THE SIGNIFICANCE OF CURRENT AND OF ORIGIN AND CONCENTRATION OF SIALIDASES FOR THE DETERMINATION OF PLASMINOGEN GROUPS

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Plasminogen (PLG) groups have been presumed first from Sachs and Szirmai (1972) on the basis of difficult diffusion-absorption tests. They were detected by Hobart (1979) and Raum et al. (1980) with an isoelectric focusing technic. Jaeger et al. (1983) have shown that PLG groups can also be determined with a simple foil electrophoresis. With a chance of exclusion of 16% the PLG groups system is highly suited for parentage testing. Until now three frequent phenotypes (PLG1, 2-1 and 2) and six rare (PLG 1-F, 1-M, 1-S, 2-F, 2-M and 2-S) have been observed (Fig.1).

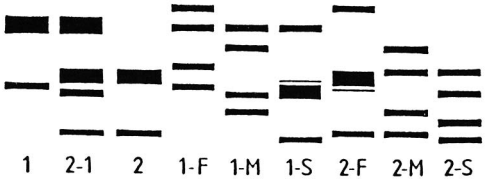

Fig.1:

In order to test the reliability of the PLG group determination in the cellulose acetate foil electrophoresis it appeared that a safe determination can be affected by various influences.

Material and methods.

Sera of the types PLG 1, 2-1 and 2 were investigated in principle according to Jaeger et al (1983) with the following variations:

First the electrophoresis chambers were filled up with tris-barbital-glucine buffer (pH 9.5)(Fig.2) and on the other hand they were filled only to a third. The partition walls were bridged within the chambers with filter paper.

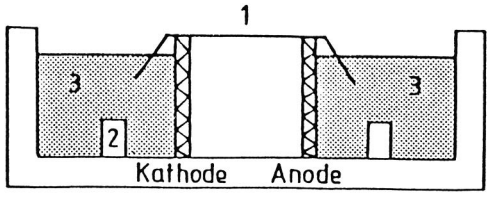

Fig.2: Electrophoresis chamber

Legend: 1:Cellulose acetate foil.2:Partition wall.3:Electrophoresis Chambers filled up without paper bridges within the chambers.

The sera were pretreated with sialidase from clostridium perfringens (CLPF)(Sigma and P-L-Biochemicals) from vibrio cholerae (VCH)(Behring) and from influenza virus (IVI) (Calbiochem), 0,4 U in phosphate buffer (pH 7.0) and 18ul serum by incubation for 18 hours at room temperature.

Finally pretreatment of sera with CLPF sialidase was carried out with 0,4 U down to 0,08 U.

Results.

If the current at a constant terminal voltage of 320 is lower than 15 mA in the beginning and 32 mA at the end of the electrophoresis the bands are not sufficiently separated. This can only be reached if cellulose

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acetate foils are used and the electrophoresis chambers are filled up with buffer without paper bridges within the chambers (Fig.3).

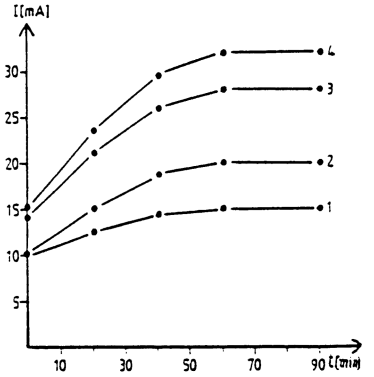

Fig.3: Change of current during electrophoresis.

Legend:1.with paper bridges Cellogel foil
2.Chamber filled up cellulose acetate foil
3.With paper bridges
4.Chambers filled up

It is very important to use special sialidases. We have found out that the pretreatment of sera with CLPF sialidase gives excellent results. Pretreatment with VCH or IVI sialidases leads independent of the incubation time and temperature to completely other and partially misleading patterns. E.g. PLG 1 looks like PLG 1-F (Fig 4) and PLG 2 looks like PLG 1-M (Fig.5).

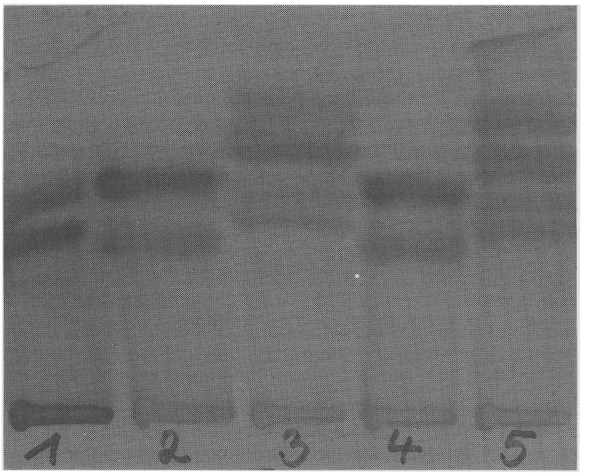

Fig.4:Pretreatment of a PLG 1 serum with different sialidase.

Legend:1: PLG 2-1 reference. Sialidase from. 2:CLPF 1. 3: VCH.4:CLPF 2. 5:IVI.

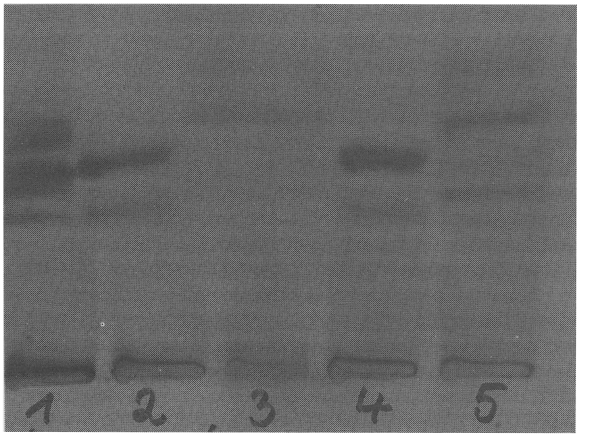

Fig.5: Pretreatment of PLG 2 serum with different sialidase

Legend:1:PLG 2-1 reference.Sialidase from 2:CLPF 1. 3:VCH. 4:CLPF 2. 5:IVI.

Surprinsingly the concentration of the sialidase seems to have no or only a minimal influence on the reliability of the PLG group determination. Pretreatment of 18ul serum with 0,4 U down to 0,08 U of CLPF sialidase leads always to a good separation of the bands of all PLG groups (Fig.6).

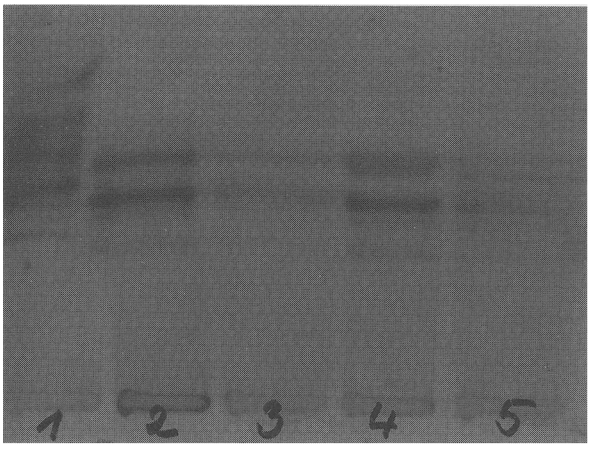

Fig.6:Pretreatment of a PLG 2-1 serum with different concentrations of CLPF sialidase.

Legend: 1:Untreated.
2:Room temp 18 hours;0,4U
3: 37°C of CLPF sialidase
4:Room temp 18 hours;0,4U
5: 37°C of CLPF sialidase

Conclusion.
In order to reliably determine PLG groups in the cellulose acetate foil electrophoresis it is necessary to take care that the current during the run increases from 15mA to 32mA by filling up the electrophoresis chambers with buffer solution. The sera should be pretreated with at least 0,08 U of CLPF sialidase per 18ul of serum for 18 hours at room temperature.

Bibliography.

HOBART MF (1979) Genetic polymorphism of human plasminogen.Ann Hum Genet (Lond) 42: 419-23.

JAEGER H, KRÜGER H-J, HUMMEL K (1983) Typisierung von PLG auf Cellogelfolien: Resultate von 97 Familien und 527 unverwandten Personen aus Süddeutschland.. Ärztl Lab 29: 195-97.

RAUM D, MARCUS D, ALPER CH (1980) Genetic polymorphism of human plasminogen. Amer J Hum Genet 32: 681-89.

SACHS V, SZIRMAI E (1972) Immunologisch nachweisbare Unterschiede in der Struktur von menschlichem Plasminogen. Bibl Haemat 37: 428-33.