Release and retention of labelled DNA in the thymus and spleen of irradiated mice

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The process of release and retention of labelled DNA in thymus and spleen of normal and irradiated (^{60}Co) mice has been studied after administration of ^{3}H-thymidine. The results indicate that the dividing fraction of lymphoid cells is more resistant to radiation than the fraction of nondividing lymphocytes. The time courses of the specific activities of DNA in thymus and spleen were different especially after irradiation with lethal doses. It is suggested that the process of depletion in the lymphoid series is probably similar for both thymus and spleen but the different cellular composition of these organs led to apparently unrelated results.

The lymphoid population from thymus and spleen is represented by the mitotically active medium and large lymphocytes and the nondividing small lymphocytes (Metcalf and Wiadrowski, 1966; Borum, 1973; Fabrikant, 1968; Hanna, 1964; Fliedner et al., 1964). Contradictory results have been reported with respect to the radiosensitivity of lymphocytes according to their size (Okada, 1970). However, some evidence suggests that the fraction of small thymocytes decreased to a smaller extent than medium and large thymocytes after irradiation with lower doses than 200 R but, in contrast, to a greater extent after larger doses (Blomgren and Révész, 1968). On the other hand, contradictory results have been noted by Gerber and Altman (1970), in earlier studies on the retention of labelled DNA in thymus and spleen after irradiation. Nygaard and Potter (1960) and Sanders et al. (1964) have found a lack of significant change from normal in the specific activity of DNA within the period of seven days after exposure. The results suggested that the labelled and nonlabelled cells were lost in a random manner. It was concluded that the dividing and nondividing cells are equally sensitive to radiation. On the contrary, the data reported by Gerber et al. (1963) show that cell-destruction did not occur in a random fashion, but the cells should be destroyed selectively. In the present study it was attempted to obtain more informations about the release and retention of labelled DNA in thymus and spleen after exposure to radiation. The results support the observation that the dividing and nondividing lymphoid cells are destroyed or removed in a selective manner.

Material and methods

Male mice of the A2G strain weighing 25 ± 2 g were given intraperitoneal injections of 25 μCi ^{3}H-thymidine (5 Ci/mmole) (Amersham, England) in 0.25 ml of 0.14 N NaCl solution. The mice were whole-body irradiated in plastic cages by a ^{60}Co therapeutic unit (Theratron 80) (FSD = 80 cm; 120 rd/min). The doserate was measured by a Siemens universal dosimeter. The animals were given a standard diet.

The DNA content in thymus and spleen was determined as indicated by Skalka et al. (1965) and Pierucci (1967). The soluble DNA fraction in 0.14 N NaCl and insoluble in 0.2 N $HClO_4$ was separated as described by Skalka et al. (1965), Pierucci (1967) and Suciu et al. (1974). The radioactivity was counted in a liquid scintillation spectrometer (Betaszint BF-5003) as has been described (Suciu et al., 1975). The radioactive measurements allowed the determination of the total activity (c.p.m./DNA/organ) and specific activity (c.p.m./mg DNA) of DNA. The values from tables and figures are means of the results obtained from six to twelve animals (± standard error). On the second and third day after exposure, the thymus and spleen from two or three mice were pooled into a single sample.

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Statistical significance:
+ = $p < 0.05$;
+ + = $p < 0.01$;
+ + + = $p < 0.001$.

Table 1. Specific activity of DNA in the thymus of irradiated and nonirradiated mice. ^{3}H-thymidine was administered one hour before exposure. The results represent specific activities as a percentage from the value of nonirradiated mice one hour after administration of the label. The figures in parantheses represent the numberof determinations (see material and methods).

Hours after exposure	0 rd	300 rd	800 rd
10	97.5 ± 11.5 (6)	168.0 ± 12.2 (6) + +	125.3 ± 13.0 (6)
24	90.3 ± 8.9 (6)	159.3 ± 10.4 (8) + +	141.4 ± 16.1 (8) +
48	67.0 ± 7.2 (6)	123.6 ± 5.1 (5) + +	140.9 ± 11.5 (5) + +
[illegible]	49.8 ± 5.3 (6)	58.6 ± 6.4 (4)	104.6 ± 8.5 (4) + + +

Results

Retention of DNA

The time course of the specific activity of DNA in thymus and spleen was determined during the period of three days after exposure to 300 and 800 rd. ^{3}H-thymidine was administered one hour before irradiation. The results indicate that in thymus the loss of nonlabelled cells significantly exceeded the loss of the dividing fraction of labelled cells (Table 1). The specific activities of DNA in spleen (Table 2) show that within the first day after exposure the loss of dividing cells was delayed as compared with the loss of the nonlabelled fraction of cells. Within the following three days, irradiation with 800 rd led to a significant decrease of the specific activity as compared with nonirradiated controls. On the other hand, after exposure with 300 rd, the specific activities of DNA in spleen were about the same in the control and irradiated groups.

In another experiment ^{3}H-thymidine was administered one hour before exposure with different doses of radiation (Figure 1). The total activity of DNA and the DNA content were determined 24 hours after irradiation in both thymus and spleen. It might be observed that the loss of the whole DNA was increased as compared with the loss of labelled DNA. This effect is more evident in thymus.

Release of labelled DNA

The release of labelled DNA into the fraction of DNA soluble in 0.14 N NaCl and insoluble in 0.2 N $HClO_4$ was determined four and ten hours after exposure with 300 rd. ^{3}H-Thymidine was administered from one hour to five days before irradiation. As shown in Figure 2, the loss of labelled DNA was enhanced if irradiation was performed one to three days after incorporation of the radioactive precursor.

Hours after exposure	0 rd	300 rd	800 rd
10	97.7 ± 8.0 (6)	154.2 ± 10.3 (6) + +	173.8 ± 12.4 (6) + + +
24	88.1 ± 10.1 (6)	109.3 ± 7.4 (8)	115.6 ± 5.5 (8) +
48	50.4 ± 2.1 (6)	53.1 ± 8.5 (5)	34.3 ± 4.7 (5) +
72	40.3 ± 4.2 (6)	34.7 ± 3.4 (4)	17.1 ± 2.2 (4) + + +

Table 2. Specific activity of DNA in the spleen of irradiated and nonirradiated mice. The same experiment as in table 1.

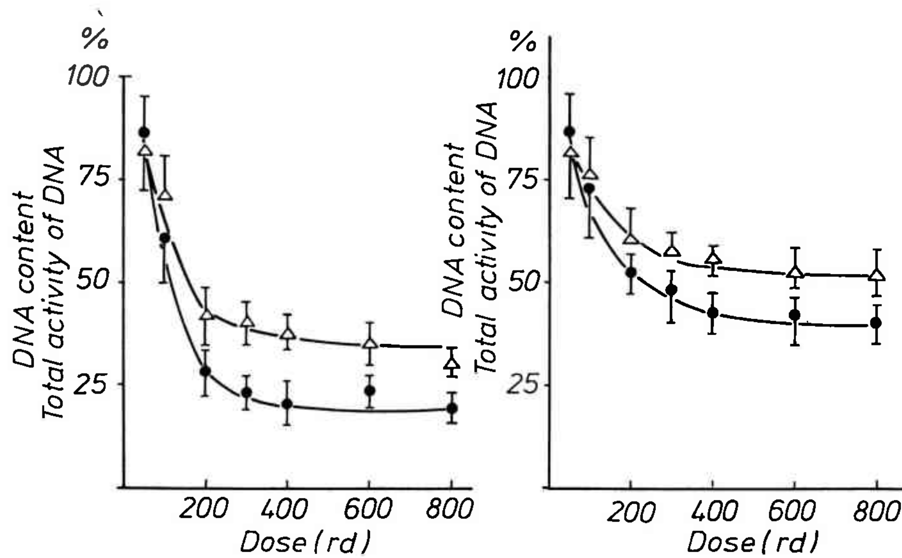

Figure 1. Effect of radiation dose on the retention of DNA (●) and on the total activity of DNA (△) in thymus (left) and spleen (right). ^{3}H-thymidine was administered one hour before exposure. DNA analysis was performed 24 hours after irradiation. The control group was sacrificed 25 hours after administration of the label.

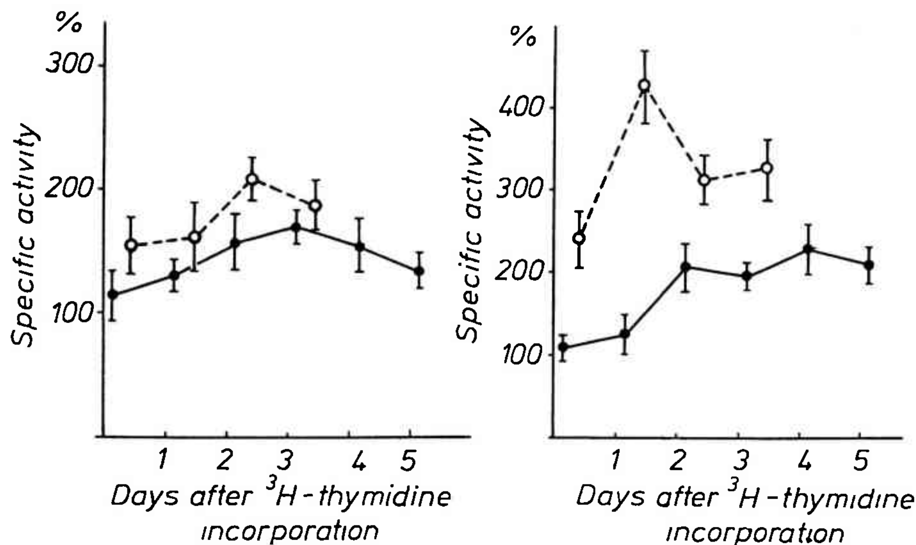

Figure 2. Specific activity of the fraction of soluble DNA in thymus (left) and spleen (right) after irradiation with 300 rd. The animals were sacrificed four hours (●) or ten hours (○) after irradiation. The results represent specific activities in the fraction of soluble DNA expressed as a percentage from the corresponding values in the fraction of unsoluble DNA.

Discussion

The results reported in Table 1 and Figure 1 clearly show that the labelled cells from thymus are more resistant to radiation than the fraction of nonlabelled cells. As ^{3}H-thymidine is incorporated only into the dividing cells, the more restistant fraction of cells appears to be that of medium and large thymocytes (Borum, 1973; Metcalf and Wiadrowski, 1966; Fabrikant, 1968). These findings support earlier cytological data reported by Blomgren and Révész (1968). Figure 2 shows that the amount of labelled DNA released into the fraction of soluble DNA few hours after exposure increased when ^{3}H-thymidine was administered from one to three days before irradiation. On the other hand, it was reported that the specific activity of DNA in thymus significantly decreased three days after exposure with 1000 R if the label was administered three days before irradiation (Gerber et al., 1963). These findings suggest that during the period of one to three days the mitotically active cells changed their response to radiation. Accordingly, the continuous transformation of medium and large thymocytes into nondividing small thymocytes was demonstrated (Borum, 1973; Metcalf and Wiadrowski, 1966; Fabrikant, 1968). It may be concluded, that the medium and large thymocytes are more restistant to radiation

than the fraction of small thymocytes. This result is in agreement with experiments demonstrating that lymphocytes dedifferentiated to lymphoblasts or stimulated by mitogens are more restistant to radiation (Gerber and Altman, 1970). It is also important to point out that the relatively small fraction of medullary thymocytes is more radioresistant than the cortical thymocytes (Blomgren, 1970; Trowell, 1961). Therefore, it is conceivable at two or three days after irradiation, when the amount of DNA retained in thymus has decreased to less than ten percent, the specific activity of DNA is largely controlled by the medullary fraction of thymocytes.

The specific activity of DNA in spleen of irradiated mice shows that within the first day after exposure the loss of the dividing fraction of cells was delayed as compared with the loss of nondividing cells (Table 2 and Figure 1). In the following three days after irradiation with 300 rd the specific activity of DNA reached the normal values. These data support the observations reported by Nygaard and Potter (1960) and Sanders et al. (1964) that in spleen the dividing and nondividing cells appear to be equally sensitive to radiation. However, our results indicate that up to three days after irradiation with a lethal dose of 800 rd, the specific activity of DNA significantly decreased as compared with that found in nonirradiated controls. A similar decrease of the specific activity of DNA in spleen was observed by Gerber et al. (1963) if the label was administered three days before irradiation with 1000 R. These experiments suggest that in spleen there existis a fraction of cells with low mitotic activity which is less affected by radiation than the lymphoid population. Accordingly, the dose-response curves of isolated spleen cells consist of two components, a radiosensitive one, which corresponds to the lymphoid fraction and a radiorestistant one, probably being represented by more restistant types of lymphocytes, reticulum cells and connective tissues (Gerber and Altman, 1970). The radiosensitive cells including the fraction of labelled lymphocytes are largely destroyed and removed from the organ up to three days after irradiation with a lethal dose. The decrease of the specific activity of DNA in spleen could be explained, therefore, by the presence of the surviving fraction of nonlabelled radioresistant cells. As previously was mentioned, the loss of labelled lymphocytes during the first day after exposure seems to be delayed as compared with the loss of nonlabelled cells, in both thymus and spleen. However, this effect is less evident in spleen (Table 2 and Figure 1), although the release of labelled DNA into the fraction of soluble DNA clearly shows that the cells labelled one hour before irradiation are less affected by radiation than the cells labelled one to three days before exposure (Figure 2). These results could be also explained in view of the fact that in spleen the fraction of nonlymphoid cells is more representative than in the thymus (Humphrey and Withe, 1970). Thus, as the lymphoid cells are removed from the spleen, the specific activity of the whole DNA is controlled in a greater extent by the nonlabelled DNA which belongs to the radioresistant fraction of cells. It is probable therefore, that with respect to the lymphoid population the process of cell depletion after exposure to radiation is similar, in both thymus and spleen.

The mean cell cycle times of dividing thymocytes (Metcalf and Wiadrowski, 1966; Fabrikant, 1968) and lymph node germinal center primitive cells (Hanna, 1964; Fliedner et al., 1964) were estimated to be in the range of five to eight hours. On these conditions, the process of cell renewal of the total lymphoid population from thymus requires about three days (Metcalf and Wiadrowski, 1966; Fabri-

kant, 1968). The dividing fraction of lymphocytes is propably transformed by asymmetrical division into nondividing small lymphocytes (Metcalf and Wiadrowski, 1966; Fabrikant, 1968). Thus, if we wish to observe the radiation response of the dividing fraction of medium and large lymphocytes it seems reasonable to resume our investigations within the period of one day after incorporation of the radioactive precursor. The experiments reported in this work demonstrate that up to one day after exposure the dividing fraction of lymphocytes is less affected by radiation than the nondividing lymphocytes.

Freisetzung und Retention von markierter DNS in Thymus und Milz von bestrahlten Mäusen

Nach Verabreichung von ^{3}H-Thymidin wurde die Freisetzung und Retention von markierter DNS in Thymus und Milz von normalen und bestrahlen (^{60}Co) Mäusen untersucht. Die R[illegible]sultate zeigen, daß die Fraktion der sich teilenden lymphoiden Zellen widerstandsfähiger [illegible] gegen Strahlen als die der sich nicht teilenden lymphoiden Zellen. Die Veränderung in der Zeit der spezifischen Aktivität der DNS war verschieden für Thymus und Milz, besonders nach Bestrahlung mit letalen Dosen. Diese Tatsache könnte so erklärt werden, daß der Prozeß der Eliminierung der lymphoiden Zellen in Thymus und Milz wahrscheinlich ähnlich verläuft, die Verschiedenheit der zellularen Zusammensetzung dieser Organe aber zu scheinbar wi[illegible]spruchsvollen Resultaten führte.

La mise en liberté et la rétention de l'ADN marqué dans le thymus et dans la rate des souris irradiées

On a étudié le processus de la mise en liberté et de la rétention de l'ADN marqué dans le thymus et dans la rate de souris normale ou irradiées (^{60}Co) après l'administration de la ^{3}H-thymidine. Les résultats montrent que la fraction des cellules lymphoïdes en division est plus résistante à l'irradiation que la fraction lymphoïde qui ne se divise pas. L'évolution avec le temps des activités spécifiques de l'ADN dans le thymus et dans la rate diffèrent surtout après l'irradiation avec des doses léthales. Nous suggérons comme explication que le processus de déplétion dans la série lymphoïde est probablement similaire dans le thymus et dans la rate, mais la composition cellulaire différente de ces organs conduit aux résultats apparemment contradictoires.

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