qua non in the production of infarction has been the chemical sterilization of the catheters.

Summary. 1. Pulmonary infarction has occurred in 19 patients with severe mitral stenosis or congestive heart failure 2½ to 79 hours following wedging of a cardiac catheter into a branch of the pulmonary artery for the measurement of pulmonary "capillary" pressure. 2. Over a period of 6 months, pulmonary infarction occurred in over half the patients studied with severe mitral stenosis. 3. In other patients without pulmonary congestion, pulmonary infarction did not occur. 4. When the catheters were sterilized by autoclaving instead of by soaking in mercurioxycyanide, pulmonary infarction did not occur in 20 consecutive patients with either severe mitral stenosis or congestive heart failure.

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Hemolysis in Hydrogen Peroxide of Erythrocytes of Premature Infants. Effect of Alpha-tocopherol.* (19408)

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György and Rose reported that administration of tocopherol prevented intravascular hemolysis and hemoglobinuria in vit. E deficient rats injected with alloxan(1). They found, also, that the red blood cells of rats deficient in tocopherol could be completely hemolyzed, while those of tocopherol-treated animals were never affected by incubation with dialuric acid or alloxantin, reduction products of alloxan(2). Since *in vitro* hemolysis was more marked with dialuric acid than with alloxantin, the authors suggested that dialuric acid might be the active moiety. Because Owens and Owens(3) had suggested that a dietary deficiency of vit. E might be responsible for retrolental fibroplasia, the erythrocytes of 14 premature infants, both with and without the disease, were tested for sensitivity to dialuric acid, using the procedure of Rose and György(4). The infants showed no evidence of E deficiency as measured by this test(5). Many premature infants do not absorb fat well, and Wright, Filer, and Mason (6) have shown that low serum tocopherol levels develop in these infants when fed diets designed to circumvent the handicap in fat absorption. Since the manifestations of E deficiency vary in different species, and in the same species at different ages(7), the possibility remained that E deficiency might exist in premature infants even though the dialuric acid test did not so indicate. The finding by György, Cogan, and Rose(8) that the red blood cells of newborn full term infants, resistant to dialuric acid, were hemolyzed by dilute solutions of hydrogen peroxide, and that this hemolysis could be prevented by alpha-tocopherol led to the present study of prematurely born infants. A significant degree of hemolysis has been found and this is consistently prevented by the feeding of alpha-tocopherol.

Subjects and methods. Observations were made in 23 premature infants, born either at the Colorado General Hospital, or admitted to its Premature Infant Center because of inadequate facilities for care at the place of birth. The infants' weights ranged from 745

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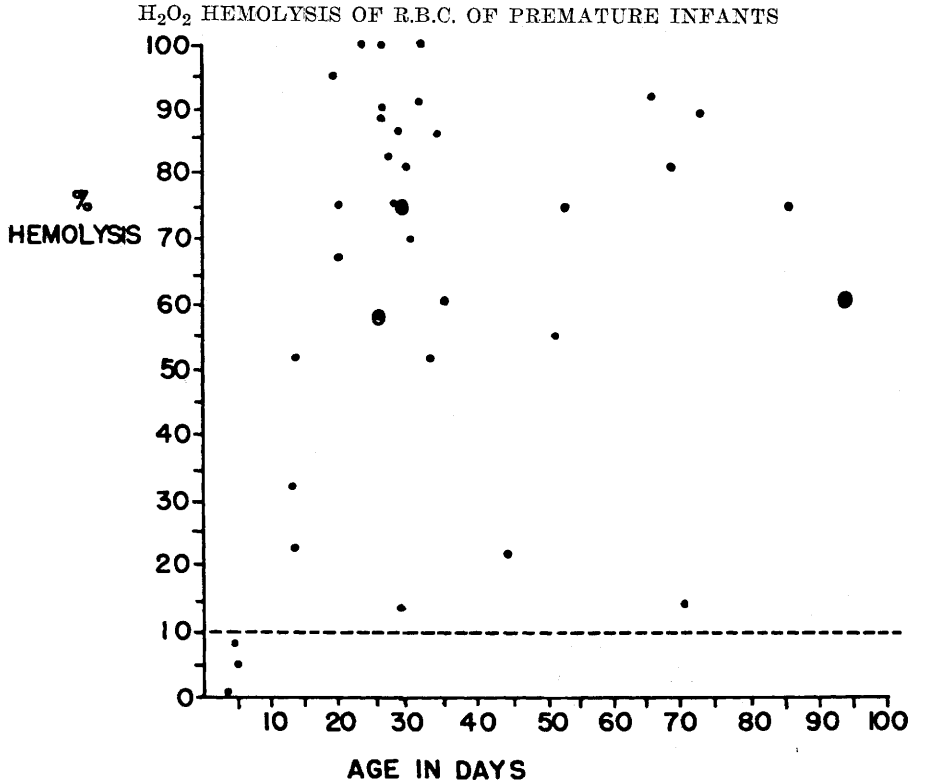

FIG. 1. Three infants, indicated by heavy dots, were fed evaporated milk. All others received partially skimmed milk. Dotted line indicates level below which all 19 observations on adults were found.

to 2000 g at the time of birth, and from 1100 to 3400 g at the time of testing. Their diets consisted, except as indicated, of a powdered preparation of partially skimmed cows' milk (Alacta) diluted with water and fortified with added carbohydrate. All but 5 infants had received supplements of ascorbic acid, 50 mg, and of vit. D, 1000 units, (Drisdol, 4 drops), daily, for 1 to 6 weeks before testing. One infant received ascorbic acid, 1.0 g in 4 divided doses on one day, for study purposes. Vit. E was given to 8 infants, either as an emulsion of alpha-tocopherol acetate, kindly supplied by the Hoffmann-La Roche Co., or as water-miscible tocopherol, Esorb (Wyeth). The hemolysing action of H_2O_2 was also tested on blood taken from 14 healthy adults, all professional hospital personnel, and 5 hospital patients.

Methods. Two-tenths ml of blood was drawn, usually from the antecubital vein, into a syringe previously rinsed with sterile saline-citrate solution, and transferred to a tube containing 2.0 ml of saline-citrate. The test was performed according to directions kindly supplied by Drs. György and Rose, with one modification, *i.e.*, 0.25 ml of 2.4% hydrogen peroxide was used, instead of 0.05 ml of 12% peroxide. This step made it possible to obtain, in duplicate or triplicate, checks within ±5%. All determinations reported herein were within this range, with the exception of 5, which were within ±6-9%. Samples of blood, from an adult and from both non-supplemented and tocopherol fed infants were generally tested simultaneously. Percent of hemolysis is calculated from the ratio of the color of the supernatant fluid in tubes containing the blood cells exposed to H_2O_2 to that in tubes in which complete hemolysis was obtained in distilled water. Readings were made at 550 μ in a Coleman spectrophotometer.

Results. Thirty-two observations in 20 in-

fants on the diet of partially skimmed milk, and single observations in each of 3 infants who had been fed an evaporated milk mixture for 8, 10, and 30 days prior to observation are presented in Fig. 1. It is seen that, in all but 8 of the observations, hemolysis in hydrogen peroxide was more than 50% of that in distilled water. Of these 8, only 3 showed less than 10% hemolysis, the level below which all the observations on the 19 adults fell. A full-term infant of 18 months with pellagra and tuberculosis also showed only 5% hemolysis.

Each of 3 premature infants whose erythrocytes showed resistance to H_2O_2 was 4 or 5 days old, and 2 of them who were followed (Subjects J and M(F)) developed increased susceptibility to H_2O_2 with increasing age (Table I). It is planned to study more newborn premature infants to permit comparison with the data of György and his coworkers on newborn full-term infants. Of 2 full-term infants studied at age 12 hours, one showed 11%, and one 47% hemolysis. The mother of the latter infant showed 2% hemolysis.

In Table I are presented data on 9 infants on whom multiple observations were made. The effect of tocopherol administration was determined in 8 of them. It is seen that in every one of 9 trials in the 8 infants, there was a prompt drop in the hemolysis to the levels found for adults. The smallest dose tried was 25 mg daily for 2 days (Subjects 4 and 9); hemolysis dropped from 83 and 91% to 10% in Subject 4, and from 89 and 72% to 0 in Subject 9. In one infant, Subject 7, the administration of 25 mg of tocopherol daily for 4 days caused a drop from 100 to 4%, with a return to 91% hemolysis on the 10th day after discontinuing tocopherol supplements. Susceptibility to H_2O_2 persisted for another 7 days, when the administration of tocopherol again reversed the test. In infant No. 8, on the other hand, the administration of 25 mg of tocopherol for 5 days was followed by a persistently negative test as long as 22 days after discontinuing tocopherol. In infant No. 9, inconsistent results were obtained after discontinuing tocopherol. The effect of the administration of tocopherol on

TABLE I. Hydrogen Peroxide Hemolysis of Erythrocytes of 9 Premature Infants—Effect of Tocopherol Administration.

Subject	Birth wt, g	Age, days	Wt, g	% hemolysis*
J. ♀	1150	4	1100	8
		13	1200	22
		20	1440	67
		28	1680	76
M. ♀	1460	5	1390	5
		19	1640	95
		26	1740	100
		27	As 1 g	
		28	1825	87
		33	1925	100
		34–41	B 50 mg daily	
		38	2240	6
		41	2240	3
M. ♂	1250	52	1460	75
		66	1790	93
		67–80	C 150 mg daily	
		73	2000	0
		80	2470	0
E. ♂	2040	27	2070	83
		32	2280	91
		32–33	C 25 mg daily	
		34	2360	10
A. ♀	1275	13	1370	52
		20	1470	75
		34	1870	86
		35–40	C 50 mg daily	
		42	2030	11
B. ♀	1475	13	1470	33
		14–27	C 150 mg daily	
		20	1580	0
		27	1720	0
C. ♂	1520	23	1650	100
		24–27	C 25 mg daily	
		29	1800	4
		37	2100	91
		39	2100	73
		44	2300	99
		45–52	B 50 mg daily	
		49	2450	2
		52	2610	1
G.A. ♂	1500	26	1790	90
		27–32	C 25 mg daily	
		33	1880	0
		42	2100	9
		52	2520	14
		55	2600	2
G.B. ♂	1440	26	1490	89
		31	1590	72
		31–32	C 25 mg daily	
		33	1600	0
		40	1820	90
		42	1800	13
		47	1950	68
		52	2080	12
		55	2370	21

* Hemolysis in H_2O_2 as compared with hemolysis in distilled H_2O.

As = Ascorbic acid. B = α-tocopherol (Esorb, Wyeth). C = Tocopherol acetate emulsion (Hoffmann-La Roche).

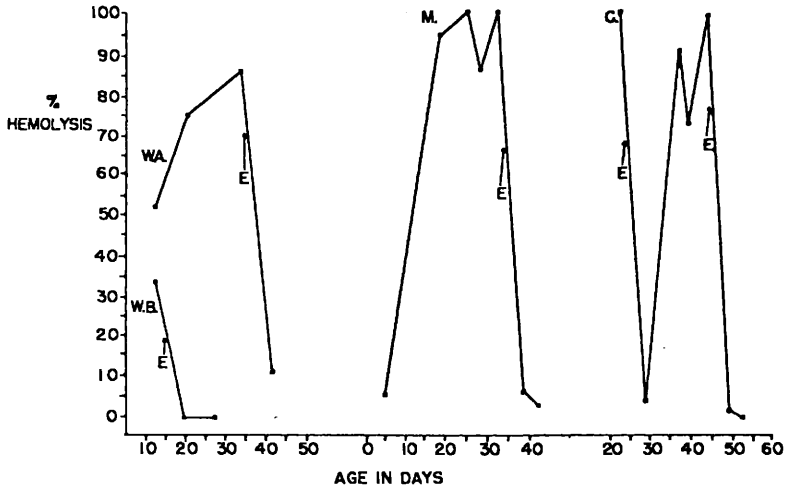

FIG. 2. Effect of α-tocopherol administration on H_2O_2 hemolysis of RBC of 4 premature infants. See Table I for dosage. Infants W. A. and W. B. were twins.

the hemolysis test in 4 of the infants is presented graphically in Fig. 2.

Discussion. The results of these studies confirm the findings of György, Cogan, and Rose(8) that susceptibility of red blood cells to dilute hydrogen peroxide can be prevented by administration of alpha-tocopherol to young infants. The effect of other substances on susceptibility to H_2O_2 remains to be studied; neither the administration of vit. D in doses of approximately 1000 units daily or ascorbic acid, 50 mg daily, prevented its development. Since ascorbic acid has been reported, along with glutathione and cysteine, as being capable of producing the *in vitro* hemolysis of the red blood cells of E-deficient rats(9), the role of ascorbic acid, a customary dietary supplement, will require investigation.

Wright, Filer, and Mason(6) have suggested, on the basis of studies of serum tocopherol levels, that the diets of premature infants should perhaps be supplemented with vit. E. They point out that premature infants may have special needs for tocopherol because of their rapid rate of growth, and the low content of tocopherol of the partially-skimmed milk mixtures widely used for their feeding. According to the data of Quaife (10), Harris(11), and Wright(6), it has been estimated that these partially-skimmed feeding mixtures contain approximately 0.04 mg of tocopherol per 100 cc, as compared with 0.10 mg % in customary evaporated milk mixtures, and 0.15 mg % in "mature" human milk. At an intake of 150-200 cc/kg/day, the tocopherol intake of a premature infant would be approximately 0.08 mg/kg as the one-half skim milk mixture, 0.2 mg/kg as evaporated milk, and 0.3 mg/kg as human milk. In 3 infants who had received an estimated 0.2 mg/kg tocopherol daily as evaporated milk for 8, 10, and 30 days before testing (Fig. 1), the tests showed hemolysis of 57, 61, and 75%. Whether a total intake of 0.3 mg/kg as human milk by premature infants would be adequate to prevent the susceptibility to hydrogen peroxide, in the light of their known defect in fat absorption, remains to be determined.

There is no evidence as yet that the susceptibility of the red blood cells of premature infants to hydrogen peroxide, and its consistent reversal by alpha-tocopherol, have physiologic or clinical significance. If this can be proven, however, then the data herein reported would support the regular addition of tocopherol to the diets of premature infants. The occurrence of edematous states during life and of hemorrhagic transudations terminally in these infants is reminiscent of the exudative diathesis reported in E deficient chicks(12,13), and the hemorrhagic state in the E deficient fetus of the rat(14).

Christensen and Dam(15) have reported

that dietary methylene blue which protects E deficient chicks against the exudative diathesis and brown coloration of adipose tissue also affords E deficient rats a marked, though not complete, protection against hemolysis of erythrocytes in dialuric acid. Antioxidants, other than alpha-tocopherol, may also be able to reverse the susceptibility of the red blood cells of premature infants to hydrogen peroxide.

Summary. Hemolysis of red blood cells in dilute hydrogen peroxide was determined for 23 premature infants. Three infants received evaporated cows' milk, and the remainder partially-skimmed cows' milk feeding mixtures. A significant degree of *in vitro* hemolysis was found and this persisted as long as the infants were observed without supplementation of their diets with tocopherol. In each of 9 trials in 8 infants the *in vitro* hemolysis was reversed by feeding alpha-tocopherol.

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Dephosphorylation of Adenosine Triphosphate by Concentrates of the Virus of Avian Erythromyeloblastic Leucosis.* (19409)

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The virus of avian erythromyeloblastic leucosis(1) occurs in high concentration in the plasma of chicks affected with the disease. Concentration and purification of the agent have been effected(2,3) by the application of ultracentrifugal procedures to such plasma passed through earthen filters. The morphology of the virus and the characters of the concentrates obtained in this way have been described(3). In the course of studies of the chemical constitution and properties of the virus, inquiry was made into any possible activity which the virus might exert on adenosine triphosphate. It was learned, that the virus concentrates and other preparations containing the virus possessed the capacity to liberate inorganic phosphorus from this material. The importance of adenosine triphosphate as a source of metabolic energy(4) and the significance of its enzymatic dephosphorylation, possibly, by the virus in the absence of living tissue *in vitro* would appear to merit the present report of findings thus far encountered.

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