Diminished albumin binding of zinc in serum of pregnant women

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Summary
1. Distribution of zinc between the metalloprotein α2-macroglobulin and albumin was determined in samples of serum obtained from twenty-three women in their third trimester of pregnancy and eighteen women who were not pregnant.
2. The decrease observed in total serum zinc in the group of pregnant women could be accounted for in large part by a decreased concentration of albumin-bound zinc.
3. The concentration of serum albumin was lower in the pregnant women, thus hypoalbuminaemia, which is often invoked to explain hypozincæmia in pathological situations, may in part account for hypozincæmia in pregnancy.
4. The affinity of albumin for zinc added to serum from pregnant women was less than that of albumin from non-pregnant women. This was determined by competition experiments between albumin and glycine for zinc. Decreased affinity of serum albumin for zinc may also contribute to the hypozincæmia associated with pregnancy.

Key words: ligands, pregnancy, serum albumin, zinc.

Introduction
Many reports have indicated that subnormal concentrations of serum or plasma zinc may be observed in pregnant women (Vikbladh, 1951; Rothe, Piskazeck & Bilek, 1960; Johnson, 1961; Mischel & Dreher, 1963; Halsted, Hackley & Smith, 1968; O’Leary & Spellancy, 1969; Halsted & Smith, 1970; Henkin, Marshall & Meret, 1971; Hambidge & Droegemueller, 1974), although a contradictory observation has been reported (Flynn, Pories, Strain & Weiland, 1973). A technique has been developed recently in our laboratory (Giroux, 1975) to determine distribution of zinc between its two major serum ligands, α2-macroglobulin and serum albumin, thereby increasing the information that may be obtained from measurements of serum zinc. This technique has been applied to a study of sera of pregnant women. The results are presented here with data comparing the affinity with which added zinc ion is bound to macromolecules in serum of pregnant and non-pregnant women.

Methods
Samples of blood were obtained from twenty-three pregnant women (mean age 25 years; range 18–37 years) on the occasion of their routine third trimester antenatal visit to their obstetrician. Blood samples were obtained by the same obstetrician from eighteen non-pregnant women (mean age 39 years; range 22–72 years). None of these control subjects used oral contraceptives. Women were randomly selected for study without conscious bias on the part of the obstetrician. Twenty of the pregnant women had no complications of pregnancy at the time of their visit; one had developed gestational diabetes and two had urinary tract infections. Four of the control subjects were postmenopausal; their serum zinc concentrations were similar to those of the other control subjects. The control subjects consulted the physician for reasons including metrorrhagia (nine subjects, in two cases secondary to complications of an intrauterine device), request for sterilization...
Blood was allowed to clot in acid-washed glass tubes and serum was obtained by double centrifugation. Aliquots for zinc, albumin, \( \alpha_2 \)-macroglobulin and caeruloplasmin assays were taken from fresh serum or from serum stored no more than 2 days at 4°C; the assays of serum non-esterified fatty acids and of albumin affinity for zinc were carried out on serum which had been stored at \(-20^\circ\text{C}\) for up to 6 weeks. To minimize zinc contamination, plastic syringes, stainless-steel needles and plastic disposable test tubes were used. Glass test tubes and pipettes were soaked in hydrochloric acid (4 mol/l) for at least 16 h, and then rinsed with water and dried before use. Water of greater than 10 MΩ cm resistivity was prepared with a Milli-Q system (Millipore, Bedford, Mass., U.S.A.).

Total serum zinc was measured by atomic absorption spectroscopy of protein-free supernatant of whole serum diluted fourfold in 5% (w/v) trichloroacetic acid (Davies, Musa & Dormandy, 1968). Serum albumin-bound zinc was similarly determined after precipitation of \( \alpha_2 \)-macroglobulin-bound zinc from whole serum by addition of polyethylene glycol 6000 to a final concentration of 10% (w/v); serum \( \alpha_2 \)-macroglobulin-bound zinc was calculated as the difference of total and albumin-bound zinc (Giroux, 1975). Albumin concentration was determined by a dye-binding assay (Doumas & Biggs, 1972) with a commercially available reagent (SpecTru BCG, Pierce Chemical Co., Rockford, Ill., U.S.A.) and by quantitative radial immunodiffusion (M-Partigen plates, Behringwerke AG, Marburg-Lahn, Germany). The concentration of \( \alpha_2 \)-macroglobulin in serum was also determined by immunodiffusion (Immuno-plate, Hyland, Costa Mesa, Calif., U.S.A.). Caeruloplasmin was measured by its oxidase activity (Schosinsky, Lehmann & Beeler, 1974). Concentration of serum non-esterified fatty acids was determined colorimetrically as their cobalt soaps (Smith, 1975). Unless otherwise specified, all reagents were obtained from E. Merck (Darmstadt, Germany). Statistical comparison of mean values was made with Student's t-test (Snedecor & Cochran, 1967).

The affinity with which macromolecules in serum bound added zinc ions was assessed by an ultrafiltration technique. Each serum sample was diluted to a final albumin concentration of 4 mg/ml (60 \( \mu \text{mol/l} \)) with buffer [morpholinopropionate sulphonic acid (0.05 mol/l)/sodium chloride (0.15 mol/l) adjusted to pH 7.5 with NaOH (2 mol/l)]. Morpholinopropionate sulphonic acid was obtained from Sigma Chemical Co. (St Louis, Mo., U.S.A.). Microlitre amounts of aqueous zinc standard (1 mg of zinc/ml) were added to increase zinc concentration in the diluted sample to 35–37 \( \mu \text{mol/l} \). A portion (4 ml) of the diluted, zinc-enriched serum was placed into each of four chambers of an eight-chambered ultrafiltration apparatus (model MMC, Amicon Corp., Lexington, Mass., U.S.A.). To these chambers were added 0, 1, 2 or 4 ml of a solution of glycine, (0.45 mol/l) in buffer. The volume in each chamber was brought to 9 ml by addition of appropriate amounts of buffer. The final concentrations of glycine in the four chambers were therefore 0, 50, 100 or 200 mmol/l. The four remaining chambers of the apparatus were used for another serum sample. Contents of the chambers were magnetically stirred over a half-hour period before ultrafiltration was initiated.

Ultrafiltration was produced through semipermeable membranes (Diaflo ultrafiltration membranes, type PM-10, Amicon Corp.) by pressurization of the chambers with nitrogen at 75 kPa. Ultrafiltrate was collected from each chamber in 1 ml fractions. The first fraction was discarded, and the second and third were analysed. Supernatants of appropriate dilutions in 5% trichloroacetic acid of diluted, zinc-enriched sera and ultrafiltrate fractions were analysed by atomic absorption spectroscopy for zinc content. After each cycle of ultrafiltration the semi-permeable membranes were removed from the apparatus and washed or replaced, as necessary. A control serum and a pregnancy serum sample were analysed at each cycle of ultrafiltration, to balance out possible variations in the technique.

Results

Zinc distribution in pregnancy and control serum

Results of clinical chemical measurements made on serum samples are presented in Table 1. The mean concentration of total serum zinc was found to be significantly lower in the group of pregnant women, compared with the control group. A difference of similar magnitude was found in mean concentrations of serum albumin-bound zinc for the two groups. The mean concentrations of \( \alpha_2 \)-macroglobulin-bound zinc also were significantly different in the
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Table 1. Zinc distribution and concentrations of serum constituents for pregnant and non-pregnant women

Mean values ± SD are shown. Statistical significance was estimated by two-tailed t-tests.

<table>
<thead>
<tr>
<th>Serum constituent</th>
<th>Control subjects (n = 18)</th>
<th>Pregnant subjects (n = 23)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total zinc (µg/l)</td>
<td>841±105</td>
<td>713±75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin-bound zinc (µg/l)</td>
<td>680±117</td>
<td>583±80</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>α2-Macroglobulin-bound zinc (µg/l)</td>
<td>161±42</td>
<td>131±41</td>
<td>&lt;0.025</td>
</tr>
<tr>
<td>Albumin (g/l)</td>
<td>40±5±3±6</td>
<td>35±5±2±7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Caeruloplasmin (units/l)</td>
<td>1.9±0.4</td>
<td>2±1±0.4</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Non-esterified fatty acids (mmol/l)</td>
<td>0.54±0.12(1)</td>
<td>0.79±0.19(2)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(1) n = 12. (2) n = 21.

two groups. Slightly more than 75% of the decrease in total serum zinc in pregnancy serum was due to a decrease in albumin-bound zinc. Mean serum albumin concentration was lower in the group of pregnant women than in the control group. Concentrations of α2-macroglobulin were not significantly different. Caeruloplasmin and free fatty acid concentrations were significantly increased in the group of sera obtained from pregnant women.

Affinity of serum macromolecules for added zinc

In the absence of any glycine added to diluted serum, essentially all zinc ions added to a final ratio of 0·6 g atom of zinc/mol of albumin were bound to macromolecules. With the ultrafiltration technique described, the concentrations of zinc in ultrafiltrates of serum to which glycine was not added were not measurably different from zero either for the group of sera from pregnant women or of those from the control group. At glycine concentrations of 50, 100 and 200 mmol/l, however, 18–89% of total zinc in the mixtures of serum, added zinc and glycine was present as small complexes, as evidenced by the passage of zinc across semi-permeable membranes rated to pass material of less than 10 000 mol wt. The data fit a linear relationship when the percentage of total zinc which was ultrafilterable was plotted as a semi-logarithmic function of the concentration of glycine (Fig. 1). A given percentage of zinc was dissociated from macromolecules with a lower concentration of glycine in the sera of pregnant women than in control sera. The two sets of data were analysed by the potency ratio method for parallel line assays as described by Colquhoun (1971). The ‘potency ratio’, which is a function of the separation of the parallel lines along the independent variable axis, was significantly different from 1·0 (P < 0·001), indicating that the affinity of macromolecules for added zinc differed between the groups of sera of pregnant and non-pregnant women.
Discussion

Our observation of reduced concentrations of zinc and albumin in sera of pregnant women is consistent with most reports in the literature (Vikbladh, 1951; Rothe et al., 1963; Halsted et al., 1968; Halsted & Smith, 1970; Horne, Howie, Weir & Goudie, 1970; Henkin et al., 1971; Hambidge & Droegemueller, 1974). This is also true of the increased concentration of cueruloplasmin (and copper), the synthesis of which is under hormonal regulation (Russ & Raymunt, 1956; von Studnitz & Berezin, 1958; Johnson, Kheim & Kountz, 1959; Evans & Wiederanders, 1968). In some of these studies a considerably greater decrease in serum zinc concentration in pregnancy was reported than we observed here.

Data obtained in rats did not suggest any specific relationship between plasma zinc concentration and either plasma oestrogen or progesterone (Sato & Henkin, 1973). At variance with this observation is the report that administration of progesterone to rats leads to increased serum zinc concentration (Boyett et al., 1968; McBean, Smith, Berne & Halsted, 1974). Increases in these hormones, as in pregnancy, have been reported to increase plasma concentration of $\alpha_2$-macroglobulin (Ganrot & Bjerre, 1967; Horne et al., 1970), although contradictory observations have also been reported (Adham, Wilding, Mehl & Haverback, 1968). Our results indicate no significant difference in mean $\alpha_2$-macroglobulin concentration in serum of pregnant and non-pregnant women. Values for serum concentration of $\alpha_2$-macroglobulin similar to those we observed have been reported for normal subjects (Adham et al., 1968; McBean, Smith, Berne & Halsted, 1974). The absolute decrease we observed in the mean concentration of $\alpha_2$-macroglobulin-bound zinc in sera of pregnant women was small. Reduced serum concentration of albumin-bound zinc was a much larger factor in the reduced concentration of total serum zinc observed in the group of pregnant women.

Among mechanisms which play a role in regulation of zinc content of plasma one is almost certainly the 'loosely bound' or 'exchangeable' pool of plasma zinc, that fraction of total zinc which is presently considered the transport form of plasma zinc, mainly comprises albumin and amino acids (Vikbladh, 1951; Parisi & Vallee, 1970; Prasad & Oberleas, 1970). $\alpha_2$-Macroglobulin is the major component of the 'firmly bound' pool (Parisi & Vallee, 1970). In normal serum 60–85% of total serum zinc is bound to albumin (Vikbladh, 1951; Boyett & Sullivan, 1970; Parisi & Vallee, 1970; Giroux, 1975).

Conditions for which hypozincæmia is a characteristic may also present with hypoalbuminaemia. This relationship was recognized early (Vikbladh, 1951) and it has been suggested, for example, that the decline in plasma zinc concentration in alcoholic liver cirrhosis is secondary to hypoalbuminaemia (Kahn, Helwig, Redeker & Reynolds, 1965). Serum zinc and albumin concentrations have been found to be correlated in such patients (Kahn et al., 1965; Boyett & Sullivan, 1970; Schechter, Giroux, Schlienger, Hoening & Sjoerdsmas, 1976).

In our group of pregnant women the mean serum albumin concentration was below that of the control group, but still within the range considered normal (Wintrobe, Thorn, Adams, Braunwald, Isselbacher & Petersdorf, 1974). It is possible to hypothesize, therefore, that pregnant women are hypozincæmic as a result of hypoalbuminaemia. The zinc-binding experiments that we carried out indicate that hypoalbuminaemia may not be the only explanation of the hypozincæmia of pregnancy. It appears that the affinity of albumin for zinc in pregnancy serum may be diminished slightly, but significantly. This effect is presumably due to serum factors that modify albumin binding of zinc. In the dissociation experiments, the ratio of zinc to albumin used was sufficiently large to make it unlikely that other serum macromolecules, present in serum in molar concentrations considerably less than that of albumin, would influence the results observed, whatever their affinity for zinc might be. Possible modifiers of albumin affinity for zinc might be non-esterified fatty acids, although we have no evidence for this. However, non-esterified fatty acids are largely albumin-bound in plasma and we observed a greater mean concentration of non-esterified fatty acids in the group of sera from pregnant women. The identity of possible modifiers of zinc binding to albumin is strictly speculative at present.

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References


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