DEMINERALIZATION OF RAW BOVINE BONES WITH ACETIC ACID CHLORINE DERIVATIVES

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It has been demonstrated 200 years ago, that bone tissue contains calcium and phosphorus, but only in 1926, the roentgen-structural analysis has shown that its crystalline structure is similar to the structure of one of the apatite-oxyapatite types — $Ca_{10}(PO_4)_6(OH)_2$ (5, 6). However, the exact identification of the bone tissue apatite is still a controversial problem (9, 10, 11, 12). The collagen, of all organic bone-tissue constituents, is doubtlessly the most significant. Its fibers in the bone plates assume the form of tiny sheaves, encircling the osteons like continuous spirals crossing each other and forming a net-like structure (13). Owing to a number of obscurities still existing in the field of mineralized biostructure, lately the interest towards the latter has assumed an important place in the research work of single investigators and scientific teams. It is universally recognized that the structure of mineralized biological structures is exclusively complex and, it can be said that it is still an unersolved problem to investigators.

It is well known that demineralization of certain biological structure is usually resorted to for a variety of scientific and practical aims. Reverse processes are also known, namely, the removal of the organic component. In demineralization, usually plates of the material investigated are used, dissolved in appropriate solvents with subsequent qualitative and quantitative determination of its composition.

In some of our works, the demineralization effect of some carbonic acids on various mineralized biostructures has been traced up (1, 2, 3, 4, 7). In the present work we set out to investigate the kinetics of the demineralization effect of chlorine derivatives of acetic acid, exerted upon raw bovine bones. The task undertaken is on one side purely practical and on the other it has theoretical orientation.

We are perfectly well aware that substitution of hydrogen atoms from the fatty radical of the acetic acids with chlorine atoms results in augmenting the degree of electrolyte dissociation of the respective carbonic acids. Thus, for instance, the dissociation constants of these particular acid have the following values: for CH_3COOH $K = 1{,}75.10^{-5}$; for $CH_2Cl.COOH$ $K = 1{,}5.10^{-3}$; for $CH\ Cl_2.COOH$ $K = 5.10^{-2}$ and for $C\ Cl_3COOH$ $K = 2.10^{-1}$. We can also expect therefrom the different demineralization effect, produced by the various derivatives.

We failed to come across literature reports, dealing with demineralization of biostructures with halogen derivatives of the acetic acid. Only Thorpe and co-workers (14) have published a report on bone demineralization with 7.5% acetic acid in combination with ultrasound effect.

In the present study, the kinetics of the process was traced up by means of processing plates of raw bovine bones with 0,1 M solutions of monochloro-dichloro- and trichloroacetic acid. The samples of the material were treated at room temperature during variable time intervals, and then other samples under identical conditions during equal time intervals. The weight loss (Δp) of the sample as related to the initial weight, and the level of Ca^{2+} transition in the dissolved part were used as kinetics index of the process.

Material and Method

1. Raw bovine bones.
2. 0,1 M solution of monochloro-dichloro- and trichloroacetic acid.
3. Benzine, 96% ethanol, ether.
4. Reagents for Ca^{2+} complexometric determination.

For experimental work, transverse sections were obtained from the shaft of the femur and treated in the fashion already described in our previous publications.

The samples were dried at 110° C, temperated and weighed until constant weight was reached. In each series three samples were taken and the results obtained are accepted as mean arithmetrical values. The samples were placed in 0,1 M solutions of the respective acids, for one hour. Then they were removed, washed out consecutively with distilled water, alcohol and ether and dried, again, temperated and weighed to constant weight. Samples of 5 ml each were taken from the solution and Ca^{2+} was determined complexometrically, according to a method elaborated at our Chair (8). Identical procedures were repeated at 2, 4, and 6 hours. Another series underwent a sixfold processing of 2 hours each, aiming to trace up the kinethics of the process for equal time intervals.

The Δp of the dissolved portion of the sample is obtained from the weight differences of the samples, before and after treatment. The Ca^{2+} amount was estimated in relation to the dissolved part of the sample.

Results

The results of the investigations performed are presented in Table 1, 2, and 3. Separately, Figs. 1, 2, 3, 4 and 5 illustrate the graphic expression of the curves, presenting the Δp and Ca^{2+} level, depending on the duration of treatment.

Table 1

	1 hour		2 hours		4 hours	6 hours		
	P/g%	Ca^{2+}/g%	P/g%	Ca^{2+}	P/g%	Ca^{2+}	P/g%	Ca^{2+}/g%
$CH_2Cl.COOH$	1,41	30,22	2,49	26,80	2,90	35,41	3,59	23,05
$CHCl_2.COOH$	1,88	32,00	3,00	36,15	4,26	30,11	5,52	33,33
$C\ Cl_3.COOH$	2,49	35,72	6,84	33,36	7,00	32,00	7,17	30,28

Table 2

Raw Bovine Bones Treated Six Times by Two Hours

	I		II		III		IV		V		VI	
	Δp/g%	Ca^{2+} g%	Δp/g%	Ca^{2+} g%	Δp/g%	Ca^{2+} g%	Δp/g%	Ca^{2+} g%	Δp/g%	Ca^{2+} g%	Δp/g%	Ca^{2+} g%
CH_2Cl . COOH	2,21	24,00	2,62	26,90	2,40	33,40	4,46	30,00	2,50	32,16	2,38	37,12
$CHCl_2$. COOH	3,18	30,12	3,30	31,50	3,10	34,08	3,12	31,83	3,20	33,00	3,20	34,00
C Cl_3 . COOH	6,55	28,16	6,90	30,00	7,20	32,15	7,00	31,55	7,00	30,05	6,82	31,47

Table 3

Summarized Changes of p, Parallel to the Increase of Time Interval in g %

	I	II	III	IV	V	VI
CH_2Cl . COOH	2,21	4,83	7,23	9,69	12,19	14,57
$CHCl_2$. COOH	3,18	6,48	9,58	12,70	15,90	19,10
C Cl_2 . COOH	6,55	13,45	20,65	27,65	34,65	41,47

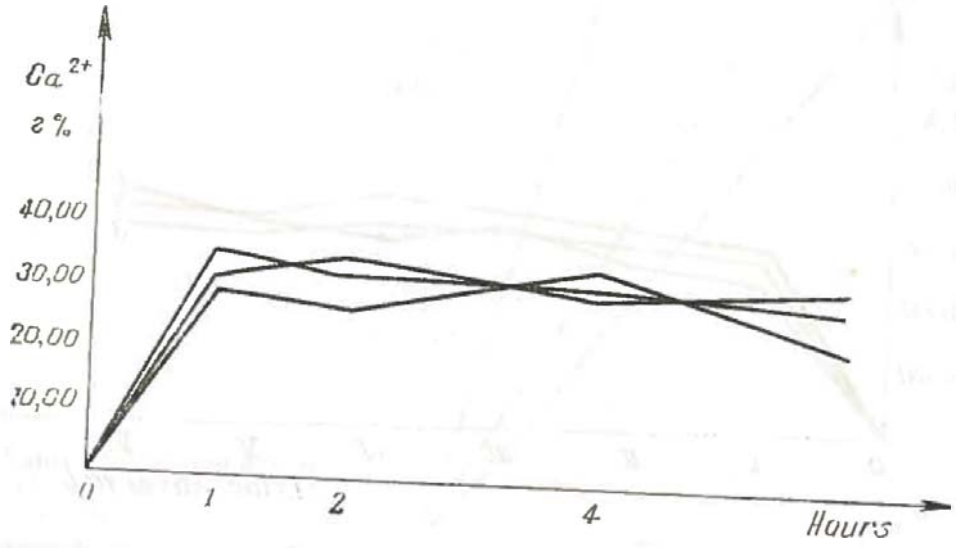

Fig. 1. Level of separated Ca^{2+} in g %
1 — monochloroacetic acid; *2* — dichloracetic acid; *3* — trichloracetic acid.

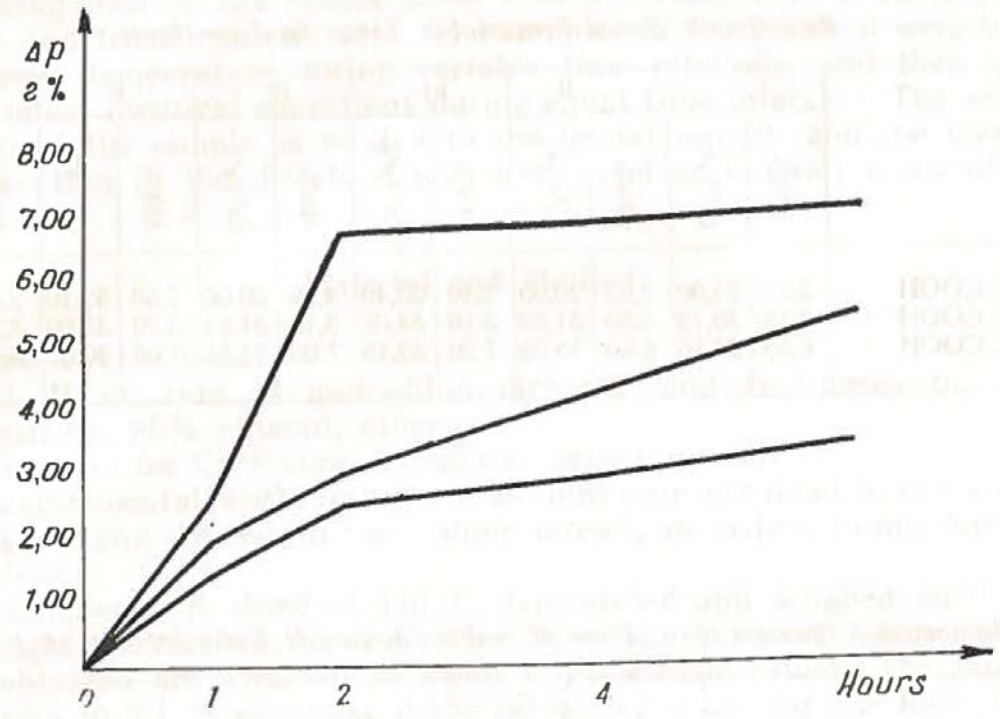

Fig. 2. Weight loss P in g %
1 — monochloracetic acid; *2* — dichloracetic acid; *3* — trichloracetic acid.

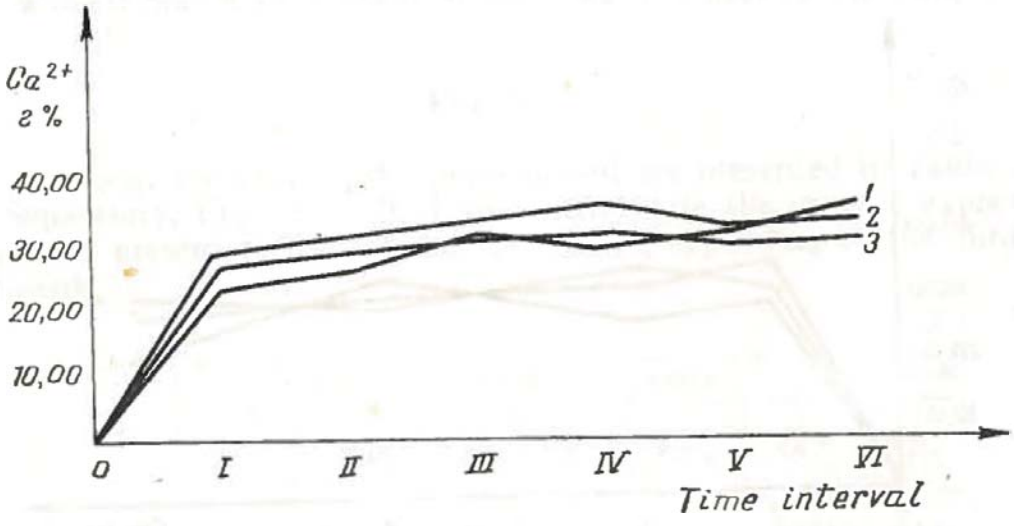

Fig. 3. Level of dissociated Ca^{2+} in g % during sixfold treatment, each lasting for 2 hours
1 — monochloracetic acid; *2* — dichloracetic acid; *3* — trichloracetic acid.

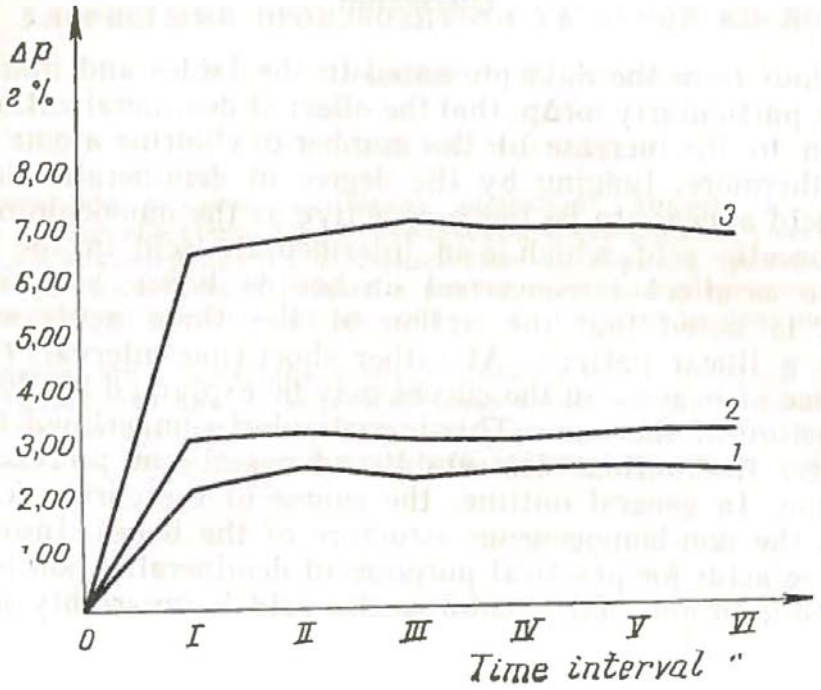

Fig. 4. Weight reduction P in g % during sixfold treatment, each lasting for 2 hours

1 — monochloracetic acid; *2* — dichloracetic acid; *3* — trichloracetic acid.

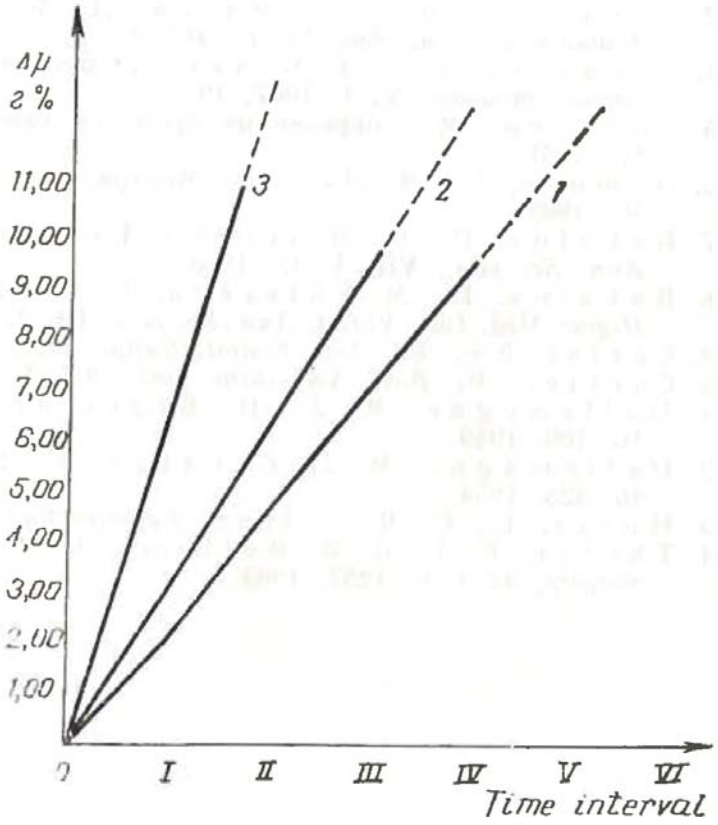

Fig. 5. Summarized changes of P, parallel to the increase of time interval

1 — monochloracetic acid; *2* — dichloracetic acid; *3* — trichloracetic acid.

Discussion

It is obvious from the data presented in the tables and from the course of the curves, particularly for Δp, that the effect of demineralization augments in proportion to the increase of the number of chlorine atoms in the fatty radical. Furthermore, judging by the degree of demineralization, the trichloracetic acid appears to be twice as active as the monochloroacetic acid. The dichloroacetic acid, which is an intermediate acid in the homologous order, as far as effect is concerned on bovine bones, assumes a middle position. It is noted that the action of the three acids with respect to time, has a linear pattern. At rather short time intervals (1—2 hours), the appearance of «waves» in the curves may be explained by the non-homogeneous structure of the bone. This is particularly underlined by the Ca^{2+} level. Stronger fluctuations are established exactly in processing of 1—2 hours duration. In general outline, the course of the curves for Ca^{2+} lays emphasis on the non-homogeneous structure of the bones. Insofar as utilization of these acids for practical purposes of demineralization is concerned, it must be pointed out that trichloroacetic acid is invariably superior.

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О ДЕМИНЕРАЛИЗАЦИИ СЫРЫХ ГОВЯЖЬИХ КОСТЕЙ ХЛОРИСТЫМИ ПРОИЗВОДНЫМИ УКСУСНОЙ КИСЛОТЫ

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РЕЗЮМЕ

Прослежен эффект деминерализации монохлор-, дихлор- и трихлоруксусной кислоты на поперечные срезы диафиза бедренной кости (сырого, говяжьего). Кислоты применялись в концентрации 0.1 М. Воздействие на образцы производилось при комнатной температуре в течение разных и равных интервалов времени. Кинетика процесса прослежена по уменьшению веса образцов (Δp) и по уровню выделенных йонов Ca^{2+} в раствор.

Учитывается, что с нарастанием числа атомов хлора в жировом радикале, растет и эффект деминерализации. В этом отношении самой активной является трихлоруксусная кислота, которая по своему действию в два раза более активна чем монохлоруксусная кислота. Дихлоруксусная кислота занимает по своему действию промежуточное положение.