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The Occurrence and Implications of Post-Mortem 'Pink Teeth' in Forensic and Archaeological Cases

Tenth Draft

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Abstract.

Of eight externally well-preserved teeth taken from four skeletons from Medieval Chichester, on sectioning six displayed a superficial similarity to teeth described in the forensic literature as 'pink teeth'. This article reviews the occurrence of 'pink teeth' in forensic specimens and describes the teeth from Chichester using a variety of analytical techniques. We conclude that, despite the similarities, the pink coloration in the archaeological specimens has a different cause from the forensic samples, and that in archaeological contexts the pinkness is probably related to post-mortem change brought about by saprophytic fungi. However the exact cause of the coloration remains unexplained. We discuss briefly the implications of this observation for dental ageing techniques and other studies of archaeological teeth.

Keywords: pink teeth, dental, forensic, archaeological, fungal, trauma.

Introduction.

Tunnelling hyphael penetration of the dentine structures in ancient teeth has been described in the archaeological science literature on a number of occasions¹⁻⁶. More recently, Lucy *et al*⁷ have described some particular instances of focal attack from the Medieval site at Chichester⁸. The observations were made on six out of a total sample of eight teeth from four individuals from the burial ground of the leper hospital on that site during an attempt to observe age related structural changes in sections of those teeth. More unusually the changes seen at Chichester included a marked ‘pinkness’ of the dentine structure. The phenomenon of ‘pink teeth’ was first described by Thomas Bell⁹ as the osseous part of the tooth (i.e., dentine) being coloured red, whilst the enamel was unaffected. This symptom has been noted many times in the forensic literature⁹⁻²¹, but Brondum and Simonson²² have pointed to a dearth of descriptions of similar ‘pink teeth’ in the archaeological literature.

The obvious questions which pose themselves at this point are: i) what are the similarities, if any, between the forensic and archaeological reports of ‘pink teeth’ , ii) can the same set of causative mechanisms be attributed to both, and iii) if not, what is the relationship between fungal hyphael penetration and pink teeth in the archaeological context?

Forensic ‘pink teeth’.

All the descriptions of ‘pink teeth’ appearing in the forensic literature have points in common. Miles and Fearnhead¹⁰ , Beeley and Harvey⁹ , Whittaker *et al.*¹¹ and Van-Wyk^{13,15} describe a red-pink coloration of the root of the teeth getting particularly deep towards the cemento-enamel junction, fading off, but still visible beneath the enamel. The pulps in fractured and sectioned teeth were described as being filled with a deep red, or pink, gelatinous material, which extended into the dentine, diminishing in intensity towards the enamel or cementum. Miles and Fearnhead¹⁰, and Van Wyk^{13,15} comment upon the variability between even adjacent teeth within the same jaw, some displaying a marked ‘pinkness’, with others being relatively unaffected.

Van Wyk^{13,15} describes similar intra tooth variation in that some were affected mainly in the coronal dentine, with others where the root dentine was mostly affected.

In the cases reported in the forensic literature two strong factors seem to contribute to the appearance of the pink colouration. Almost all authors report a time delay between death and the formation of pink teeth. Where cause of death was known the reports from the forensic literature suggest individuals with pink teeth have died as a result of great physical trauma such as being shot. In many cases where there is no direct evidence for a violent death the cause of death is attributed to asphyxia. The presence of moisture in the environment in which the body was found has also been cited as a contributing factor.

Camps²³, reporting the forensic evidence from the Christie case, explains that one of the victims, Beryl Evans, had been interred after autopsy in 1949. The pathologist at the time, Dr. R.D. Teare, had not noted the presence of pink teeth. After Christie's confession in 1953 the body of Beryl Evans was exhumed for further post-mortem examination, and it was at this time that it was noticed that the roots of the teeth were pink. The autopsy concluded that Beryl Evans had died by strangulation.

Beeley and Harvey⁹ describe five cases where pink teeth have been apparent. Two were men aged 30 and 21 whose bodies were recovered from the sea approximately 35 days and 90 days after death respectively. The third was a 40 year old man who had been shot and buried under stones in a wet culvert for 94 days before being discovered. A fourth man of 25 years of age had crashed a motor vehicle into a river, the body being recovered 30 days later. The fifth was a 31 year old woman who had taken a barbiturate overdose and was found 46 days post-mortem with vomit in her throat.

Brondum and Simonsen²² published a survey of the circumstances of death where pink teeth had been observed. The study showed that pink teeth are relatively common, occurring in 26 out of 119 forensic cases considered. Of these 26, 21 were men whose bodies had been found in sea water anything from a few days to eight months after they had first gone missing. Three were men who had been found hanging by the neck, and two were men who had been poisoned.

Van Wyk^{13,15} also comments upon the frequency of pink teeth in forensic cases; in his work he says that out of 200 corpses examined between 1985 and 1989, 21 displayed pink teeth. Apparently nine were drowned at sea, three were stabbed to

death and four died from having a lighted petrol-filled tyre placed around their necks. The other four were too decomposed to determine the cause of death. In all cases the interval between death and examination was greater than five days.

The relationships between the three main controlling variables which appear to contribute to pink teeth - time since death, manner of death and presence of water, have been explored in a series of experiments. Whittaker *et al.*¹¹ used a group of anaesthetised golden hamsters, one half of the group being strangled, the other half given a barbiturate overdose. Half of each group was then placed in soil and the other half in seawater. The onset of pink pigmentation was observed between two and three months later, and came about faster and more intensely in the strangled animals. Animals left in brine developed pink teeth faster than those buried in soil.

Kirkham *et al.*¹² poisoned an anaesthetised female dog with carbon monoxide. The mandible was resected and divided into two. Half was left in a bag with 15g of soil and water, the other half was placed under a cloth in a fume cupboard. After 14 days the half of the mandible in soil and water displayed pink teeth and the half in the fume cupboard did not. Gas chromatography of a blood sample immediately post mortem showed an 81% saturation of carbon monoxide, fluid from the teeth of the pink specimens showed a 41% saturation after 14 days. No details are available for the teeth which are reported as not being pink.

Beeley and Harvey⁹ describe an experiment in which an anaesthetised cat was placed in a centrifuge and killed by the action of the machine, in an attempt to replicate the conditions reported for an aircraft pilot who had survived in combat by performing an inverted loop and who subsequently developed pink teeth. The cat later developed pink teeth, but no details exist of the time period or storage conditions of the animal after death.

There is no single satisfactory explanation for pink teeth in the forensic cases outlined above, but it does seem likely that blood products, particularly haemoglobin, may be responsible. Haemoglobin and its derivatives have been detected in pink teeth by isoelectric focusing^{9,12,16,20}. The presence of increased amounts of iron in pink dentine over normal dentine, presumably from haemoglobin and its decay products, has been confirmed by Ikeda *et al.*²⁰ using semi-quantitative EDAX analysis. The presence of haemoglobin in dentine could be accounted for by the sudden rise in blood pressure during a highly traumatic action, such as being strangled or choking to

death on vomit, which can lead to rupture of the arterial system in the tooth and result in the presence of erythrocytes in the pulp. Sudden death can also lead to the failure of coagulation mechanisms^{24, 25}, so that blood retains its fluid properties post mortem. The time delay observed between death and red blood products getting into the dentine may be explained by the fact that erythrocytes average 7.5µm in diameter whilst dentinal tubules are only 3µm in diameter so pink dentine can only occur after the breakdown of erythrocyte cell walls (haemolysis) to allow haemoglobin and breakdown products such as porphyrins¹⁶ to filter into the dentine. Takahashi and Williams²⁵ suggest that the rate for haemolysis is at a maximum at temperatures less than 10°C, which could explain the increase in the rate seen in cold conditions such as at sea. In the case of Kirkham's¹² experiment the release of free haemoglobin proteins into the blood could be a result of inter-vascular haemolysis caused by carbon monoxide (Alistair Bavington M.D. *pers. comm.*).

Archaeological pink teeth.

Miles and Fearnhead⁹ describe a superficial brown-red staining which appeared to have penetrated from outside the tooth in dentine from archaeological teeth from St. Brides Church, Fleet Street, London. This prompted Brondum and Simonsen²² to declare that there was no evidence to suggest that archaeological teeth would display pink dentine. However the sections reported here from Chichester are more convincing. Lucy *et al.*⁷ describe six sections (Table 1), coming from two different teeth from each of three individual skeletons, where there has been a distinct pink colouration of the dentine extending from the wall of the pulpal chamber out into the primary dentine (see Figure 1 and Figure 4). Two sections from two different teeth in a fourth skeleton were unaffected and appeared to be exactly the same as equivalent sections from modern teeth.

It was this observation which encouraged us to question if there was any similarity between these teeth from Chichester and those observed in the forensic literature.

Experimental methods and procedures.

Initial sections were embedded in an easily removed silicon substrate and sawn longitudinally with an annular saw to produce buccal-lingual sections of 300µm in thickness.

EDAX: Two of the pink 300µm sections (sections 1 and 6, in Table 1) and a similarly prepared modern control were mounted on 12mm aluminium SEM stubs, carbon coated, and then examined by electron stimulated x-ray emission to see whether the increased amount of iron reported by Ikeda *et al.*²⁰ could be detected in the archaeological example.

The modern control had the expected phosphorus and calcium peaks from the calcium phosphates which make up most of the inorganic structure of dentine. Other elements detected were aluminium, copper and zinc in extremely small amounts. Iron existed in quantities very close to the minimum detectable level (estimated as 0.2% in this matrix, Dr. P. Budd, *pers. comm.*).

The pink archaeological specimen showed a very similar spectrum to the modern one with large peaks for phosphorus and calcium, and with iron being barely detected²⁶.

Light microscopy: Two of the sections displaying pink dentine (sections 3 and 5, in Table 1) were mounted in epoxy resin. One face was ground down using graded abrasives until flat and scratch free. This face was then stuck using the same epoxy resin to a standard microscope slide, and the process repeated on the exposed slide until a flat, scratch free section of 90µm to 110µm in thickness was produced.

Examined with a light microscope and crossed-polars at various magnifications the section yielded the following observations. Enamel appeared as it would on a modern tooth. The pink areas showed no evidence of normal dentinal structure, that is no tubules could be seen within it (see Figure 1) and were more opaque than expected from modern dentine; non-pink areas did display dentinal tubules (see Figure 2). The pink areas displayed no birefringent properties typical of dentine; instead the pink areas appeared to be 'cloud like' and impossible to bring into focus. The non-pink areas did display birefringent properties typical of modern dentine.

The interface between the pink and non-pink areas consisted of a gradation of one into the other. At higher magnification this could be seen as the usual tubules of normal dentine with a structure of short, non-birefringent lines running along the tubules, or parallel to them, and cutting across orthogonal to the tubules (see Figure 3).

UV microscopy: A 300µm section displaying pink dentine (section 2, in Table 1) and a modern control prepared in a similar manner were examined under a reflected ultra-violet light microscope. With a UV wavelength of 350 nm a faint blue-white fluorescence was emitted from dentine in the modern control. No such fluorescence could be detected from the pink areas on the archaeological specimen, but fluorescence of a similar colour and intensity was emitted from the non-pink areas.

Discussion.

A clear difference between the archaeological pink teeth and those described as pink teeth in the forensic literature is the external appearance. Although the pink teeth from Chichester showed no obvious external signs of damage or having been affected in any way by their burial environment, neither did they show any external sign of pink dentine. The forensic literature clearly states that pink dentine is visible by external inspection, although a case could be made for any pigmentation present originally in the outer layers of the tooth roots having been leached out over the course of time.

The very low levels of iron seen in the pink areas of archaeological sections are not by necessity an indication that iron was not present when the pink dentine was formed, merely that the elevated levels of iron are no longer present. Iron as a pigment could be present in extremely small amounts to give the light shade of colour seen in these teeth. Ikeda *et al.*²⁰ give no estimate of the quantity of iron found in their pink teeth, but they do publish their spectra which indicate that iron is present in some quantity above the minimum detectable level. The low quantities of iron being detected in both the control and pink sections in our samples would lead us to assume

that iron, and therefore the products of haemoglobin, are not responsible for the pink pigmentation seen in the Chichester teeth.

More revealing are the sections viewed under the light microscope. Sognaes¹, Werelds², Poole and Tratman³ and Bell *et al.*⁴ describe archaeological teeth which have had tunnelling changes in the dentine but where the enamel has been intact. Both Sognaes¹ and Werelds² describe tunnels made in dentine by what they presume to be saprophytic fungi. Werelds² reports the observation of some fungi in dentine from 300 year old teeth from Abbaye de Vivegnis, Vivegnis, France, and states that these fungi were of a type he describes as *actinomycetales*. Poole and Traitman³ are more detailed in their description of their Palaeolithic/Mesolithic teeth from Aveline's Hole. Four of the teeth showed extensive networks of tunnels through the dentine suggesting the invasion of the dentine from the pulp by fungal mycelium (Poole and Traitman's plates 9 and 10 show a strong similarity to Figure 3 in this article). They comment that the organisation of the network reminded them of the perpendicularly branching mycelium of an *actinomycete* and also comment upon the loss of birefringence which they account for by the preferential removal of collagen. Bell *et al.*⁴ using back-scattered electron imaging observed similar destructive patterns in the dentine of various soil-buried archaeological teeth. The descriptions of the microscopic appearance of altered dentine given above closely matches the microscopic appearance of the dentine from the Chichester teeth described as pink, but the other authors fail to state the overall colour of the affected areas.

Hackett⁵ examined 170 specimens of bone from Southern England, America, Indonesia and Australia and found four main classes of tunnelling. The first were those described as Wedl tunnels, after the Nineteenth Century microbiologist C. Wedl who observed them in 1864. These tunnels were irregular in path and had no mineral deposition associated with them. Next were linear longitudinal tunnels which are described as being somewhat more regular in path than the Wedl tunnels, following the osteon structure of the bone, but displaying a certain amount of redeposited mineral material. Interestingly enough Hackett⁵ describes these as sometimes having a pinkish tint. Budded tunnels are wider than either Wedl tunnels, or linear longitudinal tunnels, and are again described as sometimes having a pinkish tinge. Lamellate foci are seen to follow the osteon structure of bone and are accompanied by a heavy loss of bone mineral and collagen.

Of the descriptions of tunnelling activity given by Hackett⁵ linear longitudinal tunnels most resemble tunnels observed in dentine described above, any differences could be accounted for by the nature of the substrate as suggested by Hackett⁵.

Piepenbrink⁶ reports on the microscopic appearance of post-mortem changes seen in bone from a medieval ossuary in Göttingen, Germany. The description is similar to those for dentine in that much of the destruction consists of tunnels which seem to have a relationship with the haversian system. Some of these samples are said to have displayed a marked reddish, black or blue-violet hue. Upon extraction with organic solvents the colour of the staining agent was found to be dependent upon pH. A notable drop in birefringence, again associated with collagen loss, occurred on all stained areas. The fungi responsible were cultured and found to be *Stachybotrys cylindrospora*, *Doratomyces stemonitis*, genus *Pythium* and genus *Rhizoctonia*. In all stained areas a yellowish-green fluorescence was seen in ultra-violet microscopy using a stimulating wavelength of 350 nm - 450 nm.

Blue-white fluorescence stimulated by 350 nm and 300 nm ultra-violet light is associated with collagen cross-links²⁷. The dentine in the modern control displayed blue-white fluorescence as did the non-pink areas of dentine from the Chichester tooth. The pink areas displayed no fluorescence indicating that collagen cross-linking was no longer present. No yellowish-green fluorescence was observed.

Conclusions.

The foregoing discussion strongly indicates that the pink teeth seen at Chichester are not caused by the same mechanism as those which appear in the forensic literature. The coincidence of the pink areas with areas of focal damage, obliterated dentine structures and very much reduced collagen cross-link concentration would suggest that the pinkness is a consequence of post-mortem decay factors, particularly focal damage caused by the tunnelling hyphae of various species of saprophytic fungi. No explanation exists as yet for the cause of the pink colouration, although the pH dependent red staining of bones seen by Piepenbrink⁶ could form the starting point. Since no yellow-green fluorescence was emitted from the Chichester teeth there is no further evidence to suggest the same organism was responsible.

The Chichester teeth were not obviously degraded in any way from the outside. The extent of alteration within the dentine was unsuspected before sections were taken, and contrasts with the teeth used by Werelds²² and Poole and Tratman²³. Soggnaes²¹ did not specify the superficial appearance of teeth used in his study, but teeth used by Bell *et al.*²⁴ and bones studied by Piepenbrink²⁶ were externally well preserved.

We believe that dentine which has suffered structural and chemical alteration has now been sufficiently reported to be regarded as a common phenomenon in archaeology. In fact few teeth with intact dentine have appeared in the literature from archaeological contexts, although this could be because workers have tended to only report unusual findings. This conclusion must modify the widely held opinion that teeth are the best preserved parts of the human body; this statement can only really be applied to enamel, which only appears to occasionally be badly affected by soil conditions². Teeth may apparently be the best preserved, but if those with superficially perfect appearance may have lost all semblance of internal structure then they cannot be said to be well preserved. The wider implications are that age estimation based upon histological features of teeth for example, Gustafson²⁸, Bang and Ramm²⁹, Johanson³⁰, may only be possible for some archaeological human remains³¹, and hopes that the tooth can be considered as a protective sealed capsule containing large fragments of genetic material may also need to be re-evaluated³².

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List of Figures.

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Figure 1. Low magnification photomicrograph of a heavily affected dental section from Medieval Chichester. Field width $\approx 10\text{mm}$, using crossed polarised light and $\frac{1}{4}\lambda$ compensation. The outside of the tooth is uppermost (A), the very affected area adjacent to the pulp chamber is at the bottom of the field (D). A relatively unaffected area remains between the two (B), with a heavily affected area protruding into the unaffected dentine (C).

Figure 2. Low magnification photomicrograph of a heavily affected dental section from Medieval Chichester. Field width $\approx 2\text{mm}$, using crossed polarised light and $\frac{1}{4}\lambda$ compensation. Here heavily affected dentine can be seen at the top of the field (A), unaffected dentinal tubules can be made out at the bottom of the field (B), and some focal damage can be seen running both parallel to, and orthogonal to the dentinal tubules (C).

Figure 3. High magnification photomicrograph of a heavily affected dental section from Medieval Chichester. Field width $\approx 300\mu\text{m}$, using crossed polarised light and $\frac{1}{4}\lambda$ compensation. Light areas are relatively unaffected dentine (A). The darker areas clearly show the tunnelling hyphae parallel, and orthogonal to, the dentinal tubule structure which runs vertically in the field (C).

Figure 4. Low magnification photomicrograph of a modern, unaffected dental section. Field width $\approx 10\text{mm}$, using crossed polarised light and $\frac{1}{4}\lambda$ compensation. The dental tubule structure runs vertically (B) with cementum being clearly visible at the top of the field (A).

List of Tables.

Table 1. Details of the eight sections taken from four skeletons from Chichester.

Skeleton Number	Sex	Age	Section number	Tooth	Condition	Notes
CH86S20	Male	30-40	1	Left maxillal 1st molar	Pink dentine	Analysed for iron content, none found
			2	Right mandibular central incisor	Pink dentine	Examined by ultra-violet microscopy
CH86S42	Female	50+	3	Left maxillal 2nd molar	Pink dentine	Examined by light microscope
			4	Left mandibular lateral incisor	Pink dentine	
CH86S74	Male	35+	5	Right maxillal 2nd molar	Pink dentine	Examined by light microscope
			6	Left maxillal central incisor	Pink dentine	Analysed for iron content, none found
CH86S108	Male	35+	7	Right mandibular 2nd molar	As modern	
			8	Left maxillal central incisor	As modern	