

## Spleen Regeneration in Mice after Gamma Irradiation and Administration of Thymosin

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### Summary

The effect of thymosin (thymic humoral factor isolated from calf thymus) on regeneration of the spleen in mice after whole-body gamma irradiation was studied. Thymosin, in varied dosages (0.1–2.0 mg/day) applied subcutaneously before and after radiation exposure, stimulated splenic regeneration as indicated by increased splenic weight, number of endogenous splenic colonies and  $^{59}\text{Fe}$  and  $^{125}\text{IUdR}$  incorporation into the spleen. A control extract of brain tissue (cerebrosin) isolated in the same way as thymosin was applied to mice to verify specificity of thymosin. After cerebrosin application, a mild increase also was observed. Whereas a near maximal effect of thymosin was reached at a dosage of 0.1 mg, a comparable response with cerebrosin required a dosage of 1.0 mg. These data suggest that administration of thymosin has both a specific and non-specific effect on splenic regeneration and proliferation of hematopoietic stem cells.

### Introduction

While the thymus is now recognized as crucial to the development and functioning of the lymphatic and immunologic systems (Metcalf 1966, Miller and Osoba 1967), its role in hematopoiesis is still unclear. Interaction between thymocytes and production of blood elements in the bone marrow was examined by Goodman and Grubs (1970) and Goodman (1971). They observed that when thymocytes syngeneic to the administered bone marrow, but allogeneic to the recipient were administered to whole-body irradiated mice, both the number of exogenous colonies and the rate of incorporation of  $^{59}\text{Fe}$  into spleen increased. Hrvák (1973) also followed the changes of endogenous colony numbers in adult mice, sublethally irradiated 2–3 months after thymectomy and demonstrated that the number of colonies in thymectomized mice were considerably lower than in irradiated mice with the intact thymus.

During the last decade it has been further discovered that the thymic gland exerts a lymphostimulatory effect (Goldstein et al. 1972; Trainin 1974; Bach et al. 1973) probably by elaborating a circulating hormone. We also observed lymphoid stimulation of thymic extract administered to whole-body irradiated mouse (Vávrová and Petyřek 1976). The present study was designed to study the effect of thymic extract (thymosin) on regeneration and hematopoiesis of the mouse spleen after whole-body irradiation. The mouse occupies a unique position among mammals in that the adult spleen is a peripheral site of red and white blood cell formation. Because stem cells are of critical importance to hematopoiesis, we determined the number of endogenous colonies on the spleens of sublethally irradiated mice (Till and McCulloch 1961) and compared the hematopoietic response of thymosin to brain extract (cerebrosin).

### Material and Methods

**Thymosin isolation:** Thymosin was isolated from frozen calf thymus according to Goldstein et al. (1975). Thymosin 5 (Vávrová and Petyřek 1976) was concentrated and desalted on Amicon HIP5 membrane and it was marked as thymosin 6. Thymosin 5 and 6 were used in biological experiments. Control extract of calf brain was isolated in the same way and it was marked as cerebrosin 6. Thymosin and cerebrosin were injected subcutaneously at amounts of 0.1, 1.0 and 2.0 mg per mouse and per day.

**Irradiation:** Female (CBA x C57Bl/10ScSn) $F_1$  mice weighing 20–25 g were exposed to 500–750 roentgen (R) of gamma rays of  $^{60}\text{Co}$ , at an exposure rate of 75–66 R/min. at 100 cm distance from the focus.

**<sup>125</sup>IUDR uptake:** DNA synthesis was assessed by <sup>125</sup>IUDR (5-iodo-2-desoxyuridine, Radiochemical Centre, Amersham, England) incorporation in the spleen of mice. The precursor was injected intraperitoneally 90 min. before sacrifice in amounts of 5  $\mu$ Ci/mouse. The uptake was expressed as percentage of the activity applied per whole organ.

**<sup>56</sup>Fe uptake:** Erythropoietic activity was determined by <sup>59</sup>Fe incorporation into the spleen. Intraperitoneal injection of <sup>59</sup>Fe citrate (0.5  $\mu$ Ci/mouse) was given four hours before killing. The uptake was expressed as percentage of activity applied per whole organ. Gamma activity of <sup>125</sup>I and <sup>59</sup>Fe was measured simultaneously by means of Berthold BF 5300 crystal scintillation counter.

**Endogenous spleen colonies (ESC):** Number ESC was determined by the method of Till and McCulloch (1961). On day 9 after irradiation the animals were sacrificed, their spleens removed and fixed for 2 hours in Bouins fixative. The number of macroscopically visible modules (colonies) was counted on parietal side of the spleen.

**Statistical evaluation:** The results were evaluated statistically by means of Student t-test, using the Cellatron C 8206 computer. The figures and table give the means and their 95% confidence intervals. The significance ( $p = 0.05$  and less) of the differences of comparable sets is indicated in the test.

## Results

In Table 1 we record the changes in weight of the spleen, ESC number, uptakes <sup>59</sup>Fe and <sup>125</sup>IUDR in the mice spleen on the 9th day after irradiation with 500 R and after thymosin treatments. The number of ESC in T group could not be determined because of great number (> 40). From the Table it is evident that the best results was reached by the application 4 days before and 3 days after the irradiation. For this reason the above mentioned method was used in further experiments.

The number of endogenous colonies is very small after the exposure to 500 R and still decreases after the higher exposure; after 750 R

exposure no ESC can be found (Fig. 1). The application of thymosin results in the increase in the ESC number, which is significant when compared with the non-treated irradiated group. The greatest thymosin effect was reached after the exposure to 500 R, where the number of colonies exceeds 40 and cannot be evaluated. The same changes were seen in other data (the spleen weight, the incorporation of <sup>59</sup>Fe and <sup>125</sup>I).

We have tried to answer the question how great the nonspecific effect of thymosin on the irradiated animals is, comparing its effect with cerebrosin effect. After the thymosin application of 0.1 mg per mouse and per day, significant increase of all observed indices was seen, but a more expressive one was seen at the application of 1mg/mouse and day. After the dosage of 2.0 mg/mouse and day only mild increase of the parameters we followed in relation to the group where 1.0 mg of thymosin was applied. A certain, but smaller stimulated effect was observed at the application of cerebrosin to irradiated mice. The cerebrosin effect at the dosage of 1.0 mg per mouse and per day is comparable with thymosin effect at the dosage of 0.1 mg (Fig. 2).

Fig. 3 demonstrates the effect of thymosin on the kinetics of changes in <sup>125</sup>IUDR uptake in the spleen of mice exposed to 500 R and 700 R. Splenic uptake was expressively decreasing 24 hours after irradiation and was further reduced on day 4. After exposure to 500 R, regeneration started on day 9 and the value on day 14 exceeded the level of intact controls; decrease was found again on day 21. After the exposure to 700 R regeneration started on day 14 and overcompensation was seen on day 21. After the thymosin application the statistically significant increase of <sup>125</sup>IUDR incorporation into the spleen occurs on 4 and 9 day (500 R) and on days 1, 4, 9 and 14 (700 R).

## Discussion

After irradiation the most notable changes occur in hemopoietic and lymphoid tissues and in peripheral blood. As a result of lymphopenia, the irradiated host has impaired immunologic capability and heightened susceptibility

Table 1 The spleen regeneration (the changes of some indices) in mice irradiated with 500 R and treated with thymosin 6

Group	Wet weight mg	Number of ESC	$^{59}\text{Fe}$ %	$^{125}\text{IUdR}$ %
I	47.09 $\pm$ 2.82	6.06 $\pm$ 1.72	2.71 $\pm$ 0.61	0.102 $\pm$ 0.028
AT <sub>1</sub>	55.13 $\pm$ 6.61	11.70 $\pm$ 5.16	3.17 $\pm$ 1.39	0.300 $\pm$ 0.130
T <sub>1</sub>	85.95 $\pm$ 7.58	> 40	11.21 $\pm$ 1.21	0.438 $\pm$ 0.086
BT <sub>1</sub>	74.39 $\pm$ 11.12	25.00 $\pm$ 8.80	10.22 $\pm$ 4.37	0.206 $\pm$ 0.086

I — irradiated animals injected with saline; T<sub>1</sub> — irradiated animals injected with thymosin (1 mg/mouse and day) 4 days before and 3 days after irradiation; AT<sub>1</sub> — irradiated animals injected with thymosin (1 mg/mouse and day) 7 days after irradiation; BT<sub>1</sub> — irradiated animals injected with thymosin (1 mg/mouse and day) 7 days before irradiation.

to infection. Moreover, after irradiation the level of thymic hormone in blood also falls sharply. At a sublethal irradiation dose in mice, the decrease in thymic hormone is, however, only transient and by six weeks concentration rises to a level comparable to that of unirradiated mice (Bach et al. 1973). The weight of lymphoid organs also diminishes after irradiation as lymphocytes are destroyed and the fragments digested and reabsorbed. By 24 hours after a high sublethal

dose of irradiation, spleen size decreases by one-half. After exposure to 500 R regeneration starts soon thereafter and peaks on day 14 (Vávrová and Petýřek 1976).

In this study we observed that after thymosin administration in irradiated mice, splenic regeneration is even faster. By day 9 after irradiation, not only does the number of endogenous colonies increase, but splenic weight and incorporation of  $^{59}\text{Fe}$  and  $^{125}\text{IUdR}$  in-

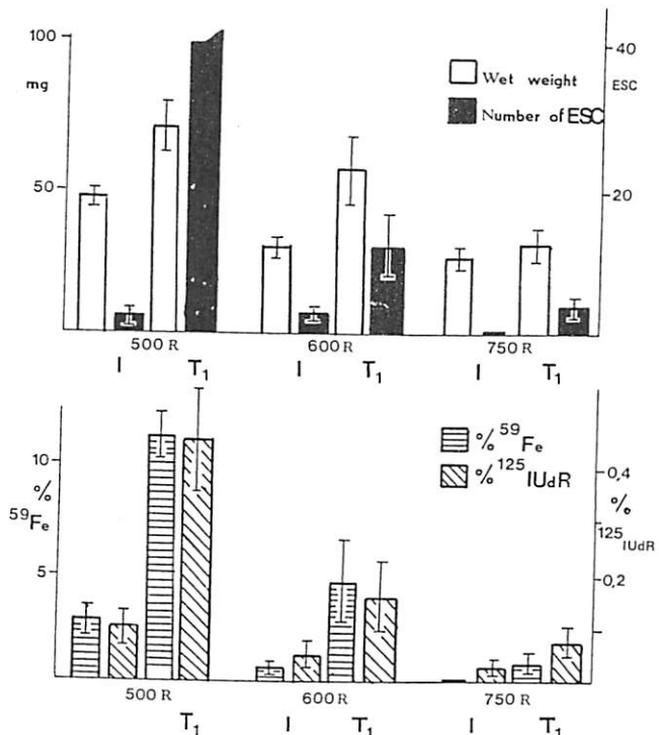


Fig. 1 Changes in wet spleen weight, number of ESC,  $^{125}\text{IUdR}$  and  $^{59}\text{Fe}$  splenic uptake on the 9th day after irradiation and thymosin 6 application. The same symbols as in Table 1

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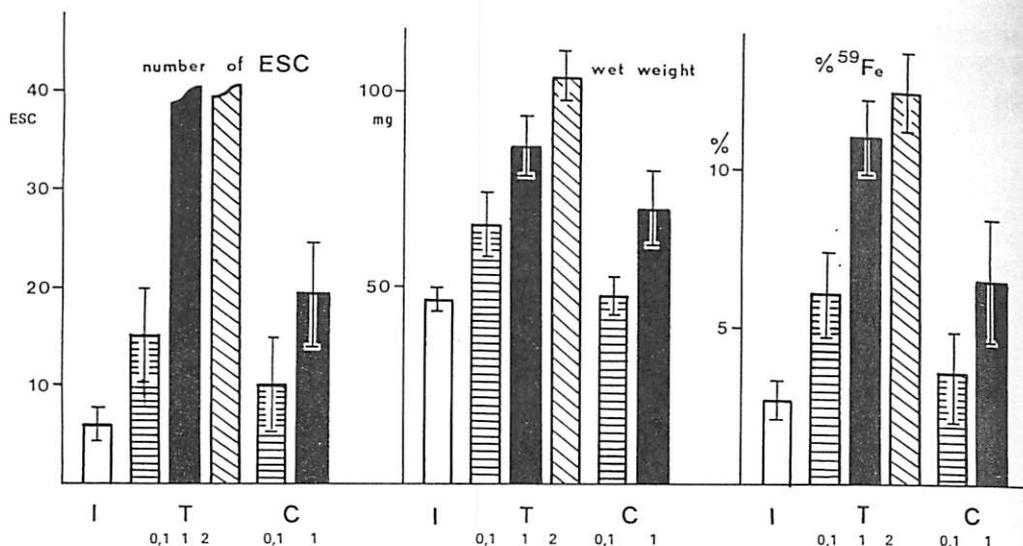


Fig. 2 Comparison of the influence of various doses of thymosin 6 and cerebrosin 6 on the mouse spleen on day 9 after the irradiation with 500 R. I – irradiated animals injected with saline; T – irradiated animals injected with thymosin, 4 days before and 3 days after irradiation; C – irradiated animals injected with cerebrosin, 4 days before and 3 days after irradiation.

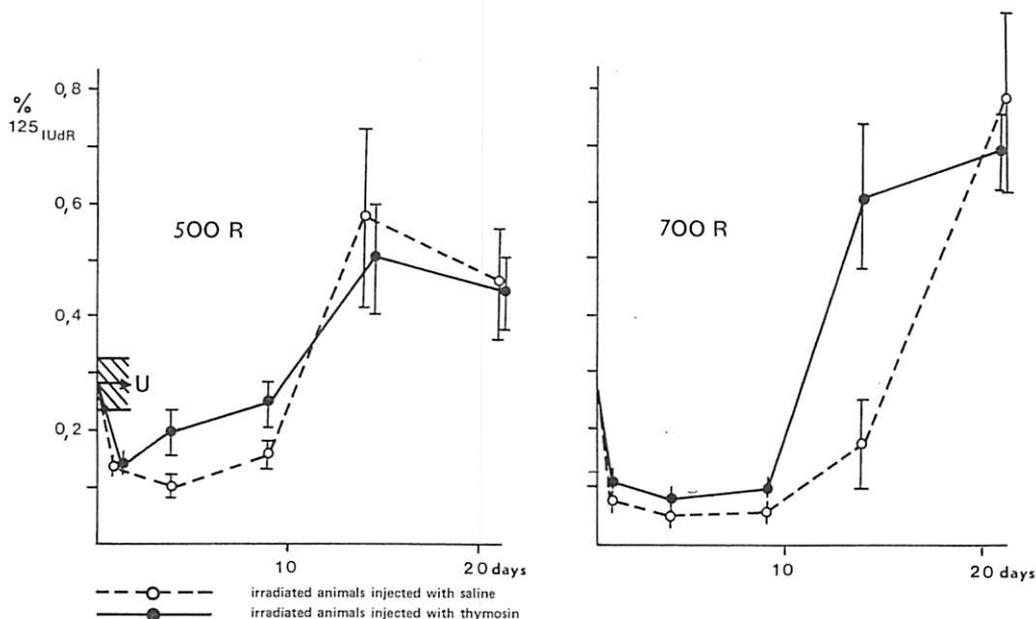


Fig. 3 Effect of irradiation and thymosin 5 application on <sup>125</sup>IUdR uptake in the spleen. Thymosin 5 (1 mg/day/mouse) was applied 4 days before and 3 days after irradiation. U – unirradiated control group.

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to spleen also rises. The maximum effect is achieved when thymosin is administered in dosage of 1.0 or 2.0 mg/mouse/day, 4 days before and 3 days after irradiation.

When comparing thymosin administration before and after irradiation divergent responses are seen. Thus,  $^{59}\text{Fe}$  incorporation is greater when thymosin was administered before irradiation while cell proliferation ( $^{125}\text{IUdR}$  incorporation) is greater after irradiation.

Löwenberg (1975) showed that the number of exogenous spleen colonies produced by fetal liver cells was doubled by an added infusion of thymocytes. This effect was also present when thymic cells were exposed to high dose irradiation in vitro prior to injection. However, when fetal liver cells were irradiated, no colonies were forthcoming. These data confirm that the additional splenic colony produced under the influence of thymic cells were neither of endogenous origin nor thymus-cell-derived, and as this author proposed splenic colonies that originated from transplanted fetal liver cells were probably stimulated by a humoral factor present in thymocytes.

Metcalf (1968) also reported that the growth of hematopoietic colonies in vitro were stimulated by the addition of thymus cells (syngeneic), but did not exclude the possibility of a non-specific immunologic factor. Since the splenic colonies, formed in sublethally irradiated mice in our experiments, were greater after thymosin administration than after cerebrosin, it is reasonable to conclude that both a specific and non-specific factor of thymic extract (thymosin) exerted a positive effect on splenic regeneration and proliferation of hematopoietic stem cells.

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