Vitamin E in neonatal hyperbilirubinaemia

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Abrams, B. A., Gutteridge, J. M. C., Stocks, J., Friedman, M., and Dormandy, T. L. (1973). *Archives of Disease in Childhood*, **48**, 721. **Vitamin E in neonatal hyperbilirubinaemia.** The polyunsaturated red cell lipids of newborn infants show a greatly increased susceptibility to autoxidation; and this may be a contributory factor to 'physiological' haemolysis. Vitamin E administered by mouth during the first week of life in relatively large doses reduced the susceptibility of red cells to autoxidation, but had no significant effect on the pattern of plasma bilirubin or haemoglobin changes.

Considerable evidence now suggests that the autoxidation of red cell lipids may be an important factor in haemolysis; and that conversely, antioxidant protection is essential for red cell survival (Mengel, 1968; Dormandy, 1971). The antioxidant protective mechanism of cells is complex and imperfectly understood, but vitamin E, a powerful antioxidant *in vitro*, undoubtedly contributes to it (Gordon and de Metry, 1952; Mackenzie, 1954). By adult standards, newborn infants are grossly deficient in this vitamin (Gordon, Nitowsky, and Cornblath, 1955; György, Cogan, and Rose, 1952); and a haemolytic syndrome which occurs in prematurely born babies around the eighth week in life responds specifically to vitamin E therapy (Hassan *et al.*, 1966; Oski and Barness, 1967; Ritchie *et al.*, 1968; Lo, Frank, and Hitzig, 1973). Chadd and Fraser (1970a, b) have shown, moreover, that haemoglobin levels are significantly higher at 8 to 10 weeks after birth in babies given vitamin E supplements.

The present investigation was undertaken (1) to study lipid autoxidation as a possible contributory mechanism in 'physiological' haemolysis of the newborn, and (2) to see if the course of this haemolysis might be influenced by vitamin E supplements to the babies' diet.

Material and methods

The relation between the susceptibility of red cells to autoxidation and plasma tocopherol levels was examined in 50 umbilical cord bloods obtained from normal deliveries and in venous blood samples obtained from 50 healthy mothers. A possible correlation between bilirubin levels, plasma tocopherol levels, and the

susceptibility of red cells to autoxidation was sought in a series of 30 normal infants studied over a period of 6 weeks from birth, and in 60 infants with 'physiological jaundice' whose serum bilirubin rose to, or above, 10 mg/100 ml during the first 10 days of life.

40 normal infants were included in the main vitamin E trial. 100 mg/day DL- α -tocopherol acetate BPC (Merck) was given daily from birth. The vitamin was administered as an oil from dropping bottles calibrated to deliver 30 mg $\pm$ 5 mg/drop at room temperature. It was given orally before feeds. Vitamin E absorption under these conditions was studied in a preliminary series of 5 infants. All blood samples were taken by heel stab into lithium heparin tubes.

The susceptibility of red cells to autoxidation was measured by an assay procedure described in detail elsewhere (Stocks *et al.*, 1972). The measurement is based on the generation of a lipid autoxidation product, malonyldialdehyde (MDA) in a standard cell preparation exposed to hydrogen peroxide for 2 hours. The normal adult range is 80 to 250 nmol/g Hb. Serum vitamin E was measured by a modification of the method of Hashim and Schuttringer (1966). The normal adult range by this method is 0.8 to 1.5 mg/100 ml. The red cell fatty acid pattern was measured by gas chromatography, using techniques based on the methods of Dodge and Phillips (1967) and Phillips, Dodge, and Rockmore (1968).

Results

Fig. 1 and 2 show the serum vitamin E levels and the susceptibility of red cells to autoxidation in expectant mothers in the last trimester of pregnancy, in cord bloods, and in normal adults. These findings are in agreement with earlier reports (Gordon *et al.*, 1955; Stocks, Kemp, and Dormandy, 1971) and indicate a high placental barrier to the transfer of vitamin E from the mother to the fetus. The high 2-hour MDA in cord and neonatal blood

FIG. 1.—Susceptibility of red cells to autoxidation (2-hour MDA) in normal adults, in pregnant women, and in normal cord bloods.

FIG. 2.—Serum vitamin E levels in normal adults, in pregnant women, and in normal cord bloods.

falls into the range found in adults suffering from severe haemolytic diseases (Stocks *et al.*, 1972).

A preliminary trial showed that 30 mg tocopherol by mouth daily had no effect either on the serum vitamin E level or on the susceptibility of red cells to autoxidation (2-hour MDA). On an increased dosage of 100 mg daily, the serum vitamin E level rose significantly to just within the normal adult range, and the 2-hour MDA was significantly reduced (though still raised by adult standard) (Table I). However, there was no effect on either

of infants with a plasma bilirubin level of 10 mg/100 ml or over was there any correlation between the bilirubin level and the 2-hour MDA.

A follow-up of normal term infants showed a slight decrease in the susceptibility of red cells to autoxidation by the 6th week of life (Table III). At 12 months the mean 2-hour MDA was 394 nmol/g Hb, i.e. still slightly above the normal adult mean.

Our results of fatty acid analyses in normal cord and normal adult red cells were in substantial agreement with those reported in earlier studies (Crowley, Ways, and Jones, 1965; Neerhout, 1968). The extremely low percentage concentration of linoleic acid in both cord blood cells and cord plasma rises sharply during the first week of life, reaching a two-fold increase by the 4th to 6th day. The overall pattern suggests the possibility of a placental barrier to the transfer of linoleic and perhaps of other polyunsaturated fatty acids similar to the barrier to tocopherol transfer. These findings will be reported in detail elsewhere.

TABLE I

Effect of tocopherol supplementation on plasma tocopherol levels and susceptibility to autoxidation of red cell lipids. Babies were given 100 mg α -tocopherol acetate daily from birth

Case no.	2-hour MDA (nmol/g Hb)		Plasma tocopherol (mg/100 ml)	
	At birth	At 1 week	At birth	At 1 week
1	885	685	0.15	0.55
2	960	560	0.20	0.90
3	830	395	0.15	0.70
4	900	830	0.30	0.70
5	850	740	0.25	0.75
Mean	885	642	0.21	0.72

the plasma bilirubin level or on Hb concentration in a controlled trial of 40 'paired' normal newborn infants, 20 of whom were given tocopherol supplement and 20 given none (Table II). Neither in our consecutive series of normal infants nor in the series

Discussion

By normal adult standards, the red blood cells of the newborn are abnormally susceptible to autoxidation and they undergo massive destruction during the first week in life. Moreover, neonatal plasma is grossly deficient in vitamin E. It might be reasonable to infer a causal relation between these facts. The main function of vitamin E is probably that of a biological antioxidant; and susceptibility to autoxidation and antioxidant activity are inversely

TABLE II

Hb and bilirubin levels in infants receiving supplements of tocopherol. The tocopherol-supplemented group received 100 mg α -tocopherol acetate daily from birth

Days from birth	Plasma bilirubin (mg/100 ml)		Hb (g/100 ml)	
	Controls	Tocopherol supplemented	Controls	Tocopherol supplemented
1	2.74 $\pm$ 0.31	2.20 $\pm$ 0.36	21.7 $\pm$ 0.45	21.7 $\pm$ 0.51
3	4.13 $\pm$ 0.16	4.35 $\pm$ 0.25	20.7 $\pm$ 0.41	20.6 $\pm$ 0.60
5	3.41 $\pm$ 0.77	2.74 $\pm$ 0.64	19.5 $\pm$ 0.50	19.6 $\pm$ 0.41

Note: Values shown are means $\pm$ SEM of 20 subjects in each group.

TABLE III

Plasma tocopherol levels and susceptibility to autoxidation of red cell lipids in 30 normal infants during the first 6 weeks of life (means $\pm$ SEM)

Days from birth	0 (cord)	3	7	42
2-hour MDA (nmol/g Hb)	749 $\pm$ 15	790 $\pm$ 17	790 $\pm$ 22	660 $\pm$ 28
Plasma tocopherol (mg/100 ml)	0.34 $\pm$ 0.31	0.29 $\pm$ 0.02	0.29 $\pm$ 0.03	0.32 $\pm$ 0.027

related. Haemolysis, moreover, is an invariable sequel of red cell autoxidation; and many haemolytic states—including the delayed haemolytic syndrome of prematurely born babies—are associated with an increased vulnerability of the red cells to autoxidative stress (Osiki and Barness, 1967; Ritchie *et al.*, 1968). Though the results of the present study do not exclude such a relation, they show that it is more complex and variable than might have been supposed. Two points in particular merit emphasis.

The increased susceptibility of neonatal red cells to autoxidation may or may not be a factor in the increased rate of cell destruction during the newborn period. A point against such a causal relation is the fact that susceptibility to autoxidation remains high for much longer than the phase of accelerated red cell destruction, diminishing gradually during the first 9 to 12 months of life. In our series, moreover, there was no correlation between susceptibility to autoxidation and either the serum bilirubin or Hb concentration during the first 2 weeks in life.

A low serum vitamin E is generally assumed to be the main cause for the increased peroxide-induced haemolysis of cord and neonatal blood. Peroxide-induced haemolysis has, in fact, been used as an indirect measure of vitamin E activity. Though the evidence for such a relation is strong, it cannot be taken for granted that vitamin E deficiency is the only or even the main cause of an increased

susceptibility to autoxidation. Both published results and our own findings show that the pattern of fatty acids in cord blood cells differs markedly from the pattern in normal adult blood; and susceptibility to autoxidation as measured by 2-hour MDA concentration could be a function of the relative degree of lipid unsaturation, i.e. of the effective substrate concentration. Haemoglobin iron, moreover, plays an essential catalytic role in autoxidation (Tappel *et al.*, 1961); and the possibility exists that the catalytic properties of fetal Hb differ from those of adult Hb. In this connexion it may be relevant that the 2-hour MDA is characteristically raised in thalassaemia major (Stocks *et al.*, 1972). Lastly, recent evidence suggests that vitamin E accounts for only a small fraction of plasma antioxidant activity (Vidláková *et al.*, 1972).

From the clinical point of view one may conclude that it is possible to raise the serum vitamin E levels and reduce the susceptibility of red cells to autoxidation by relatively large vitamin E supplements during the first week of life; but in our series this has had no significant effect on either the Hb or on the serum bilirubin levels. It may be noted, however, that even on vitamin E supplements susceptibility to autoxidation remained severely abnormal by adult standards, and that the serum vitamin E levels barely reached the lower limit of what is regarded as the normal adult range (Stocks *et al.*, 1971).

Two further questions must remain open. First, it is possible that vitamin E might influence the natural course of abnormal, as distinct from 'physiological', hyperbilirubinaemia. Second, the course of neonatal haemolysis and hyperbilirubinaemia might be influenced by large doses of vitamin E given to the mother during late pregnancy. There is some evidence that the effect of the vitamin on susceptibility to autoxidation is a delayed indirect one, e.g. it might affect the fatty acid composition of the red cells in the course of their development rather than after their release into the circulation. Clinically, such an effect could be achieved in the newborn only by overcoming the high placental barrier to the transfer of the vitamin by administering large doses to the mother. It may be recalled that, though phenobarbitone is now given to babies suffering from hyperbilirubinaemia, statistical justification for this practice rests mainly on studies in which the drug was administered to the mothers in the last trimester of pregnancy (Trolle, 1968a, b; Maurer *et al.*, 1968; Ramboer, Thompson, and Williams, 1969).

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