completely inhibited G6PD activity. By extrapolation it could be determined that 50% inhibition of G6PD and acid phosphatase occurred at final concentrations of approximately $4 \times 10^{-5} M$ and $10^{-3} M$, respectively. The value for half inhibition of G6PD was similar to that reported for a commercial enzyme preparation[13].

Table II. Effect of TPB on reactions of cytoplasmic enzymes

TPB added (M)	G6PD Δ A/340 nm	% Activity	Acid phosphatase Δ A/410 nm	% Activity
10^{-2}			0.016	4
5×10^{-3}			0.040	10
3×10^{-3}			0.064	16
2×10^{-3}	0.000	0	0.228	56
10^{-3}	0.006	5	0.364	90
5×10^{-4}	0.012	9	0.392	97
3×10^{-4}	0.028	21	0.403	100
2×10^{-4}	0.030	23		
1.5×10^{-4}	0.044	33		
10^{-4}	0.090	68		
5×10^{-5}	0.122	92		
10^{-5}	0.130	99		
None	0.132	100	0.404	100

A membrane-free 1:20 aqueous lysate was prepared from a 50% suspension of thrice-washed erythrocytes. For G6PD, 1.0 ml of aqueous TPB solutions were added to replicate cuvettes containing 1.0 ml of 0.19 M *tris* buffer, pH 8.0; 0.1 ml of 0.3 M $MgCl_2$; 0.4 ml of water; 0.2 ml of 0.002 M TPN and 0.2 ml of lysate. Reaction was started immediately by adding 0.1 ml of 0.02 M glucose-6-phosphate and changes in absorbance at 25 °C were recorded at 1 min intervals. Δ A indicated corresponds to 5 min. For acid phosphates, 1 ml of TPB in 0.1 M acetate buffer, pH 5.0, was added to 0.5 ml of lysate, followed by 1.0 ml of 0.016 M *p*-nitrophenyl phosphate. Slight precipitation was noted with the two highest concentrations of TPB. The reaction was stopped with 10% trichloroacetic acid and absorbance was determined at 410 nm in supernatants alkalinized with 0.25 M NaOH.

The foregoing experimental results demonstrate that that the interaction of TPB with human erythrocytes is not limited to the cell membrane. These findings are in contrast to the effects of proteases[14], tannic acid[10] and cephalothin[15] which readily convert normal erythrocytes into ACHE-deficient cells without affecting the activity of intracellularly-located enzymes. The observation that G6PD activity is diminished when intact cells are exposed to TPB lends support to the concept that this agent traverses biological membranes and would explain the loss of activity of intracellular enzymes and of other metabolic characteristics described for TPB-dissociated cells[16].

Resumen. El tratamiento de glóbulos rojos humanos con concentraciones no-hemolíticas de borotetrafenilo causa una reducción irreversible en las actividades de la glucosa-6-fosfato dehidrogenasa, una enzima citoplasmática y de la acetilcolinesterasa, ubicada en la superficie externa de la membrana eritrocitaria. El tratamiento casi no tiene efecto alguno sobre la fosfatasa ácida.

F. HERZ and E. KAPLAN

Departments of Pediatrics, Sinai Hospital, Belvedere Avenue at Greenspring, Baltimore (Maryland 21215, USA), and School of Medicine, Baltimore (Maryland 21205, USA),
19 July 1971.

[13] J. B. STANBURY and J. V. WICKEN, Biochim. biophys. Acta *178*, 403 (1969).
[14] F. HERZ, E. KAPLAN and J. H. STEVENSON JR., Nature *200*, 901 (1963).
[15] F. HERZ, E. KAPLAN and D. A. SEVDALIAN, Acta Haemat. *41*, 94 (1969).
[16] This work was supported by NIH Grant No. HD 01461 and by the Baltimore Rh Typing Laboratory, Inc.

Biochemical and Immunological Comparison of Phenotypes of Horse Serum Transferrins

The polymorphism of transferrins, the iron-binding globuline of blood serum, has been investigated for many different animal species. Six homozygous phenotypes have been observed in horse serum by starch gel electrophoresis: 3 fast migrating phenotypes (Tf D/D, Tf F/F and Tf H/H), and 3 slow migrating ones (Tf M/M, Tf O/O and Tf R/R)[1]. Three migrating bands containing different amounts of protein of identical sedimentation coefficient have been found for each phenotype[2].

Experimental. The samples of horse serum were obtained from patients of the veterinary hospital in Berne. The different phenotypes were prepared by gel filtration and ion-exchange chromatography[3]. Amino acids analyses of phenotypes Tf D/D and Tf R/R were carried out by the method of SPACKMAN et al.[4], in a Beckman Spinco analyzer. The acid hydrolysis was carried out for 24, 48 and 72 h in 6 N HCl in evacuated sealed tubes.

For the analyses of tryptic digest and peptide maps, the phenotypes Tf D/D, Tf F/F and Tf R/R were treated according to HARRIS et al.[5]. The tryptic digests, the electrophoresis and the chromatographies were carried out by the method of EPPENBERGER et al.[6]. Antibodies to the purified phenotypes Tf D/D, Tf F/F and Tf R/R were prepared in rabbits[2]. The micro complement fixation analyses were performed according to the description of LEVINE et al.[7].

Results. The amino acids composition of the phenotypes Tf D/D and Tf R/R are shown in the Table. Both phenotypes have almost identical compositions as far as the neutral amino acids are concerned. It is interesting to note that there are more acidic residues in the phenotype Tf D/D than in Tf R/R and more basic residues in the phenotype Tf R/R than in Tf D/D. These results are in accordance with the electropherograms which show a greater electrophoretic mobility for Tf D/D than for Tf R/R. These results confirm our early assumption that the different phenotypes are due to small differences of the global electric charge of the molecules[2].

The tracing of the acidic, basic and neutral peptide maps are shown in the Figure 1. The acidic fingerprintings

[1] M. BRAEND and C. STORMONT, Nord. VetMed. *16*, 31 (1964).
[2] A. BAER, V.M.D. Thesis, Berne (1968).
[3] A. BAER, Experientia *25*, 28 (1969).
[4] D. H. SPACKMAN, W. H. STEIN and S. MOORE, Analyt. Chem. *30*, 1190 (1958).
[5] J. I. HARRIS and R. N. POHAN, J. molec. Biol. *13*, 878 (1965).
[6] H. M. EPPENBERGER, D. M. DAWSON and N. O. KAPLAN, J. biol. Chem. *242*, 204 (1967).
[7] L. LEVINE and H. VAN VUNAKIS, *Methods in Enzymology* (Academic Press, New York 1967), vol. 11, p. 928.

Amino acid composition of different types of transferrin

Amino acid residue	Horse		Rabbit[a]	Human[b]
	Tf D/D	Tf R/R		
Lysine	56	61	45	60
Histidine	23	23	17	20
Arginine	32	34	29	27
Aspartic acid	79	76	74	80
Threonine	36	35	29	27
Serine	42	43	59	39
Glutamic acid	81	75	69	63
Proline	37	39	62	31
Glycine	51	57	53	48
Alanine	63	64	49	56
Half Cystine	33	34	28	39
Valine	51	49	47	43
Methionine	5	6	6	8
Isoleucine	20	19	18	15
Leucine	61	58	58	59
Tyrosine	27	28	25	22
Phenylalanine	27	28	26	28

The results are expressed as the number of amino acid residues per molecule (molecular weight 80.000). [a] W. WEEN and J. WILLIAMS, J. Biochem. *108*, 69 (1968). [b] H. G. VAN AYK and B. LEIJNSE, Biochim. biophys. Acta *160*, 126 (1968).

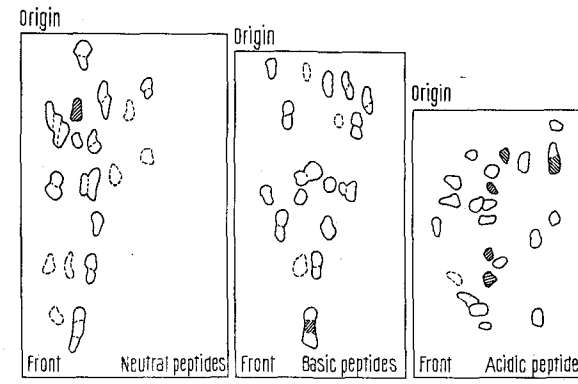

Fig. 1. Tracing of the neutral, basic and acidic peptide maps of the homozygous phenotypes Tf D/D, Tf F/F and Tf R/R. The neutral fingerprintings of each phenotype have been superimposed to match up the peptides. The same process has been used to compare the basic and the acidic peptides. The hatched spot of the tracing of the neutral and the basic peptides is present in the phenotype Tf R/R only. Both hatched spots in the upper part of the tracing of the acidic peptides are present in the phenotype Tf D/D only. The lowest hatched spot of the same tracing is missing from the phenotype Tf D/D; the 2 other hatched spots (middle of the tracing) are present in the phenotype Tf R/R only.

of the phenotypes Tf D/D, Tf F/F and Tf R/R have been superimposed to match up the peptides. The same process has been used in order to compare the basic and the neutral peptides. The differences which appear between the 3 phenotypes are very small. The fingerprintings of the basic and neutral peptides of Tf D/D and Tf F/F are identical. Tf R/R shows one common acidic peptide with Tf F/F which is missing from Tf D/D, Tf R/R and Tf D/D each have 2 more acidic peptides than Tf F/F has, and these peptides are different from each other. The total number of peptide spots is not in very good agreement with the number of lysine and arginine residues determined by the amino acids analyses (Table). As there are less peptides than would have been expected, one has to admit the presence of more small peptides in some big spots that have not been separated by the solvents used for the electrophoresis and the chromatography.

If the horse transferrin molecules would contain 2 identical subunits, the number of peptides would be about half of what has been found. The results of the fingerprintings suggest strongly that these serum protein molecules are not polymers.

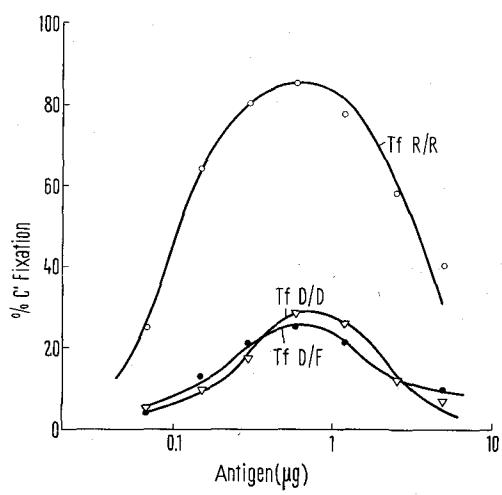

Fig. 2. Immunological cross reactivity of the phenotypes Tf R/R, Tf D/D and Tf D/F with an antiserum against Tf R/R, measured by the technique of the micro complement-fixation.

The immunological cross reactivity of the phenotypes Tf R/R, Tf D/D and Tf D/F, with an antiserum against Tf R/R is shown in Figure 2. Both heterologous phenotypes present an almost identical reaction; that suggests that Tf D/D and Tf F/F are strongly similar antigens. On the contrary, the differences observed with the homologous phenotype and both heterologous phenotypes is very well pronounced.

These results show that the structural differences between the phenotypes Tf D/D and Tf F/F are extremely small but are much more important between Tf D/D and Tf R/R than between other phenotypes. The results obtained suggest that a phenotype is different from another by some amino acid substitutions or deletions only, because if there were larger structural modifications there would be correspondingly larger immunological differences as revealed by the very sensitive method of micro-complement fixation.

These genetic modifications are relatively very small for a molecule of about 720 residues ordered most probably in a single polypeptide chain, and they did not alter the biological function[8].

Résumé. Différents phénotypes homozygotes des transferrines du sérum équin ont été comparés par analyses de leurs caractéristiques biochimiques et immunologiques. Les compositions en acides aminés des différents phénotypes sont très voisines; les différences immunologiques déterminées par la micro-fixation du complément sont plus prononcées.

A. BAER[9] and B. SCHWENDIMANN

Institute of Biochemistry,
University of Neuchâtel (Switzerland), 1 July 1971.

[8] This work has been supported by a grant from Le Fonds National Suisse pour la Recherche Scientifique.

[9] Present address: Eidg. Forschungsanstalt für Milchwirtschaft, CH-3097 Liebefeld (Switzerland).