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Effect of Cortisol on the Release and Retention of Labelled DNA in the Thymus and Spleen of Mice

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With 2 Figures

Summary

The lymphocytolytic effect of different doses of cortisol was studied in the thymus and spleen of mice previously injected with ^{3}H-thymidine. The results indicate that in thymus the fraction of labelled cells was more resistant to cortisol than the unlabelled cell population. The release of DNA into the fraction of DNA soluble in 0.14 M NaCl was delayed suggesting that cortisol controls indirectly the lymphocytolytic process.

Up to 24 hours after administration of cortisol the loss of labelled spleen cells significantly exceeded the loss of unlabelled cells. The time course of the release of labelled DNA into the fraction of DNA soluble in 0.14 M NaCl indicates that a fraction of labelled DNA was rapidly removed from the spleen after injection of cortisol.

Glucocorticoids act as lymphocytolytic agents in both normal and adrenalectomized mice (Dougherty, 1952; Stevens et al., 1966; Blomgren and Svedmyr, 1971). In the dividing fraction of lymphocytes the DNA synthesis is inhibited by an all or none manner (Lang et al., 1967). It is therefore probable, that a representative fraction of dividing lymphocytes is destroyed and removed from lymphoid organs after administration of glucocorticoids. We report here the results of an experiment designed to differentiate the effect of cortisol on the dividing and nondividing fraction of cells from thymus and spleen.

Material and Methods

Male mice of the A2G strain weighing 25 ± 2 g were intraperitoneally injected with 25 µCi of ^{3}H-thymidine (Amersham, England, 5 Ci/mM) in 0.25 ml 0.14 M NaCl solution. Different doses of cortisol acetate suspended in 0.5 ml 0.14 M NaCl solution were administered intraperitoneally one hour after the in vivo labelling.

All the mice were sacrificed by cervical dislocation. The DNA content was determined in thymus and spleen following the method of Skalka et al. (1965) by the diphenylamine colour reaction (DeFrance and LePecq, 1961). The DNA fraction soluble in 0.14 M NaCl and insoluble in 0.2 M $HClO_4$ was isolated as described by Skalka et al. (1965), Pierucci (1967), and Suciu et al. (1974).

The radioactivity was measured with a Betaszint (BF-5003) liquid scintillation spectrometer as has been described elsewhere (Suciu et al., 1975, a). The radioactive measurement allowed the determination of the total activity (c.p.m./DNA/organ). The values from Table and Figures are means of the results obtained from six to eight mice (± standard error).

Statistical evaluation of data was made according to Student's "t" test.

Results

Mice were injected with different doses of cortisol one hour after administration of ^{3}H-thymidine. The DNA content and the total activity of DNA were determined in thymus and spleen (Table 1) at 24 hours after administration of steroid. The retention of labelled DNA in thymus was significantly increased compared with that of the whole DNA. By contrast, the results indicate that in spleen the loss in the fraction of labelled cells significantly exceeded the loss of nonlabelled cells.

Table 1 Retention of DNA and total activity of DNA in thymus and spleen at 24 hours after administration of cortisol and 25 hours after injection of ^{3}H-thymidine

	Cortisol (mg/kg body weight)	DNA*) (% ± S.E.)	Total activity**) (% ± S.E.)	
Thymus	2.5	93.1 ± 8.2	85.8 ± 6.7	
	12.5	71.4 ± 7.6	70.1 ± 6.3	
	20.0	48.7 ± 7.3	62.0 ± 7.2	+
	30.0	35.8 ± 5.0	46.5 ± 5.9	++
	40.0	24.2 ± 3.4	35.2 ± 5.3	++
	100.0	19.9 ± 2.8	30.4 ± 4.1	+++
	160.0	16.5 ± 3.3	23.5 ± 3.4	+++
Spleen	10.0	83.6 ± 5.8	77.4 ± 9.1	
	20.0	76.3 ± 8.5	72.8 ± 6.4	
	30.0	66.0 ± 5.9	59.7 ± 5.6	+
	40.0	58.9 ± 6.1	51.3 ± 3.5	+
	100.0	53.6 ± 5.6	43.8 ± 5.4	++
	160.0	52.4 ± 4.7	40.2 ± 3.6	++

*) The amount of DNA (µg/organ) was expressed as percentage from the value of control animals

**) The results represent total activities expressed as percentage from the value determined in normal mice one hour after administration of ^{3}H-thymidine. Statistical significance: + = $p < 0.05$; ++ = $p < 0.01$; +++ = $p < 0.001$

The time course of the release of DNA into the fraction of DNA soluble in 0.14 M NaCl was determined in thymus and spleen up to 24 hours after administration of cortisol. It can be seen from Fig. 1 that in thymus the amount of soluble DNA increased approximately linearly in time. At 10 and 24 hours after administration of 160 mg/kg body weight cortisol the release of soluble DNA increased significantly in the spleen. The effect of a dose of 32 mg/kg body weight cortisol was relatively less evident.

The results included in Fig. 2 indicate that the fraction of labelled DNA soluble in 0.14 M NaCl is more representative than the fraction of unlabelled DNA, both in thymus and spleen. However, whereas in thymus there is an almost linear increase of labelled and unlabelled DNA into the fraction of soluble DNA, a marked release of labelled DNA was determined in spleen within the period of 8 to 10 hours after administration of cortisol.

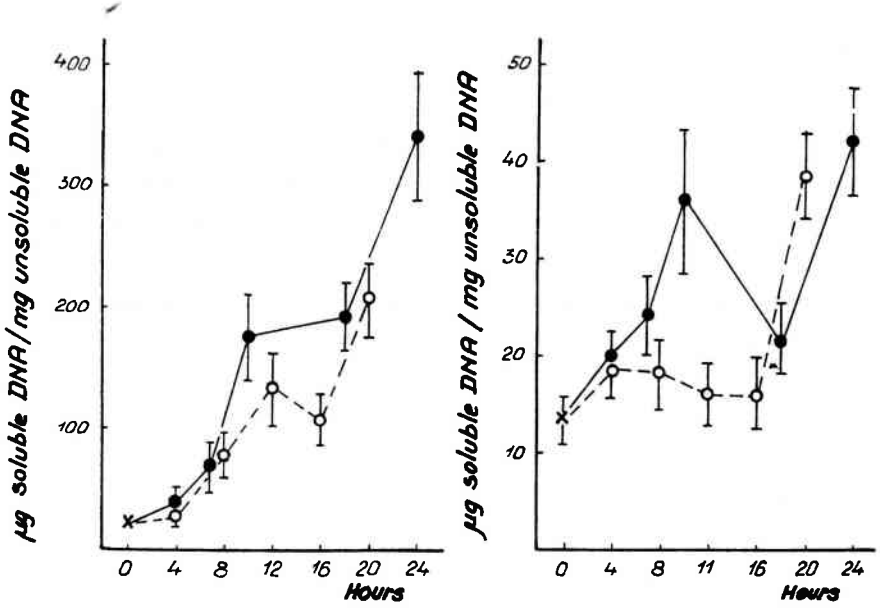

Fig. 1 Time course of the release of thymic (left) and splenic (right) DNA into the fraction of DNA soluble in 0.14 M NaCl. Administration of 32 mg/kg body weight cortisol (○) and 160 mg/kg body weight cortisol (●)

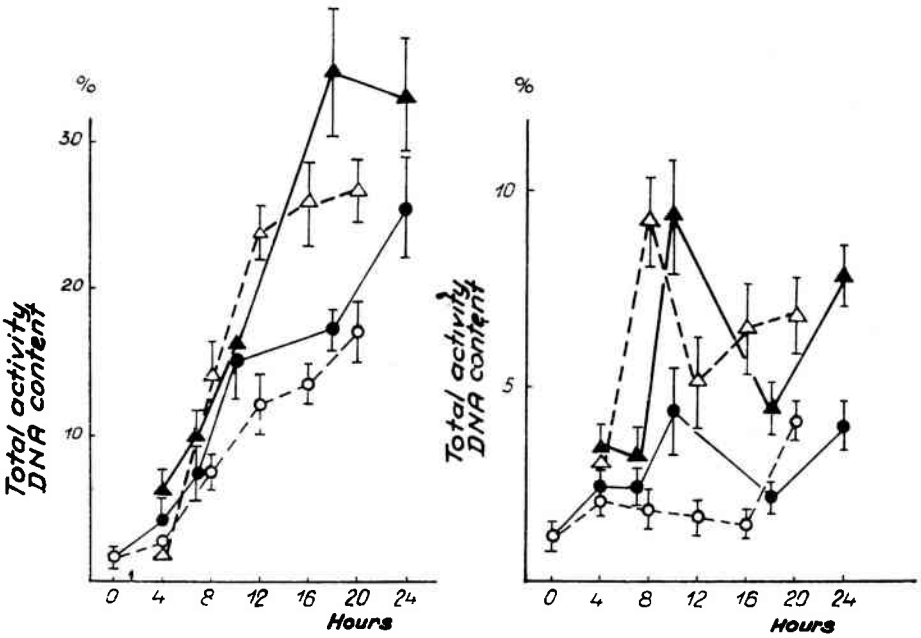

Fig. 2 Total activity and DNA content into the fraction of DNA soluble in 0.14 M NaCl in thymus (left) and spleen (right). ^{3}H-Thymidine was injected one hour before administration of cortisol. The results were expressed as percentage of total activity and DNA from the values determined in the whole organ. Total activity (△) and DNA content (○) after administration of 32 mg/kg body weight cortisol. Total activity (▲) and DNA content (●) after injection of 160 mg/kg body weight cortisol

Discussion

In earlier studies on the retention of DNA in thymus, spleen and small intestine the loss of previously labelled DNA has been equated with loss of labelled cells from the tissue (Nygaard and Potter, 1960; Sanders et al., 1964; Suciu et al., 1975 a; Suciu et al., 1976). The cell cycle times of medium and large

thymocytes (Metcalf and Wiadrowski, 1966) and germinal center primitive cells (Fliedner et al., 1964; Hanna, 1964) were estimated to be in the range of 5 to 8 hours. The dividing fraction of lymphocytes is rapidly transformed into nondividing small lymphocytes, probably by asymmetrical division (Metcalf and Wiadrowski, 1966). ^{3}H-Thymidine is incorporated only into the dividing fraction of lymphocytes (Metcalf and Wiadrowski, 1966). Accordingly, our experiments were performed during the period of one day after the in vivo labelling of DNA with ^{3}H-thymidine. The data indicate that 24 hours after administration of cortisol the surviving fraction of dividing thymocytes is more representative than the fraction of mature small thymocytes (Table 1). Similar results have been reported with respect to the radiosensitivity of thymus and spleen lypmhocytes according to their size (Blomgren and Révész, 1968) and labelling capacity (Suciu et al., 1976).

It was reported that ionizing radiations (Skalka et al., 1965; Pierucci, 1967; Suciu et al., 1974) and alkylating agents (Matyášová and Skalka, 1966) induce the disorganization of nuclear structures in the lymphoid population. This effect is indicated by the increased amounts of extractable and soluble DNA isolated in the process of homogenization of lymphoid organs in strong salt solutions. The in vitro lymphocytolytic effect of cortisol was also evidenced as an increase in non-sedimentable DNA in whole cell lysates (Giddings and Young, 1974) or as the release of DNA into the incubation medium (Haynes and Sutherland, 1967). By contrast with the effects of ionizing radiations and alkylating agents, within the period of 4 to 8 hours after administration of cortisol relatively low levels of soluble DNA were determined in both thymus and spleen (Fig. 1). It is therefore probable, that the process of lymphocytolysis is comparatively delayed after administration of cortisol. These findings are consistent with the idea that cortisol controls indirectly the lymphocytolytic process (Hofert and White, 1965, 1968). However, this assumption must be further examined taking into account that nuclear receptors have been detected for cortisol in thymus cells (Abraham and Sekeris, 1973).

The time course of the release of labelled DNA into the fraction of soluble DNA suggests that in thymus the dividing fraction of cells is more sensitive to the lymphocytolytic effect of cortisol (Fig. 2). However, the loss in the whole fraction of unlabelled DNA significantly exceeded the loss of labelled DNA at 24 hours after administration of cortisol (Table 1). These results may be reconciled if one considers the possibility that the process of lymphocytolysis of dividing thymocytes required a longer period of time or that the damaged dividing thymocytes were lost more slowly from the organ. It is therefore probable that different mechanisms might be implicated in the dying process of dividing and nondividing thymocytes.

The results indicating the release of labelled DNA in spleen (Fig. 2) support the assumption that a representative fraction of dividing cells was more sensitive to cortisol than the nondividing cells. This fraction of labelled cells appears to be rapidly removed from the organ and therefore induces the significant decrease of the total activity of DNA retained in spleen (Table 1). At 24 hours after administration of 160 mg/kg body weight cortisol histological preparations stained with haematoxylin-eosin have shown a decrease of the white pulp in spleen, especially

in the lymphoid follicles area. The perivascular sheats were less influenced. These findings are coincident with observations about an increased resistance to cortisol of T lymphocytes compared with B lymphocytes (R a f f , 1973). Thus, it is probable that the decrease of the total activity of DNA in spleen after administration of cortisol (Table 1) is mainly produced by the loss of dividing B lymphocytes.

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