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Availability of Vitamin E in the Newborn Infant.* (19931)

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The erythrocytes of rats deficient in vit. E may be hemolyzed by dialuric acid(1,2) *in vivo* and *in vitro*. They are also susceptible to the hemolyzing effect of dilute solutions of hydrogen peroxide(3). Vit. E given *in vivo* or added to suspensions of red cells *in vitro* prevents the hemolysis by dialuric acid as well as by hydrogen peroxide.

The hemolytic action of hydrogen peroxide differs in several respects from that of dialuric acid. Hemolysis of cells deficient in vit. E is complete in 15 to 30 minutes with dialuric acid, while with hydrogen peroxide hemolysis progresses only slowly and even after prolonged incubation is usually less marked than with dialuric acid. Moreover, the red blood cells of newborn infants show increased susceptibility to hydrogen peroxide and at the same time complete resistance to dialuric acid. In adults hemolysis of red blood cells by hydrogen peroxide rarely exceeds 5%(3).

The question arose whether the hemolysis in the blood of newborn infants by hydrogen peroxide might be related to vitamin E deficiency and whether addition of vit. E either in the test tube to a suspension of red blood cells, or through ingestion by the newborn infant, or, prenatally, by the pregnant mother, would restore the normal resistance of the erythrocytes toward hydrogen peroxide.

Experimental. Observations were made on normal newborn infants in the Nursery of the Hospital of the University of Pennsylvania. Normal adults served as negative controls while vit. E deficient rats were used as positive controls. *Blood* was collected in 0.9% NaCl-1% sodium citrate solution (about 15 drops of blood in 2 ml) in a 15 ml centrifuge tube. The suspension was centrifuged, the supernatant fluid withdrawn, and the cells made up in a 2.5% suspension (estimating the volume of cells from the graduations on the tube) with a mixture of equal parts of normal saline and phosphate buffer, pH 7.4.† After incubation at 37°C for one hour the tubes were again centrifuged, the supernatant fluid withdrawn, and a 5% suspension of the cells in normal saline made.

* Preliminary report given at Meeting of the Am. Pediat. Soc., Atlantic City 1951, see *Am. J. Dis. Child.*, 1951, v82, 237.

† Phosphate buffer, pH 7.4:

	ml
Potassium monophosphate, 0.2 M	25.0
Sodium hydroxide, 0.2 M	19.7
Water to make	100.0

Into 3 centrifuge tubes were measured 0.25 ml cell suspension, 0.20 ml phosphate buffer and 0.05 ml 12% hydrogen peroxide (1 volume of 30% H_2O_2 + 1.5 volumes phosphate buffer). A fourth tube containing no peroxide (0.25 ml phosphate buffer) served as a negative control. All tubes were incubated at 37° for 15 minutes and allowed to stand at room temperature for 2¾ hours. To the negative control and to 2 of the tubes containing peroxide was added 4.5 ml saline-buffer solution. The tubes were mixed by inversion and centrifuged. The color of the supernatant liquid was measured in a Klett-Summerson photometer using the No. 54 filter. Four and one half ml of distilled water was added to the remaining tube to cause complete hemolysis of the cells and the color read in the photometer. Better checks, between duplicate determinations, may be obtained by using the peroxide in more dilute solution, 0.25 ml of 2.4% H_2O_2 instead of 0.05 ml of 12% H_2O_2, as suggested by Gordon and de Metry(4). The percent of hemolysis caused by hydrogen peroxide was calculated from the ratio of the average reading of the tubes with saline-buffer and that of the completely hemolyzed tube, both readings being corrected by subtracting the reading of the negative control. In studies in which the effect of tocopherol *in vitro* was tested the desired amount of tocopherol was added before the first incubation.

Results. The hemolysis test with hydrogen peroxide was carried out first on normal infants on the 1st, 2nd, and 5th days after birth. Neither the mothers nor the infants received supplements of vit. E. The results together with positive and negative controls are summarized in Table I.

In a further group of 17 newborn infants the hydrogen peroxide test was carried out before and after addition of tocopherol to a suspension of red cells. The degree of hemolysis before treatment was found to be 36 ± 4.5% which was reduced after addition of tocopherol to the red blood cell suspension(3) to 0.6 ± 0.4%, indicating complete and regular response.

The effect of tocopherol on hemolysis by hydrogen peroxide was demonstrable also when the tocopherol was given by mouth to the newborn infants. In 11 normal infants hemolysis on the first day was 42 ± 6.1%. After feeding of 50 mg of water-miscible mixed tocopherols (Esorb, Wyeth) the hemolysis fell next day to 6 ± 2% and on the 5th day to 3 ± 1%.

TABLE I. Hemolysis of Red Cells by Hydrogen Peroxide in Normal Untreated Newborn Infants.

Type of cells	No. of determinations	Hemolysis (%)
Newborn infant, 1st day	30	27 ± 3.9
2nd	30	31 ± 4.3
5th	28	16 ± 4.4
Human adult	75	3 ± .6
Rat (vit. E deficient)	77	79 ± 1.6
Rat (treated with vit. E)	33	9 ± 2.0

TABLE II. Hemolysis of Red Blood Cells by Hydrogen Peroxide in Normal Infants Born of Mothers Receiving Prenatal Vitamin E Medication.

Type of cells	No. of determinations	Hemolysis (%)
Newborn infants, mothers receiving Vit. E (150 mg daily)		
1st day	16	22 ± 4.3
2nd	16	25 ± 3.3
5th	12	6 ± 2.5
Newborn infants, mothers receiving No vit. E		
1st day	15	25 ± 5.5
2nd	15	36 ± 5.9
5th	12	14 ± 6.9
Normal adults	41	2 ± .2
Rats (E-deficient)	39	82 ± 2.0

Similar results were previously recorded in rats(5,6). The blood of newborn rats is, as a rule, largely hemolyzed by dialuric acid as well as by hydrogen peroxide. Tocopherol not only in the test tube but also *in vivo* through diaplacental transfer of tocopherol given to the pregnant animal (5 mg daily) prevents hemolysis. We have studied the response to tocopherol through attempted diaplacental transfer in two series of observations on newborn infants. In the first group we gave the mother by mouth during the last 2 to 4 weeks of pregnancy 150 mg of water miscible mixed tocopherols (Esorb, Wyeth) daily. The results are summarized in Table II. There was no difference in the degree of hemolysis of the two groups on the first day, and the reduction of hemolysis on the 5th day in the group of newborn infants born of mothers receiving supplements of vit. E was

not statistically different from that of the control group.

In a second smaller series of observations the mothers were given 500 mg of mixed tocopherols daily during the last 2 to 4 weeks of pregnancy. Even with this larger dose of tocopherol the "prophylactic" approach proved to be ineffective. In the treated group, comprising 10 mothers, the rates of hemolysis in the newborn infants showed the following average figures: 1st day, 47 ± 11; 2nd day, 24 ± 5; 5th day, 29 ± 9.

In the control group (8 mothers without treatment) the corresponding figures were: 1st day, 70 ± 6; 2nd day, 47 ± 9; 5th day, 27 ± 7.

Thus, in newborn infants, the diaplacental protection observed in rats was not achieved even when the mother was given very high doses of vitamin E. This result is in contrast to the prompt effect of tocopherol on hemolysis of erythrocytes by hydrogen peroxide when given directly to the newborn human infant by mouth.

Discussion. The susceptibility of the red blood cells of newborn infants to dilute solutions of hydrogen peroxide is analogous to the susceptibility of erythrocytes of newborn rats to hydrogen peroxide as well as to dialuric acid. Since vitamin E *in vitro* and *in vivo* restores normal resistance of the red blood cells, the conclusion appears to be warranted that newborn rats and newborn infants suffer from "physiological" vit. E deficiency. Gordon and de Metry confirmed these observations on premature infants(4). Furthermore, the analytical data of Moyer(7) and of Wright, Filer and Mason(8) are in good accord with the results of the hemolysis tests.

The hemolysis test has the advantage of measuring the biologically active portion of total tocopherol(9). The protein bound tocopherol is unavailable for the hemolysis test (2).

The positive hemolysis test in newborn rats may be reversed not only through direct contact of vit. E with the erythrocytes but also by giving vit. E to the pregnant mother. The placental barrier is apparently less easily traversed in case of the human fetus. In the statistical average no definitely positive results were obtained even after daily doses of 500 mg of mixed tocopherols given the mother during the last few weeks of pregnancy. In contrast direct medication was promptly effective in the newborn infant. On the basis of observations made in rats, prophylactic medication of vit. E was proposed(5) via the pregnant mother in conditions such as Rh incompatibility or in diabetes. In the light of the present findings it appears that such approach might not be successful unless the amount of vit. E required to prevent hemolysis in diabetic pregnancy(5,10), or hepatic injury(5) and edema of the placenta(11,12) in Rh incompatibility is less than the level necessary for reversal of the positive hemolysis test with hydrogen peroxide. No explanation can be given for the very high threshold of human placenta for vit. E.

Summary. Red blood cells of newborn infants are hemolyzed by dilute solutions of hydrogen peroxide. This hemolysis may be prevented by vit. E *in vitro* or *in vivo*. However, even with high daily doses of vit. E (500 mg of mixed tocopherols) given the mother during the last weeks of pregnancy the hemolysis test in the newborn remained unaltered.

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