received daily intraperitoneal injection of 2 I.U. GH and 17 the corresponding volume (0.2 ml) of solvent for GH during the last 12 days of the experimental period. Age at beginning of experiment: 30 days.

RESULTS

Effect of growth hormone

Adult animals

Primary experiments. The weights of the animals during the experiment are given in Table 12. On day No. 28 the mean weights of the animals in the different sets ranged from 283.0 to 293.7 grams.

The computer analysis is based on values from 12 animals and the simplified analysis on values from 16.

The results of analysis of covariance are given in Tables XXVI—XXVII and summarised (page 46) in Tables 13—14. It is seen that the factors S, I and P and their interactions are the chief sources of variation. To the exceptions the same explanations may apply as by the normal animals.

The statistical analysis was carried further to include polynomial regression. This was based on the means of the values recorded for the 10 measuring points in each of the sets, right and left side separately, and for each of the points in the sets on the right side. Analysis with the aid of F-test revealed that apposition could be satisfactorily described with a second degree polynomial. The calculated values for apposition of dentine, the number of animals and the standard deviations are given in Tables XXVIII—XXXI. If these values are compared with the corresponding values in normal 5-month old animals (Tables III and V), a general pattern will emerge. The rate of dentine apposition in the GH-treated animals was, as a rule, lower than in the untreated animals during the period before hormone treatment, but during this treatment it rose and was finally higher. This finding was the same whether the values were analysed for each measuring point separately or whether the values formed at all 10 points were pooled before analysis. This means that this investigation failed to show that dentine formation corresponding to any particular measuring point is

Table 12. *Mean weights and weight ranges in grams during experiment. 4-month old animals. Treatment with growth hormone during latter half of experimental period. Primary experiments.*

Day	0	12	24	28
Mean weight	257.1	253.6	291.4	291.2
Range	242—276	240—275	269—316	274—310

Tables 13—14. *Summary of result of analysis of covariance. 4-month old animals. Treatment with growth hormone during latter half of experimental period. Primary experiments.*

Table 13.

Source of variation	Set 3—4		4—5	
	Right	Left	Right	Left
T	—	—	—	—
S	***	***	***	***
P	***	***	***	***
TS	***	***	***	***
TP	—	—	—	**
SP	***	***	***	***
TSP	***	***	***	***

Table 14.

Source of variation	Set 3		4		5	
	Right	Left	Right	Left	Right	Left
T	—	—	—	—	—	—
I	***	***	**	*	***	***
P	***	***	***	***	***	***
TI	—	***	***	***	***	***
TP	—	***	**	***	—	**
IP	***	***	***	***	***	***
TIP	***	***	***	***	***	***

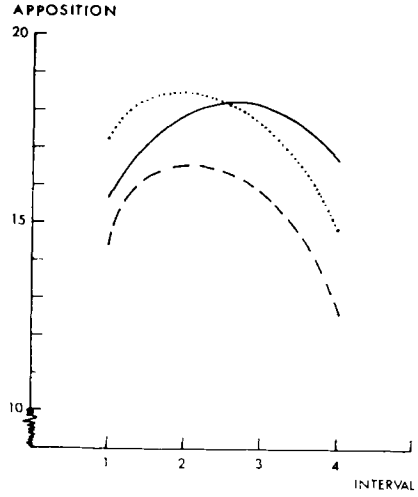


Fig. 46. Calculated variation of dentine apposition. Set 4, 10 measuring points taken together. ····· 5-month old normal animals. ——— 4-month old animals, treated with growth hormone during latter half of experimental period. – – – – 4-month old animals treated with cortisol during latter half of experimental period. Primary experiments.

more susceptible than others to GH. To save space, it was not considered necessary to illustrate the findings graphically so completely as in the normal animals. A representative curve for normal apposition and, for comparison, the corresponding curve for animals under the influence of GH are given in Fig. 46, from which it is clear that the 2 curves are very similar in shape; they are only somewhat apart. This interspace may reflect a true difference between the 2 categories of animals but it may also depend upon an altered rate of eruption or a systematical error in the positions of the sections.

The findings were analysed by the simplified method. Apposition during both an 8-day (3) and a 4-day (4) interval in 5-month old untreated animals was compared with that during the corresponding intervals in animals treated with GH. No significant differences were found.

When the two 8-day intervals were compared with each other in the same category of animals, it was found that in the untreated animals there was a highly significant decrease of the rate of dentine apposition during interval 3 compared with that during interval 1, but no such difference was found in the GH-treated animals.

When the two 4-day (2 and 4) intervals were compared with each other in the normal category and in the category of GH-treated animals, it was found that in the normal animals the rate of apposition of dentine tended to be somewhat lower during interval 4 than during interval 2, but the difference was not significant. In the GH-treated animals the rate of dentine apposition was higher during interval 4, when the animals were given GH, than during interval 2 when no treatment was given. The difference was almost significant.

Control experiments. The mean weights and weight ranges are given in Table 15. The data were analysed by the simplified method, and the numbers and means of the measuring values for untreated, GH-treated and solvent-treated animals are given in Tables XXXII—XXXIV. This analysis is based on values from all the animals. In the analysis only the mean values for the groups of classes with midpoints from 3 to 38 inclusive were used, because these groups showed the least variation around mean values. The standard deviations in the different categories were close to each other, and for the calculations one and the same estimate, 0.8, was used. The standard deviation of mean values was calculated by dividing 0.8 by the square root of the number of observations (n).

Comparison of pairs of categories of animals (no treatment versus treatment with GH, treatment with GH versus treatment with solvent.

Table 15. *Mean weights and weight ranges for different experimental categories. 4-month old animals. Control experiments.*

Body weight (mean and range). Adult animals, control experiment.

Day	Treatment				
	None	GH	Solvent for GH	Cortisol	Suspension medium for cortisol
0	226.5	230.1	229.3	231.5	227.8
	207—247	211—254	212—254	211—264	210—245
12	246.0	248.1	244.9	249.5	244.4
	222—263	233—265	227—271	228—299	228—267
24	257.9	300.5	258.9	195.8	254.3
	240—274	282—314	240—274	164—222	241—275
28	258.1	291.2	260.1	188.4	259.7
	239—273	266—314	238—278	161—216	241—281

and no treatment versus treatment with solvent) revealed no significant differences in any of the 4 intervals.

When the intervals were compared with each other in the same category of animals, *i.e.* 1 versus 2, 2 versus 3 and 3 versus 4, the untreated animals showed a significant increase in the rate of apposition of dentine during interval 2 compared with interval 1. Otherwise no

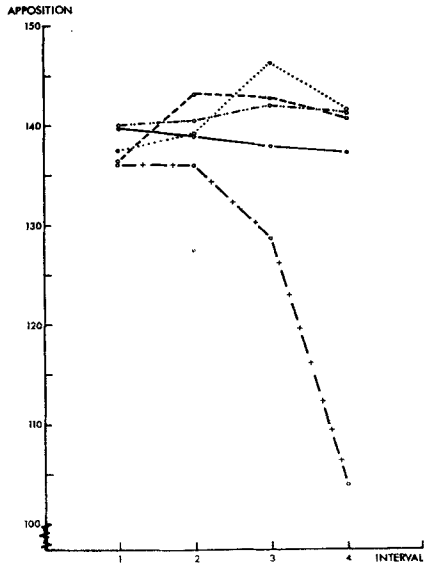


Fig. 47. Dentine apposition under varying circumstances. 4-month old animals. Control experiments. o----o no treatment. o···o···o treatment with growth hormone during latter half of experimental period. o-·-·-o treatment with solvent for growth hormone during latter half of experimental period. o—+—+o treatment with cortisol during latter half of experimental period. o——o treatment with suspension medium for cortisol during latter half of experimental period. N.B. Y-axis: sums of the observed mean values from groups of classes with midpoints from 3 through 53, irrespective of number of observations.

differences were found. The difference between intervals 1 and 2 cannot be satisfactorily explained. It may be due to chance.

In animals treated with GH no difference was found between intervals 1 and 2. The rate of apposition of dentine was highly significantly greater during interval 3 than during 2 and highly significantly *lower* during interval 4 than during interval 3.

In animals given solvent for GH no significant differences were found between the intervals.

The results are illustrated in Fig. 47.

Young animals

The mean weights and ranges are given in Table 8 (page 59).

Since extensive analysis of covariance had been performed for normal adult animals, normal young animals and hormone-treated adult animals and shown the same general pattern it was not considered necessary to perform this analysis for every set of the GH-treated young animals. It was thought sufficient to perform it for sets 4—5 and 6 right side, *i.e.* one interval in different sections and different intervals in one section, respectively. The choice of the right side was arbitrary and consistent with the earlier choice of the right side for certain statistical calculations. The detailed results are given in Table XXXV and in summary in Table 16. The findings agree well with those made earlier.

Polynomial regression was performed for the above-mentioned sets. Also in this category of animals apposition of dentine could be satisfactorily (F-test) described with a second degree polynomial. The results for all 10 measuring points taken together are given in Table

Table 16. *Summary of result of analysis of covariance. Young rats treated with growth hormone during latter half of experimental period.*

Source of variation	Set 4—5 Right	Source of variation	Set 6 Right
T	—	T	—
S	***	I	***
P	***	P	***
TS	***	TI	***
TP	**	TP	**
SP	***	IP	***
TSP	***	TIP	***

XXXVI and for each of the points in set 4—5, right side, in Table XXXVII. The corresponding standard deviations are given in Tables XXXVIII and XXXIX, respectively. The general pattern is the same as earlier.

The data from normal, GH-treated and solvent-treated animals were analysed by the simplified method. Comparisons were made only between intervals of equal length, *i.e.* 1 versus 3 and 2 versus 4. The material is presented in Tables XL—XLII. The groups of classes with midpoints above 38 were not included because of the increase in variation in the higher groups of classes. The standard deviations were estimated at 0.7 for intervals 2 and 4 and at 1.1 for intervals 1 and 3. The standard deviations for the mean values were obtained by dividing these figures by the square root of the number of measuring values (n).

When the 3 categories, normal (page 56), GH-treated and solvent-treated, were compared with regard to intervals 1 and 2 (no treatment) it was found that no differences existed between the 3 categories. For this comparison each of the intervals 1 and 2 were compared in the following way: normal versus solvent-treated, normal versus GH-treated and solvent-treated versus GH-treated. During intervals 3 and 4 (treatment intervals) the rate of apposition of dentine was highly significantly greater in GH-treated animals than in those given solvent, but — surprisingly — those animals given solvent had a highly significantly greater rate of apposition than those not treated. The results are illustrated in Fig. 48.

When no-treatment and treatment periods were compared with each other in the same category of animals, the following results were obtained.

In normal animals there was (as mentioned earlier) a highly significant decrease in the rate of apposition of dentine during intervals 3 and 4 compared with the rate during intervals 1 and 2, respectively.

In animals treated with GH there was no decrease during interval 3 (treatment with hormone) compared with interval 1 (no treatment). The number of observations was, however, comparatively small. The decrease during interval 4 (treatment with GH) compared with interval 2 (no treatment) was also smaller than in the normal animals and was not significant.

In animals given solvent for GH there was a highly significant decrease during intervals 3 and 4 compared with intervals 1 and 2, respectively. Thus, the findings were the same as in the normal animals.

The effect of the administration of the preparation of porcine growth

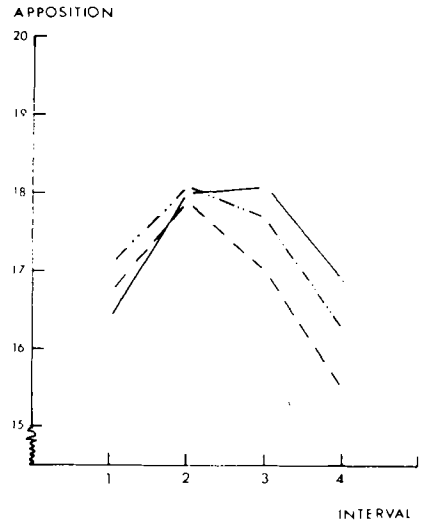


Fig. 48. Observed variation of dentine apposition in young animals. Those for intervals 2 and 4 have been doubled. o----o normal animals. o - —o animals treated with growth hormone during latter half of experimental period. o-···o animals treated with solvent for growth hormone during latter half of experimental period. The curves are comparable only along the vertical axis. *Cf.* Tables XL—XLII.

hormone used here may be *summarised* as follows. In adult animals the rate of apposition was highly significantly increased only when the treatment-free period was compared with the period of treatment in the same category of animals. In young animals an increased rate of apposition was found during the period they were given solvent for growth hormone. During the period when growth hormone was given a highly significant increase was detected both within the category of hormone-treated animals and when this category was compared with the category treated with the solvent.

Effect of hydrocortisone

Adult animals

Primary experiments. The weights of the animals are given in Table 17.

During the measurements it was found that the outline of the pulp chamber was abnormally wavy and irregular (Fig. 49), and it was difficult or impossible to measure the distances between fluorescent bands. Already at this stage of the investigation the rate of apposition of dentine appeared reduced.

The material available for statistical analysis was less complete than in the other categories. The computer analysis was based on values from 8 animals and the simplified analysis on values from 15.

Table 17. *Mean weights and weight ranges in grams during experiment. 4-month old animals. Treatment with cortisol during latter half of experimental period. Primary experiments.*

Day	0	12	24	28
Mean weight	258.1	256.4	203.5	191.8
Range	240—276	237—273	182—222	180—218

The results of the analysis of covariance (Table XLIII) are summarised in Table 18. In set 4, right side, there was one exception to the earlier rules; the factor interval was not found to be significant and the factor point (P) was only almost significant. Otherwise the results were essentially the same as before.

Polynomial regression was performed for the 10 points taken together. F-test showed a second degree polynomial to be acceptable. The results are given in Tables XLIV—XLV. The rate of apposition of dentine was decreased (Fig. 46, page 78). When the individual measuring points were to be analysed it was found that the material, being small,

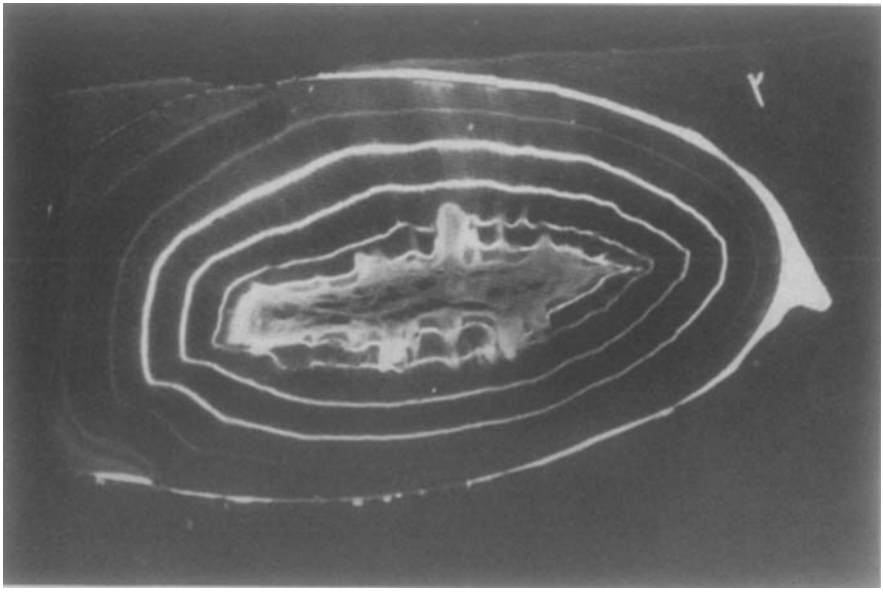


Fig. 48. Micropalatograph of section from an animal given cortisol. Control experiments. Observe the wavy and irregular demarcation against the pulpal chamber. 87×.

Table 18. *Summary of result of analysis of covariance. 4-month old rats. Treatment with cortisal during latter half of experimental period.*

Source of variation	Set 4	5	
	Right	Right	Left
T	—	—	—
I	—	***	***
P	*	***	**
TI	***	***	***
TP	—	—	—
IP	***	***	***
TIP	***	***	***

would not allow an analysis for each of the points. This analysis was therefore confined to measuring point 2. The results are given in Tables XLVI—XLVII.

A simplified analysis, based upon observations made in 15 animals, was performed and when the two 4-day intervals, 2 (no treatment) and 4 (treatment with cortisol) were compared, it was found that the rate of apposition of dentine was highly significantly decreased during cortisol treatment.

Control experiments. One of the cortisol-treated animals died from unknown cause on the third day of treatment. The weights are given in Table 15 (page 80).

Simplified analysis was performed and the number of observations and the mean values for animals treated with cortisol and for animals treated with suspension medium are given in Tables XLVIII—IL. The results are based on observations made in all surviving animals. In the analysis only the mean values from the group of classes with mid-points from 3 including 38 were used, because these groups showed the least variation. The estimated standard deviations were 0.8, and 0.8, respectively.

When the animals treated with the suspension medium for cortisol were compared with the untreated animals no difference was found between apposition during intervals 1 and 2. During intervals 3 and 4 the animals given suspension medium showed a significant decrease in rate of apposition.

When the 4 intervals in the category given suspension medium were compared with those in the category treated with GH-solvent no significant difference was found for any interval.

When the cortisol-treated animals were compared with those given suspension medium regarding the periods before treatment, it was found that apposition during interval 1 in the cortisol-treated group was significantly lower than in the solvent-treated group, while intervals 2 showed no significant difference. No satisfactory explanation can be offered for this. Anyhow, during the 6-day period immediately preceding treatment the 2 categories were without detectable difference. When intervals 3 and 4 in the 2 categories were compared, it was found that the rate of apposition of dentine was highly significantly lower during hormone treatment than during administration of the suspension medium.

When the different intervals were compared with each other, in the same category of animals, *i.e.* 1 versus 2, 2 versus 3 and 3 versus 4, no differences were found in the category of animals given suspension medium for cortisol.

Animals treated with cortisol showed a highly significantly decreased rate of dentine formation during interval 3 compared with 2. The rate of dentine formation was also highly significantly lower during interval 4 than during 3 (Fig. 47, page 80).

Functional age of formative cells and response to hormone

When the analyses were made it was presumed that if the hormones had any effect it would be detectable even if groups of odontoblasts of different ages (=different midpoints of classes) were investigated together. The choice of the groups of classes to be included in the analysis was made mainly for statistical reasons. A possible refinement would be to investigate the response in each group of classes compared with that in corresponding untreated groups. If such a comparison is to be made, it should be noted that the only place where treatment is strictly comparable to no-treatment is at the transition between the 2 periods; in the present investigation between intervals 2 and 3. If the effect of treatment is small compared with the width of the groups of classes selected, it can also be made at the transition between intervals 3 and 4 without introducing any error of importance. But if the effect of treatment is substantial, age will not correspond to the thickness of the dentine layer in the same way as earlier, and then the comparison cannot be made or it will have to be corrected for the influence of treatment before the place of comparison.

That the influence of treatment can vary with the age of the cells

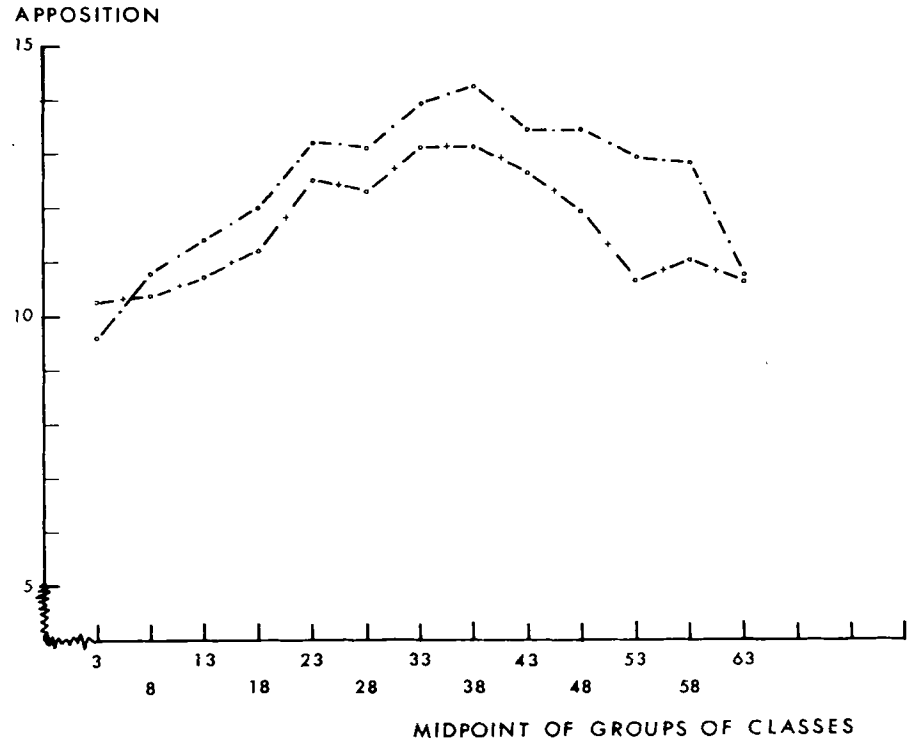


Fig. 50. Observed apposition of dentine during interval 3 (6 days, control experiment) in normal animals (o—·—o) and in animals given cortisol (o—+—o). Those odontoblasts with the highest appositional rate seem to be affected most.

is illustrated in Figs. 50—51. Fig. 50 corresponds to interval 3 and it is seen that those cells with the highest appositional rate seem to be the ones affected most by the administration of cortisol. Fig. 51 corresponds to interval 4 and the result is the same as earlier, only more accentuated and the older cells are also affected. The younger ones seem to be more resistant to cortisol.

Summarising, a retarding effect of the suspension medium was found but in animals given cortisol the rate of apposition of dentine is highly significantly lower during treatment than in normal animals and in those given the suspension medium for the hormone. This finding is the same whether the 2 experimental categories are compared with each other or treatment and no-treatment periods in the cortisol-treated category.

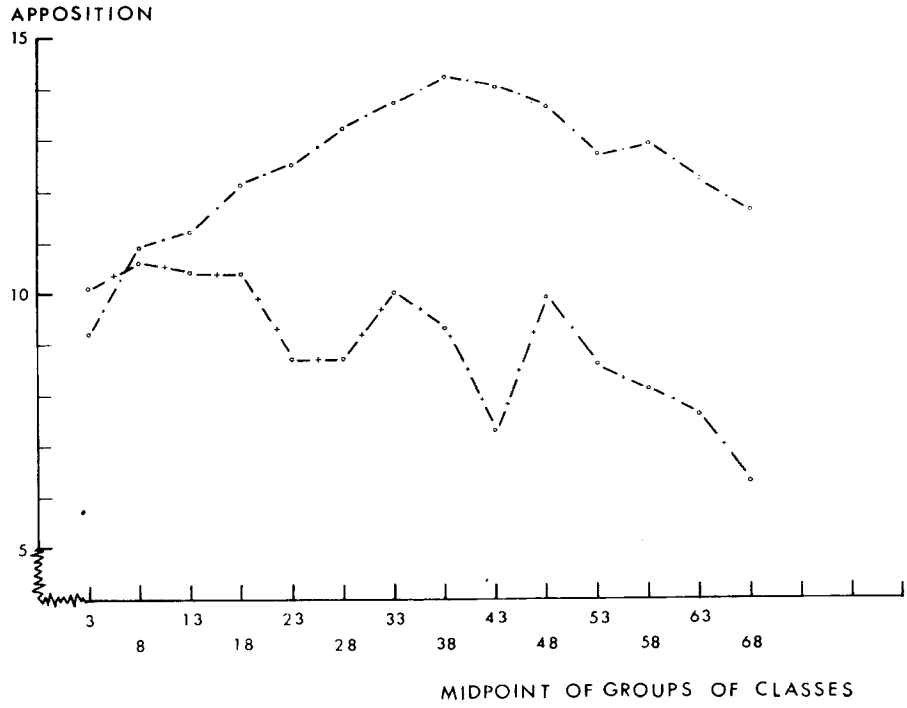


Fig. 51. Interval 4 in the same categories of animals as in Fig. 50. Young cells seem to be affected least.

DISCUSSION

To begin with, it should be stressed that the purpose of the experiments was not to make an extensive endocrinological investigation of the effects of GH and cortisol on the rate of incisor dentine formation, but simply to find out whether the formation rate could be accelerated or retarded by the administration of hormones (GH and cortisol). As the pattern of apposition was known only for normal animals, it was natural to give excessive doses of the hormone to intact animals. The effects of removal of the actual endocrine gland(s), and the effects of substitution therapy would have required investigations of dentine apposition in hypophysectomised or adrenalectomised animals.

Growth hormone

Earlier investigations of the effect of pituitary GH on dental and

skeletal tissues of intact white rats have been made after long periods of treatment with GH. Baume, Becks & Evans (1954) gave adult rats GH for 436 days and found an increase in tooth size. When Asling & Evans (1961) described the effects on skeletal development of GH in excess "to normal growing female rats starting at 81 days of age", they considered administration of GH for 30 days a short-term experiment. When studying the induction of gigantism in rats by GH, Asling, Simpson & Evans (1965) administered the hormone for 8 months or more. The period of hormone treatment used in the present investigation, 12 days, was therefore comparatively short. This must be borne in mind in the appraisal of the results.

In *adult animals* the primary experiments indicated that GH had an accelerating effect on dentine formation. In the control experiments the rate of dentine formation was greater during the period when GH was given than during the period without treatment when these 2 periods were compared with each other in the same category of animals. The difference was highly significant when intervals 2 and 3 were compared. However, the increase did not continue during interval 4 but a drop occurred as compared to interval 3. Still, the rate was somewhat higher than during the period with no treatment.

In *young animals* the pattern seems somewhat more distinct. When the apposition in 3 categories: normal, GH-treated and solvent-treated animals, were compared regarding the period preceeding treatment they were found not to differ significantly from one another. When the second parts of the experimental period, *i.e.* the intervals when treatment was given, were compared it was found that the rate of apposition of dentine in the solvent-treated animals was highly significantly greater than in normal animals and that the rate was highly significantly greater in the GH-treated animals than in the solvent-treated.

When the periods without and with treatment were compared in each category of animals, it was found that both normal and solvent-treated animals showed a highly significant decrease in the rate of apposition of dentine during the intervals corresponding to treatment. When the animals were given GH, this decrease did not occur and no significant differences were found.

The highly significant difference found between normal and solvent-treated animals was unexpected. Several tentative explanations may be offered, but no definitive answer can be given. The route of administration may play a role in spite of earlier investigations denying this possibility (Greenspan et al. 1949). It may be due to one or more

constituents of the solvent. It is possible that the handling of the animals every day during the experimental period is the cause. It has been claimed that the more rats are handled the tamer and quieter they become and then probably grow more quickly (Lane-Petter 1963). This assumption may be supported by the fact that the effect of solvent was only seen in young animals which can be expected to be more sensitive than adults. It has been claimed that children subjected to stress grow taller and it seems certain that stimulation of rats during infancy may cause increase in the rate of growth (Whiting, Landauer & Jones 1968).

With the method developed measurements can be performed with high precision. This fact and the possibility that the test system is easily influenced by various factors, especially in young animals, may explain the unexpected finding.

It is not easy definitely to decide whether GH has an effect or not. Even if highly significant differences were found, the absolute differences between the values measured were not so large (Tables XXXII—XXXIV, XL—XLII). However, and this may be the important finding, in all of the 3 categories of animals treated with GH; adult animals, both primary and control experiments, and young animals the results of GH-treatment point in the same direction and indicate that GH has an accelerating effect on upper incisor dentine formation in the intact female white rat. The conclusion may be drawn that it is highly probable that a true and not an apparent effect was found. The results are in conformity with those obtained by earlier investigators who used longer experimental periods and less precise methods.

Hydrocortisone

A retarding effect was demonstrable already in the histologic sections (Fig. 49, page 84). Also the values measured (Tables XLVIII—IL) clearly demonstrated that a decrease occurred and the final proof was produced by the statistical analysis, which showed a highly significant decrease during cortisol treatment. Young odontoblasts seem to be less responsive than older ones (Figs. 50—51, pages 87—88).

Again the vehicle was found to have an effect in the same direction as the hormone. In the interpretation of the fact that the decrease, compared with that in normal animals, was significant but not highly significant, it should be borne in mind that no difference was found between the effect of suspension medium for cortisol and that of the sol-

vent for GH. The suspension medium is more complex than the solvent for GH, and it is possible that it can disturb the formation of dentine. Earlier investigators (Brånemark & Goldie 1967, Brånemark, Goldie & Lindström 1967) have found deleterious effects of the suspension medium for a corticosteroid preparation when locally applied to experimental animals. The effect was found attributable to sorbitol.

At present the finding can only be noted; no definite explanation can be offered and further investigations are desirable. Anyhow, the difference between animals given suspension medium and those given cortisol was so great that no doubt should exist that cortisol itself in the dosage employed has a profoundly retarding effect on the rate of incisor dentine apposition.

The present investigation has not shown how the hormones exert their effects. A very complicated chain of events may be at work. The effects found are connected with the administration of the hormones. Similar effects were obtained by administration of the solvent, respectively the suspension medium, but the effect of the hormones were highly significantly different from that of the suspension medium or the solvent alone.

VII.

TENTATIVE COMPARISON BETWEEN APPOSITION OF INCISOR DENTINE AND LAMELLAR BONE

INTRODUCTION

Garn, Lewis & Blizzard (1965) compared dental and skeletal development in some endocrine disturbances in man. They found that the retardation or acceleration of development was less pronounced in dental than in skeletal tissues; in dental tissues only half as much or one-fourth of that in skeletal tissues. In congenital adrenal hyperplasia Bergstrand & Filipsson (1967) preliminarily reported a similar finding; dental development was less advanced than skeletal development.

In the present investigation an attempt was made to compare the formation of dentine and of lamellar bone.

MATERIAL AND METHODS

As mentioned above, the femur and the tibia on each side had been extracted and stored in the cold. For the investigation the right femur and tibia were selected from the following experimental categories: 5-month old normal adult animals, 4-month old animals given GH or cortisol (primary experiments) and the 4-month old adult animals used for control experiments. Only those animals where the investigation of the dentine had shown no missing fluorescent bands were used.

From the middle third of the unembedded femur and the tibia, 3 transverse sections were taken from each bone, and ground according to the method described by Frost (1958). After passing through absolute alcohol and xylol they were mounted in DePeX. All together 411 histologic sections, 127 from untreated, 75 from GH-treated and 209 from cortisol-treated animals were investigated.

RESULTS AND DISCUSSION

In the specimens from untreated animals neither on the endosteal nor on the periosteal side, was bone formation present on the whole circumference of the bone at the same time. Parts of the circumference showed bone formation and at these places fluorescent bands were visible (Fig. 52).

A possible source of error should be mentioned. Though only 5 injections of OTC have been given, 6 fluorescent bands were visible. This can be explained by the observation that during the grinding of sections OTC is dissolved from the bone and is present in the water during grinding and is absorbed to the surface of bone. The same finding can be made in dentine. If a section from a tooth from an animal given no injections of OTC is ground with the aid of equipment previously used for the grinding of sections from animals given OTC, a fluorescent band will be found in the boundary between dentine and the pulp chamber. If a section from an animal given no injections of OTC is ground with

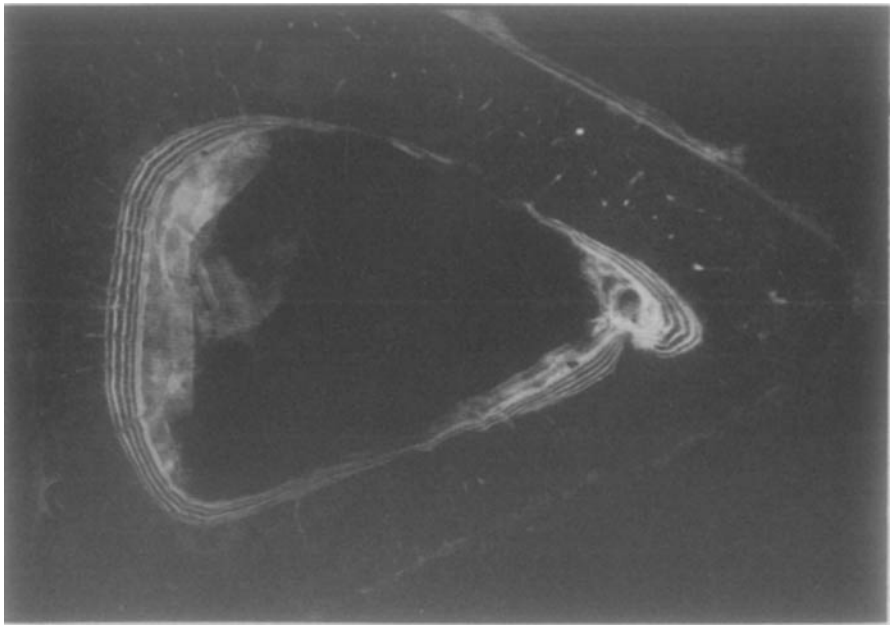


Fig. 52. Transverse section from tibia. 4-month old normal rat. On endosteal side: 5 fluorescent bands (6 days' interval). What appears as the 6th band is an artefact. 37×.

the aid of completely fresh equipment, no fluorescent band will be found.

It was very soon realised that good quantitative measurement of the rate of lamellar bone formation could not be made. This was because the fluorescent bands did not regularly occur round the whole periphery of the bone with the result that it was not possible to make measurements at corresponding parts of the sections from different animals. It is theoretically possible to overcome this difficulty by making comparisons between untreated and treated periods in the same section at randomly selected points where bone formation occurred during the whole experimental period. But in practice this seemed inconvenient because even if a point was selected and measurements were made along the corresponding radius, the rate of apposition during periods of equal length varied widely even in normal animals. Also the variation between different points on the circumference was considerable. It may be possible to make a very great number of measurements in untreated and, for example, hormone-treated animals, and then com-

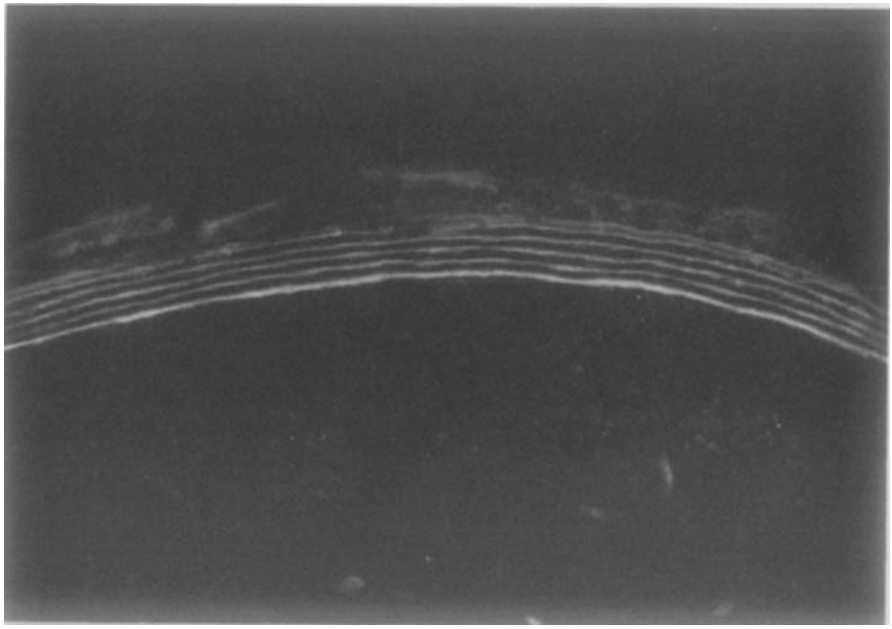


Fig. 33. Transverse section from femur. 4-month old normal rat. Periosteal side. Five fluorescent bands visible (6 days' interval). 147×.

pare the quotients between periods of treatment and no treatment, but it is very doubtful whether such comparisons would be really informative.

Sections were nevertheless made from bones from the animals in the primary experiments and examined in the hope that it would reveal substantial differences, if any, between periods of treatment and no-treatment. It was found that normal and GH-treated animals behaved alike and no certain difference could be detected between them.

In animals given cortisol at most 3 fluorescent bands could be detected. In every one of the untreated and of the GH-treated animals 5 bands could always be seen in at least one histologic section from every animal. For purposes of control 3 sections were also taken from the femur and the tibia on the left side of cortisol-treated animals (primary experiments) and here, too, at most 3 fluorescent bands were seen.

The findings were the same in the animals in the control experiments. Normal and GH-treated animals behaved alike. Five fluorescent bands were found in at least one histologic section from each animal, *i.e.*

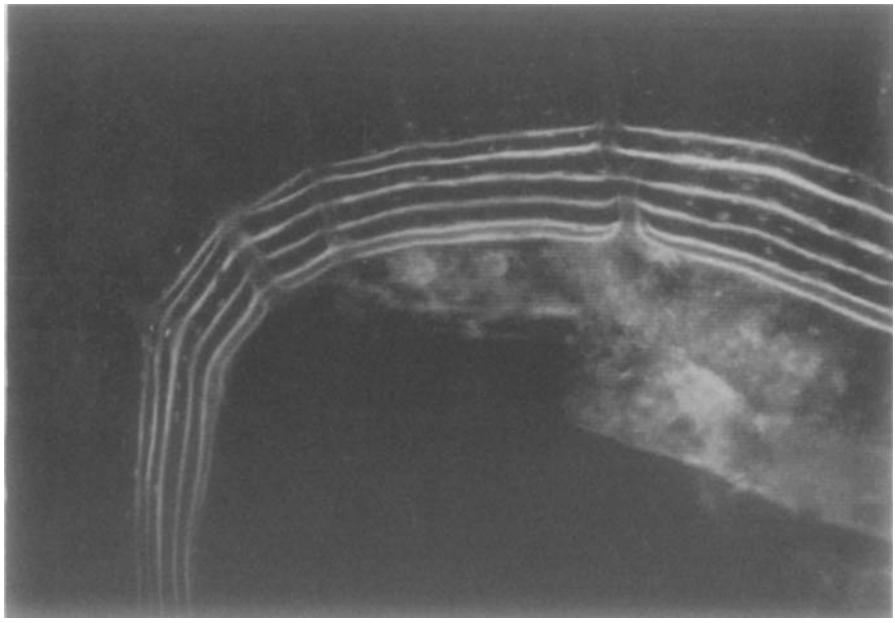


Fig. 54. Transverse section from tibia. 4-month old normal rat. Endosteal side. Five fluorescent bands visible (6 days' interval) (6th band artefact). 147×.

bone formation occurred during the whole experimental period (Figs. 53—54). In the animals given cortisol at most 3 bands could be detected on the endosteal and periosteal sides (Fig. 55). Animals given solvent for GH or suspension medium for cortisol exhibited the same features as the untreated and the GH-treated animals.

The result indicated that during the treatment with cortisol no formation of bone occurred or was so low that the chronologically last 3 fluorescent bands could not be separated. However, the last band was not so thick that it could be suspected of consisting of 3 confluent bands (Fig. 55). It is also remotely possible that the bands seen were not the ones resulting from the first 3 injections of OTC, but from the last injections. In view of the findings in untreated animals this explanation was considered improbable. The most probable explanation is that during treatment with cortisol in very large doses lamellar bone formation completely stops.

One might imagine that during cortisol treatment bone is formed, but its mineralisation is so defective that it is very soft and is lost

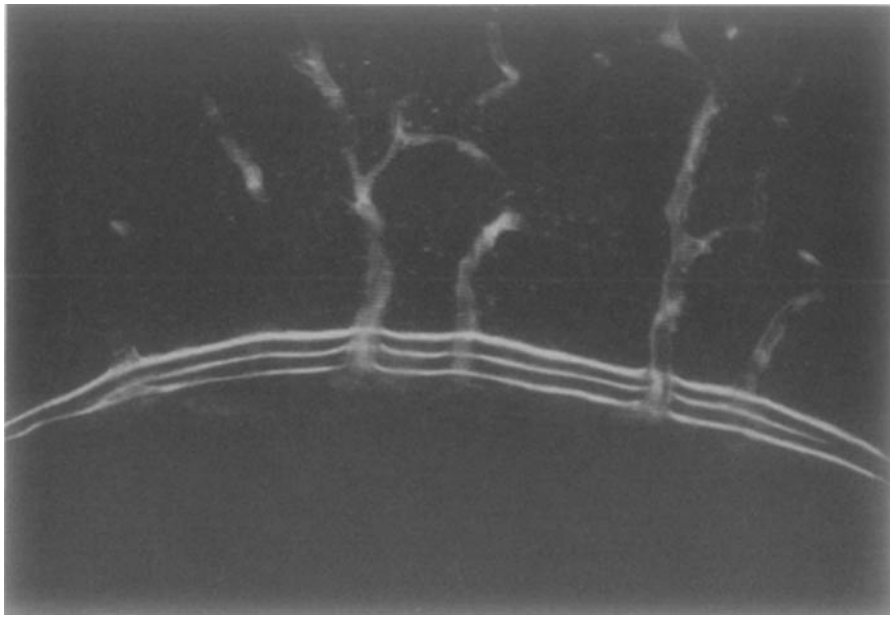


Fig. 55. Transverse section from femur. Endosteal side. 4-month old rat treated with hydrocortisone. Only 3 fluorescent bands visible (6 days' interval). 147×.

during the preparation of sections. This explanation may, perhaps hold for the bone formed on the periosteal side but seems less likely for that on the endosteal side, where the last formed bone appears to be more protected. The findings in cortisol-treated animals were the same on both the periosteal and endosteal side. To rule out this possible source of error, some sections from the left side of animals used in the control experiments were embedded in Ward's Bioplastic and then ground, but the finding was the same as in unembedded sections.

The results may be *summarised* as follows. It is not possible to measure the rate of peri- and endosteal bone apposition in the femur and the tibia in adult rats accurately by performing linear measurements between the fluorescent bands. The short-term administration of GH has no certain effect. Administration of very large doses of cortisol seems to result in complete arrest of lamellar bone formation. This last finding parallels the simultaneous retardation of incisor dentine formation.

Concerning this parallelism in mode of reaction it may be mentioned that the rate of longitudinal growth from the proximal growth plate of tibia has been studied in normal, solvent-treated and GH-treated young animals used in the present investigation. GH was found to accelerate this growth highly significantly (Ahlgren & Hansson, unpublished observations).

SUMMARY

In the course of personal preliminary studies of bone healing and lamellar bone formation under the influence of the growth hormone it was thought that dentine might serve as a "test tissue" for investigations of hormonal influence. The chemical and submicroscopic structure of dentine is similar to that of bone and in normal dentine no resorption occurs.

According to the literature, rat incisor dentine normally forms at a constant rate and is very sensitive to influences. Personal preliminary investigations failed to wholly confirm this view. A re-investigation was considered necessary.

Using oxytetracycline as a marking agent a technique was devised for measuring the linear rate of apposition of dentine in different parts of the upper incisors from the apex to the incisal edge, and from different places on the circumference. Statistical analysis of the values measured (mostly with the aid of computer) was performed. The rate of incisor dentine apposition varied in a regular but more complicated manner than earlier known. From the apex to the incisal edge of the incisor 3 phases could be recognised: a rising one, a maximum one and a falling one. Studies on female rats of different ages revealed the same general pattern but in young animals the course of events was faster. Based on the results of the computer analysis a technically simpler, but not less informative, statistical analysis was devised. The "functional age" of the odontoblasts could be related to the rate of apposition of dentine. Young and old cells produced less dentine per unit of time than did those of intermediate age.

The apposition of dentine is briefly compared with other types of growth and with the rate of bone formation in a Haversian system. It

is suggested that different types of growth can be described with S-shaped curves.

Administration of pituitary growth hormone in excess produced a slight but highly significant increase in the rate of apposition of dentine as investigated in adult and young intact female rats.

Administration of hydrocortisone in excess produced a highly significant decrease in the rate of dentine apposition as investigated in adult female rats. Young cells tended to be less sensitive.

In an attempt to compare the rate of formation of dentine with that of lamellar bone in adult female rats it was found that the use of oxytetracycline as a marking agent would not allow precise quantitative determination of the linear rate of formation of periosteal and endosteal lamellar bone. A direct numerical comparison between the rate of formation of dentine and of lamellar bone was therefore not possible. Both types of tissue were susceptible to hydrocortisone, which suppressed the rate of formation of dentine and caused apparently complete cessation of formation of lamellar bone.

ACKNOWLEDGEMENTS

Sincere thanks go to Professor G. Wiberg and Professor K.-Å. Omnell for invaluable guidance, encouragement and constructive criticism throughout the investigation.

For generous help acknowledgement is due to Docent L. I. Hansson, Miss H. Möller, Mrs. L. Olofsson, Mr. A. Herm and Miss I. Hundertmark.

The investigation was supported by grants from the Faculty of Medicine, University of Lund, Österlunds Stiftelse, Malmö, Konung Gustaf V:s 80-årsfond, Stockholm, Försäkringsaktiebolaget Eir and the Swedish Medical Research Council (Proj. Nos. B 67—17 X—2031—01 and B 68—17 X—2031—02).

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APPENDIX

Tables I—II. *Analysis of covariance. 5-month old normal rats.*

Table I.

Source of variation	Set 3—4				4—5					
	Right		Left		Right		Left		Right and Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	36.1	9	36.3	8	14.2	6	1.9	6	9.6	10
S	1811.2	3	1135.5	3	577.6	4	574.5	4	908.1	4
P	352.9	9	376.1	9	73.7	9	73.0	9	115.3	9
TS	23.7	27	27.0	24	4.4	24	4.8	24	4.7	40
TP	3.6	81	2.5	72	1.5	54	1.0	54	1.3	90
SP	51.1	27	45.6	27	12.9	36	14.8	36	20.9	36
TSP ...	2.8	243	2.3	216	1.3	216	1.0	216	1.2	360
R	0.6	399	0.6	359	0.5	349	0.4	349	0.4	549

Table II.

Source of variation	Set 3				4				5			
	Right		Left		Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	16.7	6	10.4	9	24.2	13	43.4	12	28.6	11	64.5	9
I	536.5	3	627.6	3	603.0	3	334.7	3	6301.6	3	4748.6	3
P	408.0	9	503.8	9	508.8	9	496.9	9	312.5	9	286.2	9
TI	8.4	18	12.4	27	38.0	39	41.9	36	16.5	33	26.2	27
TP	3.5	54	6.0	81	7.2	117	6.3	108	6.6	99	5.4	81
IP	20.0	27	25.5	27	46.3	27	45.0	27	67.3	27	61.8	27
TIP	2.8	162	2.5	243	3.9	351	3.3	324	3.9	297	3.6	243
R	1.0	279	1.2	399	1.5	559	1.2	519	1.5	479	1.2	399

Table III. *Calculated values for dentine apposition for 10 measuring points taken together. 5-month old normal rats. Figures in brackets denote number of animals.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
—	—	6.5	6.3	14.1	14.2	17.2	16.7	19.5	19.2
13.7	14.1	9.0	8.9(7)	16.6	16.3	18.4	18.0(12)	17.2	17.6
17.9	17.6(9)	9.4(7)	9.4	18.1(7)	17.6(10)	17.6(12)	17.6	13.3(12)	13.8(10)
17.0(10)	17.0	7.7	7.8	18.4	18.2	14.8	15.5	7.7	8.0
11.1	12.4	4.0	4.1	—	—	—	—	—	—

Table IV. *Standard deviations of values in Table III.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1.0	1.0	0.6	0.6	1.2	1.0	0.9	0.9	0.9	1.0

Table V. *Calculated values for dentine apposition at each of the 10 points in each of the sets. Right side. 5-month old normal rats. Figures in brackets denote number of animals. Cf. Table XXV.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 3—4	19.2	15.3	19.0	14.8	11.7	12.8	12.8	11.8	9.7	12.6
	22.1	18.4	21.0	19.0	16.5	14.7	17.5	14.2	15.7	18.9
	19.2 (18)	18.3 (18)	18.8 (17)	18.4 (17)	16.4 (16)	13.3 (16)	17.7 (12)	13.0 (14)	16.0 (16)	18.0 (17)
	10.8	15.2	12.4	12.8	11.3	8.6	13.4	8.3	10.6	9.7
Set 4—5	9.7	7.3	8.4	6.7	5.2	5.9	5.5	5.3	4.5	5.9
	11.1	8.9	10.9	9.6	7.9	7.5	8.6	7.3	7.7	9.2
	10.5 (11)	9.1 (12)	11.1 (11)	10.1 (12)	8.5 (12)	7.5 (13)	9.6 (11)	7.6 (11)	8.6 (11)	9.9 (11)
	7.8	8.0	9.0	8.3	7.2	6.0	8.6	6.2	7.1	7.8
	3.0	5.6	4.6	4.1	3.8	3.0	5.5	3.0	3.2	3.0
Set 3	19.3	14.8	17.3	13.6	11.2	12.0	12.4	12.6	10.2	13.0
	21.2	16.7	20.2	17.3	14.9	15.0	15.2	14.4	12.5	15.3
	22.4 (14)	17.9 (15)	21.7 (14)	19.4 (14)	16.8 (12)	15.9 (11)	17.5 (8)	15.0 (7)	15.2 (11)	17.9 (12)
	22.7	18.4	21.8	20.0	17.2	14.6	19.3	14.4	18.6	21.1
Set 4	21.3	17.3	21.5	18.4	15.4	15.7	15.6	15.3	13.5	16.2
	22.5	18.8	21.4	19.9	17.3	15.2	17.6	14.9	16.7	19.5
	20.4 (16)	18.8 (16)	20.2 (16)	19.4 (15)	16.9 (15)	14.0 (15)	18.4 (14)	13.6 (15)	17.0 (15)	19.2 (15)
	14.9	17.3	17.8	16.9	14.3	12.2	18.1	11.4	14.6	15.4
Set 5	23.9	18.9	22.4	21.5	18.6	16.4	18.8	16.3	17.7	21.0
	19.2	18.5	18.9	18.4	16.3	13.3	17.5	13.5	16.0	18.4
	13.1 (14)	15.9 (16)	14.4 (14)	14.0 (15)	12.4 (14)	10.0 (15)	15.1 (15)	10.2 (15)	12.2 (15)	13.0 (15)
	5.6	11.3	8.9	8.2	7.1	6.5	11.6	6.2	6.4	4.9

Table VI. *Standard deviations of values in Table V.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set										
3—4	0.6	0.3	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.6
4—5	0.5	0.3	0.4	0.4	0.3	0.3	0.3	0.3	0.3	0.5
3	0.6	0.3	0.5	0.5	0.5	0.5	0.7	0.6	0.4	0.5
4	0.7	0.3	0.5	0.5	0.5	0.6	0.5	0.5	0.5	0.8
5	0.7	0.4	0.7	0.6	0.6	0.4	0.6	0.5	0.6	0.7

Table VII. *Means of measured values for dentine apposition at point 2 for interval 3 (8 days) in groups of 5 classes. 5-month old normal rats.*

Midpoint of groups of classes	Number of observations (n)	Mean	SD
3	22	13.5	
8	10	14.6	
13	19	15.1	
18	16	15.7	
23	11	17.5	
28	12	17.7	$0.8 : \sqrt{n}$
33	13	18.5	
38	10	18.5	
43	10	18.6	
48	10	18.5	
53	11	18.3	
58	11	17.5	
63	14	16.4	$1.8 : \sqrt{n}$
68	9	16.0	
73	5	14.6	
	Total 183		

Table VIII. *Means of measured values for dentine apposition at point 2 for intervals 2, 4 and 5-pulp (4 days) in groups of 5 classes. 5-month old normal rats.*

Midpoint of groups of classes	Number of observations (n)	Mean value	SD
3	44	6.6	
8	33	7.3	
13	28	7.5	
18	23	7.9	
23	20	8.3	
28	31	8.6	
33	25	9.0	$0.6 : \sqrt{n}$
38	21	9.4	
43	21	9.7	
48	19	9.5	
53	40	9.0	
58	26	9.2	
63	34	9.1	
68	19	9.1	
73	30	8.7	$1.2 : \sqrt{n}$
78	24	7.0	
83	36	5.6	
88	18	5.4	
93	7	4.6	
	Total 499		

Tables IX—X. *Analysis of covariance. 10-month old rats, low weight.*

Table IX.

Source of variation	Set 3—4				4—5			
	Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	22.9	6	55.1	5	0.4	2	11.5	5
S	1167.9	3	1034.1	3	232.0	4	325.3	4
P	162.2	9	216.2	9	19.1	9	53.7	9
TS	65.1	18	54.4	15	15.2	8	20.0	20
TP	6.7	54	4.1	45	2.1	18	1.2	45
SP	24.8	27	27.2	27	2.7	36	8.7	36
TSP	3.8	162	3.2	135	1.4	72	1.0	180
R	0.8	279	0.6	239	0.6	149	0.3	299

Table X.

Source of variation	Set 3				4				5			
	Right		Left		Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	15.5	6	17.6	6	50.4	8	90.4	5	196.4	7	5.3	5
I	364.9	3	360.7	3	1056.0	3	512.1	3	4270.2	3	3252.2	3
P	270.8	9	339.5	9	183.6	9	156.9	9	109.0	9	151.7	9
TI	24.2	18	26.8	18	68.8	24	61.5	15	20.8	21	25.3	15
TP	6.0	54	6.5	54	9.3	72	6.4	45	11.3	63	10.1	45
IP	12.9	27	9.9	27	22.9	27	14.2	27	24.4	27	18.1	27
TIP	2.3	162	2.3	162	3.9	216	3.2	135	4.4	189	3.5	135
R	1.2	279	0.8	279	1.4	359	1.1	239	1.8	319	1.3	239

Table XI. *Calculated values for dentine apposition for 10 measuring points taken together. 10-month old rats, low weight. Figures in brackets denote number of animals.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
—	—	6.7	7.0	14.0	13.9	17.4	17.1	18.6	18.2
14.6	14.0	8.5	8.5	16.3(7)	16.6(7)	18.5(9)	17.9(6)	15.6(8)	14.7(6)
17.8(7)	17.6(6)	8.6(3)	8.3(6)	17.4(7)	17.6(7)	17.0(9)	16.6(6)	11.7(8)	10.7(6)
16.4(7)	16.3(6)	6.9	6.5	17.3	16.9	12.9	13.0	6.9	6.2
10.6	10.1	3.5	3.0	—	—	—	—	—	—

Table XII. *Standard deviations of values in Table XI.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1.2	1.3	1.0	0.7	1.1	1.1	1.1	1.2	1.3	1.3

Tables XIII—XIV. *Analysis of covariance. 11-month old normal rats.*

Table XIII.

Source of variation	Set 3—4				4—5			
	Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	59.0	9	21.9	7	3.3	4	10.3	3
S	1293.2	3	716.3	3	327.5	4	278.9	4
P	181.0	9	160.5	9	23.4	9	23.2	9
TS	24.7	27	20.8	21	8.8	16	3.3	12
TP	5.0	81	6.7	63	1.7	36	1.5	27
SP	30.6	27	30.5	27	7.1	36	5.9	36
TSP	3.3	243	2.9	189	1.3	144	0.9	108
R	0.6	399	0.5	319	0.5	249	0.4	199

Table XIV.

Source of variation	Set 3				4				5			
	Right		Left		Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	85.6	7	24.1	7	24.7	9	31.5	10	99.2	8	103.4	9
I	503.2	3	598.2	3	340.6	3	424.4	3	3244.7	3	3999.2	3
P	225.7	9	227.6	9	148.1	9	166.2	9	107.6	9	176.7	9
TI	16.1	21	15.9	21	27.8	27	17.7	30	26.1	24	17.7	27
TP	7.9	63	8.5	63	9.0	81	9.2	90	7.4	72	13.9	81
IP	13.2	27	11.0	27	29.0	27	14.8	27	15.9	27	30.8	27
TIP	2.5	189	2.2	189	3.7	243	2.5	270	5.9	216	6.2	243
R	1.2	319	1.0	319	1.3	399	2.0	439	1.5	359	1.3	399

Table XV. *Calculated values for dentine apposition for 10 measuring points taken together. 11-month old normal rats. Figures in brackets denote number of animals.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
—	—	7.1	6.6	14.0	13.5	16.7	16.5	17.3	17.5
14.7	14.8	8.9	8.9	16.3(8)	16.1(8)	18.2	17.8(11)	16.0(9)	16.1
17.9	18.0	8.9(5)	9.2(4)	17.5(8)	17.5(8)	17.7(10)	17.1(11)	12.8(9)	12.8(10)
17.0(10)	17.7(8)	7.1	7.6	17.7	17.9	15.1	14.4	7.7	7.4
11.9	13.7	3.6	4.0	—	—	—	—	—	—

Table XVI. *Standard deviations of values in Table XV.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
0.8	0.9	0.6	0.7	1.0	0.9	0.8	0.7	1.0	1.0

Tables XVII—XVIII. *Analysis of covariance. Young normal rats. Age at beginning of experiment: 30 days.*

Table XVII.

Source of variation	Set 3—4				4—5			
	Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	74.4	10	60.6	8	11.2	14	20.8	11
S	387.9	3	240.7	3	1133.9	4	665.9	4
P	277.7	9	282.0	9	95.1	9	90.0	9
TS	19.8	30	23.7	24	8.8	56	12.0	44
TP	3.6	90	3.2	72	1.3	126	1.6	99
SP	44.7	27	21.1	27	23.7	36	8.3	36
TSP	2.8	270	2.5	216	1.0	504	1.2	396
R	0.6	439	0.7	359	0.4	749	0.5	599

Table XVIII.

Source of variation	Set 5				6			
	Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	3.2	4	5.8	2	56.1	7	22.9	7
I	691.2	3	874.5	3	5140.8	3	2515.5	3
P	110.9	9	66.5	9	75.5	9	180.6	9
TI	36.6	12	17.3	6	45.5	21	23.6	21
TP	4.7	36	2.5	18	5.3	63	6.9	63
IP	28.3	27	15.0	27	54.6	27	26.0	27
TIP	5.5	108	5.7	54	5.6	189	4.7	189
R	1.2	199	1.4	119	1.5	319	1.5	319

Table XIX. *Calculated values for dentine apposition for 10 measuring points taken together. Young normal rats. Age at beginning of experiment: 30 days. Figures in brackets denote number of animals.*

Set 3—4		4—5		5		6	
Right	Left	Right	Left	Right	Left	Right	Left
—	—	7.9	8.0	15.7	15.1	18.6	16.8
15.9	16.2	9.7	9.7	19.7	20.8(3)	20.5	20.5
18.9(11)	19.1(9)	9.8(10)	9.8(10)	19.3(5)	20.2	17.0(8)	18.6(8)
19.3	20.0	8.0	8.3	14.4	13.1	8.1	11.2
17.1	18.9	4.5	5.1	—	—	—	—

Table XX. *Standard deviations for values in Table XIX.*

Set 3—4		4—5		5		6	
Right	Left	Right	Left	Right	Left	Right	Left
0.8	0.9	0.5	0.5	1.3	1.5	1.0	1.0

Table XXI. *Calculated values for dentine apposition at each of the 10 measuring points in set 4—5, right side. 15 young normal rats.*

Measuring point	1	2	3	4	5	6	7	8	9	10
	10.9	7.9	10.5	8.6	6.8	6.4	6.8	7.0	6.0	7.3
	11.3	9.1	11.8	10.7	8.9	8.6	8.4	8.8	8.7	9.9
Set 4—5	10.2	9.0	11.4	10.8	9.3	9.0	8.9	8.9	9.4	10.3
	7.8	7.6	9.2	8.8	8.0	7.7	8.2	7.5	8.0	8.5
	3.9	5.0	5.1	4.9	5.0	4.6	6.2	4.4	4.7	4.5

Table XXII. *Standard deviation for the values in Table XXI.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 4—5	0.4	0.3	0.3	0.4	0.3	0.2	0.3	0.3	0.3	0.3

Table XXIII. *Means of measured values for dentine apposition at point 2 for interval 3 (8 days) in groups of 5 classes. Young normal rats.*

Midpoint of groups of classes	Number of observations (n)	Mean	SD
3	22	14.9	
8	16	16.6	
13	21	17.9	
18	19	18.8	1.0 : $\sqrt{n}$
23	18	18.4	
28	24	17.2	
33	17	15.4	
38	8	13.8	
	Total 145		

Table XXIV. *Means of measured values for dentine apposition at point 2 for interval 4 in groups of 5 classes. Young normal rats.*

Midpoint of groups of classes	Number of observations (n)	Mean	SD
3	17	6.4	
8	20	7.6	
13	9	8.0	
18	18	8.7	0.6 : $\sqrt{n}$
23	13	9.2	
28	15	9.4	
33	18	9.2	
38	23	8.0	
43	25	6.1	
48	29	4.9	
53	8	4.3	1.0 : $\sqrt{n}$
	Total 195		

Table XXV. *Means of measured values at each of the measuring points in the 5 sets, right side only. 5-month old normal animals. Cf. Table V, which gives the corresponding values, calculated with the polynomial.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 3—4	19.3	15.4	18.9	14.8	11.8	12.8	13.0	11.9	9.9	12.9
	21.8	17.9	21.1	18.9	16.1	14.6	16.8	14.1	15.1	18.0
	19.5(18)	18.8(18)	18.7(17)	18.6(17)	16.8(16)	13.4(16)	18.4(12)	13.3(14)	16.7(16)	18.9(17)
	10.7	15.0	12.3	12.9	11.3	8.5	13.2	8.4	10.4	9.5
Set 4—5	9.8	7.5	8.5	6.8	5.4	5.8	5.8	5.2	4.8	6.4
	10.6	8.7	10.6	9.3	7.4	7.9	7.7	7.4	6.9	7.9
	11.2(11)	8.9(12)	11.3(11)	10.2(12)	8.8(12)	7.3(13)	10.2(11)	7.9(11)	9.1(11)	10.8(11)
	7.3	8.7	9.0	8.5	7.3	5.9	8.5	5.8	7.4	7.7
	3.1	5.4	4.5	4.0	3.6	3.1	5.3	3.2	3.1	2.7
Set 3	19.2	14.9	17.1	13.6	11.2	11.7	12.3	12.1	10.3	13.0
	21.3	16.7	20.6	17.3	15.0	15.8	15.8	15.7	12.4	15.2
	22.2(14)	18.0(15)	21.4(14)	19.4(14)	16.7(12)	15.1(11)	17.0(8)	13.7(7)	15.4(11)	18.0(12)
	22.7	18.4	21.9	20.0	17.2	14.9	19.5	14.9	18.5	21.0
Set 4	21.3	17.3	21.4	18.4	15.5	15.7	15.6	15.4	13.4	16.3
	22.5	18.9	21.8	20.0	17.1	15.1	17.3	14.7	16.8	19.1
	20.4(16)	18.8(16)	19.9(16)	19.3(15)	17.1(15)	14.1(15)	18.7(14)	13.9(15)	16.9(15)	19.7(15)
	14.9	17.4	17.9	16.9	14.3	12 1	18.0	11.3	14.7	15.3
Set 5	23.7	18.8	22.1	21.4	18.4	16.1	18.5	16.1	17.5	20.5
	19.9	18.9	20.0	18.8	16.9	14.0	18.3	14.1	16.7	20.0
	12.4(14)	15.6(16)	13.3(14)	13.7(15)	11.9(14)	9.3(15)	14.4(15)	9.5(15)	11.5(15)	11.4(15)
	5.7	11.5	9.3	8.4	7.3	6.7	11.9	6.4	6.7	5.5

Tables XXVI—XXVII. *Analysis of covariance. 4-month old rats. Treatment with growth hormone during latter half of experimental period.*

Table XXVI.

Source of variation	Set 3—4				4—5			
	Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	20.5	6	30.8	4	5.8	4	13.0	2
S	849.1	3	841.4	3	321.0	4	200.7	4
P	228.5	9	117.9	9	26.1	9	15.2	9
TS	36.2	18	42.1	12	6.7	16	19.0	8
TP	2.2	54	2.7	36	1.5	36	2.9	18
SP	30.2	27	26.7	27	9.9	36	5.2	36
TSP	2.7	162	1.8	108	1.2	144	1.2	72
R	0.5	279	0.5	199	0.4	249	0.5	149

Table XXVII.

Source of variation	Set 3				4				5			
	Right		Left		Right		Left		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	0.9	3	26.8	2	15.2	9	25.8	9	53.5	9	147.0	8
I	524.5	3	448.7	3	307.3	3	372.1	3	2719.8	3	2517.8	3
P	176.7	9	118.3	9	230.4	9	261.9	9	171.5	9	160.9	9
TI	4.4	9	8.6	6	67.2	27	86.4	27	102.4	27	70.4	24
TP	2.6	27	4.5	18	5.9	81	7.6	81	6.3	81	7.4	72
IP	13.4	27	8.2	27	33.0	27	29.0	27	41.6	27	29.7	27
TIP	2.7	81	1.3	54	3.7	243	4.2	243	5.3	243	4.5	216
R	0.9	159	0.7	119	1.1	399	1.0	399	1.4	399	1.2	359

Table XXVIII. *Calculated values for dentine apposition for 10 measuring points taken together. 4-month old animals. Treatment with growth hormone during latter half of experimental period. Figures in brackets denote number of animals.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
—	—	7.0	6.4	12.8	12.3	15.6	15.2	18.1	17.8
14.1	13.9	8.9	8.9	15.7(4)	15.2(3)	17.8(10)	17.7(10)	17.5(10)	17.6(9)
18.1(7)	18.4(5)	9.0(5)	9.5(3)	17.7	17.2	18.1	18.2	14.8	15.0
17.9	17.9	7.2	8.1	18.7	18.5	16.7	17.0	9.9	9.8
13.5	12.5	3.5	4.8	—	—	—	—	—	—

Table XXIX. *Standard deviations of values in Table XXVIII.*

Set 3—4		4—5		3		4		5	
Right	Left	Right	Left	Right	Left	Right	Left	Right	Left
1.1	1.3	0.7	1.0	1.5	1.5	0.9	1.0	1.2	1.1

Table XXX. *Calculated values for dentine apposition at each of the 10 points in the sets. Right side. 4-month old animals. Treatment with growth hormone during latter half of experimental period. Figures in brackets denote number of animals.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 3—4	18.5	14.3	18.2	14.7	11.9	12.4	12.2	12.4	10.6	13.1
	22.2	17.4(10)	21.2	19.2(9)	16.5(8)	15.9(7)	16.6(7)	15.6(7)	16.3(7)	18.2(9)
	21.2(10)	18.5	20.2(10)	19.3	17.1	15.2	17.8	15.0	17.1	18.7
	15.5	17.4	15.0	15.1	13.7	10.2	15.9	10.6	13.0	14.5
Set 4—5	9.2	7.2	8.7	7.0	5.8	6.2	6.1	6.2	4.9	6.3
	10.7	8.9	10.6	9.3	8.1	7.9	8.4	8.0	7.7	9.2
	10.2(7)	9.3(7)	10.6(7)	9.6(7)	8.6(7)	7.9(5)	9.0(5)	8.1(5)	8.6(6)	9.7(7)
	7.6	8.5	8.4	7.9	7.1	6.2	8.1	6.4	7.4	8.0
	2.9	6.4	4.3	4.4	3.8	2.9	5.6	2.9	4.1	3.9
Set 3	17.5	13.5	15.9	12.7	9.7	10.3	10.7	11.4	9.2	11.9
	19.6	15.1	19.8(7)	16.1(6)	13.7(5)	14.2(5)	13.9(4)	14.0(4)	12.3(4)	14.4(6)
	21.1(7)	16.9(8)	21.2	18.3	16.1	16.2	16.8	15.5	15.2	17.6
	22.0	18.9	20.1	19.5	16.7	16.1	19.3	15.9	17.7	21.4
Set 4	19.2	15.5	19.9	16.5	14.4	14.8	14.3	15.2	12.0	14.2
	21.6	17.7	20.7	18.9	16.1(10)	15.8	16.7	16.2	15.9	18.0
	20.7(10)	18.9(10)	20.0(10)	18.9(10)	16.5	15.3(10)	18.0(10)	16.1(10)	17.4(10)	19.2(10)
	16.4	19.1	17.8	16.7	15.6	13.3	18.0	14.9	16.7	17.7
Set 5	21.5	18.1	20.5	19.2	17.7	15.5	17.8	15.7	16.3	18.3
	20.4	18.8	18.5	18.2	17.5	14.4	17.5	14.9	16.5	18.5
	15.7(10)	17.1(10)	14.8(10)	15.4(10)	14.7(10)	12.0(10)	16.4(10)	12.5(10)	14.2(10)	15.3(10)
	7.4	13.2	9.6	10.9	9.2	8.1	14.7	8.5	9.5	8.7

Table XXXI. *Standard deviations for the values in Table XXX.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 3—4	0.8	0.4	0.6	0.8	0.7	0.6	0.8	0.5	0.8	0.9
4—5	0.7	0.6	0.5	0.5	0.5	0.4	0.5	0.4	0.4	0.6
3	0.8	0.4	0.6	0.7	1.0	0.8	0.6	0.7	0.7	0.6
4	1.0	0.5	0.5	0.7	0.8	0.7	0.7	0.7	0.8	0.8
5	1.3	0.9	0.9	1.1	1.0	0.8	1.1	1.0	0.9	1.1

Table XXXII. *Means of measured values in different intervals and groups of classes. 4-month old animals. No treatment. Control experiments.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean
3	17	10.1	13	9.9	16	9.9	19	9.7
8	13	10.5	7	11.0	11	10.6	12	11.6
13	13	11.5	11	11.5	15	11.4	17	11.1
18	15	11.7	11	12.1	5	12.6	8	12.4
23	11	12.3	5	13.8	10	13.6	15	13.1
28	11	13.5	13	13.6	15	14.1	10	13.3
33	16	13.8	7	14.3	3	15.0	5	14.0
38	3	12.7	3	15.0	11	14.9	13	14.4
43	13	13.3	13	14.4	9	14.1	10	14.4
48	8	13.4	10	13.9	7	13.6	7	13.4
53	5	13.6	3	13.7	6	13.0	10	13.3
58	—	—	12	12.9	13	13.3	14	13.5
63	2	13.0	6	13.3	10	13.1	8	10.9
68	1	11.0	—	—	10	10.9	8	10.9
73	—	—	1	9.0	9	9.3	11	9.4
78	—	—	2	8.5	2	6.5	3	6.7
83	—	—	—	—	1	12.0	4	8.2
88	—	—	—	—	1	5.0	—	—
Total	128	—	117	—	154	—	174	—

Table XXXIII. *Means of measured values in different intervals and groups of classes. 4-month old animals. Treatment with growth hormone during latter half of experimental period. Control experiments.*

Midpoint of groups of classes	Interval	1		2		3		4	
		No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean
3		15	9.6	15	9.9	12	10.0	8	10.2
8		12	10.7	13	10.9	13	11.5	19	10.9
13		13	11.1	9	11.3	10	11.6	9	11.6
18		12	12.0	13	11.9	7	12.7	11	11.6
23		8	12.9	9	12.8	13	13.3	14	12.6
28		15	12.9	9	13.4	8	14.4	7	13.7
33		9	14.0	8	14.1	7	15.0	11	14.4
38		13	13.5	12	14.0	6	14.5	10	14.3
43		11	14.0	9	13.6	9	14.4	7	14.1
48		7	13.3	8	13.6	8	14.8	8	14.2
53		8	13.5	13	13.7	11	14.1	7	14.0
58		2	11.5	10	12.6	6	13.5	10	13.9
63		—	—	8	13.0	10	12.8	6	12.7
68		—	—	7	11.0	10	12.3	17	11.2
73		—	—	1	11.0	9	10.3	6	9.2
78		—	—	—	—	1	9.0	10	7.6
83		—	—	—	—	1	7.0	3	7.0
88		—	—	—	—	—	—	1	7.0
	Total	125	—	144	—	141	—	164	—

Table XXXIV. *Means of measured values in different intervals and groups of classes. 4-month old animals. Treatment with solvent for growth hormone during latter half of experimental period. Control experiments.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean
3	12	9.7	15	9.7	19	9.9	11	9.7
8	7	10.4	8	10.9	15	11.2	20	11.0
13	15	11.3	13	11.8	19	11.3	16	11.1
18	10	12.0	11	12.1	8	12.6	11	12.3
23	5	13.2	10	12.6	15	13.2	21	12.8
28	14	13.7	14	13.9	16	13.9	9	12.9
33	8	14.1	6	14.0	5	14.0	11	14.4
38	6	13.8	11	14.1	15	13.9	10	14.7
43	8	13.7	13	14.4	14	14.0	14	14.4
48	5	13.4	10	13.2	5	13.6	9	14.1
53	3	14.7	9	13.8	16	14.3	12	13.8
58	2	13.0	7	14.0	15	13.4	13	13.2
63	1	12.0	5	13.2	13	12.6	9	11.3
68	1	12.0	5	12.8	11	10.4	16	12.1
73	1	7.0	2	10.5	6	11.3	8	10.7
78	—	—	—	—	7	10.3	5	8.2
83	—	—	2	11.0	3	7.7	4	5.7
88	—	—	—	—	—	—	1	8.0
Total	98	—	141	—	202	—	200	—

Table XXXV. *Analysis of covariance. Young animals. Treatment with growth hormone during latter half of experimental period.*

Source of variation	Set 4—5 Right m.sq.	d.f.	Source of variation	Set 6 Right m.sq.	d.f.
T	16.4	22	T	32.9	14
S	1370.5	4	I	4203.1	3
P	146.3	9	P	256.1	9
TS	13.7	88	TI	55.5	42
TP	1.5	198	TP	5.6	126
SP	16.8	36	IP	66.3	27
TSP	1.2	792	TIP	4.0	378
R	0.5	1149	R	1.4	599

Table XXXVI. *Calculated values for dentine apposition for 10 measuring points taken together. Young animals. Treatment with growth hormone during latter half of experimental period.*

	Set 4—5 Right	6 Right
Section or interval	—	16.8
	8.3	20.5(12)
	10.1	18.8
	10.2(10)	11.7
	8.5	—
	5.2	—

Table XXXVII. *Calculated values for dentine apposition at each of the 10 measuring points, set 4—5, right side. 23 young animals. Treatment with growth hormone during latter half of experimental period.*

Measuring point	1	2	3	4	5	6	7	8	9	10
Set 4—5 right side	10.4	8.6	10.2	8.9	7.4	7.4	7.2	7.4	6.8	8.0
	11.9	9.5	11.9	10.9	9.4	9.1	9.1	9.3	9.3	10.2
	11.6	9.4	11.8	11.2	9.9	9.3	9.6	9.5	10.1	10.6
	9.4	8.1	9.9	9.7	8.7	8.1	8.6	8.1	9.0	9.1
	5.5	5.8	6.3	6.4	6.1	5.5	6.1	4.9	6.2	5.9

Table XXXVIII. *Standard deviations of values in Table XXXVI.*

Set 4—5 Right	6 Right
0.5	0.9

Table XXXIX. *Standard deviations for values in Table XXXVII.*

Measuring point	1	2	3	4	5	6	7	8	9	10
	0.4	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3	0.3

Table XL. *Means and numbers of measured values in different intervals and groups of classes. Young animals. No treatment.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean
3	27	15.6	18	7.2	22	14.9	17	6.4
8	17	17.8	17	8.3	16	16.6	20	7.5
13	1	17.0	13	8.8	21	17.9	9	8.0
18	—	—	21	9.5	19	18.8	18	8.7
23	—	—	12	9.9	18	18.4	13	9.2
28	—	—	9	9.9	24	17.2	15	9.4
33	—	—	—	—	17	15.4	18	9.2
38	—	—	—	—	8	13.8	23	8.0
43	—	—	—	—	—	—	25	6.1
48	—	—	—	—	—	—	29	4.9
53	—	—	—	—	—	—	9	4.3
58	—	—	—	—	—	—	1	3.0
Totol	45	—	90	—	145	—	197	—

Table XLI. *Means and numbers of measured values in different intervals and groups of classes. Young animals. Treatment with growth hormone during latter half of experimental period.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean
3	53	15.5	36	7.2	39	15.4	32	7.2
8	12	17.4	40	8.3	33	17.4	21	8.1
13	1	19.0	41	9.2	32	19.2	29	8.7
18	—	—	34	9.7	37	19.4	25	9.5
23	—	—	25	10.0	33	19.8	28	9.9
28	—	—	4	9.7	30	18.2	21	9.7
33	—	—	1	10.0	28	17.0	35	9.7
38	—	—	—	—	5	15.6	27	8.9
43	—	—	—	—	1	14.0	42	6.9
48	—	—	—	—	—	—	45	5.7
53	—	—	—	—	—	—	11	4.4
58	—	—	—	—	—	—	—	—
Total	66	—	181	—	238	—	316	—

Table XLII. *Means and numbers of measured values in different intervals and groups of classes. Young animals. Treatment with solvent for growth hormone during latter half of experimental period.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean	No. of observations	Mean
3	24	15.8	18	6.6	16	14.6	16	7.2
8	15	18.5	20	8.0	22	16.6	6	7.5
13	—	—	14	8.9	17	18.2	13	8.0
18	—	—	15	9.8	18	18.9	11	8.7
23	—	—	12	10.4	12	19.7	15	9.6
28	—	—	10	10.6	18	18.8	13	9.4
33	—	—	—	—	12	17.2	12	9.4
38	—	—	—	—	8	15.2	17	9.0
43	—	—	—	—	1	17.0	16	6.7
48	—	—	—	—	—	—	18	6.1
53	—	—	—	—	—	—	5	5.2
58	—	—	—	—	—	—	2	4.5
Total	39	—	89	—	124	—	144	—

Table XLIII. *Analysis of covariance. 4-month old animals. Treatment with cortisol during latter half of experimental period.*

Source of variation	Set 4		5			
	Right		Right		Left	
	m.sq.	d.f.	m.sq.	d.f.	m.sq.	d.f.
T	32.1	3	151.4	5	22.2	3
I	250.9	3	1255.9	3	1036.2	3
P	34.3	9	39.8	9	34.5	9
TI	97.8	9	102.6	15	57.8	9
TP	11.7	27	10.1	45	9.2	27
IP	29.1	27	31.3	27	21.9	27
TIP	8.1	81	8.0	135	5.9	81
R	1.2	159	1.9	239	1.7	159

Table XLIV. *Calculated values for dentine apposition for 10 measuring points taken together. 4-month old animals. Treatment with cortisol during latter half of experimental period.*

	Right	Left
Set 4	14.4 16.5 15.9 (4) 12.6	
Set 5	16.4 16.7 14.5 (6) 9.7	17.3 17.5 15.1 (4) 10.2

Table XLV. *Standard deviations of values in Table XLIV.*

Set 4	5	
Right	Right	Left
1.6	1.5	1.4

Table XLVI. *Calculated values for dentine apposition. Measuring point 2, right side. 4-month old animals. Treatment with cortisol during latter half of experimental period. Figures in brackets denote number of animals.*

Set 3—4	3	4	5
12.8 15.8 16.3 (7) 14.4	12.0 14.6 15.1 (6) 13.6	15.1 17.4 16.4 (8) 12.2	17.5 17.5 15.9 (11) 12.5

Table XLVII. *Standard deviations of values in Table XLVI.*

Set	3—4	3	4	5
	0.7	0.7	0.8	0.7

Table XLVIII. *Means and numbers of measured values in different intervals and groups of classes. 4-month old animals. Treatment with cortisol during latter half of experimental period. Control experiments.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean
3	13	9.8	17	10.0	18	10.3	18	10.1
8	18	10.4	14	10.8	7	10.4	9	10.6
13	12	10.6	6	11.5	12	10.7	14	10.4
18	17	11.7	16	12.0	11	11.2	10	10.4
23	12	12.4	11	12.2	8	12.5	6	8.7
28	15	12.8	10	13.1	9	12.3	8	8.7
33	15	13.8	13	13.9	9	13.1	7	10.0
38	6	13.7	11	13.6	9	13.1	6	9.3
43	7	13.4	10	13.5	7	12.6	3	7.3
48	2	14.0	12	13.2	14	11.9	9	9.9
53	—	—	5	12.2	9	10.6	5	8.6
58	—	—	6	13.0	5	11.0	8	8.1
63	—	—	1	13.0	11	10.6	7	7.6
68	—	—	—	—	2	9.5	3	6.3
73	—	—	—	—	2	9.0	2	6.5
78	—	—	—	—	—	—	2	5.5
83	—	—	—	—	—	—	—	—
88	—	—	—	—	—	—	—	—
Total	117	—	132	—	133	—	117	—

Table IL. *Means and numbers of measured values in different intervals and groups of classes. Treatment with suspension medium for cortisol during latter half of experimental period. Control experiments.*

Midpoint of groups of classes	Interval 1		2		3		4	
	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean	No. of observations (n)	Mean
3	16	10.1	11	10.0	5	9.6	4	9.2
8	10	11.0	9	11.1	10	10.8	14	10.9
13	12	11.1	14	11.5	11	11.4	6	11.2
18	12	11.9	10	12.6	8	12.0	9	12.1
23	11	12.9	8	12.6	10	13.2	13	12.5
28	11	13.7	9	13.3	9	13.1	6	13.2
33	9	13.7	7	14.1	10	13.9	7	13.7
38	11	13.9	10	13.9	6	14.2	9	14.2
43	7	13.9	6	13.8	9	13.4	7	14.0
48	8	13.6	6	13.2	8	13.4	9	13.6
53	2	14.0	10	13.0	8	12.9	7	12.7
58	2	12.5	5	11.8	9	12.8	7	12.9
63	1	12.0	4	12.7	6	10.7	10	12.2
68	—	—	3	11.3	9	10.7	5	11.6
73	—	—	—	—	2	11.0	14	8.6
78	—	—	1	9.0	3	10.7	5	6.6
83	—	—	—	—	1	9.0	4	8.0
88	—	—	—	—	—	—	2	7.0
Total	112	—	113	—	124	—	138	—