K = condition, W = weight, L = length)[16] which accompany the different growth rates resulting from differences in ration sizes, temperatures, or growth hormone administration[8]. Rainbow trout in the 20–30 cm length range did, however, have mosaic fibers of smaller diameter in winter than in summer[10]. This is an especially significant observation since it was made not on wild populations, but on fish from 2 hatcheries in which food availability and temperature were essentially constant all year, and consequently in which condition (K) did not change significantly with season as it would have done under natural conditions[10]. The possible adaptive significance of this phenomenon in reducing winter protein requirements has already been considered[10].

From our studies[8,9] and others[5,7], it seems that high growth rates and possible attainment of large final size may be inversely related to mean fiber diameter. This would imply that some, at least, of the observed interspecific differences in growth rate among fish are consequences of inherent differences in ability of muscle to continue to recruit new fibers.

The suggestion that the length range of 20–30 cm is of special significance for the characteristics of muscle growth in rainbow trout could be further investigated for fish in this size range by: a) administering large doses of bovine growth hormone and insulin, both of which stimulate growth in fish[8,17], to trout receiving ad libitum rations; b) feeding a 'superdiet' of rations very high in proteins, minerals and vitamins; c) a combination of (a) and (b).

Finally, we note that the rainbow trout appears to be an excellent subject for experimental studies of vertebrate growth. The somatic growth of the species is highly labile – i.e. responsive to differences in food, temperatures, etc., while the predominant mosaic muscle mass is relatively simple in structure and its characteristic growth dynamics are now broadly accounted for.

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Degradation of type I collagen fibrils synthesized by human dental pulp cells in explant culture exposed to *Actinomyces viscosus*. Electron microscope immunotyping[1]

H. Magloire, J. Dumont, J.A. Grimaud, A. Joffre, G. Benay and D. Herbage

Laboratoire de Biologie Bucco-Dentaire, Faculté d'Odontologie, rue G. Paradin, F-69372, Lyon cedex 2 (France), Institut Pasteur de Lyon, ERA 819, rue Pasteur, Lyon (France), and C.M.E.A.B.G., Faculté des Sciences, Villeurbanne (France), 12 January 1981

Summary. Actinomyces viscosus Be 66, added to pulpal cells in culture, does not cause apparent cellular damage. The extracellular matrix consists of altered collagen fibrils and thin filaments, immunochemically identified as type I collagen. They probably represent the first steps of collagen degradation.

In carious dentine, the demineralization process is associated with degradation of the organic matrix, probably caused by the microbial enzymes present in the lesion[2,3]. This proteolysis has also been described in the pulpal tissue (free of bacteria) beneath advanced[4] or early dentine lesion[5]. Thus, the disruption of pulpal collagen fibrils might be correlated with the microbial enzymes found in carious dentine. Indeed, many strains of oral bacteria have been shown to cause disintegration of collagen fibrils or films in vitro[6,7]. However, little attention has been given to the proteolytic activity of *Actinomyces viscosus*, a gram positive microorganism isolated from deep human carious dentine[8] or root surface caries[9]. In the present paper, the collagenolytic activity of *Actinomyces viscosus* on the matrix synthesized by human dental pulp cells in explant cultures is described.

Material and methods. Explant cultures, obtained from children's permanent tooth germs removed for orthodontic reasons, were grown in Leighton tubes and suspended in Eagle basal medium supplemented with 10% calf serum, penicillin, streptomycin and ascorbic acid as described previously[10]. The cultures, incubated 3 weeks at 37 °C were suspended in Eagle medium without antibiotics 2 days before the inoculation with bacteria. Other cultures, without bacteria, served as controls. The strain *Actinomyces viscosus* Be 66, kindly supplied by Prof. Edwardsson

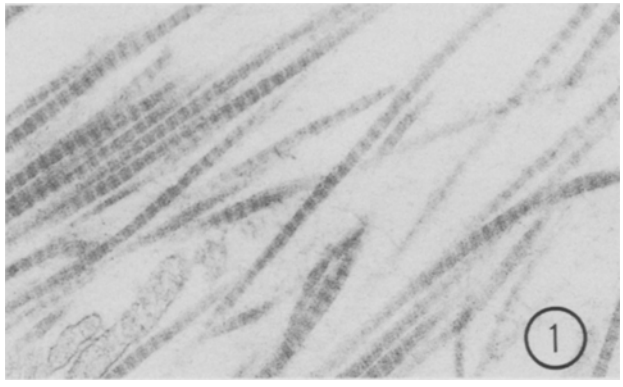

Figure 1. Typical collagen fibrils elaborated by the cultured cells. × 40,000.

(University of Malmö, Malmö, Sweden) was isolated from human carious dentine. The lyophylisate was anaerobically grown (Gas pak BBL) and 1 ml was incubated on VF agar plates (Institut Pasteur, Paris, France) supplemented with 0.5% (w/v) glucose at 37 °C for 24 h. Finally, the exponential cultures, originating from a colony incubated as mentioned above, were harvested, washed twice and suspended in Eagle medium without antibiotics to give a final concentration of about 3×10^6 bacteria per ml. It should be noted that no other concentrations were used in this experiment. 2 ml of this suspension were introduced into the growth in each Leighton tube. After the exposure time (2 h) the medium was drained out and grown as mentioned above, and served as a control for the viability of the bacteria. The cultured cells (treated and control) were routinely fixed for electron microscopy in glutaraldehyde (2%)-cacodylate (0.15 M) solution, washed, post-fixed, dehydrated and embedded as mentioned previously[10]. The sections were routinely contrasted, before examination with a Philips 300 electron microscope.

Antisera against type I collagen were prepared in rabbit from normal or fibrotic human livers as described previously[11]. For direct immunoperoxidase labelling, the treated cultures were extensively washed in a cacodylate buffer, fixed, washed again and subjected to the peroxidase conjugated anti-type I antibodies. The bound peroxidase complexes were routinely visualized according to Graham and Karnovsky[12]. The sections were observed with no further contrast. Control samples were incubated with non-immune serum obtained from the rabbits before their immunization with type I collagen; or immune serum previously placed in contact with an excess of the corresponding type I collagen.

Results. In control cultures (fig. 1), the uniformly wide collagen fibrils (around 50 nm in diameter) appeared singly or formed bundles of fibrils surrounded by a finely fibrillar material. In longitudinal sections, the typical cross-banding of collagen was clearly visible. On examining sections of treated cultures, no typical features of cell impairement were noted (fig. 2a). The mitochondrial matrix, cristae and limiting membranes appeared to be normal. The well-developed rough endoplasmic reticulum suggested a good physiological function. Concerning the extracellular matrix, attention was centred on the presence of large numbers of fine filaments, less than 15 nm wide and of undetermined length, apparent between fragments of collagen fibrils (fig. 2b). No cross-striation could be detected. Collagen fibrils often exhibited frayed ends and seemed to disintegrate longitudinally into fine filaments without periodicity. In figure 2c, the peroxidase deposits, following the anti-type I collagen labelling procedure, appear closely bound to the filaments, with no periodical arrangement throughout the filament length. In control sections, no labelling was observed. The growth of the bacterial suspension in relation to the cultured cells suggested that the bacteria were well preserved.

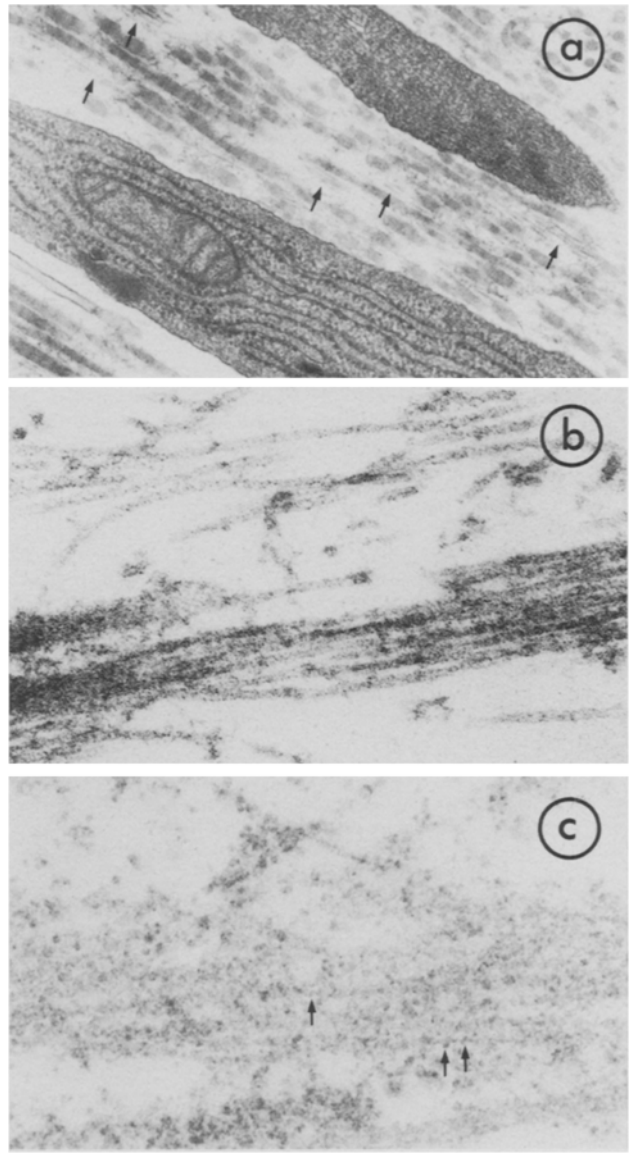

Figure 2. Cultures exposed to *Actinomyces viscosus*. *a* No cellular damage is apparent. Bundles of fine filaments are present (→). × 30,000. *b* Higher magnification of the non striated microfibrils. × 100,000. *c* Electron immunoperoxidase labelling of type I collagen. The peroxidase deposits are arranged throughout the fibrillar length (→). × 100,000.

Discussion. In the present study, we have investigated the ultrastructural changes accompanying in vitro 'infection', by *Actinomyces viscosus*, of the collagenous matrix elaborated by dental pulp cells in culture. These typical cross-banded fibrils were recently identified as type I collagen[11,13] which forms the main part of dentine and pulp matrix[14]. In comparison with the control samples, it was found that *Actinomyces viscosus* did not cause apparent cellular damage. The discrepancy between the present results and the cytotoxic effects of crude extracts from dental plaque on cells in vitro[15] may be due either to the short contact between bacteria and cells or to the bacterial species used here. Recently, it was reported that *Streptococ-*

cus faecalis inhibited the growth of HeLa cells in vitro whereas *Streptococcus mutans* stimulated it[16]. Nevertheless, the extracellular matrix appeared to be disturbed, with fragmentation of collagen fibrils and the presence of a large number of non-striated microfilaments. Similar bundles of fine filaments, closely bound to collagen fibrils, have been described in periodontal diseases[17] or in the pulpal border zone of teeth affected by early caries lesions[5]. They were then considered to be oxytalan fibers, a pre-elastic component. In this investigation, the use of anti-type I collagen antibodies, the specificity of which has been thoroughly established elsewhere[11], clearly demonstrates the collagenous nature of these filaments. In addition, the non-periodical arrangement of peroxidase deposits is different from previous data[11,13], suggesting changes in the molecular order or helicity of collagen. Such splitting of fibrils probably represents the 1st stage of extracellular collagen degradation. Sometimes, this degradative process occurs within the vacuolar apparatus of various cell types such as fibroblasts in vivo or in vitro[18]. In the present experiment, phagocytosis of collagen fibrils was never found, although it exists under physiological conditions[10]. So, the collagen fibrils elaborated by dental pulp cells in culture might have been altered partly by the direct action of *Actinomyces viscosus* and partly by the possible activation of a latent collagenase detected in various explant culture systems[19]. The use of tissue culture as a model, therefore, most closely approximates the in vivo state of the tissue. In this regard, our results lead us to believe that *Actinomyces viscosus* may actively participate in the destruction of the organic matrix of the dentine or the pulp beneath carious lesions.

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Cellular damage and recovery of the early developing mouse eye following low dose irradiation. I. X-rays on day 8 of gestation

I. Balla, C. Michel and Hedi Fritz-Niggli[1]

Strahlenbiologisches Institut der Universität Zürich, August-Forel-Strasse 7, CH-8008 Zürich (Switzerland), 22 December 1980

Summary. The eye region of mouse embryos, irradiated with 90 rad X-rays on day 8 of gestation, was examined 24 h later for cellular damage. Besides the overall developmental retardation, the radiation insult decreased the proliferation rate and altered the mitotic phase ratio. Due to the limited extension of necrotic zones, a reduced number of dead cells was found in the irradiated optic vesicles.

The high radiosensitivity of the developing eye has been confirmed in several experimental studies. Recently, Michel and Fritz-Niggli[2] observed an enhanced rate of microphthalmia in mouse fetuses after irradiation of early organogenetic stages with low doses. Concerning the microscopical investigation of the embryonic eye, only insufficient data exist as yet with regard to the cellular changes following low dose-application.

The aim of this study was to examine the influence of X-irradiation on the cellular damage and regeneration capacity of the early eye primordium. Thereby, particular attention was given to the primary biological events (chromosome damage, mitotic delay and cell death) as the possible causes of malformations.

Material and methods. Female virgin NMRI mice, 2.5 months old, were mated overnight with fertile males of the same stock. The day when a vaginal plug was found was designated as day 0 of gestation. On day 8, pregnant mice were divided into 3 groups: 1. unrestrained, 2. sham-irradiated, 3. irradiated. The sham-irradiated group, consisting of animals restrained in a plexiglass phantom during the total dose exposure time, served as control to the irradiated one. The exposed group was whole-body irradiated with a single dose of 90 rad (200 kV, 12 mA, 1 mm Al + 0.5 mm Cu, HVL 0.93 mm Cu, 40 cm target-to-object distance and dose rate 47.5 R/min).

The embryos were examined histologically on day 9 using the following parameters: relative eye size, mitotic activity and phase distribution, anaphase aberrations and cell death. At this developmental stage, the neural tissue consists of a homogenous population of undifferentiated neuroblasts. The 24-h time lapse between irradiation and observation corresponds to 2 division cycles of the cells under study, with a total generation time of 10.5 h[3]. The relative eye size was defined as the number of sections through the eye region multiplied by 5 µm (section thickness). The mitotic index was determined by counts of all mitotic figures and non-proliferating cells in the 1st section of the optic vesicles with continuous stalk lumen. The anaphase aberrations and cell death were recorded by