CHANGES IN OPTICAL DENSITY AND VISCOSITY OF HEMOLYZATES UNDER THE INFLUENCE OF ULTRAVIOLET IRRADIATION AND HEAT

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If a 1% hemolyzate is irradiated with ultraviolet light, the bright red color of the liquid soon acquires a brownish tint. This phenomenon is associated with the formation of methemoglobin [5].

In this paper, the changes are described which take place in the optical density and viscosity of hemolyzates following exposure to ultraviolet irradiation and also to heat.

EXPERIMENTAL METHOD

The hemolyzates were obtained and prepared as follows. Blood obtained from a man or animal was mixed with a solution of Richter's heparin in the proportion of one drop to 3 ml blood. The erythrocytes were then washed three times in a centrifuge with physiological saline (at 1500 rpm). After decantation of the liquid, a 1% hemolyzate was prepared from the residue in medinal-veronal buffer pH 8.4 (0.5 ml of packed erythrocytes to 50 ml buffer), and this was then centrifuged at 2500 rpm to remove the stroma of the erythrocytes. The hemolyzate was divided into two parts: one part remained unirradiated (control) while the other was exposed for 60 min to irradiation by the full spectrum of a PRK-2 lamp (35 cm from the source). Altogether four hemolyzates were used: unirradiated unheated, unirradiated heated, irradiated unheated, and irradiated heated. The hemolyzates were kept in the refrigerator at 0°.

The optical density of the hemolyzates was measured by means of the FEK-M photoelectric colorimeter. The viscosity of the hemolyzates was determined at constant temperature (20°) by means of an Ostwald's viscosimeter (capillary tube 0.6 mm in diameter). To simplify the calculations, all the values of the optical density were multiplied by 1000, and those of the viscosity by 10,000.

EXPERIMENTAL RESULTS

The mean values of the optical density of the hemolyzate (M) and the significance of the difference are shown in Table 1.

It is clear from Table 1 that the process of irradiation itself produced a significant increase in the optical density of the hemolyzate. The action of heat was different: the unirradiated hemolyzate gave no significant increase in optical density, whereas in the irradiated hemolyzate the optical density was increased four times by heating.

Changes of this type may be explained by assuming that the irradiated hemolyzate, when heated for 15 min at 58°, not only turns the liquid brown, but also makes it turbid. At the same time, the unirradiated hemolyzate remained unchanged in color and transparency by comparison with its original state after heating. The sensitivity of the irradiated hemolyzate to heat thus discovered is in agreement with reports in the literature. L. A. Blyumenfel'd [1], for example, found that methemoglobin is less stable in relation to denaturing agent than oxyhemoglobin. The phenomenon of clouding was evidently associated with the protein part of the methemoglobin, i.e., with globin.

The results of the investigation of the viscosity of the hemolyzates, together with the significance of the differences obtained, are given in Table 2. This shows that irradiation caused a significant decrease in the relative viscosity of the original hemolyzate. Subsequent heating of the irradiated hemolyzate caused a still greater decrease in relative velocity.

It may be concluded from the results obtained that changes take place in the molecular weight of hemoglobin during irradiation of the hemolyzate.

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TABLE 1. Characteristics of Optical Density of Hemolyzates (Multiplied by 1000)

Hemolyzate		Groups	Optical density	P
Unheated	Unirradiated n = 23	1	29.8 ± 2.4	$P_{1-2} < 0.001$
	Irradiated n = 23	2	85 ± 3	$P_{2-4} < 0.001$
Heated	Unirradiated n = 15	3	37.3 ± 4.1	$P_{1-3} > 0.1$
	Irradiated n = 15	4	334 ± 2.1	$P_{3-4} < 0.001$

TABLE 2. Mean Values of Relative Viscosity of 25 Hemolyzates and Evaluation of Significance of Differences (M±m)

Index	Type of hemolyzates				Significance of difference		
	Unheated		Heated		Mean values compared	n	P
	Original M_1	Irradiated M_2	Original M_3	Irradiated M_4			
Mean relative viscosity ($\eta \cdot 10{,}000$)	10,390 ± 29.3	10,314 ± 22.6	10,356 ± 26.3	10,280 ± 27.3	M_1 and M_2	25	0.05014
					M_1 and M_4	25	< 0.02
					M_1 and M_3	25	0.4

TABLE 3. Molecular Weight of Hemoglobin

Treatment of hemoglobin	Molecular weight
Original	68,010
Irradiated (unheated)	49,780
Heated (unirradiated)	59,700

To reflect the relationship between viscosity and molecular weight of a substance, the general equation of Shtaudinger [6] is used:

$$[\eta] = K \cdot M^a,$$

where $[\eta]$ is the reduced viscosity; K a constant; M the molecular weight; and a a value characterizing the coagulation of macromolecules in solution.

It follows from Shtaudinger's equation that the increase or decrease in viscosity reflects an increase or decrease in the molecular weight of the particle, although this relationship is not one of direct proportion [2]. In the present case, this conclusion cannot be drawn without qualification. As has been shown [4], the difference between the original and the irradiated hemoglobin is that the latter, when heated, quickly changes into a hydrophobic state and is precipitated in an isoelectric buffer (pH 6.8), and in such conditions changes in viscosity cannot reflect changes in molecular weight of the irradiated and subsequently heated hemoglobin. On the other hand, unirradiated hemoglobin, heated in the same conditions, does not become turbid and remains a hydrophilic colloid, not precipitated in an isoelectric buffer. The original irradiated hemoglobin also remains transparent and hydrophilic.

On the basis of this argument, a provisional calculation was made of the molecular weight of the hemoglobin only for the hemolyzates given in Table 3. In these conditions the molecular weight of the original hemoglobin (from sources in the literature) was taken as 68,000 and the value of a in Shtaudinger's formula as 0.7, the mean of the values given for different polymers [3]. The constant M was determined by substituting the known value of the molecular weight of the original hemoglobin in Shtaudinger's formula. Its value was 0.00001615.

As Table 3 shows, irradiation itself modified the hemoglobin molecule and lowered its molecular weight. So far as the heated (unirradiated) hemoglobin is concerned, since no significant decrease could be observed in the viscosity, it can only be suggested that a slight decrease may take place in its molecular weight when an unirradiated solution of hemoglobin is heated in the conditions of these experiments.

LITERATURE CITED

1. L. A. Blyumenfel'd, Hemoglobin and the Reversible Combination with Oxygen [in Russian], Moscow (1957).
2. S. S. Voyutskii, A Course in Colloid Chemistry [in Russian], Moscow (1964).
3. V. I. Kasatochkin and A. G. Pasynskii, Physical and Colloid Chemistry [in Russian], Moscow (1960).
4. F. S. Okolov and E. F. Sivets, Uchen. Zapiski Kubansk. Med. Inst., No. 2, 322 (1963).
5. C. Sonne, Strahlentherapie, 28, 45 (1928).
6. G. Shtaudinger, Macromolecular Organic Compounds [in Russian], Moscow-Leningrad (1935).