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New Methods of identifying Serum Siderophilin using Radioactive Iron

In earlier investigations of the immunoelectrophoretic identification of the iron-binding protein siderophilin or transferrin, pure preparations of the protein have been used[1–3]. The preparation of siderophilin is, however, difficult in spite of the fact that methods have been perfected to do this; for example, precipitation with rivanol[2,4]. Wuhrmann and Jasinski[8] were able to show siderophilin in paper electrophoresis by labelling it with radioactive iron. Paper electrophoresis, however, only allows the classification of siderophilin into a specific group of serum proteins, that is, the β-globulins.

In the present experiments, radioactive iron was used for detecting siderophilin in immunoelectropherograms of sera. The method made possible not only the classification of siderophilin into a specific group of proteins, but also the exact identification of the siderophilin arch.

Precipitating sera were obtained from rabbits weighing about 3,500 g, which were immunized with fresh rat and horse sera. Bentonite was used as adjuvant[5]. Adjuvant was mixed with an equal volume of serum. Three days after the last (fifteenth) injection, the serum levels of precipitins were checked in the immunized animals, which were then bled. The sera (with merthiolate added in the proportion 1 : 10,000) were stored at −20° C. Equine precipitating serum No. 512 (from the Pasteur Institute, Paris) was used in the study of human sera.

The isotope iron-55 (half-life 2·94 years) as the chloride in weak solution of hydrochloric acid (Radiochemical Centre, Amersham) was used. To 1 ml. of serum, 0·2 μc. of iron-55 was added, and the mixture was incubated in the refrigerator for 1 h.

The method of Scheidegger[6], as modified by Hirschfeld[7] using agar gel, was adopted. Mixtures of sera with iron-55 were separated electrophoretically for 2·5 h. The precipitating sera described above were used to precipitate proteins. The preparations were washed and dried and, after making autoradiograms, were stained with amide black.

Foton–Roentgen 'Super Films' were used for autoradiography. The preparations obtained by immunoelectrophoresis were covered by roentgen films of dimensions equal to those of the preparation and were exposed for 4 weeks.

After saturating the serum with radioactive iron, immunoelectrophoretic preparations and their autoradiograms were obtained and compared with each other. In the autoradiograms, iron-binding protein gives bright arches.

In Fig. 1, the immunoelectrophoretic pattern of normal human serum (*b*) and the autoradiogram of the same preparation (*a*) are illustrated. The autoradiogram contains a single bright arch, which is an exact reflexion of the arch of iron-binding protein precipitated in the immunoelectropherogram. The arch lies in the β_1-globulin region and is situated near the ditch containing the precipitating serum. Its localization in our experiments is analogous to

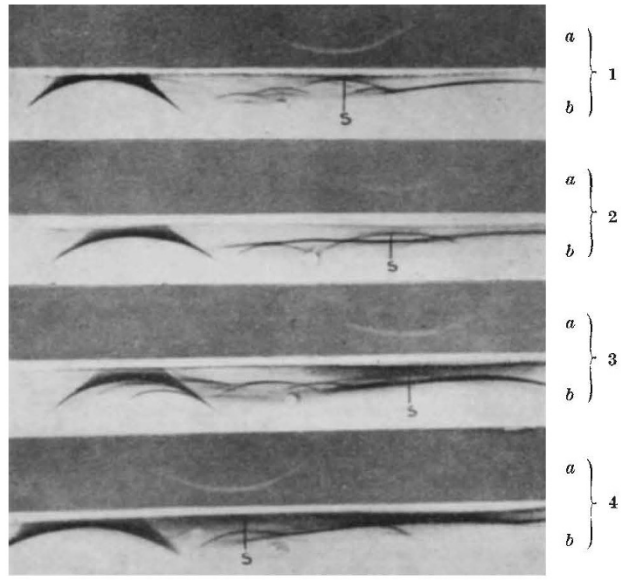

Fig. 1. *a*, Autoradiogram of siderophilin of human serum; *b*, immunoelectropherogram of normal human serum

Fig. 2. *a*, Autoradiogram of siderophilin of horse serum; *b*, immunoelectropherogram of normal horse serum

Fig. 3. *a*, Autoradiogram of siderophilin of rat serum; *b*, immunoelectropherogram of normal rat serum

Fig. 4. *a*, Autoradiogram of siderophilin of sheep serum; *b*, immunoelectropherogram of normal sheep serum

that obtained by Grabar and Burtin[1], Polonovski *et al.*[3], and Kistler *et al.*[2], who utilized pure preparations of siderophilin.

Siderophilin in the autoradiogram of horse serum is also situated in the β_1-globulin region (Fig. 2), and its arch is identical with the β_1-globulin arch in the immunoelectropherogram nearest the ditch containing the immune serum.

The siderophilin in rat serum shows different electrophoretic mobility, lying in the β_2-globulin region (Fig. 3). In the same way as in the preceding serum preparations, the arch of siderophilin of rat serum lies nearest the ditch containing precipitin.

Preliminary experiments showed that the electrophoretic mobility of sheep-serum siderophilin corresponds to that of the α_2-globulins (Fig. 4).

For technical reasons, the isotope iron-55 (half-life, 2·94 years), which has very weak radiant energy, was used in our experiments, which made necessary long exposure of the photographic films. The exposure time can be shortened to several days[8], using the isotope iron-59 (half-life, 47 days), which has much higher energy of β-radiation than iron-55.

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