FROM THE DIVISION OF RADIOBIOLOGY, RESEARCH INSTITUTE OF NATIONAL DEFENCE, SUNDBYBERG, SWEDEN.

THYMIC SHIELDING IN IRRADIATED MICE

by

B. JÄRPLID

Protection from external irradiation has been a valuable technique in investigating haematopoietic tissues in mice in reference to function, interrelationships and roles in radiation leukaemogenesis. Bone marrow and spleen have been thoroughly investigated in these respects. Although the greatest interest during recent years has been focused on the thymus, radiation protective investigations on this organ have not been reported in mice. This is probably because of the inaccessibility of the thymus within the thoracic cavity. In some experiments in rats (STAPLES et coll. 1966) and rabbits (VELDMAN 1970, NIEUWENHUIS 1971) the thymus has been shielded together with the sternal and vertebral bone marrow with a lead shield outside the thorax. In the present experiments efforts have been made to protect the thymus in mice without protection of any other tissue, including bone marrow of the thoracic vertebrae.

The *equipment* (Figs 1, 2) consists of three main parts, an operating board (ABC), two thymic shields (D) and an angled stand (E). The plastic operating board consists of a base plate (A) with a groove (B) for the mouse and an arrangement (C) for stretching the mouse and fixing the shield (D). The base plate is sized 2 mm × 105 mm × 150 mm. The groove has a semicircular cross-

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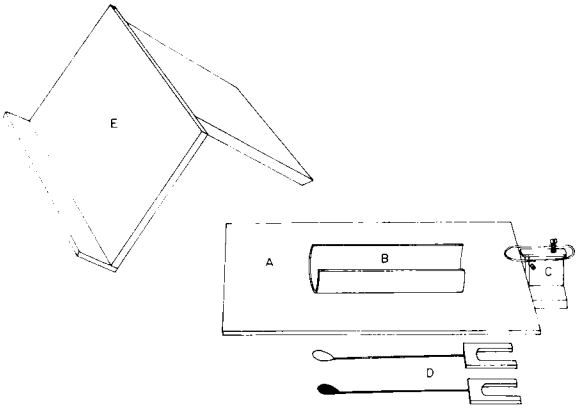

Fig. 1. Equipment for thymic shielding. A — Base plate. B — Groove for the mouse. C — Arrangement for stretching and fixing the mouse. D — A proper shield of platina (black) and a sham shield of silver-coated plastic. E — Stand for the base plate forming an angle of 50° with the horizontal plane.

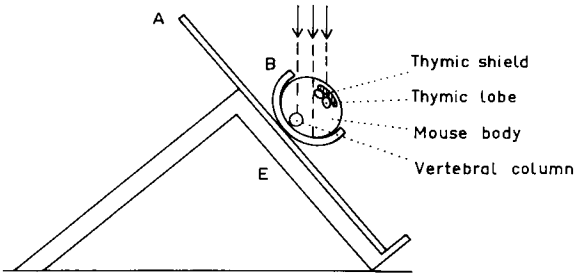

Fig. 2. The equipment for thymic shielding with a mouse in position for irradiation.

section with an inner diameter of 24 mm and wall thickness of 2 mm. The horizontal surface at the top of B is sized 12 mm × 22 mm and is located 21 mm above the base plate. At this surface the shield (D) is fixed with a screw, a spring and a piece of metal. The shield (D) is composed of a hollowed plastic plate, sized 1.5 mm × 12 mm × 22 mm, a 0.8 mm thick bar of steel, and the thymic shield proper of either a plastic (sham shield) or a platina material. The thymic shield is crescent-shaped in cross-section and is sized 5 mm × 9 mm. The thickness of the shield is 2.3 mm. To avoid static electricity, the plastic shield is covered with a thin layer of silver. The platina shield is estimated to afford protection corresponding to about 4.3 mm lead. The protective effect of the plastic shield is negligible. The plastic stand (E) for the base plate forms an angle of 50 degrees with the horizontal plane (Fig. 2).

Technique. For the experiment the mouse was anaesthetized with an intraperitoneal injection of pentobarbital sodium (Mebumal) and placed in a dorsal position in the groove (Fig. 3). A rubber band was affixed to the teeth and the

Fig. 3

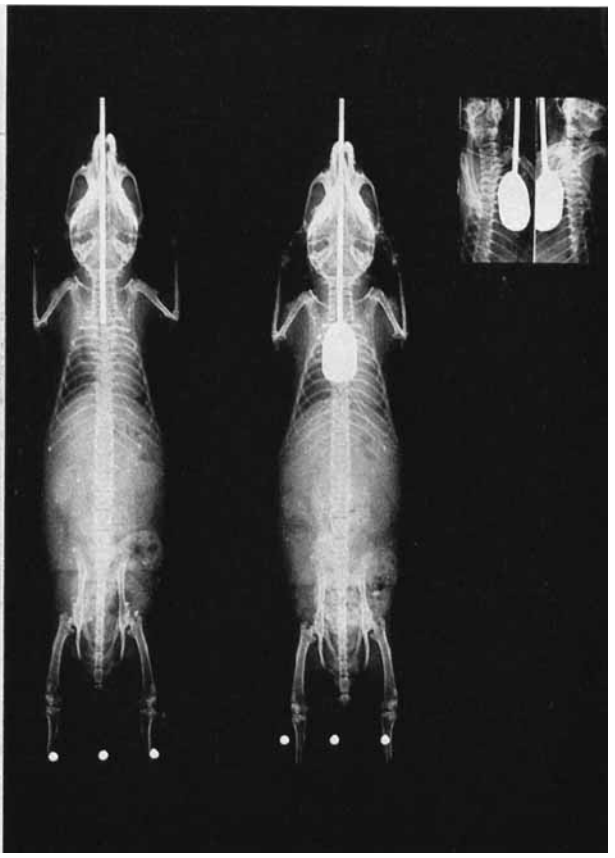

Fig. 4

Fig. 3. Anaesthetized mouse, fixed and equipped with the thymic shield for irradiation.

Fig. 4. Left and middle: Dorsoventral films of mice with a thymic shield of plastic (sham-shielding, left) and platina (middle), respectively. Right: Shielded area of thymic-shielded mouse in left and right position as for irradiation. No protection of the vertebral column.

animal was stretched backwards to get the vertebral column as straight as possible and fixed with tapes over the hindlegs and tail. The thymus was exposed by the method of SJÖDIN et coll. (1963). The bisected sternum was opened with a forceps and the shield was inserted along the ventral surface of the thymus so as to cover part of both lobes, leaving the shield bar passing through the sternal slit. The skin wound was closed around the bar and the fore legs were fixed along the edges of the plastic tube. The base plate with the mouse was then placed on the plastic stand for irradiation from an angle of 40 degrees with the transversal plane through the animal (Fig. 2). The animal was irradiated in two

Table

Total weight of thymus (mg) 1 to 6 days after irradiation with 600 R. n for each sample = 5. Mean± standard error

Days after irradiation	Thymus, shielded	Thymus, sham-shielded	Controls, untreated
—	—	—	70.54±2.19
1	33.24±1.79	33.64±1.34	—
2	25.10±2.14	19.04±1.18	—
4	20.70±1.26	12.14±0.83	—
6	25.30±1.65	13.02±0.59	—

phases, one on each side, with a short interruption for turning the base plate with mouse. After irradiation the animal was examined roentgenologically for checking the position of the shield. A dorsoventral film showed the position of the shield in the median plane (Fig. 4). To ensure that the vertebral column had not been protected, films were also exposed of the shielding area with the animal in position as for irradiation above (Fig. 4). For that purpose small films (about 1 cm × 3 cm) were placed just beneath the thorax of the animal in the groove. The films were developed and animals with the shield not in proper position were excluded.

After roentgenography the sternum was opened again, the shield was removed and the cutaneous wound was finally closed with clips.

Preliminary experiments. In preliminary experiments irradiations were performed with a roentgen apparatus, Müller MG 300, operating at 260 kV and 12 mA. The radiation was filtered by 0.5 mm Cu and 0.5 mm Al. The focal distance was 25 cm and the dose rate 250 R/min. One hundred and ten two-month-old C_{57}Bl female mice were divided into two equal groups and irradiated with 2 × 300 R. During the irradiation the thymus was shielded in one group with platina and in the other with plastic (sham-shielding). The day for irradiation was called day 0. Twenty mice seryed as untreated controls. The animals were subjected to blood examination and killed in groups of five at 1, 2, 4, 6, 8, 10, 12, 16, 20, 24 and 30 days after the irradiation. The thymus, spleen and brachial, inguinal and mesenteric lymph nodes were weighed and fixed in Stieve's fluid (Romeis 1948) together with sternum for histologic investigation. The number of mononuclear cells in the peripheral blood was counted in a Bürker chamber.

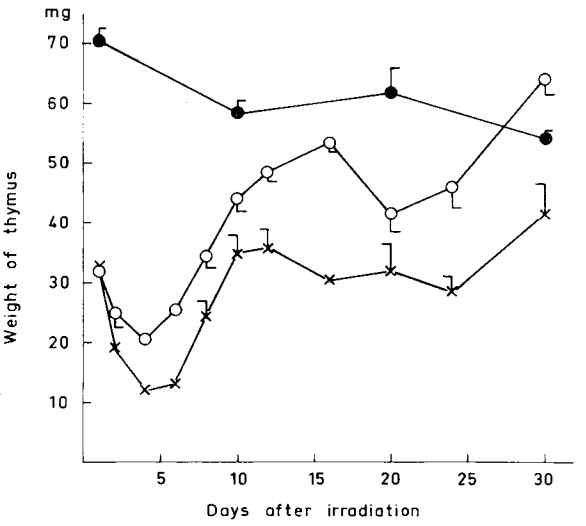

Fig. 5. Total weight of thymus of female $C_{57}Bl$ mice after irradiation with 600 R. ● untreated controls, ○ shielded thymus, × sham-shielded thymus. n for each sample = 5. Mean ±SE.

Results

Thymus. The size of the shielded part of the thymus was difficult to state exactly. It may be estimated, however, to have been about 20 to 30 per cent, judging from the difference in weight decrease of the thymus between shielded and sham-shielded animals and from the extension of the histologically preserved areas of the thymus in the shielded animals. The histologic observations and the mean weight of the thymus with its standard error 2, 4 and 6 days after irradiation (Table) indicate that this shielded part of the thymus was of similar order of magnitude between different individuals in the same group.

During the first phase of lymphocyte depletion, days 1 to 4 (JÄRPLID 1968), the weight of the thymus in the sham-shielded animals decreased to almost 60 per cent of that of the shielded animals by day 4 (Fig. 5, Table). Shielded thymus had then a faster and greater increase of weight and also more evident second phases of depletion and regeneration (JÄRPLID 1968) than the sham-shielded thymus.

Histologically the well known initial phases of destruction and depletion of lymphocytes (MURRAY 1948) were seen in all unshielded and sham-shielded parts of the thymic cortex. The shielded areas seemed to be well preserved and were in general rather sharply delimited from the unshielded areas (Fig. 6). On the 4th day a regeneration started in these irradiated areas in four out of five animals with a platina shield and in one out of five animals with a plastic shield. Two days later the regeneration in these areas was of similar appearance in both

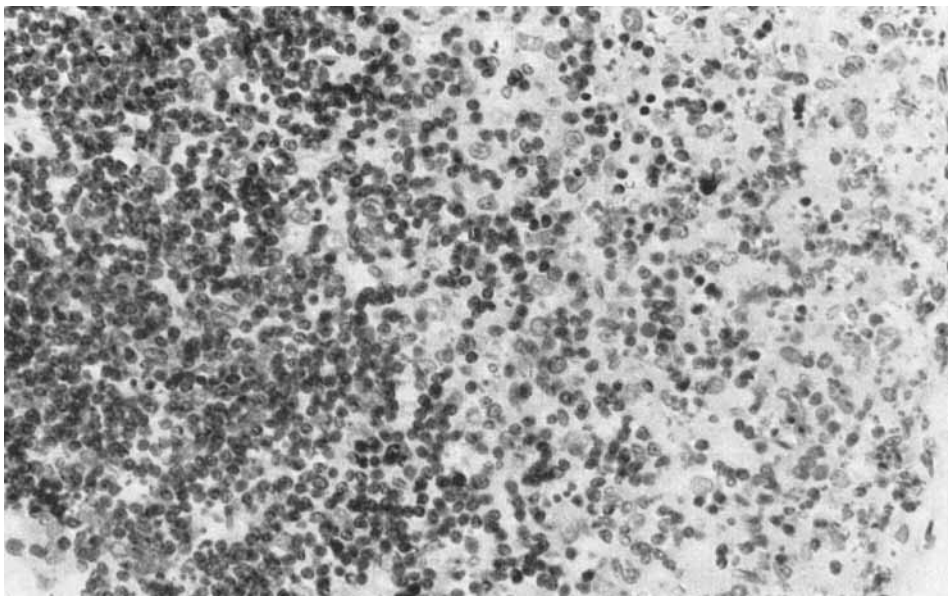

Fig. 6. Thymus one day after irradiation with 600 R. Left: Rather well preserved part of cortex shield with platina. Right: Sham-shielded part of cortex with destruction and depletion of lymphocytes. Haematoxylin and eosin, × 250.

groups. On day 8 the whole of the thymus appeared normal again. A slight degree of cortical thinning was seen during the period 16 to 24 days in the sham-shielded animals and during the period 20 to 24 days in animals with a thymic shield. After 30 days the histologic appearance of the entire thymus was normal again.

Spleen. The changes in weight of the spleen predominantly reflected the development in the red pulp (Fig. 7). The initial destruction and depletion of cells was of similar order of magnitude in the two groups. In two animals with a shielded thymus the extramedullary haematopoiesis started on day 6 and in all other animals on day 8. The extent of this extramedullary haematopoiesis was somewhat greater in the shielded than in the unshielded animals. There was no difference in cellularity of the white pulp, with the possible exception of day 6. On this day the centrofollicular parts seemed to be somewhat more depleted in the unshielded than in the shielded group. The entire spleen had a normal histologic appearance again on days 20 to 24.

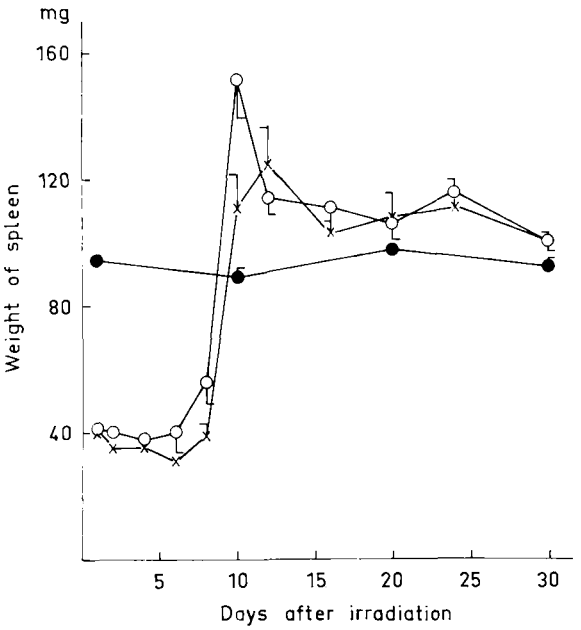

Fig. 7. Weight of spleen after irradiation with 600 R. ● untreated controls, ○ shielded thymus, × sham-shielded thymus. n for each sample = 5. Mean ± SE.

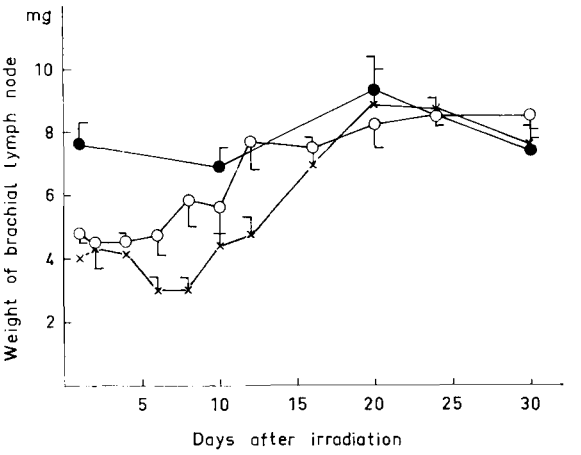

Fig. 8. Weight of brachial lymph node after irradiation with 600 R. ● untreated controls, ○ shielded thymus, × sham-shielded thymus. n for each sample = 5. Mean ± SE.

Lymph nodes. After the initial decrease in weight the increase in weight of the lymph nodes was faster in the group with a thymic shield than in the unshielded group (Fig. 8). Histologically no notable differences between the two groups could be detected. However, as the paracortical zone (a thymus-dependent area, PARROT et coll. 1966) is a dominating part of the lymph node in the mouse, the

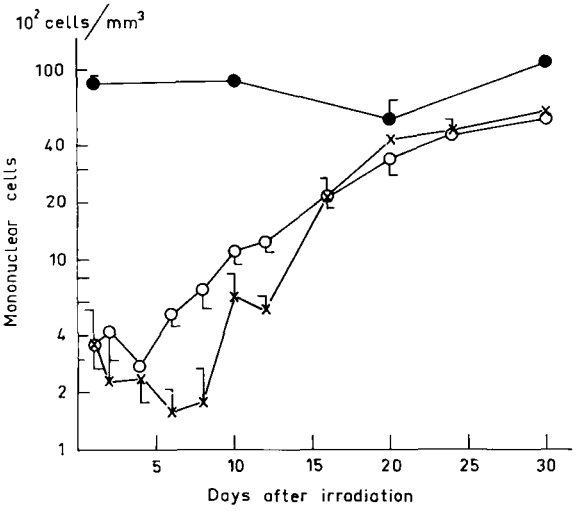

Fig. 9. Number of mononuclear cells in the peripheral blood after irradiation with 600 R. ● untreated controls, ○ shielded thymus, × sham-shielded thymus. n for each sample = 5. Mean ± SE.

difference in weight probably reflects differences in the cellularity mainly of this part.

Bone marrow. During the different phases of destruction, depletion and regeneration both groups had a similar histologic appearance.

Peripheral blood. Protection of the thymus seemed to lead to a higher number of mononuclear cells (mainly lymphoid cells) in the peripheral blood 6 to 12 days after irradiation (Fig. 9).

Comments

The most important differences between shielded and sham-shielded animals appeared in the weight and histology of the thymus. The findings in extrathymic tissues indicate that partial shielding of the thymus during irradiation promotes the regeneration of other lymphoid organs, including the so called thymus-dependent areas, and increases the number of lymphoid cells in the peripheral blood. These results confirm the central role of the thymus for the lymphoid tissues and for the circulating pool of lymphocytes.

The size of the shielded part of the thymus could only be grossly estimated from the degree of weight decrease and from the extension of the histologically observed damage. The experiments indicate, however, that the method of shielding of the thymus, when further developed, may be a valuable new technique for experimental investigations of the thymus and its relation to other haematopoietic tissues.

Acknowledgement

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SUMMARY

A method for shielding of the thymus in mice during external irradiation is described. The results of the experiments indicate that thymic shielding during roentgen irradiation promotes the regeneration of extrathymic lymphoid tissues and the recovery of the number of lymphoid cells in the peripheral blood.

ZUSAMMENFASSUNG

Es wird eine Methode beschrieben, um den Thymus von Mäusen während externer Bestrahlung zu schützen. Die Ergebnisse der Untersuchungen deuten darauf hin, dass ein Schutz des Thymus während der Röntgenbestrahlung die Regeneration der ausserhalb des Thymus gelegenen lymphoiden Gewebe und die Erholung der lymphoiden Zellanzahl im peripheren Blut beschleunigt.

RÉSUMÉ

L'auteur décrit une méthode de protection du thymus des souris au cours de l'irradiation externe. Les résultats d'expérience montrent que la protection du thymus au cours de l'irradiation roentgen favorise la régénération des tissus lymphoïdes extrathymiques et la restauration du nombre des cellules lymphoïdes dans le sang périphérique.

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