THE EFFECT OF ULTRASOUND AND TEMPERATURE ON THE COLLOID PROPERTIES OF HUMAN SERUM

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EXPERIMENTAL METHOD

For these experiments we used an ultrasound generator producing 2000 kc with an intensity of 2 W/cm^2 of radiator. A cylinder made of very thin glass, 2.5 cm in diameter and 10 cm high, was used. The cylinder was covered with a rubber stopper, through which was passed a long thermometer bulb, so that its tip was immersed in the serum to be irradiated. The readings of a thermometer placed in a medium irradiated with ultrasound are slightly increased. However, we regarded this slight error as acceptable, for the important thing in these experiments was that the temperature should not be underestimated.

Into this apparatus was poured 1 ml of the test serum, obtained on the previous day, and the stopper with the thermometer was then applied. The cylinder was fixed on to a stand above the radiator so that the oil spray produced by the generator when in operation just reached the bottom of the glass vessel. To prevent the temperature from rising, iced water was used, and the vessel with the oil was immersed in it.

The protein concentration in the original and irradiated sera was determined by a refractometric method, the clotting time of the serum was measured at 62° [1] and the relative viscosity of the serum was determined by Hess's method at 20°.

EXPERIMENTAL RESULTS

Protein concentration. The protein concentration in twelve irradiated sera varied from 6.6 to 9.35%. All the differences between the properties of the irradiated and control sera were unaccompanied by any change in the protein concentration.

TABLE 1. Mean Clotting Times of Sera (in minutes)

Original sera				Irradiated sera				Significance of difference	
clotting time								M_2-M_1	
n	M_1	$\pm\sigma_1$	$\pm m_1$	n_1	M_2	$\pm\sigma_2$	$\pm m_2$	t	P
12	58	13,6	3,9	29	74	25,0	4,63	2,67	<0,05 >0,02

Clotting time of the serum. The clotting time of 1 ml of serum, obtained from the blood of a healthy person, is 40-75 min at 62°. Exposure to ultrasound for a period of 25-30 min led to a decrease in these times (Table 1).

The results in Table 1 show that the difference between the mean thermal clotting times of the untreated and treated sera (M_2-M_1) was significant, although it was brought about by the combined action of high-frequency ultrasound and the raised temperature arising during irradiation by the ultrasound.

The results given in Table 2 show the original values, the effect of heating alone (temperature control) and the

TABLE 2. Effect of Ultrasound Waves on the Clotting Time of Sera (in minutes) Compared with the Temperature Factor

Clotting times of											
original sera				sera heated to 37-56° for 15-60 min				sera exposed to ultrasound at 37-56° for 15-60 min			
n_1	M_1	$\pm^{\sigma} 1$	$\pm^{m} 1$	n_2	M_2	$\pm^{\sigma} 2$	$\pm^{m} 2$	n_3	M_3	$\pm^{\sigma} 3$	$\pm^{m} 3$
20	53	16	3,55	20	66	24	5,3	20	68	24,6	5,35

TABLE 3. Relative Viscosity of Unirradiated and Irradiated Sera, Taking Account of the Temperature Control

Expt. No.	Protein (in %)	Initial relative viscosity of serum	Heating temperature in control and irradiated samples	Period of heating or irradiation (in min)	Relative viscosity of heated sera	
					unirradiated	irradiated
1	7,0	1,500	32—40°	30	1,483	1,424
2	6,64	1,669	39°	60	—	1,620
2	6,64	1,669	50°	60	—	1,621
2	6,64	1,669	52°	60	—	1,663
3	7,7	1,845	52—54°	15	1,910	1,880
4	8,49	1,800	50—55°	15	2,100	2,180
5	6,87	1,750	54—58°	30	1,670	2,450
5	6,87	1,750	55—56°	30	1,530	2,800
6	7,85	1,900	55—57°	15	—	2,760
7	6,12	1,650	62°	30	2,900	~*
8	6,93	1,77	62°	30	4,400	~
9	6,8	1,8	62°	30	4,200	~
10	6,4	1,78	62°	30	4,100	~

*Gel formation and syneresis were observed here.

effect of exposure to ultrasound, accompanied by the same increase in the temperature of the serum. The results of the three series of experiments represent mean values.

Statistical analysis showed that heating led to an almost statistically significant change in the indices (factor of error 0.07), whereas the action of ultrasound alone, taking into consideration the temperature control, had no effect.

The results were different in those cases in which the temperature of the serum rose during exposure to ultrasound to 62° (this was achieved by not cooling the oil). In six experiments performed at 62°, the clotting time of the serum after exposure to ultrasound varied between 20 and 70 min (mean 49 min), whereas in the matched controls the clotting time ranged between 46 and 110 min (mean 80 min).

The increase in the rate of clotting of the serum irradiated at 62° presented certain remarkable features. When these sera were allowed to stand, a dense mass was formed on the glass of the vessel. The liquid part became separated from the coagulated protein. The protein concentration in the fraction separating after exposure to ultrasound for 37, 50, and 90 min fell to 1,97-0,6%. Hence, the increase in the rate of coagulation of the serum taking place at 62° under the action of ultrasound appeared, in fact, in the same temperature range in which thermal denaturation of proteins occurs. This process, as we know, consists of the opening out of the protein particles, which change from a globular into a fibrillary state, and of the fixation of water between the interwoven fibrillary protein particles. In this way a gel is formed. If during this period the protein is in an ultrasonic field, its architectonics is disturbed and syneresis takes place.

Relative viscosity of the sera. The results given in Table 3 illustrate the effect of heating on the viscosity of the sera (temperature control), and also the effect of exposure to ultrasound, accompanied by the same increase in the temperature of the serum.

The order of these experiments was that the serum was first irradiated by ultrasound (taking note of the temperature), and then another sample of the same serum was heated to within the same limits of temperature as during irradiation.

From a comparison of the results given in Tables 1-3 the following conclusions may be drawn:

1. When the temperature of the serum during exposure to ultrasound did not exceed 54°, the viscosity varied in the neighborhood of the initial value.

2. The action of ultrasound waves, as such, in causing a significant increase in viscosity (taking account of the temperature control), could be observed when the temperature of the serum rose during exposure to 58-62°, i.e., to the range of temperature at which protein particles begin to open out and to change from a globular to a fibrillary state.

SUMMARY

Concentration of protein, coagulation time of serum at 62°C (after F. S. Okolov, 1947) and viscosity after Hess were determined for the assessment of changes occurring in the serum under the influence of ultrasound. The action of ultrasound, with due consideration of the temperature, does not cause any changes in the properties of the serum, if the temperature of the serum is less than 56°C. Only when the temperature increases up to 62°C can the effect of ultrasonic vibrations be shown. In this case an accelerated coagulation of serum accompanied by syneresis is observed.

LITERATURE CITED

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