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SOME STUDIES ON COLLAGEN IN THE SKIN OF THE GUINEA PIGS

Asuman SİYAHHAN* – Nevzat BABAN* – Şenol ERTÜKOĞLU** – Hafize UZUN*

SUMMARY

Neutral (1% NaCl), Alkaline (0.1 M NaHCO3), and acidic (0.1 M Citricacid-sodium citrate buffer (CASC) of pH 2.6 and ionic strength of 0.6) solutions were passed through the skin of the guinea pigs under vacuum, and the effect of the solutions on the tissue was studied.

After passing neutral and alkaline solutions through the skin, some decrease in the connective tissue fibrils has been observed. But, upon passing CASC through the skin, some increase is noted in the connective fibrils, and they became somewhat thicker.

If the 0.1 M NaHCO3 and CASC solutions are passed consequently through the skin, a significant increase in size and thickness of the fibrils is observed.

As already known, CASC can dissolve pre-keratin and 0.1 M NaHCO3 can precipitate it. But, for the collagen fibrils increased significantly in size and thickness in our experiments, it can be deduced that the consecutive interaction of NaHCO3 and CASC has the opposite effect on collagen in comparison with pre-keratin.

Another effect of the above process on the skin was the formation of micro bullae which is made up from the remnants of the cells.

Key words: CASC, sodium bicarbonate, collagen, keratin, micro bullae.

* Department of Biochemistry, Istanbul University, Cerrahpaşa Medical Faculty, İstanbul – TURKEY.

** Department of Histology and Embriology, Istanbul University, Cerrahpaşa Medical Faculty, İstanbul – TURKEY.

INTRODUCTION

Collagen is one of the basic proteins in the connective tissue and is synthesized from procollagen, which is an intermediary product of its anabolic pathway. On the other hand keratin is another basic protein in the epitelial tissue and prekeratin is an intermediary product of its synthetic pathway (1).

Prekeratin can be extracted readily by 0,1 M citric acid-sodium citrate buffer (CASC) of pH 2,6 and ionic strength of 0,6.In this buffer 0,1 M $NaHCO_3$ can precipitate this dissolved prekeratin (2).

As it is known collagen in a sense opposite in structure from α-keratins in this study, by passing these solutions through the skin by vacuum, their action on collagen and procollagen has been investigated.

MATERIALS AND METHOD

MATERIALS:

1- 10 guinea-pigs were used in this study.The guinea pigs were killed by decapitation under anesthesia and their skins were stripped immediately.Then, each skin is freshly cut into 3 pieces of equal size.

2- 1 % NaCl solution

3- 0,1 M $NaHCO_3$ solution

4- 0,1 M citric acid-sodium citrate (CASC) buffer of pH 2,6 and ionic strength of 0,6.

a) *0,1 M sodium citrate:*

21.00g citric acid. $1H_2O$ was dissolved in 200 ml n NaOH in a 1 liter volumetric flask and was made up to volume with the distilled water.

b) *Preparation of CASC buffer (pH 2,6 ; ionic strength 0,6):*

36,4 ml solution (a) was made up to 100 ml with 0,1 n HCl (2)

5- Histologic dyes and solutions:

4 % formalin solution, ethyl alcohol, toluol, paraffin, hematoxylin-eosin, Van Gieson.

6- Reichert microtome.

7- Zeiss photomicroscope.

8- Vacuum pump: A Hickman type vacuum pump ($1,2 \times 10^{-7}$mm Hg) was used.

METHODS:

1. Guinea pig skins were streched on glass funnels (r=3,5 cm) in such a way that their hairy sides point towards the inside of the funnels and the skins were attached tightly to the funnels with a string.

Then, some solutions of CASC,0,1 M $NaHCO_3$, 1% NaCl were put into becher respectively.The skins streched on the funnels were immersed in the solutions of and by the application vacuum to the thin part of the funnel, the solutions were passed through the skins (Figure:1).

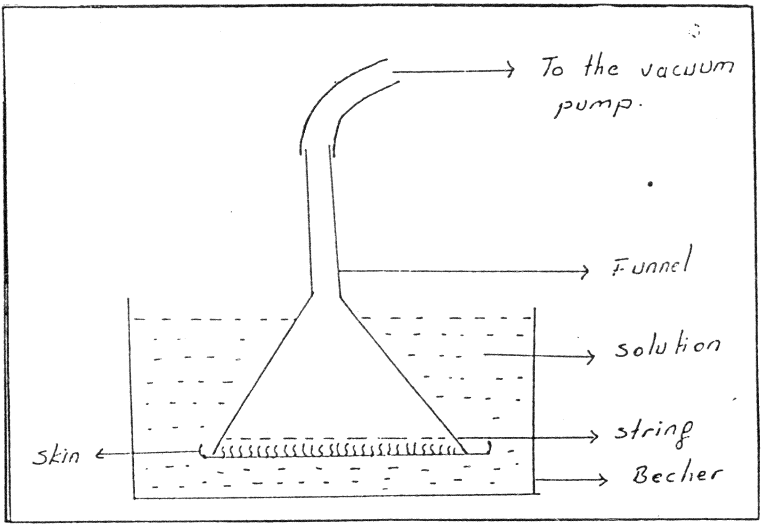


Figure 1: *The application of vacuum to the skin*

HISTOLOGICAL METHOD:

2- Samples of tissues from the removed skin are fixed in 4 % formalin solution as promptly as possible.

After passing through the classical alcohol and toluol solutions, the pieces are embedded in paraffin blocks and cut using a Reichert microtome.Then histological sections are stained with hematoxylin-eosin and Van Gieson dyes in usual manner (3). Stained specimens are examined microscopically and their pictures were taken by a Zeiss photomicroscope.

RESULT AND DISCUSSION

By passing different solutions (CASC, 0,1 M $NaHCO_3$,1 % NaCl, 0,1 M $NaHCO_3$ and then CASC respectively) through the skins different results have been observed.

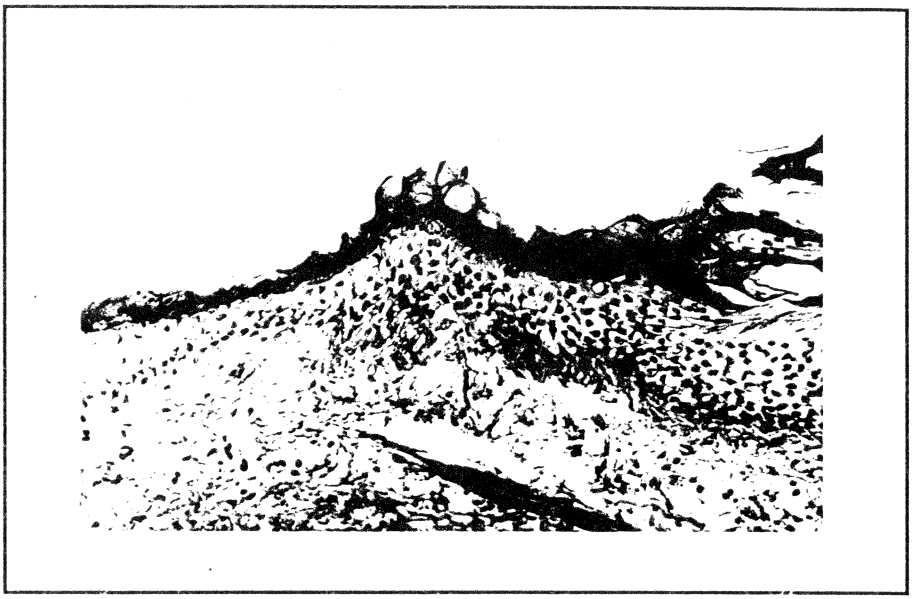
1- 1 % NaCl has been used as a neutral solution and its result has been used to check the effect of the other solutions.After examining the histological specimens of this group the formation of some microscopical bullae in the stratum lucidum has been observed (4). Some of these bullae were arranged like prayer beeds in some parts and constracted clusters in some others (Picture:1).

Epidermal tissue was entirely edematic.The collagen fibers and granules of the stratum granulosum were normal in size and quantity (Picture:2).

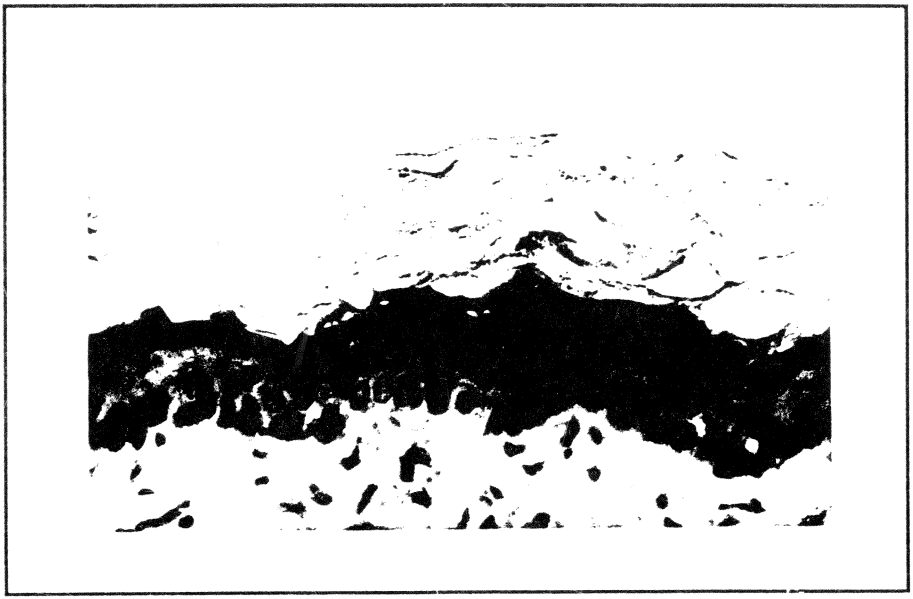
2- pH 2,6 CASC buffer caused some increase in the number of collagen fibers.Their size was some what thicker than normal (Picture:3).

3- 0,1 M $NaHCO_3$ solution caused a decrease in the number of the connective tissue elements, especially in the collagen fibers (Picture:4).

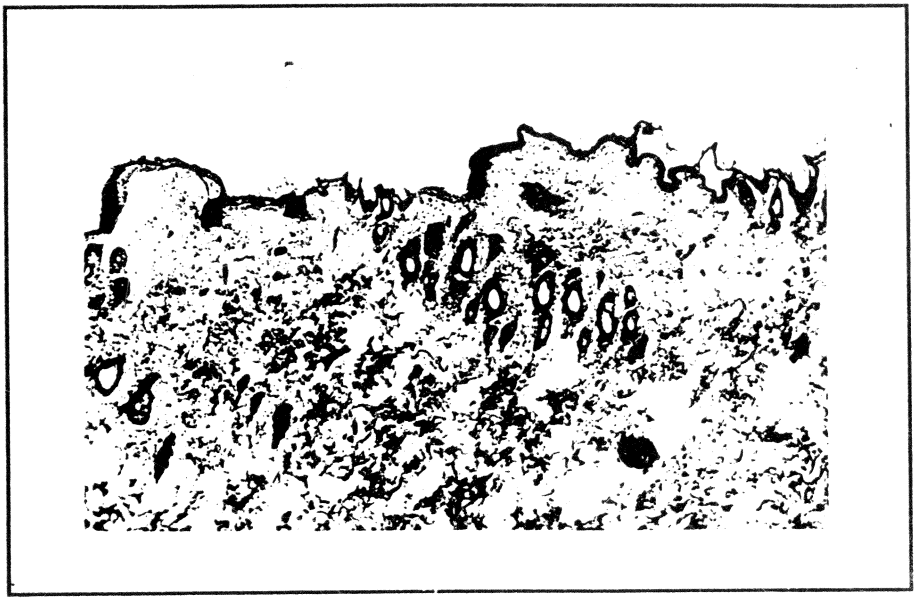
4- Passing 0,1 M $NaHCO_3$ and pH 2,6 CASC solutions through the skins consecutively (15 minutes for each solution) caused an important increase in size and number of the collagen fibers (Picture:5).



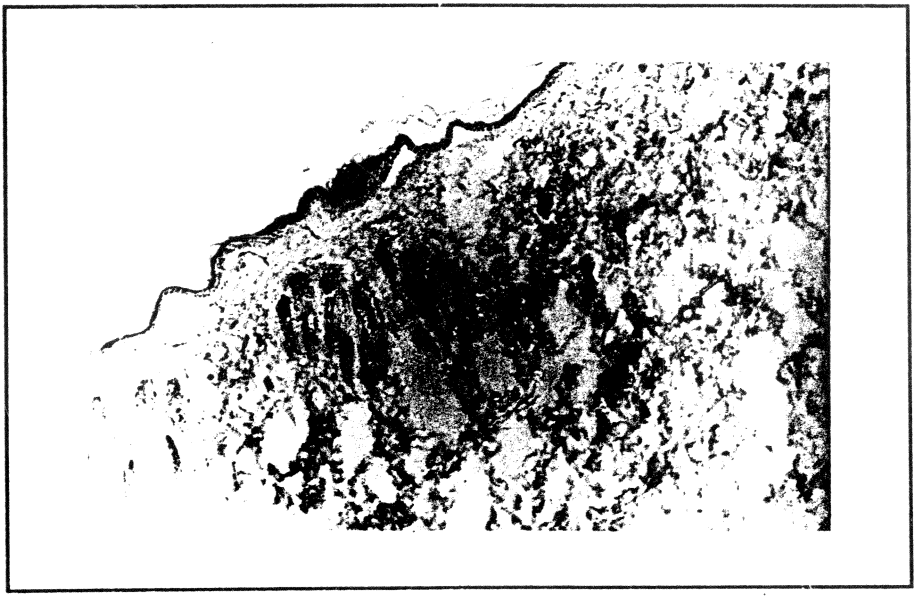
Picture 1: *The microscopic view of the bullae.*



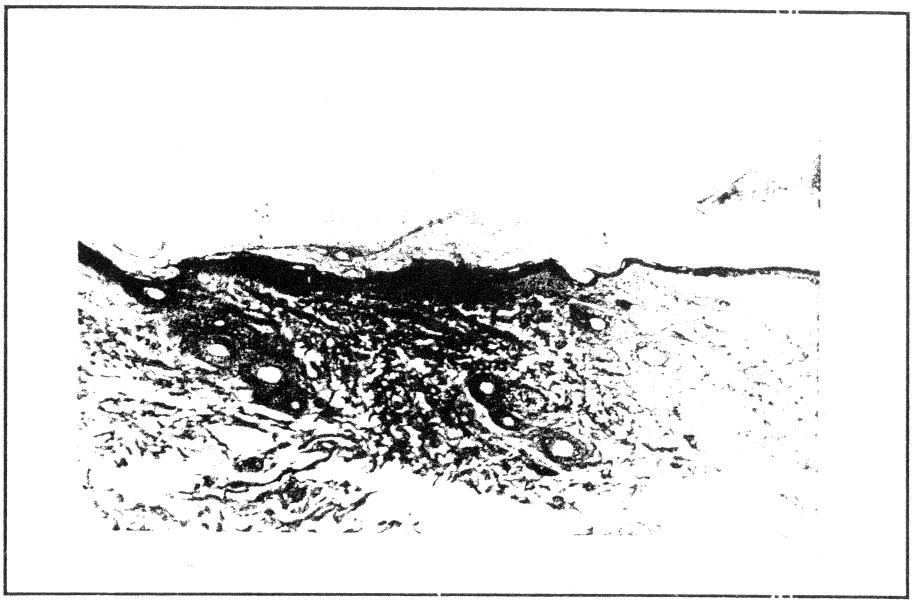
Picture 2: *Normal view of collagen fibers and stratum granulosum.*



Picture 3: *The increasing of the size and the number of collagen fibers by the action of CASC buffer.*



Picture 4: *The decrease of the connective tissue elements by the action of 0.1 M $NaHCO_3$.*



Picture 5: Consecutive action of 0.1 M $NaHCO_3$ and CASC buffer. Important increase in size and number of the collagen fibers are seen.

CONCLUSIONS

1- Although pH 12,6 CASC buffer solution can dissolve and extract prekeratin, it can not dissolve collagen. On the contrary it caused some limited increase in size and number of the collagen fibers.

2- However passing 0.1 M $NaHCO_3$ through the skin and CASC consecutively caused a marked increase in size and number of the collagen fibers.

3- According to the findings mentioned above it can be conclude that 0,1 M $NaHCO_3$ solution accelerated the formation of collagen from procollagen and the CASC caused an important increase in this transformation.

REFERENCES

1-Rawn, J.D., Biochemistry, Neil Patterson Publishers, Carolina Biological Supply Company Burlington, North Carolina, 1989, pp:86-94.

2-Matoltsy, A.G., Prekeratin, Nature, 201, 1130-1131 (1964).

3-Lillie, R.D., Histopathologic technic and practical histochemistry. Third edition, McGraw-Hill Book Comp. NewYork, (1965) pp:539-543.

4-Ostrovskii, A.A and Leve, O.I. Changes in the microstructure of the human epidermis following local vacuum exfoliation, Arkh. Anat, Histol. Embriol. 101 (9-10): 55-59 (1991).