OBSERVATIONS ON THE USE OF ISOELECTRIC FOCUSING FOR SUBTYPING IN THE TRANSFERRIN (Tf) SYSTEM

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In 1978 Kühnl and Spielmann, and simultaneously Thymann, identified 2 alleles of TfC by the application of isoelectric focusing. Further work by Kühnl and Spielmann (1979) revealed the TfC^3 gene, a further subdivision of TfC^1, and pretreatment of serum samples with ferric chloride by Weidinger (1980) improved the visualisation of the bands. This gave a possible total of 6 phenotypes at the TfC locus. Weidinger also calculated the theoretical exclusion rate to be approximately 19 % and the system is now widely used for paternity testing.
In this laboratory, over the past 2 1/2 years a total of 3255 serum samples have been tested using ammonium ferrous sulphate pretreatment (Constans 1980).
From this survey (fig. 1) gene frequencies were calculated as approximately TfC^1 0,79, TfC^2 0,15, TfC^3 0,05, TfB 0,008 and TfD 0,001 which agree well with other published figures.

This distribution and the relative stability of the system observed in serum samples, would prove useful for blood stain identification if typing were possible.

In 1979 Hoste reported disappointing results but used no pretreatment of stains and in 1983 Pascali reported increased sensitivity after pretreatment of sera with 2-Mercaptoethanol. These solutions, i.e. Mercaptoethanol and ferrous and ferric ammonium sulphate, were investigated as a possible means of bloodstain typing.
Parallel studies on 230 paired sera and bloodstains were

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investigated using ultra-thin (0,15 mm) polyacrylamide gel isoelectric focusing (pH 4-6).
Fixation in sulphosalicylic acid was followed by staining in Coomassie Blue. Serum samples were diluted 1:9 with the extraction solutions, ferrous and ferric ammonium sulphate (0,35 %) and 0,18 M 2-Mercaptoethanol and incubated overnight at 4° C.

Results

Pretreatment with ferrous and ferric salts produced clear results in both cases but the bands were always more intense using the ferrous salt.

The band pattern was also observed to be different (fig.2). The ferrous salt produced two bands for each allele, a stronger anodal and weaker cathodal band, whereas the ferric salt showed activity only in the cathodal region. The identification of homozygotes from serum samples typed after ferrous salt extraction gave no problems. Heterozygotes however were sometimes difficult to interpret because the cathodal band of the 2 allele, in 2-1 or 3-2 phenotypes was always weak and sometimes absent. In these cases the relative intensity of the other bands was of great importance, and provided the only means of avoiding a false interpretation.

After storage at -20° C serum samples could, in most cases be typed without loss of activity for two to three months.

The typing of bloodstains proved to be not so straightforward because this relative intensity of the bands was not consistent. Consequently identification of the phenotype was only possible by reference to the presence or absence of bands. As the 2 band was always weaker in a

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heterozygote, it was sometimes possible to identify a 1 band from a 2-1 phenotype but with no activity in the 2 region. As no reference could be made to the relative band intensity this would pass unnoticed and a false result obtained. Although this was not often the case, it was more noticeable in older stains and occured occasionally in fresh stains.

Treatment with Mercaptoethanol did not improve the band resolution. It was also found that in stored alcohol samples bandswere sometimes observed which could be misinterpreted as B or D variants.

These observations show that although stain grouping was possible and in most cases the correct phenotype identified, errors could be made in the interpretation. Until this is resolved it would be better to use this system cautiously in casework.

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Transferrin

	Münster (N=3255)	Weidinger (N=184)
C^1	0,79	0,78
C^2	0,15	0,15
C^3	0,05	0,07
B	0,008	0,005
D	0,001	-

Fig.1: Tf gene frequencies in Germany

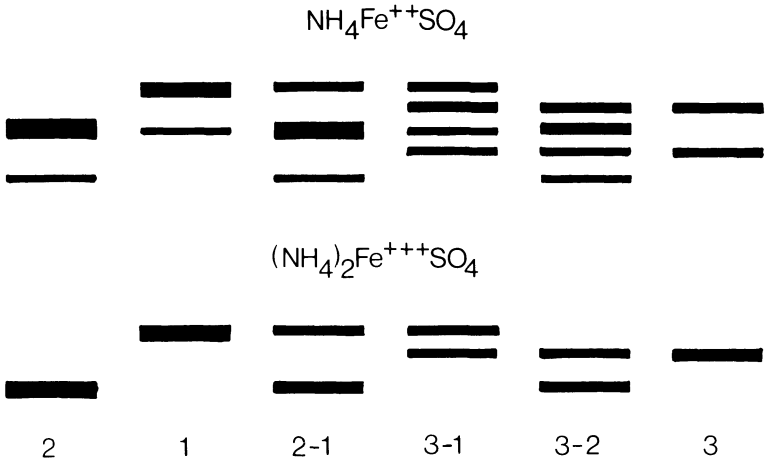

Fig. 2: Diagrammatic representation of Tf band patterns after pretreatment

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