Table II. Laboratory findings (mean and range) before and after incubation of the post-occlusion blood samples in 5 subjects and in 1 patient given ^{125}I-labelled fibrinogen

	Serum FDP/fdp (μg/ml)	Fibrinogen (mg/100 ml)	Thrombin time (sec)	Radioactivity in serum (cpm/ml)
Before incubation	<10	305 (210–375)	28 (19–33)	1900
After incubation	<10	310 (210–380)	27 (19–31)	1940

conditions comparable to those of the dilute clot lysis. The determinations listed in Table II and the electrophoretic studies were performed on plasma obtained from blood before and after incubation. In the samples containing ^{125}I-fibrinogen, the radioactivity was determined in the serum after clotting the plasma with thrombin.

The mean values and range for the laboratory determinations before and after venous occlusion are shown in Table I. Significant shortening of euglobulin lysis time and of the dilute whole blood clot lysis time occurred in each of the post-occlusion samples. These findings are in accordance with those previously reported[12]. However, the level of FDP/fdp in serum did not change, being 2 μg/ml, in all samples. Similarly, the radioactivity in the serum remained unchanged, confirming the fact that no unclottable fibrinogen derivatives were formed as a result of the fibrinolytic activity induced by venous occlusion. The fibrinogen concentration was increased following occlusion, being out of proportion to the degree of hemoconcentration as measured by the hematocrit, an observation previously made by NILSSON and ROBERTS[12].

The effect of incubation of plasma samples on these measurements is shown in Table II. No changes in fibrinogen, thrombin time, FDP/fdp or serum radioactivity occurred. These findings indicate that the fibrinolytic activity did not result in any degradation of fibrinogen into its unclottable derivatives.

It has been shown in our laboratory that electrophoresis of plasma in SDS-acrylamide gel (3.5%) results in separation of fibrinogen into 2 bands, the major portion being of higher molecular weight, the LMWF representing 25–35% of the total clottable protein[13]. The proportion of LMWF was not affected either by venous occlusion nor by incubation of the blood samples in vitro. The Figure shows the densitometric scans of gels after electrophoresis of plasma and serum from a single representative subject. The pattern before occlusion (A), after occlusion (B) and after incubation of the post-occlusion blood (C). No increase in the percent of LMWF to the clottable protein occurred, in fact a slight decrease was found. The mean percentage values of LMWF in plasma of all subjects before and after occlusion was 32% (range 31–34%) and 28% (range 26–30%) respectively. The mean percent of LMWF in plasma after incubation for 24 h at 37 °C was 27% of the total fibrinogen (range 19–31%). These findings indicate that the fibrinolytic activity did not result in any conversion of high into LMWF even after 24 h of incubation of the plasma rich in plasminogen activator. The heterogeneity of fibrinogen in man, represented by the presence of LMWF, does not seem to be the result of direct fibrinogenolysis and may rather be due to an indirect pathway of fibrinogen catabolism such as 'endocytosis' postulated by REGOECZI[14].

The difference in susceptibility of fibrinogen and fibrin to proteolytic degradation have been attributed to several factors. The binding of plasmin by antiplasmins, such as α_2-macroglobulin, was shown to inhibit the enzymatic activity with respect to fibrinogen but not fibrin degradation[15]. Secondly, the type of plasminogen activator appears to be a significant factor by which fibrinolysis rather than fibrinogenolysis occurs as demonstrated by CAMIOLO et al.[16] in a study comparing tissue activator with urokinase and streptokinase. By whatever mechanism, circulating fibrinogen appears to be effectively protected from proteolysis induced by naturally occurring plasminogen activators. It is therefore likely that FDP/fdp in serum are the products of fibrin breakdown as previously suggested[4, 5].

Zusammenfassung. Nachweis, dass nach Venenokklusion bei 10 gesunden Probanden erhöhte Fibrinolyse, nicht aber Fibrinogenolyse auftrat.

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The Protective Effect of L-DOPA on Heinz Body Formation in G6PD Deficient Red Cells

A number of chemicals including Acetylphenylhydrazine (APH) have been found to produce Heinz body formation in normal human red cells, and in erythrocytes of glucose-6-phosphate dehydrogenase (G6PD) deficient patients. The same compounds were shown to generate free hydrogen peroxide (H_2O_2) in red cells[1]. It is generally accepted that Heinz body formation involves the oxidative denaturation of hemoglobin mediated by H_2O_2[2]. On the other hand, the red cell enzymes, catalase and glutathione peroxidase, play a major role in protecting hemo-

globin from oxidation; glutathione peroxidase which employs reduced glutathione (GSH) as a hydrogen donor, requires reduced triphosphopyridine nucleotide (NADPH) as a co-factor. In erythrocyte (G6PD) deficiency, the NADPH generating system is defective. Thus, the use of a compound which can significantly reduce levels of H_2O_2, may be able to protect the G6PD deficient red cell from Heinz body formation. 3,4 Diphenylhydroxylamine (L-DOPA), a naturally occuring biological amino acid, which removes H_2O_2 during oxidation to the quinone and indolequinone form[3] was then tested in an attempt to replace the ineffective reducing mechanism in the G6PD deficient red cell, and offer additional protection against the formation of Heinz bodies.

Fresh, whole, heparinized blood was obtained from normal volunteers and 3 patients with severe G6PD deficiency. The effect of varying concentrations of L-DOPA on Heinz body formation was carried out incubating in the dark, 0.1 ml whole blood aerobically at 37 °C in a 0.07 *M* phosphate buffer, pH 7.5, supplemented with 8.0 m*M* glucose and various 'oxidizing' reagents, such as APH 10^{-2} *M*, ascorbic acid 10 m*M* and sodium azide 4 m*M* in a total volume of 2.0 ml. Following a 4 h incubation, the red cells were stained with crystal violet and the percentage of Heinz bodies was determined in 200 consecutive cells. The Heinz bodies were rated 1 to 4 according to the following criteria: 1+, small granules less than 5 per cell; 2+, small granules greater than 5 per cell; 3+, large granules less than 5 per cell; and 4+, large granules greater than 5 per cell.

Table I. The effect of L-DOPA on Heinz body formation following 4 h incubation with APH 10^{-2} *M* in G6PD deficient cells, pH 7.5

Test drugs concentration Incubation mixture	Heinz body formation (%)			
	1+	2+	3+	4+
APH	18	15	25.5	41.5
L-DOPA 10^{-3} *M*	0	0	0	0
APH, L-DOPA 10^{-3} *M*	13.5	5	17.5	0.5
APH, L-DOPA 10^{-4} *M*	15	8	19	3
APH, L-DOPA 10^{-5} *M*	28	12.5	21.0	38.5
APH[a], L-DOPA 10^{-3} *M* (pH 7.0)	5		12	
APH[a], L-DOPA 10^{-3} *M* (pH 8.2)		15	15	55

[a] Varying pH of the final incubation mixture

Table II. Effect of L-DOPA 10^{-3} *M* on Heinz body formation following 4 h incubation with 'oxidizing' reagents in G6PD deficient red cells pH 7.5

Test drug concentration Incubation mixture	Heinz body formation (%)			
	1+	2+	3+	4+
Ascorbic acid (10 m*M*)	33	67		
Ascorbic acid (10 m*M*) + L-DOPA	12.5	13.6		
Na azide 4×10^{-3}*M*	0	0	0	0
APH + Na azide		21	23	56
APH + Na azide + L-DOPA	8	32	10	15
Na azide + ascorbic acid	22	78		
Na azide + ascorbic acid + L-DOPA	32	68		

L-DOPA protected G6PD deficient red cells from Heinz body formation (Tables I and II). Following incubation with 10^{-2} *M* APH, 41.5% type-4 Heinz bodies were decreased to 0.5%, and 15% type-2 were lowered to 5% following incubation with L-DOPA. Thus a total of 56.5% of the cells with a significant Heinz body formation were reduced to only 5% with L-DOPA. Ascorbic acid induced 67% type-2 Heinz bodies which likewise were corrected to 13.5% with L-DOPA. The maximum effect was obtained with 10^{-3} *M* of L-DOPA at pH 7.0–7.5 and disappeared at pH 8.2. Normal red cells were also protected by L-DOPA although the effect was not as dramatic due to a lower number of Heinz bodies.

Following incubation of G6PD deficient red cells with 10^{-2} *M* APH and 4 m*M* sodium azide, a catalase inhibitor[4], the protective effect of L-DOPA was partially reduced (Table II). The combined used of two catalase inhibitors, ascorbic acid 10 m*M*[4] and 4 m*M* sodium azide, completely inhibited the protective effect of L-DOPA.

The finding that L-DOPA protects against in vitro Heinz body formation following incubation of G6PD deficient red cells with various 'oxidizing' agents suggests that L-DOPA may not be related to hemolysis in certain G6PD deficient patients, as was previously suggested[5]. To the contrary, the ability of the red cell to metabolize L-DOPA may offer an important alternative pathway for the removal of H_2O_2 from the G6PD deficient red cells. Thus, in the present system, dopa may be oxidized to the quinone at a high rate with the subsequent consumption of excess H_2O_2 which has been previously generated by 'oxidizing' drugs (APH, ascorbic acid[3]).

This mechanism is further elucidated by the data demonstrating the ability of catalase inhibitors, sodium azide and ascorbic acid[4], to block the protective effects of L-DOPA; for, it is conceivable, that L-DOPA in the presence of H_2O_2 may activate the 'peroxidatic' component of catalase[6,7] by acting as enzyme substrate as well as co-factor[3] and it is by this mechanism that H_2O_2 is dissipated. Studies are in progress to elucidate the clinical efficacy of L-DOPA in the treatment of G6PD deficiency.

Résumé. Le L-DOPA empêche la formation de «Heinz bodies» résultant de l'incubation des globules rouges normaux et des globules rouges privés de G6PD avec un grond nombre d'oxydants. Le L-DOPA a eu le plus d'effet à 10^{-3} *M* et à un PH de 7.0–7.5. L'incubation avec les inhibiteurs de la catalase, l'acide ascorbique et l'azide de sodium bloque l'effet protecteur du L-DOPA.

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