Effects of ultraviolet irradiation on the electrophoretical typing of serumprotein polymorphisms

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INTRODUCTION

For many years a therapy has existed using the retransfusion of blood after ultraviolet irradiation (the socalled "ultraviolet autologous blood irradiation") (Wiesner 1967, Wiesner et al. 1986). The mechanisms of this therapy are still poorly understood. Up to now an extensive clinical study is still missing. Hence this therapy can rightly be regarded as a matter for scientific dispute.
The present study has the aim to investigate the effects after ultraviolet irradiation using a defined part of the human blood - the serum proteins. For that purpose standard methods for genetic typing were applied. These methods (e.g. isoelectrofocusing) are able to recognize very small differences in the structure or charge of proteins.
We hope to have thus made a contribution to understanding the effects of ultraviolet irradiation of the blood.
Moreover, a study of this type might provide information for the forensic serologist concerning the analysis of blood stains.

MATERIAL AND METHODS

Fresh serum samples were treated and analyzed. The treatment by ultraviolet irradiation was carried out in quartz cuvets with FMR 10 equipment (Manufacturer: VE Kombinat Präcitronik, Dresden, GDR, Hg-lamp HNU 6; maximum emission at 254 nm).
The periods of irradiation were 1, 5 and 10 minutes. The results of genetic typing were compared before and after irradiation.

The following serum protein polymorphisms were typed:

C3, TF	typing by agarosegel electrophoresis (AGE)
BF, GC, HP, PI and TF	including the subtypes typing by isoelectrofocusing (IEF) in polyacrylamide gel (0.5 mm) carried out with the "Multiphor" equipment (LKB, Bromma, Schweden). For technical details see Patzelt (1984), Prokop and Göhler (1986).

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For the exclusion of thermal effects we performed experiments in which serum samples were heated without using ultraviolet irradiation (42°C, max. 20 minutes). It was revealed that heating did not produce the effects of irradiation.

RESULTS AND DISCUSSION

The results are compiled in table 1. We could observe a reduction of the protein bands examined after ultraviolet irradiation using electrophoresis or isoelectric focusing as separation methods. This reduction may reach the degree of total disappearance of the protein zones (e.g. after 10 min. of irradiation). These results are like those obtained after different physical or chemical treatments for protein denaturation. These denaturations are due to changes of charge or structure of proteins.

Our results and the evidence obtained in this way for the different stability of proteins are in agreement with many other well-known facts in protein chemistry or in forensic serology.

The most important effects were found for the PI system. With all PI phenotypes tested, ultraviolet irradiation produced the same changes: all bands (zones 2, 4, 6, 7 and 8) were shifted to the anodic side (table 2, fig. 1). The distance of the shift corresponds to the distance between subtype M1 and M2 (corresponds to 0.01 pH units of the isoelectric point). We wish to underline this change because of the possibility that "new" phenotypes are produced by ultraviolet radiation (e.g. from PI M2 the "new" type "M1").
From this we conclude that there is a small change in the protein structure. Changes of glycolization have to be considered. We know that the different zones of PI after IEF are due to the different content in sialic acid (Yoshida and Wessels 1978). But anodic PI bands contain a larger amount of sialic acid. For that reason the reported effects of irradiation cannot be explained by the loss in sialic acid.
For forensic serological practice we have to continue these investigations. The next steps will be the analysis of blood and of blood stains after ultraviolet irradiation.

SUMMARY

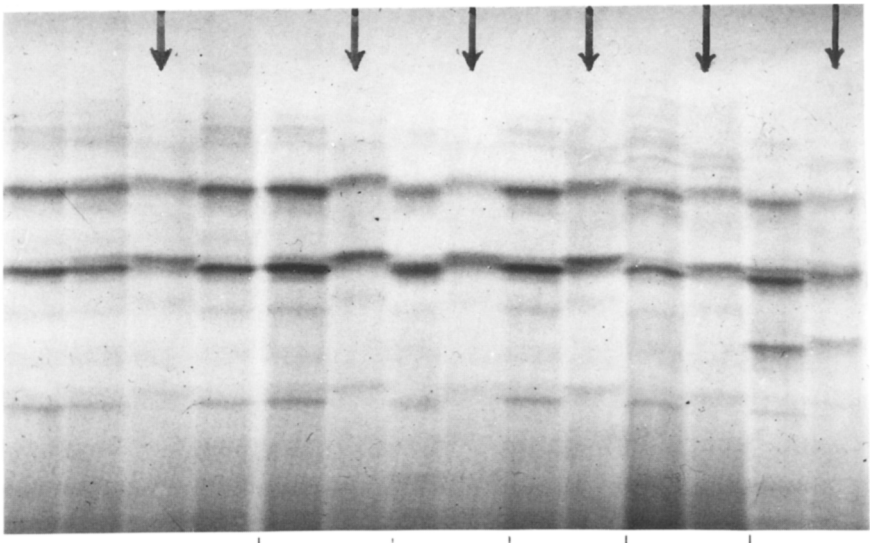
Short-term ultraviolet irradiation (5 or 10 min. with UVC) of serum samples is able to produce marked changes in the typing of genetic serum protein polymorphisms (C3, TF, GC, HP, BF, PI). For most protein bands varying degrees of reduction were observed which even went as far as their total disappearance. In the PI system small but important changes of the IP are obtained because "new" phenotypes arise from the original type (e.g. M1 instead of M2).

Table 1. Effects of UV irradiation of serum samples on the typing of genetic polymorphisms

System	Method	Irradiation (min)	Effects on the specific protein bands
TF	AGE	1	distinctly reduced
		5, 10	strongly reduced or disappeared
C3	AGE	1	distinctly reduced
		5, 10	strongly reduced or disappeared
GC subtypes	IEF	1	unimportantly reduced
		5, 10	strongly reduced
TF subtypes	IEF	1	unimportantly reduced
		5	distinctly reduced
		10	strongly reduced
HP subtypes	IEF	10	distinctly reduced
BF subtypes	IEF	10	disappeared
PI subtypes	IEF	1	normal bands
		5, 10	all bands slightly shifted to the anode

Table 2. Effects of UV irradiation (10 min.) of serum samples on PI typing

Original PI type	IEF results after irradiation
M1	all bands slightly shifted to the anode
M1 M2	
M1 M3	
M3	
M3 S	
M3 M2	→ "new" phenotype like M1 M3
M2	→ "new" phenotype like M1



0 = Original
2,5,10 = UV Irradiation (min.)
42° = Heating (20 min.)
↓ = Conversion

Fig. 1. Effects of UV irradiation of serum samples on PI typing (IEF)

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