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SERUM ANTIOXIDANT ACTIVITY IN PREMATURE BABIES

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Autooxidation of bovine-brain homogenate proved to be a good model to determine the antioxidant capacity of sera. It was measured in parallel with the level of ceruloplasmin and apotransferrin in sera of 35 healthy children and 20 premature babies (gestational age 28-33 weeks). Antioxidant capacity was very low in premature babies with a nadir on the 4th postnatal day. Lipid-peroxidation of bovine-brain homogenate could be inhibited in vitro by the addition of exogen antioxidant. In this assay MTDQ-DS, Cavinton®, DPA-HCl, vitamin E, A, C showed different antioxidant activity.

INTRODUCTION

During the study of lipid peroxidation in tissue homogenates it was observed that serum could inhibit this process /1,6/. Serum proteins, particularly ceruloplasmin and apotransferrin (with ferroxidase activity and ability to bind ferric ion) have high antioxidant activity similarly to other biological fluids (cerebrospinal and synovial fluid, colostrum). It is well known that variations in serum antioxidant activity have considerable clinical relevance /3,5/. That was the main reason for a standard biological antioxidant assay developed by Stocks et al /3/.

Spontaneous autooxidation of bovine-brain homogenate proved to be a good model to study lipid peroxidation in tissues and to investigate the inhibition effect of several biological fluids and different drugs. Using this method Sullivan and Newton demonstrated that the antioxidant capacity of new-born infants' cord sera increased with birth weight, but was much

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lower than in the adult /4/.

The purpose of our study was to evaluate the antioxidant capacity in premature babies. First we measured the antioxidant capacity of sera in parallel with the level of ceruloplasmin and apotransferrin in 35 healthy infants and children from the age 1 day to 13 years (normal controls). Then we determined these parameters in 20 premature babies (gestational age 28-33 weeks) on the 1st, 4th, 7th and 14th day of their postnatal life together with blood-gase parameters (pO_2, pCO_2, pH). The premature babies were patients in our perinatal intensive centre. Although they have received similar medical treatment they constituted a rather inhomogenous and non-healthy population. In addition we investigated in vitro the inhibition effect of different drugs (D-penicillamine.HCl, Cavinton, vitamin E, C, A, MTDQ-DS, salicylic acid and D-mannit) on the lipid peroxidation of bovine-brain homogenate.

METHODS

The antioxidant activity was determined by the modified method of Stocks et al /3/.

The compounds tested were as follows: D-penicillamine·HCl (Knoll AG), Cavinton (vinpocetine, Richter), D-mannit (Merck), salicylic acid, ascorbic acid (Reanal), MTDQ-DS were solved in 1/15 mol phosphate buffered saline (pH 7.4), vitamin A (Egis) in oleum helianthi and vitamin E (Fluka) in water-ethanol (1:1) containing 10 % Tween-20.

The concentration of ceruloplasmin and apotransferrin was determined by using a Beckman Immunochemistry Analyzer type II. nephelometer.

RESULTS

In accordance with the results of Sullivan and Newton /4/ we have found that antioxidant capacity in 35 healthy children

(ages from 1 day to 13 years) increased with age. In case of normal control children (ages from 1 day to 4 weeks) the volume of serum needed for maximal inhibition of bovine-brain autooxidation (v_0) is high (62 $\pm$ 6 μl), i.e. antioxidant capacity is low. The antioxidant capacity increases during the first 4 months of age, then remains unchanged until 13 years of age (average value of v_0: 15 $\pm$ 2 μl) (Fig. 1).

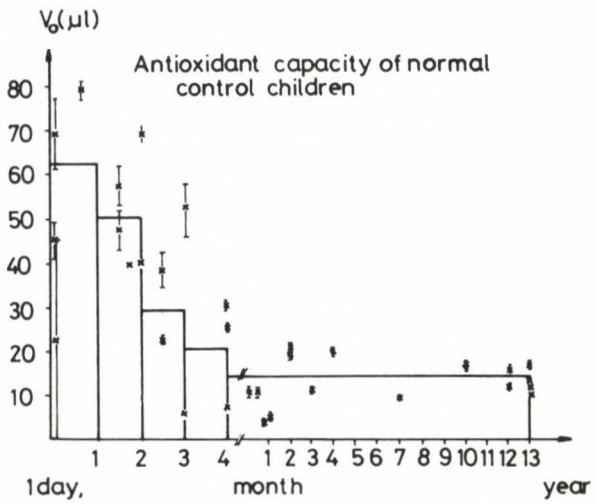

Fig. 1. Antioxidant capacity of normal control children

Premature babies with a gestational age of 28-33 weeks, and a birth weight of 720-2700 g had very low antioxidant activity. Antioxidant activity increased with gestational age and birth weight (Figs. 2,3). We have found a characteristic postnatal change of antioxidant capacity in a group of 9 premature babies with gestational age of 28 to 33 weeks and birth weight of 1200-2000 g. The nadir of antioxidant capacity was on the 4th postnatal day (Fig. 4). As during the first 4 days they often suffered from hypoxia, acidosis and asphyxia, this low antioxidant capacity was probably due to several factors. Together with the other parameters, antioxidant capacity shows that the 4th day is a critical period for premature babies.

On the basis of our results, antioxidant capacity of serum

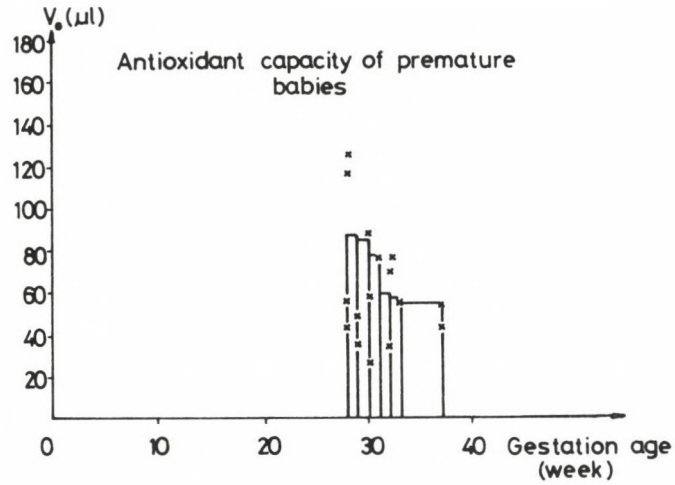

Fig. 2 Antioxidant capacity of premature babies

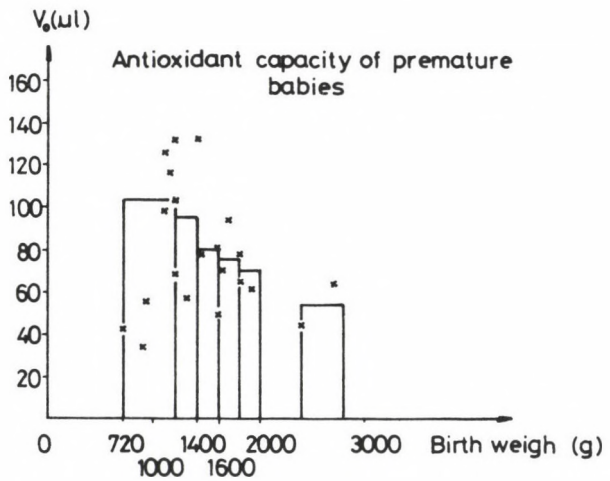

Fig. 3 Antioxidant capacity of premature babies with different birth weight

seems to have considerable clinical relevance in premature infants. Serum apotransferrin concentration in premature babies has a maximum value on the 4th postnatal day. Ceruloplasmin level is increasing during the first 14 days of life but declines on the 4th day (Fig. 5).

Stocks et al /3/ and Sullivan /4/ described that lipid

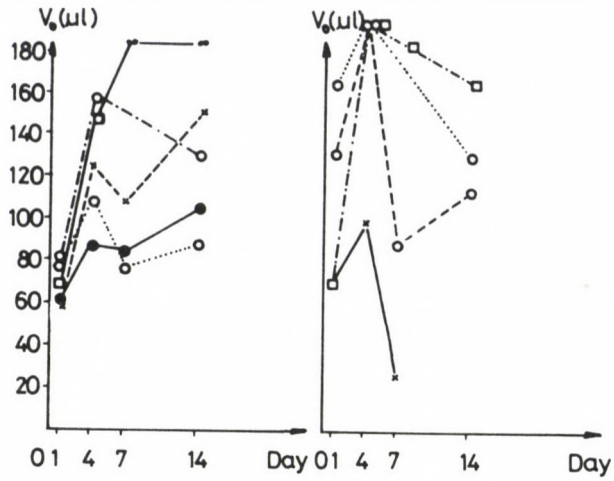

Fig. 4 Antioxidant capacity of 9 premature babies during the first 14 days of life

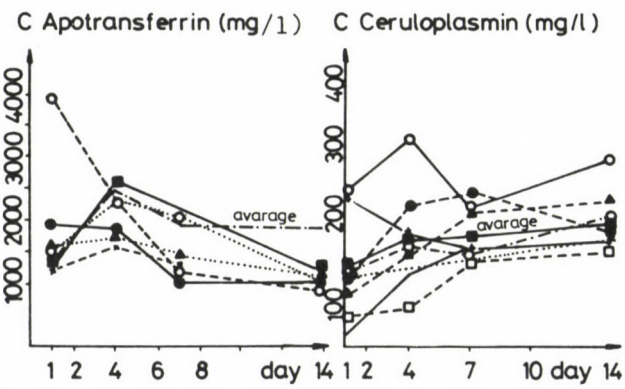

Fig. 5 Serum apotransferrin and ceruloplasmin level in premature babies during the first 14 days of life

peroxidation of bovine-brain homogenate in vitro can be inhibited by the addition of exogen antioxidant (vitamin E, DPA, desferoxamine mesylate and iron chelating reagents).

We measured residual autooxidation in the presence of vitamin E, A, C, Cavinton, D-penicillamin·HCl, MTDQ-DS, salicylic acid and D-mannit. Inhibitor concentration at 50 % inhibition ($C_{1/2}$) is a characteristic of drugs, with good antioxidant effect (Figs. 6,7,8).

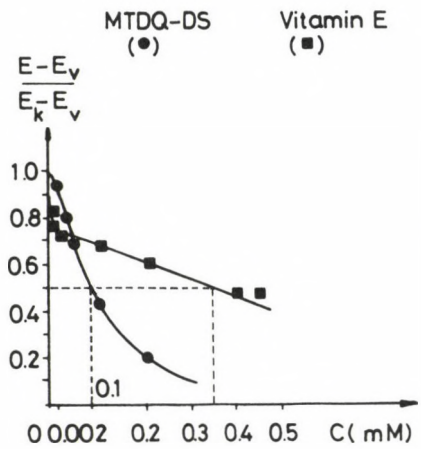

Fig. 6 Inhibition effect of MTDQ-DS and vitamin E on the autooxidation of bovine-brain homogenate

	$C_{1/2}$ (mM)
MTDQ-DS	0.08
vitamin E	0.35
Cavinton	0.50
DPA·HCl	0.56
vitamin A	2.55
vitamin C	2.60

As the vitamin E could be solved only in water-ethanol Tween-20 solution, and vitamin A in oleum helianthi, we have measured the inhibition effect of the solvents too, but we have found no substantial inhibition at this low concentration range.

Using our in vitro system salicylic acid (0.1-4.4 mM) and D-mannit (1-50 mM) did not show any antioxidant effect.

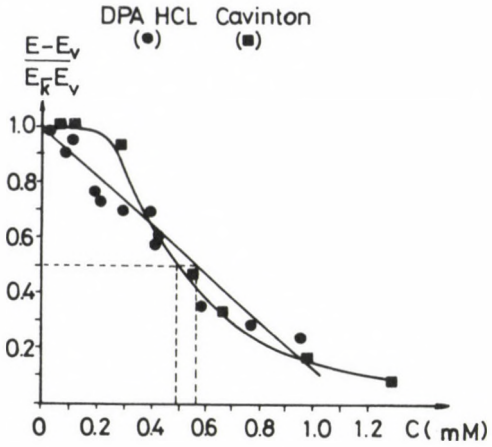

Fig 7 Inhibition effect of DPA·HCl and Cavinton on the autooxidation of bovine-brain homogenate

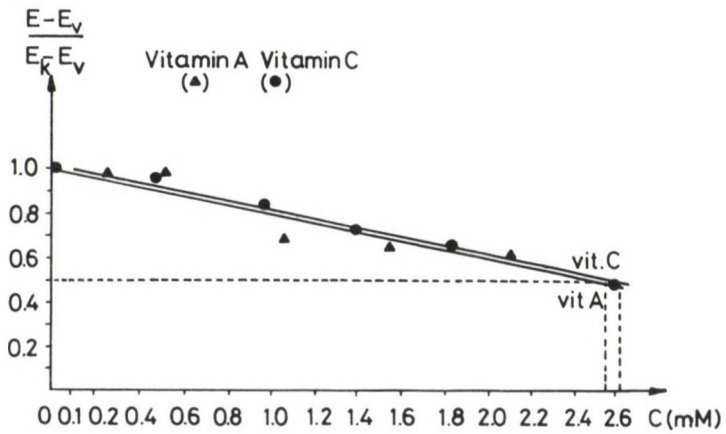

Fig 8 Inhibition effect of vitamin A and vitamin C on the autooxidation of bovine-brain homogenate

DISCUSSION

As the speed of lipidperoxidation depends on iron-ion concentration, we could determine the autooxidant capacity only in those sera which did not suffer haemolysis. Probably iron-ions liberated from haemoglobin cause erroneously high lipid peroxidation in bovine-brain homogenate. This fact limited the precision of v_0 value (SE 10 %) derived by linear regression (correlation coefficient 0.967-0.999).

Among other authors Sullivan and Newton /4/ have emphasized the central importance of increased plasma concentration of iron. In accordance with their results we have proved that the antioxidant capacity in premature babies is low and depends upon the birth weight and gestational age. During the first 14 days of postnatal life this parameter is changing and depends on several factors requiring further investigations. Antioxidant capacity seems to be characteristic for the general conditions of premature babies.

In our in vitro investigations we have found that vitamin E, A, C, Cavinton, DPA·HCl, MTDQ-DS are effective inhibitors of lipid autooxidation.

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