HAPTOGLOBIN SUBTYPING by POLYACRYLAMIDE GEL ISOELECTRIC FOCUSING of SERUM, HEMOLYZED BLOOD and BLOODSTAINS

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INTRODUCTION

The aim of the present investigation is to compare two alternative methods for the isolation of haptoglobin: ion-exchange chromatography and immunoprecipitation. The possibility of Hp-subtype determination with immuno-precipitation in hemolytic sera, blood for alcohol testing (NaF stabilized blood) and bloodstains has been investigated too.

MATERIAL AND METHODS

Preparation of the Haptoglobin
Ion-exchange chromatographie (according to Patzelt and Schröder 1985). Absolute non-hemolytic sera are needed. We used a Serva Cellulose-Ion-exchanger DEAE-SS.
Immunoprecipitation (according to Scherz et al. 1990 and Dimo-Simionin et al. 1990). For the preparation from serum we dealt with the originally quoted amounts. The double amount was used with hemolytic sera stored up to 10 months at -18 centigrade and blood for alcohol testing (0.08 g NaF per 8 ml blood for stabilization) stored up to 10 weeks. The bloodstains (cotton textile 5x5 mm in dimension soaked with blood) had been set off with 50 µl aqua dest. at first and then treated as the hemolyzed blood. Most of the used anti-human Hp originated from in house production, the rest came from Atlantic Antibodies (Cat. No. 81952 G).
Hp-cleavage (according to Pastewka et al. 1973 or Constans and Viau 1975).

Isoelectric focusing has been carried out in polyacrylamide flat gels, 260 x 125 x 0,5 mm in dimension, using an LKB Multiphor set. The gel (T = 5%, C = 3%) contained the following carrier ampholytes (LKB ampholine): 0.6 ml 5-7, 0.2 ml 6-8, 0.3 ml 5-6 and 0.1 ml 3.5-10. After 45 minutes of prefocusing the samples were placed onto the gel surface, using 0.5 x 1.0 cm strips of filterpaper, and allowed to stay there 1 h. Focusing took 3 h 30 min, with maximum electric values of 1,600 V, 10 mA, 10 W. Anolyt 1M H_3PO_4, catolyt 1M NaOH.
Visualization with Serva Blue (1 g Serva Blue + 180 ml ethanol + 100 ml formalin + 420 ml aqua dest.).

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

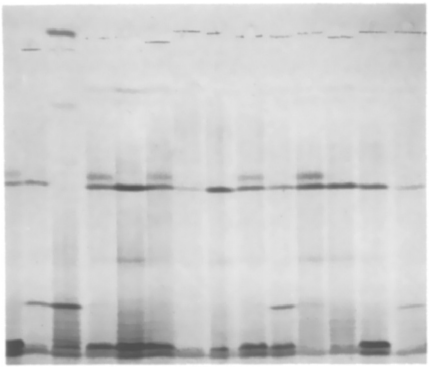

Fig. 1. Hp-subtypes prepared through ion-exchange chromatographie from sera. From left to right: 1S-2FS, 2FS, 2FS, 2FS-1F, 1S-2FS, 2FS-1F, 2FS, 2FS, 2FS-1F, 2FS, 2FS-1F, 1S-2FF, 1S-2FS, 2FS-1F. The anode is at the top

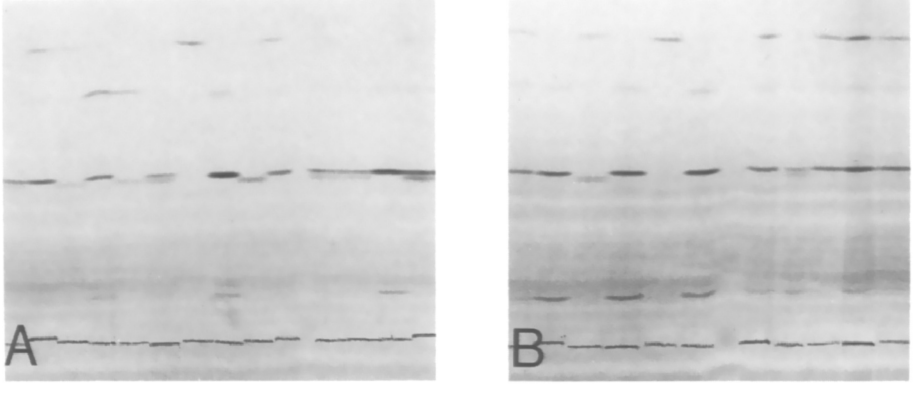

Fig. 2. Hp-subtypes prepared through immunoprecipitation
A) from sera. From left to right: 2FS-1F, 1S-2FS, 1S-1F, 2SS-2FS, 2SS-1F, 2FS-1F, 1S, 2FS, 1F, 1S-2FS, 2FS-1F, 2FS-1F, 2FS, 2FS-1F.
B) from hemolytic sera: 1S-2FS, 2FS, 1S-1F, 2FS, 1S, 2FS, 1S-2FS, 2FS-1F, 1S-2FS, 1S-2FS, 1S-2FS. The anode is at the top

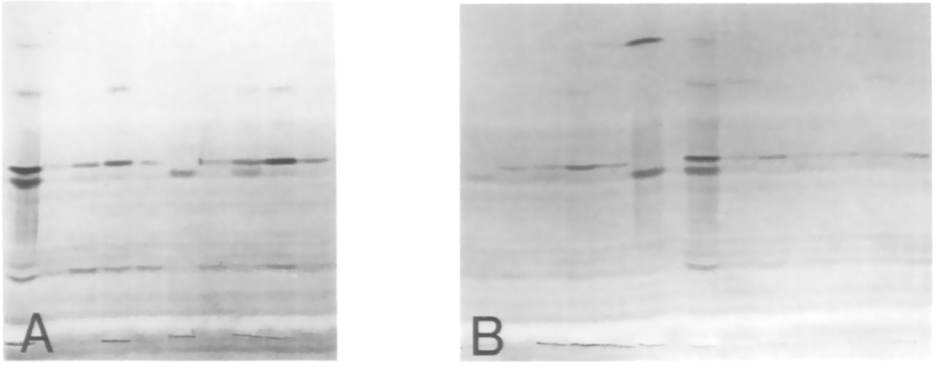

Fig. 3. Hp-subtypes prepared through immunoprecipitation
A) from NaF stabilized blood. From left to right: Control 2FS-1F 2FS, 2FS, 2FS, 2FS, 2FS, 1F, 2FS, 2FS-1F, 2FS, 2FS.
B) from blood stains. From left ro right: 1F, 2FS, 2FS-1F, 2FS, 1S-2FS, control 1S-1F and 2FS-1F, 2SS-2FS, 2FS, 2FS-1F, 1S-2FS, 2SS-2FS, 2FS

RESULTS AND DISCUSSION

Figure 1 shows the visualization of the Hp-subtypes from sera using ion-exchange-chromatography, Fig. 2A using immunoprecipitation. No discrepancies between the two methods are to be observed. The cheaper method is ion-exchange-chromatography though it demands absolute non-hemolytic sera. Figure 2B shows the Hp-subtypes from hemolytic sera, Fig. 3A from blood for alcohol testing and Fig. 3B from bloodstains, all after immunoprecipitation. To prove the existence of Hp-subtypes in a bloodstain it must be considerable large and fresh according to our practical knowledge.
As result of our analyses we ascertain that the ion-exchange chromatography suits good to the routine analysis of sera. The immunoprecipitation is the method of choice for the haptoglobin subtyping of hemolyzed blood and bloodstains.

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