Further Studies on Plasma Proteins, Lipids, and Dye- and Drug-Binding in a Child with Analbuminemia

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A previously reported patient with analbuminemia was re-investigated after 4 1/2 years, at age 6. The serum albumin concentration was 150 mg/L by radioimmunoassay. Most of the observed increase in total plasma protein over the 4 1/2 years was attributable to gamma-globulin. Concentrations of total and high-density lipoprotein cholesterol were increased; the esterified/free ratio and the lecithin-cholesterol acyltransferase activity were both normal. Albumin is apparently not essential for binding of lysol-ecithin generated by the acyltransferase-catalyzed reaction. The binding of bromphenol blue suggested that analbuminemic serum has about 25% of normal binding capacity for bilirubin (more than expected in a patient with analbuminemia), which may explain why newborns with this disorder do not develop kernicterus. Binding by the patient’s plasma of diazepam (1020 mg/L) and warfarin (1040 mg/L), which bind primarily to albumin, as well as of propranolol (1.05 g/L), which binds primarily to α1-acid glycoprotein, was also studied. The proportions of free diazepam (14.4%) and warfarin (4.8%) were about 10-fold normal. In contrast, the proportion of propranolol in the free form was decreased (4.5%). Evidently, other plasma proteins are partly compensating for the deficiency of albumin.

Analbuminemia (or, more precisely, hypoalbuminemia) was first described by Bennhold et al. in 1954 (1). Only 13 other patients with this condition, including our own, have been described so far (2–13). Follow-up of these patients is desirable because this “experiment of nature” can help us to understand the physiological role of albumin and the compensatory mechanism operating in its absence. Rather remarkably, the individuals deficient in albumin show only minor clinical and biochemical abnormalities. A parallel to the human condition, an analbuminemic strain of rats, was recently described (14). These animals grow, reproduce, and have a life span identical to their normal litter mates. While the loss of the plasma oncotic pressure, to which albumin ordinarily contributes about 80%, can be compensated for by an increase in other small protein molecules and a decrease in blood pressure, little is known about compensation for the binding and transport functions of albumin.

We investigated the changes in plasma proteins with development of a previously described child with analbuminemia, specifically, changes in plasma lipoproteins and in lecithin-cholesterol acyltransferase (LCAT; EC 2.3.1.43) activity. To assess the bilirubin-binding capacity of the patient’s plasma, we used bromphenol blue, addressing ourselves to the question of perinatal jaundice and its treatment in this particular patient. The capacity of the patient’s plasma to bind drugs ordinarily bound by albumin and α1-acid glycoprotein was also studied.

Materials and Methods

This child was the subject of a previous report at age 11/2 years (10). Blood specimens collected, after fasting, into EDTA-containing Vacutainer Tubes (Becton-Dickinson, Rutherford, NJ 07070) were put on ice and centrifuged in a refrigerated centrifuge. Analyses for lipids and most of the protein analyses were done immediately. Plasma was frozen at −70 °C in aliquots to be used for additional analyses.

Plasma proteins. Quantitative protein electrophoresis of serum was done on cellulose acetate. High-resolution protein electrophoresis on preformed agarose gels (Panagel slide; Millipore Biomedica, Freehold, NJ 07728) was carried out according to the manufacturer’s instructions. Plasma albumin, transferrin, α1-antitrypsin, ceruloplasmin, C3, C4, IgA, IgM, IgG, α1-acid glycoprotein, α2-macroglobulin, and haptoglobin were determined by radial immunodiffusion. Haptoglobin was typed by starch-gel electrophoresis (15). The alpha-fetoprotein concentration was measured by radioimmunoassay (Abbott Laboratories, North Chicago, IL 60064). Albumin was also determined by radioimmunoassay, by a modification of the method of Miles et al. (16). Plasma protein immunoelectrophoresis was done according to Irwin and Campbell (17).

Bromphenol blue binding studies. Using a method described by Hertz (18), we studied the binding of bromphenol blue (BPB) by plasma, to determine the number of available bilirubin binding sites. A solution containing 8 mg of BPB per liter was prepared in phosphate buffer (0.05 mol/L, pH 7.3). BPB binding was determined with a double-beam spectrophotometer for normal, hypoalbuminemic, and analbuminemic plasma. The relative binding capacity was assessed from the differences of absorbance at the peak at 615 nm and the trough at 585 nm.

Drug-binding studies. The plasma protein binding of diazepam, warfarin, and propranolol was studied in duplicate at therapeutic total drug concentrations by an equilibrium dialysis method described previously (19). These drugs were chosen as representative of classes of drugs that bind to the tryptophan-binding site on human serum albumin (20) (diazepam), to the acidic drug site on albumin (21) (warfarin), and to both α1-acid glycoprotein and lipoproteins (22) (propranolol).

Lipid studies. Plasma total and free cholesterol and triglycerides were determined as described previously (23). High-density lipoprotein (HDL) cholesterol was determined by manganese chloride and heparin precipitation (24). LCAT activity was determined by the Stokke–Norum method (25), including different amounts of albumin in the assay (final albumin concentration ranged from 10 to 50 g/L). Human serum albumin (Connaught Laboratories, Toronto, Ontario) was used in these studies. Lipoprotein electrophoresis on

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Nonstandard abbreviations: LCAT, lecithin-cholesterol acyltransferase; BPB, bromphenol blue; HDL, LDL, high- and low-density lipoproteins, respectively.

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agarose and on polyacrylamide gel was performed by previously described methods (23).

Results

Plasma proteins. Table 1 summarizes the findings at age 31 days, 1 1/2, 3 1/2, and 6 1/2 years of age. Only trace amounts of albumin were found after immunoelectrophoresis; its concentration by radial immunodiffusion was unchanged at approximately 100 mg/L over the 6 1/2-year period. Albumin concentration at 6 1/2 years of age was 150 mg/L by radioimmunoassay. Electrophoresis showed increases in all α₁, α₂, β, and γ-globulins, the values for immunoglobulins being higher than the normal range for his age group. Increases in the concentration of numerous proteins (in comparison with a control) were observed and are summarized in Table 2. However, concentrations of alpha-fetoprotein and C3 and C4 components of complement were normal, and haptoglobin (phenotype 2-2) decreased. Many of the above changes are well demonstrated by high-resolution protein electrophoresis (Figure 1).

Plasma lipoproteins and LCAT activity. As Table 3 summarizes, hypercholesterolemia has been present since birth. The increase in plasma cholesterol seen over the six years of follow-up probably reflects, as with the immunoglobulins, a normal developmental pattern. The proportion of free and esterified cholesterol as well as plasma triglycerides and LCAT activity (expressed as milligrams of cholesterol esterified per hour) are all within normal limits. There is an apparent decrease in LCAT activity in the presence of albumin and also the "initial fractional rate of esterification" (% of cholesterol esterified per hour) is low.

HDL cholesterol is increased. On both cellulose acetate and agarose gel electrophoresis an increase in the β band was observed.

BPB-binding study. The difference in absorbance at 615 nm reflects the degree of BPB binding in each of the three specimens: normal, nephrotic, and analbuminemic plasma containing 45, 24, and 0.15 g of albumin per liter, respectively (Figure 2). Despite the almost complete absence of albumin in our patient's serum there was still a shift in the BPB spectrum, resulting in differences of absorbance that were approximately one-fourth of normal (one-half of the change with nephrotic serum). If albumin were the only protein binding BPB, the analbuminemic serum's binding capacity would be that of about 12 g of albumin per liter.

Drug-binding studies. Results of the drug-binding studies are shown in Table 4, along with typical reported values for proportions of unbound drugs in normal individuals. The unbound portion of diazepam was 14.37% of the total, 11.5-fold higher than normals. Free warfarin was also increased, to 4.78%, 8.5-fold greater than average normal values. Free propranolol was 4.45%, less than the low end of the reported normal range.

Table 2. Serum Proteins in a Patient with Analbuminemia

<table>
<thead>
<tr>
<th>Protein, mg/L, at age</th>
<th>31 d</th>
<th>1 1/2 yr</th>
<th>3 1/2 yr</th>
<th>6 1/2 yr</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>1000</td>
<td>8500</td>
<td>11 000</td>
<td>14 400</td>
<td>5500–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16 000</td>
</tr>
<tr>
<td>IgA</td>
<td>250</td>
<td>1400</td>
<td>16 700</td>
<td>25 600</td>
<td>500–2000</td>
</tr>
<tr>
<td>IgM</td>
<td>230</td>
<td>1350</td>
<td>18 800</td>
<td>13 700</td>
<td>300–1100</td>
</tr>
<tr>
<td>Albumin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>150</td>
</tr>
<tr>
<td>α₁-Antitrypsin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4350</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>310</td>
<td>640</td>
<td>530</td>
<td>550</td>
<td>200–350</td>
</tr>
<tr>
<td>Transferrin</td>
<td></td>
<td></td>
<td>6500</td>
<td>5200</td>
<td>2000–4000</td>
</tr>
<tr>
<td>C₃</td>
<td></td>
<td>1750</td>
<td>18 500</td>
<td>18 000</td>
<td>1000–2500</td>
</tr>
<tr>
<td>C₄</td>
<td></td>
<td></td>
<td>320</td>
<td>320</td>
<td>160–450</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td></td>
<td></td>
<td>190</td>
<td></td>
<td>200–2350</td>
</tr>
<tr>
<td>α₁-Acid glycoprotein</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1190</td>
</tr>
</tbody>
</table>

* Normal range for six-year-old child.

Table 3. Plasma Lipids and LCAT Activity in a Patient with Analbuminemia

<table>
<thead>
<tr>
<th>Conc, mg/L, at age</th>
<th>1 yr</th>
<th>3.5 yr</th>
<th>6.5 yr</th>
</tr>
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<tbody>
<tr>
<td>Total cholesterol</td>
<td>2680</td>
<td>3200</td>
<td>3900</td>
</tr>
<tr>
<td>Free cholesterol</td>
<td></td>
<td>860</td>
<td>960</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td></td>
<td>910</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>860</td>
<td>900</td>
<td>650</td>
</tr>
<tr>
<td>LCAT</td>
<td></td>
<td>18 a</td>
<td>30 b</td>
</tr>
</tbody>
</table>

* LCAT activity expressed in milligrams of free cholesterol esterified by 1 ml of plasma in 1 h, Stokke-Norum assay (25). b Decrease in free cholesterol over 1 h of incubation at 37 °C as measured by enzymic procedure with Abbott automatic analyzer (ABA-100).
Discussion

The finding of increased gamma-globulins, transferrin, \( \alpha_1 \)-antitrypsin, \( \alpha_2 \)-macroglobulin, and ceruloplasmin are consistent with previous reports on other patients with analbuminemia (11).

The data on plasma haptoglobin concentrations are not uniform; although some analbuminemic patients had increased haptoglobin concentrations, our patient, similarly to one described by Weinstock et al. (11), had a low haptoglobin concentration and the same phenotype (Hp 2-2). The significance of this finding is not known but is apparently unrelated to the analbuminemia, the relatives of Weinstock’s patient having had normal albumin but low haptoglobin concentrations.

The increase in the synthesis of proteins of low molecular mass compensates, by approximately 50%, for the loss of the colloid osmotic pressure that results from the absence of albumin. As previously pointed out (10), this fact, together with low blood pressure, partly explains the lack of significant edema in most analbuminemic individuals.

The catabolism of albumin (10) and other plasma proteins might also be influenced by their concentration or by oncotic pressure of plasma. Thus both increased protein synthesis and decreased catabolism may contribute to “compensatory” plasma protein changes in analbuminemia.

High-resolution protein electrophoresis and analytical ultracentrifugation were used to search for possible unusual proteins. No additional or unusual bands were observed on agarose gel electrophoresis (Figure 1). In contrast to Keller et al. (6), who reported the presence of small amounts of 0.7, 2.1, and 3.3S protein fragments on several occasions in the serum of their patient, we could not demonstrate the presence of low-molecular-mass fragments. The pattern we obtained on analytical ultracentrifugation was very similar to that found in a patient with nephrotic syndrome and severe hypoaibuminemia (unpublished observation).

A previously reported inverse relationship between concentrations of albumin and serum cholesterol (28) was presumed to be related to the serum colloid osmotic pressure. These findings are supported by the studies in analbuminemic rats and in perfused liver from nephrotic rats. Although in analbuminemic rats both the plasma cholesterol and triglycerides are increased (14), only the serum cholesterol concentration is high in most analbuminemic individuals.

Both increased synthesis and decreased catabolism may contribute to the increased concentration of the low-density lipoprotein (LDL) and therefore to the hypercholesterolemia in our patient. The finding of a normal ratio of free/esterified cholesterol and a normal value for LCAT activity is of interest. Obviously albumin is not necessary for binding lysolecithin generated by the LCAT reaction.

Some analbuminemic individuals may be at risk for cardiovascular disease because of the hypercholesterolemia. A 12-year-old girl described by Boman et al. (12) had both corneal arcus and retinal lesions. Montgomery et al. (3) reported an analbuminemic patient with hypercholesterolemia who suffered from a cerebral vascular accident. The 12-year-old girl had low HDL cholesterol (300 mg/L), whereas our patient has a high HDL-cholesterol concentration (900 mg/L) and a “low risk” LDL/HDL cholesterol ratio (27).

The study of BPB binding suggested that the patient’s serum has approximately 25% of the normal bilirubin binding capacity. If we assume that BPB binding sites are similar to those of bilirubin (18), as much as 50 mg of bilirubin per liter could be bound by the patient’s plasma proteins. A large proportion of newborns do not exceed that concentration of bilirubin, and therefore it is not too surprising that kernicterus has not been reported in a patient with analbuminemia. We were unable to demonstrate the presence of alternative BPB and bilirubin-binding proteins, using an electrophoretic technique similar to Goule et al. (13). Our results tended to support their findings that bilirubin apparently exists in unbound form when present at greater than 10 mg/L (results not shown). We cannot, however, completely exclude the possibility of a loose association between bilirubin and some of the other patient’s plasma proteins (or lipoproteins?).

In normal pharmacological situations albumin is the major binding protein of both diazepam (28) and warfarin (29). Accordingly, the unbound fraction of these representative drugs was greatly increased in the analbuminemic plasma. The proportion of drug that was unbound was approximately that predicted from an albumin concentration one-tenth that of normal (35–55 g/L). This indicates that plasma proteins other than albumin are serving a significant transport role, and if this same kind of adjustment occurs for endogenous ligands as well, may be one contributor to the lack of clinical sequelae in this disorder. The 11.5- and 8.5-fold increases in unbound diazepam and warfarin, respectively, show low affinity and a high degree of binding-site saturation in the analbuminemic plasma. The proportion of unbound drug will therefore be very dependent on total drug concentration. Fluctuations of the free drug concentration over a dosing interval will be greater, which in turn will result in a more rapid onset of drug action, a greater peak response, and the earlier appearance of toxicity (30). These effects may be even more exaggerated for albu-

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Table 4. In Vitro Plasma Protein Binding of Representative Drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Diazepam concn, ( \mu g/L )</th>
<th>Warfarin concn, ( \mu g/L )</th>
<th>Propranolol concn, ( \mu g/L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analbuminemic</td>
<td>1020</td>
<td>1040</td>
<td>1050</td>
</tr>
<tr>
<td>Analbuminemic free fraction (and SD), %</td>
<td>14.37 (0.35)</td>
<td>4.78 (0.10)</td>
<td>4.45 (0.16)</td>
</tr>
<tr>
<td>Typical av. free fractions, %</td>
<td>1.25*</td>
<td>0.56*</td>
<td>5–11.8*</td>
</tr>
</tbody>
</table>

* Abel et al. (19).  b Sager et al. (22).
min-bound drugs such as salicylates, which, in usual doses, are present at much higher drug/albumin ratios than either diazepam or warfarin. Displacement of drugs from plasma protein-binding sites and interaction between endogenous ligands such as bilirubin and fatty acids and drugs will also increase at a greater fractional saturation of drug.

Propranolol binds specifically to $\alpha_1$-acid glycoprotein, and the proportion of unbound drug varies inversely with the concentration of this plasma protein. Propranolol also associates nonspecifically with plasma lipoproteins (22). Sager et al. (22) found that the concentration of $\alpha_1$-acid glycoprotein ranged from 2240 down to 530 mg/L as unbound propranolol increased from 5.0 to 11.5% of total drug. The lower concentrations of free drug in this child, therefore, cannot be accounted for solely by high $\alpha_1$-acid glycoprotein concentrations, but may be due to concomitant increases in LDL and HDL.

References