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 Ca²⁺, it activates the extrinsic blood-coagulation pathway. Wijngaards 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 recombinants with binary phospholipid mixtures consisting of phosphatidylethanolamine/phosphatidylserine or phosphatidylcholine/phosphatidylserine. Hence the anionic properties of these lipid mixtures are 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 0.2–10 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 linolenic acid; (h) microsomal fraction + unoxidized linoleic acid. cerebral cortex; liver; brain.

structure and even to the inactivation of membrane-bound enzymes (Shimada et al., 1977).

This investigation was supported in part by the British Heart Foundation.


**Eosinophil membrane changes during interaction with antibody-coated non-phagocytosable surfaces**

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Eosinophils play a major role in resistance to helminth infections, even though they constitute only a minor proportion of peripheral blood leucocytes. Although they are phagocytic cells, they have a particular ability to interact with and damage large antibody-coated helminths, which are too big to be phagocytosed. In this reaction, the eosinophils attach themselves closely to the helminth surface in a process termed "frustrated phagocytosis" and release the contents of their lysosomal granules onto the parasite. Lysosomal enzymes, and notably the toxic eosinophil major basic protein, are then present in a sufficiently high local concentration to damage the underlying helminth (Butterworth et al., 1980).

Membrane changes which occur when eosinophils interact with large non-phagocytosable, antibody-coated targets have been investigated in a model system. Layers of agar in which tetanus toxoid antigen, diluted 10-fold (The Lister Institute, Worts Causeway, Cambridge CB2 1IP, U.K.) and human anti-tetanus immunoglobulin, diluted 10-fold (The Wellcome Foundation, London) were pretreated with lactoperoxidase (20 pg/ml), glucose oxidase and glucose, and [125]iodide (100 μCi/ml). The radioactive proteins were solubilized in 4% (w/v) SDS and 20% (v/v) mercaptoethanol and separated by SDS-polyacrylamide-gel electrophoresis.

Four major newly accessible proteins were detected on the eosinophil membrane by this method. Proteins 1 (mol.wt. 80000) and 4 (46000) were found to be radioactive even when eosinophils were incubated in suspension without antigen-antibody complexes, and are believed to be a consequence of membrane turnover or rearrangement in the unstimulated cell. Proteins 2 (mol.wt. 58000) and 3 (55000) were only found to be radioactive after the eosinophils had interacted with the antibody-coated agar layers. They were not found in the absence of antibody. Protein 2 was not labelled when ECF was present and it may therefore be blocked by, and perhaps be a receptor for, ECF.

The appearance of protein 3 at the cell surface precedes, and is not a consequence of, degranulation. It is seen within 15 min of interaction, whereas degranulation takes several hours. It is seen when degranulation is inhibited either with dibutyryl cyclic AMP and theophylline, or by incubation at 4°C instead of at 37°C.

Conversely, the labelling of protein 3 was enhanced by cytochalasin D (10 μg/ml) and this was associated with a threefold increase in degranulation. Attachment of eosinophils to helminths or to the agar layer can be achieved with concanavalin A instead of immunoglobulin, but this attachment does not lead to degranulation. Protein 3 does not appear under these conditions. The appearance of protein 3 seems to be necessary to lead to subsequent degranulation.

We conclude that the interaction of eosinophils with large, antibody-coated surfaces induces the appearance of a protein of mol.wt. 55 000 at the cell surface and that the rearrangement of this protein leads to degranulation.

This work was supported by The Wellcome Trust and by The Nuffield Foundation.