over, in the cortical cytoplasm, thin filaments usually arranged in large bundles. running parallel to the long axis of the cell, were often detectable (figure 2). The filaments measured 50–70 Å in diameter and often the bundles showed a cross-striated appearance due to the presence of electron-dense bodies throughout their length. Numerous microtubules were also visible.

In indirect immunofluorescence, the cells of the regenerating tissue displayed an intense reaction to human anti-smooth muscle antibodies (figure 3). Staining appeared more intense than in the controlateral tendon (figure 4). The fluorescent cells were uniformly distributed both in the peripheral part and in the centre of the regenerating tissue. No fluorescence was detectable when normal human sera were employed in the same test.

Discussion. In a previous study[10] tenocytes were shown to have morphological and immunochemical characteristics of contractile cells. These findings suggested that tenocytes may be considered myofibroblasts and tendon a contractile organ. Our observations demonstrate that contractile structures are also present in the cells of the granulation tissue during the early stages of tendon regeneration in an amount by far larger than in normal tendon.

In adult tenocytes, the contractile apparatus has been considered to be involved in the modulation of the contractile and retractile activity of the muscle-tendon functional unit[10]. Since during the early stages of the regeneration this modulation is temporarly interrupted, contractile structures could then play a determinant role in a) the process of adhesion, movement and orientation of the newly differentiated cells, and b) in the retraction of the regenerated tissue. These new functions could require a contractile apparatus more developed than that necessary for the normal modulatory activity. A system of cytoplasmic contractile filaments similar to those described in this study has been observed in amoeba[13] and in cultured chick embryo fibroblasts[14], where they seem to be critical for cell-adhesion and mobility. It is likely that such contractile structures play a similar role in the myofibroblasts of regenerating tendon. In fact, between the 2 ends of a cut tendon, a large gap is formed which is initially filled with fibrin clot and blood cells. Successively, cells of the peritendineous sheaths proliferate and progressively invade the blood clot[15,16]. The main role of the contractile apparatus could, therefore, be related to the ameboid movements which allow the cells of the peritendineous sheats to invade and substitute the initial blood clot. At this stage, a retraction of the newly formed tendon probably occurs. The contractile apparatus might at this time play an important role, as has been shown to be the case in the wound contraction described by Gabbiani et al.[4,5,7]. Only at later stages when the cells of the regenerated tissue acquire the morphological and functional characteristics of adult tenocytes[17], the fibrillar system would assume the function played in normal tendon.

12 E. M. Tan, J. clin. Invest. *46*, 735 (1967).
13 P. D. Pollard and S. Ito, J. Cell Biol. *46*, 267 (1970).
14 J. F. Perdue, J. Cell Biol. *58*, 265 (1973).
15 T. Skoog and B. H. Persson, Plast. reconstr. Surg. *13*, 384 (1954).
16 F. Postacchini, E. Ippolito and C. Morlacchi, G. ital. Ortop. Traumat. *1*, 415 (1975).
17 F. Postacchini, P. G. Natali, L. Accinni, E. Ippolito, C. De Martino, in preparation.

Erythropoiesis and plasma tocopherol levels in irradiated mice

R. Kobric and R. M. Roy

Department of Biological Sciences, Sir George Williams Campus, Concordia University, Montreal (Canada),
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Summary. Plasma protein and tocopherol concentrations, haematocrit and 59-iron incorporation into erythrocytes have been measured in vitamin E-deficient and supplemented mice before and after exposure to 500 R of 260 kVp X-ray. Supplemented animals had greater haematocrit, plasma tocopherol and protein levels initially. After irradiation plasma tocopherol concentration decreased drastically in the vitamin E-supplemented mice.

The haematopoietic system is particularly sensitive to ionizing radiation[1]. In view of the role of alpha tocopherol in protecting cell membranes from peroxidation[2], protecting red blood cells from hemolysis[3,4] and its role in haematopoiesis[5], the tocopherol status of an organism may be expected to have a great influence on the radiation-response of that organism.

Several studies have been carried out to test the modifying influence of vitamin E on radiation damage[6–9] and the results have been conflicting. This may be due to the fact that tissue tocopherol levels are not readily increased by injection of some forms of tocopherol[10] and furthermore normal lab diets are sufficiently high in tocopherols that further supplementation with vitamin E has little effect

1 E. B. Harris, in: Manual on Radiation Haematology, p. 99. IAEA technical report, Vienna 1971.
2 A. L. Tappel, Ann. N. Y. Acad. Sci. *203*, 12 (1972).
3 E. W. Prince and J. B. Little, Radiat. Res. *53*, 49 (1973).
4 A. Hoffer and R. M. Roy, Radiat. Res. *61*, 439 (1975).
5 C. D. Fitch, Ann. N. Y. Acad. Sci. *203*, 172 (1972).
6 T. J. Haley, E. F. McCulloh and W. G. McCormick, Science *119*, 126 (1954).
7 B. H. Ershoff and C. W. Steers, Jr, Proc. Soc. exp. Biol. Med *104*, 274 (1960).
8 U. K. Alekperov and D. D. Akundova, Genetika *10*, 12 (1974).
9 D. D. Akundova and U. K. Alekperov, Izv. Akad. Nauk SSSR *1*, 3 (1973).
10 H. Newmark, W. Pool, J. C. Bauernfeind and E. De Ritter, J. pharmac. Sci. *64*, 655 (1974).

Changes in blood parameters following X-irradiation of vitamin E-supplemented and vitamin E-deficient mice

Time post irradiation (days)	Haematocrit (%) Supplemented		Deficient	Plasma protein (mg/ml) Supplemented		Deficient	Serum tocopherol (μg/ml) Supplemented	Deficient	59-Iron activity (cpm × 10^{-3}) Supplemented		Deficient
0	40 ± 2.3	*	35 ± 2.2	46 ± 13	*	30 ± 8	30	1.0	195 ± 10		182 ± 24
2	36 ± 2.2		35 ± 2.3	47 ± 7		43 ± 14	18	0.9	40 ± 22		54 ± 25
4	34 ± 2.4		30 ± 4.3	43 ± 8		38 ± 9	15	0.9	0		0
6	27 ± 1.7		29 ± 4.3	36 ± 6	*	30 ± 5	10	0.9	105 ± 95		127 ± 50
8	27 ± 1.7		27 ± 2.5	46 ± 5	*	37 ± 6	10	0.9	138 ± 86		97 ± 69
10	27 ± 3.8		23 ± 3.3	43 ± 12		41 ± 13	9	0.9	132 ± 64	*	241 ± 55

* Significant ($p < 0.05$) differences between supplemented and deficient groups compared by t-test. SD are indicated in the table ($n = 5$).

with respect to radiation response. In addition to modifying the initial radiation damage, it is conceivable that tocopherol levels post irradiation may influence recovery processes. Therefore this study will evaluate the combined influence of these 2 effects on blood parameters in irradiated mice.

Materials and methods. After weaning, male Swiss albino mice (Canadian Breeding Farm and Laboratories Ltd) were maintained on vitamin E-deficient and vitamine E-supplemented (200 mg dl-α-tocopherol per kg diet) rations for 110 days prior to irradiation as previously described[4]. Food and water were provided ad libitum. Haematocrit, plasma tocopherol and 59-iron incorporation into red blood cells were measured in groups of 5 animals before exposure to 500 R of 260 kVp X-ray at 20 R/min generated from a Muller MG-300 X-ray machine and at 2-day-intervals for 10 days post-irradiation. Animals were injected i.p. with 0.7 μCi $^{59}FeCl_3$ in 0.9% NaCl (NEN) 2 days prior to sacrifice. Blood was removed in a heparized syringe by cardiac puncture. Blood cells were washed twice by centrifugation and the protein content determined by Biuret test. Plasma was combined from 5 animals for serum tocopherol determination using the Desai modification[11] of the Quaife and Harris method[12].

Results. Haematocrit decreased following irradiation (table). Vitamin E-supplemented animals had significantly higher pre-irradiation haematocrit but this decreased by 2 days post irradiation ($p < 0.05$, t-test). Thereafter, there were no significant differences in haematocrit between vitamin E-supplemented or deficient animals.

Plasma protein levels were significantly higher in vitamin E-supplemented mice before irradiation and from day 6 to 8 after irradiation. However, the most drastic vitamin effect was in changes in plasma tocopherol concentration. Before irradiation, the tocopherol-supplemented group had 30 times the concentration of the deficient group. Following irradiation, the tocopherol concentration in the supplemented animals declined 3fold by the 6th day whereas no decline was observed in the deficient animals. Incorporation of 59-iron into erythrocytes was not significantly different under the 2 dietary regimes before irradiation. After irradiation, the uptake of 59-iron declined to zero by the 4th day and thence recovered in both groups; on the 10th day there was a significant ($p < 0.05$) increase in uptake by the vitamin E-deficient group.

Discussion. The initially lower haematocrit in vitamin E-deficient mice is consistent with observations that tocopherol reduces fragility and plays a role in haeme synthesis[3–5]. The increased plasma protein concentration of tocopherol supplemented mice may be consistent with reports of increased humoral immunity with vitamin E supplementation[13,14].

The rapid decline in plasma tocopherol concentration in vitamin E-supplemented mice after irradiation may reflect reduced consumption of test-diet as some anorexia would be expected at the dose employed. Kayden and Bjornsen[15] reported a 50% reduction in plasma tocopherol concentration in humans fasting for 64 h following prior ingestion of 3 g tocopherol daily. Also, intestinal absorption of ingested diet may be impaired following irradiation. It is also possible that plasma tocopherol is mobilized into radiosensitive tissues damaged by the radiation stress. It seems unlikely that the decline reflects in situ destruction of tocopherol since similar declines were not observed in the animals on deficient diet.

The very similar response of the vitamin E-supplemented and deficient groups to radiation seems to indicate that tocopherol supplementation does not reduce damage to the erythropoietic system nor does it enhance its recovery. Although it is conceivable that some difference in the rate of recovery between day 4 and day 6 may occur or that the response may differ after 10 days, the results are somewhat unexpected in view of reports[3,4,8,9] of significant radioprotection by vitamin E in several test-systems. It is well established that reducing oxygen tension in tissues reduces radiosensitivity. It is possible that the slight anaemia of vitamin E-deficient mice reduces the oxygen effect and thereby masks any protection or enhanced recovery in vitamin E-supplemented animals.

11 I. D. Desai, Can. J. Physiol. *46*, 819 (1968).
12 M. L. Quaife and P. L. Harris, J. biol. Chem. *56*, 499 (1944).
13 R. P. Tengerdy and R. H. Heinzerling, Ann. N. Y. Acad. Sci. *203*, 177 (1972).
14 R. H. Heinzerling, R. P. Tengerdy, L. L. Wick and D. C. Lueker, Infect. Immun. *10*, 1292 (1974).
15 H. J. Kayden and L. Bjornson, Ann. N. Y. Acad. Sci. *203*, 127 (1972).