The Effect of Vitamin E on the Preservation of Blood¹

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By observing the effects of tocopherylacetate (Toc-ac) and tocopheryl glycolate (Toc-glyc) on the stored citrate-phosphate-dextrose (CPD) blood and the blood added with EDTA-K₃, the following results were obtained.

1. By adding 10, 20, and 50 mg, respectively, of Toc-ac per 100 ml or 20 and 50 mg Toc-glyc per 100 ml, a daily decline of osmotic resistance of erythrocytes in the CPD-stored blood could be suppressed to a certain extent.

The range of effects revealed that 50 mg per 100 ml was the greatest in effectiveness and that Toc-ac and Toc-glyc had almost the same effectiveness.

2. It was found that the preservative action of Toc-ac and Toc-glyc for the osmotic resistance of erythrocytes was not due to the action of the solvent in tocopherol.

3. With respect to the specimens added with 10 or 20 mg per 100 ml of Toc-ac, more erythrocytes with normal morphology were observed on the 7th day after the blood sampling, and in the former there was no difference from the one added with the solvent. That is, the residual rate of normal erythrocytes in both cases was somewhat higher, and the solvent itself seemed to have more or less preservative effectiveness in a comparatively short period. In the specimens added with 20 and 50 mg of Toc-glyc per 100 ml, the residual rate of erythrocytes with normal morphology showed the same high value on the 6th day after the blood sampling, when compared with the group without addition and the group added with the solvent.

4. Both in the CPD blood added with 10, 20 and 50 mg per 100 ml of Toc-ac, 20 and 50 mg per 100 ml, respectively, of Toc-glyc and in the EDTA blood added with 50 mg per 100 ml of Toc-ac, the decrease of the number of leucocytes after the blood sampling was suppressed to a certain extent. Moreover in the latter group, there was neither the relative decrease of granulocytes in the differential count of leucocytes nor the increase of lymphocytes observed for 6 hours after the blood sampling.

5. Tocopherol added group showed the tendency of a hourly decrease of thrombocytes depressed in the EDTA blood. Furthermore, the preservative effect on the morphology of thrombocytes in the same group was comparatively good.

¹ Following abbreviations are used: Toc-ac, α -tocopherylacetate; Toc-glyc, α -tocopherylglycolate; CPD, citrate-phosphate-dextrose

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Some reports have been published on the effects of tocopherol on the preservation of osmotic resistance of erythrocytes (1-4), and on the preservation of stability under mechanical stimulus (5) or hyperbaric oxygenation (6). Since the authors had been interested in the preservation of an hourly stability of blood cells treated with an anticoagulant, it was considered an interesting and practical question whether the effects of tocopherol are useful for the preservation of various types of blood cells.

As the results of the studies employing Toc-ac and Toc-glyc, the vitamin was proved to be effective on the preservation of leucocytes and thrombocytes in part as well as erythrocytes.

MATERIALS AND METHODS

1. Bloods

The blood was collected from normal persons of both sexes, aged 20-32. CPD (7) and EDTA-K₃ were used as anticoagulants. In the case of citrate-phosphate-dextrose (CPD), 36 ml of blood was collected under a germ-free condition and mixed with 14 ml of CPD solution, the mixture being sealed up in sterilized vials, 2 ml each, and preserved at 4°. The blood was discarded every time after use and never used again. EDTA-K₃ was dissolved as a 2.5% solution, 0.1 ml of which was dried in a vial mixed and with 2 ml of blood.

2. Methods

Osmotic resistance of erythrocytes — It was determined by Dacie's method (8) for 3 to 4 weeks at intervals of 4 to 7 days.

Number of erythrocytes and leucocytes — Calculation was made according to the authors' method (9,10) employing the Toa microcell counter Model 1.

Hemoglobin value — Van Kampen's method was used (11).

Hematocrit [packed cell volume] — The microhematocrit method was used.

Number of thrombocytes — Calculation was made by Brecher-Cronkite's method (12) modified partially (13).

Differential count of leucocytes — In the ordinary Giemsa staining, 5 sheets of smear were prepared, and 100 leucocytes were classified by 5 technicians, and the percentage was obtained on an average.

Morphology of erythrocytes — The CPD blood specimen was diluted to the suitable concentration with the subject's own blood plasma, and was put into a Bürker-Türk hemocytometer to classify the normal round erythrocytes as Type 1, the slightly crenated ones as Type 2, the crenated ones as Type 3, and the swollen oval ones as Type 4 (14).

Both Toc-ac and Toc-glyc used in the present experiments were the products of Eisai Co. Ltd. Tokyo.

RESULTS

1. Evaluation of Osmotic Resistance of Erythrocytes

Effect of solvent — As there is a report about the maintaining effect of the solvent of tocopherol on the osmotic resistance of erythrocytes, the blood specimens of a normal person added with 50 mg per 100 ml of Toc-ac or Toc-glyc or those added with the same amount of the solvent contained in Toc-ac or Toc-glyc were compared with the controls, *i.e.*, the identical specimen on the 4th day after the blood sampling not added with Toc-ac, Toc-glyc or solvent, and the specimen collected from the same person immediately before the experiment with no addition. As shown in Fig. 1, the grade of hemolysis in the groups of solvent was clearly higher than the controls, and the difference in hemolysis was more marked when compared with the tocopherol added group.

FIG. 1 Effect of tocopherol and solvent on osmotic resistance of erythrocytes

●—●, control (4th); △—△, solvent (4th); ▲—▲, tocopherol (4th); ○—○, fresh blood.

FIG. 2 Rate of hemolysis (Toc-ac, 10 mg per 100 ml) —, 0.85 % NaCl; ---, 0.60 % NaCl; —, 0.50 % NaCl; ●, control; ○, tocopherol.

Addition of 10 mg Toc-ac per 100 ml of CPD Blood.

In the case of 0.85 % saline — Hemolysis was hardly observed until the 17th day after the blood sampling in the control group without addition of Toc-ac (Group C). On the 24th day, the grade of hemolysis was 29 ± 15.5 % in Group Toc-ac, and on the 31st day, 70.8 ± 4.4 % in Group C and 45 ± 17 % in Group Toc-ac, the results obtained on the 31st day showing the statistical significance between the two groups (Fig. 2).

In the case of 0.60 % saline — The measurement was conducted from the 17th day, and the grade of hemolysis in Group C and Group Toc-ac was 19.2 ± 17.2 and 3.8 ± 5.7 %, respectively, on the 17th day, 46.0 ± 11.2 and 43.5 ± 17.2 % on the 24th day, and 84.0 ± 4.1 and 58.2 ± 23.3 % on the 31st day, showing no significance in each case but the tendency of less hemolysis in Group Toc-ac.

In the case of 0.50 % saline — At the time of blood sampling there was hardly any significance between Group C (3 ± 3 %) and Group Toc-ac (2 ± 1 %), and the grade of hemolysis in both groups showed a steady increase as seen in 23.5 ± 32.7 % in Group C and 9.7 ± 12.6 % in Group Toc-ac on the 15th day, 30.5 ± 15.5 and 28.5 ± 6.9 % respectively, on the 17th day, and 93.4 ± 3.7 and 74.9 ± 12.6 % on the 31st day. The result showed a significantly less grade of hemolysis in Group Toc-ac than in Group Toc-glyc.

Addition of 20 and 50 mg per 100 ml of Toc-ac

In the case of 0.85 % saline — The experiment was carried out for 3 weeks. The grade of hemolysis in Group C, Group Toc-ac added with 20 mg of Toc-ac, and Group Toc-ac added with 50 mg was in the range of 2 ± 3 %. There was hardly any significance among the groups.

In the case of 0.60 % saline — The grade of hemolysis before the 15th day after the blood sampling remained within 5 % in each group. On the 15th day, Group C showed 5.0 ± 1.6 %, Group Toc-ac-20, 3.0 ± 1.1 %, and Group Toc-ac 50, 2.8 ± 0.3 %. There was a significance with the fiducial limits of 95 % between Group C and Group Toc-ac-50. On the 21st day, the significance between Group C and Group Toc-ac became more marked, Group C showing 12.3 ± 1.3 %, Group Toc-ac-20, 3.1 ± 1.1 %, and Toc-ac-50, 2.6 ± 0.7 %. There was a significance with the fiducial limits of 99 % between Group C and each of Group Toc-ac-20 and Group Toc-ac-50.

In the case of 0.50 % saline — At the time of blood sampling, Group C, Group Toc-ac-20 and Group Toc-ac-50 showed 8.1 ± 3.5 , 9.0 ± 5.4 , and 12.5 ± 9.6 %, respectively; on the 5th day, 9.4 ± 3.2 , 10.1 ± 6.0 and 4.8 ± 1.5 %, on the 7th day, 8.9 ± 2.7 , 6.8 ± 2.2 , and 6.6 ± 2.6 %; and on the 12th day, 8.1 ± 2.8 , 6.9 ± 3.8 , and 4.5 ± 1.3 % without a large difference among them, but on the 15th day, the difference became larger among them as shown in 17.9 ± 6.9 , 9.9 ± 3.7 , and 6.9 ± 4.7 %, respectively. On the 21st day, Group C showed 35.0 ± 10.6 %, Group Toc-ac-20, 15.7 ± 10.0 % and Group Toc-ac-50, 12.8 ± 5.8 %. There was a significance with the fiducial limits of 98 % between Group C and Group Toc-ac-20 and with the limits of 95 % between Group C and Group Toc-ac-50.

In the case of 0.44 % saline — The measurement was carried out from the 7th day when Group C showed 7.0 ± 2.2 , Group Toc-ac-20, 4.0 ± 1.2 , and Group Toc-ac-50, 3.6 ± 1.5 %; on the 13th day, 39.7 ± 21.4 , 16.3 ± 10.9 , and 32.3 ± 13.9 %, respectively; on the 15th day, 58.3 ± 15.9 , 46.7 ± 19.4 , and 44.2 ± 15.8 %; and on the 21st day, 67.2 ± 16.7 , 50.6 ± 17.4 , and

FIG. 3 Rate of hemolysis
(Toc-ac, 20, 50 mg per 100 ml)

●, control; ○, 20 mg per 100 ml; ⊙, 50 mg per 100 ml.

47.0 ± 13.7 %, showing no statistical significance but less decline of osmotic resistance was found in the group added with Toc-ac. In this experiment, the result obtained after the 13th day, except for the findings in the case of 0.44 % saline on the 13th day, showed the tendency of lower grade of hemolysis in Group Toc-ac-50 as compared with Group Toc-ac-20 (Fig. 3).

Addition of 20 and 50 mg per 100 ml of Toc-glyc

In the case of 0.85 % saline — The experiment was carried out for 3 weeks. There was no larger significance among the range of 1.8 ± 4.1 % in Group C, 2.7 ± 0.5 % in Group Toc-glyc-20, and 3.5 ± 2.6 % in Group Toc-glyc-50; the increase of the grade of hemolysis in the group added with Toc-glyc being suppressed as compared with the group without addition of the vitamin.

In the case of 0.60 % saline — The grade of hemolysis in each group remained within 5 % at its maximum until the 15th day after the blood sampling. On the 21st day, Group C showed 12.3 ± 1.3 %; Group Toc-glyc, 5.0 ± 1.7 %; and Group Toc-glyc-50, 2.7 ± 0.5 %.

In the case of 0.50 % saline — The grade of hemolysis at the blood sampling in Group C, Group Toc-glyc-20 and Group Toc-

FIG. 4 Rate of hemolysis
(Toc-glyc, 20, 50 mg per 100 ml)

●, control; ○, 20 mg per 100 ml; ⊙, 50 mg per 100 ml.

glyc-50 was 8.0 ± 3.5 , 3.4 ± 3.6 , and 7.9 ± 3.7 %, respectively; on the 5th day, 9.4 ± 3.5 , 6.2 ± 4.4 , and 5.2 ± 3.7 %; on the 13th day, 8.1 ± 2.8 , 5.1 ± 1.9 , and 6.8 ± 2.4 %; on the 14th day, 17.9 ± 7.7 , 8.2 ± 2.1 , and 4.7 ± 1.9 %; and the 21st day, 35.0 ± 13.4 , 18.7 ± 7.5 , and 12.3 ± 8.1 % (Fig. 4).

2. *Number of Erythrocytes, Hemoglobin Value, and Hematocrit Value (packed cell volume)*

In the case of EDTA blood — Studies for 24 hours after the blood sampling revealed the following results: The number of erythrocytes (unit; $10^4/\text{mm}^3$) at the blood sampling was 417 ± 12.9 in Group C, and 421 ± 19.4 in the group added with Toc-ac-50. The number after 2, 4, 6, and 24 hours was 410 ± 16.5 , 416 ± 22.5 , 410 ± 22.7 , 413 ± 21.2 respectively in Group C and 415 ± 11.7 , 415 ± 18.9 , 403 ± 23.6 , and 415 ± 20.4 , respectively in Group Toc-ac-50. The hemoglobin value at the blood sampling was 12.2 ± 0.9 in Group C and 12.2 ± 0.9 g/100 ml in Group Toc-ac-50. The value after 2, 4, 6, and 24 hours was 12.0 ± 0.9 , 12.4 ± 1.0 , 12.6 ± 1.0 , and 11.8 ± 1.1 g/100 ml, respectively, in Group C; 12.0 ± 0.9 , 12.8 ± 0.8 , 12.6 ± 1.0 , and 11.4 ± 1.0 g/100 ml in Group Toc-ac-50. The hematocrit value at the blood sampling, after 2, 4, 6, and 24 hours was 37.0 ± 2.2 , $38.5 \pm$

2.2, 38.3 ± 2.4 , 37.4 ± 2.4 , and 38.3 ± 2.6 %, respectively, in Group C; 37.7 ± 2.6 , 38.3 ± 2.5 , 36.7 ± 2.5 , 37.3 ± 2.6 , and 37.5 ± 2.3 % in Group Toc-ac-50.

In the case of CPD blood — The number of erythrocytes (unit; $10^4/\text{mm}^3$) at the blood sampling was 365 ± 10 in Group C and 344 ± 41 in Group Toc. Until the 31st day, the number showed fluctuations, seemingly without any significant rise and fall, between 343~312 in Group C and 332~320 in Group Toc.

3. Morphology of Erythrocytes

CPD blood of normal persons was prepared, and with the blood plasma collected from the same person the optimal volume of the CPD blood was diluted to make the sufficient concentration to distinguish erythrocytes separately one by one, and classified in a Bürker-Türk hemocytometer. According to the standard by Nakao *et al.* (4), normal erythrocytes with a round disc form were classified as Type 1, those with a slightly

FIG. 5 Types of erythrocytes

crenated form as Type 2, those with a crenated form as Type 3, and those with a swollen oval form as Type 4 (Figs. 5-6).

10 and 20 mg of Toc-ac, and 20 and 50 mg of Toc-glyc were added per 100 ml of blood,

Control

Toc-ac 10 mg per 100 ml

Control (solv.)

Toc-ac 20 mg per 100 ml

FIG. 6 Erythrocytes (7th day)

respectively. There were control specimens without addition and the groups added with the same amount of the solvent containing Toc-ac and Toc-glyc, respectively.

CPD blood was measured in sterilized vials, 2 ml each, and the blood was discarded every time after use. As is seen from Tab. 1, there was no difference between Toc-ac-10 group and Toc-ac-20 group until the 4th day after the blood sampling; on the 7th day, Type 1 in the group without addition of the vitamin showed the decrease, while Toc-ac-20 group was constant in value, and the groups added with the solvent and 10 mg of tocopherol showed mean values. On the 10th day, the residual rate of normal erythrocytes was lower than on the 7th day, but the Toc-ac-20 group showed the highest residual rate. Toc-glyc was also tested once on the 6th day after the blood sampling. While in the control group without addition of the vitamin the residual rate in Types 1, 2, 3 and 4 was 54.9; 18.3; 31.3, and 3.5 %, respectively; in the Toc-glyc-20 group, 81.5; 8.7; 9.0, and 0.8 %; and in Toc-glyc-50 group 83.6; 7.6; 8.9, and 0 %, showing the higher residual rate of normal erythrocytes in the group added with Toc-glyc. When 50 mg of Toc-ac was added per 100 ml to the identical specimen for comparison, the

residual rate in Types 1-4 was 78.2; 9.3; 11.4 and 1.4, respectively, showing approximately the same results as in the group added with 50 mg of Toc-glyc per 100 ml. When the solvent containing in 20 and 50 mg of Toc-glyc, respectively, was added, the results were almost the same as those in the group without addition. The residual rate of normal erythrocytes was noticeably lower than those added with tocopherol.

4. Number of Leucocytes

In the case added with 10 mg of Toc-ac per 100 ml — The number of leucocytes at the blood sampling was 4,900/mm³, and on the 4th day that in Group C and Group Toc-ac was 4,300 and 4,200, respectively; on the 7th day, 2,900 and 4,100; on the 11th day, 2,100 and 3,400; on the 24th day, 2,000 and 3,500; and on the 31st day, 1,500 and 2,800, respectively. The figure showed the marked tendency to decline from the 7th day in the Group C, and from the 11th day in Group

Exp	Type	Days				
		1	4	7	10	14
Control (none)	I	84.4	81.2	42.6	14.1	2.9
	II	9.6	9.1	15.8	12.7	15.2
	III	6.1	7.0	41.7	73.2	73.1
	IV	0	0	0	0	8.8
Control (solvent)	I	82.0	78.0	51.0	28.6	3.6
	II	9.4	10.9	25.8	32.1	13.1
	III	8.5	11.1	23.3	39.4	70.2
	IV	0	0	0	0	13.2
Toc-ac 10 mg per 100 ml	I	83.0	80.5	51.4	30.0	11.4
	II	12.0	11.0	25.7	33.9	26.3
	III	5.0	8.5	22.9	35.6	57.6
	IV	0	0	0	0	13.1
Toc-ac 20 mg per 100 ml	I	85.2	84.0	79.0	45.0	15.4
	II	11.3	10.9	13.0	28.6	25.3
	III	3.6	5.1	7.6	26.3	50.1
	IV	0	0	0	0	9.2

FIG. 7 Tocopherol effect on leucocyte numbers
●, control; ○, tocopherol.

Toc-ac. There was a marked difference in the extent of decline between the two groups, and the tocopherol-added group showed a less declining tendency (Fig. 7).

In the case added with 20 and 50 mg of Toc-ac per 100 ml—The number of leucocytes at the blood sampling in Group C, Group Toc-ac-20 and Toc-ac-50 was 6,200; 6,000 and 5,900, respectively; on the 5th day, 4,100; 4,400, and 4,700; on the 12th day 3,500; 3,900 and 4,800; on the 15th day, 3,700; 4,100 and 4,300; and on the 21st day, 3,100; 3,100 and 3,900, respectively, showing a less daily declining tendency in the tocopherol group.

In the case added with 20 and 50 mg of Toc-glyc per 100 ml—The number of leucocytes at the blood sampling in Group C, Group Toc-glyc-20, and Group-Toc-glyc-50 was 6,200; 6,200 and 5,900, respectively; on the 6th day, 4,700; 4,800 and 4,800; on the 12th day 3,500; 4,500 and 4,500; and on the 21st day 3,100; 3,200 and 3,200, respectively, showing a marked partial suppression of decline on the 12th day but no difference in decline on the 21st day.

In the case added with 50 mg of Toc-ac per 100 ml of EDTA-K₃ blood—The number of leucocytes at the blood sampling in Group C, and Group Toc-ac was 6,740 and 6,420; after 2, 4, 6, 24 and 48 hours, they were 6,360; 5,660; 5,840; 5,700 and 3,840, respectively, in Group C; in Group Toc-ac they were 6,380; 6,000; 6,180; 6,500 and 5,180, respectively, showing a marked suppression of the decline in the latter group (Fig. 7).

5. Morphology of Leucocytes

EDTA-K₃-added blood was used for the study. Lymphocytes in Group C at the blood sampling, after 2, 4, 6, and 24 hours were 38.7; 34.3; 40.4; 46.5 and 81.7 %, respectively, showing an hourly rise of relative appearance. No significant alteration in Toc-ac-50 Group was observed until after 6 hours, but the same extent of high appearance as in the control group was shown after 24 hours. Segmented leucocytes were 57.1; 50.9; 51.2; 48.5 and 16.3 %, respectively, in the control group; and 51.4; 57.5; 61.5; 57.1 and 16.7 % in the tocopherol group. While in the control group the

appearance of segmented leucocytes gradually declined, contrary to the case of lymphocytes, the value in Group Toc was almost constant until 6 hours, except for the slight difference of value immediately after the blood sampling.

6. Number of Thrombocytes

The number of thrombocytes in 4 examples observed at the blood sampling, after 2, 6 and 24 hours was 22.8 ± 3.9 , 22.0 ± 4.4 , 21.6 ± 2.6 and 18.8 ± 3.7 , (unit: $10^4/\text{mm}^3$), respectively, in the control group; 23.4 ± 2.3 , 24.8 ± 3.9 , 24.4 ± 4.7 and 23.3 ± 2.6 in Toc-ac-50 group. The hourly decline seemed to have been suppressed in the latter. When examined under a phase contrast microscope, not only the ghosts of erythrocytes were of less deformity but also the morphology of thrombocytes was preserved in a good form (Fig. 8).

Tocopherol/added Thrombocytes

Control

FIG. 8 Tocopherol effect on thrombocyte

DISCUSSION

As described above, the authors have reported the effects of tocopherol esters upon the number of various types of blood cells. Weinstein (1) was the first to report the effects of tocopherol to inhibit the time-dependent decline of osmotic resistance of erythrocytes in stored blood using tocopherol phosphate. Luczak and Wolf (2,3), and Körner-Kleine (4) have reported the same results using Toc-ac. As the authors' object was not only to pursue the physiological activities of tocopherol but also to make tocopherol contribute to the application of routine clinical test, it was planned to study the minimum volume of tocopherol required or its optimum volume in order to avoid undesirable phenomenon such as the increase of viscosity following addition of the vitamin as well as to obtain desired results. Toc-glyc was also used for the study, and at the same time the relation to the solvent of tocopherol (fat-soluble surface active agent) was also studied, since Reber (15) in recent years conducting experiments with natural hemolysis rate of stored blood as an indicator, reported that the solvent alone inhibited markedly. Although in the present experiments CPD blood sampling was carried out in a germ-free condition, it cannot be denied that there might have been some degree of pollution in the process of experiments such as measuring into glass vials. Conserving temperature was also relatively high. Even under such circumstances, the authors obtained the same results as those by Luczak *et al.* (3), which showed that it was not the solvent itself but tocopherol itself that was effective in preserving the osmotic resistance of erythrocytes, contrary to the skeptic opinion of Reber (15). There was a difference of the grade of hemolysis observed between the control and the group added with 10 mg of Toc-ac per 100 ml on the 15th day in the case of 0.5 % saline; on the 17th day in the case of 0.6 % saline; and on the 25th day in the case of 0.85 % saline. The difference between the two was much greater on the 30th day.

When 20 or 50 mg of Toc-ac per 100 ml was used, the effect was more marked in the latter, as the experiment was conducted with the identical blood under the identical conditions. It is difficult to compare Toc-ac-10 group with Toc-ac-20 or Toc-ac-50 group, because the conditions of the experiment were not identical. However, the comparison between the result of Toc-ac-10 group with 0.5 % saline on the 17th day and the results of Toc-ac-20 and Toc-ac-50 groups with 0.5 % saline on the 20th day showed that the grade of hemolysis of the former was 9-10 % higher. As it is obvious from the entire process that the grade of hemolysis increased daily, it is easily inferred that Toc-ac-20 and -50 groups have the greater suppressive effects on the hemolysis.

The effects of the addition of 20 and 50 mg of Toc-glyc per 100 ml were almost the same as those of the addition of 20 and 50 mg of Toc-ac per 100 ml just mentioned above, and the effect of 50 mg per 100 ml was greater.

As for the mechanism of the effects of tocopherol on erythrocytes, the influence upon the membrane may be considered. In this respect, it is interesting to note the results obtained by Kleine (16) who showed the suppression of the decline of osmotic resistance of erythrocytes even after washing the tocopherol-added blood with physiological saline. It is said that of all the lipids contained in the membrane of erythrocytes (17) tocopherol suppresses the peroxidation of highly unsaturated fatty acids and prevent the degeneration of stroma protein and the decline of lipid metabolism of the tissue (18). On the other hand, Lucy and Dingle (19) reported that since long isoprenoid compounds had an anti-hemolytic action, the function and the structural properties of tocopherol esters mentioned above could participate in the anti-hemolytic activities. Therefore, it seems natural that Toc-ac and Toc-glyc have produced almost the same effects, since the two compounds have similar long-chained isoprenoid structure. There was no difference in the value of erythrocytes in CPD blood itself by the addition of the vitamin. It may not be strange

that no difference was observed in about 30 days in the present experiment, because the erythrocyte life span is about 120 days (20). However, the fact that tocopherol esters preserve the osmotic resistance of erythrocytes, seems to have brought forth a new problem in the preservation of blood.

With respect to the morphology of erythrocytes, Toc-ac was effective in the preservation of morphology, when 10 mg per 100 ml was used, and it was more effective when 20 mg was used. It was found that the effectiveness until the 10th day corresponded to that on the 4-7th day in the case without addition. At the same time, it was found that Toc-glyc had the same effect. Recently, the abnormalities of the morphology of erythrocytes due to hemolytic anemia (21) and the abnormal metabolism of lipids (22) have attracted attention. On the other hand, it was considered that the senescence of normal erythrocytes was due to the decline of the levels of proteins and fats which are not synthesized by erythrocytes and to the resulting decline of the levels of glycolytic substances (23). It is said that in senescent erythrocytes the contents of total lipids, phospholipids, and cholesterol decline (24) and the surface of the membrane becomes electron-microscopically thin and smooth (25). It has already been pointed out that the preservation of osmotic resistance of erythrocytes by tocopherol depends on the preservation of the lipid metabolism. Jacob and Lux (26) reported on phospholipids that in tocopherol-deficient erythrocytes the destruction of phosphatidylethanolamine containing much unsaturated fatty acids was marked. Accordingly, it is conceivable that the addition of tocopherol contributes to the preservation of the morphology of erythrocytes through the suppression of these disturbances of lipid metabolism.

On the other hand, it has been reported by Nakao *et al.* that the preservation of the morphology of erythrocytes depends largely on the ATP content of erythrocytes. The report said that when the ATP content fell to below 1/2 of the normal content, the erythrocytes became crenated; below 1/10, it became smooth

and round, and when ATP was recovered to the normal level by adding adenine and inosine, the normal morphology was recovered (14).

While Jacob and Lux (26) reported that there was no difference in various energy-rich metabolites when tocopherol-deficient erythrocytes were disturbed by the addition of peroxide, there is another report that tocopherol is related more or less to the energy metabolism. For example, in tocopherol deficiency the disturbance of oxidative phosphorylation and the reduction of the glycogen levels in liver and heart muscle occurred (27), and by administration of tocopherol the storage of glycogen was observed (28). It was also reported that in tocopherol deficiency there was a disturbance of the enzyme converting glucose-1-phosphate to glucose 6-phosphate (29). It is one of the interesting questions to be solved how the effects of energy metabolism and the addition of tocopherol are correlated to erythrocytes.

Though it is well known that the number of leucocytes decreases hourly in ACD-stored blood or in EDTA blood, the mechanism is not yet clarified. Recently, lysosome has been attracting the attention, and the presence of lysosomes in various leucocyte cells including granulocytes and lymphocytes was observed (31). It is naturally conceivable that the emigration of lysosome necessarily causes the degeneration and destruction of leucocytes, and it is reported that this emigration is more or less related to the peroxidation of membrane lipids (32). It has already been mentioned that the preservative effects of tocopherol on the membrane of erythrocytes depend on the peroxidation of unsaturated fatty acids. To connect this concept with the results obtained by the authors is a very interesting problem.

Lastly, with respect to the effects on the preservation of thrombocytes, the authors' results showed no significance in the value at the blood sampling presumable due to the collection of venous blood, but more or less suppression of hourly reduction by adding tocopherol. Thrombocytes are caused to agglutinate by thrombin (33). Zierler *et al.* (34) showed

that Toc-phosphate had an antithrombin effect in blood coagulation. Recently, the authors observed that the thrombocyte aggregation caused by addition of ADP seemed to be somewhat depressed by adding a certain amount of Toc-ac or Toc-acetophosphate (35). These mechanisms remain to be elucidated. However, it may be conceivable that the Ca^{2+} chelating action of EDTA used as an anticoagulant in the experiment and the antioxidative action of tocopherol seems to act at least synergistically.

The results obtained showed the usefulness of tocopherol for the preservation of blood cells suggesting the possible clinical use in future.

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