Resistance of Red Cells to Dialuric Acid of Premature Infants with Retrolental Fibroplasia.* (18701)

STANLEY COHEN, JAMES P. DEMETRY, AND HEINZ HERRMANN.

From the Department of Pediatrics, University of Colorado School of Medicine, Denver, Colo.

The etiology of retrolental fibroplasia in premature infants is not known. It has been suggested, however, that an increased incidence of the disease occurred following (a) oxygen therapy, (b) the administration of iron, (c) the administration of water-miscible vitamin preparations(1), and (d) the use of certain detergents(2). Owens and Owens(3) reported that a deficiency of vit. E might be the primary factor in the incidence of retrolental fibroplasia. Callison and Orent-Keiles(4) noted abnormalities of the eye occurring in young vit. E-deficient rats.

The experiments of Rose and György(5) furnish a sensitive method for the detection of the initial stages of vit. E deficiency in the rat. *In vitro* the erythrocytes from deficient rats were readily hemolyzed by compounds such as dialuric acid and alloxantin, and this hemolysis was prevented by the addition of alpha-tocopherol either to the diet or *in vitro*. Using this procedure, we have investigated the possibility that retrolental fibroplasia in premature infants may be correlated with a deficiency of tocopherol.

Experimental. Premature infants admitted to Colorado General Hospital during the latter half of 1950 were studied. All infants were fed partially skimmed cow's milk-carbohydrate mixtures with added vit. A and D. None received any added vit. E in the diet. Blood samples were obtained by heel pricks from 5 normal premature infants and from 9 infants showing varying degrees of retrolental fibroplasia. The erythrocytes were tested for sensitivity to hemolysis by dialuric acid employing the procedure of Rose and György(5). For the hemolysis test 10 drops of blood were collected in 2 ml of a solution containing 0.9% of sodium chloride and 1% of sodium citrate. The blood was centrifuged, the supernatant fluid removed and 0.9% sodium chloride added to make a 5% suspension of the blood cells. To 0.25 ml of this suspension in a small tube were added 0.18 ml of phosphate buffer and 0.07 ml of a 0.1% solution of dialuric acid in the buffer. The tubes were incubated for 15 minutes at 37°C, after which they were kept at room temperature and observed for at least 4 hours. A control tube without dialuric acid was run with the test sample. The validity of the procedure for the detection of a deficiency of vit. E in rats was confirmed. One group of 4 rats was fed a diet free from vit. E (General Biochemicals); a second group of 4 animals was fed a stock commercial diet. In 46 days the red blood cells obtained from the deficient animals were almost completely hemolyzed by dialuric acid, whereas those from the control animals showed no hemolysis. These results are included in Table I.

Results. We have never observed any hemolysis of the erythrocytes of infants by dialuric acid. The red blood cells from normal premature infants and from infants with retrolental fibroplasia showed complete resistance to hemolysis by dialuric acid in the concentrations used (Table I).

Rose and György also studied the litters of rats receiving a stock diet and found that immediately after parturition the erythrocytes of the newborn animals were hemolyzed by dialuric acid. This sensitivity to dialuric acid disappeared within several days.

We have examined 5 samples of cord blood obtained from full term infants. No hemolysis was noted. We have also examined the

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4. Callison, E. E., and Orent-Keiles, E., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 295.

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TABLE I (a). Hemolysis of Red Cells from Vit. E-Deficient Rats by Dialuric Acid.

Exp. No.	Diet	Days after diet started	Hemolysis in 4 hr
1	E-deficient	8	0
		21	0
		46	complete
2	Purina Lab. Chow	46	0

Four animals were used in each experimental group.

TABLE I (b). Resistance of Red Cells to Dialuric Acid of Premature Infants with Retrolental Fibroplasia. No hemolysis in 4 hr.

Subject	Birth wt, g	Age when tested, days	Wt when tested, g	Retrolental fibroplasia
Ma.	880	88	2250	e*
Mi.	1008	84	2310	a
C.	1035	79	2600	a
G.	1148	52	1990	e
G.	1148	75	2370	e
A.	1150	86	2250	a
J.	1268	76	2560	e
L.	1277	64	2400	0
Al.	1305	51	2130	0
F.	1365	28	1460	e
F.	1365	63	2450	r
W.	1417	75	2240	a
Jo.	1470	15	1540	0
R.	1644	57	2860	a
Z.	1750	52	2950	0
Re.	2197	20	2095	0

* e = early; a = advanced; r = regressed.

blood from guinea pig embryos obtained by Caesarian section. No hemolysis was detected in the three litters examined. The guinea pigs had been fed a stock diet of Purina Rabbit Pellets supplemented with lettuce.

Discussion. We have found no correlation between the occurrence of retrolental fibroplasia in premature infants and a deficiency of vit. E as measured by the hemolysis test. It should be mentioned, however, that even if a vit. E deficiency existed in the premature infant, it might not be detected by the procedure employed, which has been validated for the rat only. Mason(6) has summarized the differences in pathology of vit. E deficiency in different species.

Summary. 1. The erythrocytes of 5 normal premature infants and of 9 with retrolental fibroplasia have been examined for a deficiency of vit. E by the hemolysis test of Rose and György. The red blood cells from all of the infants were resistant to hemolysis by dialuric acid. 2. We have confirmed the validity of the hemolysis test for a deficiency of tocopherol in the rat.

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Lethal Infection with Coxsackie Virus of Adult Mice Given Cortisone. (18702)

EDWIN D. KILBOURNE AND FRANK L. HORSFALL, JR.
From the Hospital of The Rockefeller Institute for Medical Research, New York City.

Coxsackie viruses are characterized by virulence for immature mice or hamsters and avirulence for adults of either species. In fact, this unique property is the single biological characteristic which defines this large group of viruses.

Recent studies in this laboratory which demonstrated increased influenza A, B, and mumps virus multiplication in embryonated eggs injected with cortisone(1), and observations of Shwartzman(2) on enhancement by cortisone of poliomyelitis infection in mice and hamsters, suggested the use of this hormone to essay an alteration in the insusceptibility of the adult mouse to Coxsackie viruses.

Materials. Viruses. The Conn. 5 strain of Coxsackie virus (previously obtained through the courtesy of Dr. Joseph L. Melnick) and the RB strain of virus, recovered in this laboratory from the stool of an 8-year-old boy

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