AN INVESTIGATION OF THE LAMELLAE IN HUMAN ACELLULAR CEMENTUM AS A POSSIBLE MEANS OF DETERMINING BIOLOGICAL AGE

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by

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ABSTRACT

In many species of non-human mammals, the number of growth lines in the dentin and cementum reliably reflects the animal's biological age. In humans, only cementum continues to be formed throughout life and usually does not undergo extensive resorption. It also has a laminated appearance. Therefore, it is possible that the growth lines in human cementum may reflect a person's biological age.

The sample investigated includes 25 teeth removed for pathological reasons, 2 teeth removed from one cadaver and 2 teeth from an ancient burial site. The teeth, embedded in Bioplastic, were sectioned at mid-root; then ground and polished, and etched in a solution of detergent and water (0.6 cc Ivory liquid detergent to 250 cc tap water) for 8-24 hours. The prepared specimens were then observed in reflected light and interference contrast. Composite photographs were made of the entire cementum thickness at one location on the tooth's circumference. Lamellae seen in each composite photograph were plotted on a graph where one axis represents the number of visible lamellae and the other axis represents the cumulative thickness of the lamellae.

Observations (x63 objective) of the etched surface revealed the presence of thin (1/3 - 3 microns) lamellae in all samples (excepting the two archeological specimens). There was no noticeable difference between the size and appearance of lamellae in pathological and cadaver teeth. Lamellae formed during the first 20 years of cementum deposition are generally more clearly defined than those formed later in life. Older teeth usually have large gaps in the sequence of lamellae seen in the outer half of the cementum thickness. No simple

one to one relationship was found between the number of lamellae present and the number of years of cementum deposition. However, teeth with 20 years cementum deposition or less have approximately 4 times as many lamellae as years of deposition.

Cementum thickness data obtained in this study was compared with that provided by Zander and Hürzeller (1958). A simple straight-line relationship does not seem to describe adequately the relationship between cementum deposition and age. For the sample examined here, cementum thickness seems to increase at a fairly constant and even rate during the first 20 years of deposition and then level off.

Teeth from the same mouth were compared for similarities in their acellular cemental lamellae patterns. None were found. Local conditions seem to have a more direct influence on the cementum than any biological rhythm which might be present.

Cementum from young female teeth was compared with that from young male teeth. No differences were found in the size and appearance of the lamellae. However, female teeth showed visible lamellae throughout the entire cementum thickness more often. Male teeth usually showed noticeable discontinuities in the sequence of visible lamellae throughout the cementum thickness.

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Dr. M. Freeman introduced me to the literature on aging non-human mammals by counting the number of growth lines in the teeth.

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INTRODUCTION

Biologists since 1950 have been using growth lines in bone and in the cementum and dentin of non-human mammal teeth as a means of determining an animal's biological age. In many species of mammals, the number of growth lines in cementum reliably reflects the biological ages of known-age specimens. Although the technique has been most widely used with mammals living in temperate and arctic climates, there is some evidence that annual growth lines are also present in the teeth of subtropical mammals (Adams and Watkins, 1967; Kenyon and Fiscus, 1963).

In humans, only cementum continues to be deposited throughout life and usually does not undergo extensive remodelling. The laminated structure of human cementum has long been known to dental researchers. It is evident, from microradiographic research (Provenza, 1964; Röckert, 1956; Soni et al., 1962) that these laminations reflect variations in the degree of calcification of cementum. They also reflect variations in collagen-fibre content (Furseth and Johansen, 1968; Scott and Symons, 1972).

Since so many other mammals can be aged by the number of growth lines in the cementum and since human cementum possesses a similarly laminated structure which increases in thickness throughout life (Zander and Hürzeller, 1958), there is a distinct possibility that a correlation exists between the number of growth lines in a person's cementum and that person's biological age. Nowhere in the literature has an attempt been made to determine if such a correlation exists. This is an investigation of just such a possibility.

Using etched cross-sections we will observe the fine structure of human cementum, search for correlations between the number of growth lines seen and the number of years of cementum deposition. Comparisons will be made between the cementum thickness data obtained and Zander and Hürzeler's (1958) paper.

Growth lines seen in male and female cementum will be compared and the cementum on teeth from the same mouth will be examined for similarities.

CHAPTER I

GROWTH LINES IN THE CEMENTUM OF NON-HUMAN MAMMALS

The possibility of determining the age of non-human mammals by the presence of growth lines in their cementum, dentin and bone has interested biologists for some time. Table 1 summarizes studies containing information on growth lines in non-human mammal cementum. It indicates whether the specimens were of known age, unknown age, or had been aged by other biological means, and indicates either that annual layering was present or that the study was inconclusive.

Information on 31 species representing 15 families indicates that the deposition of cementum results in a pattern of annual layers in 17 species (10 families). The presence of annual layers in the remaining 14 species could not be verified because of the unavailability of known-age specimens.

Table 2 summarizes each research paper separately providing more information on the specimens and the techniques used and includes a précis of the findings.

There is some variety in the way cementum grows amongst species studied. For instance, cementum on certain teeth of some animals is deposited mainly at the apex of the root (e.g. Beaver molars) while in others, deposition occurs more evenly over the entire root (e.g. Black bear canine). In some species (e.g. Red notcules, Grizzly bears, Grey seal, Grey rat and White-tailed deer) narrower cemental layers are deposited with increasing age while in other species (e.g. Large mouse-eared bat, Hawaiian monk seal and Beaver) the layers deposited remain constant throughout life. Yet in all species studied, the cementum was arranged in layers.

Table 1. List of Species Studied

Order	Family	Species	Aged	Known age	Unknown age	Annual layers.
INSECTIVORA	Soricidae	Common shrew (Sorex araneous)	x			?
CHIROPTERA	Vespertilionidae	Red notcules (Nyctalus noctala)		x		yes
		Large mouse-eared bat (Myotis myotis)	x			?
CARNIVORA	Ursidae	Black bear (Ursus americanus)		x	x	yes
		Grizzly bear (Ursus horribilis)		x	x	yes
	Procyonidae	Raccoons (Procyon lotor)		x		yes
	Canidae	Coyotes (Canis latrans)		x		yes
		Arctic fox (Alopex lagopus)		x		yes
	Mustelidae	Sea otter (Enhydra lutris)	?	?	?	?
		Sable (Martes zibellina)	x			yes
		American mink (Mustela vison)	?	?	?	?
PINNIPEDIA	Phocidae	Crabeater seal (Lobodon carcinophagus)			x	?
		Ringed seal (Phoca hispida)			x	?
		Grey seal (Halichoerus grypus)		x		yes
		Harbour seal (Phoca vitulina)		x		yes
		Hawaiian monk seal (Monachus schauinslandi)			x	?
RODENTIA	Sciuridae	California ground squirrel (Spermophilius beecheyi)		x		yes
	Castoridae	Beaver (Castor canadensis)		x		yes
	Muridae	Field mouse (Apodemus agrarius)		x	x	yes
		Grey rat (Rattus norvegicus)	x			?
		Common hamster (Cricetus cricetus)	x	x		yes
		Muskrat (Ondatra zibethicus)	x			?

Table 1 cont'd.

Order	Family	Species	Aged	Known age	Unknown age	Annual layers.
ARTIODACTYLA	Cervidae	Moose (*Alces alces*)	x	x		yes
		Scottish red deer (*Cervus elephas*)		x	x	yes
		White-tailed deer (*Odocoileus virginianus*)		x	x	yes
		Barren-ground caribou (*Rangifer tarandus*)	x	x	x	yes
	Bovidae	Bison (*Bison bison*)			x	?
CETACEA	Physeteridae	Sperm whale (*Physeter catodon*)			x	?
	Monodontidae	Beluga (*Delphinapterus leucas*)			x	?
	Delphinidae	Common dolphin (*Delphinus delphis*)			x	?
		Pilot whale (*Globicephala melaena*)			x	?

Key:

Aged - specimen's age determined by body weight, body length, tooth eruption, tooth wear, zygomatic breadth, development of sex organs, etc., or some combination of these features.

Known age - Specimen's biological age known exactly.

Unknown age - determination of specimen's age was not possible.

In decalcified stained specimens each layer consisted of one wide light-staining and one thin dark-staining band. On undecalcified polished specimens the layer was comprised of one wide low-reflecting (translucent) and one thin highly-reflecting (opaque) band. Subdivisions were usually seen within the wider light-staining band which were less darkly stained that the thin dark-staining band. These subdivisions are sometimes referred to as rut lines. See Table 2 for complete descriptions of the appearance of the layers for each species and also the magnifications at which they were observed.

In studies where known-age specimens were available, the annual deposition of cementum seemed to be one wide light-staining band and one thin dark-staining band. In the case of grizzly bears, this is most certainly the case, since teeth removed from the same bears on successive years showed one additional cemental layer (i.e. one light- and one dark-staining band) each year (Craighead et al., 1970).

Some researchers thought that variations in the calcification of cementum were the direct result of metabolic variations during an animals annual cycle. For instance, the period of fasting and moult in seals was thought to correlate with the formation of the translucent band in the cementum (Mansfield and Fisher, 1960). On the other hand, no direct correlation was found between such a marked metabolic disturbance as hibernation in bears and the deposition of a distinct cemental band (Stoneberg and Jonkel, 1966). It is now thought by some that deposition of the different cemental bands is controlled by hormonal or endocrinal activity (Craighead et al., 1970).

The proposal that deposition of cemental bands is not directly subject to simple external environmental controls receives support from research on the Hawaiian monk seal and the California ground

Table 2. Summary of Papers on Growth Lines in

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Order - INSECTIVORA Family - Soricidae				
Klevezal & Kleinenberg (1969)	Common shrew (Sorex araneous)	40 individuals (aged by weight sexual devel., & tooth wear) Cementum of all teeth.	Decalcified sections, hematoxylin stained.	Transmitted light, various magn. from x60 to x100.
Order - CHIROPTERA Family - Vespertilionidae				
Klevezal & Kleinenberg (1969)	Red notcules (Nyctalus noctula)	11 individuals (known age), canine cementum.	Decalcified transverse sections, stained with hematoxylin.	Transmitted light.
Klevezal & Kleinenberg (1969)	Large mouse-eared bat (Myotis myotis)	? (aged by weight & tooth wear); cementum of unidentified teeth.	?	?
Order - CARNIVORA Family - Ursidae				
Marks & Erickson (1966)	Black bear (Ursus americanus)	54 (known age), upper canine cementum.	Decalcified sections, variously stained.	Transmitted & reflected light, dissection microscope.
Stoneberg & Jonkel (1966)	Black bear (Ursus americanus)	3 (known age), canine cementum.	Decalcified sections (10μ) hematoxylin & eosin stained.	Transmitted light, magnif. x?
Mundy & Fuller (1964)	Grizzly bear (Ursus horribilis)	29 (unknown age), cementum of lower 3rd molar.	Ground thin sections mounted in Canada balsam.	Reflected light, magn. x40.
Craighead, Craighead & McCutcheon (1970)	Grizzly bear (Ursus horribilis)	8 (known age) 4 (unknown age), cementum of 4th premolar,	eosin stained. Decalcified thin sections hematoxylin &	Transmitted light, magn. x?

Non-human Mammal Cementum.

Findings

Common shrew probably lives for no more than 2 yrs. Cheek teeth of over-wintered animals have two broad, light-staining layers of cementum separated by thin, dark-staining layers at the apex of the root. (No information on cervical cementum.) Incisor cementum does not show this layering because "deposition of cementum here evidently only begins during the second year of life of the individual".

Clearly visible layers present which are "evidently annual". After the second year, cementum layers become more narrow and counting is very difficult.

Layers exist in the cementum which are "evidently annual". Width of layers in this species does not diminish with age and even increase somewhat in thickness. Identical number of layers were found in dentin and cementum of the same individual.

Review of age determination methods. Upper canine cementum layer criteria found most accurate. Whelping occurs in Feb. and permanent canines begin erupting in Sept. First layer of cementum is deposited in second year of life. Annual sequence of deposition of one translucent and one opaque cemental zone after this. Technique proved reliable for 1-10 year old animals.

Cementum showed wide, light-staining bands alternating with narrow, dark-staining bands. One wide and one narrow band represented one year's growth. Summer-killed specimens have wide light band on the outside and fall killed specimens have narrow dark band on the outside. When using canines, one year must be added to number of annual layers present since canine eruption doesn't begin untill 9th month of life. Formation of these layers does not seem to be related to metabolism.

Unstained cementum in reflected light consisted of wide bands of light (translucent) cementum alternating with thin, dark (opaque) bands. Spring-killed bears had light bands outermost and fall-shot bears had dark bands outermost. Yearly layer probably consists of one light and one dark layer. Number of layers present in cementum had positive correlation with zygomatic width of skull. Layers become narrower with age.

Permanent 4th molar erupts bewteen 5 & 6 months of life and cementum deposition begins. Dark-staining layer of cementum deposited between Sept. & May. Light-staining cemental layer deposited between June & Sept. Annual increment of one light- & one dark-staining cemental layer per yr. is readily detected in grizzlies of ½ - 6½ yrs. old, & with less (cont'd)

Table 2

References	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Craighead et al. continued.				
		Family - Procyonidae		
Grau, Sanderson & Rogers (1970)	Racoons (*Procyon lotor*)	54 (known age), incisor cementum.	Decalcified, stained sections.	Transmitted light.
		Family - Canidae and Mustelidae		
Linhart & Knowlton (1967)	Coyotes (*Canis latrans*)	30 (known age), canine cementum.	Decalcified sections 16μ thick stained with Paragon.	Transmitted light, magn. x40 & x100.
Klevezal & Kleinenberg (1969)	Arctic fox (*Alopex lagopus*)	30 (known age), canine cementum.	Decalcified sections, hematoxylin stained.	Transmitted light, magn. x60.
	Sea otter (*Enhydra lutris*)	?	Decalcified sections, hematoxylin stained.	Transmitted light, Magn. x?
	Sable (*Martes zibellina*)	35 (known age), canine cementum.	Decalcified sections, hematoxylin stained.	Transmitted light, "high magnification".
	American mink (*Mustela vison*)	?	?	?
Order - PINNIPEDIA		**Family - Phocidae**		
Laws (1958)	Crabeater seal (*Lobodon carcinophagus*)	76 (unknown age?), canine cementum.	Polished transverse sections.	Transmitted light, magn. x?
McLaren (1958)	Ringed seal (*Phoca hispida*)	750 (unknown age), canine cementum.	Ground thin sections.	Reflected & transmitted light, magn. x?

continued.

Findings

certainty in animals 9½ to 10½ years old. No direct correlation found between deposition of cementum and nutrition or hibernation. Cause is probably hormonal or endocrinal.

Comparison of different methods of aging racoons indicates that counting incisor cemental layers is accurate for aging individuals from 1-4 years of age but tends to underestimate the age of animals more than 4 years old. Suture closure and tooth wear are better criterion for aging animals older than 4 years. First dark-staining layer of cementum begins to be deposited in Feb. One light- and one dark-staining cemental layer deposited each year thereafter. Dark-staining layers are narrower.

Permanent lower canines erupt at 4-5 months and root-tip opening closes at 8-9 months. First dark-staining cemental layer forms between 18-23 months of life. One dark- and one light-staining layer form each year afterwards. 1st transparent layer covers the root at 18 mons.

In all specimens "the number of layers in the cementum coincided precisely with the ages of the individuals in years".

Found annual layers in cementum.

Found annual layers in cementum.

Found annual layers in cementum.

"Distinct layers seen by transmitted light in thin sections of the cementum show a similar general pattern to the dentinal layers and also provide confirmation of the age determination based on the dentine layers".

"Dense cementum bands show a one to one correlation with dense dentine bands in younger seals and do not decrease with in thickness with age".

Table 2

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Hewer (1960)	Grey seal (Halichoerus grypus)	4 (known age), canine cementum.	Longitudinal section.	Transmitted light, magn. x?
Hewer (1964)	Grey seal (Halichoerus grypus)	295 (unknown age), canine cementum.	Ground longitudinal thin sections.	Transmitted light, magn. x30.
Mansfield & Fisher (1960)	Harbour seal (Phoca vitulina)	1 female (known age), upper right canine cementum.	Ground thin cross-section.	?
Kenyon & Fiscus (1963)	Hawaiian monk seal (Monachus schauinslandi)	1 male & 1 female (unknown age), upper right canine cementum.	Polished longitudinal sections.	Reflected light.

Order - RODENTIA Family - Sciuridae

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Adams & Watkins (1967)	California ground squirrel (Spermophilus beecheyi)	22 (known age), 3rd lower molar cementum.	Decalcified sections.	Transmitted light, magn. x128.

continued.

Findings

One 26 year old female showed 25-26 cemental rings. One 6 mon. old animal showed 6 clear and complete rings. One 6½ month old animal showed part of the first annual ring. One 7½ months old animal showed part of the first annual ring. Other younger specimens showed that cementum is first deposited 3-4 months after birth in 2 uneven bands that later coalesce. Main difficulty with using cemental bands to estimate age is that the earlier ones, i.e. before sexual maturity, are broad without sharply defined edges. The lines which define these annual deposits are probably deposited during the moult when fasting occurs. Therefore, visible rings will give you age in terms of number of moults not number of years lived. It is necessary to have information about length of time between birth and first moult before you can know age in years.

Cementum first appears at 3-4 months. Very active deposition of cementum is characteristic of the second part of the first year of canine tooth growth. Cementum has prevented the dentin from increasing the length of the tooth by the end of the 1st year. Cementum begins to be laid down on the apical end of the root, approximately from the neonatal line to the apex. Gradually, cementum is deposited further up the root and eventually covers the entire root surface. Cementum continues to be deposited throughout life but in thinner and thinner layers (esp. after sexual maturity). There is considerable individual variation in thickness of the layers. Each layer which consists of one wide dark and one narrow light band, most probably represents one year of cementum deposition - tested and found accurate on a few known-age specimens.

Tooth revealed well-defined layers made up of a wide band of opaque cementum and a narrow band of translucent cementum. Translucent cementum is deposited in spring and early summer and may represent a period of minimal feeding during the breeding and moult. Betweem 18 & 20 layers were counted in the cementum of this 19½ year old specimen.

Non-migratory seal living under more uniform conditions than most pinnipeds. Annual variation in day length less than for most species. Annual temp. variation from 66°F. in winter to 78°F. in summer. Both animals were adults. No external annuli were present on canine roots, but the thick cementum showed layering. Layers do not decrease in thickness with age - same as in ringed seal. Pulp cavity fills early, probably at about 4-5 years but cementum continues to be deposited. Counting the dense bands in the cementum indicated an age of 20 yrs for male & 11 yrs. for the female. Tooth wear information tended to support these ages.

Dark-staining cemental layer deposited in winter and light-staining cemental layer deposited in summer. Method only reliable for the first 4 years of life. Later layers are very irregular and difficult to interpret.

Table 2

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
		Family - Castoridae		
Van Nostrand & Stephenson (1964)	Beaver (Castor canadensis)	42 (known age), lower molar cementum.	Polished longitudinal sections.	?
Larson & Van Nostrand (1968)	Beaver (Castor canadensis)	? molar cementum.	?	?
Klevezal & Kleinenberg (1969)	Beaver (Castor fiber)	? cementum of cheek teeth	Polished sections.	Reflected light, magn. x18 & x13.
		Family - Muridae		
Klevezal & Kleinenberg (1969)	Fieldmouse (Apodemus agrarius)	80 (aged by tooth wear) & 3 (known age), molar cementum.	Decalcified thin sections, hematoxylin stained.	Transmitted light, magn. x60.
	Grey rat (Rattus norvegicus)	63 (aged by tooth wear), molar cementum.	Decalcified sections, hematoxylin stained.	Transmitted light, magn. x?
	Common hamster (Cricetus cricetus)	10 (known age) and 13 (aged by tooth wear & body dimensions), molar cementum.	Decalcified thin sections, stained by hematoxylin.	Transmitted light, magn. x60.
	Muskrat (Ondatra zibethicus)	20 (aged by tooth development), molar cementum.	Decalcified thin sections, hematoxylin stained.	Transmitted light, magn. x60.
Order- ARTIODACTYLA		Family - Cervidae		
Sergeant & Pimlott (1959)	Moose (from Nfld.) & (from B.C.) (Alces alces)	64 (aged by tooth eruption & wear) 1 (known age) 1st lower incisor cementum.	Polished thin sections	Reflected & transmitted light, magn. x10 & x40.

continued.

Findings

Authors assume that animals born in May-June. First incremental cemental layer deposited at 2½-3 years of age - i.e. between April and Nov. Broad summer layer and usually a narrower lighter layer deposited in winter makes up one year's deposition.

Aging techniques are evaluated and the combined techniques of tooth eruption and annuli are deemed most accurate. Other techniques are accurate only in separating the 0-1 year old animals from those more than 1 year old.

Annual layers found in cementum. These become especially clear after the 4th year and their thickness does not diminish with age. Additional small streaks within the annual layers can be seen at higher magnifications.

Annual layers found in cementum. That is, overwintered animals had 2 thick light-staining layers of cementum separated by one narrow dark-staining band of cementum thought to represent cementum deposition during 2 summers and 1 winter respectively. Animals which are thought to be in their first summer showed no dark-staining band in the cementum. "Occasionally, besides the main (dark-staining) band , supplementary streaks are seen which are not so brightly stained".

Layers seen in cementum. Number of narrow darkly-staining bands in the cementum coincided with the number of "adhesion lines" in the angular extension of the lower jaw. After the third layer, the layers in the cementum become very narrow and hard to distinguish.

Cementum forms in layers. The number of layers present plus one year gives the age of the animal. "It is possible that in hamsters, no cementum forms on the roots of the teeth during the first year of life and that the deposition of cementum begins during the second year." No 1 yr. old specimens were available to test this hypothesis, (Perhaps the molars don't erupt right away.)

Cementum very thin even in old animals. Layers are narrow and difficult to distinguish. Number of layers in cementum is one less than the animals age - as with hamsters.

Cementum showed regular alternations of opaque & translucent growth zones formed by periodic concentrations of the cell bodies of cementoblasts. A full annual layer probably consists of 1 opaque & 1 translucent layer. The known-age 3½ yr. old moose showed 3½ annual layers. Opaque cementum is probably deposited in the summer and fall & translucent cementum in winter and spring. Ages determined for younger animals tended to agree with age arrived at by other methods but ages of older animals were consistently higher using the new method.

Table 2

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Mitchell (1963)	Scottish red deer (Cervus elephas)	2 (known age) & ?(unknown age), 1st lower molar cementum.	Polished sections	Reflected light, magn. x20.
Gilbert (1966)	White-tailed deer (Odocoileus virginianus)	10 (known age), 1st incisor cementum.	Decalcified sections, hematoxylin stained.	Transmitted light, magn. x100 to x400.
Ransom (1966)	White-tailed deer (Odocoileus virginianus)	16 (known age), 1st lower molar cementum.	Polished cross-sections	Reflected light & dissecting microscope.
Gilbert & Stolt (1970)	Maine white-tailed deer (Odocoileus virginianus)	682 (unknown age), incisor cementum.	Decalcified sections, hematoxylin stained.	Transmitted light, magn. x?
McEwan (1963)	Barren-ground caribou (Rangifer tarandus)	100 (approx.? known age), 1st incisor cementum.	Decalcified 7μ thick sections, hematoxylin stained.	Transmitted light.
Reimers & Nordby (1968)	Norwegian reindeer (Rangifer tarandus)	37 (known age) &1064 (unknown age, 1st incisor cementum.	Decalcified sections (30μ) stained with Mayer's acid haemalum.	Transmitted light.

Family - Bovidae

Reference	Animal	No. of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Novakowski (1965)	Bison (Bison bison)	97 (unknown age), lower 4th pre-molar cementum.	Polished & etched sections.	?

continued.

Findings

Cementum has alternating opaque and translucent layers. These layers are most easily seen and counted in the thick cementum pads between the roots of molariform teeth. Opaque layers are composed of aggregations of cementoblast cells while translucent layers contain fewer cells and more fibrous material. First molars are functional at 4-6 months. First opaque layer is deposited at 2 years, therefore age is determined by number of opaque cemental layers plus 1 year. One 2 year, 7month stag and one 17 year, 4 month stag confirmed these findings.

Cementum is composed of alternating dark- and light-staining layers. Cementum deposition begins before eruption and first layer is a light-staining one. Therefore, counting dark-staining layers will give you the animals age in years, one year's deposition consisting of a light-plus a dark-staining layer. Technique tested on 10 known-age specimens and confirmed.

First light cemental layer deposited in the 1st summer and first dark layer deposited in the first winter of life. Deposition of alternate light and dark layers continues throughout life but older animals have thinner layers.

Results of aging the same deer in the field by tooth-wear, and in the laboratory by cemental annuli are compared. Tooth-wear determinations tended to overestimate the number of deer in the 5½ year and up age class (by tooth annuli) and underestimate the number of deer in the less than 5½year age class (by tooth annuli). True ages were unknown.

Cementum is acellular and consists of alternating dark- and light-staining zones. Dark-staining zones are thought to be rest lines. Deposition of cementum on incisor begins around the time of eruption, i.e. 8 months. First dark-staining zone is deposited 2nd winter after birth. Growth lines after the 7th rest line become irregular with larger light-staining zones, and rest lines vary in thickness and shape.

One broad light- and one thin dark-staining layer of cementum is deposited each year. First dark line or "juvenile rest line" formed in the first winter of life, Another rest line being deposited every winter thereafter. Broad light layers are probably deposited from June until late autumn. Additional thin dark lines per year in males probably represent "rut lines".

Different criteria used for age determination were compared, i.e. cemental deposition, eye-lense weight, incisor wear and dressed carcass weight. Sequence of eruption is well-known and works for the first 4 years of life. Cementum deposition on PM4 begins at 4 years of age. PM4 erupts at 3½ years of age with the root still open. Dentin continues to be deposited until 4½ years of age. Ist deposit of cementum was a wide opaque layer possibly indicating a complete summer of rapid deposition. In 5½ year old animals, cementum showed one wide opaque layer, one thin translucent layer and another wide opaque layer. It is possible to conclude that one opaque and one translucent layer equals one complete year's deposition.

Table 2

Reference	Animal	No, of Specimens & Teeth Used	Preparation of Tissue	Viewing Method
Order - CETACEA Sub-order - Odontoceti				
Klevezal & Kleinenberg (1969)	Toothed whales (Odontoceti)	? cementum of all teeth.	Polished sections & thin sections.	Reflected & transmitted light.

continued.

Findings

Clear layers in cementum exist in the teeth of the bottle-nosed dolphin (Tursiops truncatus), pilot whale (Globicephala melaena), Beluga (Delphinapterus leucas), and sperm whale (Physeter catodon). The number of these layers corresponds to the number of layers present in the dentin. Layers also exist in the cementum of the common dolphin (Delphinius delphis) but are very thin and hard to count.

squirrel. Hawaiian monk seals are non-migratory and live in a fairly uniform climate; yet the structure of their cementum resembles that of other pinnipeds, especially the Ringed seal. The specimens studied were of unknown age and therefore the results are inconclusive. However, the ages estimated by counting cemental layers are supported by tooth wear information (Kenyon and Fiscus, 1963).

The California ground squirrels which were studied were of known age being raised in captivity. They are reported to show annual cemental layers for the first four years of life, with subsequent layers being much harder to distinguish (Adams and Watkins, 1967).

In conclusion we may say that the formation of identifiable annual layers in cementum appears to be widespread amongst non-human mammals living in areas of marked seasonality. There is also evidence to suggest that non-human mammals living in areas of more uniform temperature and light conditions form similar annual layers in their cementum. Formation of the bands which make up the annual layer cannot be definitely correlated with metabolic changes, even when those changes are as drastic as hibernation. Consequently, one might reasonably expect to find the same sort of growth lines in the cementum of human teeth.

CHAPTER II

HUMAN CEMENTUM

The Nature of Cementum

Cementum is mesodermal in origin. It functions mainly to anchor the tooth in its alveolus (Provenza, 1964:289; Sicher, 1962:165). However, healthy cementum also compensates for occlusal wear by maintaining the length of the tooth, allows for readjustment of the principal fibres of the periodontal membrane during vertical eruption and mesial drift of the tooth, and prevents the downgrowth of epithelial tissue which would result in expulsion of the tooth (Gottlieb, 1942; Kerr, 1961:184-185).

Cementum is the least hard of the three calcified dental tissues and more closely resembles bone than dentin or enamel. The chief organic component of cementum is collagen, which comes in two varieties. One type, the principal fibres of the periodontal membrane (Scott and Symons, 1972:253) occurs as bundles of fibres produced by fibroblasts in the periodontal membrane. The ends of these fibres are embedded in the cementum, roughly perpendicular to the root surface. These embedded portions are called Sharpey fibres (Scott and Symons, 1972:246). The other type of collagen, presumably produced by cementoblasts (Provenza, 1964:296; Selvig, 1965;431-432) occurs as fibrils filling the spaces between the Sharpey fibres. These matrix fibrils form a dense meshwork of randomly arranged fibrils lying roughly parallel to the dentin surface. Both types of collagen are embedded in a ground substance of glycoprotein (Provenza, 1964:292), the nature of which is not well understood.

The dominant inorganic substances present are calcium, magnesium

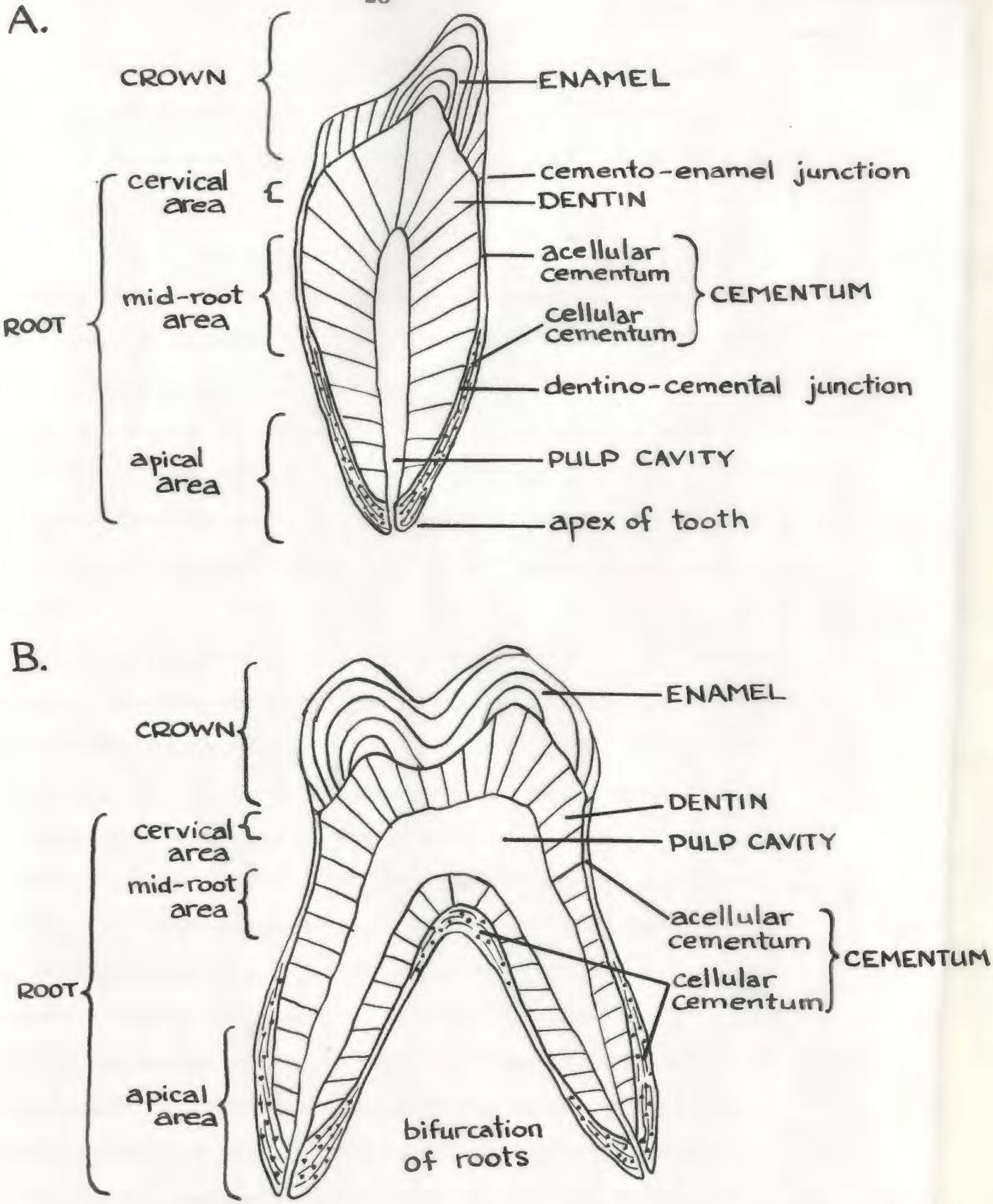


Fig. 1: Cross-section of (A.) single and (B.) double-rooted teeth, identifying tissues and anatomical areas.

and phosphorus with traces of copper, fluorine, iron, lead, potassium, silicon, sodium and zinc (Provenza, 1964:292). Calcium and phosphorus occur as the mineral form hydroxyapatite, $Ca_{10} (PO_4)_6 (OH)_2$ (Scott and Symons, 1972:163).

Two types of cementum, acellular and cellular, are usually distinguished and are commonly described in the following way (Akiyoshi and Inoue, 1963; Awazawa, 1963; Held,1951; Provenza, 1964:294-298; Scott and Symons, 1972:244-246; Sicher, 1962:170-174; Soni et al., 1972). Acellular cementum is thinner, is more homogeneously mineralized, contains more densely packed Sharpey fibres, contains no embedded cells and is found mainly on the cervical half of the root (Figure 2). Its main purpose is thought to be anchoring the tooth firmly in its socket.

Cellular cementum is thicker, is more irregularly mineralized, contains embedded cells and is found mainly on the apical one third of the root and the bifurcation of roots in multirooted teeth (Figures 1 and 3). Both types of cementum have a laminated appearance.

Structural differences between these two types of cementum are thought to result partly from the conditions under which each is formed. Cellular cementum is formed generally on the more mobile apical end of the root. It is deposited quickly and in greater quantity leaving less time for calcification. Furthermore, mineralization begins not only a few microns from the cementoid surface but also between and around the cementoblasts themselves, which often become trapped in the tissue they are so rapidly forming.

Conversely, acellular cementum forms more slowly, in less quantity, and on a part of the root which is subjected to different stresses. This cementum calcifies more throughly and the transition between

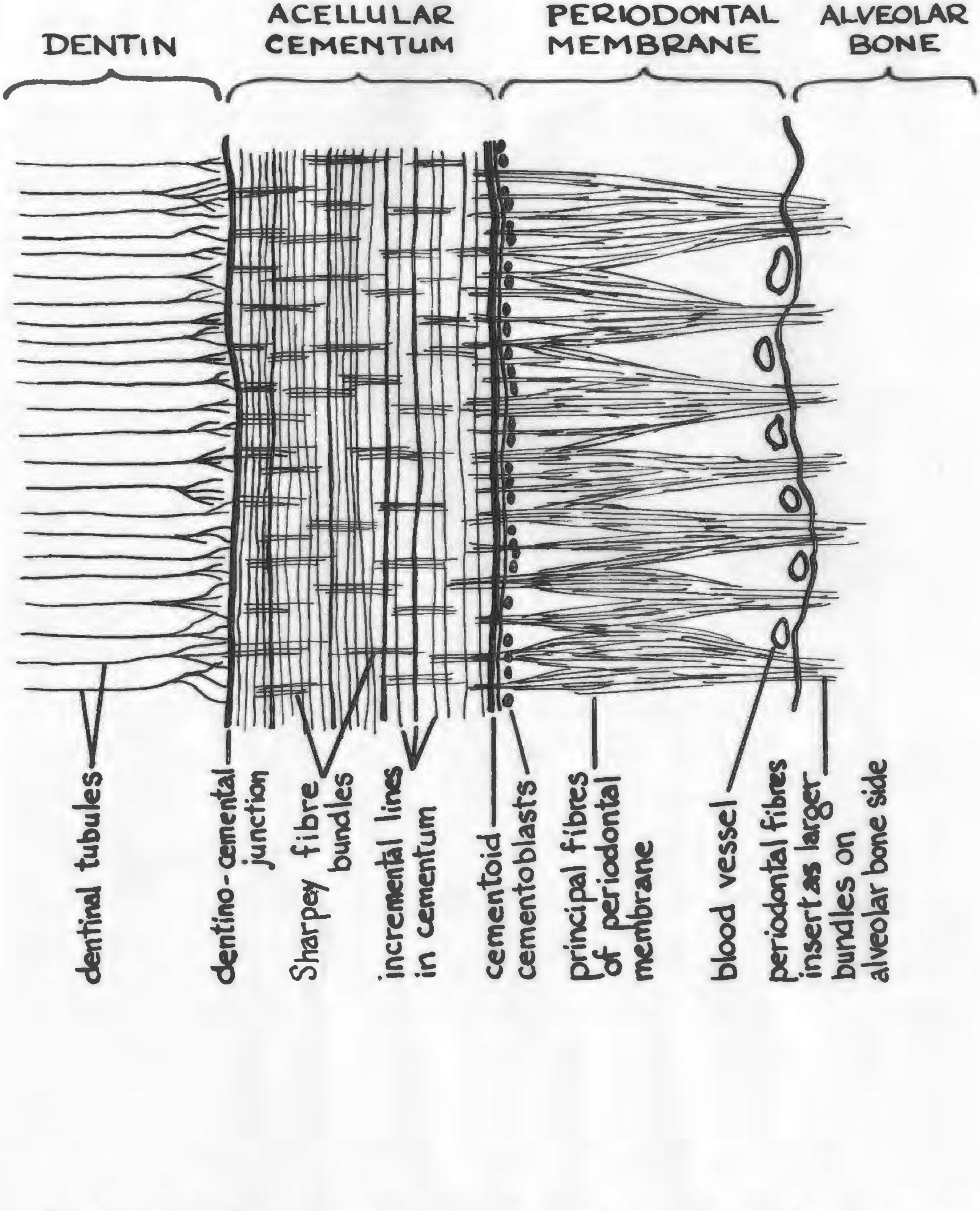


Fig. 2: Acellular cementum enlarged to show fine structure and the nature of the attachment of the periodontal membrane (proportions of structures not to scale).

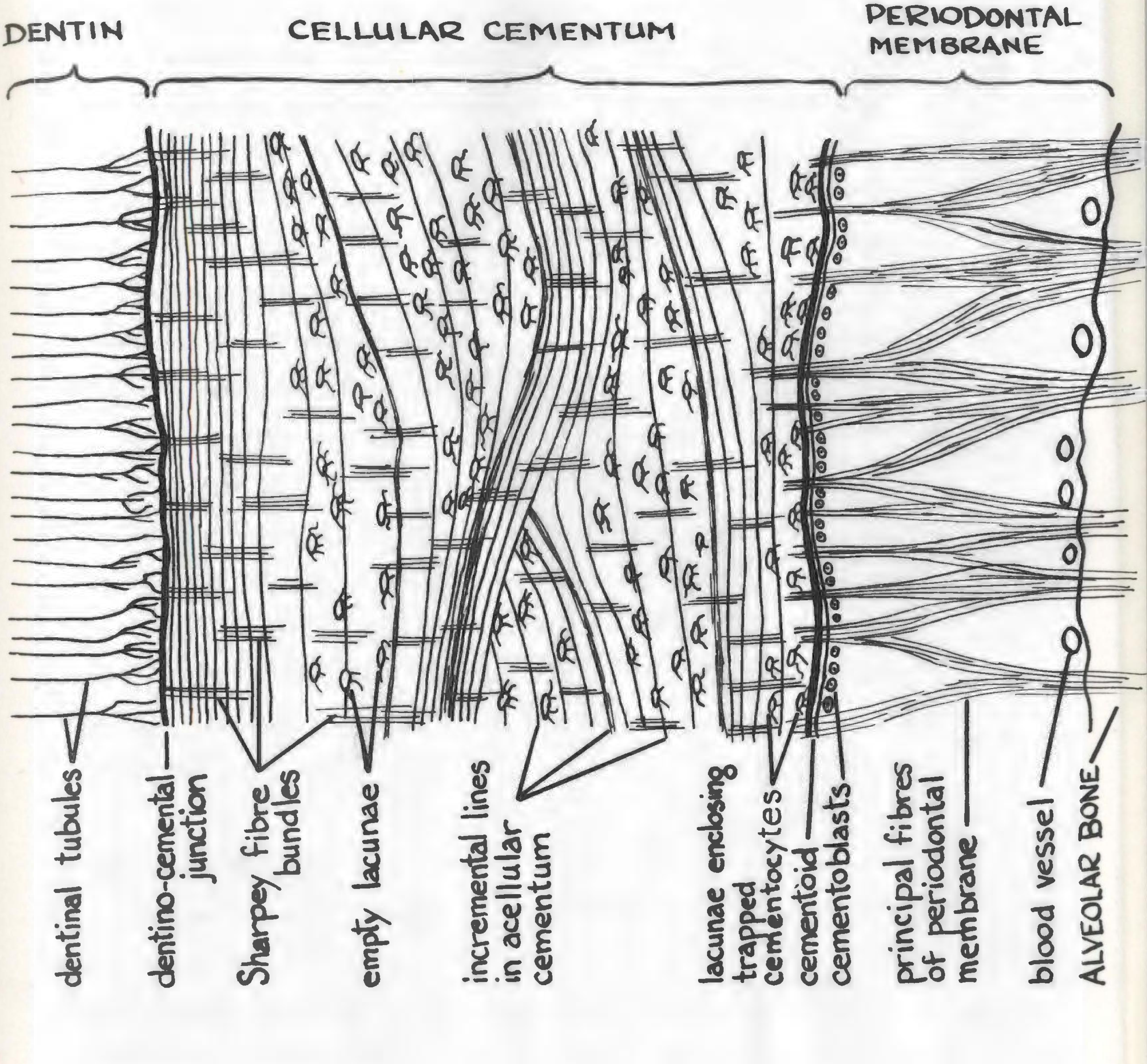


Fig. 3: Cellular cementum enlarged to show fine structure and the nature of the attachment of the periodontal membrane (proportions of structures not to scale).

cementoid and calcified cementum is better defined than in cellular cementum.

Therefore, human cementum forms a layer over the root surface, thinnest at the cervix and thickest at the apex and bifurcation of roots in multirooted teeth (Figure 1). The thickness of cementum varies greatly. Measurements found in the literature range from a minimum of 1 micron (Hopewell-Smith, 1920:63) to a measurement of 712 microns (Soni et al., 1962:374). Several measurements falling between these two extremes have also been recorded by Box (1922:186), Rautiola and Craig(1961:117), Sicher (1962:170) and Selvig (1965:135).

Zander and Hürzeler (1958) conducted systematic measurements of the thickness of cementum from different areas on teeth from individuals whose ages were known. They found that location of measurement and age of the specimen were important in determining the thickness of cementum. Therefore, it may be concluded that measurements of cementum thickness are useless for comparative purposes when the location of the measurement and the identity and age of the tooth are not given.

Cementum Formation

Cementum formation has been described by Provenza (1964:292-297) and Sicher (1962:165-170) and can be summarized in the following way. Enamel and coronal dentin are formed before the onset of eruption. As the tooth begins to erupt, Hertwig's epithelial sheath proliferates, outlining the shape of the root. Formation of the root dentin then begins immediately and rapidly. When dentin on the root surface becomes calcified, Hertwig's epithelial sheath begins to disintegrate allowing cells from the future periodontal membrane to migrate through the sheath towards the calcified dentin surface. These cells become cementoblasts which line up along the calcified dentin surface and are responsible for the formation of cementum matrix and matrix fibrils.

The cementoblasts begin to produce matrix fibrils and cementum matrix which are deposited together on the dentin surface. Small bundles of fibrils, which will become the principal fibres of the periodontal membrane, also migrate towards the dentin surface as Hertwig's epithelial sheath fragments. Their ends become embedded in the forming cementoid and these portions will become Sharpey fibres. When a small amount of cementoid has been formed, mineralization begins.

Small apatite crystals first appear on or between the collagen fibrils and are especially evident on the surface of the Sharpey fibres. The interiors of the Sharpey fibres mineralize more slowly than their outer layers or than the cementum matrix. Apatite crystals grow rapidly and achieve maximum size between 1 and 4 microns from the cementoid surface. Acellular cementum is usually mineralized fairly uniformly and it is not uncommon for the Sharpey fibres to be completely mineralized. On the other hand, cellular cementum is more unevenly mineralized with the interiors of the Sharpey fibres often unmineralized and the embedded cementocytes always unmineralized.

As eruption progresses and the tooth emerges, deposition of cementum c o ntinues as described. However, the Sharpey fibres slowly become larger and more closely packed in the cementum as the tooth becomes fully functional. When this happens, the increase in size and density of the Sharpey fibres stops but cementum continues to be formed. Whether this deposition occurs rhythmically, intermittently or at a constant and even rate is in dispute.

The cementum formed beside the dentino-cemental junction is sometimes thought to differ from the rest of cementum but according to Selvig (1964) and Akiyoshi and Inoue (1963) these differences only reflect differences in tooth function which can also appear in cementum formed much later on.

Selvig (1964) observed the formation of cementum on the teeth of mice using unerupted, erupting and functioning teeth. He observed that the cementum deposited nearest the dentino-cemental junction was composed mainly of a meshwork of matrix fibrils (produced by cementoblasts) in a calcified matrix. Only single fibrils or small bundles of parallel fibrils, produced by fibroblasts of the periodontal membrane, ran from the adjacent periodontal membrane into the cementum matrix.

Teeth from older animals revealed that the more functional the tooth becomes, the larger and more numerous are the Sharpey fibres. When active eruption stops and the tooth becomes functional, size and number of the Sharpey fibres present in cementum also stabilizes.

Akiyoshi and Inoue (1963) made similar observations on human teeth when they studied the structure of cementum of teeth at various functional stages. Sharpey fibre size (i.e. diameter of bundle) and density (i.e. number of bundles per unit area of cementum) was seen to increase as the tooth emerged and came into occlusion. In normally functioning teeth, the size and density of Sharpey fibres remained constant. But teeth which had become non-functional showed a decrease in the size and density of Sharpey fibres embedded in the cementum. Therefore, cementum deposited nearest to the dentino-cemental junction seems to differ from that deposited when the tooth is in normal occlusion only in its functional structure.

Growth of Cementum

Information discussed in this section is summarized in Table 3. The idea that cementum continues to be formed as long as the tooth is functional and healthy is an early one (Broomell, 1898:700). This idea was based on the observation that older teeth possessed thicker cementum than younger teeth (Box, 1922:185; Tomes, 1923:119). After this early research, the idea that cementum deposition continues throughout life was sometimes treated as fact (Held, 1951:53; Kronfeld, 1938:1453). Gottlieb (1942), however, was the first to provide sound arguments for the functional value of continuous cementum deposition. He said that the continuous deposition of cementum would:

1) stop downgrowth of epithelial tissue and eventual expulsion of the tooth,
2) maintain a good attachment with the periodontal membrane and
3) prevent cementum resorption.

Zander and Hürzeler (1958) were the first to test the continuous deposition theory quantitatively. They measured the thickness of the cementum of 233 single-rooted teeth with healthy supporting tissue from people ranging in age from 11 to 76 years and concluded that the thickness of cementum increases in a straight-line relationship with age. According to them, cementum deposition is continuous throughout life and the amount deposited does not decrease with age.

The papers written after Zander and Hürzeler's work generally assume that cementum grows continuously throughout life (Kerr, 1961:183; Scott and Symons, 1972:244; Sicher, 1962:169-172). Selvig (1965;434)

Table 3. Summary of Papers Concerning Growth of and Growth

Author	Identification used for lamellae	Seen in cellular or acellular cementum.	Explanation.
Broomell (1898)	lamellae, striated markings.	Seen in both; wider & more numerous in cellular cementum.	Periods of activity & rest during cementification.
Hopewell-Smith (1920)	layers, lamellae, incremental lines.	Seen in both; thicker and more numerous in cellular cementum.	Lamellae are probably the "calcified remains of the original connective tissue which possesses a different refractive index from the rest of the matrix".
Box (1922)	layers, lamellae.	Seen in both; thicker at apex than in gingival area.	None
Tomes (1923)	lamellae, incremental lines.	Lamellae thinner at cervical area & thicker at apex but number about the same.	Suggests intermittent formation of the tissue.
Mummery (1924)	laminations, incremental lines, laminae.	Acellular cementum usually not laminated, cellular cementum is laminated.	Represent lines of incremental growth as in bone.
Dewey (1926)	lamellated.	?	None
Kronfeld (1938)	incremental lines.	?	None
Gottlieb (1942)	—	—	—
Held (1951)	lamellae	Cellular cementum only.	Lamellae caused by rhythmic variation in calcification; thin dark-staining bands are poorly calcified.

Lines in Human Cementum (papers arranged in chronological order).

Remarks on continuous growth of cementum.	Samples and preparation	Viewing method and magnification.
Cementum formation continues as long as the tooth is functional & healthy.	Deciduous & permanent teeth, decalcified (& stained?) thin sections.	Transmitted light, magn. from x40 to x1000.
Cementum is generally thicker on older individuals and incremental lines more numerous.	Various unidentified teeth, mostly showing abnormal conditions of cellular cementum, prepared by grinding & left unstained or stained by various methods.	Reflected? light at magn. from x40 to x400.
Cementum thin in childhood and increases with age.	Various unidentified teeth, preparation generally not described.	?
Cementum thin on newly erupted teeth, thicker on adult tooth and still thicker on tooth of aged person.	Sample unspecified, ground sections and perhaps decalcified stained sections.	?
None	Various teeth occasionally identified, sometimes shown as ground sections (Weil process) but usually preparation not described.	Transmitted (and reflected?) light, magn. from x50 to x700.
"There is nearly always a layer of precementum in sound teeth."	Teeth from 20 people, preparation not specified.	Transmitted? light, magns. from x36 to x230.
Cementum deposition is continuous throughout life, one layer being deposited on top of the other. "Usually this is indicated by the presence of incremental lines.".	Various human teeth usually unidentified, & 1 fox molar, preparation of human teeth not specified.	Transmitted? light, magn.?
Cementum deposition continuous because: stops downgrowth of epithelium; maintains attachment with periodontal membrane; prevents cementum resorption.	Various unidentified human teeth, also sheep teeth and a pig tooth, preparation unspecified.	Transmitted? light, magnification?
Cementum forms continuously by more or less rhythmical apposition of lamellar tissue.	Various unidentified human teeth decalcified & variously stained.	Transmitted? light, at magn. from x26 to x1000.

Table 3

Author	Identification used for lamellae	Seen in cellular or acellular cementum.	Explanation.
Henry & Weinmann (1951)	incremental lines.	Seen in both; closer together in acellular cementum.	None
Röckert (1956)	light & dark bands	Seen in cellular cementum.	Bands caused by rhythmic mineralization.
Gustafson & Persson (1957)	layers	?	"layers" are optical illusions & really represent points at which Sharpey fibre bundles change direction.
Zander & Hürzeller (1958)	-	-	-
Kerr (1961)	laminae	Seen in both.	None.
Sicher (1962)	incremental lines	Seen in both.	Indicates periodic formation of cementum throughout life.
Soni et al. (1962)	laminated pattern, laminae.	Seen in both. Acellular cementum had less distinct and narrower laminae.	Laminated pattern reflects the rhythm of formation of all calcified tissue.

continued.

Remarks on continuous growth of cementum.	Samples and preparation	Viewing method and magnification.
Since incremental lines in acellular cementum are often continuous with those in cellular cementum & the thickness between incremental lines is greater in cellular cementum, then more cementum was formed in one area than another during the same time period.	261 teeth from 15 human dentitions. Tooth's age identity & orientation given with each figure. Teeth were decalcified & stained with hematoxylin & eosin.	Transmitted light at magnifications from x22 to x120.
None	Various human teeth, gives age & sex but not identity of each tooth.	Microphotography & microradiography both at x64.
Cementum deposited each time fibre bundles change direction. Deposition is not rhythmic or continuous.	2 unidentified human teeth, preparation unspecified.	Polarization microscope (transmitted light?) at magnifications from x80 to x370.
Cementum thickness measurements confirmed quantitatively that cementum thickness increases in a straight-line relationship with age, i.e. cementum deposition is continuous throughout life & amount deposited does not decrease with age.	233 single-rooted healthy teeth aged 11-76 years, decalcified & cross-sectioned from apex to cemento-enamel junct. stained with hematox. & eosin.	Transmitted light with overhead projector at x25 magnification.
Continuous deposition of cementum allows for: 1)reattachment of periodontal fibres after reorientation of tooth,2) maintenance of vitality of attachment as cementum ages, 3) compensation of widened periodontal space & 4) resorption prevention.	Various unidentified specimens, preparation unspecified.	Transmitted? light, magn. unspecified.
Continuous deposition necessary to maintain: a vital surface layer & attachment of periodontal fibres, also compensates for eruptive movements.	Various unidentified specimens, preparation unspecified but some information on certain useful stains.	Transmitted? light, magn. unspecified.
None.	4 human teeth , ident. & area of tooth given for each figure, undecalcified ground sections (10-20 u thick).	Microradigraphs at x50 & x90 magn.

Table 3

Author	Identification used for lamellae	Seen in cellular or acellular cementum.	Explanation.
Provenza (1964)	lamellae, incremental lines, imbrication lines, resting lines.	Seen in both. More lamellae in cellular cementum than acellular cementum.	Calcification proceeds with "cyclic activity"producing a pattern of lamellae subdivided by imbrication lines. Resting lines between lamellae indicate where cementum formation stopped & recommenced.
Selvig (1965)	incremental lines, resting lines.	Acellular cementum has numerous incremental lines. Cellular cementum consists of layers separated by resting lines or layers of acellular cementum.	None.
Furseth & Johansen (1968)	alternating bands, incremental lines.	?	Lamellar appearance caused by alternating bands of high & low mineral content.
Scott & Symons (1972)	incremental lines, layers.	Seen in both.	Lamellated appearance of acellular cementum results from layers of collagen-fibre-containing material being separated by layers of fibre-free amorphous substance.

continued.

Remarks on continuous growth of cementum.	Samples and preparation.	Viewing method and magnification.
Cementum deposited in thick layers (lamellae) which are calcified rhythmically. A new lamella is added when cementum surface loses its "vitality". Lamellae are separated by resting lines.	Various human teeth sometimes identified in illustrations but mostly not. Decalcified & stained variously or ground thin sections.	Transmitted light, phase contrast, electron microscopy & microradiography, magn. x35 to x39,000.
All specimens studied contained zone of small mineral crystals at cementum surface. This supports continuous cementum formation theory, but rate of formation may vary greatly with time & location.	Various human teeth sometimes identified. Ground thin sections, silver shadowed negative collodion replicas, and decalcified ultrathin sections.	Microradiography, light microscopy & electron microscopy at magn. from x35 to x140.
None	100 human teeth (molars, premolars & incisors) with carious cementum & 25 teeth with sound cementum. 50µ thick sections.	Microradiography at magn. from x35 to x140.
"Formation of cementum continues throughout life."	Various unidentified teeth mostly prepared into ground thin sections. Silver stain used.	Light microscopy at magn. from x6.5 to x580.

provides some objective evidence for the continuous growth of cementum by stating:

> "The observation that all specimens in the present study contained a zone of very small mineral crystals at the surface tends to support the concept that the cementum is formed continuously."

However, Selvig qualifies his support of the continuous deposition theory by saying that his evidence does not preclude "that the rate of formation may vary greatly with time and from one region to another". That the amount of cementum deposited does vary from one region to another is supported by Henry and Weinmann's (1951.282) observation that:

> "Since incremental lines in acellular cementum are often continuous with those in cellular cementum and the thickness between incremental lines is greater in cellular cementum than acellular cementum, then more cementum was formed in one area than another during the same period."

Provenza (1964:300-302) briefly proposed a different version of the continuous growth theory. He suggests that cementum is periodically deposited in relatively thick "lamellae" which are calcified rhythmically and therefore display a number of "imbrication lines" throughout their thickness. The "lamellae" are separated by "resting lines". He also believes that the width of the lamellae vary, depending on the severity of the stimulus which initiates cementum deposition. The nature of this stimulus is not specified by Provenza. Whether he thinks cementum is deposited in the same way on both acellular and cellular cementum is not clear, but the photograph (Fig. 9-17) used to illustrate his lamellae, resting lines and imbrication lines is of cellular cementum.

Gustafson and Persson (1957) proposed another theory about the growth of cementum. They suggested that the formation of cementum occurs at erratic intervals stimulated by the need for the principal fibres of the periodontal membrane to be reimbedded after reorientation of the tooth in its alveolus.

According to their theory, the principal fibres of the periodontal membrane must be embedded in cementum to a certain depth for secure attachment. The fibres also must emerge at right angles to the cementum surface. Tooth movement reorients the fibres and they lose their tensile strength. New cementum of the same depth as the previous layer must then be deposited over the reoriented fibres in order for them to again emerge at right angles to the cementum surface. Since the position of the tooth in its alveolus changes a number of times throughout life, an equal number of principal fibre adjustments are necessary. Furthermore, since the amount of cementum deposited each time is the same, the resulting structure of the whole thickness of cementum appears laminated. However, these laminations are more apparent than real, since they are defined by a change in direction of Sharpey fibres rather than a difference in degree of calcification of cementum.

This theory is interesting and would be more convincing if the proponents' prime(and only illustrated) example did not fail to illustrate the authors' claim. In the photographs (their Figures 2 and 3) there are several laminations clearly visible through which the Sharpey fibres run in a straight line. Personal observations of cemental layers do not support this theory. Although Sharpey fibres do change direction throughout the thickness of cementum, incremental lines occur most often where such changes do not.

We may conclude, then, that the available information concerning cementum growth supports some form of deposition of cementum throughout life. Whether this deposition is uninterrupted or periodic, uniform or patchy, occurs in a spontaneous and rhythmic fashion or is evoked by mechanical and/or chemical stimuli remains to be discovered.

Cemental Lamellae

The information discussed in this section is summarized in Table 3, column 2. Lamellae have been observed in cementum by most dental researchers. These lamellae were seen at various magnifications in both acellular and cellular cementum and a number of terms have been used to describe them; i.e. striated markings, lamellae, layers, incremental lines, laminae, light and dark bands, laminar deposits, imbrication lines, resting lines and alternating bands. Because none of the research is specifically concerned with investigating cemental lamellae, these terms have usually been applied casually, often used interchangeably and seldom defined.

Kerr (1961:183), Scott and Symons (1972:245) and Sicher (1962:172) note the presence of laminations in both acellular and cellular cementum. Broomell (1898:703-706) and Hopewell-Smith (1920:65) observed laminations in both types of cementum but thought the laminations were thicker and more numerous in cellular cementum. Henry and Weimann (1951:282) and Tomes (1923:119) have all seen laminations in both types of cementum and agree that cellular cemental laminations are thicker. They think that the number of laminations is roughly the same in both acellular and cellular cementum. Selvig (1965:426-427) thinks that acellular cementum is "characterized by numerous incremental lines running parallel to the root surface" while cellular cementum consists of layers "separated by x-ray dense resting lines or layers of acellular cementum". Mummery (1924:290) thought that cellular cementum was laminated while acellular cementum usually was not. Held (1951:56) and Röckert (1956:18) have observed laminations in cellular cementum only. Provenza (1964:300-302) describes cellular cementum as consisting of "lamellae" marked off from one another by "resting lines" and subdivided by "imbrication lines". It is not clear whether he thinks this scheme also describes acellular cementum but he does think there are more lamellae present in cellular cementum than acellular cementum.

The proliferation of terms used for the layers seen in human cementum probably results from the variety of viewing techniques and specimen preparations used by the authors named above. None of these researchers has reported a thorough investigation of cemental lamellae to try to resolve the differences of opinion. For further comments on this subject see Chapter 3, page 49.

Theories proposed to account for the presence of cemental lamellae may be summarized as follows:

1) cemental lamellae represent periods of activity and rest in cementum formation (Broomell, 1898:700; Tomes, 1923:119; Sicher, 1962:172; Soni et al., 1962:377).
2) cemental lamellae represent the rhythmic calcification of cementum, poorly calcified bands alternating with well calcified bands (Furseth, and Johansen, 1968:1197-1198; Held, 1951:67; Provenza, 1964:292-300; Röckert, 1956:18; Soni et al., 1962:374).
3) cemental lamellae represent layers of collagen-fibre-containing material alternating with layers of fibre-free amorphous substance (Furseth and Johansen, 1968:1200; Scott and Symons, 1972:245).

4) Cemental lamellae are optical illusions, really representing the point at which all Sharpey fibres change direction (Gustafson and Persson, 1957).

These categories need not be mutually exclusive. However, how they are interrelated is not clear.

In conclusion, we have seen that the thickness of human cementum increases, either continuously or sporadically, throughout life. We have also seen that human cementum has a laminated appearance. The remainder of this thesis is concerned with observing the layers present in human cementum and looking for correlations between the number of layers present and the age of the tooth.

CHAPTER III

MATERIALS AND METHOD

The 29 single-rooted teeth used in this investigation are listed and described in Table 4. Since the crown end and the apex of the root represent the two extremes in cementum thickness, it was decided to investigate the cemental lamellae at mid-root, which according to Zander and Hürzeler (1956:1037) very closely reflects the average increase in cementum thickness for the whole root. Single-rooted teeth were chosen because they provide a larger area of acellular cementum at mid-root.

Thorough investigation of lamellae in cellular cementum will not be undertaken here because the structure of cellular cementum is more complicated than that of acellular cementum (see Chapter 4, page 53 for details).

Specimens listed in the series from 1 to 94 were extracted for reasons of unspecified poor dental health, and therefore constitue a pathological sample. Pathological teeth were obtained either from the General Hospital, Forest Road, St. John's (Dr. Josephson), or from various St. John's dentists as indicated in Table 4. One tooth, number 47, came from Dr. C. M. Calder, a dentist in Nain, Labrador. More teeth were collected than were used. All teeth were examined visually and those showing obvious gross pathology were rejected. The 25 teeth used in this study represent teeth which displayed little or no visible pathology.

Healthy teeth <u>in situ</u> at death are difficult to obtain and only two such teeth (1968-1 and 1968-2) from one cadaver are present in this sample. The biological ages of the people from whom the above teeth

Table 4. Description of Sample

Tooth Number	Identity	Source	Age	Sex	Donated By
1	I	h.e. 5-642-72	50	M	Dr. Josephson, St. John's
3	C	h.e. 5-642-72	50	M	Dr. Josephson, St. John's
4	C	h.e. 5-642-72	50	M	Dr. Josephson, St. John's
15	C	h.e. 5-439-72	17	F	Dr. Josephson, St. John's
20	C	dentist's extraction	25	M	Dr. G. Anthony, St. John's
21	PM	dentist's extraction	20	F	Dr. G. Anthony, St. John's
23	C	h.e. 5-362-72	26	M	Dr. Josephson, St. John's
31	I	h.e. 5-440-72	18	M	Dr. Josephson, St. John's
32	C	h.e. 5-331-72	25	F	Dr. Josephson, St. John's
33	I	h.e. 5-643-72	22	M	Dr. Josephson, St. John's
42	I	dentist's extraction	55	M	Dr. B. L. Bowden, St. John's
47	C	dentist's extraction	26	F	Dr. C. M. Calder, Nain
57	I	dentist's extraction	50	M	Dr. C. M. Calder, Nain
68	I	h.e. 5-362-72	32	M	Dr. Josephson, St. John's
69	C	h.e. 5-643-72	22	M	Dr. Josephson, St. John's
70	C	h.e. 5-331-72	18	M	Dr. Josephson, St. John's
71	I	h.e. 5-516-72	28	M	Dr. Josephson, St. John's
72	I	h.e. 5-588-72	25	F	Dr. Josephson, St. John's
74	I	h.e. 5-477-72	53	M	Dr. Josephson, St. John's
78	I	h.e. 5-306-72	31	M	Dr. Josephson, St. John's
79	PM	h.e. 5-439-72	17	F	Dr. Josephson, St. John's
82	PM	h.e. 5-381-72	24	F	Dr. Josephson, St. John's
83	PM	h.e. 5-588-72	25	F	Dr. Josephson, St. John's
84	PM	h.e. 5-588-72	25	F	Dr. Josephson, St. John's
94	PM	h.e. 5-568-72	67	M	Dr. Josephson. St. John's
1968-1	I	cadaver	50	M	Dr. K. O. McCuaig, U. of T., Toronto
1968-2	C	cadaver	50	M	Dr. K. O. McCuaig, U. of T., Toronto
18B	first upper I	miscellaneous tooth Port aux Choix burial	?	?	Dr. S. Jerkic, M.U.N., St. John's
47B	C	miscellaneous tooth Port aux Choix burial	?	?	Dr. S. Jerkic, M.U.N., St. John's

h.e. - hospital extraction

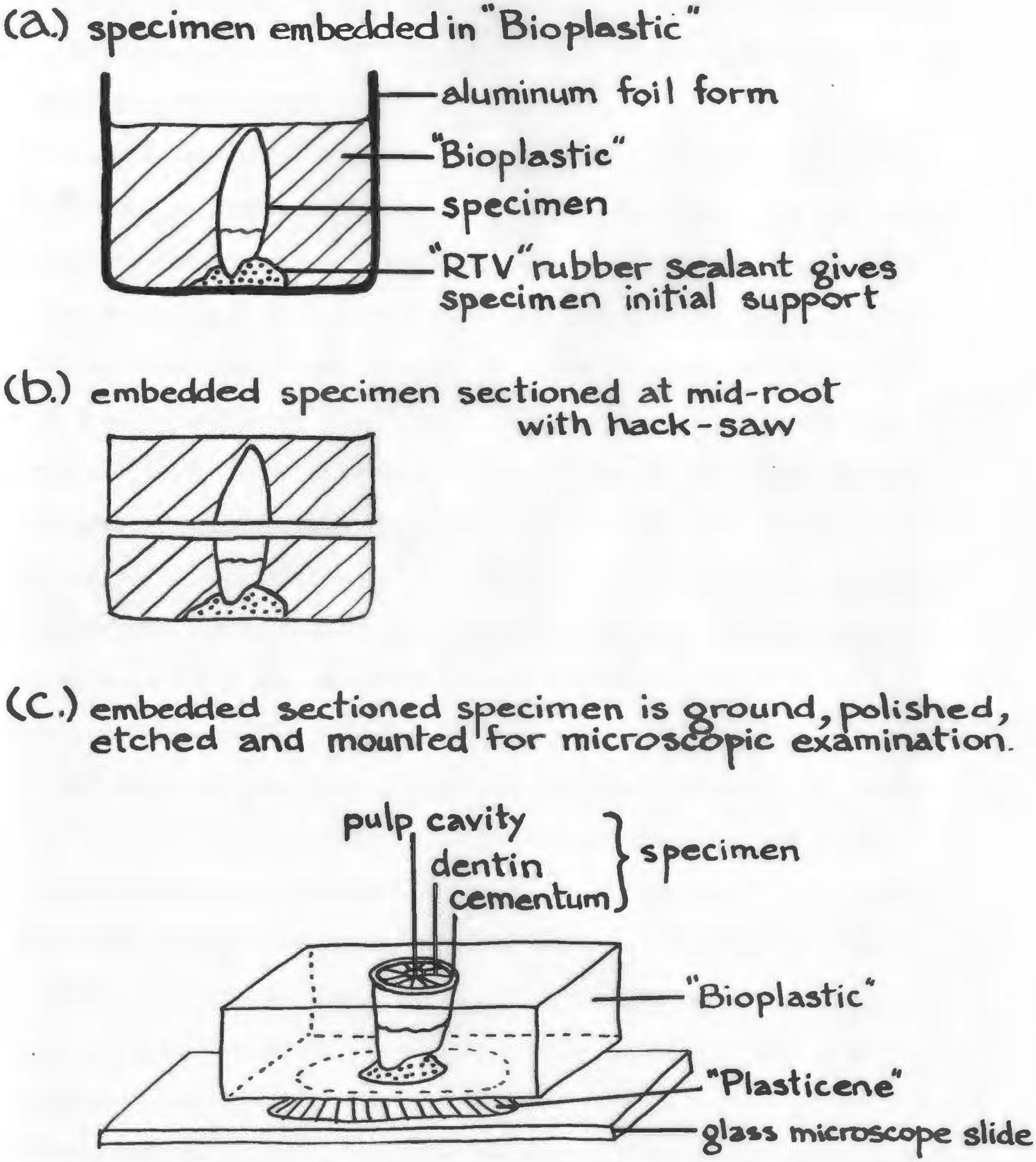


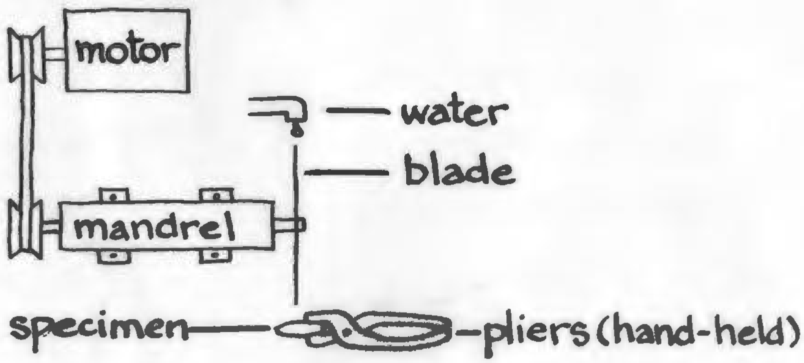
Fig. 4: Preparation of specimens embedded in "Bioplastic"

were removed is known. Teeth 18B and 47B are of unknown age and come from two burials at the Port aux Choix archeological site where they occurred as miscellaneous teeth.

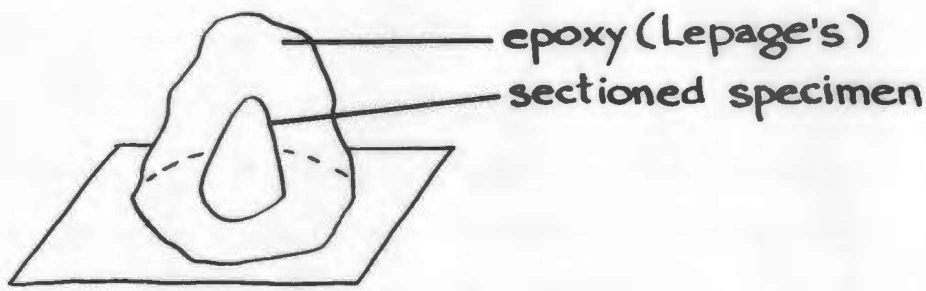
In preparation for examination, teeth in the series from 1 to 94 were embedded in blocks of clear plastic (Ward's Bioplastic), cross-sectioned at mid-root with a hack saw, then polished and mounted as illustrated in Figure 4. However, it was found to be easier to use a jeweller's circular saw which can cut a bare tooth root into the desired sections without fracturing the tooth root in any way (see Figure 5). Teeth 1968-1, 1968-2, 18B and 47B were sectioned at mid-root with a jeweller's saw. The resultant sections were coated with enough plastic (LePage's Epoxy) to ensure stability during polishing. By this method, both longitudinal sections and cross-sections can be prepared on the same specimen at the same time.

Sectioned and embedded specimens were ground on 3-M Co., Wetordry Tri-M-ite paper, in grit sizes 320, 400 and finally 600, using water as a lubricant. This prepared the sectioned cementum surface for polishing with Geonite diamond compounds in grit sizes of 6 microns, 1 micron and finally 1/4 micron, using oil as a lubricant on fine cloth surfaces. The specimen was washed carefully between grits during both grinding and polishing to prevent mixing of grits and scratching of the specimen surface.

Mixing of grits is not a serious problem during grinding because the grits are attached to the grinding paper. However, the polishing compounds enter every crack and in spite of careful and repeated washings between grit sizes, scratches are usually present on the sectioned cementum surface after the final polish. These scratches are readily identified and easily distinguished from cemental (a.) specimen sectioned (across or longitudinally) with a jeweller's saw.



(b.) sectioned specimen coated with epoxy to provide stability during polishing and prevent cracking as tooth dries.



(c.) coated sectioned specimen is ground, polished, etched and mounted for microscopic examination.

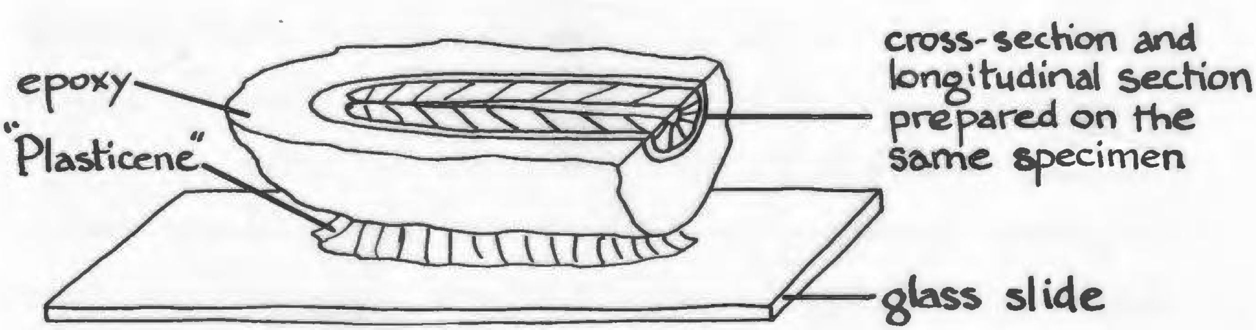


Fig. 5: Preparation of specimens coated with epoxy

laminations.

After final polishing and thorough washing with soap and water, the cementum surface was examined under reflected light with interference contrast. It appears (ignoring the scratches) as a highly polished smooth surface distinguished from the dentin and Bioplastic by the dentino-cemental junction which appears as a slight depression and the outer edge of cementum which appears as a steep decline from the level of the cementum surface to the level of the plastic embedding material. Ward's bioplastic always polished down faster than cementum and dentin and often shrank away from the cementum surface and for these reasons its use was abandonned in favour of LePage's Epoxy. The Epoxy shrinks less, polishes away at the same rate as cementum and is non-reflective even after polishing, thus reducing glare under the microscope. The specimen was then mounted level in plasticene on a glass slide and immersed in etching solution for 8-24 hours. The etching solution consisted of tap water and detergent in the ratio of 250 cc. tap water to 0.6 cc. Ivory liquid detergent and had an acid ph of 4.5 (7.0 is neutral).

Other mild etches such as very weak solutions of hydrochloric acid, acetic acid, phosphoric acid, nitric acid, Coca Cola and ordinary soap were tried. While all these solution etched, the action was too violent and indescriminant. The best of these unsuccessful etches is the acetic acid etch which reveales the same surface structure as that revealed by the detergent etch only less clearly. As with all the other unsuccessful etches, the polished surface has the appearance of being attacked too violently even though the specimen had been in contact with the etch for only a few seconds. Washing the polished tooth in an ultrasonic cleaner for a few minutes produced a better etch

Fig. 6: Reichert microscope fitted with reflected-light interference contrast and photographic attachments.

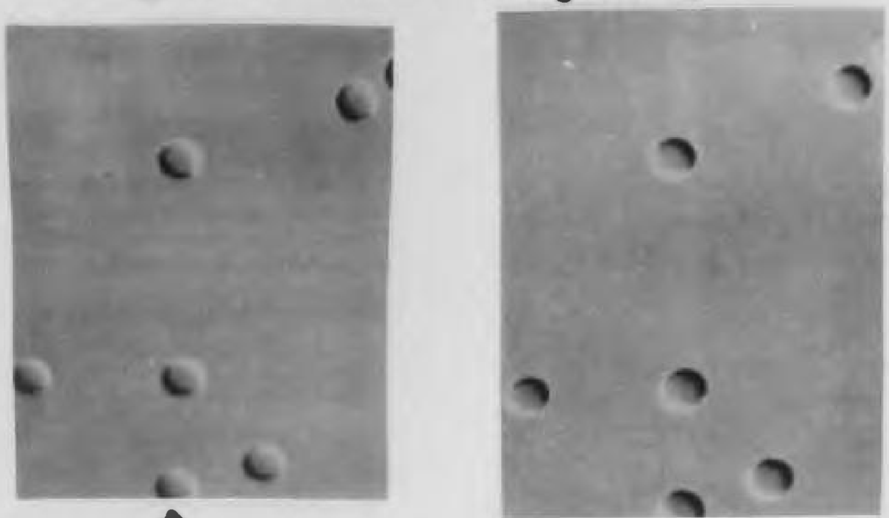
than the acetic acid solution but coarser than that achieved with the detergent solution. The tooth, however, was cracked by the high frequency agitation and after a few treatments began to disintegrate.

The etched cementum surface was observed with a Reichert microscope (Figure 6) fitted with reflected light and an interference contrast attachment. Figure 7 (adapted from Gabler and Herzog, no date) demonstrates how the interference contrast technique reveals surface topography with great clarity. The unstained erythrocytes are almost invisible in ordinary transmitted light but are clearly seen in transmitted light with interference contrast. Although these examples are of interference contrast used with transmitted light and transparent specimens, the visual effect is the same with reflected light and an opaque specimen.

Objectives of x12.5 and x32 magnification were most useful for a general scanning of the etched cementum section and the x63 objective is best for examining the surface topography of the etched cementum in detail. A x100 oil immersion objective was also used occasionally to clarify details. But since the specimen has to be coated with a fine metallic film before an image results and since this higher magnification does not reveal cemental lamellae finer than those seen with the x63 objective, it was rarely used.

The term "cemental lamellae" (lamella being the diminutive of lamina) is used here to indicate the smallest cemental layer resolveable under a light microscope. All the other terms listed in Table 3, column 2 which have been used to describe layering in cementum probably refer to the same phenomenon seen at different magnifications. Cemental lamellae merge at magnifications lower than x63 objective. Although plainly visible, the layers appear to be

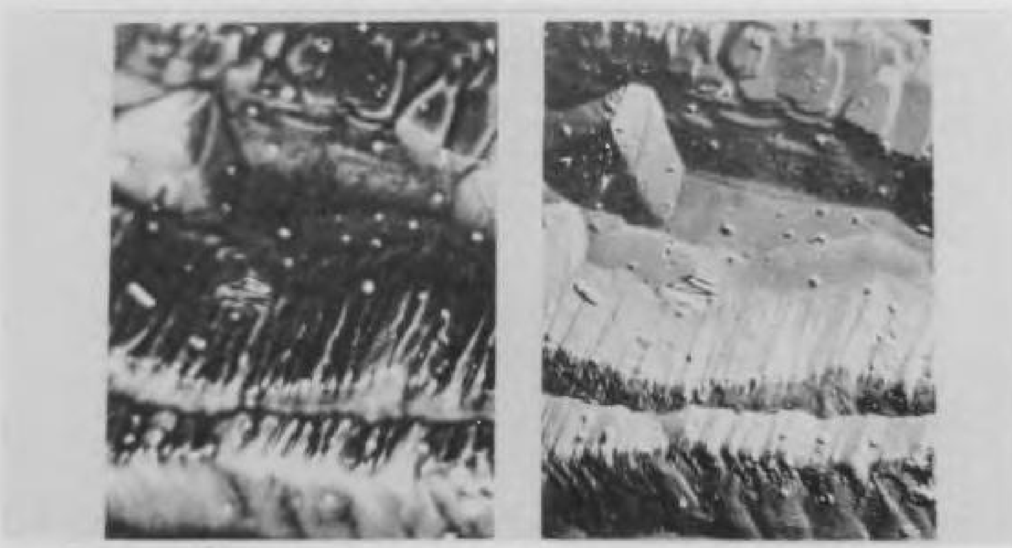
Unstained erythrocytes
(100x oil immersion objective)



high contrast position

contrast reversed

Replica of the surface of a calcite crystal.
(40x objective)



negative phase contrast

compared with

interference contrast

Fig. 7: Manufacturer's examples of effect of Reichert transmitted-light interference contrast attachment.

fewer in number with each decrease in magnification. No further subdivisions of the layers are seen with objectives of higher power than x63.

Cementum was photographed at locations where it seemed most normal, i.e. where the cementum was of about average thickness for that particular tooth section and where the cemental laminations were best defined. These attributes are most often seen in cementum at the corner of a tooth root rather than on any of its four sides. The reasons for this are as follows. Cementum on the mesial and distal sides of the tooth root is often very much thicker than on the labial or lingual sides. When the root has distinct vertical grooves, as seen in some single-rooted premolars, the mesial and distal sides often contain pockets of cellular cementum. Conversely, labial and lingual cementum is often extremely thin. The compromise between these two extremes is usually to be found in the area where the labial or lingual sides merge into the mesial or distal sides.

Photomicrographs of the etched cementum sections were taken using a Reichert photographic tube attachment and a Zenit SLR or Asahi Pentax K2 camera body. Film used was Kodak Plus-X, 135 ASA. Prints were made on Kodak Polycontrast RC print paper.

Very often when using the x63 objective, the entire thickness of cementum could not be accomodated in the field of view. Also, since the Bioplastic embedded specimens always developed a slope during polishing, several focuses had to be made when viewing the etched specimen and each photographed. It was then possible to make a composite picture of the entire cementum thickness.

Using these composite pictures, graphs were made plotting the number of clearly visible lamellae against the cumulative thickness of the lamellae. The slopes of the resulting lines were then examined in various ways to determine whether any correlation exists between the number of lamellae and the number of years of deposition of cementum. Cementum thickness measurements obtained here were compared with those of Zander and Hürzeler (1956). Teeth from the same mouth were examined to see if similarities exist in the appearance of the lamellae and finally, male teeth were compared with female teeth to see if the cemental lamellae show any sex-related differences.

CHAPTER IV

OBSERVATIONS

As stated in the last chapter, polished cross-sectioned cementum has a very shiny surface. The only visible landmarks are the outer surface of cementum, the dentino-cemental junction, an occasional scratch and any Sharpey fibres which defy polishing. The plastic embedding material often shrinks away from the cementum surface leaving an open space between the cementum and plastic which picks up polishing debris. Occasionally, bits of periodontal membrane which remain attached to the cemental surface can also be seen embedded in the plastic.

After etching, the cross-sectioned cementum developes a more detailed topography. The main landmarks are still the dentino-cemental junction and the outer surface of the cementum. However, between these two margins, numerous lamellae have etched out of the polished surface. A thorough investigation of cellular cementum has not been included in this study because the kind of lamellae which are present in acellular cementum are not so clearly seen in cellular cementum. They are usually clearly visible only in the thin layer of cell-free cementum often found in cellular cementum next to the dentino-cemental junction.

Incremental layers are found in cellular cementum which differ from the lamellae seen in acellular cementum in three ways. Firstly, compared with acellular cemental lamellae, these layers are much more poorly defined when observed through a x63 objective. The lines that seem to define each layer at a lower magnification (x32 objective) look like discontinuous aggregations of thin (½ - 2 microns wide) short layers when seen at a higher magnification (x63 objective). At the higher magnification it is often impossible to locate and identify the boundaries of a layer seen clearly at a lower magnification.

Secondly, the incremental layers in cellular cementum are generally much wider than acellular cemental lamellae. The width can vary considerably both within each layer and from layer to layer while the opposite is true of acellular cemental lamellae.

Thirdly, not all the layers extend around the whole circumference of the root. Often, one or several layers cover only a small patch on the root's surface. In cross-sections or longitudinal sections, these layers appear as lenses enclosed within more extensive layers of cellular cementum.

Perhaps the finest layers seen (x63 objective) as discontinuous aggregations in cellular cementum are similar to the lamellae seen in acellular cementum although they are very distorted due to the way cellular cementum is deposited. However, it was thought that including an investigation of the finest layers seen in cellular cementum would only confuse matters, since these layers are much more difficult to trace than acellular cemental lamellae. They also show much more variability in appearance and number than acellular cemental lamellae. Therefore, unless otherwise specified, all the following observations are made on cross-sections of acellular cementum.

The acellular cemental lamellae, which can be seen most clearly with a x63 objective, run roughly parallel to each other and to the two cemental boundaries. They cross the Sharpey fibres approximately at right angles. These same lamellae can also be seen on the etched surface using ordinary reflected light and on acetate replicas of the etched surface using transmitted light. However, they are most clearly observed on the etched specimen when reflected light with an interference contrast attachment is used (see Figures 8 - 12).

Lamellae can be seen in some part of the cementum thickness in all secions examined except those made from the archeological samples 18B and 47B. Since it is possible that mineral replacement occurred while the teeth were buried, the absence of visible lamellae on the etched specimens cannot be taken as proof that lamellae were never present in these specimens. All we can say with certainty is that this particular etching solution did not reveal lamellae in the cementum of these two prehistoric samples. It is possible, however, that different etching solutions might yield better results on archeological samples.

In the remaining 27 samples, the lamellae vary in thickness from 1/3 to 3 microns but are most commonly between 3/4 and 1 1/2 microns thick. The width of each lamella remains almost constant from one edge of the photograph to the other, except where obstacles such as poorly calcified Sharpey fibres are traversed. Where they can be seen clearly, the lamellae do not merge or branch. There is no noticeable difference in the structure of the lamellae seen in pathological and cadaver teeth.

Lamellae show great variability of expression. Some are not very clearly defined and their course is difficult to follow. This is true even in cementum where the lamellae are generally well defined. In such teeth there is always a scattering of poorly defined lamellae, or sections of cementum where lamellae can barely be detected at all.

Lamellae are usually better defined in cementum formed early in life than that formed later. Except for the first approximately

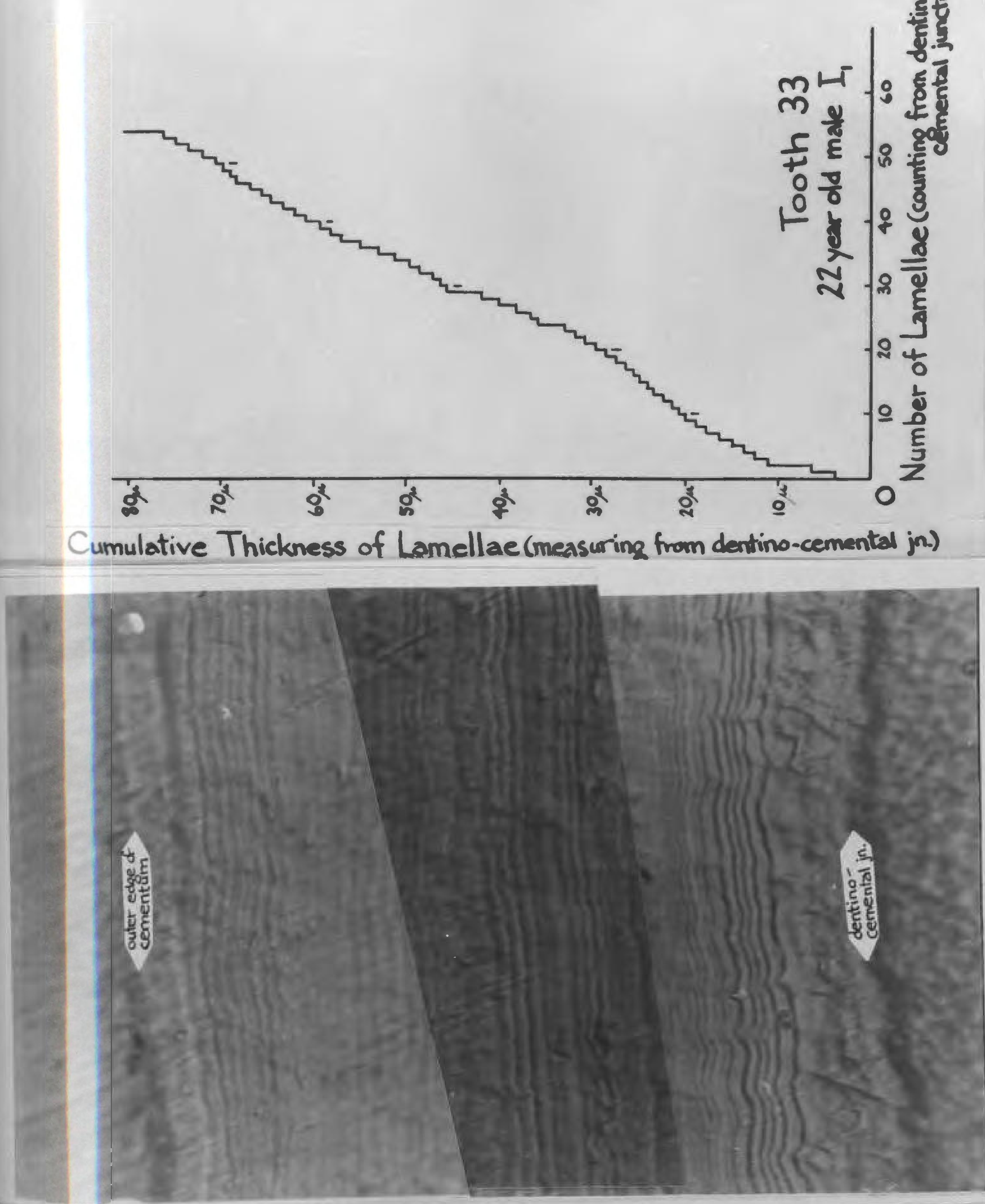


Fig. 8: Composite photomicrograph of cementum used to prepare graph of number versus cumulative thickness of lamellae.

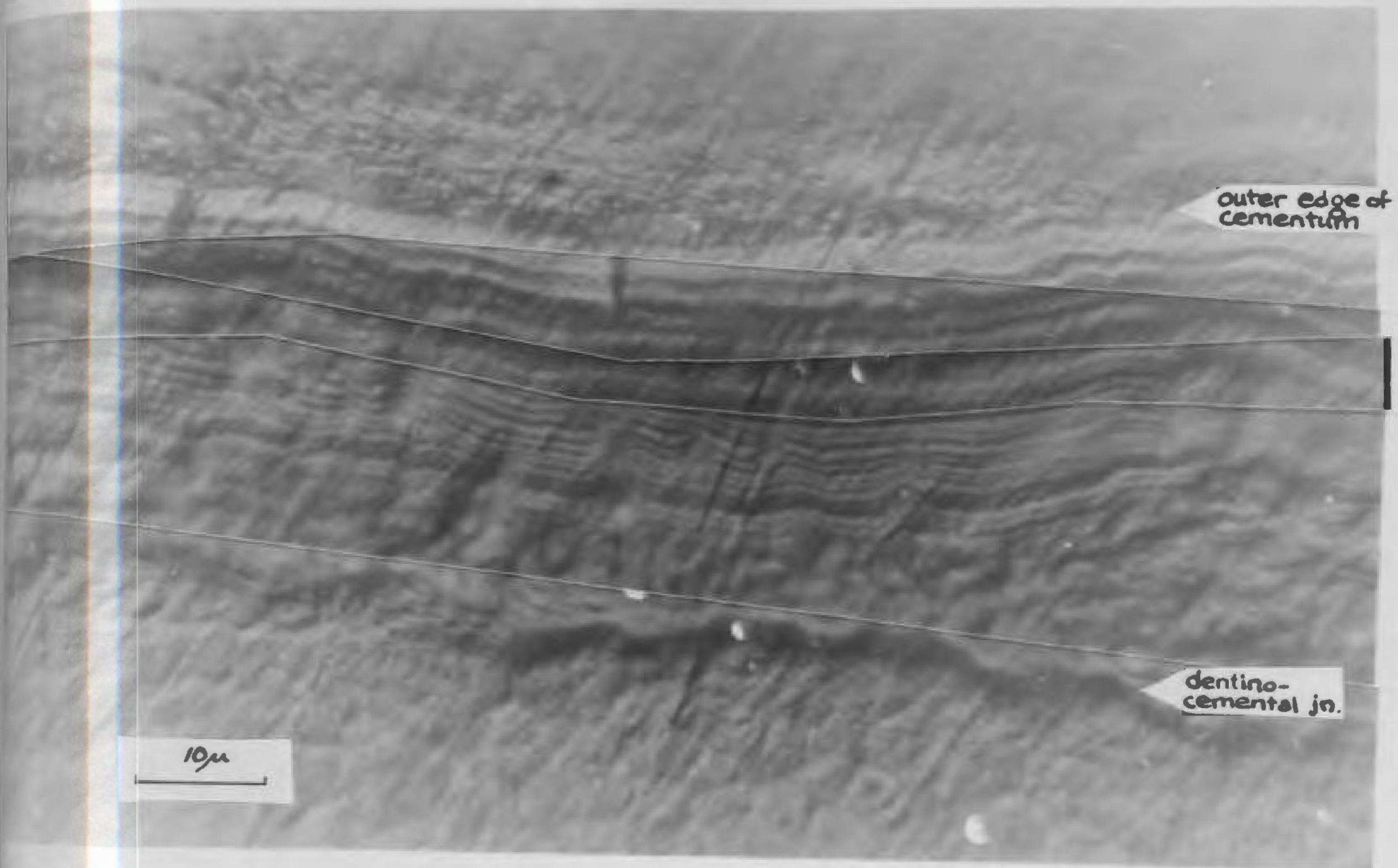


Fig. 9 : Composite photomicrograph of cementum of tooth 79
17 year old female PM^2

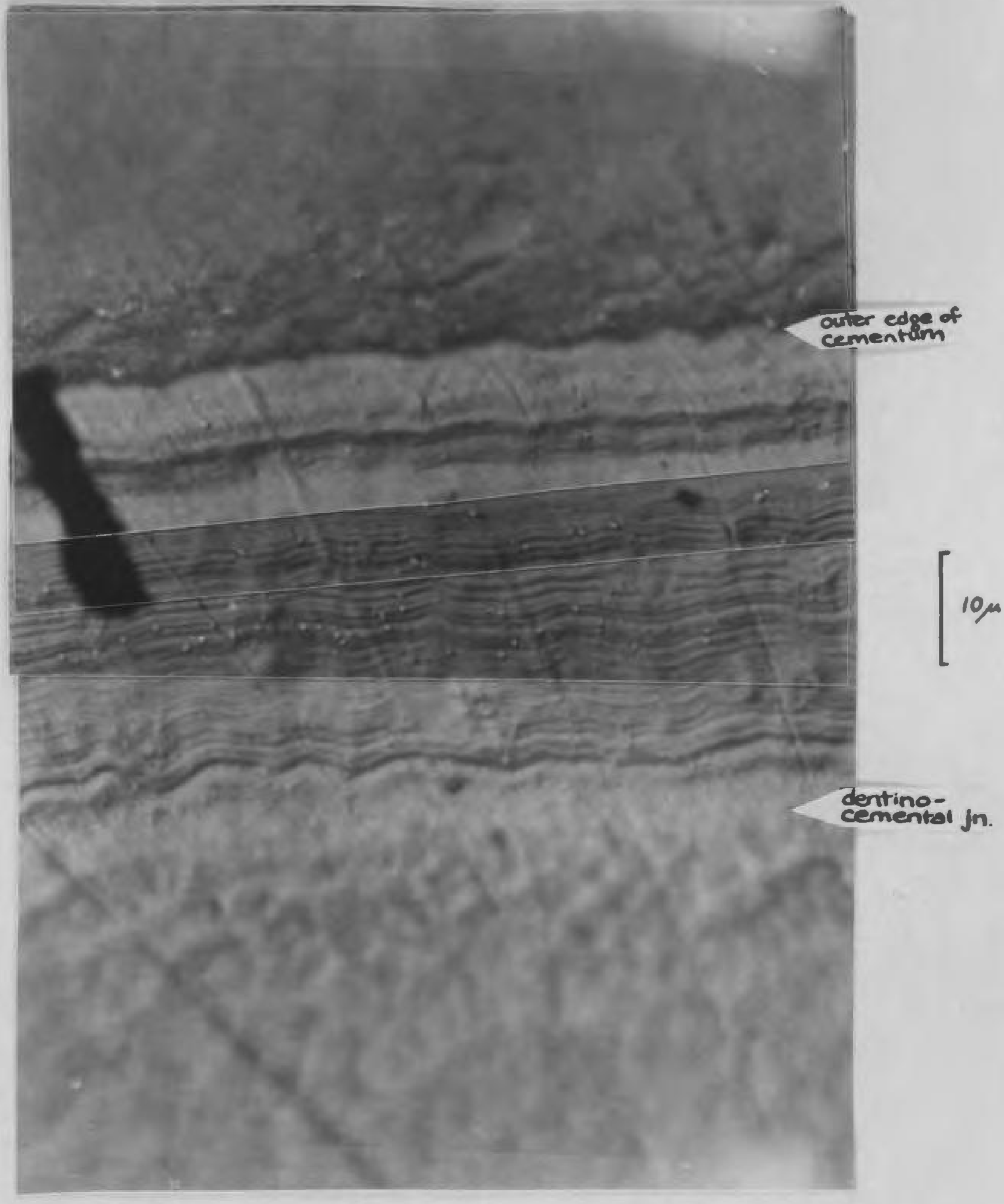


Fig.10: Composite photomicrograph of cementum of tooth 83
25 year old female PM^1

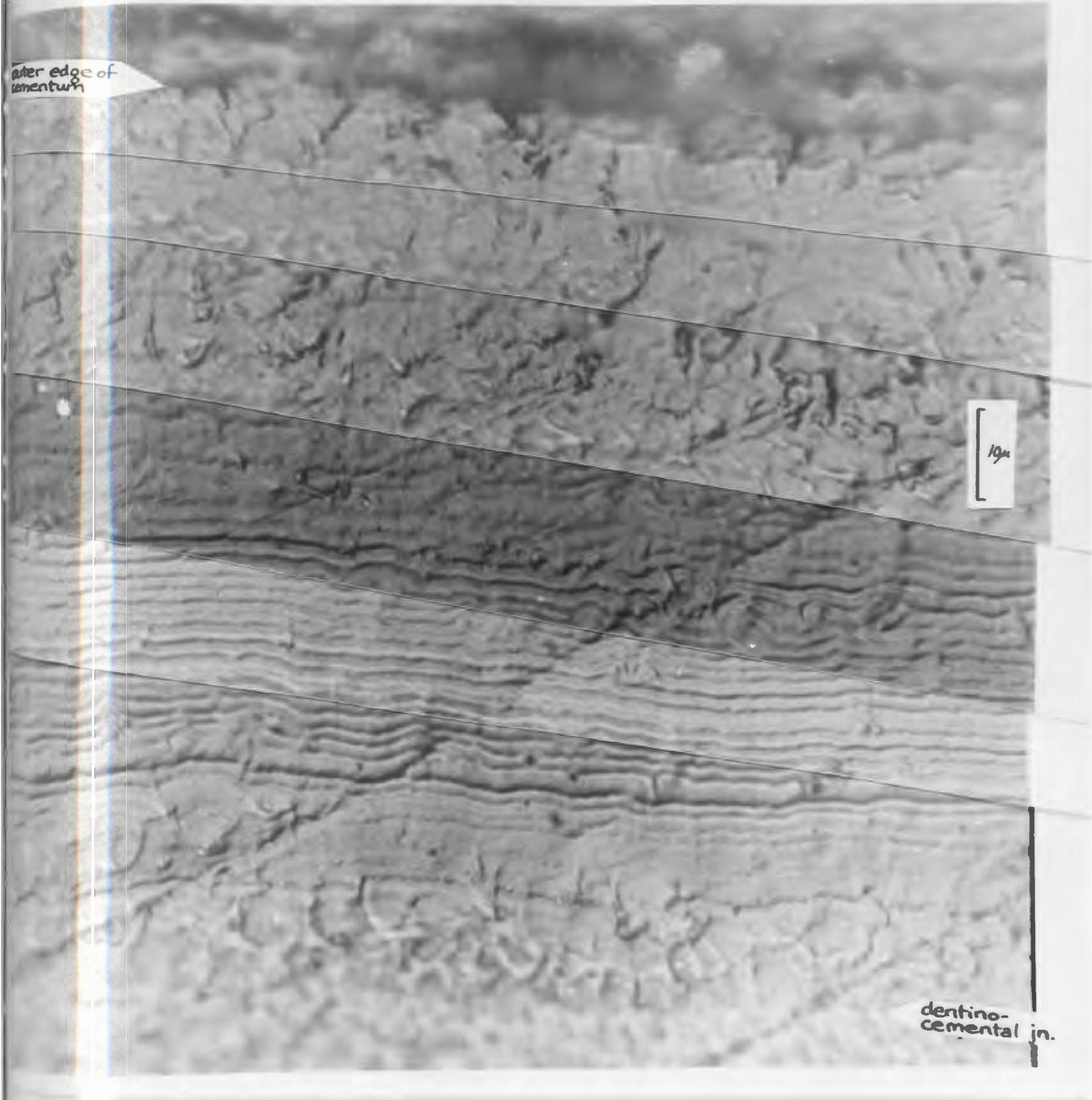


Fig. 11: Composite photomicrograph of cementum of tooth 71 28 year old male ¹I

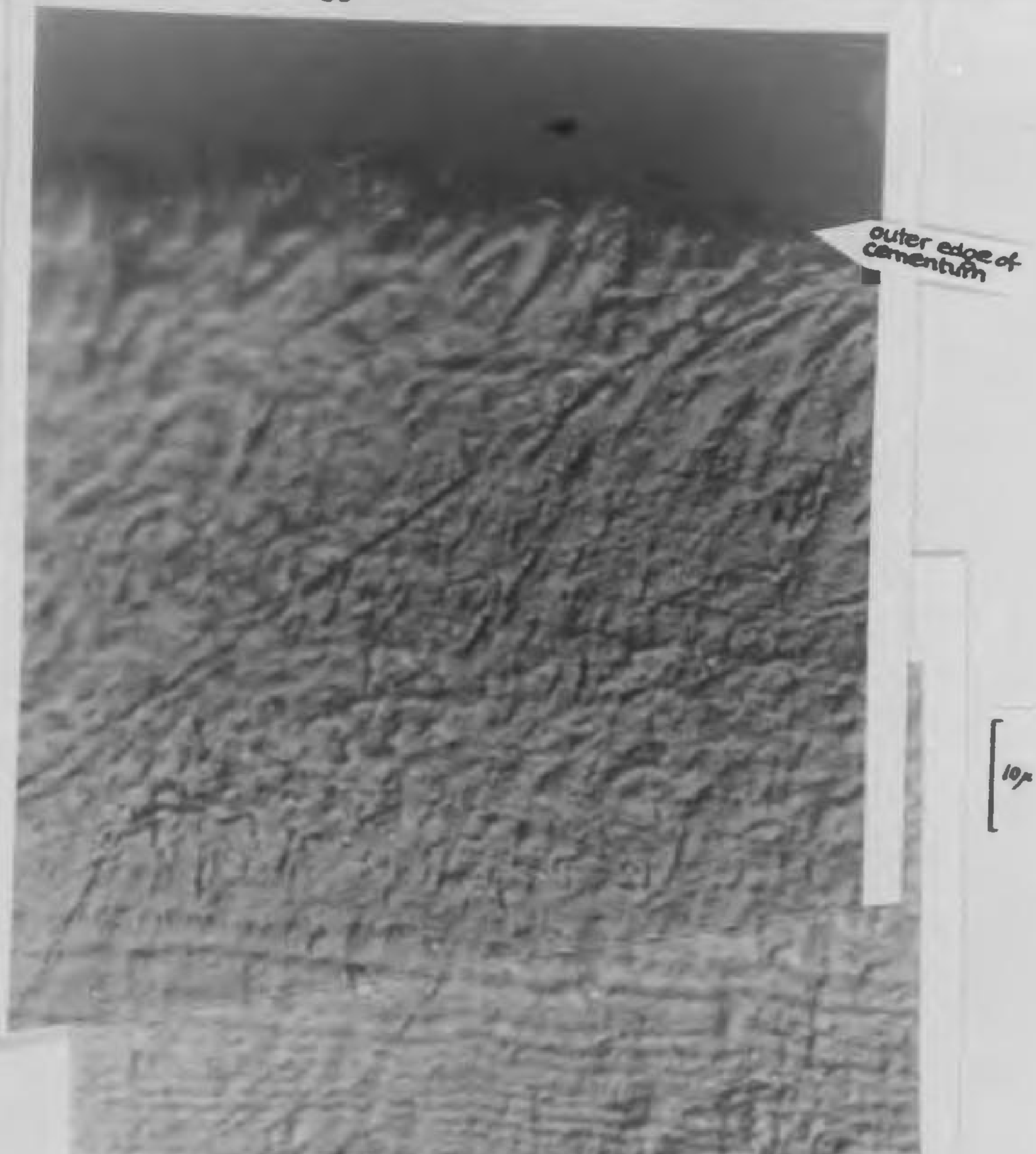


Fig. 12 : Composite photomicrograph of cementum of tooth 1968-1
55 year old male I^1

5 microns next to the dentino-cemental junction, cementum on teeth from younger individuals (up to 25 years of cementum deposition) almost always show lamellae throughout the entire cementum thickness (see Figure 8). The cementum from the teeth of older people almost always has an outer thickness of cementum which shows no lamellae at all or only an occasional lamella(see Figure 12). The etched surface of this outer cementum which shows few lamellae is generally rougher than that of the inner cementum where lamellae can be seen more clearly. This overall roughness is unlike the localized roughness caused by the presence of uncalcified Sharpey fibres. The latter occur throughout the cementum thickness. Their dimensions and presence seem to be unrelated to the presence or absence of lamellae, the degree of definition of lamellae or the overall roughness or smoothness of the cementum surrounding these structures.

The presence of uncalcified Sharpey fibres does disrupt the lamellae to some extent in that the lamellae tend to bulge, wave or curve temporarily when passing through them. The straightest lamellae can be seen where the uncalcified Sharpey fibres are very faintly expressed. But the degree of definition of the lamellae (i.e. the clarity of their boundaries) is not affected by the presence or absence of uncalcified Sharpey fibres, their size or degree of expression.

The number of lamellae clearly seen in the photographs of etched cementum does not equal the number of years of cementum deposition for any of the 27 teeth in this sample. Moreover, straight observation of the photographs is unsatisfactory as a means of counting lamellae. In most sections there are simply too many faintly defined lamellae or areas where lamellae are not visible at all.

A more useful way of counting the lamellae present is to plot the lamellae onto a graph with one axis representing the number of lamellae actually seen in the cementum and the other axis representing the cumulative thickness of the lamellae (see Figure 8). In this way, the approximate thickness of each lamella is represented on the graph. One can also see where, in the cementum thickness, the clearly visible lamellae occur and where the areas showing no lamellae occur.

Separate graphs for each tooth (except the two archeological samples) are included in this chapter (see Figures 13-30). They are arranged chronologically by age. Graphs of teeth from the same mouth are arranged together in one figure, so the pattern of cemental lamellae can be compared more easily.

In concluding we can say that the existence of identifiable cemental lamellae has been demonstrated. We can also say that there is not a simple one to one relationship between the number of years of cementum deposition and the number of lamellae present. More complex correlations between the number of cemental lamellae and the number of years of cementum deposition will be examined in the following chapter.

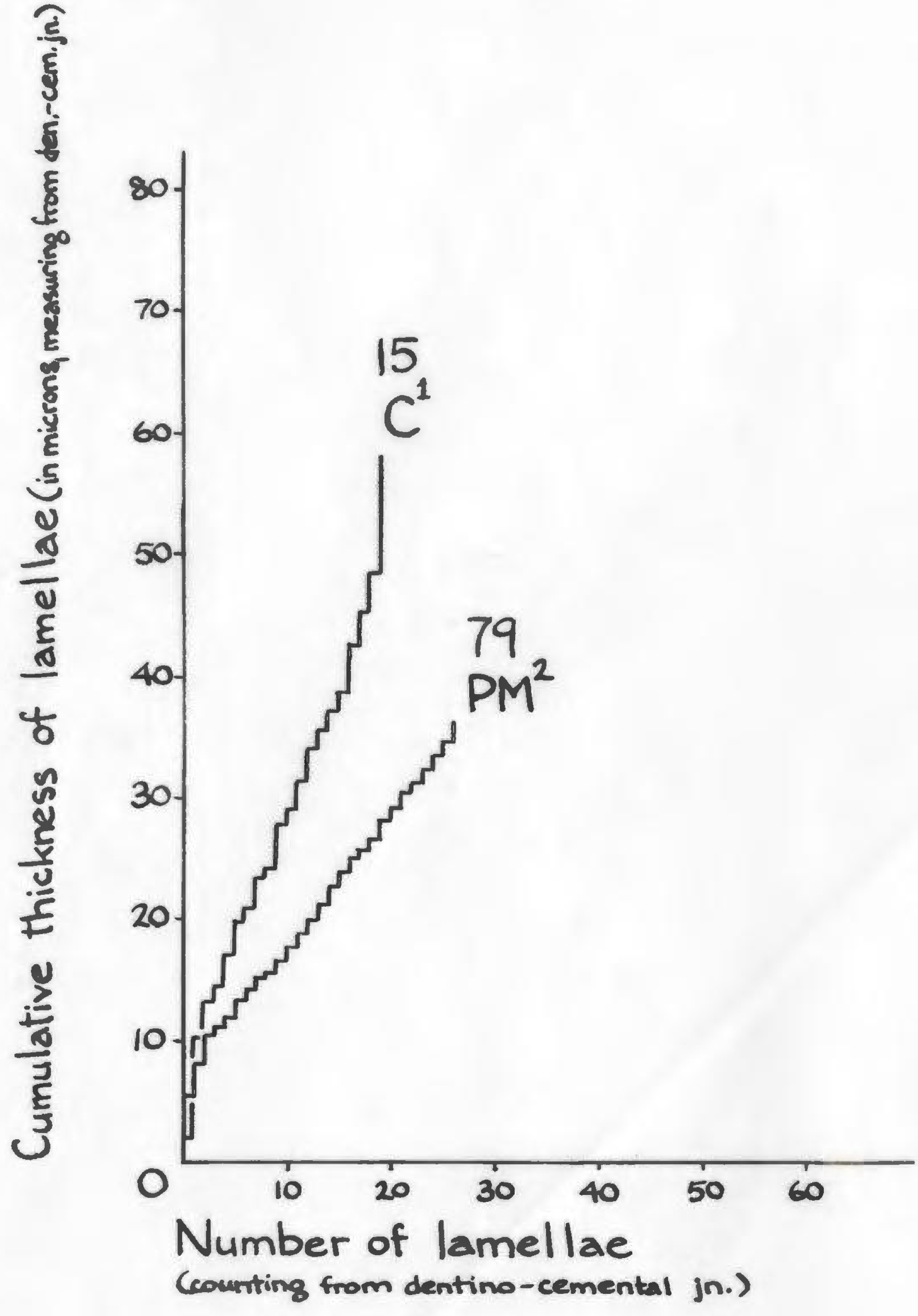


Fig13 : Number vs. cumulative thickness of lamellae in cementum of tooth 79 and tooth 15
17 year old female

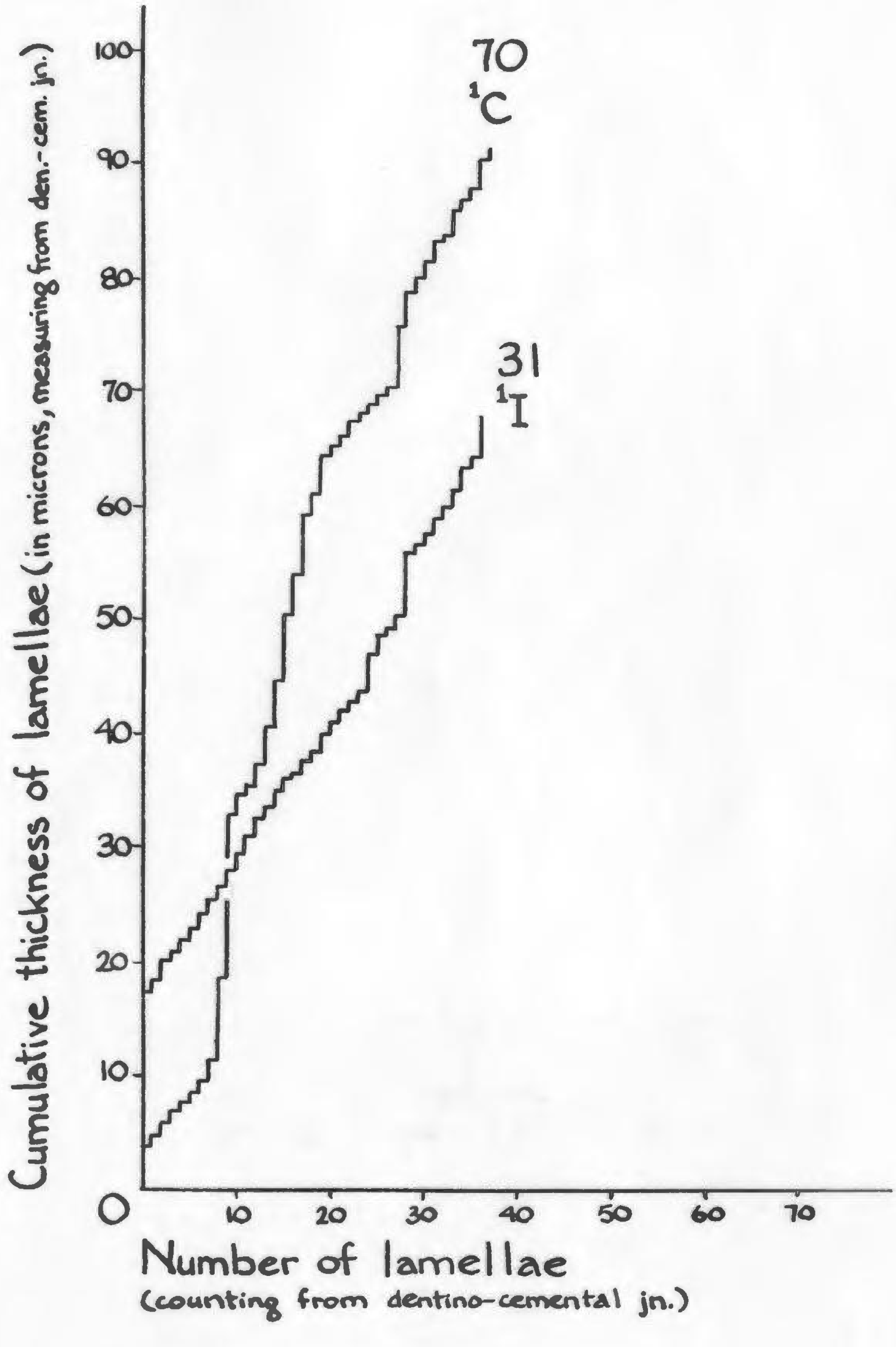


Fig.14: Number vs. cumulative thickness of lamellae in cementum of tooth 70 and tooth 31
18 year old male

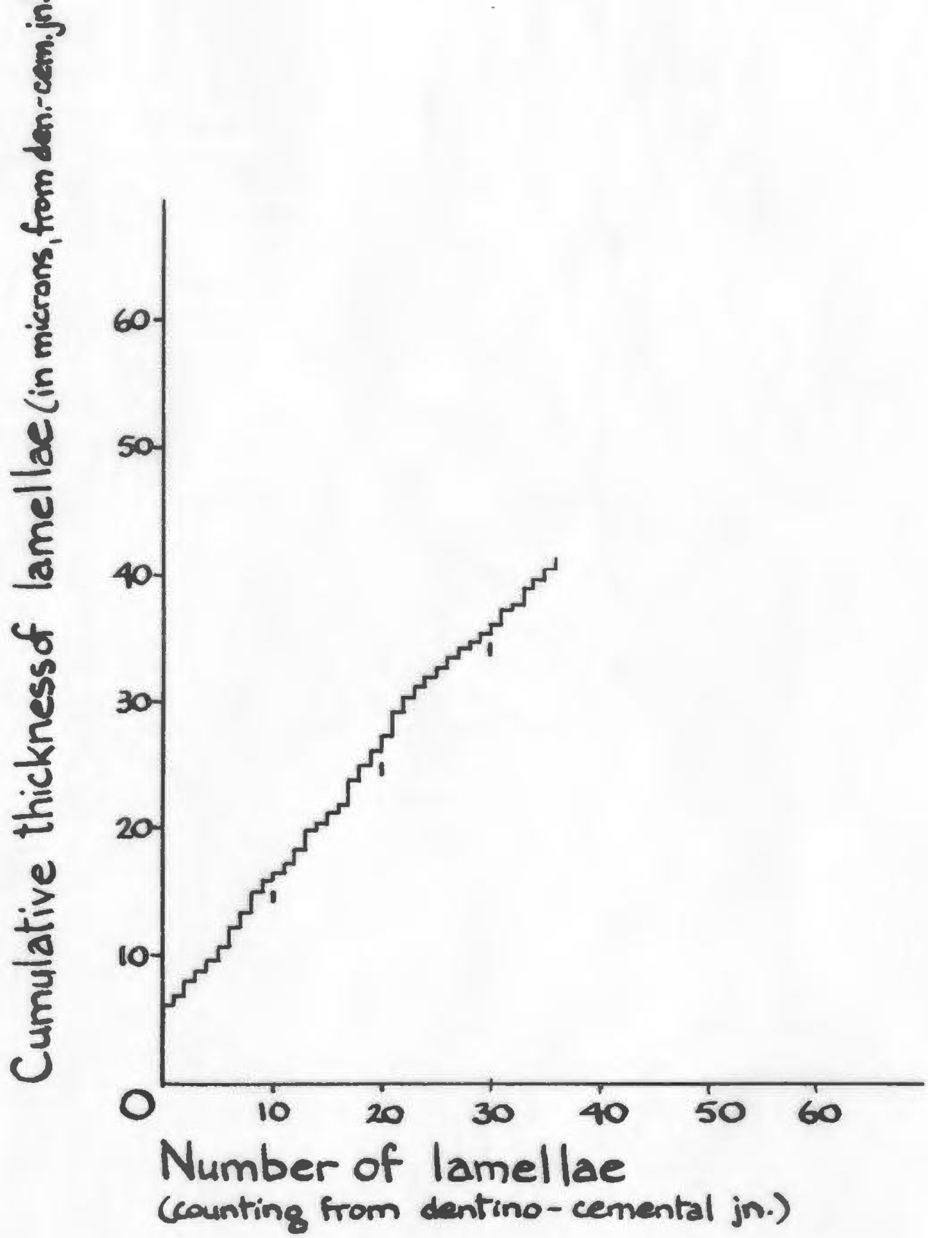


Fig. 15: Number vs. cumulative thickness of lamellae in cementum of tooth 21
20 year old female [1]PM

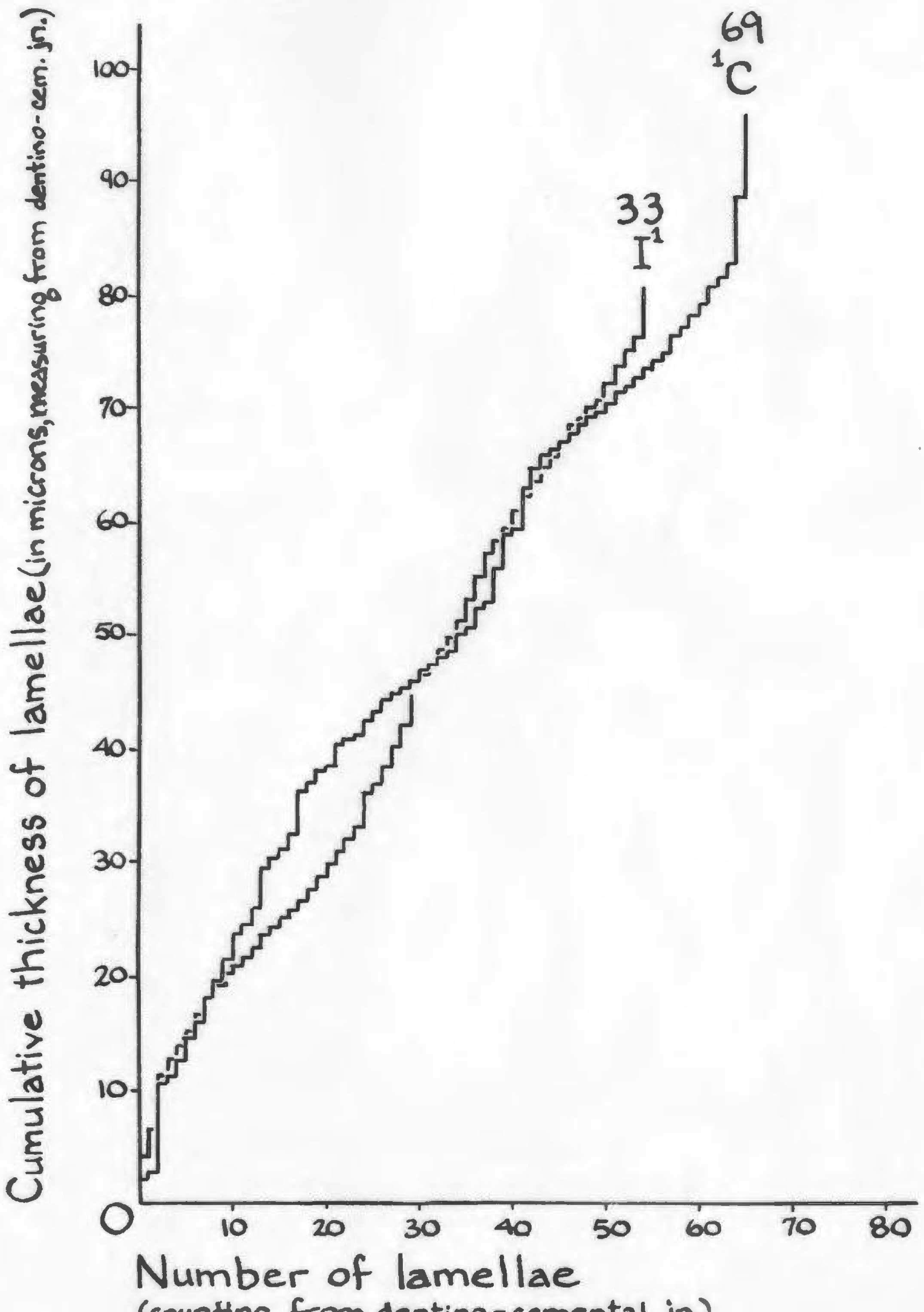


Fig. 16: Number vs. cumulative thickness of lamellae in cementum of tooth 33 and tooth 69
22 year old male

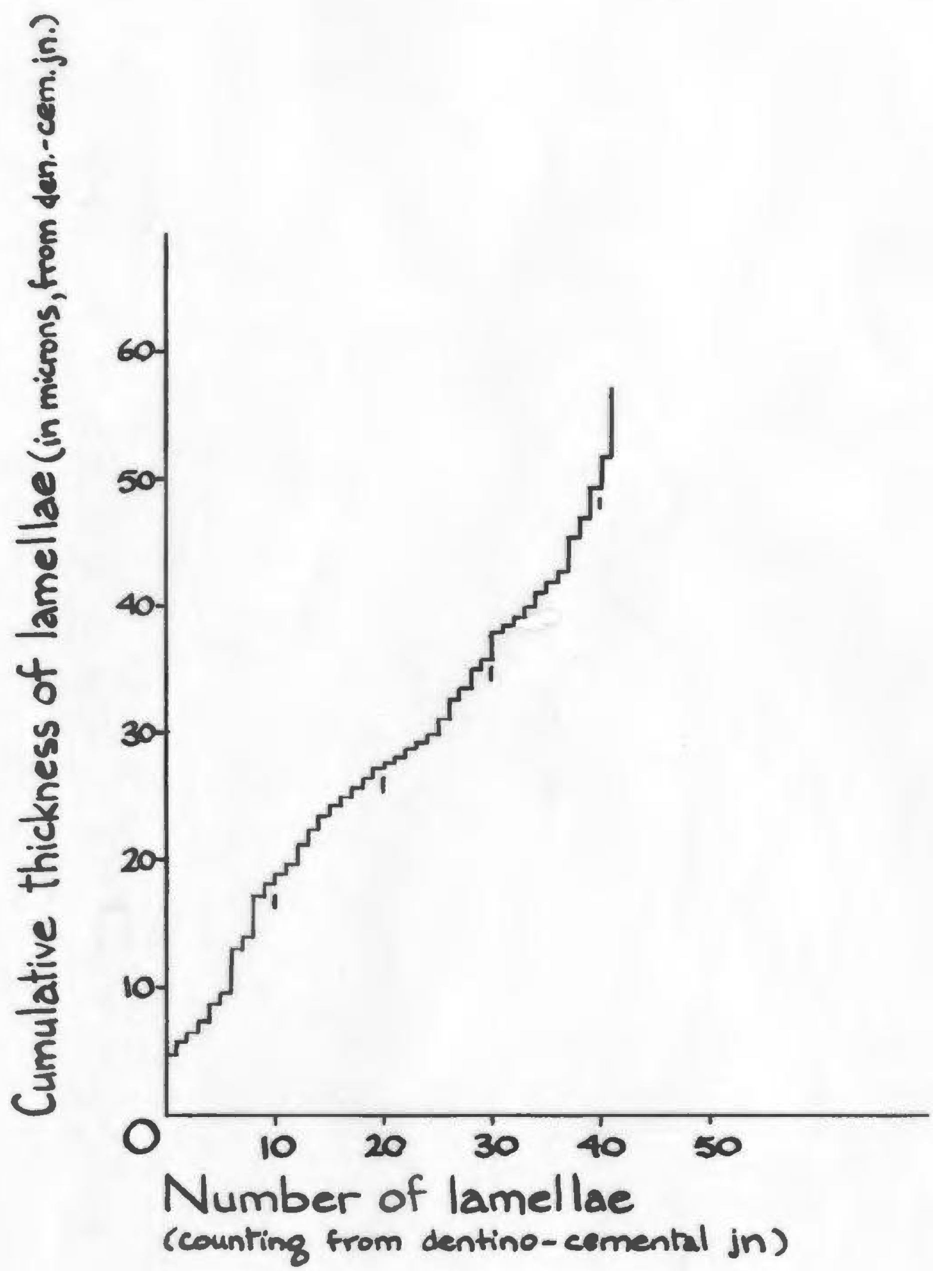


Fig. 17: Number vs. cumulative thickness of lamellae
in cementum of tooth 82
24 year old female ^{2}PM

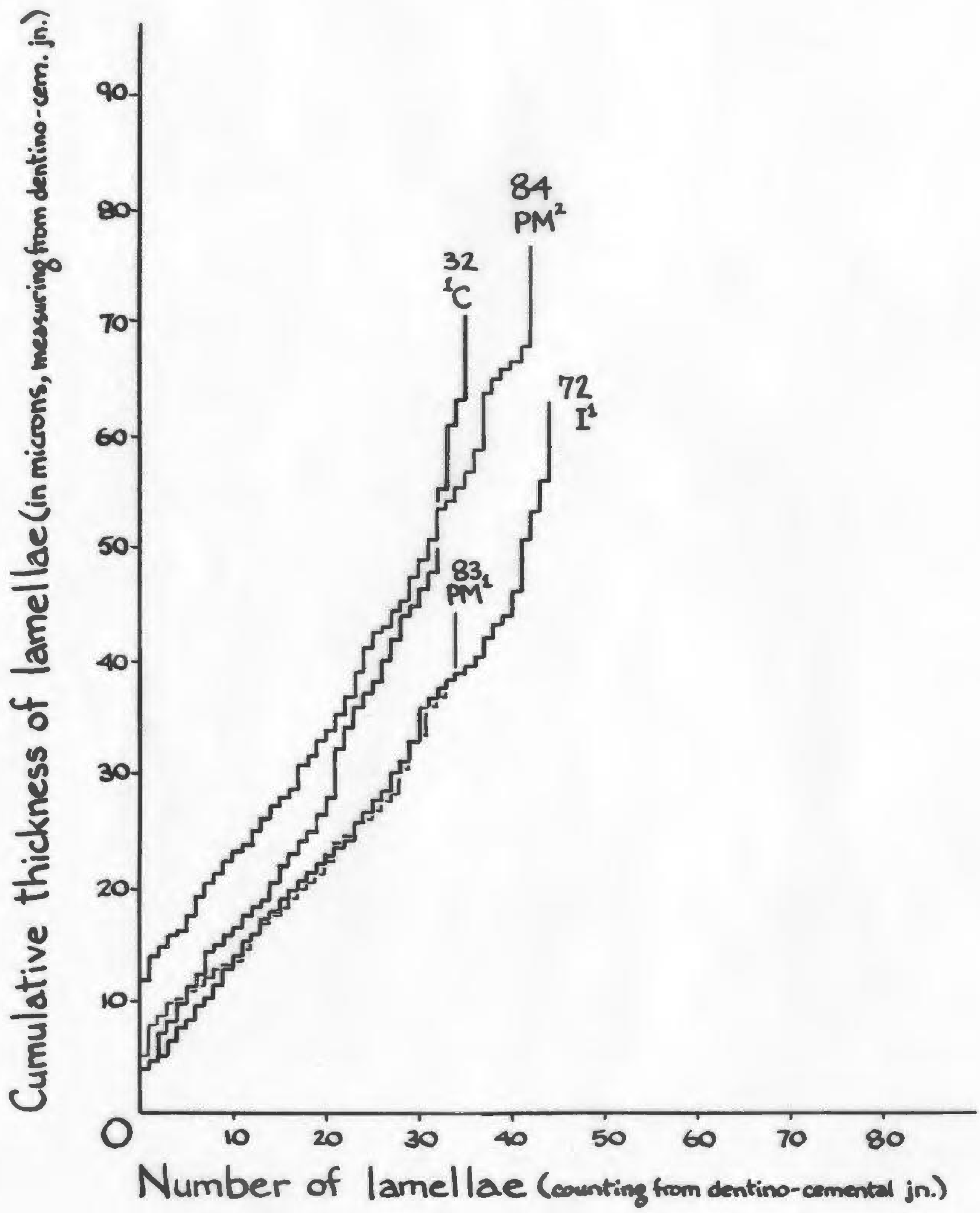


Fig. 18 : Number vs. cumulative thickness of lamellae in cementum of teeth 32, 72, 83 and 84
25 year old female

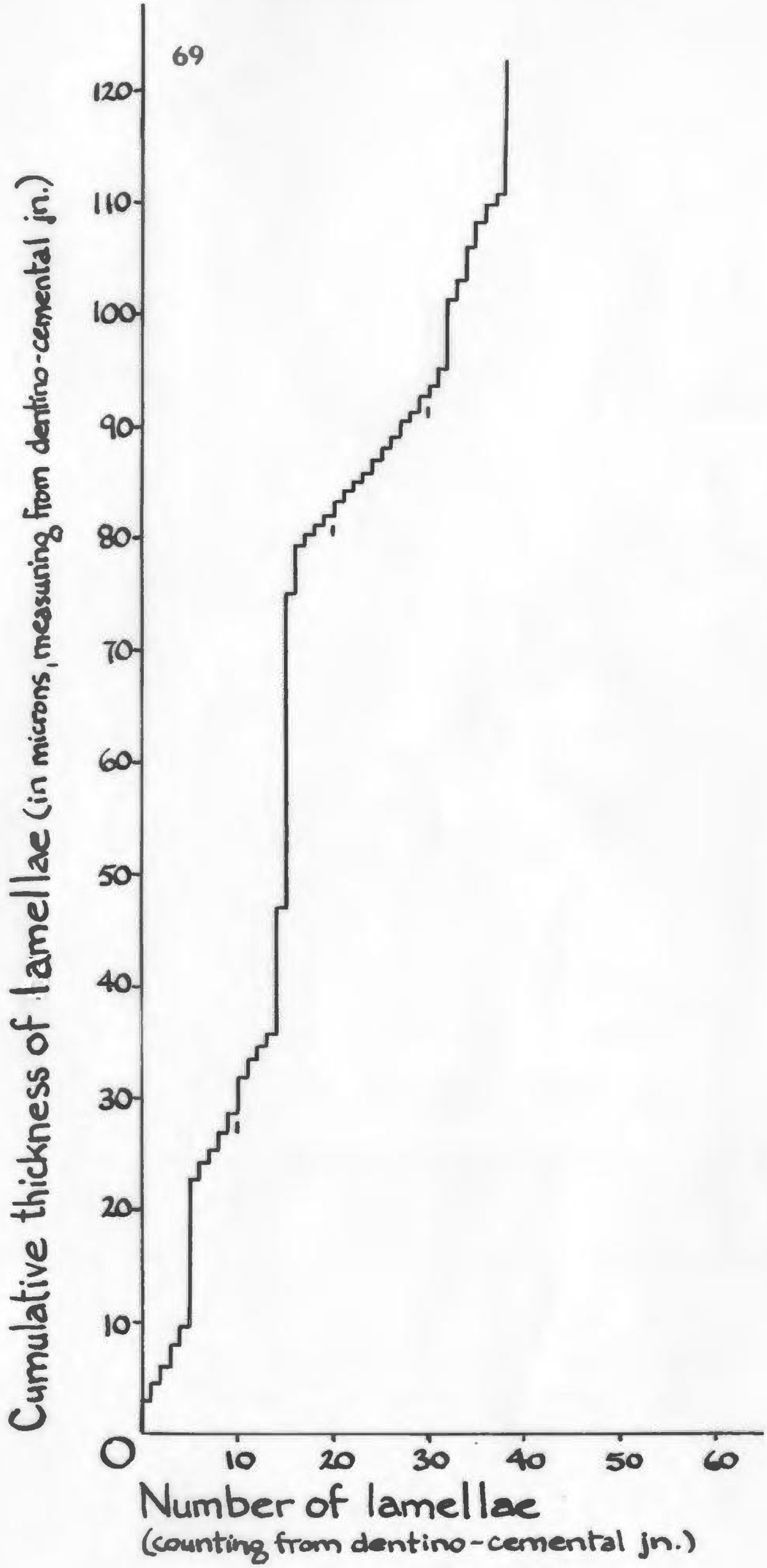


Fig.19: Number vs. cumulative thickness of lamellae in cementum of tooth 20
25 year old male C^1

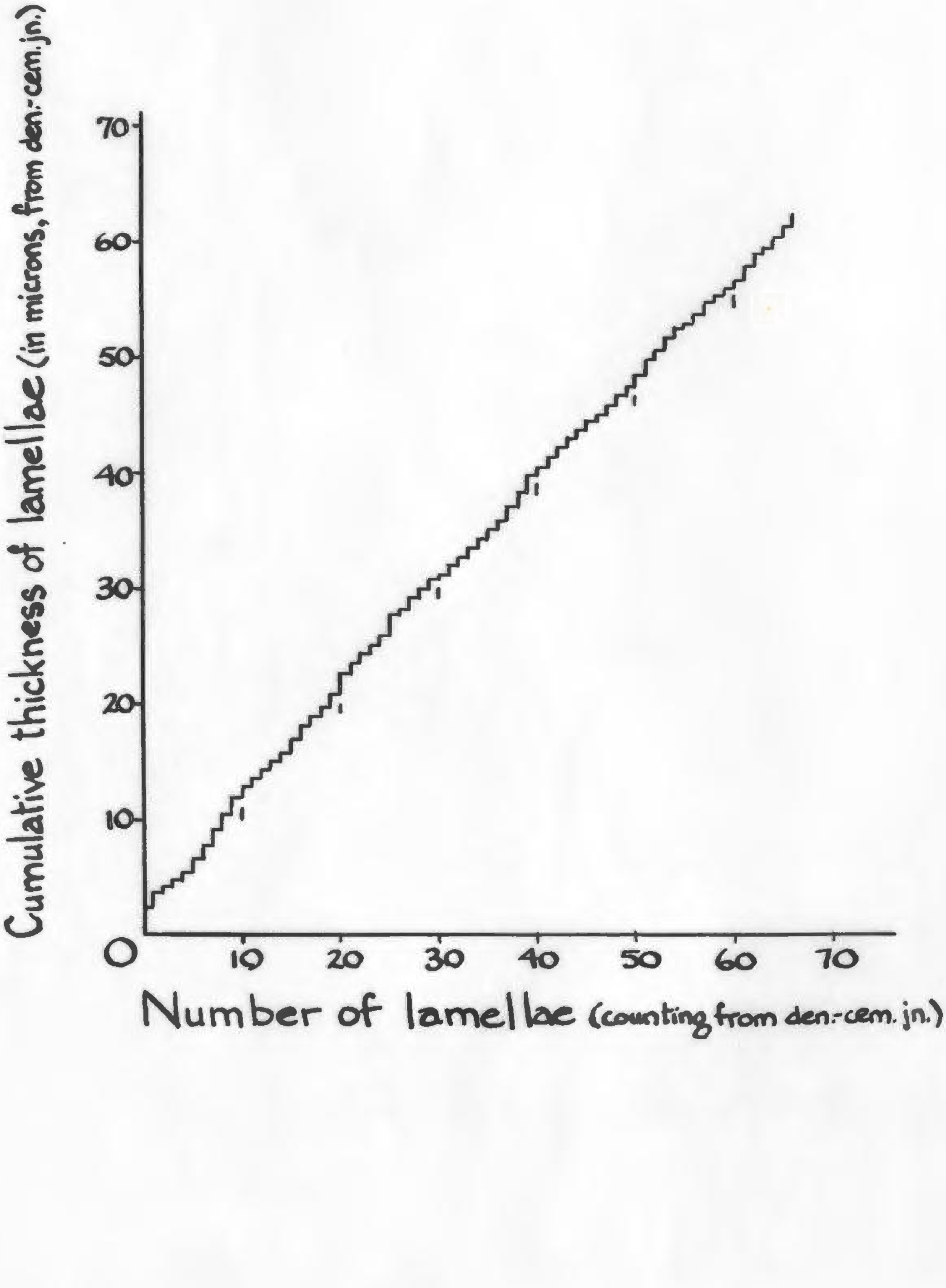


Fig.20: Number vs. cumulative thickness of lamellae in cementum of tooth 47
26 year old female C_1

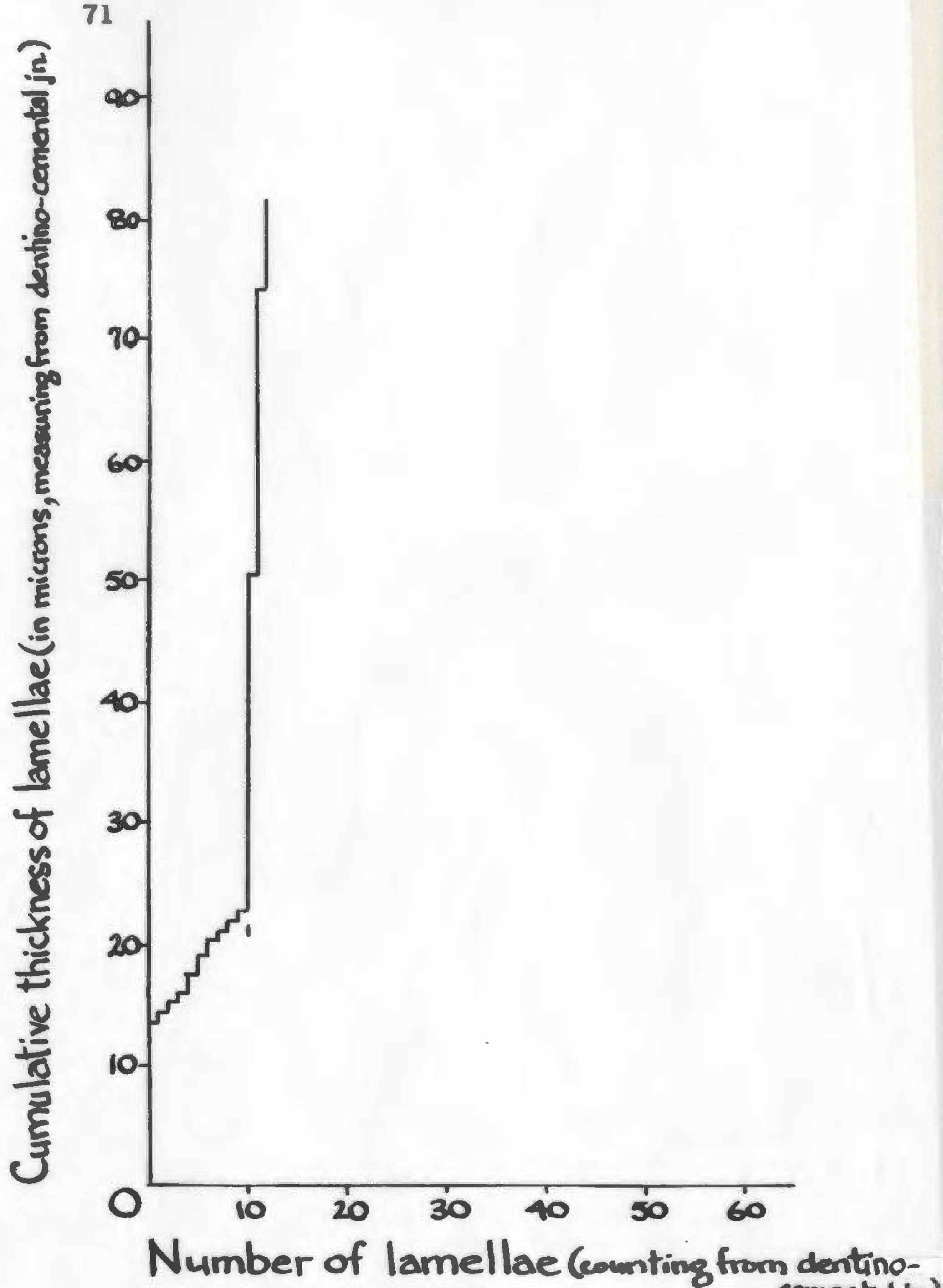


Fig. 21: Number vs. cumulative thickness of lamellae in cementum of tooth 23
26 year old male C^1

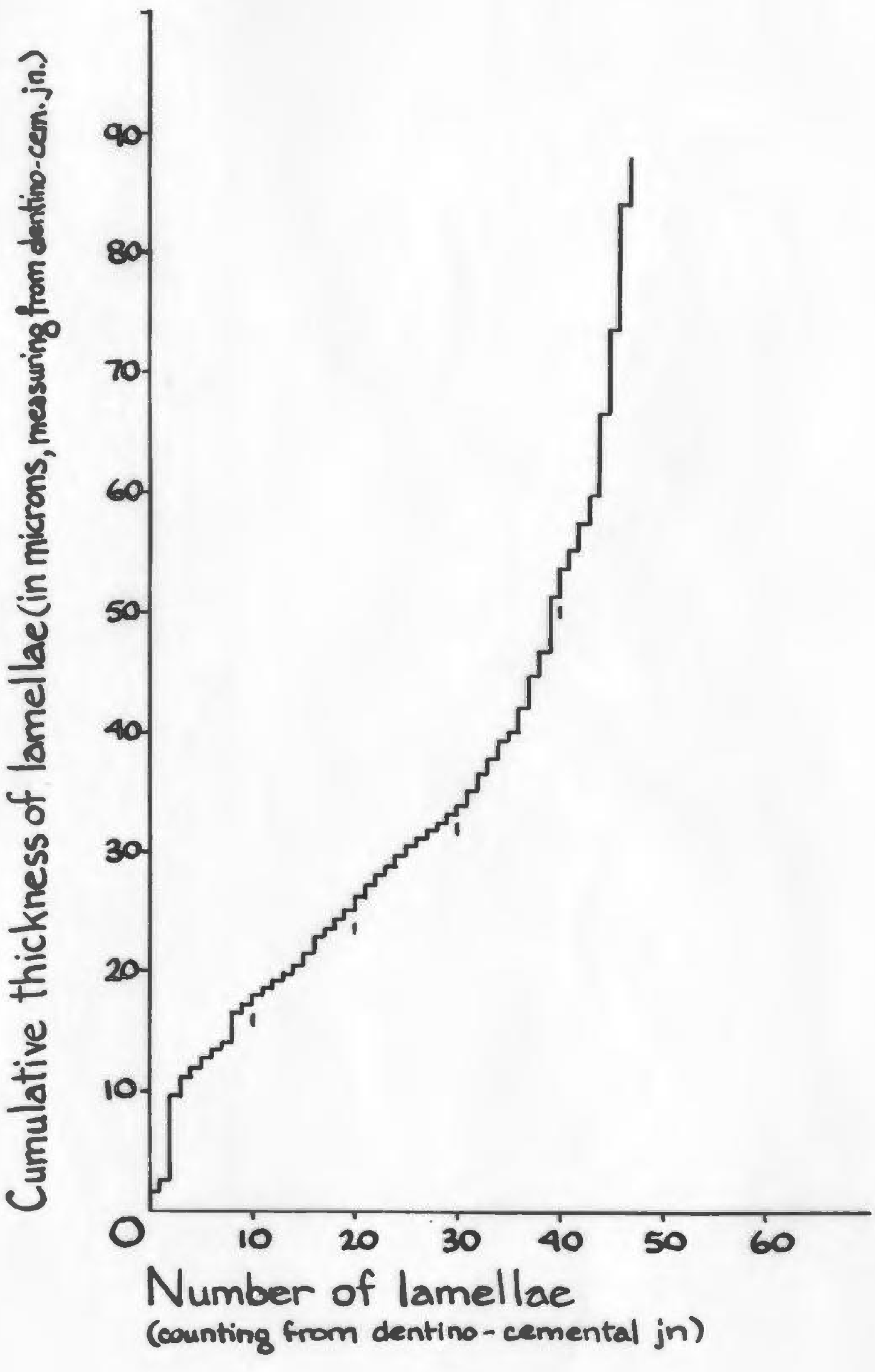


Fig.22: Number vs. cumulative thickness of lamellae in cementum of tooth 71
28 year old male ^{1}I

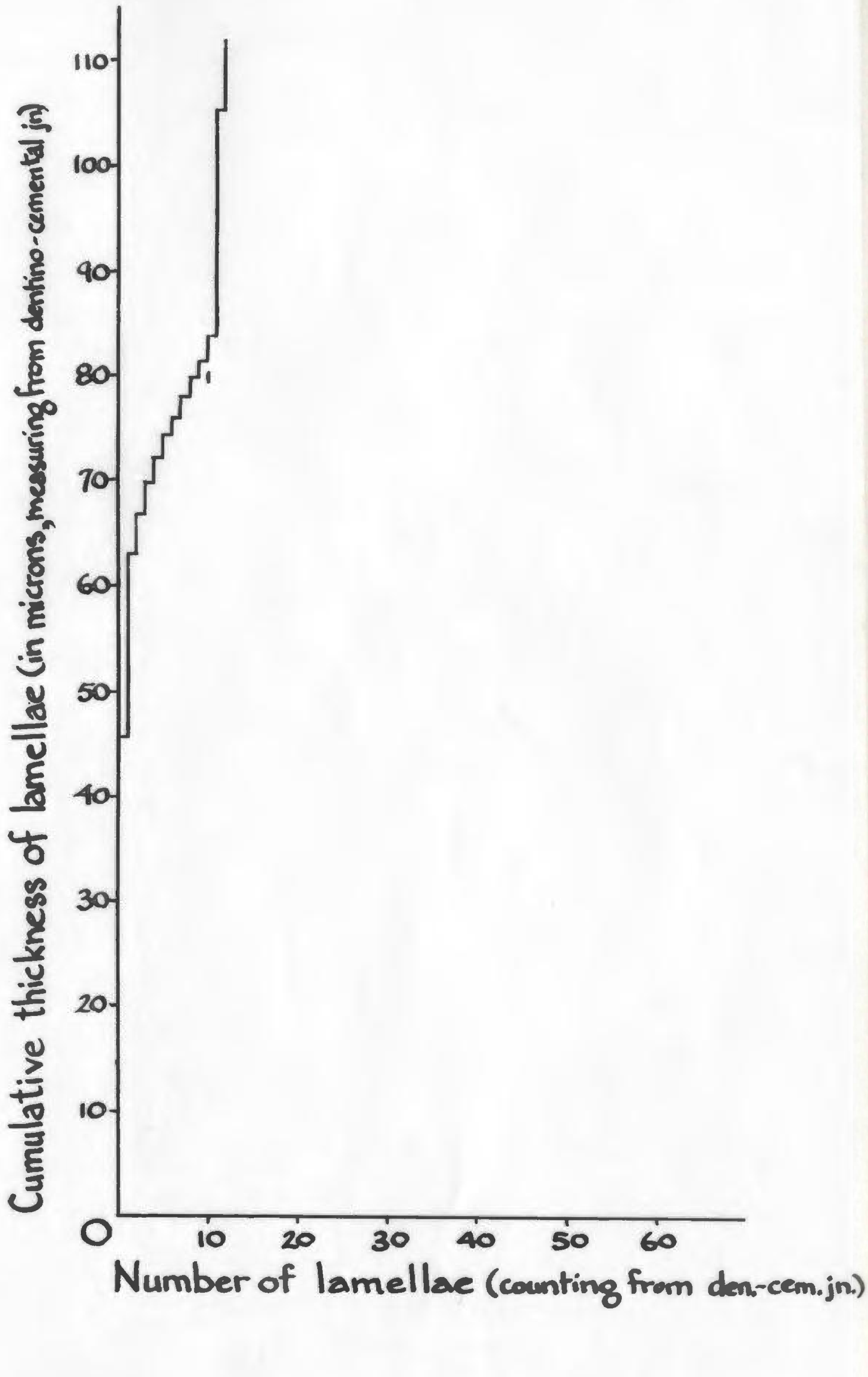


Fig. 23: Number vs. cumulative thickness of lamellae in cementum of tooth 78
31 year old male I^2

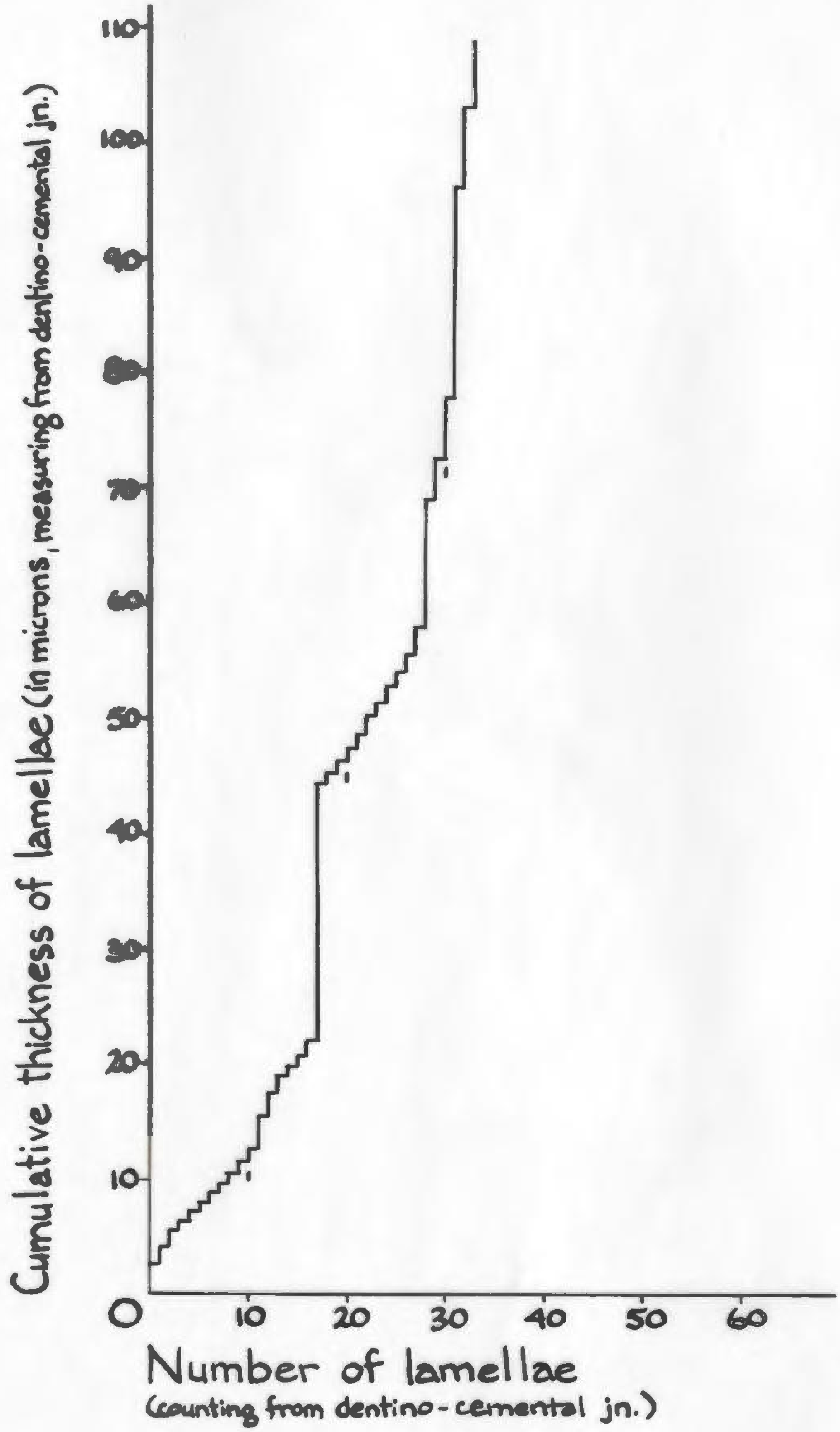


Fig.24: Number vs. cumulative thickness of lamellae in cementum of tooth 68
32 year old male ¹I

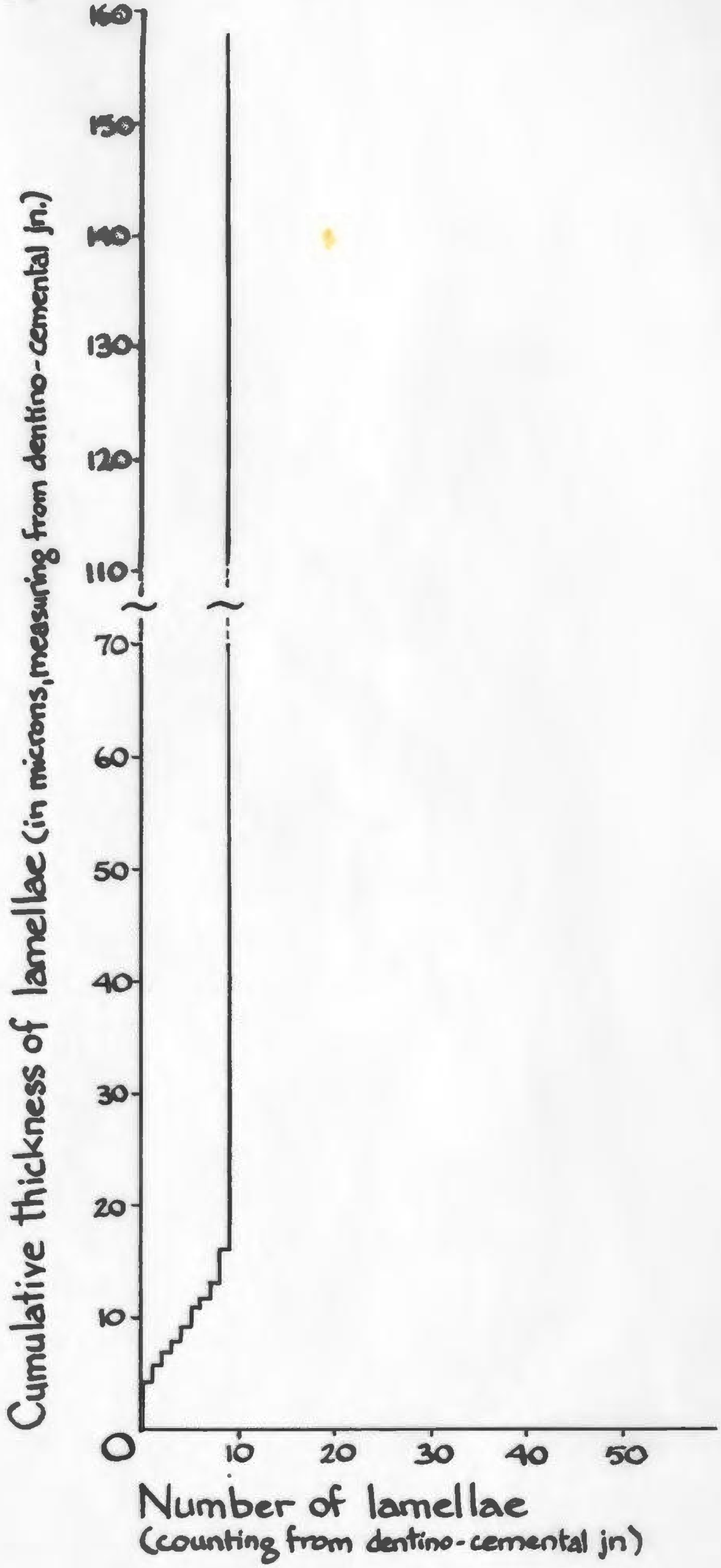


Fig. 25: Number vs. cumulative thickness of lamellae in cementum of tooth 57
50 year old male ^{1}I

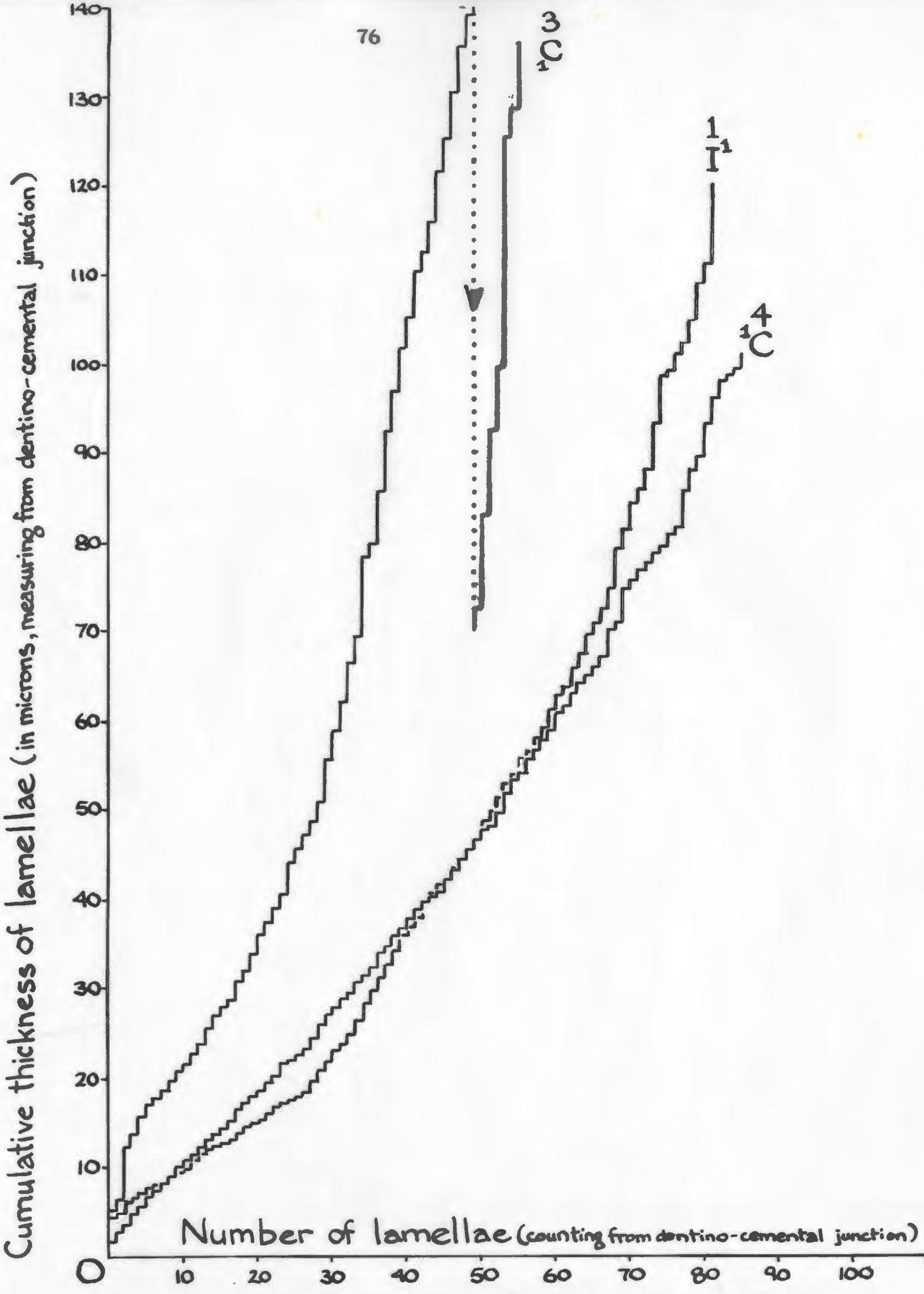


Fig.26: Number vs. cumulative thickness of lamellae in cementum of teeth 1, 3 and 4
50 year old male

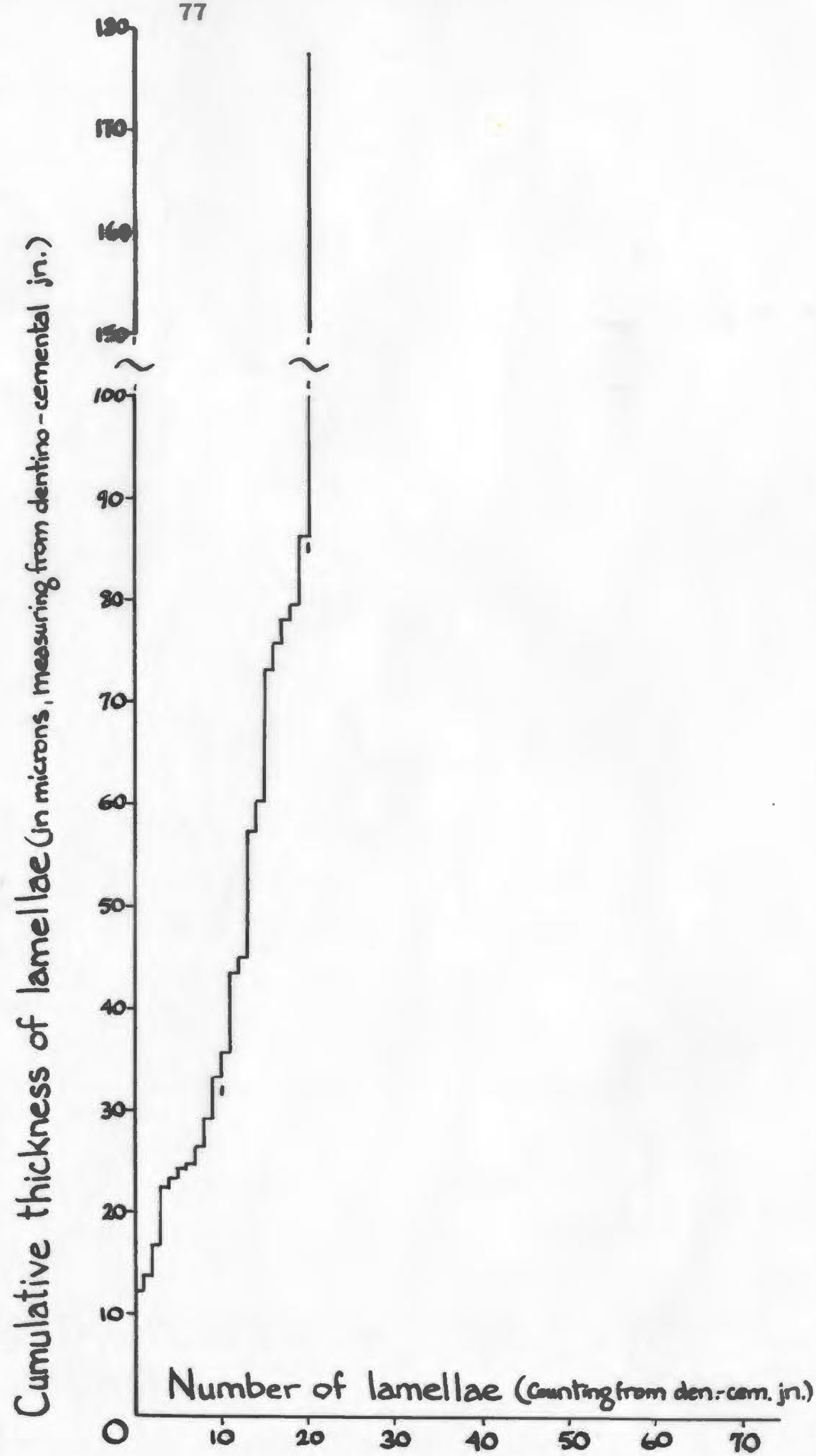


Fig.27: Number vs. cumulative thickness of lamellae in cementum of tooth 74
53 year old male C^1

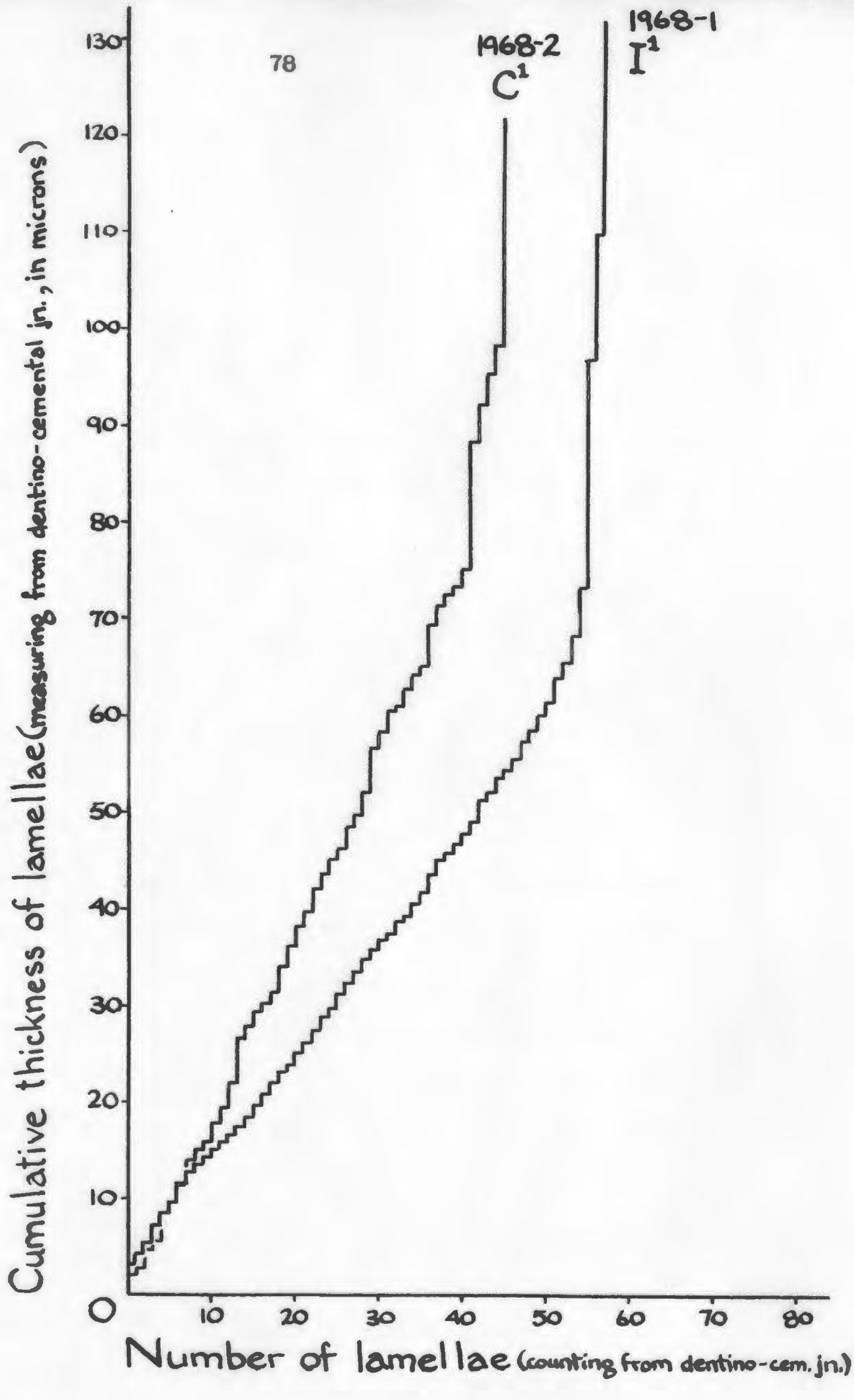


Fig.28: Number vs. cumulative thickness of lamellae in cementum of tooth 1968-1 and tooth 1968-2 55 year old male

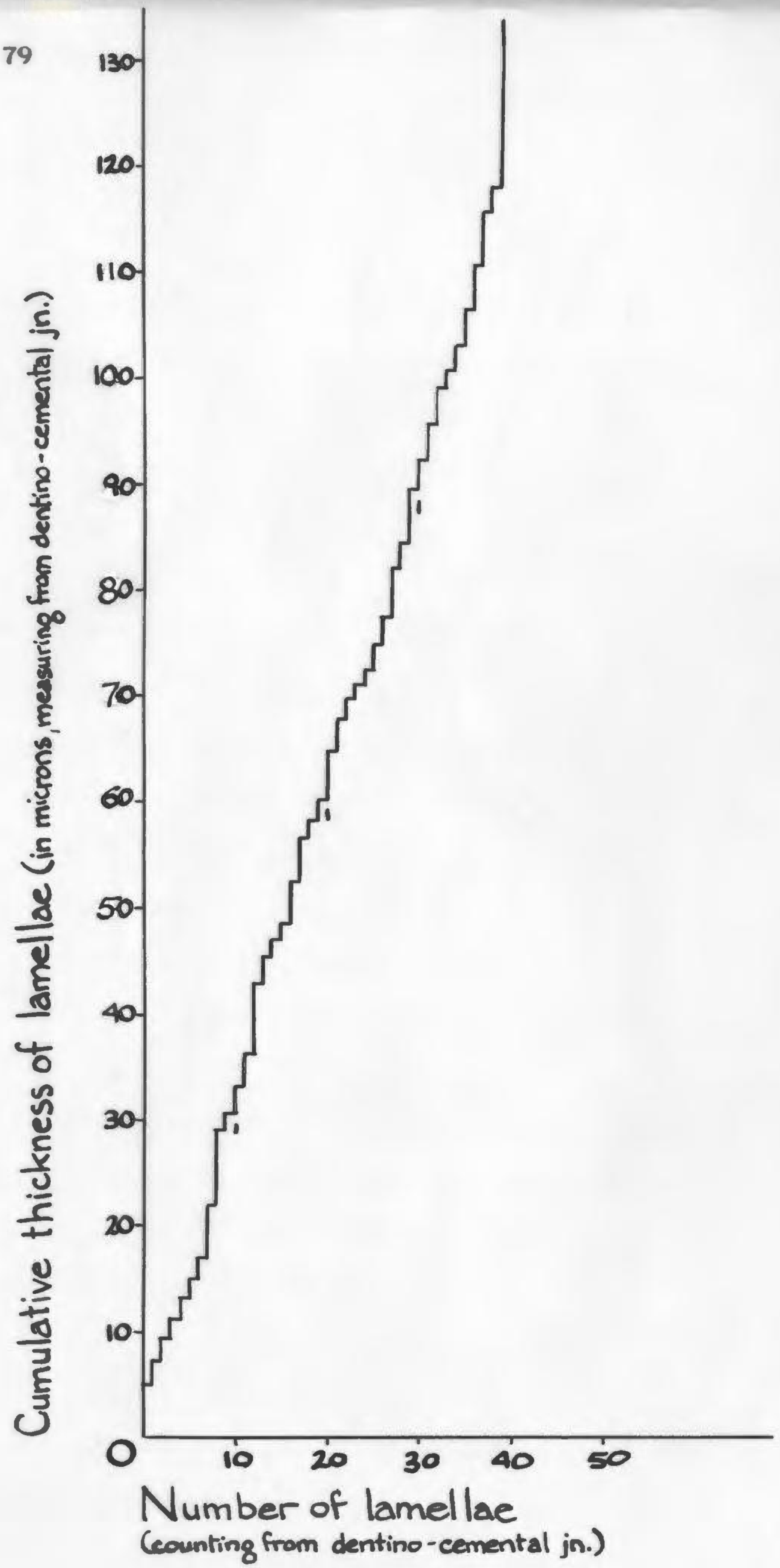


Fig.29: Number vs. cumulative thickness of lamellae in cementum of tooth 42 55 year old male I^2

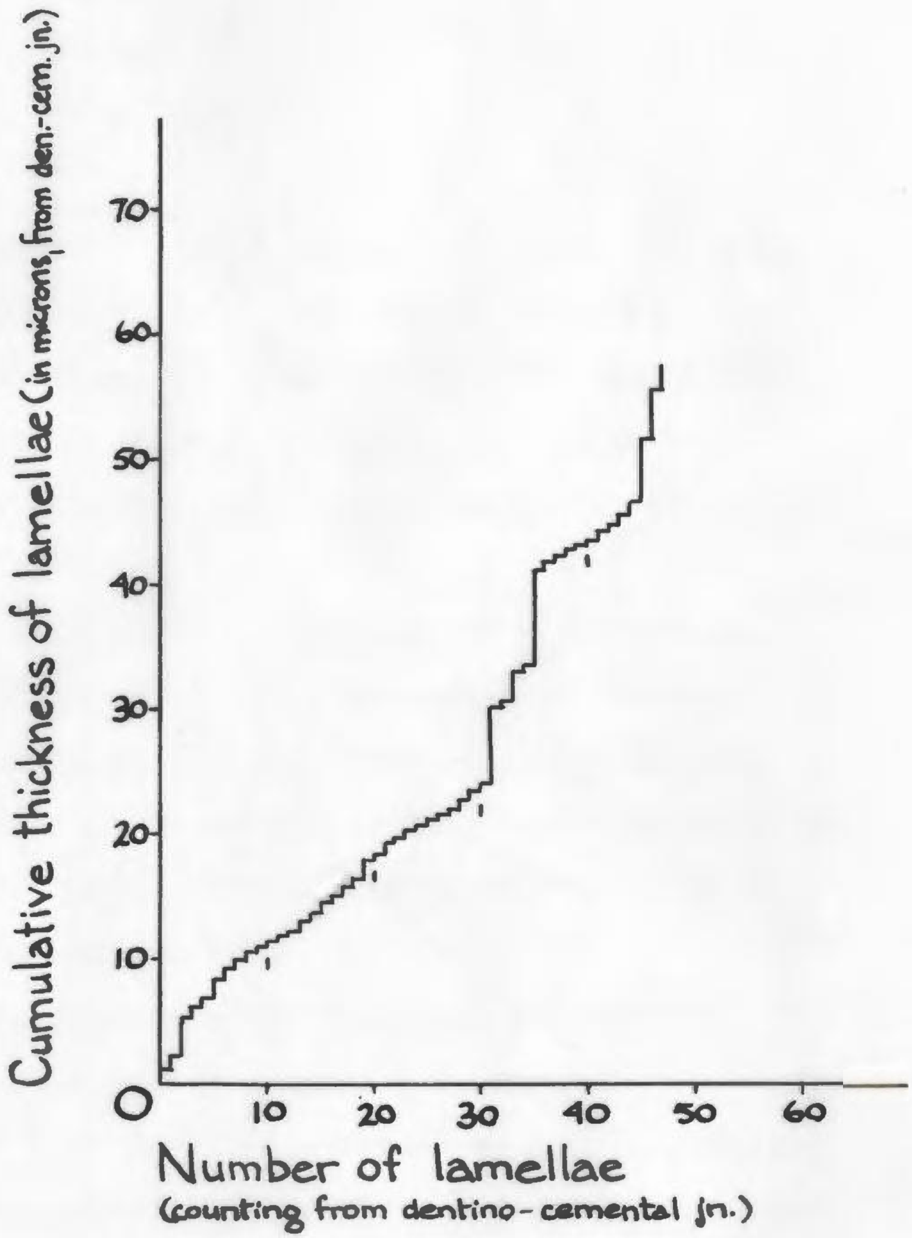


Fig. 30: Number vs. cumulative thickness of lamellae in cementum of tooth 94
67 year old male PM^1

CHAPTER V

DISCUSSION AND CONCLUSIONS

In Chapter 4 we demonstrated that lamellae are present in human acellular cementum. A simple one to one correlation between the number of lamellae present and the number of years of cementum deposition does not exist. However, this observation does not preclude the existence of a more complex relationship between these two factors.

In searching for a correlation between the number of cemental lamellae and a person's biological age, the numbers of lamellae actually seen in the photographs and recorded on the graphs (Figures 13-30) are plotted against the numbers of years of cementum deposition in Figure 31. Numerical values for the lamellae actually seen in each tooth are given in Table 5, column 6.

Observing Figure 31, we can see that a tooth's age cannot be predicted from a simple count of the lamellae present. However, the data points for the first 20 years of cementum deposition do suggest some relationship between the number of years of cementum deposition and the number of lamellae. This is not true for older age groups. The solid line in Figure 31 is a least squares line (constrained to pass through zero - Youden, 1951:47) for the data points in the first 20 years of deposition. We find that an average of 3.06 (standard deviation = 0.31) lamellae would have to be deposited per year for the data points to fall on that line. If a similar least squares line is drawn for the female data points only (the dashed line in Figure 31), the resulting number of lamellae per year is 3.13 (standard deviation = 0.22).

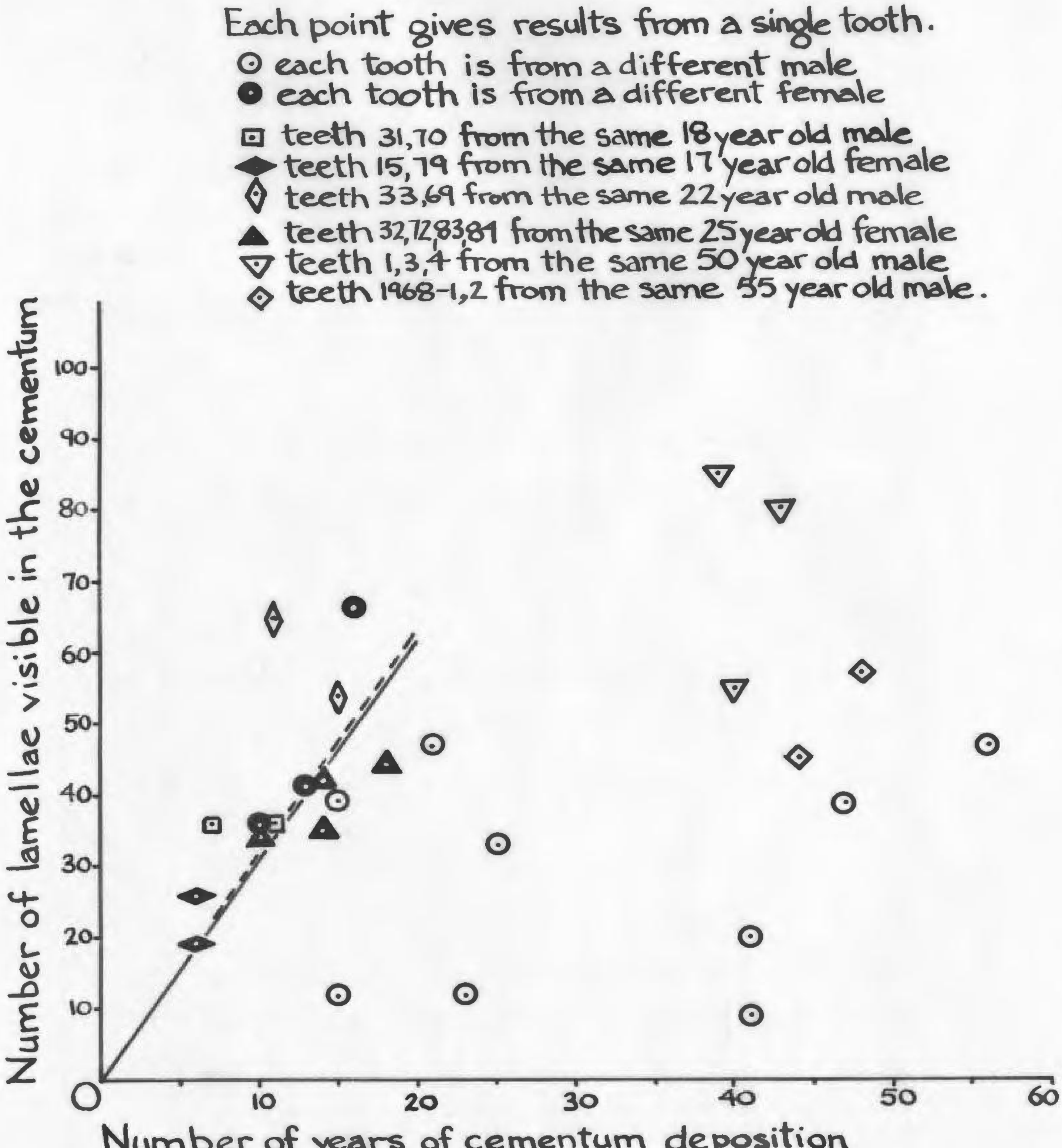


Fig. 31: Number of years of cementum deposition vs. number of visible lamellae in cementum.

Table 5. Numerical Values for Data Used in Figures 31 - 35.

Tooth number	Age of person	Sex	Identity of tooth	Years of deposition	cementum thickness in microns	Number of visible lamellae.	Number of estimated lamellae (corrected for large jumps)	Average lamella thickness in microns	Number of lamellae estimated from cementum thickness ÷ av. lamella thickness.
79	17	F	PM	6	36.3	26 (4.3 p.a.)	34 (5.6 p.a.)	1.0765	33 (5.6 p.a.)
15	17	F	C	6	57.9	19 (3.1 p.a.)	27 (4.5 p.a.)	2.1053	27 (4.5 p.a.)
70	18	M	C	7	91.7	36 (5.1 p.a.)	63 (9.0 p.a.)	0.9393	97 (13.9 p.a.)
31	18	M	I	11	67.6	36 (3.2 p.a.)	57 (5.1 p.a.)	1.1470	58 (5.3 p.a.)
21	20	F	PM	10	41.3	36 (3.6 p.a.)	42 (4.2 p.a.)	0.7471	55 (5.5.p.a.)
33	22	M	I	15	80.6	54 (3.6.p.a.)	66 (4.4 p.a.)	0.8962	89 (5.9 p.a.)
69	22	M	C	11	99.7	65 (5.9 p.a.)	86 (7.8 p.a.)	0.7968	125 (11.3 p.a.)
82	24	F	PM	13	57.5	41 (3.1 p.a.)	58 (4.4 p.a.)	0.8501	67 (5.2 p.a.)
32	25	F	C	14	70.5	35 (2.5 p.a.)	52 (3.7 p.a.)	1.0932	64 (4.6 p.a.)
84	25	F	PM	14	76.9	42 (3.0 p.a.)	62 (4.4 p.a.)	0.9194	83 (5.9 p.a.)
72	25	F	I	18	62.7	44 (2.4 p.a.)	63 (3.5 p.a.)	0.9705	64 (3.5 p.a.)
83	25	F	PM	10	45.8	34 (3.4 p.a.)	51 (5.1 p.a.)	0.7708	59 (5.9 p.a.)
20	25	M	C	15	122,8	39 (2.6 p.a.)	80 (5.3 p.a.)	1.0158	120 (8.0 p.a.)
47	26	F	C	16	62.6	66 (4.1 p.a.)		0.7729	80 (5.0 p.a.)
23	26	M	C	16	81.7	12 (0.7 p.a.)	67 (4.1 p.a.)	1.0634	76 (4.8 p.a.)
71	28	M	I	21	88.1	47 (2.2 p.a.)	72 (3.4 p.a.)	0.7690	114 (5.4 p.a.)
78	31	M	I	23	112.0	12 (0.5 p.a.)	50 (2.1 p.a.)	1.7712	63 (2.7 p.a.)
68	32	M	I	25	108.9	33 (1.3 p.a.)	96 (3.8 p.a.)	0.8646	126 (5.0 p.a.)
57	50	M	I	42	157.6	9 (0.2 p.a.)	138 (3.2 p.a.)	1.1538	136 (3.2 p.a.)
3	50	M	C	40	206.5	55 (1.3 p.a.)	140 (3.5 p.a.)	0.9328	221 (5.5 p.a.)
4	50	M	C	39	100.8	85 (2.1 p.a.)	99 (2.5 p.a.)	1.1312	89 (2.2 p.a.)
1	50	M	I	43	119.9	80 (1.8 p.a.)	101 (2.3 p.a.)	0.5275	227 (5.2 p.a.)
74	53	M	C	42	177.7	20 (0.4 p.a.)	114 (2.7 p.a.)	1.7738	100 (2.3 p.a.)
1968-1	55	M	I	48	132.1	57 (1.1 p.a.)	113 (2.3 p.a.)	0.7625	173 (3.6 p.a.)
1968-2	55	M	C	44	121.7	45 (1.0 p.a.)	67 (1.5 p.a.)	1.0888	111 (2.5 p.a.)
42	55	M	C	47	139.0	39 (0.8 p.a.)	68 (1.4 p.a.)	1.9361	69 (1.4 p.a.)
94	67	M	PM	56	57.5	47 (0.8 p.a.)	86 (1.5 p.a.)	0.5835	98 (1.7 p.a.)

However, Figure 31 incorporates only the lamellae which are clearly visible. As was noted, these lamellae most often occur in the inner half of the cementum of older teeth while the outer half of the cementum shows far fewer lamellae. In spite of that, it is possible to suppose that there really are lamellae in the outer half of the cementum and in the short width of cementum next to the dentino-cemental junction which often shows no lamellae.

To test this supposition, curves in Figures 13-30 were corrected for any large jumps (discontinuities) of more than 4 microns which appear throughout their courses. The method of correcting the curves proceeds as follows.

If the first 5 or 10 microns of cementum show no visible lamellae, the curve is projected backwards to the dentino-cemental junction from the nearest area of visible lamellae. The slope of the curve produced by the area of visible lamellae gives the slope for the line being drawn backwards to the dentino-cemental junction. Proceeding throughout the number of visible lamellae versus cementum thickness curves, each jump of more than 4 microns is corrected similarly. That is, the slope produced by the innermost neighbouring area of visible lamellae gives the slope for the line projected through the area of the discontinuity. When another area of clearly visible lamellae is reached, the curve dictated by the lamellae seen in the photograph is resumed only somewhat displaced to the right of its original position. This proceedure continues to the outer edge of the cementum thickness. The corrected line then dictates the number of lamellae estimated if the deposition of cemental lamellae proceeds similarly in areas of no obsearveable lamellae and areas of clearly visible lamellae. Numerical values for the

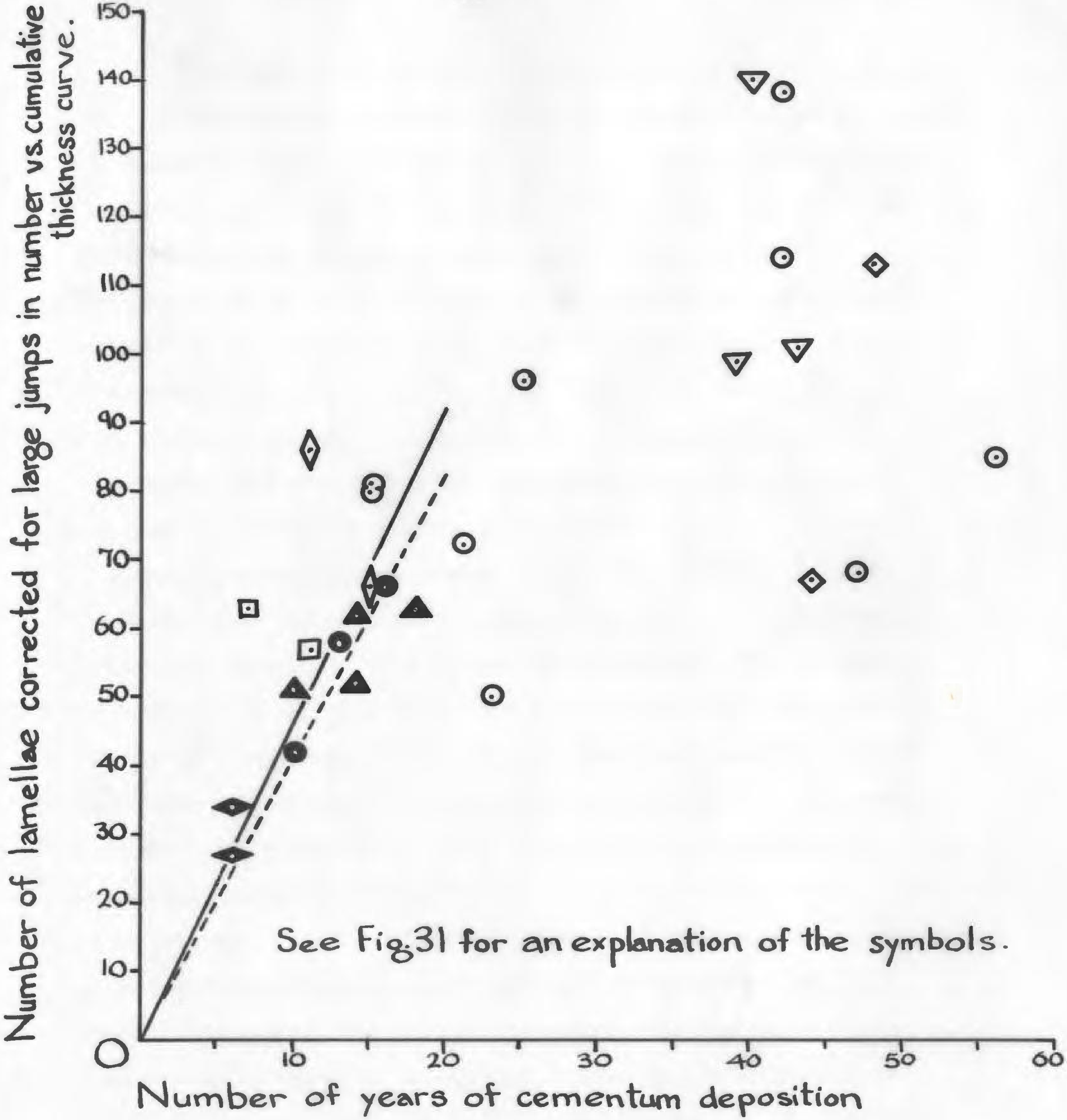


Fig. 32: Number of years of cementum deposition vs. number of lamellae corrected for large jumps in the number vs cumulative thickness curve.

number of estimated lamellae, corrected for large jumps, for each tooth plotted against the number of cementum deposition for each tooth. Data points for the first 20 years of deposition are less scattered than in Figure 31. The solid line in Figure 32 is a least squares line (constrained to pass through zero - Youden, 1951:47) for the data points in the first 20 years of deposition. We find that an average of 4.61 (standard deviation = 0.30) lamellae would have to be deposited per year for the data points to fall on that line. If a similar least squares line is drawn for the female data points only (the dashed line in Figure 32), the resulting number of lamellae per year is 4.13 (standard deviation = 0.18).

However, increasing the number of lamellae present by correcting for large jumps in the visible lamellae versus cumulative thickness curves does not tighten the cluster of data points for the teeth in older age groups. The data points for these older teeth still scatter and fall below the values that would be expected if lamellae deposition continues at the same rate throughout life. This seems to indicate that there really is a difference in the way cementum deposition proceeds with age.

Another way of estimating the numbers of lamellae which may be present but unobserveable is to calculate the average lamella thickness for each sample, using the largest portion of cementum thickness which shows lamellae clearly (see Table 5, column 8 for numerical values). Next divide cementum thickness by the average lamella thickness value. The resultant numbers of lamellae (see Table 5, column 9 for numerical values) are plotted against the number of years of cementum deposition in Figure 33. The results are similar to those of the previous figure. For the first 20 years of

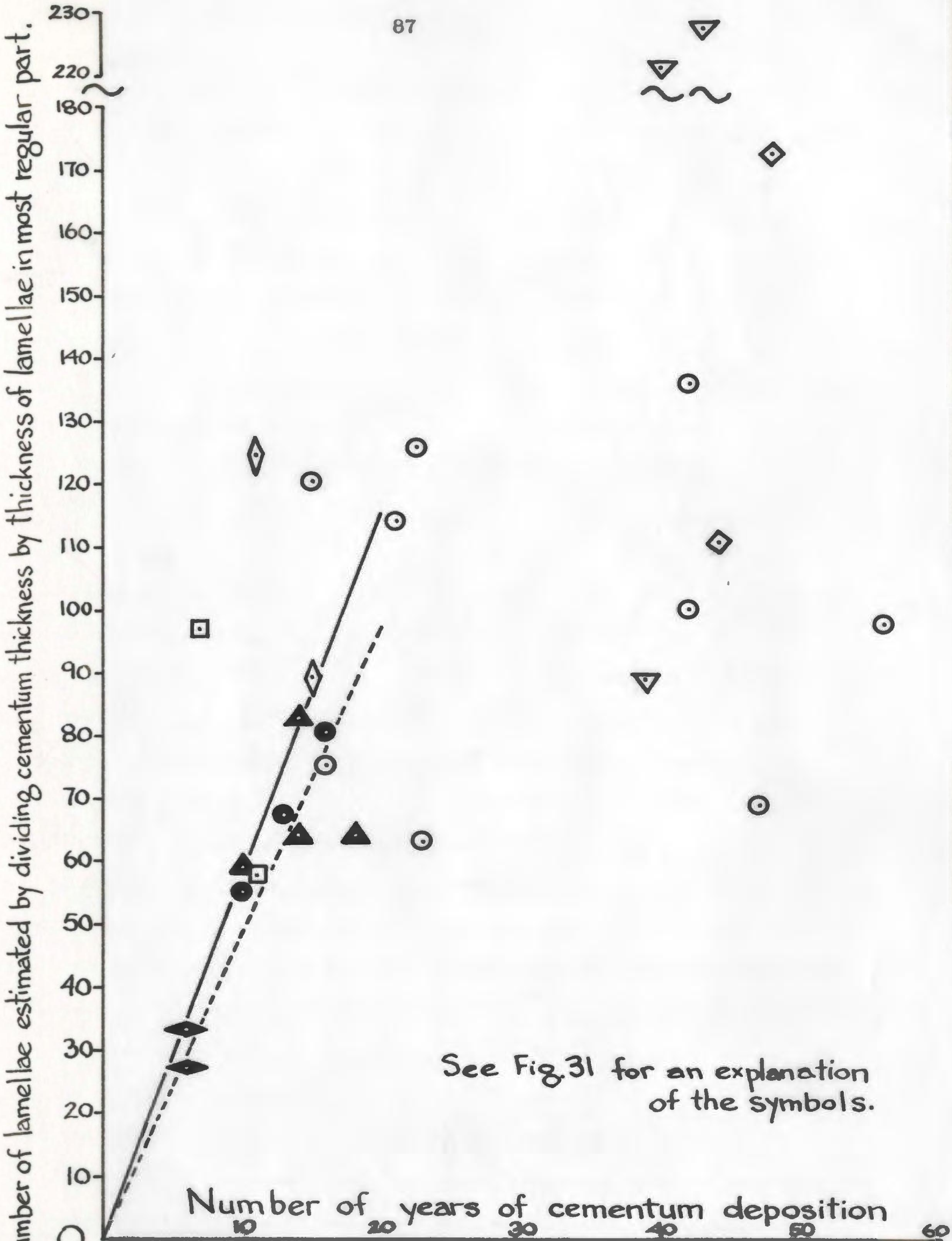


Fig.33: Number of years of cementum deposition vs. number of lamellae estimated from cementum thickness

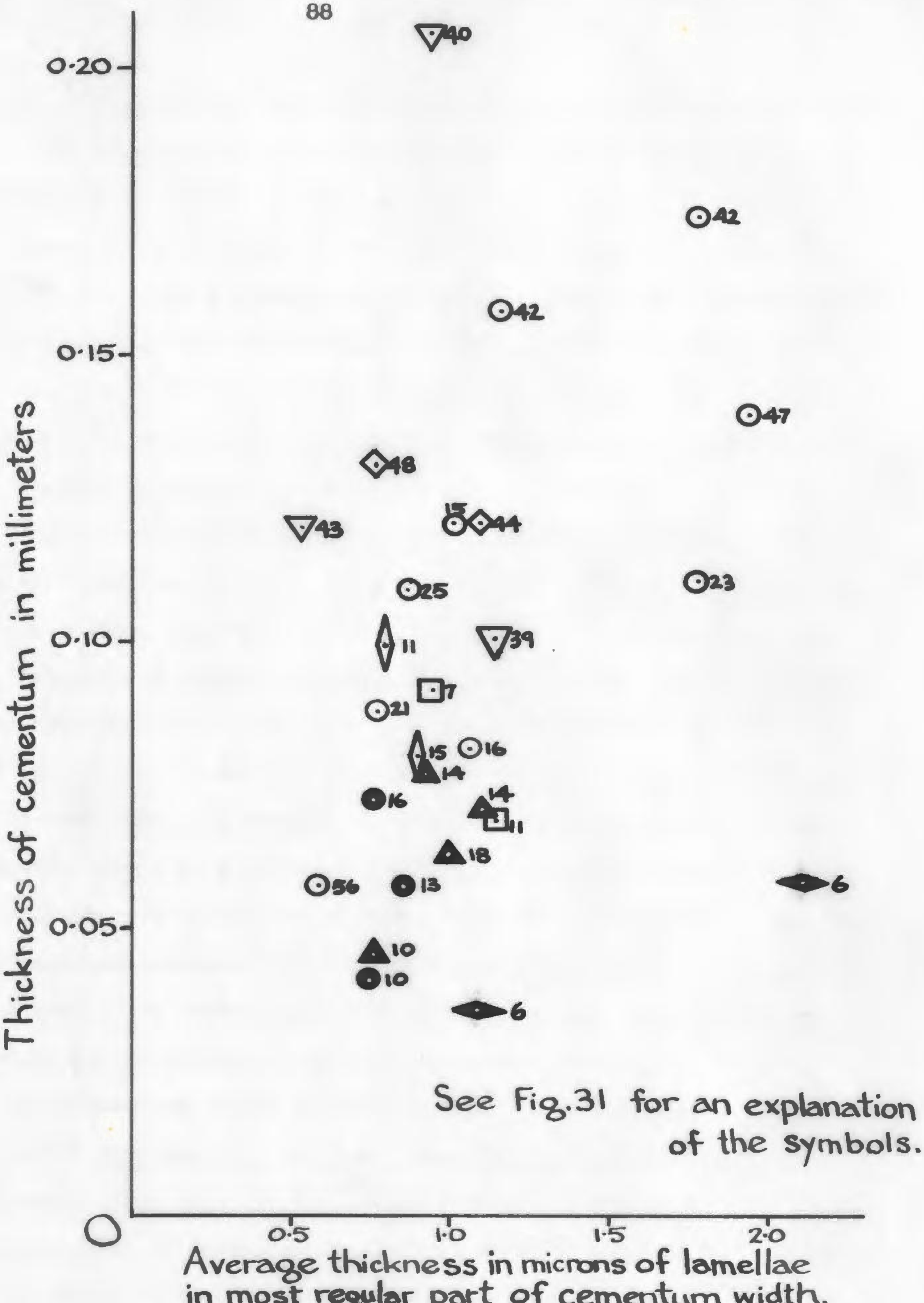


Fig.34: Average thickness of regular lamellae vs. thickness of cementum.

cementum deposition, the data points tend more or less upward. The solid line in Figure 33 is a least squares line (constrained to pass through zero - Youden, 1951:47) for the data points in the first 20 years of deposition. We find that an average of 5.77 (standard deviation = 0.56) lamellae would have to be deposited per year for the data points to fall on that line. If a similar least squares line is drawn for the female data points only (the dashed line in Figure 33), the resulting number of lamellae per year is 4.86 (standard deviation = 0.29).

Again, data points for the older age groups are scattered and fall short of the expected, if lamellae deposition continues at the same rate throughout life.

When average lamella thickness (as calculated above) is plotted against cementum thickness in Figure 34, the average lamella thickness looks suspiciously uniform. For instance, two teeth with almost the same thickness of cementum and almost identical average lamella thicknesses differ dramatically in years of cementum deposition - 44 years and 15 years. Yet it is the older, slightly thinner cementum which has the slightly larger average lamella thickness. The reverse would be expected if the same number of lamellae were deposited each year in both teeth.

It is possible that the slope of the lines suggested by the data points in Figures 31-33 reflects only the increase in cementum thickness with age. In Figure 35, cementum thickness is plotted against years of cementum deposition and this suspicion is confirmed. Data points for the older age groups are less scattered than in Figures 31-33 but they still fall below values expected if cementum deposition continues at the same rate throughout life. Cementum

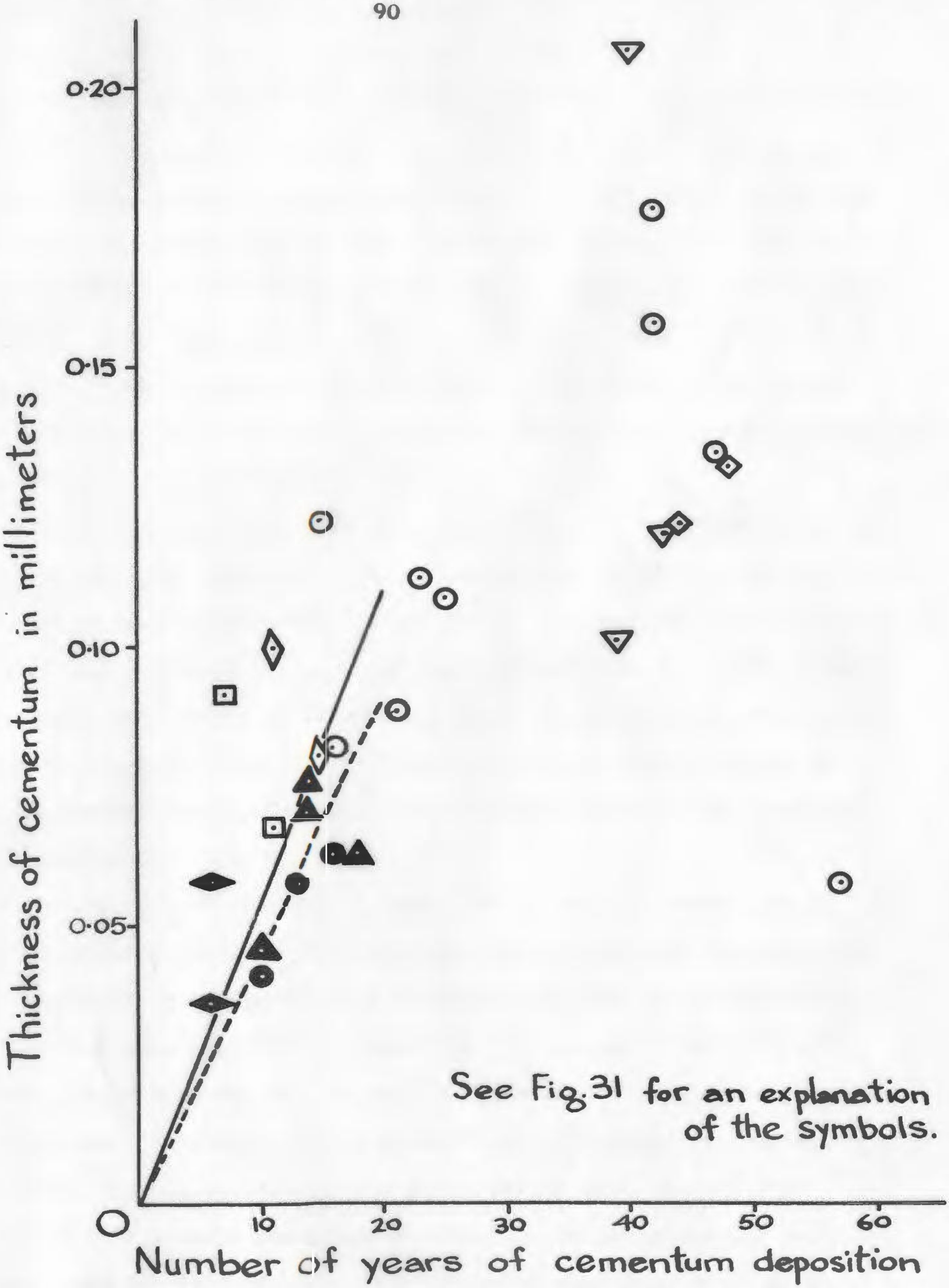


Fig.35: Number of years of cementum deposition vs. thickness of cementum.

deposition seems to differ (at least in volume) in the older age groups.

The solid line in Figure 35 is a least squares line (constrained to pass through zero - Youden, 1951:47) for the data points in the first 20 years of deposition. We find that an average of 5.49 (standard deviation = 0.52) microns of cementum would have to be deposited per year for the data points to fall on that line. If a similar least squares line is drawn for the female data points only (the dashed line in Figure 35), the resulting number of microns of cementum deposited per year is 4.52 (standard deviation = 0.39).

The observation that cementum deposition seems to lessen with age contradicts the findings of Zander and Hürzeller (1958:1043) who conclude that cementum deposition occurs in a straight line relationship with age throughout life. Zander and Hürzeller do not provide their original data points so that comparisons with their original data cannot be made. Their Figure 4 (page 1041) shows what is possibly a regression line for their data on cementum thickness at mid-root plotted against age.

It should be noted that a regression line is the result of a statistical technique which involves fitting the <u>best</u> straight line to a set of data (Spiegel, 1961:248-252; Reichmann, 1973:134-136). This does not mean that a straight line is the only sort of line which would fit the data or that the data is not considerably scattered. It should also be noted that the dashed lines on either side of and closest to the straight (regression?) line in their Figure 4 represents the standard deviation of the <u>means</u> and not the standard deviation for the original data points.

Baird (1962:33) provides an equation ($S_m = \frac{s}{\sqrt{n}}$) for calculating standard deviation of original data points when only the standard deviation of the mean and the number of data points are known.

Working backwards from the information contained in Zander and Hürzeller's Tables I and II, the standard deviations and means for each age group were reconstructed and are plotted in Figure 36. The standard deviations and means for cementum thickness measurements presented in this report are also plotted in Figure 37.

Since Zander and Hürzeller calculated average cementum thickness for the entire tooth circumference at mid-root, their thickness values differ from the ones used in plotting Figure 37. The thickness measurements for the teeth investigated in this report are taken from photographs of one small area of cementum at mid-root. As explained in Chapter 3, this area is usually on the corner of the tooth where the cementum is mid-way in thickness between the extremes found on the labial/lingual (thin) and mesial/distal (thick) sides of the tooth.

In Figure 37, there were only two samples in the 31-40 age group so the mean for this age group was not calculated. There were also only four measurements in the 41-50 age group. Since all four were from 50 year old people, these measurements were added to the five measurements properly belonging in the 51-68 year age group to make one larger sample. The original data points for Figure 37 are plotted in Figure 38.

The standard deviations reconstructed for Zander and Hürzeller's age groups (Figure 36) are as large as, or larger than, the standard deviations for the age groups in Figure 37. We can then assume that their original data points were at least as scattered as the data points presented in Figure 38. It is therefore not at all clear that Zander and Hürzeller's original data points dictated a straight line relationship between cementum thickness and age. This point is

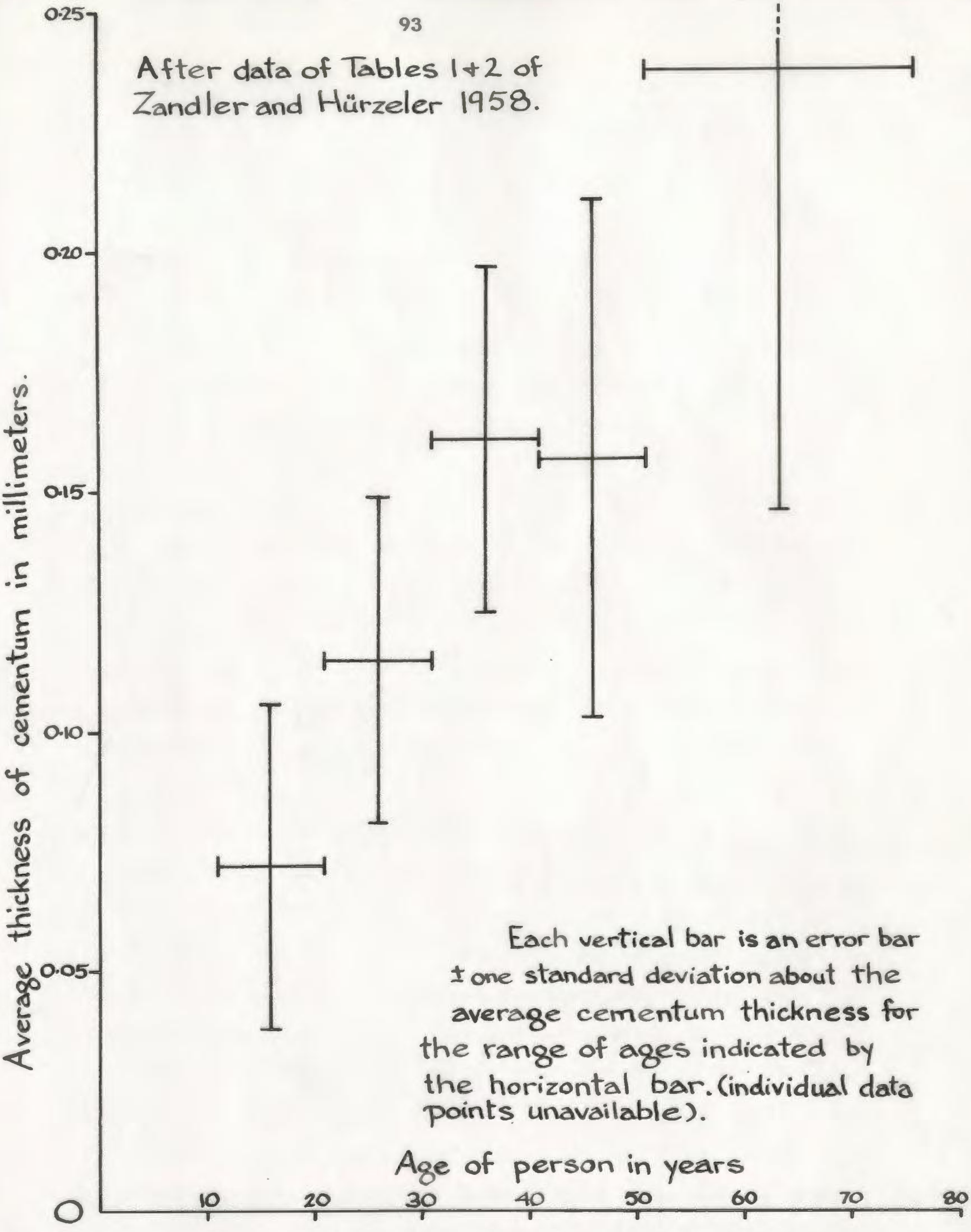


Fig. 36: Person's age vs. cementum thickness from Zandler and Hürzeler

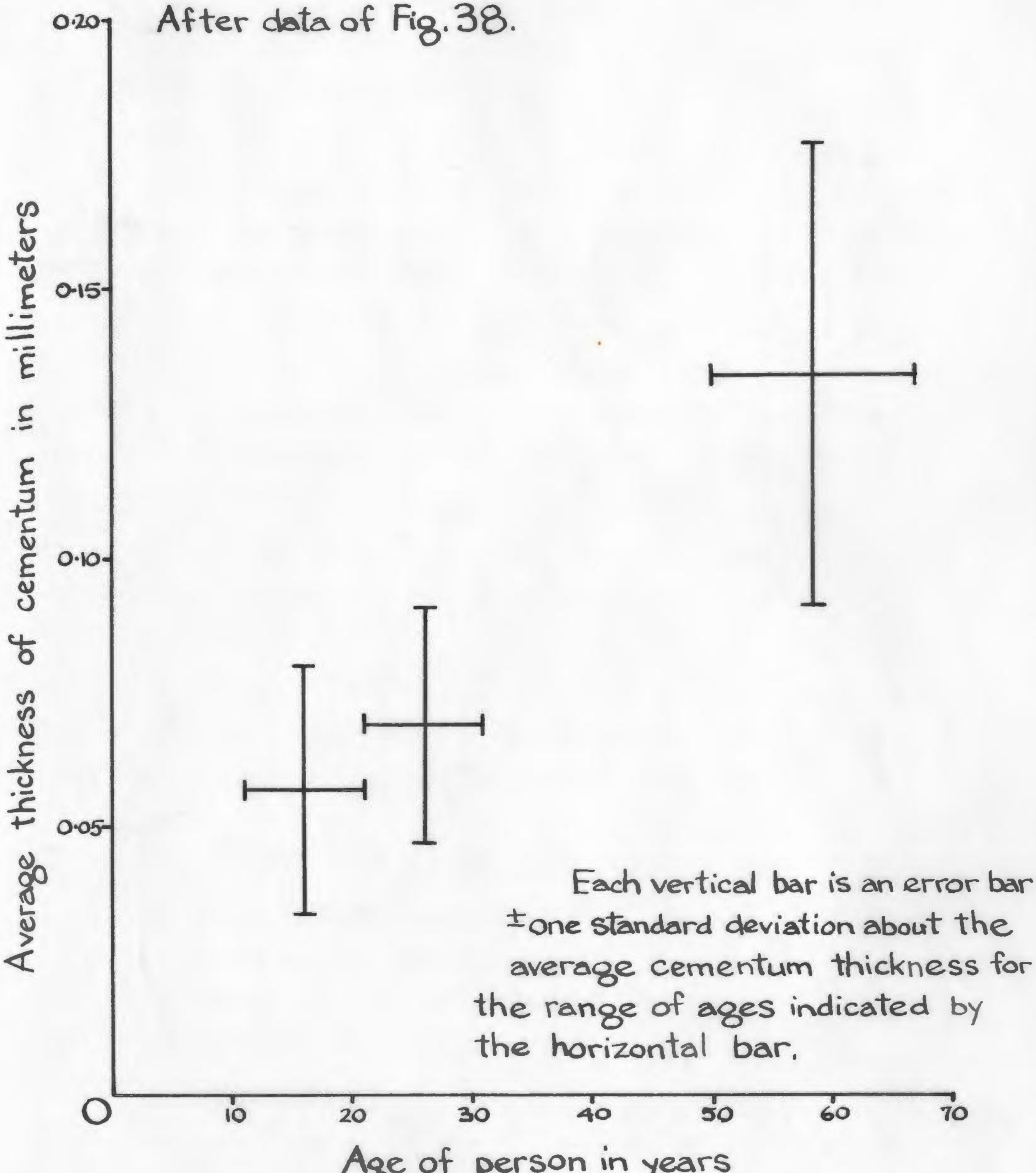


Fig. 37: Person's age vs. cementum thickness from data of present study plotted for ease of comparison with Zandler and Hürzeler.

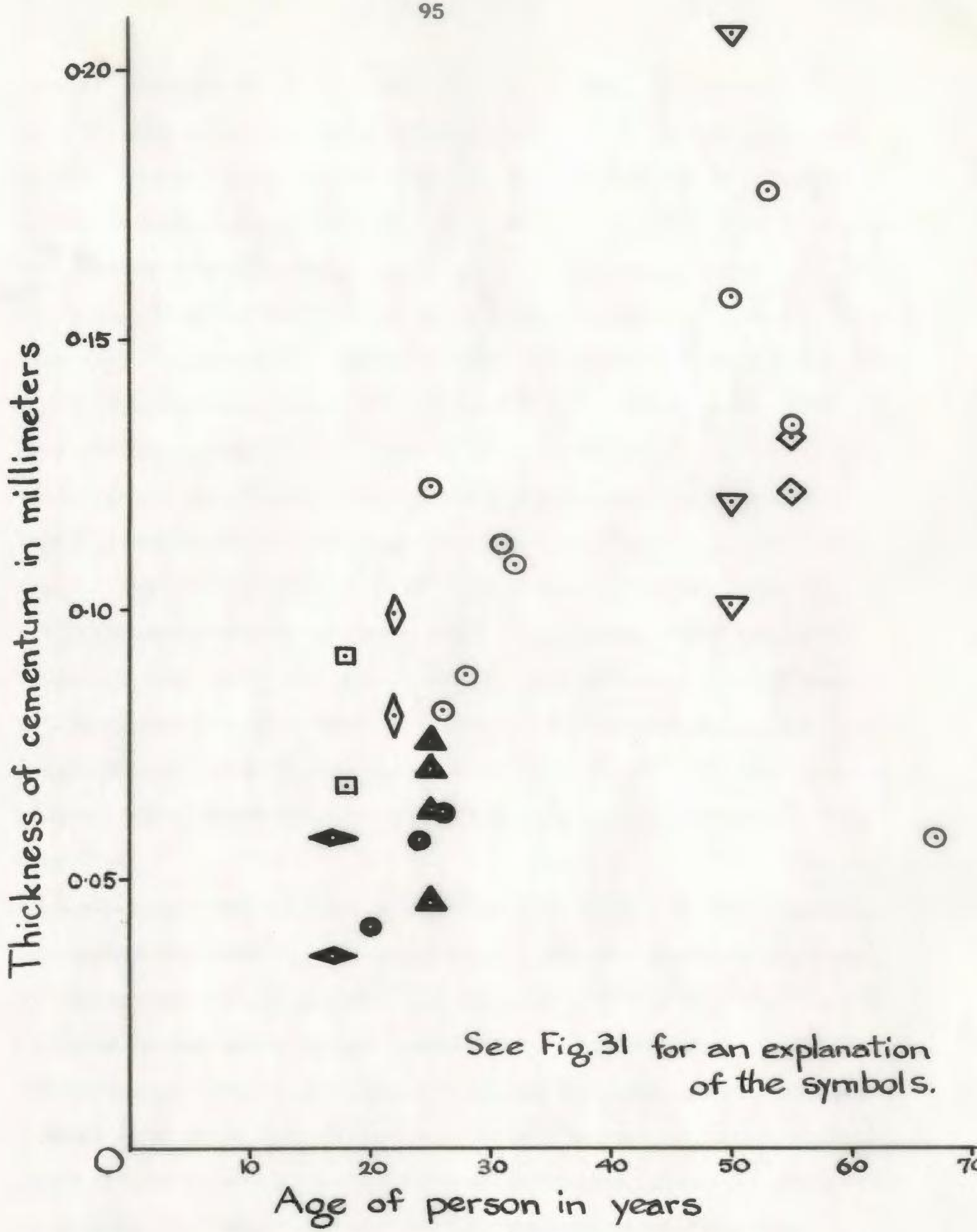


Fig. 38: Age of person vs. thickness of cementum; plotting the individual data points from present study.

further illustrated by the fact that the straight line shown in their Figure 4 does not pass through zero, or any value on the age axis. Instead it passes through a value on the cementum thickness axis. Does this mean that their samples had, on eruption, a thickness of cementum equal to approximately 10 years of deposition?

It was not possible to discover how much cementum is present at the time of tooth eruption. Newly erupted teeth are difficult to obtain and measurements of cementum thickness at the time of a tooth's eruption are unreported. However, since formation of the major part of a tooth's root begins only with the onset of eruption, such a rapid accumulation of cementum would be surprising. Furthermore, the part of the root which is formed first (just below the crown) has the thinnest cementum even in young teeth (see Zander and Hürzeller's Table II, page 1037) which presumeably have not erupted sufficiently to expose the cervical cementum and thus stop deposition. One would expect the cervical cementum to be at least as thick as that at mid-root if rapid cementum deposition occurs during the tooth's initial eruption.

Another possible explanation is that a simple straight line does not adequately describe the relationship between cementum thickness and age. Figures 31-33 and 35 all suggest a fairly constant rate of cementum accumulation during roughly the first 20 years of cementum deposition and then a leveling off during the later years. A least squares line drawn for all the data points on each of these graphs would not pass through zero. However this same thickness information as plotted in Figure 37 superficially suggests a straight line relationship between cementum thickness and age, with the straight line having its origin at some value on the cementum thickness axis.

If the original data points were absent, it would be impossible to determine whether a straight line drawn to accomodate all the means in Figure 37 had been dictated by the original data points or fitted to them. This is the problem one encounters in Zander and Hürzeller's paper.

Also, plotting the cementum thickness values against age of the person rather than number of years of cementum deposition distorts the distribution of data points because teeth that erupt at different ages (and therefore have had more or less time to accumulate cementum) are lumped together. This distortion is seen when one compares Figure 35 with Figure 38. Plotting the cementum thickness values against age of person also introduces the uncertainty about where any line describing the relationship between cementum thickness and age should have its origin. When cementum thickness is plotted against number of years of cementum deposition there is no problem since the line must start at zero.

Graphs of cemental lamellae from the teeth of younger females (Figures 13,15,17,18) can be compared with those from the teeth of males of similar age groups, that is from 17-28 years (Figures 14,16, 19,21,22). Males and females from older age groups cannot be compared because no teeth from older females are present in this sample. The graphs made from male cementum generally show more jumps (discontinuities) than those made from female cementum. There does not seem to be any recognizeable difference in the actual appearance of visible lamellae in male and female cementum. However, it does appear that cemental lamellae are more likely to be visible throughout the entire thickness of cementum in female teeth than in male teeth.

The teeth examined in this report include six individuals who are represented by more than one tooth. The graphs for these teeth are assembled as sets (see Figures 13, 14, 16, 18, 26, 28). Similarities do not exist in the distribution, the width and the degree of definition of lamellae for teeth in each set. It would not be possible to sort the samples accurately into sets by examining the graphs and the photographs since cementum deposition seems to proceed slightly differently for each tooth in the set. Also the thickest cementum does not always belong to the tooth with the most years of cementum deposition for each set. It would appear that local conditions have a very strong influence on the way cementum is deposited on each tooth. However, the fact that cemental lamellae occur with such regularity in spite of local influences is interesting.

In concluding this report, the following statements can be made:

1) Cemental lamellae do exist in human acellular cementum. They can be seen in both transmitted and reflected light in replica and on polished etched specimens respectively. The type of etch used is critical in revealing the lamellae without destroying them.
2) No simple relationship exists between the number of visible lamellae and the number of years of cementum deposition. However, cementum deposition seems to proceed fairly regularly for approximately the first 20 years of cementum deposition. It may be that approximately 4 lamellae are deposited per year during this early period.
3) For the sample examined in this report, the relationship between cementum thickness and age does not appear to be a straight line. Rate of accumulation of cementum seems to level off after about 30 years of deposition.

4) Teeth from the same mouth cannot be identified by the appearance of the lamellae. There are no common landmarks in the sequence of lamellae which can be used to identify all the teeth from one mouth.
5) The appearance of visible lamellae does not seem to differ in male or female cementum. However, it seems that cemental lamellae are more likely to be visible throughout the entire cementum thickness in female than in male teeth. Since there were no older female teeth present in this sample it was not possible to determine whether this trend continues throughout life.
6) While most non-human mammals in the temperate zone reportedly show annual layers in their cementum, human cemental lamellae do not seem to accrue at a regular rate annually. Whether the layering phenomenon in human cementum is completely different from that found in other mammals or whether it is simply no longer synchronized with a particular time unit is not clear.

Since the deposition of annual layers in the cementum is so widespread among non-human mammals it would not be surprizing to find at least a trace of this phenomenon in human cementum. Human cemental lamellae might be caused by a biological rhythm similar to that suspected of causing annual layers in other mammal species, only weaker and no longer synchronized to a particular time period. However, it is difficult to argue that cemental lamellae are the expression of a biological rhythm when the lamellae in teeth from the same mouth look so different.

On the other hand if the lamellae seen in human cementum are a different phenomenon from the annual layering seen in the non-human mammals then is <u>Homo sapiens</u> the only mammal possessing such cemental lamellae? It would be interesting to see what the cementum of the anthropoid apes looks like. It would also be interesting to make a thorough examination of ancient teeth of Homo sapiens and his predecessors.

Although no lamellae were observed in the two archeological samples examined in this study we cannot conclude that no lamellae were ever present in them. It is possible that remineralization could occur in teeth which have been buried. In this case a different method of preparing the cementum for observation might yield more information on its fine structure.

However, since most of the teeth studied here were pathological, it would be necessary to repeat this investigation with a sample of healthy specimens to see whether the results apply to healthy teeth as well. There was no obvious difference in the size or appearance of lamellae in the cementum of the pathological teeth and the cadaver teeth examined. Therefore, any differences found on examining a sample of healthy teeth are more likely to be differences in the presence or absence of lamellae rather than in the size or appearance of them.

We may thus conclude that teeth cannot be biologically aged by the number of lamellae present in their acellular cementum. However cementum thickness seems to increase fairly constantly during approximately the first 20 years of deposition. After this time, cementum deposition seems to level off.

There are no similarities in the appearance of the lamellae in the cementum of teeth from one mouth. However, the cementum from the younger females seems to show visible lamellae throughout its entire thickness more often than the cementum of younger males.

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