The Interaction of Protamine with Human Blood Plasma Proteins

The biological role of many important macromolecules depends primarily on the internal architecture of the molecule, but it may also be influenced by the formation of complexes with other macromolecules. Complex of fibrinogen and protamine was described by RICE et al.[1] and suggested by GODAL[2] as influencing the coagulation rather than neutralization of heparinoids. Protamine is capable of influencing also the activities of some enzymes, e.g. the muscle phosphorylase[3] and lipoprotein-lipase[4,5].

The experimental approach consisted in the electrophoretical separation[6,7] of blood plasma proteins in agar gel for various detection purposes. Protamine hydrochloride (SPOFA-Prague) was added to serum directly, or it was used as a precipitation agent in 1% solution for filling the grooves, similarly as is done in the detection of immunoelectropherograms. For application to the agaroelectropherograms we used the detection plate into which 1% protamine solution was allowed to diffuse for 1 h. The plate was then attached by sandwich technique.

Agaroelectrophoretic analysis of human plasma or serum proteins reveals by this way 3 turbidity zones in plasma (Figure 1) and 2 in serum. Comparison shows that the 3rd zone with the mobility of β_2 to γ-globulins belongs to fibrinogen, the precipitation of which with protamine was already described earlier[1].

When 2 mg of protamine were added to 1 ml of serum or plasma, we could find low-density lipoproteins (in contrast to the addition of heparine) slowing down on the immunoelectropherograms (Figure 2). Precipitation lines of the other fractions did not show any altered mobility.

By methodical analogy to immunoelectrophoretic analysis, using 1% protamine solution instead of antiserum, 2 turbidity precipitation lines in serum and 3 in plasma were gained (Figure 3). After removing the superfluous components which had not reacted during the usual manipulation, both anodically situated lines can be stained with Sudan black and Amido black 10B. This demonstrates that these precipitation lines belong to α_1-lipoprotein (HDL) and α_2-lipoprotein (LDL). The cathodic line belonging to fibrinogen is stained with Amido black 10B only incompletely.

As shown, low-density lipoproteins combine at pH > IP with both polyanions[8], e.g. heparine and polycations, e.g. protamine. It would be difficult to interpret that macromolecules of opposite charge combine with the same proteid only from the view-point of electrostatic forces. Therefore RICE's assumption[1] that also dispersion short range forces are remarkably involved seems to be valid. Also relatively selective precipitation by protamine of 3 out of about 20 main plasma-protein fractions, all having the same negative charge under these experimental conditions, seems to be dependent not only on electrostatic forces.

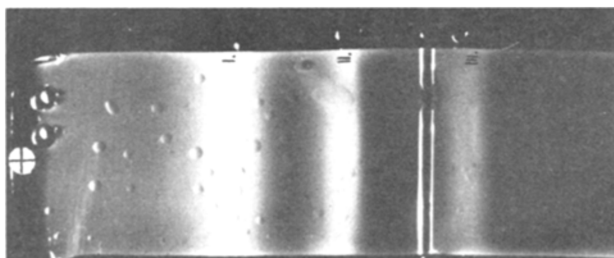

Fig. 1. Electrophoretic analysis of human blood plasma was performed in agar gel and attached to the detection plate containing protamine. 3 turbidity zones (I, II, III) demonstrate that the interaction took place. In the case of blood serum only zones I and II are formed.

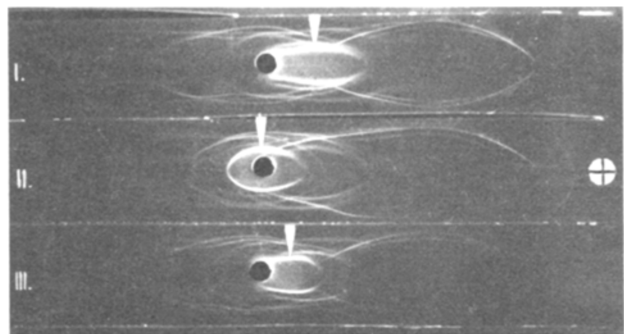

Fig. 2. Immunoelectrophoretic analysis of human blood serum (Antiserum E.L.1 – Human, Budapest, Hungary) alone (strip III), serum with protamine added (strip II) and serum with heparin added (strip I). Arrows show the position of the precipitation line of α_2-lipoproteins (LDL).

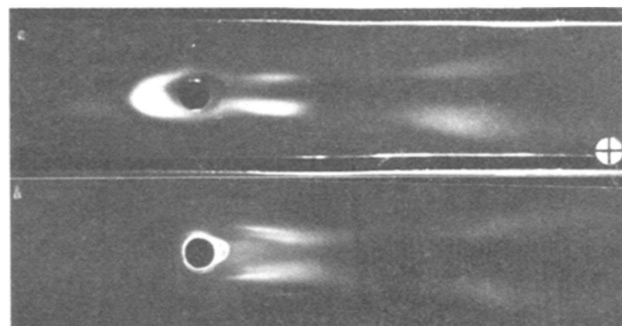

Fig. 3. Analysis of human plasma proteins using a methodical analogy of immunoelectrophoresis (protamine solution in grooves). 3a denotes the analogy of precipitation lines alone and 3b the result of staining with Sudan black.

The facts described might help as the starting point for more accurate knowledge on the mechanism of activation or inhibition of lipoprotein lipase. It seems probable that other precipitating or complex forming agents than protamine can be used. These agents can be applied electrophoretically separated protein fraction by the way of filling them, instead of antisera, into the grooves in the methodical analogy of immunoelectrophoresis to acquire information otherwise not easily accessible.

Zusammenfassung. Es wurden Interaktionen von Protamin mit vorher in Agar-Gel elektrophoretisch getrennten plasmatischen Eiweisskörpern untersucht. Neben der schon früher bekannten Interaktion mit Fibrinogen wurden auch solche mit beiden Klassen der plasmatischen Lipoproteine gefunden.

C. LOŠTICKÝ

Institute of Haematology and Blood Transfusion, Praha 10 (Czechoslovakia), 16 May 1969

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