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Behaviour of adenylic and pyridinic compounds in gingival tissue after a short-term exposure to air

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SUMMARY

Biochemical variations of adenine and pyridine compounds in human gingival grafts during the period between excision and implantation have been studied. These groups of compounds are considered as «indicators» of the metabolic and energetic status of the living cells. Adenylic compounds such as ATP, ADP and AMP are involved in numerous metabolic processes as «modulators» of allosteric enzymes. NAD+ and NADP+ are involved in the carbohydrate metabolism as co-factors of many reactions of oxydoreduction. The exposure to air of the gingival tissue induces modifications in the energy state of the cells as well as in the ox-reox system. No variation is detectable in the intermediates of the pyridine compounds cycle.

KEY WORDS:

Human gingival tissue - Adenylic compounds - Pyridinic compounds - HPLC

RÉSUMÉ

Dans des gencives humaines prélevées pour des greffes, ont été étudiées, à certains intervalles de temps entre le prélèvement et la greffe, les variations biochimiques des composés adényliques et pyridiniques, qui sont les «indicateurs» des conditions énergétiques et métaboliques du tissu. Des composés comme l'ATP, l'ADP et l'AMP participent à de nombreux processus métaboliques comme «modulateurs» des enzymes allostériques. NAD+ et NADP+ participent au métabolisme des carbohydrates comme co-facteurs de nombreuses réactions d'oxydoréduction. Une brève exposition de la gencive à l'air provoque des changements dans le métabolisme des cellules et du système d'oxydoréduction. Il n'y a pas de variation notable dans les composés intermédiaires du cycle pyridinique.

MOTS-CLÉS:

Gencive humaine - Composés adényliques - Composés pyridiniques - HPLC

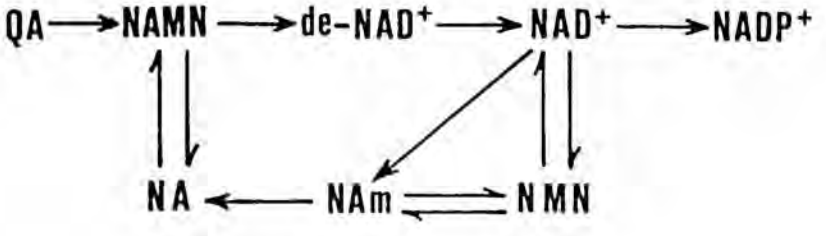
INTRODUCTION

The free gingival graft has been generally performed for the purpose of increasing the dimension of the attached gingiva and the extension of the vestibular fornix. In recent years it has been reported that an effective treatment consists in covering the denuded root surfaces created by gingival recession, gingival cleft and pocket elimination (Sullivan & Atkins, 1968; Ward, 1974; Yukna et al., 1977) with the creeping attachment following graft (Matter & Cimasoni, 1976).

Preservation of the structural specificity of free gingival grafts is one of the prerequisites for the success of graft implantation.

Most investigations on this topic deal mainly with the morpho-structural variations of the free gingival graft in the various phases of healing (Hartman et al., 1977; Duluc & Boudat, 1978; Bernimoulin & Schroeder, 1980) and with histological features of the tissue in the transition area of the graft (Gordon et al., 1968). No data are available concerning the possible biochemical variations which might occur in the human gingival tissue during the intervening period of time between excision and implantation. A study of the behaviour of adenylic and pyridinic compounds of the human gingival tissue during this interval of time might provide important information on its energetic and metabolic condition. The two groups of compounds are considered as «indicators» of the metabolic and energetic «status» of the living cells. This definition derives from the following evaluations. Adenylic compounds such as ATP, ADP and AMP are involved in numerous metabolic processes as «modulators» of allosteric enzymes. The energy state of the cell depends on the ratio of concentrations between ATP, ADP and AMP: the energy charge of the cell will be at its maximum value if a high concentration of ATP is present and at its minimum value if the adenylic nucleotides are mostly represented by AMP. Moreover, very recently, a role of adenine nucleotides as «neurotransmitters» and «neuromodulators» has been proposed (Figueira and Ribeiro, 1985). NAD^+ and $NADP^+$, as cofactors of numerous ox-redox reactions, are involved in carbohydrates, lipids, proteins and energy metabolism. Therefore the levels of the two coenzymes must be closely regulated and steadily maintained. The cycle of the pyridine intermediates (Fig. 1) involved with biosynthesis and catabolism of NAD^+ and $NADP^+$ plays a decisive role in the regulation of intra-cellular concentration of the two coenzymes.

Fig. 1: Cycle of pyridine compounds in animal cells.
Fig. 1: Cycle des composés pyridiniques dans les cellules animales.



In a previous paper (Fonzi et al., 1987) we have described a HPLC method for the separation and the identification of adenylic and pyridinic compounds in the normal gingival tissue. The purpose of the present paper was to apply the above-mentioned method to the study of the behaviour of adenylic and pyridinic compounds in the human normal gingival tissue frozen immediately after excision and after 5 and 15 minutes of exposure to air.

MATERIALS AND METHODS

Nicotinamide (NAm) and nicotinic acid (NA) were purchased from Merck, Darmstadt, Germany; quinolinic acid (QA) from Fluka AG Buchs, Switzerland; nicotinamide-adenine-dinucleotide (NAD), nicotinate mononucleotide (NAMN) nicotinate-adenine-dinucleotide (de-NAD), adenosine monophosphate (AMP) and adenosine diphosphate (ADP) from Sigma Chemical Co, USA; nicotinamide mononucleotide (NMN), adenosine triphosphate (ATP) and nicotinamide-adenine-dinucleotide phosphate (NADP) from Biochemia Boehringer Mannheim, Germany. Filters 0.45 μm Millex HV millipore were from Millipore Corp. Bedford MA, USA. All the other reagents were commercial products of analytical grade.

Tissue preparation — For this study 30 subjects were selected from patients requiring a gingival graft procedure. Following infiltration analgesia, each graft was obtained from an appropriate donor site of attached gingiva and from each graft a small sample was taken for the biochemical study. Ten samples, to be used as controls, were frozen at −25 °C immediately after the preparation of the graft, ten where kept for 5 minutes at room temperature and the last ten were kept for 15 minutes at room temperature.

Adenine and pyridine compounds extraction — The gingival tissue, after weighing, was homogenized in Turrax with two 0.6 PCA volumes and centrifuged at 20,000×g for 20 minutes. The supernatant was very carefully neutralized with cold KOH to a pH 6.5. After 15 minutes at 5 °C, $KCLO_4$ was removed by centrifugation at 20,000×g for 20 minutes and the supernatant was filtered through a 0.45 μm Milipore filter and analyzed by HPLC.

High performance liquid chromatography — A Beckman Mod. 332 Liquid Chromatograph equipped with a Mod. 420 Controller Microprocessor, a Mod. 160 Detector with a recorder and 250×4.6 mm column Supelcosil LC-185 μm with a stainless steel-guard column (20×4.6 mm i.d.) packed with

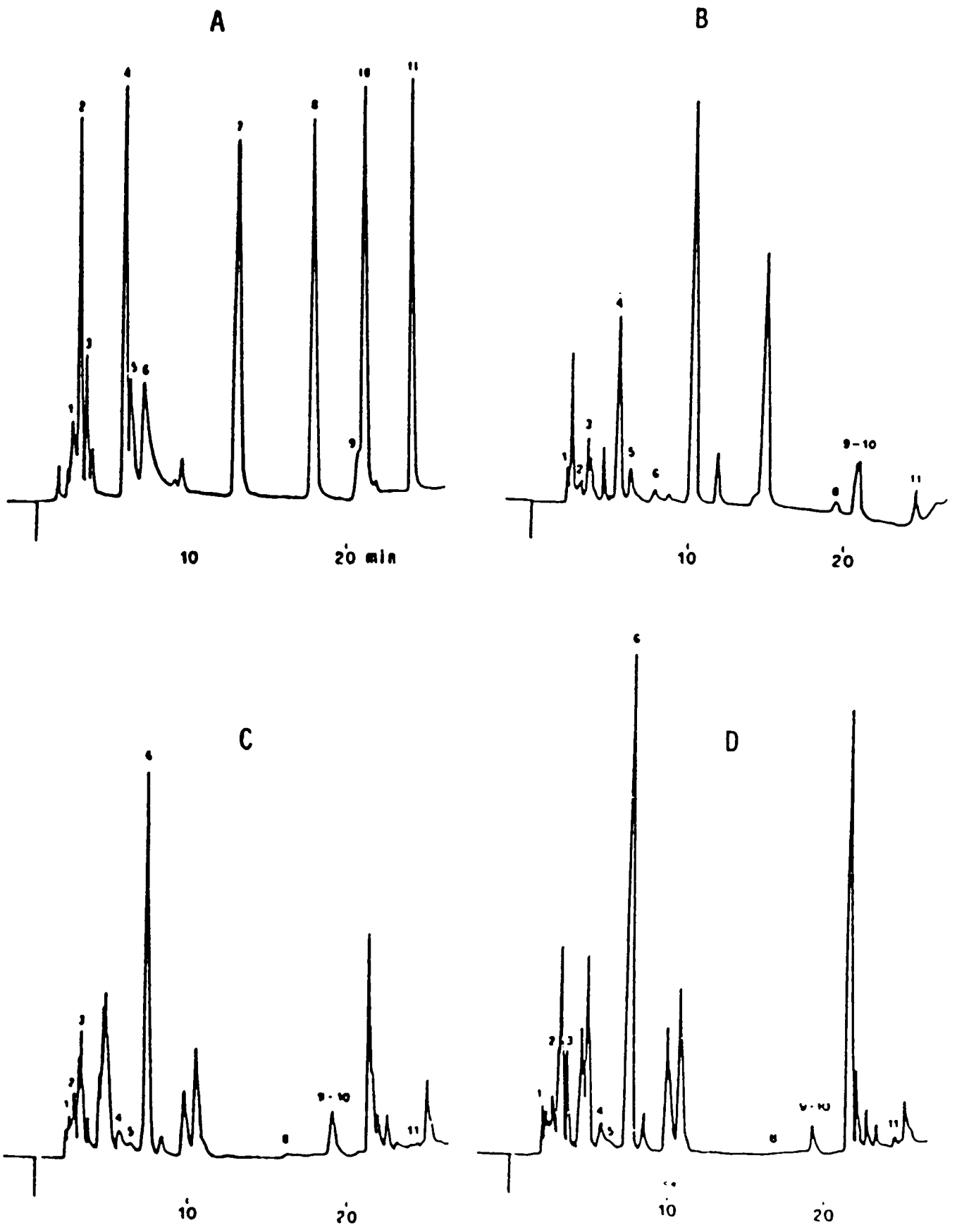


Fig. 2: HPLC chromatograms — A standards compounds: 1) QA; 2) NAMN; 3) NMN; 4) ATP; 5) NA; 6) ADP; 7) AMP: 8) $NADP^+$; 9) NAm; 10) de-NAD^+; B, C, and D: gingival tissue immediately frozen and exposed to the air 5 and 15 minutes respectively.

Fig. 2: Chromatogrammes HPLC. A: composés standards: 1) QA; 2) NAMN; 3) NMN; 4) ATP; 5) NA; 6) ADP; 7) AMP: 8) $NADP^+$; 9) NAm; 10) de-NAD^+; B, C, and D: tissue gingival congelé immédiatement et exposé à l'air pendant respectivement 5 et 15 minutes.

pellicular reversed phase were used. The elution was performed using a gradient already described (Stocchi et al., 1984). The injection volume was 20 μl equivalent to 500-1,000 pmoles of each standard compound and to approximately 3 mg of gingival tissue. The optical density range was 0.04 and was monitored at 254 nm. The HPLC analysis was carried out at room temperature. Methanol (spectral quality) was from Merck. All liquids were prefiltered through 0.45 μm Millipore filter. Nucleotide standards were prepared as 1 mM stock solutions in double-distilled water, and diluted before use. Stock solution were maintained at −20 °C.

RESULTS AND DISCUSSION

Fig. 2A shows the HPLC chromatograms of the standard compounds separated in less than 25 minutes: only NAm and de-NAD coelute. In Fig. 2B, C and D are reported the patterns of adenylic and pyridinic compounds of the gingival tissue fragments frozen immediately after excision and after an exposure to air of 5 and 15 minutes respectively. The HPLC chromatogram of Fig. 2B shows the typical condition of a metabolically active animal cell. ATP is the most quantitatively represented adenylic compound: the high ratio value ATP/ADP (which is of the same magnitude as in that of the rat liver) (Reiss et al., 1984) provides the cell with a large availability of high energy bonds. AMP is undetectable in this condition. NAD^+ is present in an appreciable amount; $NADP^+$, whose concentration is reported to be lower than NAD^+ in other animal tissue (Martelli & Ricci, 1964), is detectable only in traces.

The two possible biosynthetic pathways of pyridine coenzymes operating in animal cells are outlined below:

1) QA → NAMN → $deNAD^+$ → NAD^+
2) NAm → NMN → NAD^+

In chromatogram 2B all compounds involved in the biosynthetic pathways 1) and 2) are present in well resolved peaks except NAm and $deNAD^+$ which are co-eluted. As the high ratio value NAm/$deNAD^+$ (30/1) reported in the literature (Reiss et al., 1984) for some animal tissues, NAm (precursor of the «salvage way») is very probably the major component of the peak 9-10. Both the increase of ADP and the decrease of ATP induce a remarkable change in the ATP/ADP ratio value in the cell tissues exposed to the air being frozen (Fig. 2C and D). This data is the consequence of the ischemic state of the cells: 5 minutes are evidently sufficient for the onset of a decrease in energy potential of the gingival tissue. Literature data correlate the ischemic condition to a decrease in the concentration of NAD^+ and a subsequent increase of its reduced form (Brosnan et al., 1970). In Figures 2C and D it is possible to observe a flattening in the spot corresponding to peak 11 (NAD^+), but this observation must be confirmed using specific and sensitive enzymatic assays to evaluate the possible variation of the NAD^+/NADH ratio. No changes in the levels of pyridine compounds involved in 1) and 2) are detectable after 5 or 15 minutes of exposure to air before being frozen; consequently the biosynthetic pathways seem to be still operating.

On the basis of our findings we can conclude that certainly after 5 minutes of exposure to air, and maybe before, the gingival tissue shows some biochemical variations concerning the energy state and ox-redox system. Future experiments are necessary to evaluate better the extent of the above mentioned variations and to obtain the optimum conditions for the preservations of the grafts from the moment of their drawing until that of implantation.

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