

hospital routine. This is the same conclusion as was reached by Illingworth *et al.* (1952) with one difference: demand feeding is inappropriate for low birthweight and small-for-dates infants who are at risk of hypoglycaemia.

Summary

In the summer of 1975 demand feeding was introduced for all babies born at the John Radcliffe Hospital, where previously babies were fed according to a rigid schedule to fit in with ward routine—clock feeding. Over a 10-day period before demand feeding was introduced details were collected about infant feeds concerning 42 normal babies whose mothers had decided to breast feed. 2 weeks after the introduction of demand feeding similar details were collected about 43 normal breast-fed babies. At the time of the observations 65% of all babies born in this hospital were being breast fed.

Comparing breast feeding patterns, there was a wider scatter of interfeed time intervals in the demand-fed group than in the clock-fed group, over the first 2 days after birth. By the end of the first week these differences were no longer present. The introduction of demand feeding presented no problems in ward management and is now the established routine in this maternity hospital.

We thank all the midwives and the mothers concerned with this study.

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Oxidative metabolism in cord-blood polymorphonuclear leucocytes

Serious bacterial infections occur more frequently in newborn infants than at older ages during childhood. The body defences of infants have been studied extensively to try to explain this increased

susceptibility to infection. Polymorphonuclear leucocytes (PMNs) provide the major defence against bacterial infection. The increase in oxidative metabolism (OxM) that accompanies phagocytosis by these cells is unquestionably required for efficient antibacterial activity (DeChatelet, 1975). Accordingly, many investigators have assessed postphagocytic OxM of PMN from newborns. Results have been contradictory (Strauss and Mauer, 1976). Most workers have failed to detect metabolic abnormalities, but decreased activity of OxM was reported in a few studies of both resting (Jemelin *et al.*, 1971) and phagocytic (Coen *et al.*, 1969; Anderson *et al.*, 1974) leucocytes from infants when compared to cells from older individuals.

Cord and venous blood from infants in the immediate newborn period contain a mixture of cells including transformed lymphocytes, young erythrocytes, and monocytes (Prindull *et al.*, 1975). These cells are metabolically active and some are phagocytic. They are present in varying numbers from sample to sample and could theoretically influence experiments intended to selectively evaluate PMN metabolism. Leucocyte suspensions rather than isolated PMNs were studied in previous reports. In our study, OxM of isolated cord-blood PMNs was assessed by two techniques. Hexose monophosphate shunt (HMS) activity was selected because an increase in the activity of this pathway characteristically accompanies phagocytosis by normal PMNs, and failure to do so is invariably associated with PMN dysfunction (DeChatelet, 1975). The kinetics of OxM were measured as the rate of light emission by chemiluminescence, an assay related to superoxide and singlet oxygen formation (Johnston *et al.*, 1975) that has not been used previously to evaluate PMNs from infants.

Materials and methods

Cord blood was collected into sodium citrate (0.38% final concentration) from the placental side of the divided umbilical cord of healthy, term infants. Contamination with maternal blood was estimated by the acid elution technique (Dacie and Lewis, 1968), and samples with >10% maternal erythrocytes were excluded. Although rarely mentioned in previous reports, this precaution should be a routine procedure when studying cord blood. Several samples consisted almost entirely of maternal erythrocytes (and presumably leucocytes) and would have been assumed, incorrectly, to represent infant blood. PMNs were isolated by standard techniques of Dextran sedimentation, Ficoll-Hypaque centrifugation, and hypotonic lysis. Final suspensions were

completely viable by dye exclusion and contained >90% PMNs. Preliminary studies of OxM and staphylococcal killing established that cells collected in this manner were functionally comparable to those collected simply by Dextran sedimentation and hypotonic lysis. Venous blood samples from healthy adults were studied simultaneously as controls. The study was approved by the Committee for Human Investigation.

Activity of the hexose monophosphate shunt (HMS) was determined by glucose-1- ^{14}C oxidation in 17 cord-blood samples and 9 simultaneously studied adult controls (Baehner *et al.*, 1970). Simultaneous glucose-1 and glucose-6- ^{14}C was not used since the increase in glucose metabolism after phagocytosis is almost entirely of the glucose-1 fraction (Iyer *et al.*, 1961). Chemiluminescence was measured in 14 cord-blood samples and 9 controls with a liquid scintillation spectrometer set in the out-of coincidence mode during the phagocytosis of opsonised zymosan at a particle-to-cell ratio of 20:1 (Johnston *et al.*, 1975). In agreement with other studies and as an important control, it was established that the luminescence detected was dependent in part on the generation of superoxide anion, because superoxide dismutase (Miles Laboratories, Elkhart, Indiana) decreased light emission by 70%, whereas heated enzyme or albumin did not.

Differences between mean values were tested for significance by Student's *t* test using logarithmic means. Values for means and standard deviations, however, are expressed as geometric numbers on the figures and in the text for purposes of clarity.

Results

HMS activity in both resting and phagocytic cells was significantly greater ($0.05 > P > 0.01$) in cord-blood PMNs as compared to controls, and the increase in HMS activity accompanying phagocytosis was nearly identical when expressed as the phagocytic mean value minus the resting mean value (1.7 vs 1.4 nmol/min per 5×10^6 PMNs in cord and adult PMNs, respectively). However, this increase was less in cord-blood PMNs compared to controls ($P < 0.05$) when expressed as the ratio of phagocytic to resting values, a method by which data are frequently reported. This ratio is designated 'phagocytic index' on Fig. 1, although we recognise that this index is influenced by factors other than phagocytosis (particle ingestion) such as the concentration of a variety of intracellular enzymes, substrates, co-factors etc., that contribute to the metabolic response.

The kinetics of chemiluminescence (light emission) are presented as curves in Fig. 2. The luminescence of resting cells could not be determined because

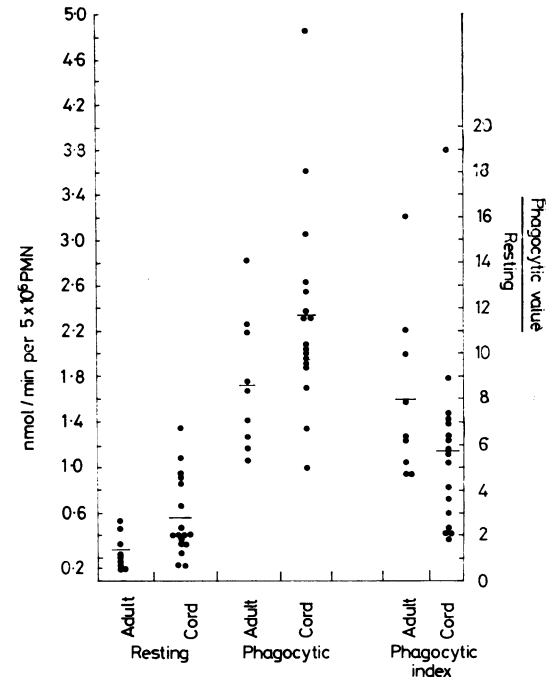


Fig. 1 HMS activity of polymorphonuclear leucocytes from 17 cord-blood samples and 9 adult controls studied simultaneously is plotted. All reaction mixtures were prepared in duplicate and each point represents the average. Means are indicated by bars. Glucose-1- ^{14}C oxidation is expressed as nmol/min per 5×10^6 PMN. The phagocytic index is the quotient of the value from phagocytic cells divided by the value from resting cells.

light emission did not exceed background. The mean values for peak luminescence (highest value at any point on the curve) from cord-blood PMNs and controls (18.0 and 20.7 cmp $\times 10^4$, respectively) were comparable ($P > 0.05$). The kinetics, however, were quite different. The chemiluminescence curves of several cord-blood PMNs fell below the normal range during the later phases of the response. Accordingly, light emission from cord-blood PMNs and controls was compared at several time points after the addition of zymosan. No difference was found after 2, 5, and 8 minutes of phagocytosis ($P > 0.05$). However, after 15 and 25 minutes chemiluminescence was significantly less from cord-blood PMNs than from controls ($P < 0.05$, $P < 0.025$, respectively).

Conclusions

The basal activity of OxM in resting cord-blood PMNs was at a higher level than that of adult con-

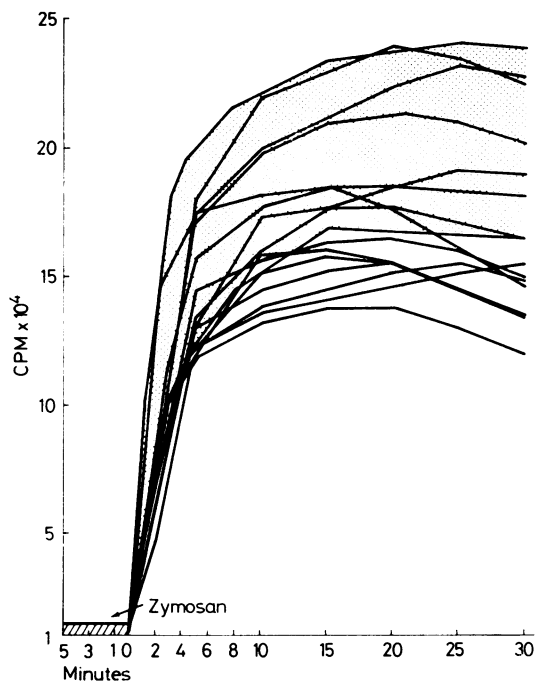


Fig. 2 Chemiluminescence of 14 cord-blood samples are plotted as individual curves. The mean and 2 SDs of 9 controls are indicated by the shaded area. Background luminescence was measured for a minimum of 5 minutes. Oposonised zymosan was added at time zero. Luminescence was quantitated at 1- to 3-minute intervals as counts per minute (cpm) $\times 10^4 / 1 \times 10^7$ PMN.

trols when determined by HMS activity. In addition, cord-blood PMNs increased OxM in response to phagocytosis in a similar manner to controls when assessed by peak chemiluminescence and HMS activity during zymosan ingestion. These findings are similar to those of prior reports using mixed leucocytes or unfractionated blood (Strauss and Mauer, 1976). Cord-blood PMNs, however, were unable to sustain the increased rate of postphagocytic OxM for as long a period of time as adult controls. This finding may indicate a deficiency of metabolic reserve in cord-blood PMNs and requires further study as a possible mechanism for the increased susceptibility to infections in newborns.

Summary

Polymorphonuclear leucocytes were isolated from cord-blood samples collected from healthy, term infants. Oxidative metabolism was assessed in these

cells by hexose monophosphate shunt activity and chemiluminescence. Basal levels of oxidative metabolism were spontaneously increased in resting (non-phagocytic) cord-blood cells as compared to adult controls. As a response to phagocytosis, cord-blood cells initiated the expected increase in oxidative metabolism and reached normal peak values of activity. However, these cells were unable to maintain the high metabolic rate for as long a time as adult controls. This aberration of leucocyte function may indicate a deficiency of metabolic reserve and could be related to the increased susceptibility of newborns to bacterial infections.

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