Bisalbuminaemia in a Spanish family: characterization of the albumin variant

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SYNOPSIS A case of bisalbuminaemia has been found in Luarca, Asturias. At least two relatives also bear the trait. The variant albumin Luarca (Al Lu, gene notation A¹Lu), the first to be found on the mainland of Spain, is described in terms of its electrophoretic mobilities and dye-binding properties. It is heat-stable but sensitive to storage, freezing, and thawing and it is the first albumin to show this property.

An electrophoretically distinct albumin variant will mostly be detected as bisalbuminaemia in the serum of its heterozygote carriers. About 50 Caucasian families with this type of abnormality have been reported, and several unpublished cases are known to exist. Their structural differences have not been worked out, but a comparison of their electrophoretic mobilities in different media and of their dye-binding properties will establish non-identities or leave identities as at least a possibility. A recent study, made by these means, found eight distinct albumin variants in eight unrelated families with bisalbuminaemia (Tárnokey, Dowding, and Lakin, 1970).

This paper describes albumin Luarca, the first albumin variant from the Spanish mainland, found in Luarca on the Asturian coast. Its electrophoretic mobilities under a number of conditions have been examined, and dye absorption tests with four dyes in three media carried out. Kohn has found instability in an alloalbumin after prolonged storage at room temperature (Schultze and Heremans, 1966), and a thermolabile albumin has also been described (Arends, Gallango, Layrisse, Wilbert, and Heinen, 1969). Albumin Luarca shows a new type of instability, to freezing and thawing as well as to storing at room temperature.

Material

Serum was taken from the index case S.P., a woman of 68 with cerebral arteriosclerosis, and two healthy relatives A.S. and M.G. Parallel experiments with albumin Kashmir (Tárnokey and Dowding, 1969) and a bisalbuminaemia from the Canary Islands (Rawstron and Mellor, 1971) were performed for comparison.

Methods

PROTEIN ESTIMATION Total protein and albumin were determined by a biuret and 28% Na₂SO₃/biuret method (Reinhold, 1953) and albumin also by elution and by scanning after electrophoresis on cellulose acetate.

ELECTROPHORESIS The following conditions were employed:

Filter paper
In Oviedo, LKB horizontal equipment, Whatman no. 1 and 3MM paper, 0.3 mA/cm in a Na-barbitone (0.055 mol)—Na acetate (0.1 mol)—starch (0.1%) buffer at pH 8.6. In Reading, A-shaped (Flynn and de Mayo) and Shandon horizontal equipment, Whatman no. 1 and 3MM paper, Na-barbitone (0.06 mol) buffer at pH 8.6, Oxoid barbitone acetate buffer (0.1 mol), pH 8.6, and, as in Oviedo, with 0.66 mA/cm on vertical and 1 mA/cm on horizontal runs.

Cellulose acetate
In Oviedo, Atom horizontal equipment, Cellogel—Chemetron strips, 0.6 mA/cm at pH 8.6 (0.0575 mol barbitone). In Reading, Shandon tank, cellulose acetate—Oxoid, 0.44 mA/cm in 0.053 mol barbitone and in 0.1 mol Oxoid buffer, both at pH 8.6.

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Received for publication 16 November 1970.
Disc electrophoresis
Seven per cent acrylamide gel in a discontinuous system of pH 8:3--8:9--8:3 (Tärnoky and Dowding, 1967).

Agar gel electrophoresis
Shandon tank; 8 x 8 cm slide covered by 9 ml of 1% (w/v) agar prepared, and used at 1-63 mA/cm, in 0-1 mol Oxoid buffer, pH 8:6.

DYE BINDING
Pre-staining for electrophoresis on filter paper, cellulose acetate, and agar
Shortly before electrophoresis 0-1 ml aliquots of serum were incubated at 37°C for 10 min with 0-04-0-05 ml of bromophenol blue (0-05%), Ponceau S (0-05%), HABA (0-05% at pH 6:2 and at pH 7:35), and bromoresol green (0-003% at pH 3:9 and at pH 7:35).

Pre-staining for disc electrophoresis
Two μg of the 0-05% dye solutions was mixed with each 0-3 mg of protein (the sample size) shortly before the run.

STABILITY
Heat stability
Sera were heated at 56°C for 30 min as recommended by Arends et al (1969) and also for two hours and checked by cellulose acetate electrophoresis and scanning before and after being heated.

Behaviour on storage, freezing, and thawing
Sera were twice frozen to -20°C and, a few hours later, thawed at 37°C. Sera were also frozen to -20°C, thawed at 37°C, and immediately frozen again, 10 times. In another experiment sera were divided, one half was frozen, rapidly thawed out but immediately frozen again, 30-35 times in the course of seven to 10 days. All experiments were checked by cellulose acetate electrophoresis and scanning.

Results
Albumin Luarca is a slow alloalbumin. It separates on horizontal electrophoresis in barbitone-acetate starch or Oxoid buffer when Whatman no. 1 filter paper is used, but the bands are close together. Under other conditions separation on paper is incomplete.

Cellulose acetate separations are the most convenient and suitable for quantitation. The index case was investigated three times in the course of three months. During this time her total protein changed from 6:2 to 8:1 and then to 7:3 g/100 ml and her albumin from 2:6--5:1--3:5 g/100 ml with marked changes in the globulin pattern, but the ratio of the electrophoretically normal albumin A to A1 Lu remained constant at 54:46-52:48--53:5:46-5. Relative mobilities of the two albumins depend on the exact experimental conditions used. Adapting the 100-mm notation of disc electrophoresis (Smith, 1968) to this medium, denoting the cathodic end of the stained pattern 0 and the advancing edge of A1 A 100, the relative electrophoretic mobility of A1 Lu (and, in brackets, the actual length of the pattern) is 94 on Cellogel (mean, 55 mm), and on Oxoid cellulose acetate 87-5 in barbitone buffer (57 mm) and 91-5 in Oxoid buffer (27 mm).

On disc electrophoresis (mean, 36 mm) A1 Lu is at 87-93 and A1 A at 93-5-100. The latter stains more strongly than the slow variant. In agar (31 mm) albumin Luarca is at 92 and on staining with excess
Ponceau S takes up slightly more than albumin A. Immuno-electrophoresis on agar with rabbit whole antiserum (Behringwerke) shows only one precipitation arc for the two albumin bands.

The dye uptake results are given in the Table. Where the dye does not stain the protein it runs ahead as a free band and is generally found around 135-145.

<table>
<thead>
<tr>
<th>Filter Paper</th>
<th>Cellulose Acetate</th>
<th>Acrylamide Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromophenol blue</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Ponceau S</td>
<td>A, Lu</td>
<td>—</td>
</tr>
<tr>
<td>HABA, pH 6.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HABA, pH 7.35</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Bromocresol green, pH 3.9</td>
<td>A, Lu</td>
<td>—</td>
</tr>
<tr>
<td>Bromocresol green, pH 7.35</td>
<td>A, Lu</td>
<td>—</td>
</tr>
</tbody>
</table>

Table  Dye uptake of serum containing albumin Luarca

A deficiency of dye added may attach itself to albumin A, the electrophoretically normal band, the slow variant Lu, or may not be protein-bound (—).

Both albumin bands are completely stable to heating at 56°C for two hours.

The instability of albumin Luarca in the cold was first seen in Oviedo while storing the serum of M.G. whose Al Lu (on Cellogel) fell to 22%, and in another sample to 29% of the total albumin. Tenfold freezing and thawing of serum S.P. did not affect the total albumin figure (4.2 before and 4.3 g/100 ml after the experiment) but altered the Al:A Al Lu ratio from 56:44 to 65:35. Again, when serum S.P. was twice frozen for some hours there was no decrease in total albumin, but the Al A: Al Lu ratio fell to 64:36. Albumin Kashmir and Rawstron and Mellor's serum, treated in the same way, showed no change.

Both storage for seven to 10 days and freezing and thawing 30-35 times over this period has caused Al Lu to merge into the normal albumin band. These results, however, are not fully reproducible since in one such experiment out of five the serum frozen 35 times was recovered with its Al Lu intact while in the aliquot kept at room temperature it had merged into the Al A band.

Comment

Besides adding to the list of known albumin variants this report raises two points.

The first concerns the choice of methods by which alloalbumins should be compared. It is clear from this two-centre study that small differences in running conditions will cause the relative electrophoretic mobility to vary and may even determine whether separation can take place. Standardized conditions for agreed test procedures, as advocated by Tarnoky et al (1970), will need stringent specification.

The second point is perhaps more fundamental. Storing, freezing, and thawing transform albumin Luarca and make it merge into Al A while the total amount of albumin remains the same. A change in the electrophoretic mobility of one albumin and not merely a decrease in its dye-binding power is involved. This suggests that when inherited albumin variants are electrophoretically distinct the difference is not, or at least not always, due directly to a single amino acid change.

We wish to thank Dr A. Telenti under whose care the index case was admitted, Dr A. Nestal for serum from relatives, Dr J. R. Rawstron for a sample of bisalbuminaemic serum, Dr K. A. Kimber for the photograph.

References


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doi: 10.1136/jcp.24.6.515

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