ULTRASOUND AND ORGANIC ACIDS INVESTIGATIONS ON MINERALIZED BIOLOGICAL STRUCTURES

Fifth Communication

DEMINERALIZATION OF BIO-STRUCTURES (BONES AND SHELLS) WITH COMPLEXON III SOLUTION IN ULTRASOUND ENVIRONMENT

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The results obtained from investigations on mineralized structures, treated with organic acids in ultrasound environment are referred to in previous publications by the authors (1, 2, 3, 4). The present work represents a continuation of studies initiated in the past. Here the results are presented of the treatment of samples of human and bovine bones, mussel valves and earth snail shells with complexon III solution in combination with ultrasonics.

As anticipated, a chemical effect was established on behalf of the complexon, manifested in decalcination, which intensified when combined with ultrasound application.

Material and Method

1. Small plates of raw bovine bones and macerated human bones.
2. Mussel (Donax venustus) valves and earth snail (Helix nemoralis) shells.
3. Benzin, ethanol 96% and ether.
4. 0.1 M complexon-III solution.

From the femoral diaphysis of bovine and human bones cross-cut sections were prepared, thick about 2 mm. They were thoroughly cleansed from the bone marrow, periosteum and cancellous parts. They were kept for 48 hours in benzin and 24 hours in 96% ethanol for fats' removal. Finally, meticulous wash-up with ether was carried out.

The specimens were dried at 110° C with weight measurements until a constant value was reached. Thereafter they were subjected to the combined effect of 0.1 M complexon-III solution (5) and ultrasound with frequency 800 kH — 20% and surface of the vibrator 8 square centimeters. Room temperature (17° C) was maintained throughout the treatment. The ultrasonic strength amounted to 2 W per square centimeter, recorded on the measuring device of the apparatus.

The weight reduction of the specimens (Δp) was measured after ultrasound treatment for 30, 60, 90 and 120 min with the samples being washed up with 96% ethanol and ether and repeatedly dried and weighed after each separate session. The weight reduction of the specimens was reckoned on the basis of the initial weight. Separately, the effect on the samples was tested, which was exerted by 0.1 M complexon III solutions alone.

Results

The results of the investigations are illustrated in tables 1 and 2. All data represent mean arithmetical values, received from three determinations.

Table 1

Mineralized Biostructures, Treated with 0.1 M Complexon III Solutions in Ultrasound Environment (Δ p in g %)

	30 min	60 min	90 min	120 min
Macerated human bones	1.00	2.19	2.69	4.60
Raw bovine bones	2.32	3.00	4.19	5.30
Donax venustus	8.53	17.65	27.43	39.83
Helix nemoralis	14.61	24.50	37.50	51.93

Table 2

Mineralized Biostructures, Treated with 0.1 M Complexon III Solutions (Controls) (Δ p in g %)

	30 min	60 min	90 min	120 min
Macerated human bones	0.48	1.16	1.58	2.16
Raw bovine bones	1.00	1.62	2.33	3.29
Donax venustus	3.68	5.70	11.02	24.00
Helix nemoralis	8.02	14.51	19.33	32.65

Table 3

Total Δ p Changes with the Increase of the Duration of Combined Influence

	30 min	60 min	90 min	120 min
Macerated human bones	1.00	3.19	5.88	10.48
Raw bovine bones	2.32	5.32	9.51	14.81
Donax venustus	8.53	26.18	53.61	93.44
Helix nemoralis	14.61	39.11	76.61	128.54

Table 4

Total Δ p Changes with the Increase of Time Interval in the Control Tests

	30 min	60 min	90 min	120 min
Macerated human bones	0.48	1.64	2.22	4.83
Raw bovine bones	1.00	2.62	4.95	8.24
Donax venustus	3.68	9.38	20.40	44.40
Helix nemoralis	8.02	22.53	41.86	74.51

Tables 3 and 4 illustrate the total Δ p alteration with the increase of the time interval in both instances.

The dynamics of the demineralization processes is more clearly outlined in the graphic expression of the results obtained. On the absiss, the time of treatment (duration) in minutes is recorded, and along the ordinate — p in g % (Figs. 1, 2, 3, 4, 5, 6).

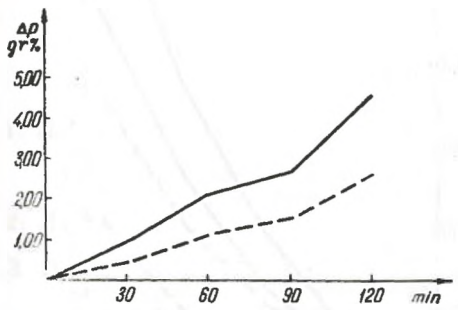

Fig. 1. Weight reduction in human bones: with dense line — under the effect of complexon III and ultrasound and with dotted line — under the effect of complexon III alone.

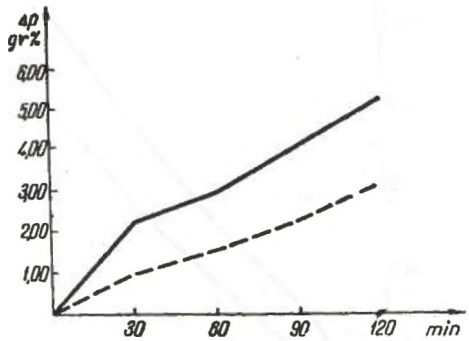

Fig. 2. Weight reduction in bovine bones: with continuous line — under the combined effect of complexon III and ultrasound and with dotted line— under the effect of complexon III alone.

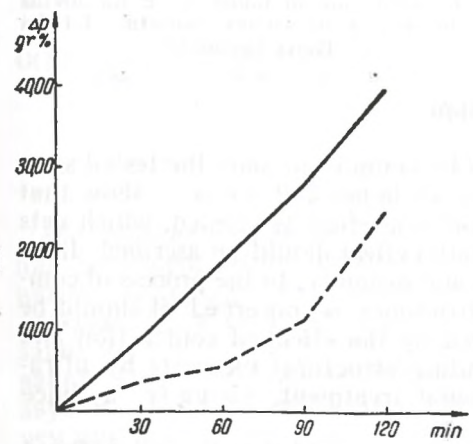

Fig. 3. Weight reduction in Donax venustus: with continuous line — under the combined effect of complexon III and ultrasound and with dotted line— under the effect of complexon III alone.

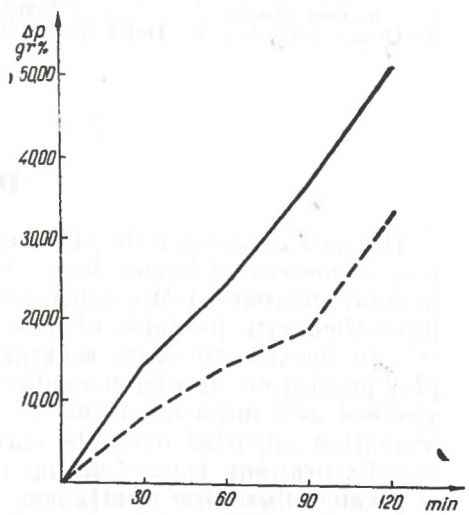

Fig. 4. Weight reduction in Helix nemoralis: with continuous line — under the combined effect of complexon III and ultrasound an with dotted line — under the effect of complexon III alone.

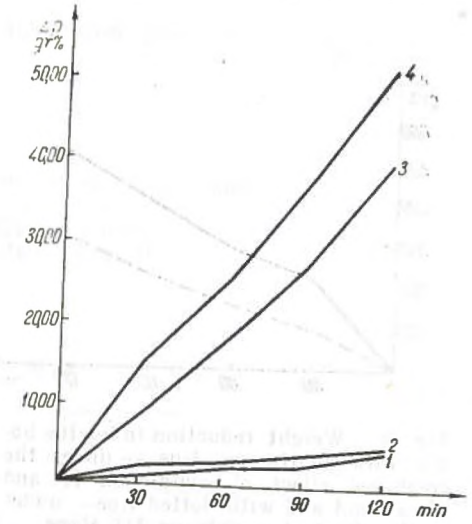

Fig. 5. Weight reduction under the combined effect of complexon III and ultrasound:
1 — human bones; *2* — bovine bones; *3*—Donax venustus; *4*— Helix nemoralis

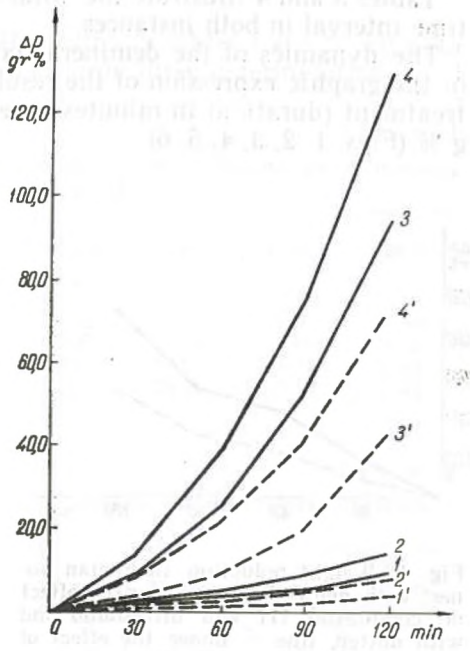

Fig. 6. Total weight reduction p with increasing the time interval: with continuous line — under the combined effect of complexon III and ultrasound and with dotted line — under the effect of complexon III alone.
1. 1'for human bones. 2. 2' for bovine bones. 3. 3' for Donax venustus. 4.4' for Helix nemoralis.

Discussion

The data concerning the effect exerted by complexon upon the tested samples — macerated human bones, raw bovine bones and shells — show that in concentration 0.1 M a substantial dissolving effect is exerted, which gets intensified with the lapse of time. The latter effect should be ascribed, firstly, to the strength of the acid (pH 3.0) and secondly, to the process of complex production. Insofar the action of ultrasonics is concerned, it should be assumed as a mechanical one, determined by the effect of contraction and relaxation imparted upon the corresponding structural elements by ultrasound vibrations. Hence, during ultrasound treatment, virtually, a twice as greater dissolving is attained.

The quantitative data about the dissolving recorded with and without ultrasound application for all four cases display a oneway direction. The degree differences, ranging from Helix nemoralis to human bone, should be explained by the variable resistance of the materials tested equally inasmuch chemical and mechanical influence is concerned. The different resi-

stance, on the other hand, is an absolute indication for the difference in the strength of the mineralized tissues under investigation.

The strength differences of the Helix nemoralis shells and Donax venustus valves as compared to the bones of vertebrates are due, in all likelihood, to the difference in the function of the mineralized organs. In Helix nemoralis and Donax venustus, the shell assumes a passive protective function, whilst in the vertebrates investigated, the long tubular bones are organs of the locomotor apparatus.

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ИССЛЕДОВАНИЯ ПРИ ПОМОЩИ УЛЬТРАЗВУКА И ОРГАНИЧЕСКИХ КИСЛОТ МИНЕРАЛИЗОВАННЫХ БИОЛОГИЧЕСКИХ СТРУКТУР

V-ое сообщение

ОПЫТЫ НА ДЕМИНЕРАЛИЗАЦИЮ БИОСТРУКТУР (КОСТЕЙ И РАКОВИН РАСТВОРОМ КОМПЛЕКСОНА III В УЛЬТРАЗВУКОВОЙ СРЕДЕ

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РЕЗЮМЕ

В сообщении приводятся результаты экспериментальных исследований деминерализации мацерированных человеческих костей, сырых говяжьих костей, раковин Donax venustus и Helix nemoralis, в 0,1 М комплексоне III в ультразвуковой среде. Устанавливается, что деминерализация всех видов образцов растет почти линейно со временем третирования. При комбинированном воздействии (комплексоном III и ультразвуком с частотой 800 кГ)все образцы растворяются в два раза лучше, чем при воздействии одного комплексона III (контроль). Эффект деминерализации более сильно выражен в раковинах, чем в костях, ввиду разных функций, которые они выполняют.