Some Metabolic and Nutritional Factors Affecting the Survival Time of Erythrocytes

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It might seem somewhat contradictory to include a discussion concerned with factors affecting the survival time of erythrocytes as part of a symposium concerned with nutritional and metabolic aspects of blood cell formation. Yet, the circulating red blood cell count, which is frequently used as the first order criterion for changes in red blood cell production, may be as much a result of modifications of the survival time as responses in the bone marrow itself. Quantitative measurement of the erythron really measures the resultant of the rates of destruction and production, and the former frequently may be masked by the compensatory ability of the bone marrow to proliferate upon demand at an increased rate. As the subject is developed it will be seen that an assay of the survival time frequently is necessary in order to elucidate the mechanism whereby the erythron changes from the normal to either an increased or decreased value.

ERYTHROCYTE SURVIVAL TIME

Mathematical Model of Survival Curves

Mathematically, death due to senescence, i.e., dependent on some characteristic such as age, would be linear when determined from a random sample of blood. On the other hand, death occurring irrespective of any certain characteristic, i.e., by chance or by random destruction, would occur in a negative exponential fashion. Obviously combinations of these two mechanisms of erythrocyte removal would result in a curve combining both linear and exponential influences. Only by determining the entire decay curve of erythrocyte survival, can a full picture of the factors at play be elucidated. We believe, therefore, that the value of this somewhat tedious procedure cannot be emphasized too much. Figure 1 illustrates the effect of an increasing rate of random destruction (increasing values of K) on survival curves, with the linear life span held constant. This is an artificially constructed mathematical model, correct in principle.

Factors Affecting Survival Time

In order to focus attention on some, but not all, of the factors which may influence red blood cell survival, a number of factors and the possible site in which these factors may be effective are given in Figure 2. This is in no sense meant to be a complete analysis of the interrelationships between erythrocyte structure and the composition that can be altered to result in changes in red blood cell survival time. It is merely an indication of the direction in which thinking and experimentation may be directed. It now has been established that the life span of the erythrocyte is a finite time, and for each given species the life span seems to be fairly fixed and subject to relatively little normal variation. Under the conditions of normal day to day living, the erythrocyte is endowed with a certain complement of constituents necessary to its life. As time passes,

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This work was supported in part by research grants from the National Institutes of Health, American Cancer Society, Muscular Dystrophy Associations and Contract AT-(40-1)-2681 with the Atomic Energy Commission.

This paper was presented at the Symposium on Nutritional and Metabolic Aspects of Blood Cell Formation held at the University of Arkansas Medical Center, Little Rock, Arkansas, on October 20, 1961, under the sponsorship of The National Vitamin Foundation, Inc.

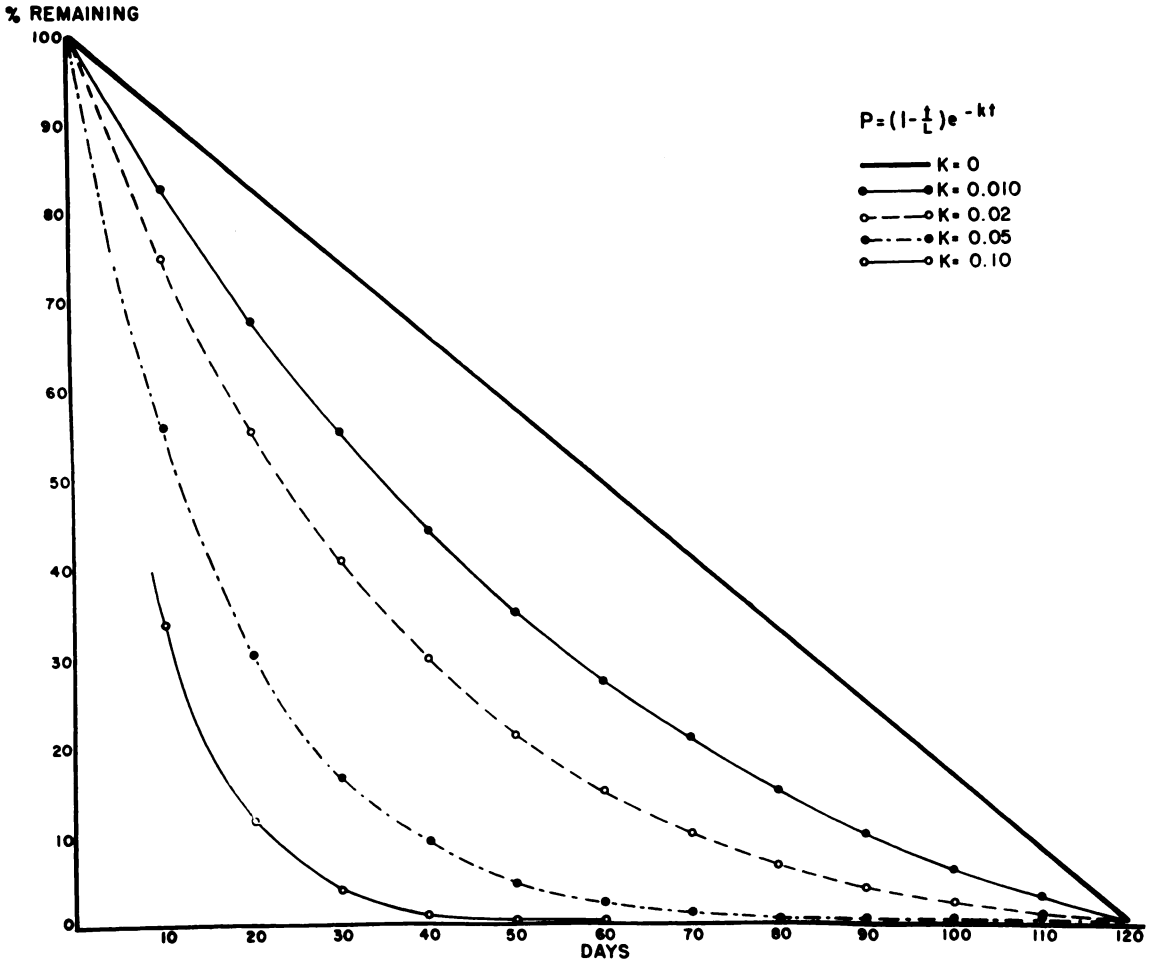

FIG. 1. Mathematical model of a family of curves expected to result from graphs relating per cent survival time with a uniform life span (L) and various values for K as formulated.

these constituents decrease until some critical material becomes reduced below the minimal requirement, and the cell dies. As a point of pure logic, one must consider the reality that a group of factors, as well as a single factor, may become limiting. The *route* of elimination or sequestration of the dead erythrocytes is not truly a part of the subject delineated for consideration in this report.

Turning again to Figure 2 one might select catalase, postulating that it is one of the general factors important in general senescence because of its role in the final steps of oxidative metabolism. If one plots the units of catalase per milliliter of blood against the maximal survival time for the same species, a distribution as seen in Figure 3 results. Some of these data are obtained from the literature; others were determined in our own laboratory. A regression line for homothermic animals alone demonstrates only a poor correlation between catalase content and survival time. Yet there seems to be sufficient correlation to suggest that the catalase content of erythrocytes, which decreases with aging of the cell,[1] may be a contributing factor in the final death and disappearance of the erythrocytes. Inclusion of the data from poikilothermic animals makes this conclusion less convincing. Yet we know that primaquine-sensitive erythrocytes contain less catalase than those with normal resistance to drugs.[2] Another enzyme constituent of the erythrocyte, cholinesterase, seems to be completely unimportant as a factor in the survival

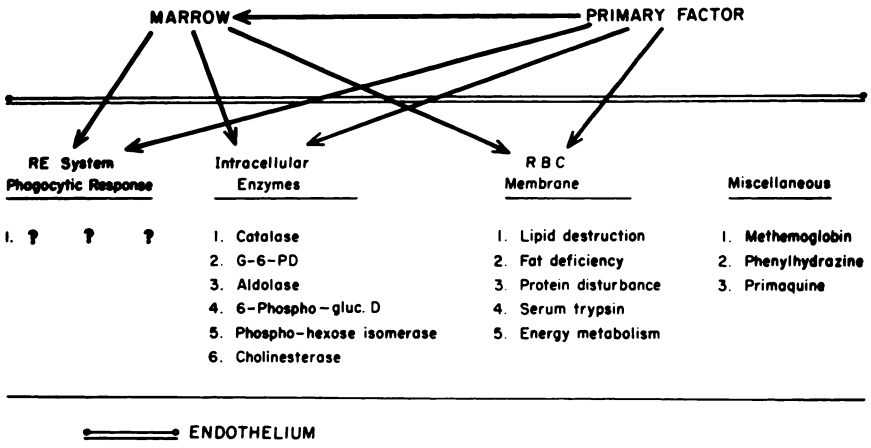

FIG. 2. Some possible interacting factors affecting erythrocyte survival time. Normal aging = summation of normal contributions and is nonrandom. Random destruction = not necessarily related to age.

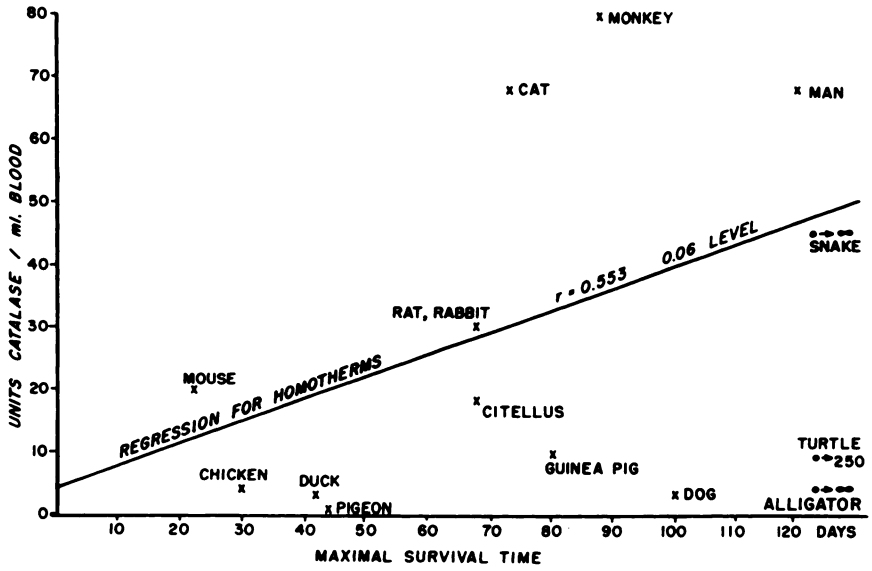

FIG. 3. Relationship between erythrocyte catalase and maximal survival time of erythrocytes of several species. Regression line mathematically fitted for homothermic species only.

of the red blood cell. This is attested to by the fact that diisopropylfluorophosphate, which combines irreversibly with cholinesterase and inactivates this enzyme, has been used in survival studies and has resulted in normal values. At the present stage of development, studies on single enzymes as factors controlling senescence of red blood cells has not been particularly rewarding.

One might conceive that the aging process and senility in the erythrocyte are determined by the same factors which govern the rate of aging in individual subjects in general. A graphic analysis of this is given in Figure 4; the relationship is poor even if one considers only the species with non-nucleated erythrocytes. Inclusion of all types of erythrocytes would render this relationship even less convincing. The

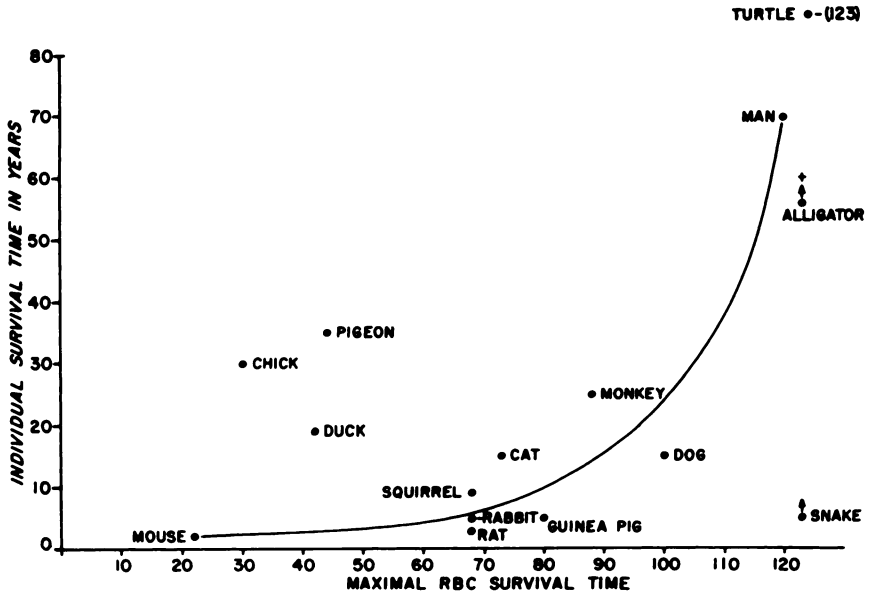

FIG. 4. Average life span of individuals related to maximal survival time of erythrocytes. Curve drawn relating coordinates of only those species with non-nucleated erythrocytes.

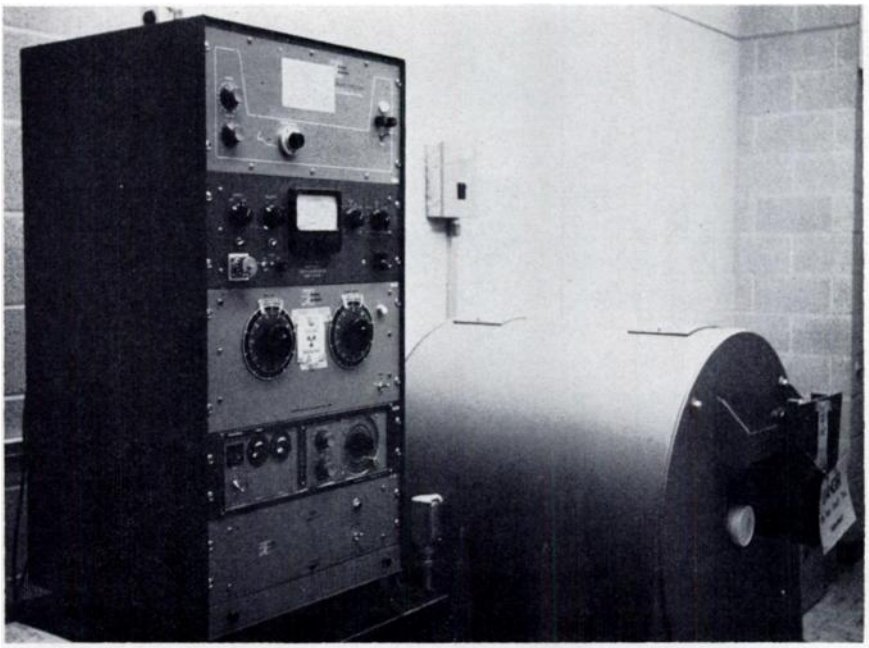

FIG. 5. The "whole body counter" of the gamma ray sensitive liquid scintillator type used in the present studies.

results of a similar analysis of the relationship between the metabolic level of the various species and the survival time of the erythrocytes of these species are about the same. A direct attack on this aspect of the problem has proved to have some merit.

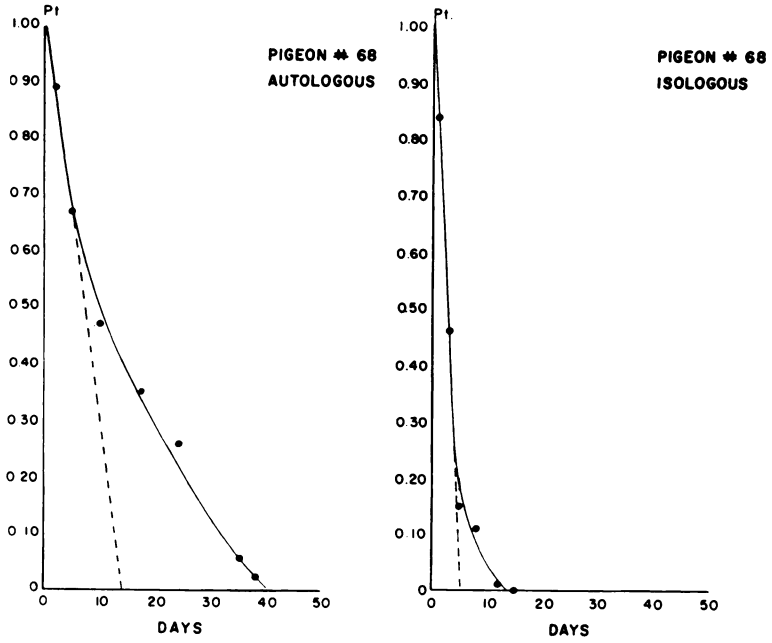

FIG. 6. Normal survival curve on the left with maximal survival of forty to forty-two days when pigeon no. 68 received its own cells. On the right a maximal survival time of only fourteen days when cells of pigeon no. 68 were transfused into another pigeon.

Methods for Determining Survival Time

A few statements should be made concerning methods. The Cr^{51} tagging technic has been used consistently, essentially as described previously,[3] except for variations in the amount of activity of the nuclide used for animals of different body sizes. In addition to removing blood samples for counting purposes at intervals after tagging, in some experiments the blood count rate for the entire animal was carried out in a "4π whole body counter" as seen in Figure 5. In interpreting red blood cell survival curves obtained by the Cr^{51} technic, two pit-falls must be borne in mind. (1) When tagged the blood must be returned autologously to the same subject from which it was drawn. This seems to be even more important in animals than in man. In animals, notably the pigeon,[4] the blood of two individuals of the same strain may demonstrate no incompatibility by usual crossmatching procedures yet, when transfused isologously into a subject other than the one from which it was drawn the survival time of erythrocytes may be shortened greatly (Fig. 6). (2) The chromium procedure is characterized by physical elution which causes the curve to depart from linearity in a fashion indistinguishable from the effect of random destruction. The quantitative role of elution, as well as random destruction, is represented by the constant (K) in the formula in Figure 1. This formulation has become rather well accepted as essentially correct for the curvilinear relationship between the percentage of remaining tagged erythrocytes and time after labeling.[5–7] It is becoming more and more apparent that K may vary from the normal value and differ between species.[3] In Figure 1, it can be seen that by using only the half-life, survival values differed widely as the result of differences in only the elution rate, although the maximal survival (L) time was fixed at 120 days. Thus again it is emphasized that it is our practice to carry survival curves to extinction to avoid misinterpretations.

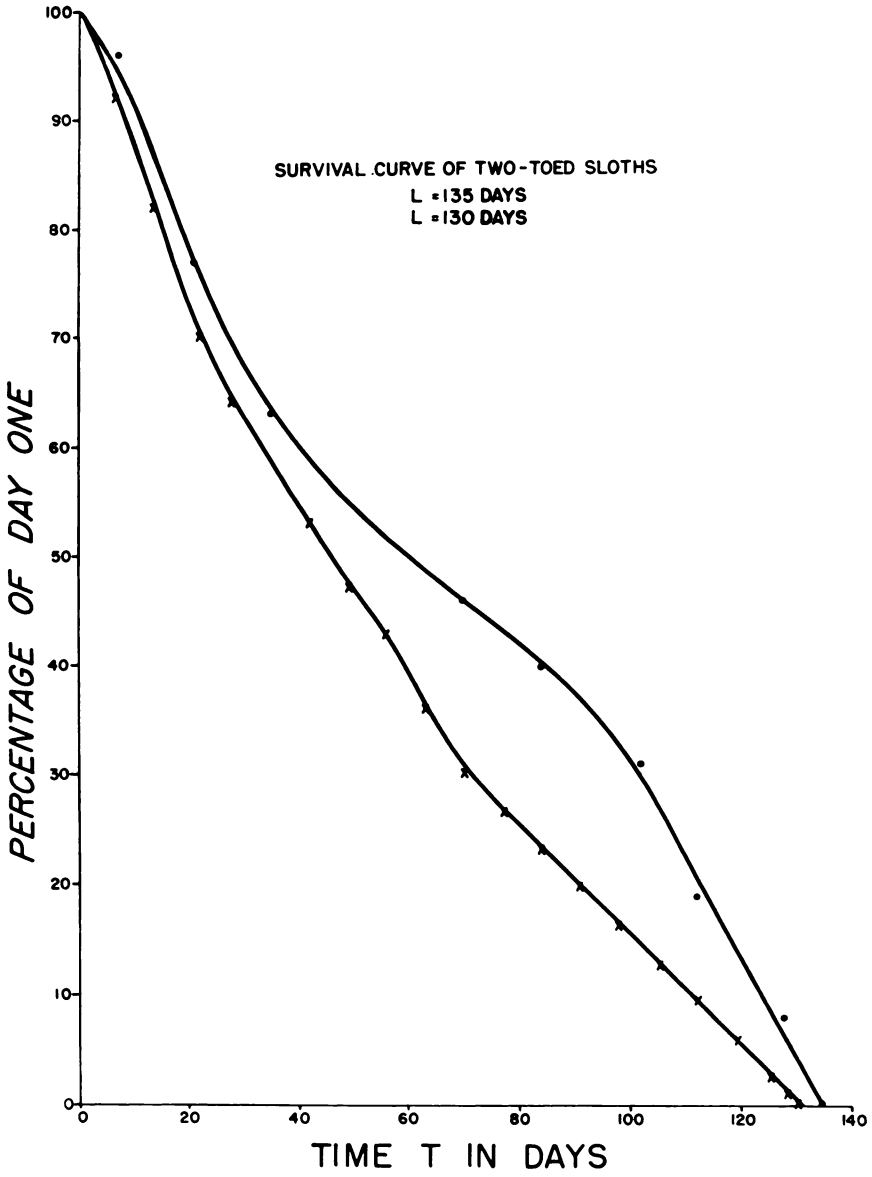

FIG. 7. Erythrocyte survival curves of two two-toed sloths (*Choloepus didactylus*).

Relationship Between Basal Metabolic Rate and Erythrocyte Survival

As a first approach to the relation of general metabolic level to red blood cell survival times, the two-toed sloths were tested because they have much slower respiratory and metabolic rates and lower body temperatures than most mammals. Maximal survival times of 130 and 135 days were obtained (Fig. 7); these can be compared with seventy to ninety days for the cat, rabbit and monkey of comparable body size. In only the horse[8] and man[7] have survival times been reported in this range. A more direct approach would be to decrease the metabolic rate by inducing hibernation. The common ground squirrel (Citellus tridecemlineatus) was used for this study; hibernation was induced by placing the animals in a top-opening refrigerator box at 4°c. Experience showed that the animals required

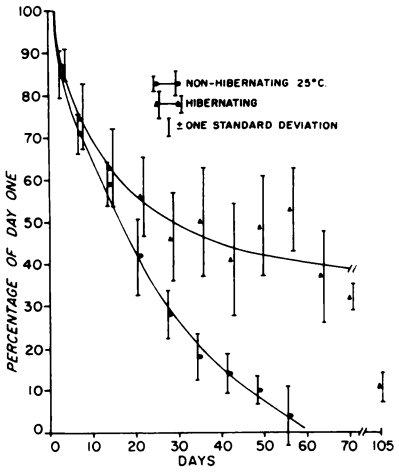

FIG. 8. Curves resulting from blood cell counts on hibernating and nonhibernating ground squirrels. On the seventieth day the hibernating animals had 30 per cent of their Cr^{51} activity remaining and on the hundred and fifth day, to the right of the broken line, the animals still retained 10 per cent.

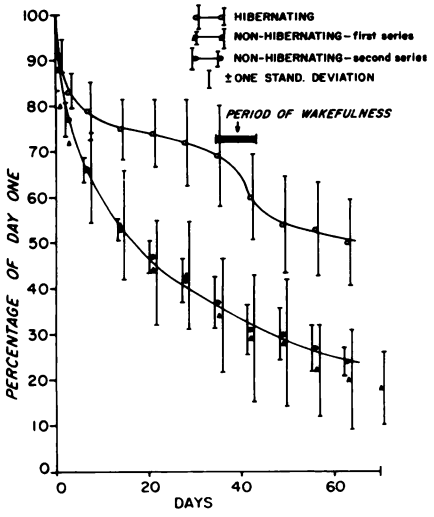

Fig. 9. Curves relating whole body counts on hibernating and nonhibernating ground squirrels. During the seven day period labeled "period of wakefulness" the hibernating animals awoke because environmental temperature temporarily rose to 27 to 30°c.

about seven days of exposure before they became torpid. Tagging procedures were carried out, therefore, after the animals had been exposed to the conditions for six days, and only an occasional animal remained awake more than one day after tagging. The red blood cell survival curves are shown in Figures

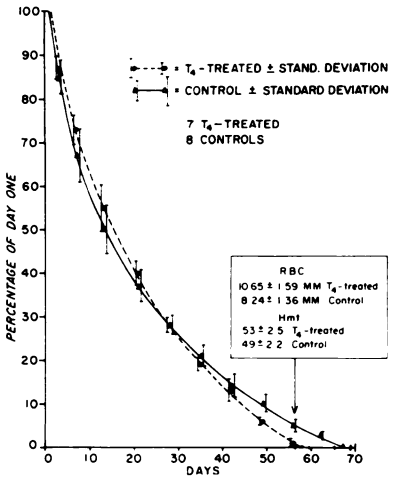

FIG. 10. Erythrocyte survival curves of normal and thyroxine-treated (T_4) rats. After the forty-fifth day, the curves and the extinction intercepts (y = 0) are statistically different.

8 and 9. From these curves one may conclude that hibernation, with its attendant decrease in metabolism, prolongs the survival time of the circulating erythrocytes. An accidental modification of the experiment resulted from a power failure permitting the cold box to warm and the squirrels to awaken. During that period (Fig. 9) the whole body counts declined more rapidly. A natural corollary to this experiment would be a study of survival time under hypermetabolic circumstances. The survival time of red blood cells of rats given injections of thyroxine (T_4) was determined[9] and compared with that of control animals (Fig. 10). The thyroxine-treated animals began losing chromium at a faster rate by the third day as determined by whole body counts, and the blood cell counts were significantly different after the forty-fifth day. The values of the intercepts of the curves with the abscissa for T_4-treated rats were significantly different from control values. This difference would have been overlooked if only the initial slopes of the curves had been considered. The polycythemia expected with T_4 treatment was observed but can in no way be related to the shorter life span of the erythrocytes.[10]

On the expectation that poikilothermic species would have the advantage of con-

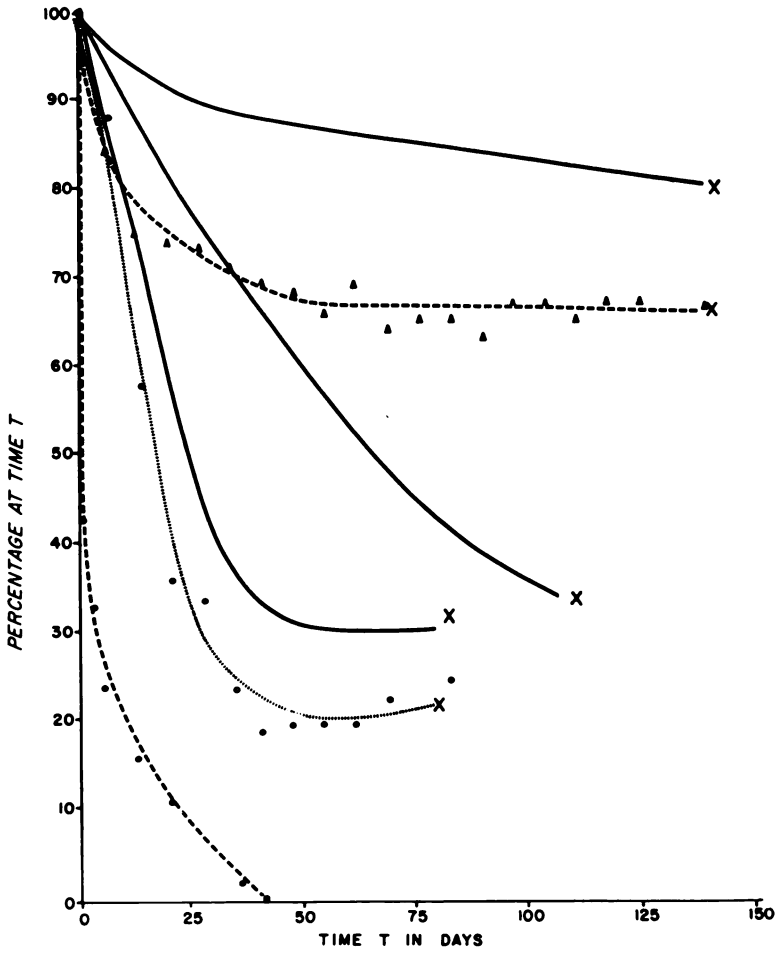

FIG. 11. Survival curves of snake erythrocytes. Whole blood used in tagging. Lower left curve and initial rapid fall in other curves represent clearance of plasma chromium not tagged to red blood cells.

trollable step gradients in body temperature and, therefore, metabolism, we turned our attention to snakes. At the present writing, only blood counts have been carried out, but the results clearly indicate (Fig. 11) that the snake (Elapha o. obsoleta) is comparable to the turtle[11,12] in that they both have a low turnover rate of red blood cells. Thus the original purpose of the study could not be accomplished because the life span of these red blood cells at room temperature is so long that further reductions in body metabolism by lowering body temperature would produce little if any detectable effect. The study does emphasize the inverse relationship between basal metabolic rate and erythrocyte life span.

Relationship Between Vitamin Deficiencies and Erythrocyte Survival

Let us turn now from general metabolic effects to specific nutritional and biochemical factors. Anemia as the earliest sign of vitamin E deficiency in the monkey was first described by Day and Dinning.[13] Since the bone marrow of such monkeys is hyperplastic, it seemed reasonable that the anemia was the result of a serious shortening of the erythrocyte life span. Studies were carried out using the Cr^{51} tagging technic, and this prediction was found to be true.[14] The results are graphically represented in Figures 12 and 13. It can be seen that after being on the diet for 300 days, the survival

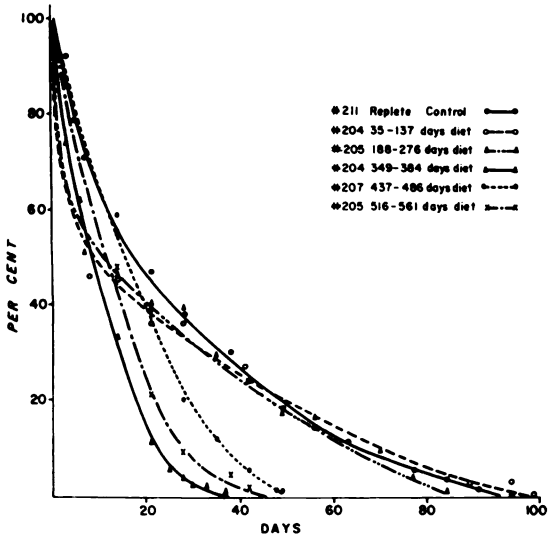

FIG. 12. Survival curves obtained from three vitamin E-deficient monkeys, two curves from monkeys on the diet prior to development of symptoms and one from a replete control monkey.

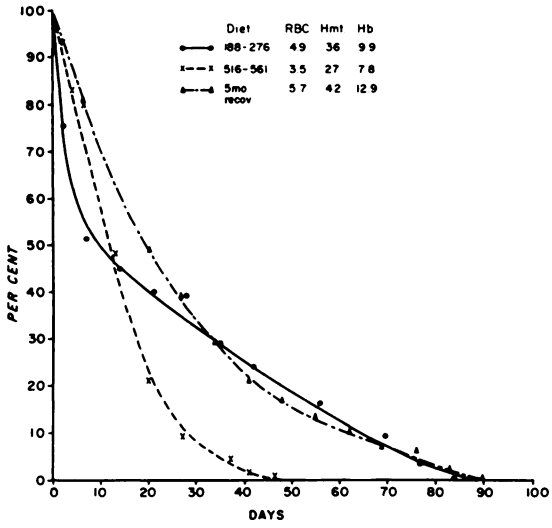

FIG. 13. Survival curves obtained from one monkey, during development of vitamin E-deficiency anemia and after complete recovery when vitamin E was restored to diet.

times had decreased from eighty-five to one hundred to as low as thirty-eight days. In Figure 13 one can see that the survival curve will become normal five months after the addition of only vitamin E to the diet. It seemed apparent that little if any effect of the deficiency could be seen on the survival curves for as long as nine

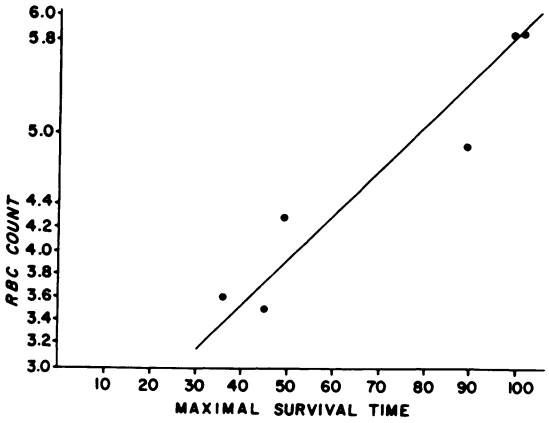

FIG. 14. Regression line relating maximal erythrocyte survival time and average erythrocyte count during time of survival study.

months, but once the anemia was imminent, the deficiency progressed rapidly. As one might expect then, a better correlation (Fig. 14) exists between the erythrocyte count and the maximal survival time, than between survival time and number of days on the diet. It was suggested that in the absence of vitamin E, a lack of the antioxidant properties of the vitamin may permit the oxidation of lipids to peroxides in the red blood cell envelope. The resulting inadequacy of the lipid skeleton in the cell membrane would increase the fragility of such erythrocytes.

Working from this hypothesis, rats were placed on a fat-free diet and tagged autologously at an appropriate time.[15] The resultant curves (Fig. 15) showed that the survival time of erythrocytes from the fat-deficient rats was significantly reduced as compared with that of the control rats. Addition of cottonseed oil to the diet restored the survival time to normal as far as maximal survival time was concerned, but did not completely eliminate some excessive random loss. The depression of red blood cell survival was maximal since there was no further decrease in survival time if rats were carried longer on the diet before tagging. Results obtained from one fat-deficient monkey suggest that this species is quantitatively and qualitatively similar to the rat in this regard.

In order to study ascorbic acid as an erythrolytic factor, guinea pigs were selected and

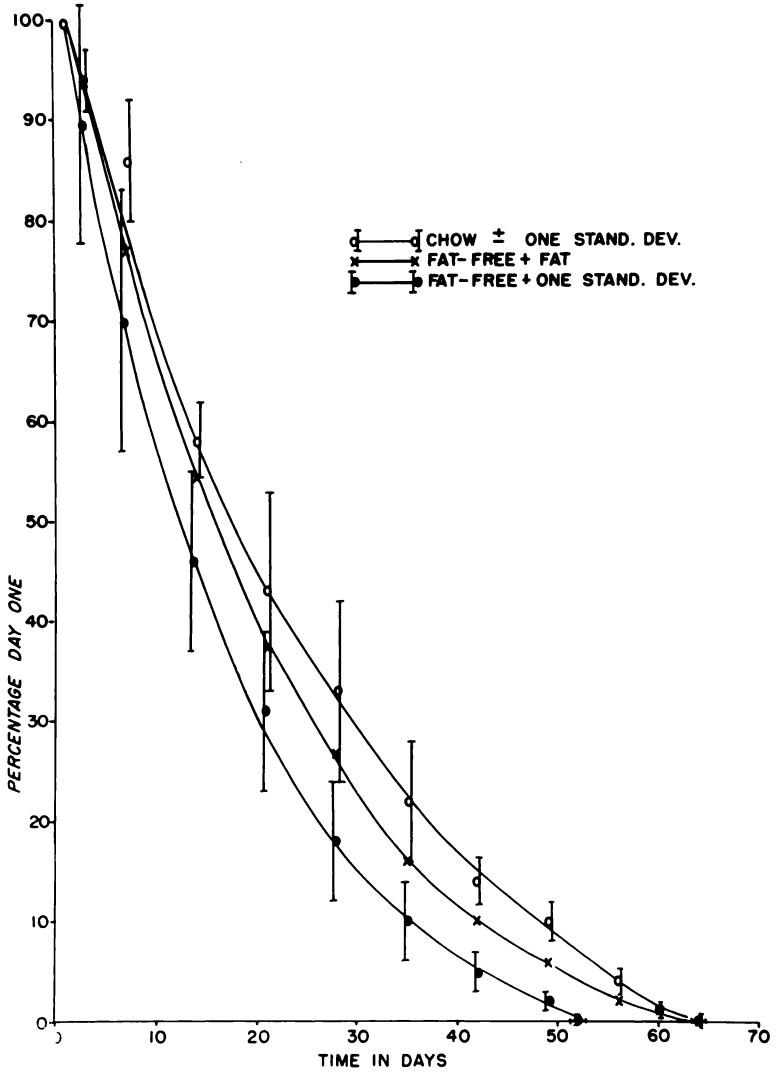

FIG. 15. Red blood cell survival curves of rats on control and fat-free diets and rats on a fat-free diet but receiving cottonseed oil as a supplement.

placed on a scorbutic diet. As soon as the deficiency manifested itself by weight loss, weakness and poor general appearance, the guinea pigs were maintained with injections of ascorbic acid in fluctuating doses. The object here, of course, was to keep the animals alive, yet as deficient as possible. Once equilibrium seemed to be established, blood from scorbutic subjects was tagged and injected autologously into scorbutic animals, and isologously into replete animals. Normal blood tagged and transfused both autologously and isologously into normal recipients failed to show any incompatibility reflected in survival times. As seen in Table I, normal erythrocytes transfused into scorbutic guinea pigs persisted for a normal life span, but scorbutic cells had a shortened life span in either scorbutic or normal recipients. It seems reasonable to conclude that vitamin C deficiency results in a structural or biochemical defect of erythrocytes which confers a survival disadvantage even in a normal physiologic environment.

SUMMARY

In addition to the studies discussed previously, there are many other factors involved in the production of erythrocytes which must

TABLE I

Red Blood Cell Survival Time in Vitamin C-Deficient Guinea Pigs

Test	Animals (no.)	Maximal Survival Time (days)	Range (days)	Half-Life (days)
Autologous Transfusion				
Normal into normal	12	82	79–84	21
Scorbutic into scorbutic	5	64	58–77	16
Isologous Transfusion				
Normal into normal	9	82	80–83	21
Scorbutic into scorbutic	4	66	60–71	14
Normal into scorbutic	2	No data*		
Scorbutic into normal	9	67	60–71	18

* Since the recipient guinea pigs died, no further data were available.

be analyzed for their role in the successful survival of the circulating corpuscle. As with all technics, studies of intravascular survival reveal only one segment of the life history of the erythrocyte. With scientific caution it must be remembered that these studies have determined the effect or lack of effect of certain agents on survival time once the erythrocyte is in circulation. Cholinesterase is apparently not a survival factor for erythrocytes in circulation, but who can say it is not a vital material in the complicated genesis of the non-nucleated erythroplast? And conversely certain hemolytic drugs may be effective only on circulating erythrocytes, bypassing the marrow in which these elements are generated.

REFERENCES

1. ALLISON, A. C. and BURN, G. P. Enzyme activity as a function of age in the human erythrocyte. *Brit. J. Haemat.*, 1: 291, 1955.
2. TARLOV, A. R. and KELLERMEYER, R. W. The hemolytic effect of primaquine. XI. Decreased catalase activity in primaquine-sensitive erythrocytes. *J. Lab. & Clin. Med.*, 58: 204, 1961.
3. MARVIN, H. N. and LUCY, D. D. The survival of radiochromium-tagged erythrocytes in pigeons, ducks and rabbits. *Acta Haemat.*, 18: 239, 1957.
4. MARVIN, H. N. Evidence of erythrocyte incompatibility in pigeons of an inbred strain using radiochromium-tagged cells. *J. Cell. & Comp. Physiol.*, 53: 13, 1959.
5. SCHIØDT, E. On the duration of life of the red blood corpuscles. *Acta med. scandinav.*, 95: 49, 1938.
6. EADIE, G. S. Studies of red cell survival. *Ann. New York Acad. Sc.*, 77: 737, 1959.
7. BERLIN, N. I., WALDMANN, T. A. and WEISSMAN, S. M. Life span of red blood cell. *Physiol. Rev.*, 39: 577, 1959.
8. CORNELIUS, C. E., KANEKO, J. J., BENSON, D. C. and WHEAT, J. D. Erythrocyte survival studies in the horse, using glycine-2-C^{14}. *Am. J. Vet. Res.*, 21: 1123, 1960.
9. AULSEBROOK, L. H. and MARVIN, H. N. Unpublished data.
10. EERNISSE, J. G. and VAN ROOD, J. J. Erythrocyte survival-time determinations with the aid of $DF^{32}P$. *Brit. J. Haemat.*, 7: 382, 1961.
11. BRACE, K. C. and ALTLAND, P. D. Red cell survival in the turtle. *Am. J. Physiol.*, 183: 91, 1955.
12. MARVIN, H. N. Unpublished data.
13. DAY, P. L. and DINNING, J. S. Anemia in vitamin E-deficient monkeys. *Fed. Proc.*, 15: 548, 1956.
14. MARVIN, H. N., DINNING, J. S. and DAY, P. L. Erythrocyte survival in vitamin E-deficient monkeys. *Proc. Soc. Exper. Biol. & Med.*, 105: 473, 1960.
15. MARVIN, H. N. and PANOS, T. C. To be published.