*Results and discussion.* The effect of neural and liver extracts on the mitoses of the neural tube is shown in figure 1. Mitotic activity is stabilized at approximately ½ of the original value from the concentration of about $5 \times 10^{-4}$ g of proteins of brain extract per embryo. Its level is not changed by the $4 \times 10^{-3}$ concentration of proteins, but it drops rapidly afterwards. With this concentration the nonspecific toxic effect is likely to predominate; this is, among other things, indicated by the virtually complete mitotic inhibition found in other tissues (fig. 2,a). The liver extract does not show any inhibitory action with lower concentrations, its toxic effect being shown first with the dose of $1 \times 10^{-2}$ g protein per embryo. Figures 2,a and b demonstrate that the inhibitory effect of the brain extract is not evident with low doses in the other studied tissues and that only hepatocytes are inhibited by liver extract.

Substances with regulating effects have been isolated from the neural tissue before. Inhibition of the development of homologous neural structures by brain extracts has been described by Török and Törö[12]. On the other hand, Tiedemann[13] reported the isolation of the neural inducing factor. The question whether the neural tissue is subject to chalone regulation is far from answered yet. It is not for example clear what the effect of high protein doses would be after longer time, or what possible morphogenic or teratogenic action the extract might have. Without considering all these aspects, the specific toxicity of the extract cannot be excluded. In spite of this, the facts obtained so far indicate the possible effect of tissue-specific and species-nonspecific inhibitors. The inhibitory influence could also be mediated by the inactivation of the so-called nerve growth factor[14] stimulating the multiplication of the embryonic nerve cells.


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0014-4754/83/020168-02$1.50 + 0.20/0


## Identification of type I collagen fibrils in human dentine. Electron microscope immunotyping[1]

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*Summary.* Using immunoperoxidase labeling with anticollagen antibodies and electron microscopy, the collagen fibrils constituting the matrix of human dentine were identified as type I collagen. Thus, the unique odontoblastic origin of dentinal collagen was confirmed.

Pulp and dentine are the 2 major components of the tooth. The former is a soft connective tissue consisting of ground substance, collagen, reticulin fibrils and various cells. The latter represents the mineralized organic matrix composed mainly of collagen fibrils and elaborated probably by the odontoblasts throughout the life of the tooth. The existence of intermolecular cross-links, generating a high degree of insolubility, has made the biochemical analysis of the dentine collagen a difficult task[2]. However, a few investigators[3-5] identified dentinal collagen as type I collagen, with traces of type I trimer ($3\alpha$1(I) chains), respectively, in bovine or rat teeth. Using immunological techniques and the light microscope, Lesot and Ruch[6] and Thesleff et al.[7] confirmed the presence of type I collagen in the dentinal matrix of mouse tooth germs. Sauk et al.[8] found that type III collagen does exist in the dentine of patients affected by osteogenesis imperfecta. However, there is very little information concerning the quality of human dentinal collagen. So, in the present paper, its identification is described, using immunoperoxidase labeling and the electron microscope.

*Material and methods.* Ten partially developed, unerupted wisdom teeth were removed from children for orthodontic reasons and immediately cracked open in a vice. For standard electron microscopy, the dentinal fragments were placed in 2% glutaraldehyde, 0.1 M cacodylate solution (pH: 7.4), washed in 0.2 M cacodylate-sucrose buffer (pH: 7.4) and progressively demineralized in $HNO_3$ (from 10 to 2%) for 2 weeks at room temperature. The specimens were then washed again, routinely postfixed in 1% osmium tetroxide, dehydrated and embedded in Epon. Thin sections were then contrasted in uranyl acetate followed by lead citrate and examined under the electron microscope.

For indirect immunostaining with antibodies using the peroxidase procedure, the fractured dentine fragments were fixed in a paraformaldehyde-cacodylate-sucrose solution as described previously[9]. After washing, the samples were progressively demineralized in $HNO_3$ (from 10 to 2%) for 2 weeks at room temperature, washed again, embedded in tissue teck II O.C.T. medium and quickly frozen.

Cryostat sections (10 µm) were incubated in monospecific antitype I and III collagen antibodies overnight at 4 °C,

washed again, reacted with goat antirabbit IgG-peroxidase conjugated and subjected to the DAB (3-3′ diaminobenzidine) procedure according to Graham and Karnovsky[10]. The sections were then osmicated, dehydrated in ethanol and observed without further contrast using a Philips 300 electron microscope.

Control samples were incubated with phosphate buffer, or non-immune serum obtained from rabbits before their immunization with type I and III collagen; or with immune serum previously placed in contact with an excess of the corresponding collagen type; or with a goat anti-rabbit IgG-peroxidase conjugate.

*Results.* According to the anti-type I collagen labeling procedure, the extracellular matrix of the demineralized dentine from human permanent teeth consists of aggregates of collagen fibrils of varying thicknesses, forming the intertubular areas.

The thicker fibrils (around 100 nm in diameter), densely arranged and shown in cross section on figure 1, constitute the intertubular matrix. In contrast with the standard sections observed under the electron microscope with routine procedure (fig. 2), these fibrils are peripherally emphasized by a thin peroxidase deposit. The fibrils forming the border of the intertubular dentine are thinner in diameter (around 50 nm) and intensely marked. The individual fibrils (from 50 to 150 nm in diameter), detected within the tubules, parallel to its long axis, appear as coarse fibrils with typical deposits regularly arranged throughout the fibrillar length, thus enhancing the periodicity of 64 nm. It should be pointed out that the micrographs were choosen without odontoblast processes within the dentinal tubules. Finally, no positive reaction could be detected with the anti-type III collagen antibodies or control procedures.

*Discussion.* In this investigation, the use of anticollagen antibodies, the specificity of which has been thoroughly established elsewhere[9], clearly demonstrates that the collagen type found in human dentine is type I collagen. No type IIII collagen could be detected with exception of some hereditary diseases affecting the human dentine[8]. Thus, the biochemical[3,5] and immunofluorescent analysis on animal teeth[6,7] have been corroborated. In addition, the intensity of the peroxidase labeling (stronger at the peripheral bor-

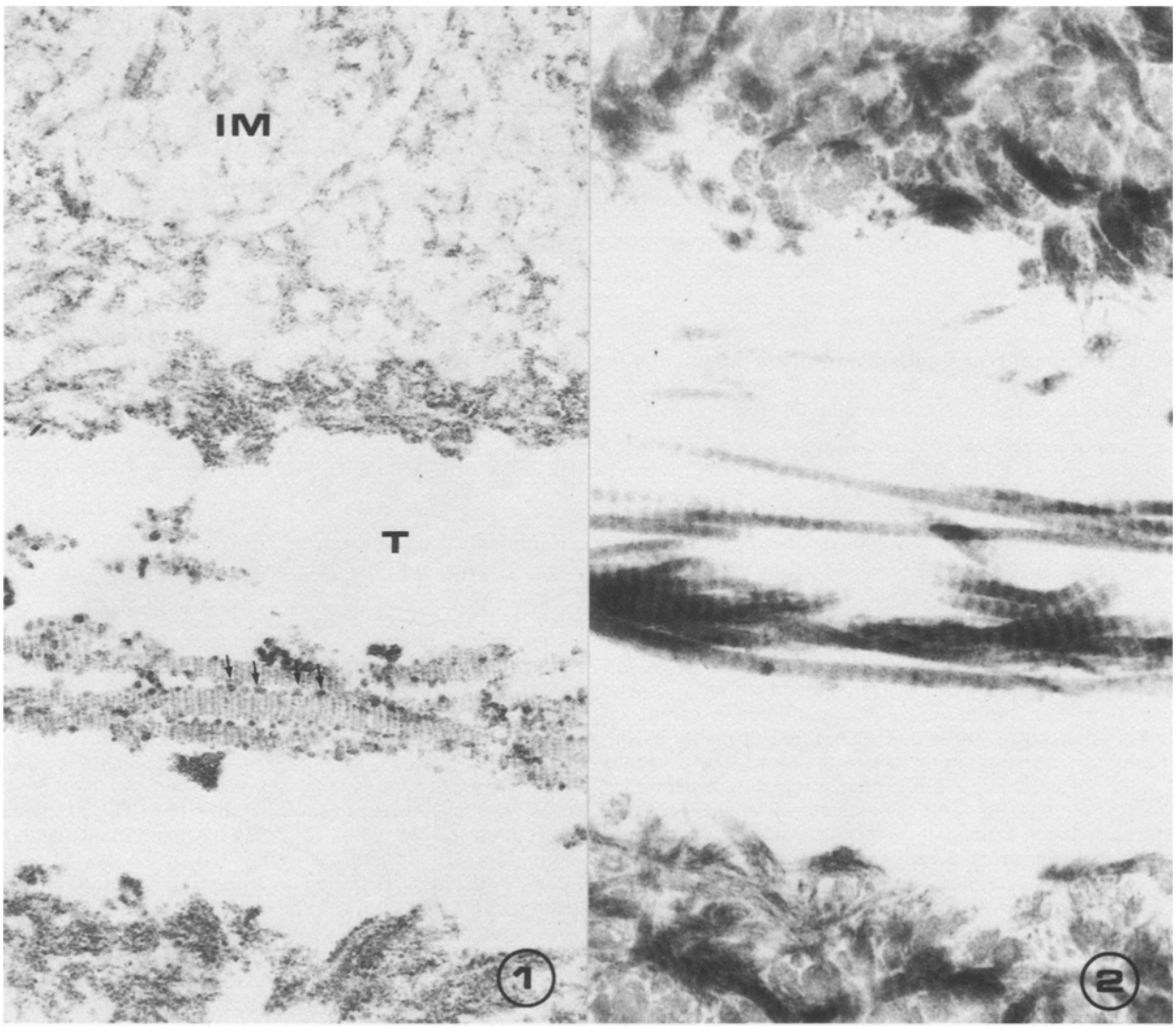


Figure 1. Electron microscope localization of type I collagen fibrils in human demineralized dentine. Longitudinal section of a dentinal tubule (T) showing the presence of peroxidase deposits around the fibrils of the intertubular matrix (IM) as well as around the individual fibrils (arrows) within the tubule. × 34,000.

Figure 2. Typical collagen fibrils of dentine matrix routinely prepared and contrasted for standard electron microscopy. × 34,000.

der of the tubules than in the rest of the tissue) might be correlated with at least in part to difficulties of antibody penetration into the dense and packed collagenous dentinal matrix.

Type I dentinal collagen consists of large and well organized cross-banded fibrils with a pattern similar to the peroxidase deposits found in previous studies[9,11]. This might indicate that the antigenic sites of collagen molecules are not altered by the demineralization process. On the other hand, the type I trimer, biochemically detected in lathyritic rat dentine[12] or normal bovine dentine[5] cannot yet be immunologically and morphologically distinguished from type I fibrils, thus leaving this question open for further investigation.

Finally, it seems obvious that odontoblasts, which were shown to elaborate type I procollagen only[6,13] are the only cells responsible for the synthesis of dentinal collagen. Thus, the dentine probably does not represent a mineralized pulp collagen matrix as the latter contains large amounts of type III collagen[14].

1 This work was supported by CNRS (RCP No. 533).

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0014-4754/83/020169-03$1.50+0.20/0


## Restoration of full mass in nerve-intact muscle grafts after delayed reinnervation[1]

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*Summary.* A rat muscle freely grafted with the motor nerve intact becomes restored to full mass and contractile function, in contrast to the reduced weight of a standard free graft. By crushing the nerve to a nerve-intact graft and delaying reinnervation, full mass is still restored. One can conclude that earlier reinnervation is not the reason for the success of nerve-intact grafts, but that it is rather due to reinnervation along preserved Schwann cell channels.

Autogenous free grafts of entire muscles undergo a sequence of degeneration and regeneration of muscle fibers before becoming functionally reintegrated with the host[2]. Yet, typical standard free muscle grafts become stabilized at weights and contractile strengths only 35–50% of those of their normal counterparts[3].

In a recent experimental model, rat extensor digitorum longus (EDL) muscles were freely grafted as before, but with the motor nerve to the muscle left intact[4]. The early development of nerve-intact grafts was identical to that of standard free muscle grafts (with no preserved nerve connections), but the nerve-intact grafts were eventually restored to normal mass and normal or near-normal contractile properties. In searching for variables that might account for the difference between the 2 types of grafts, no difference was found in the number of muscle fibers between standard grafts, nerve-intact grafts or control muscles, but the muscle fibers in nerve-intact grafts were considerably larger than those of standard grafts. A major difference between standard and nerve-intact grafts was the time of formation of functional neuromuscular junctions. in standard grafts, neuromuscular junctions showed demonstrable function around the end of the 3rd postoperative week, whereas in nerve-intact grafts, after the initial degeneration of the ischemic terminal portions of the nerves, neuromuscular transmission was recorded 8 days after transplantation. On the basis of these results, it was suggested that the success of nerve-intact grafts might be due to the earlier restoration of functional neuromuscular junctions in these grafts. This idea was in accord with the earlier work of Hall-Craggs and Brand[5], who found improved muscle regeneration after they had previously crushed the motor nerve and allowed earlier access of the regenerating nerve fibers to the muscle.

We tested the timing hypothesis by designing the nerve-intact-crush model. In this, the EDL muscle was grafted with the motor nerve intact, but the sciatic nerve was also crushed so that regenerating nerve fibers innervated the graft at the same time after grafting (21–24 days) as reinnervation occurs in standard EDL grafts. If timing of reinnervation were the critical variable, one would expect the mass of nerve-intact-crush grafts to be less than that of nerve-intact grafts and similar to that of standard grafts.

*Methods and results.* This experiment was conducted on 59 male 175–200 g Sprague-Dawley and Wistar rats. All animals were anesthetized with ether. In experimental legs the EDL muscle was grafted with an intact nerve, and the sciatic nerve was crushed with forceps (fig. 1, left). Silver stained (Palmgren) preparations and confirmatory electromyographic studies, conducted as in our previous study[4],