EFFECT OF LOW-FREQUENCY ULTRASOUND ON ELECTROPHORETIC PROPERTIES AND THERMOSENSITIVITY OF HUMAN SERUM PROTEINS

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Low-frequency ultrasound (23.5 kHz) of relatively low intensity (2 W/cm^2) by its action on the blood serum does not cause irreversible denaturation of its proteins, but modifies their electrophoretic properties, increasing the number of protein particles with average electrophoretic mobility. Proteins of the irradiated sera are more resistant to thermal denaturation.

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In the investigation described below, changes in electrophoretic and certain other properties of the proteins were studied after exposure of the blood serum to low-frequency ultrasound. This problem, although extremely important, has not been adequately dealt with in the Soviet and Western literature [2]. Information on this matter for high-frequency ultrasound, moreover, is contradictory and scanty [10, 11].

EXPERIMENTAL METHOD

Healthy human serum was irradiated in a flask with ultrasound of a frequency of 23.5 kHz and intensity 2 W/cm^2. The intention was that the temperature of the serum would not rise above 25-30° during irradiation. To exclude any effect of heat on the proteins, a control serum was heated to the sonication temperature.

The composition of the serum protein fractions was studied by electrophoresis on paper using Sel'kov's [7] principle of double electrophoresis as modified by the writer [each serum (control and irradiated) was fractionated twice – in the native state and after heating to 62° for 36 min]. The conditions for electrophoresis for the heated and unheated sera were identical (fractionation in the same bath).

Other determinations carried out on each serum included the protein concentration by a refractometric method, pH, clotting time by Okolov's method [6], relative viscosity and temperature coefficient of viscosity [1], optical density of the protein solution, and the content of SH-groups in it.

EXPERIMENTAL RESULTS

Irradiation with ultrasound caused marked changes in the electrophoretic properties of the serum proteins. The content of proteins which acquired the electrophoretic properties of α_2- and β-globulins clearly increased (Table 1), while the content of γ-globulins fell appreciably. The content of albumins was unchanged. With an increase in the duration of exposure to ultrasound, the tendency of the changes in the protein fractions became clearer.

Levinson and Fedin [5], who exposed serum to high-frequency ultrasound, observed similar changes in the electrophoretic properties of its proteins. Consequently, the changes in electrophoretic properties of the serum proteins produced by low- and high-frequency ultrasound were identical in principle. After sonication of the sera, changes took place in the relative proportions of the protein fractions, with a marked tendency toward electrophoretic homogenization [8], i.e., toward an increase in the number of protein particles with average electrophoretic mobility.

The changes discovered in the electrophoretic properties of the serum proteins after sonication are not specific for ultrasound alone, for changes of essentially the same type are observed in proteins following exposure of the serum to other factors [4, 12].

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TABLE 1. Changes in Serum Protein Fractions after Sonification

Fractions	Control sera (M_1)	Irradiated with ultrasound				P for M_1-M_2
		total (M_2)	1 h	2 h	3–4 h	
Globulins						
γ	18,1	14,2	18,0	14,3	10,2	0,05
β	11,9	15,9	13,1	15,7	18,1	0,05
α_2	6,8	10,2	9,0	10,5	10,8	0,001
α_1	4,0	4,2	3,9	4,5	4,8	0,1
Albumins	52,9	55,0	55,0	56,0	56,1	0,1
No. of observations (n)	13	13	3	6	4	

TABLE 2. Effect of Sonication of Sera on Sensitivity of Its Proteins to Heat (in % of total protein content)

Sera	n	Content of albumins			P for M_1-M_2	Protein content in first 2 cm from starting line			P for M_3-M_4
		in unheated sera (A_1)	in heated sera (A_2)	index of decrease after heating (A_1-A_2)		in unheated sera (a_1)	in heated sera (a_2)	index of increase after heating (a_1-a_2)	
Control	13	59,1	12,3	$M_1=46,8$	0,02	4,6	54,6	$M_3=50,0$	0,001
Irradiated with ultrasound	13	55,5	17,7	$M_2=37,8$		3,9	21,8	$M_4=17,9$	

It should be emphasized that the doses of ultrasound which were used did not cause denaturation of the proteins. After irradiation of the sera with ultrasound, nothing more than modifications [9] of the serum proteins was observed (lowering of viscosity while the pH remained unchanged, the proteins did not lose their solubility in the isoelectric zone; no increase in optical density was found; the number of SH-groups was reduced and not increased, as happens during denaturation).

Electrophoresis of the sera heated to 62° did not reveal the clear demarcation into 4-5 fractions observed during electrophoresis of the native sera. The proteins of the heated sera were divided fairly clearly into two groups: those with low mobility (nearer to the starting line) and those with higher. The latter group of proteins corresponded to remains of the albumin fraction of the native serum. Differences in the sensitivity of the serum proteins to heating were particularly marked as regards the increase in the content of protein settling in the first 2 or 3 cm of the paper (from the starting line) during electrophoresis of the heated sera compared with the unheated, native sera. To assess the changes in thermosensitivity of the proteins after irradiation with ultrasound, this index was used together with the decrease in content of proteins of the albumin fraction after heating the native sera.

As would be expected, the proteins of the control sera were very sensitive to heating (Table 2): four-fifths of the total albumin lost its previous electrophoretic mobility after heating and settled mainly near the starting line.

The proteins in the sera irradiated with ultrasound were more resistant to heating, and for this reason the content of albumin which lost its mobility after heating was much smaller than in the control sera: 2.7 times less protein than in the control sera settled near the starting line.

The decrease in thermosensitivity of the proteins demonstrated by electrophoresis on paper after exposure to ultrasound was confirmed by measurement of the clotting times of the sera. Control sera after heating clotted on the average in 61.8 min, compared with 97-175 min for sera irradiated for 1-4 h, respectively.

At the same time, it was shown that irradiation of the sera with ultrasound lowers their relative viscosity and their temperature coefficient of viscosity, reflecting a decrease in the state of aggregation of the material [3]. It may accordingly be considered, that the observed de-aggregation of the serum protein particles is one of the main causes for the decrease in thermosensitivity of its proteins.

It can be concluded from the results of this investigation that low-frequency ultrasound of relatively low intensity (2 W/cm^2), by its action on serum does not cause irreversible denaturation of its proteins, but can modify their electrophoretic properties, producing an increase in the number of protein particles with average electrophoretic mobility. Exposure to ultrasound causes the proteins to lose their natural high sensitivity and to become more resistant to heat, as can be shown relatively easily if the principle of measured thermal denaturation is used in conjunction with electrophoretic investigation. The use of electrophoresis on paper with this additional refinement makes it possible to study, at low cost of serum and time, not only quantitative changes in the fractional composition, but also qualitative changes developing in the proteins under the influence of ultrasound, a highly important matter because qualitative changes are known to take place frequently much sooner than quantitative changes and to disappear after them.

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