5. Kilgore, W. W., Greenberg, J., *J. Bact.*, 1960, in press.

6. Mandell, J. D., Greenberg, J., submitted to *Nature*.

7. Saroff, H. A., Evans, R. L., *Biochim. Biophys. Acta*, 1959, v36, 511.

8. McKay, A. F., *J. Am. Chem. Soc.*, 1949, v71, 1968.

9. ———, *Chem. Rev.*, 1952, v51, 301.

10. Davis, B. D., Mingioli, E. S., *J. Bact.*, 1950, v60, 17.

11. Szybalski, W., Bryson, V., in *Origin of Resistance to Toxic Agents*, M. G. Sevag, R. D. Reid, O. E. Reynolds, eds., Academic Press, N. Y., 1955, pp 20-41.

Received July 21, 1960. P.S.E.B.M., 1960, v105.

## Erythrocyte Survival in Vitamin E-Deficient Monkeys.* (26145)

HORACE N. MARVIN, JAMES S. DINNING AND PAUL L. DAY†

*Departments of Anatomy and Biochemistry, University of Arkansas Medical Center, Little Rock*

Anemia as the earliest sign of Vit. E deficiency in the monkey was first reported by Day and Dinning(1). The anemia was slightly macrocytic, and was accompanied by a granulocytosis as evidence of marrow activity. These findings suggested that the anemia occurring might be the result of an increased rate of erythrocyte destruction. Results of an experiment designed to test this hypothesis are the subject of this report.

*Methods.* Monkeys of the *rhesus* type which had been on a diet deficient in Vit. E for varying periods of time were selected for this study. The composition and preparation of the diet have been described(2).

Erythrocytes were tagged with radio-active chromium according to the principles set forth by Gray and Sterling(3) and Ebaugh *et al.*(4) and modified for application to small animals by Marvin and Lucy(5). The one departure from the technic as described was use of the brachial vein rather than cardiac puncture for obtaining blood for tagging, and use of the ear for obtaining repeated samples. Complete blood counts were made at intervals, also from the ear veins. Radioactive chromium ($Cr^{51}$) was determined in a scintillation well counter using a thallium-activated sodium iodide crystal.

*Results and discussion.* Survival curves of 6 studies on 4 monkeys are shown in Fig. 1. Monkey #211 served as a replete control, having never been given an E-deficient diet. Its erythrocyte count was 5.8 million per cu mm and hemoglobin and hematocrits were normal. Maximal survival time was 98 days; the curve was symmetrical with no apparent changes in rate of chromium loss. This normal value agrees well with that of 100 ± 20 days reported by Harne, *et al.*(6) using the consecutive reticulocyte peak method.

Monkey #204 had been on the deficient diet only 35 days when its cells were tagged and injected. On the 137th day of the dietary regimen, no activity could be detected; a maximal survival time of 102 days. During this time, erythrocyte count was 5.7-5.9 millions per cu mm. Monkey #205 had an 88 day survival time when measured between the 188th and the 276th day on the deficient diet. During this time red cell count decreased from 5.55 to 4.24 millions per cu mm. This is possibly not a significant, but certainly a suggestive, shortening of the life span.

On the other hand, after 349 or more days on the diet, survival was shortened to 49, 45, and 35 days in monkeys 207, 205, and 204, respectively. Erythrocyte counts were between 3.02 and 3.98 millions during the time these determinations were being made.

Five months after addition of adequate daily supplements of tocopherol were given monkey #205, red cell count had risen from 3.07 to 5.85 millions. Life span of the eryth-

* Supported by research grants from Nat. Inst. Health, Am. Cancer Soc., and Muscular Dystrophy Assns. of America, Inc.

† Present address: Food and Drug Administration, Dept. of HEW, Washington, D. C.

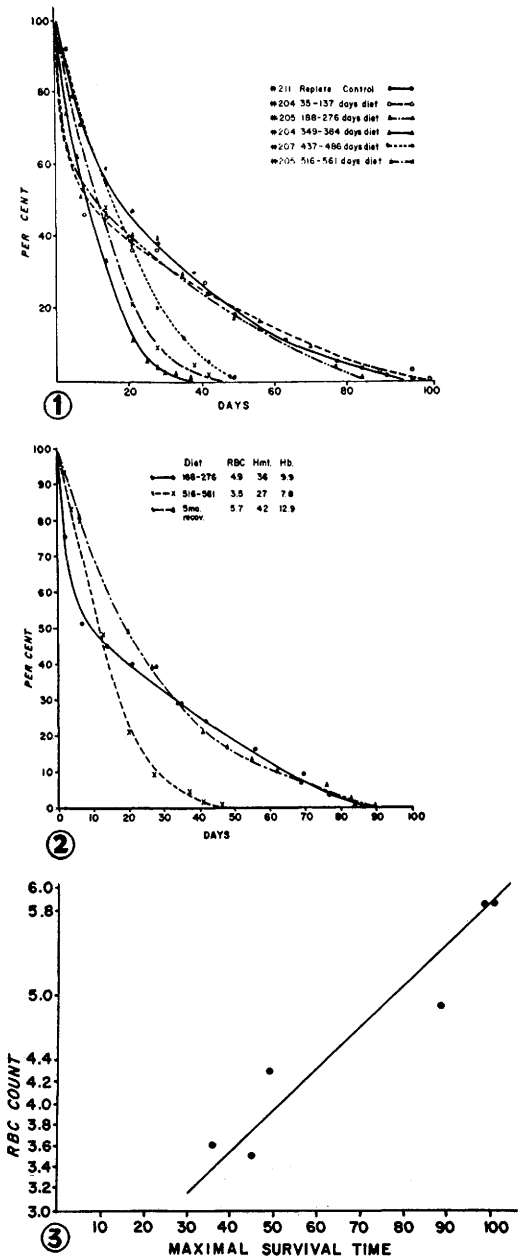


FIG. 1. Curves showing disappearance of $Cr^{51}$ from blood of 4 monkeys. Ordinate gives ratio of $Cr^{51}$ content at various times after tagging to $Cr^{51}$ content on day after tagging. Abscissa indicates time after tagging. Monkeys 204 and 205 were tested twice.

FIG. 2. Curves showing effects of vit. E-deficiency and replacement therapy on disappearance of $Cr^{51}$ from the blood of Monkey #205. Coordinates the same as Fig. 1.

FIG. 3. Regression line relating maximal erythrocyte survival time on abscissa, and avg erythrocyte count during time of survival study on the ordinate.

rocytes of this recovered monkey was 91 days (Fig. 2).

Curves in Fig. 1 show rather poor correlation between life span and time on the deficient diet. If, however, one plots lifespan against erythrocyte count, a respectable regression line can be drawn by inspection (Fig. 3).

The deficiency of Vit. E may alter the erythrocyte in such way that its longevity is impaired. The erythrocytes may thus die linearly due to a premature senescence. On the other hand, E-deficiency may result in a random destruction of erythrocytes regardless of age. A combination of these two mechanisms must certainly be considered. As pointed out by Sheets, *et al.*(7) removal of cells by a combination of random destruction and senescence can be represented by the formulation

$$N_t = N_o \left(1 - \frac{t}{T}\right) e^{-kt}$$

where $N_o$ and $N_t$ are numbers of cells initially and at time t, T is mean survival time and K is a constant characterizing rate of random destruction. With the chromium technic, K is a measure also of rate of elution. Rearrangement of the formula, solving for K(5) makes possible calculation of this constant. When values obtained are tabulated and inspected, it is apparent that rate of random loss of chromium (random destruction plus elution) persists longer at a higher level in the severely deficient monkeys than in those normal or only slightly deficient ones. This would substantiate the suggestion that the anemia of E-deficiency in the monkey is partly hemolytic(2).

The mechanism whereby this random "hemolytic" destruction takes place is of interest. György and Rose(8) reported that the erythrocyte of E-deficient rats could be hemolyzed in dialuric acid, but this agent did not appear to have a differential hemolytic effect on erythrocytes of premature infants (9). Such erythrocytes could, however, be hemolyzed by hydrogen peroxide(10), and this hemolysis was inversely proportional to plasma tocopherol levels(11). Horwitt *et al.* (12) showed that *in vitro* exposure of eryth-

rocyte stroma to hydrogen peroxide increased color production with 2-thiobarbituric acid; presumably reflecting an increased peroxide content. According to Deuel(13) peroxidation of unsaturated fatty acids at the ethene locus is followed by formation of hydroxy-keto-acids and finally cleavage of the chain. If this occurs in the lipid of the erythrocyte envelop and stroma, greater fragility and hemolysis might well result. The well documented antioxidant effect of alpha tocopherol (14) could prevent the action of hydrogen peroxide and other oxidizing agents *in vitro*. Oxidizing substances *in vivo* which could alter erythrocyte lipids in absence of Vit. E are not presently defined, but hydrogen peroxide as a metabolic transient might well be one of them.

It has also been shown that blood fat exerts a hemolytic effect(15) even at normal post-prandial levels(16). The diets used in our experiments, and generally used in studies of Vit. E deficiency, contained relatively large amounts of fat. Replacement with alpha tocopherol, without changing fat content of the diet, reduced the excessive rate of erythrocyte loss. It is possible, therefore, that Vit. E may interfere with hemolytic action of the ingested fat. If the binding of the fat by the erythrocyte is the critical point, as has been suggested(15), and this binding is oxidation-dependent, the antioxidant effect of Vit. E may be important. Further work is indicated before the mechanism is elucidated.

*Summary.* Survival of $Cr^{51}$ tagged erythrocytes, autologously transfused, is severely shortened in monkeys maintained on Vit. E-deficient diets. Maximal survival times of 35, 45, and 49 days were obtained after monkeys had been on the diet for 349 or more days, as compared with about 100 days for replete controls. The shortened survival time, associated with anemia, could be reversed upon addition of Vit. E. The mechanism is discussed.

1. Day, P. L., Dinning, J. S., *Fed. Proc.*, 1956, v15, 548.

2. Dinning, J. S., Day, P. L., *J. Exp. Med.*, 1957, v105, 395.

3. Gray, S. J., Sterling, K., *J. Clin. Invest.*, 1950, v29, 1604.

4. Ebaugh, F. G., Emerson, C. P., Ross, J. F., *ibid.*, 1953, v32, 1260.

5. Marvin, H. N., Lucy, D. D., *Acta Haem.*, 1957, v18, 239.

6. Harne, O. G., Lutz, J. F., Zimmerman, G. I., Davis, C. L., *J. Lab. Clin. Med.*, 1945, v30, 247.

7. Sheets, R. F., Janney, C. D., *J. Clin. Invest.*, 1951, v30, 1272.

8. Rose, C. S., Gyorgy, P., *Blood*, 1950, v5, 1062.

9. Cohen, S., DeMetry, J. P., Herrmann, H., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 134.

10. Gyorgy, P., Cogan, G., Rose, C. S., *ibid.*, 1952, v81, 536.

11. Gordon, H. H., Nitowsky, H. M., *Nutr. Symp. Ser.*, 1956, v12, 83.

12. Horwitt, M. K., Harvey, C. C., Duncan, G. D., Wilson, W. C., *Am. J. Clin. Nutr.*, 1956, v4, 408.

13. Deuel, H. J., Jr., in *The Lipids, Vol. I Chemistry*, New York, N. Y., Interscience Publishers, Inc., 1951, 157.

14. Olson, R. E., *Ann. Rev. Biochem.*, 1959, v28, 467.

15. Greisman, S. E., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 725.

16. Freeman, L. W., Loewy, A., Johnson, V., *Am. J. Physiol.*, 1944, v140, 556.

Received July 29, 1960. P.S.E.B.M., 1960, v105.