Protein concentrations in milk from mothers of preterm and term infants

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Several authors have recently reported that milk from mothers of preterm infants contains up to 20% more nitrogen than does milk from mothers of term infants (Atkinson et al., 1978, 1980; Schanler & Oh, 1980; Gross et al., 1980). It is not yet clear whether the additional nitrogen is present as protein or as other nitrogen-containing compounds. This report describes the investigation of the concentrations of nine proteins in the milk from mothers of term and preterm infants, and also in the colostrum from mothers of term infants.

Milk samples were obtained from 20 mothers of term infants and 20 mothers of preterm infants, by complete expression of one breast into sterile containers. A sample was removed for analysis and the remainder was given to the infant as required. Colostrum was obtained by the expression of a few drops. Samples were stored at -20°C, and all assays were performed on skimmed milk. Preterm and term samples were paired for days after delivery. All protein concentrations were estimated by radial-immunodiffusion assays (Mancini et al., 1964), except lysozyme, for which a lysoplate assay was used (Osserman & Lawlor, 1966).

Table 1 shows the concentrations of the nine proteins measured in milk, including the standard deviation and the results of a t-test comparing the two groups of mothers. The mean concentrations of the three milk-specific proteins, lactoferrin, lysozyme and α-lactalbumin, showed no significant difference between preterm and term mothers. For four of the serum proteins there was similarly no significant difference between the two groups of mothers, although in all cases the preterm mothers had higher mean concentrations. The concentration of immunoglobulin A, however, was significantly increased in preterm mothers, and plasminogen was only observed in the two preterm mothers who consistently showed high concentrations of the other serum proteins.

It was also noted that for all the serum proteins examined, as well as for lactoferrin and α-lactalbumin, the standard deviation of the results was greater among the preterm mothers.

A series of colostrum samples was obtained antenatally from five pregnant women, who subsequently delivered at term. The samples were assayed for vitamin-D-binding globulin, transferrin and albumin. Four of the women showed decreasing concentrations of these proteins in successive samples. Each protein, however, did not decrease in concentration at the same rate, which suggests that the results were not related to a general decrease in concentration caused by the expression of the colostrum.

The histology of the mammary gland in animals has revealed very tight cell contacts in the mature lactating gland (Pitelka, 1978). However, Linzell & Peaker (1974) have demonstrated in the goat that during pregnancy there is much greater permeability in the mammary epithelia, believed to be a result of leakage at the cell junctions. Thus before parturition there may be intercellular leakage of the plasma, which surrounds the epithelial cells, into the lumina of the mammary alveoli.

This mechanism has not been demonstrated in the human mammary gland, but we propose that the decreasing concentrations of vitamin-D-binding globulin, transferrin and albumin in antenatal colostrum illustrate the gradual exclusion of these and other serum proteins from entry into milk via the junctions between cells. In the mature gland it is believed that serum proteins may only enter the milk by means of a regulated intracellular transport system. However, in mothers delivering preterm the mammary gland may not be fully developed for mature lactation at the time of parturition. Therefore, although the mechanisms for synthesis, transport and secretion of proteins by the epithelial cells function normally, under the influence of prolactin and oxytocin, there may also be leakage of proteins and other nitrogen containing compounds between the cells.

### Table 1. Concentrations of proteins measured in 40 specimens of milk from mothers of preterms and term infants

<table>
<thead>
<tr>
<th>Protein</th>
<th>Preterm Mean (μg/ml)</th>
<th>Preterm SD (μg/ml)</th>
<th>Term Mean (μg/ml)</th>
<th>Term SD (μg/ml)</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactoferrin (%) of standard</td>
<td>16.6 ± 7.5</td>
<td>14.8 ± 4.3</td>
<td></td>
<td></td>
<td>P &gt; 0.1</td>
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<tr>
<td>α-Lactalbumin (mg/100ml)</td>
<td>803 ± 330</td>
<td>857 ± 219</td>
<td></td>
<td></td>
<td>P &gt; 0.5</td>
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<tr>
<td>Lysozyme (mg/100ml)</td>
<td>6.0 ± 2.0</td>
<td>5.0 ± 2.0</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
</tr>
<tr>
<td>Albumin (mg/100ml)</td>
<td>66.5 ± 33.2</td>
<td>59.5 ± 13.7</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
</tr>
<tr>
<td>Transferrin (mg/100ml)</td>
<td>2.7 ± 2.4</td>
<td>2.0 ± 1.4</td>
<td></td>
<td></td>
<td>P &gt; 0.2</td>
</tr>
<tr>
<td>Vitamin-D-binding globulin (mg/100ml)</td>
<td>2.8 ± 1.2</td>
<td>2.2 ± 0.9</td>
<td>P &gt; 0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α1-Antitrypsin (mg/100ml)</td>
<td>9.4 ± 7.3</td>
<td>5.4 ± 3.0</td>
<td></td>
<td></td>
<td>P &gt; 0.1</td>
</tr>
<tr>
<td>Immunoglobulin A (mg/100ml)</td>
<td>86.2 ± 22.9</td>
<td>62.2 ± 19.1</td>
<td></td>
<td></td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Plasminogen (mg/100ml)</td>
<td>1.2</td>
<td></td>
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</table>

* Measured as percentage of a colostrum standard.

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This hypothesis would account for the higher nitrogen concentration in preterm milk, and also for the greater variability in concentrations, since leakage, unlike synthesis and transport, would not be regulated.

We thank Sister S. O'Connor for help with the collection of the milk samples.


Comparative studies on thromboplastin in various tissues and of factors that modify its procoagulant activity

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The clot-promoting activity of various tissue extracts is attributed to the presence of thromboplastin. This factor is a lipid–glycoprotein complex, and, on interaction with factor VII in the presence of Ca++, it activates the extrinsic blood-coagulation pathway. Wijngaard et al. (1977), Nemerson et al. (1970) and Pitlick et al. (1970) reported that the isolated protein component of lung and brain thromboplastin (apoprotein III) regained its full activity in recombimants with binary phospholipid mixtures consisting of phosphatidylethanolamine/phosphatidylserine or phosphatidylcholine/phosphatidylserine. Hence the anionic properties of these lipid mixtures is a requirement for optimum clotting activity as well as the presence of unsaturated fatty-acyl chains, since complete saturation resulted in inactive preparations.

In the present study, the clotting activity of thromboplastin in various rat tissues and subcellular particles therefrom were surveyed by a similar approach to that of Astrup (1965) for brain and liver tissues. Rat brain and liver homogenates together with their microsomal fractions were obtained by the method described by Guarnieri et al. (1976) and Kamath et al. (1972) respectively. Adipose-tissue homogenates were also prepared from mesenteric, subcutaneous and epididymal fat-pads as described by Giercksky (1977). It was found that the brain microsomal fraction possessed a greater amount of coagulative activity than the equivalent fraction obtained from liver, whereas the activities of the microsomal fraction and homogenates prepared from adipose tissue surpassed those for both of the other tissues.

The higher coagulative activity of thromboplastin of some microsomal fractions may be accounted for by the variable phospholipid content of different tissues (Tamai et al., 1974) and would be additional to the control exerted by the normal asymmetric distribution of negatively charged phospholipids across the lipid bilayer profile of cell membranes (Zwaal et al., 1977). However, in order to account more completely for differences in the clotting activity of the various tissues of the same species such as the rat, we also examined their susceptibility to lipid peroxidation under optimum conditions. Brain and liver microsomal preparations of the rat were obtained and ferrous sulphate (0.02 mM), ascorbic acid (0.07 mM), NADH (0.6 mM), unsaturated fatty acids (Placer et al., 1966) as well as their extracted water-soluble autoxidized products were used to initiate and enhance the lipid-peroxidation process (Barrowcliffe et al., 1975).

The results obtained (Fig. 1) indicated that brain microsomal preparations were less susceptible to peroxidation than was a similar fraction from liver. Furthermore, when ferrous sulphate and ascorbic acid were used at higher concentrations (0.4–0.8 mM) and (0.3–10 mM) respectively there was a further loss of 60% of the thromboplastin activity. Finally the inclusion of 10 mM-ascorbic acid resulted in its complete inactivation. When the effects of various concentrations of malonaldehyde, the main product derived from lipid peroxidation, were tested for any inhibitory effect towards thromboplastin of a rat brain microsomal preparation, it had no effect on the coagulative activity of thromboplastin in the microsomal fraction at 0.2–1.0 μM, whereas at higher concentration (20 μM) it inhibited 40% of the clotting activity.

In order to minimize the destructive effects caused by lipid peroxidation, it may be necessary to include antioxidants during the isolation of functional proteins such as apoprotein III, since this process may lead eventually to the destruction of membrane.


Fig. 1. Peroxidized lipid in rat brain and liver microsomal fractions

Microsomal fractions (0.1–0.4 mg of protein/ml) were incubated aerobically for 30 min at 37°C. The concentration of malonaldehyde formed was measured as described by Placer et al. (1966). (a) Microsomal fraction; (b) microsomal fraction + 0.02 mM-ferrous sulphate; (c) microsomal fraction + 0.07 mM-ascorbic acid; (d) microsomal fraction + 0.6 mM-NADH; (e) microsomal fraction + extract from oxidized linolenic acid; (f) microsomal fraction + unoxidized linolenic acid; (g) microsomal fraction + extract from oxidized linoleic acid; (h) microsomal fraction + unoxidized linoleic acid.

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