INHIBITION OF PROTEIN SYNTHESIS IN TENDON CELLS BY EXTRACTS FROM EXPERIMENTAL GRANULATION TISSUE

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1. Introduction

When working with the synthesis of collagen in detached, matrix-free tendon cells of chick embryos we became interested in the effect of the recombination of the cells with the extracellular matrix of the connective tissue, in this case extracted from the experimental granulation tissue of rat. Recently, Balazs and Darzynkiewics [1] as also Wiebkin and Muir [2,3] have elaborated the effects of hyaluronate and other glycosaminoglycans on the synthetic and other functions of connective tissue cells. Here we report the effect of the extracellular matrix on the synthesis of collagen and especially, on its transfer from the cells to the medium.

2. Materials and methods

The experimental granulation tissue was induced in male adult albino rats by a subcutaneous implantation of viscose-cellulose sponges [4,5] and harvested after three weeks. The minced granulomas were homogenized with Ultra Turrax-homogenizer into 0.3 M Tris–HCl buffer, pH 7.8, containing 0.0015 M $CaCl_2$, and centrifuged at +4°C for 30 min at 20 000 *g*. The supernatants were added to the incubation media.

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The matrix-free cells were isolated from the tendons of 17-day old chick embryos employing trypsin and bacterial collagenase as described by Dehm and Prockop [6]. The cells were counted and their vitality was tested by Trypan blue. About $1-3 \times 10^6$ cells were incubated with [^{3}H] proline (The Radiochemical Centre, Amersham, England) in 3 ml of modified Krebs-Ringer medium [7], buffered with 20 mM HEPES, with the addition of 0.5 ml of the described granuloma extract which contained about 1 mg protein. The medium contained also 990 μg of cold proline in 3.0 ml (2.87 mM) while in 0.5 ml of granuloma extract added there was only 8 μg of proline. Into the control samples the same amount of protein was added as bovine serum albumin in the extraction buffer. After two hours the incubation was stopped with the addition of cycloheximide (270 μg) and α,α′-dipyridyl (400 μg).

The medium and the cells were separated by centrifugation (350 *g* for 12 min) and the pellet washed with fresh medium containing 1 mM α,α′-dipyridyl and 100 μg/ml cycloheximide. The washings were discarded. The medium and the disintegrated cells suspended in water were dialyzed separately with carrier proline against running tap water. The dialyzates were hydrolyzed in 6 M hydrochloric acid at 130°C for 3 hr and acid evaporated over a boiling water bath. Aliquots were taken for the determination of the total protein radioactivity as also for the assay of the radioactivity of hydroxyproline [7–9]. The results were calculated in per cent of the respective control values.

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3. Results and discussion

The main findings are shown in table 1. The incorporation of proline to collagen is decreased in cells to 49% and in medium to 12%. The effect is proportional to the amount of granuloma extract added to the medium. The disturbed extrusion of labelled collagen to medium is demonstrated especially by its concentration ratio in medium/cells (table 2). The share of collagen in total protein is much decreased in medium but in cells no difference is observed.

There is no effect on the granulation-tissue slices (data not presented), perhaps because the slices contain the active extracellular materials in situ. The experimental evidence did not support the hypothesis that collagen would be broken down in the medium in the presence of granuloma extract. The medium from a control experiment was divided into two parts and one incubated further with granuloma extract but no breakdown of collagen was observed.

McGee, O'Hare and Patrick [10] mention that extracts of granulation tissue from healing wounds, but not those of normal skin, stimulated collagen synthesis in cultured fibroblasts. In our hands, extracts from fatty and CCl_4-injured liver stimulated collagen synthesis both in the embryonal chick tendon cells and in granuloma slices but more data are necessary for conclusive statements (unpublished work). As indicated above, various cells and tissues differ in their response to factors affecting collagen synthesis.

The number of cells and cell aggregates, measured first as a matter of routine, was found to increase during the incubation in the presence of granuloma extract, in the average to 5.5-fold (table 1). No particles whatever could be demonstrated in the granuloma extracts which thus prevent the formation of the clusters from the tendon cells. This disaggregating effect and the suppression of the collagen secretion did not run in parallel in the various samples.

Table 1
Effect of granuloma extract on the incorporation of radioactive proline into matrix-free embryonal tendon cells

Experiment	Cells		Medium		No. of particles
	Total protein	Collagen	Total protein	Collagen	
Resp. control	100.0	100.0	100.0	100.0	100
With granuloma extract	42.1 ± 5.4***	49.3 ± 6.8***	35.7 ± 9.1***	11.6 ± 2.9	550 ± 210*

The figures (mean ± S.E.M.) express the incorporation in per cent of the respective control (n=7). Statistical significance:
* $2P < 0.05$,
*** $2P < 0.001$.

Table 2
Effect of granuloma extract on the distribution of labelled collagen and other proteins in matrix-free tendon cells and incubation medium

Experiment	Collagen/total protein		Collagen in medium/cells
	Cells	Medium	
Resp. control	0.585 ± 0.084	0.851 ± 0.118	2.117 ± 0.405
With granuloma extract	0.663 ± 0.079	0.311 ± 0.095***	0.446 ± 0.097**

Means ± S.E.M. of the ratios are given (n=7). Statistical significance:
** $2P < 0.01$:
*** $2P < 0.001$.

Preliminary experiments were made on the effects of granuloma extracts on the activities of the marker enzymes of the isolated plasma membranes of the cells from granulation tissue. There was an increase on the activity of 5′-nucleotidase, a slight decrease in Na,K-activated ATPase and no effect on leucyl-β-naphtylamidase. The granuloma extract itself contained these enzymes (16.4, 34.3 and 5.7 μmole substrate degraded/hr/ml of extract, respectively). Some glycoproteins have been purified from the granuloma extract and found similarly effective on the synthesis of collagen but the crude extract was much more active. The inhibitor can be carbohydrate also [11].

The granuloma extract disturbs the secretion of collagen and it remains to be studied whether the secretion rate regulates the synthesis of collagen or vice versa.

4. Conclusion

The buffered extracts from the experimental granulation tissue suppress the synthesis of collagen and of other proteins in matrix-free embryonal chick tendon cells. The secretion of collagen from the cells to the medium is affected especially. The granuloma extracts prevent also the cluster formation of the tendon cells during the incubation.

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References

[1] Balazs, E. A. and Darzynkiewicz, Z. (1973) in: Biology of Fibroblast (Kulonen, E. and Pikkarainen, J., eds.) pp. 237–252, Academic Press, London and New York.
[2] Wiebkin, O. W. and Muir, H. (1973) in: Biology of Fibroblast (Kulonen, E. and Pikkarainen, J., eds.) pp. 231–236, Academic Press, London and New York.
[3] Wiebkin, O. W. and Muir, H. (1973) FEBS Lett. 37, 42–46.
[4] Viljanto, J. and Kulonen, E. (1962) Acta Path. Microbiol. Scand. 56, 120–126.
[5] Lampiaho, K. and Kulonen, E. (1967) Biochem. J. 105, 333–341.
[6] Dehm, P. and Prockop, D. J. (1971) Biochim. Biophys. Acta 240, 358–369.
[7] Aalto, M., Pikkarainen, J., Lampiaho, K. and Kulonen, E. (1973) Biochem. J. 132, 663–671.
[8] Aalto, M., Rõnnemaa, T. and Kulonen, E. (1974) Biochim. Biophys. Acta 342, 247–253.
[9] Juva, K. and Prockop, D. J. (1966) Anal. Biochem. 15, 77–83.
[10] McGee, J. O'D., O'Hare, R. P. and Patrick, R. S. (1973) Nature New Biology 243, 121–123.
[11] Waldman, A. A., Marx, G. and Goldstein, J. (1974) Biochim. Biophys. Acta 343, 324–329.