ON ELECTROPHORETIC RESOLUTION AND DENSITOMETRIC DETERMINATION OF APO-TRANSFERRIN AND IRON-BOUND TRANSFERRIN

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The iron in the blood plasma exists in a conjugated form with transferrin. It is believed that in normal plasma transferrin is not fully saturated with iron; about one third of it is present as iron-transferrin complex (Fe-Tf) and the remainder as iron-free form (apo-transferrin, Apo-Tf) (Rath and Finch (1949)). However, up to present, there is no satisfactory means of determining such a ratio of Fe-Tf versus Apo-Tf directly, and therefore in the practical clinical blood analysis, the Fe-Tf is expressed by the amount of "serum iron" and the Apo-Tf level is, for example, represented as the "iron-binding capacity of serum" (Rath and Finch (1949)).

The present authors found that, upon polyacrylamide gel electrophoresis (Raymond and Weintraub (1959); Hermans et al. (1960)) of diluted serum, the Fe-Tf complex and the Apo-Tf appear as two distinct bands, of which proportion can be measured by densitometry almost quantitatively, and the results obtained are briefly reported here.

Gel block (8 × 28 × 0.2 cm; for 4 samples) was prepared by polymerization of 5 g/dl solution of Cyanogum 41 (consisting of 95% acrylamide and 5% N,N'-methylene-bis acrylamide) in a Tris-EDTA-borate buffer of pH 9.2 (containing 80.66 g of Tris, 10.46 g of EDTA-2Na and 6.3 g of boric acid in 8 litres) in the

presence of 0.08 ml of β-dimethylamino-propionitrile (DMAPN) and 0.08 g of ammonium persulfate. Serum to be examined was diluted to give final concentration of total serum proteins of 1 g/dl in order to obtain satisfactory resolution of the transferrin bands. Such a diluted serum was applied to a slot (1.0 × 12.0 × 1.5 mm; 18.0 μl in volume) and electrophoresis was carried out in the above-mentioned Tris-EDTA-borate buffer in a current of 22 - 26 mA and at 400 - 500 V for 2.5 - 3 hours. During the run, the gel block was cooled by circulating tap water in contact with the outer surface of the bottom of plastic trough which accomodated the gel. After electrophoresis, protein bands were stained by amidoblack 10B.

A typical electrophoretic pattern of diluted serum from a normal individual is cited in Fig. 1-(1). It is apparent that a faster moving band (F band) and a slower moving band (S band) are clearly distinguishable and also that the former is considerably thicker than the latter. When known amounts of ferrous ammonium sulfate had previously been added to the serum under examination, the proportion of these two bands proved to change distinctly. Thus, as is clear from Fig. 1-(2), -(3) and -(4), parallel to the increase in the amounts of inorganic iron added to the serum (50, 100 and 300 μg of iron per dl serum, respectively), the F band tends to diminish and the S band tends to increase its color density after staining, and at a level just a little over saturation (Fig. 1-(4); 300 μg iron per dl serum), the F band disappears completely.

The amounts of these Fe-Tf and Apo-Tf can be represented by the areas of the peaks in the densitograms of the stained electropherograms, as shown in Fig. 2. Thus, the ratio of Fe-Tf versus total Tf, representing the degree of iron saturation

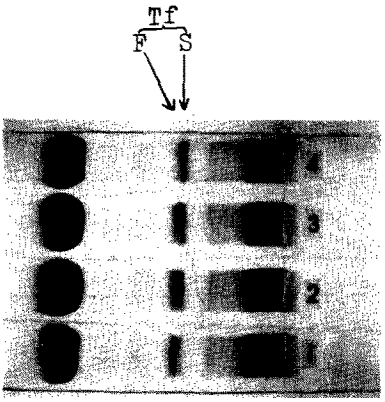

Fig. 1. Polyacrylamide gel electrophoresis of human serum after addition of varying amounts of inorganic iron. (Experimental conditions; see the test).
1: Without addition of iron
2: 50 μg/dl iron added
3: 100 "
4: 300 "

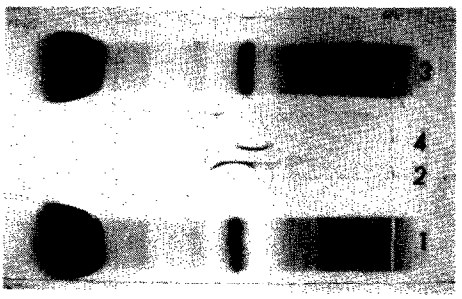

Fig. 3. Polyacrylamide gel immunoelectrophoresis of iron-bound (Fe-Tf) and Iron-free transferrin (Apo-Tf). Condition of the electrophoresis is the same as in Fig. 1.
1: Apo-Tf
2: Precipitin line by the reaction of Apo-Tf with anti-human-transferrin rabbit serum
3: Fe-Tf
4: Reaction of Fe-Tf with anti-human-transferrin rabbit serum

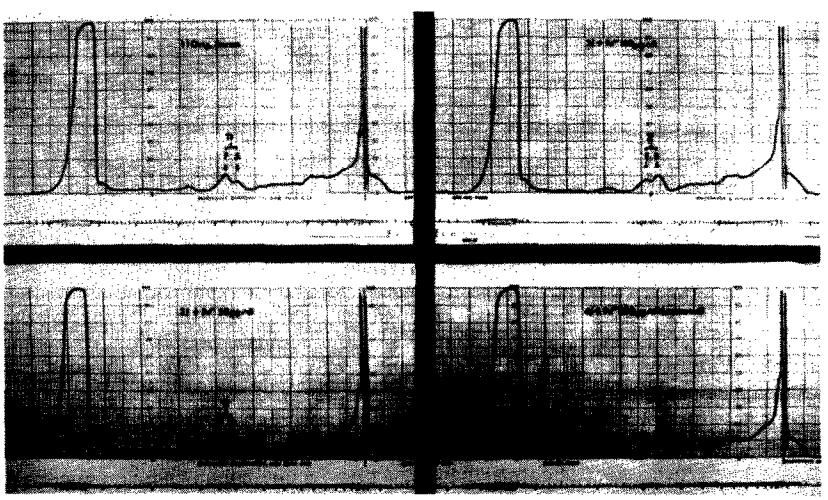

Fig. 2. Densitometry of the electrophoretic patterns of Fig. 1.

of Tf, was calculated from the areas as indicated in TABLE I.

TABLE I. Degrees of iron saturation of transferrin (Fe-Tf/total Tf) determined by densitometry

Exp.	Fe added (μg Fe per dl serum)	Difference	Fe-Tf/total Tf from Fig. 2	Difference
(1)	0		0.350	
		50		0.10
(2)	50		0.451	
		50		0.09
(3)	100		0.540	
		200		0.46
(4)	300		1.000	

If, however, the serum specimen had previously been dialyzed thoroughly against a EDTA solution (0.005 M, pH 4.8) in order to remove the bound iron, the electrophoretic pattern showed only the F band, the S band being completely absent in this case. When such dialyzed preparation was subjected to an equilibrium dialysis (Jone and Perkins (1965)) against ferrous ammonium sulfate solution (10 mg iron per litre of Tris-EDTA-borate buffer of pH 9.2), the F band was replaced by the S band, indicating the recombination of iron on the Tf molecule.

The fact that these two bands really correspond to two distinct molecular species of Tf, of which the slower moving one (S band) does contain iron while the faster moving one (F band) does not, was further confirmed by the following tracer experiment with radio-active iron as well as by immunoelectrophoresis on polyacrylamide gel (Antoine (1962)). Serum was mixed with $^{59}FeSO_4$ (0.15 microcurie per one slot) and was then subjected to zone electrophoresis on polyacrylamide gel as mentioned above. Radio-autography of the electropherogram thus obtained showed the existence of one irradiated band just in a position

corresponding to the S band. Furthermore, immunoelectrophoresis employing anti-human-transferrin rabbit serum (Behringwerke) revealed the formation of respective precipitin line with respect to each of the S and F bands, indicating that both of them are immuno-chemically identifiable to be transferrin (Fig. 3).

Such an electrophoretic resolution of Fe-Tf and Apo-Tf was also observed in the case of urinary transferrin excreted in certain pathological conditions, including nephrosis and pregnancy toxicosis. In most of these cases, however, the Fe-Tf was found to be only a trace, namely, the majority of urinary transferrin was found to consist of Apo-Tf.

The success in resolving the iron-bound and iron-free forms of transferrin by a simple zone electrophoretic technique and subsequent densitometric determination of the degree of iron saturation of transferrin in a given serum will contribute much to the biochemical diagnosis of such disorders as those due to iron deficiency and other impaired iron metabolism. Investigations on this line of approach will be reported later.

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