Preparation of Phenotypes of Horse Serum Transferrins by Gel Filtration and Ion-Exchange Chromatography

The transferrins of horse serum show a well-established polymorphism. There are 6 homozygous phenotypes. Each one shows 2 bands on starch gel electrophoresis. The first band (anodic) is wider and more coloured than the second one (cathodic). The space between those bands is smaller for the 3 faster migrating phenotypes (Tf D/D, Tf F/F and Tf H/H) than for the 3 slower migrating ones (Tf M/M, Tf O/O and Tf R/R)[1].

Experimental. In vivo about $^1/_3$ of the serum transferrins is saturated with iron. The remaining part is found as iron-free transferrins, that is apotransferrins (Apo-Tf). It is important, before analysing a serum, to saturate the Apo-Tf with iron because the electric charge of the Apo-Tf is different from that of the Fe-Tf. To that effect 300 γ of iron as ferrous ammonium sulfate were added to 100 ml of serum.

4 μC of ^{59}Fe were added to 1 ml of serum to identify by radioactivity the transferrins during their separation[2]. The identification was accomplished by autoradiography of the electropherograms. A Kodak film Kodirex 'no screen' was exposed for 14 h for the starch gel electrophoresis. The film was exposed for 36 h for the electrophoresis on cellulose strips and immunoelectrophoresis (micromethod of SCHEIDEGGER). The film was developed in Kodak D-19 B at 20 °C.

After a first separation of the serum components by gel filtration on Sephadex G-200, the transferrin fractions were pooled and subjected to ion-exchange chromatography on Sephadex DEAE A-50. The elution followed a NaCl gradient.

Antibodies against the isolated transferrins were obtained by immunizing 3 rabbits for each phenotype. 2 ml of buffer containing 30–50 γ of proteins emulsified in 2 ml of complete Freund adjuvant (Difco) were injected intradermally into the 2 rear foot pads. An injection of 70–90 γ of antigen was made i.v. 12 days later. The rabbits were killed 8 days after the second injection.

Results. The elution curve of a whole horse serum during filtration on Sephadex G-200 shows 3 main peaks corresponding to those described by FLODIN and KILLANDER for human serum[3]. The first peak contains the macroglobulins and the lipoproteins, the second the 7-S-γ-globulins and the third the albumins. The transferrins are eluted between the second and the third peak (see Figure 1).

The elution of each phenotype by ion-exchange chromatography on Sephadex DEAE A-50 proceeds at different NaCl molarities. Homozygous phenotypes can be separated from a heterozygous serum providing the fast moving band of the slow phenotype does not move faster than the slow moving band of the fast moving phenotype. It is therefore impossible to separate an unresolved

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[3] P. FLODIN and J. KILLANDER, Biochim. biophys. Acta *63*, 403 (1962).

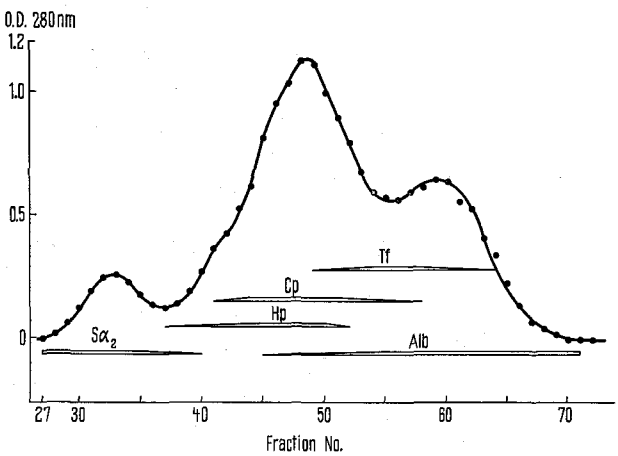

Fig. 1. Filtration of a whole horse serum on Sephadex G-200. Column: 4 × 60 cm. Elution rate: 15–18 ml/h. Buffer: *Tris*-HCl 0.1 *M*, pH 8.1 plus NaCl 0.2 *M*. Sα_2, slow α_2-macroglobulins; Hp, haptoglobins; Cp, ceruloplasmins; Tf, transferrins; Alb, albumins.

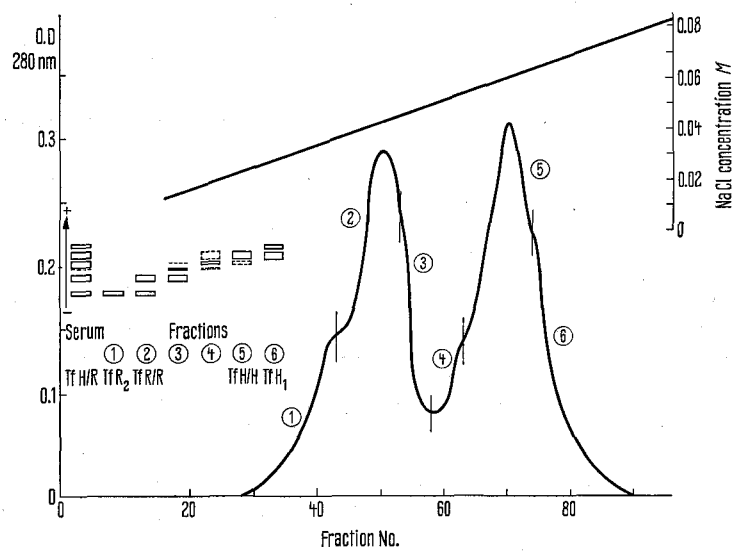

Fig. 2. Elution pattern of a heterozygous phenotype (Tf H/R) by ion-exchange chromatography on Sephadex DEAE A-50. Column: 1 × 18 cm. Buffer: *Tris*-HCl 0.1 *M*, pH 8.1. The NaCl concentration is indicated in the upper right part of the Figure. In the left part is shown a schematic representation of the pattern of a starch gel electrophoresis of the same material as it appears coloured with amido-black. The fractions 1–6 corresponds to the fractions indicated by the same numbers of the curve.

binary mixture of the following types: Tf M/M, Tf O/O and Tf R/R, but all other combinations are separable. One can also partially separate both bands of a phenotype by choosing an adequate NaCl gradient. The absorbancy curve of the eluted fractions shows in this case 2 shoulders on each side of a main peak. The first one corresponds to the cathodic band and the second one to the anodic band. The main peak is a mixture of both bands. In Figure 2 can be seen the elution curve of a heterozygous phenotype, Tf H/R. In this case, the curve shows 2 main peaks, the first corresponding to Tf R/R and the second to Tf H/H instead of 1 main peak as in the case of a homozygous phenotype. It is interesting to note that a third very narrow band appears at the very end of the chromatogram for each phenotype, whatever its type. This band is also visible on the pherogram of a whole serum saturated with iron. The iron-binding capacity of the compound contained in this band has been demonstrated[4]. Therefore each phenotype of the horse serum transferrins exhibits not 2 but 3 bands by starch gel electrophoresis. Some characteristics of these bands will be reported elsewhere.

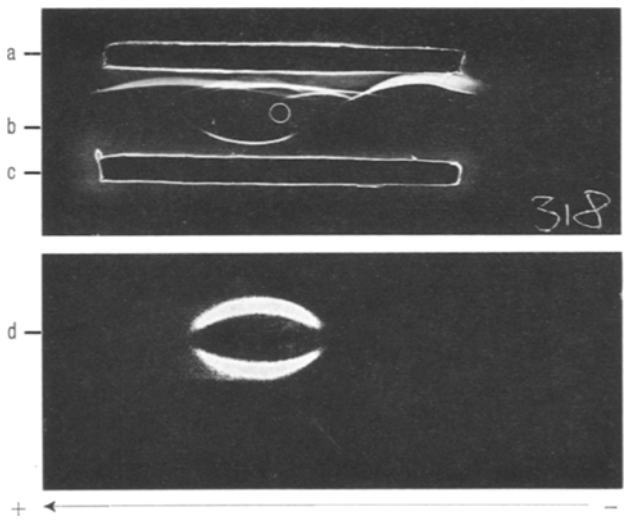

Fig. 3. Immunoelectrophoresis of a whole horse serum (micromethod of SCHEIDEGGER) and its autoradiogram as described in the text.

Immunologically, a band of purified transferrin shows only one precipitine line with an immunserum against a whole horse serum. An antiserum prepared against a purified transferrin shows likewise only 1 precipitine line with a whole serum (see *b* and *c* of Figure 3). This is illustrated by immunoelectrophoresis in Figure 3 where *b* is the circular dish containing the antigen (whole horse serum labeled with 4 μC of ^{59}Fe/ml). Dish *a* contains an immunserum against the pool of transferrins fractions mentioned above. An immunserum against pure transferrin is contained in dish *c*; *d* is an autoradiogram of the same experiment. The circular dish is barely visible but the position of the inferior line is exactly the same as that of the line between *b* and *c*, thus demonstrating that the latter corresponds to the transferrin.

One can obtain a larger amount of almost pure transferrins by precipitating the serum, diluted with 1 volume of physiological NaCl and adding enough ammonium sulfate to bring the final concentration to 56% in ammonium sulfate. This precipitation is substituted for the gel filtration and ion-exchange chromatography is replaced by a batch operation. The transferrins thus obtained are still contaminated by 2 albumins which can be eliminated by column chromatography[5,6].

Résumé. Les bandes des différents phénotypes de transferrines (Tf), identifiées par amido-électrophorèse, ont été purifiées par filtration sur gel et chromatographie échangeuse d'ions. Des anticorps de lapin ont été préparés contre plusieurs de ces phénotypes.

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Influence of Season and Activity on Sodium Content of Bones and Plasma of the Bat, *Myotis lucifugus*

A number of workers have noted changes in plasma levels of certain minerals during the hibernal siege, but there are few reports concerning fluctuations in bone levels of these elements. Furthermore, no attempt has been made to determine what effects simple inactivity might have upon hibernators' skeletons if the animals were not hibernating. This study was part of an attempt to clarify the status of major minerals in a hibernator, *Myotis lucifugus*, during natural hibernation and in 2 activity states while kept under laboratory conditions.

Methods. Hibernating bats were captured in a southern Indiana cave at 3 stages of the hibernating season (early, November; deep, February; late, April). In the laboratory they were housed in a moist, cold (8 ± 2 °C), darkened room until sacrifice shortly after capture. Summer bats were captured in June from an attic colony and sacrificed immediately. Bats designated 'winter free-flight' and 'winter restricted' were procured from a colony in early fall. The 'free-flight' bats were weighed, sexed, coded, and placed into a screened cage of 450 ft^3 flying space. The cage was maintained in a room at 35 °C with a relative humidity of 20%. The 'winter restricted' bats were housed in the same room, but were restricted in movement by placing them in a confined space of 100 in^3. Animals designated as 'summer free-flight' and 'summer restricted' were obtained from a colony in June and housed in the same manner as the 'winter free-flight' and 'winter restricted' groups, respectively. All bats were fed mealworm (*Tenebrio molitor*) larvae, and water was provided ad libitum.