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Morphological Changes in the Lymphoid Organs Induced by Diphenylhydantoin Sodium (DPH)

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Summary. In rats injected with DPH the morphological changes induced in the thymus and lymph nodes were studied. In the thymus, features suggesting block of cellular differentiation were found, and in lymph nodes depletion of the paracortical zone and intense plasma cell hyperplasia could be observed. The correlation of these findings with the functional changes in the immunological response induced by the drug, and the possible implications of these changes in the induction of lymphoma are discussed.

Key words: Hydantoins – Immunosuppression – Lymphoma.

Introduction

Lymphadenopathy is a common event in patients taking hydantoin derivatives as anticonvulsants for variable periods of time (Saltzstein et al., 1959; Lennert, 1961; Gams et al., 1968; Morgenfeld et al., 1970; Palmer, 1974). The histological aspects of the affected lymph nodes are variable, but hyperplasia of the reticulum and plasma cells, and cellular infiltration with neutrophils and eosinophils are the most common features.

Usually, the normal node architecture is not effaced (Saltzstein et al., 1959; Gams et al., 1968); however, in some cases, necrosis and/or fibrosis can change this picture. Even more important, atypical reticulum cells may be found, giving a lymphoma-like pattern (Gams et al., 1968; Palmer et al., 1974).

As most patients with hydantoin-induced lymphadenopathy experience prompt regression of all nodal enlargement after withdrawal of the offending drug, this syndrome is called pseudolymphoma (Hyman et al., 1966; Gams et al., 1968; Palmer, 1974). There are nevertheless at present in the literature 12 cases of true lymphoma in which a causal relationship to the use of hydantoins has been proposed (Aisenberg, 1973; Iashima, 1974).

These drugs also induce immunological deficiencies, the most important being depression of cellular immunity (Sorrel et al., 1971; Grob et al., 1972;

Sorrel et al., 1975); lower values of circulating IgA (Grob et al., 1972; Sorrel et al., 1975; Seager et al., 1975) and occurrence of antinuclear antibodies (Alarcon-Segovia et al., 1972; Beernink et al., 1973).

There are few experimental studies dealing with hydantoin-induced morphological changes in lymphoid organs (Kaslaris, 1953; De Srulijez et al., 1963; Krüger, 1970). The present paper is intended to analyse these changes in the rat.

Materials and Methods

Sixty female rats (Wistar strain), weighing about 200 g each, were divided into 6 groups of 10 animals: groups I (1 mg DPH/100 g of weight), groups II (5 mg DPH/100 g of weight) and groups III (control groups: injected with the carrier solution for the drug). For each dosage, one group was treated for 2 months (I_2, II_2, III_2) and the other for 4 months (I_4, II_4, III_4).

The drug used was sodium 5-5′ diphenylhydantoin (DPH) dissolved in a mixture of propyleneglycol, ethanol and water. Every animal received daily subcutaneous injections in the abdominal area. All were weighed at the beginning and at the end of the experiment. The thymus and mediastinal and axillary lymph nodes were excised for study.

Organ Weight. Thymus and mediastinal lymph nodes were carefully dissected and freed from connective tissue. The thymus and the whole set of mediastinal nodes were then weighed separately.

Histological Techniques. (1) Imprints, made from each organ, were stained with Leishman's stain. The percentage of thymic lymphoblasts was established by counting 500 cells. (2) Pieces of every organ were fixed in Bouin's fixative, and sections stained with hematoxylin-eosin in the usual way; additionally, formalin-calcium fixed material was stained with methyl green-pyronine (Lillie, 1965), silver impregnation for reticulin fibres (Gomori, 1937) and cells were made (Hadler, 1962).

Table 1. Thymus. Analysis of variance: weight (cubic root), percentage of cortex and lymphoblasts (angular transformation), lobular area (square root)

	Degrees of freedom	Weight F	% Cortex F	Lobular area F	% Lymphoblasts F
Dose I, II, III	2	5,640**	44,932***	7,450**	77,092***
Periods $I_2+II_2+III_2$, $I_4+II_4+III_4$	1	2,873 NS	12,980***	0,075 NS	17,002**
Dose × periods	2	1,167 NS	17,611***	0,152 NS	2,675 NS
Total dosage in periods I_2, II_2, III_2, I_4, II_4, III_4	5	3,297**	27,613***	3,057**	35,314**
Residual	54				
Mean		5,113	49,659	3,194	21,211
Standard deviation		0,389	1,536	0,241	1,552
Coefficient of variation		7,62%	3,09%	7,57%	7,32%

NS = not significant, ** = significant, *** = highly significant

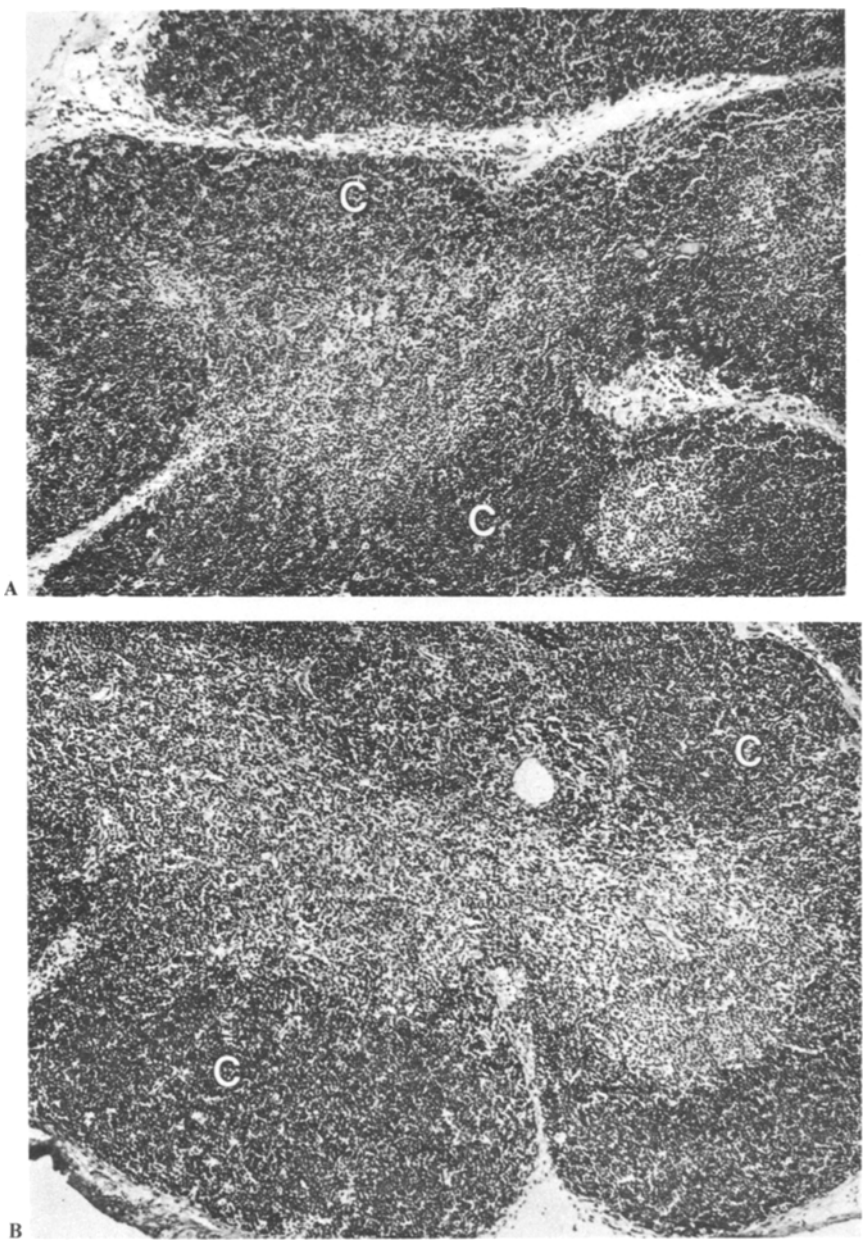

Fig. 1. Thymus. **A** Group III_4 (control), **B** group II_4 (treated). Cortex (*C*) is widened in treated rat (**B**). (H × E, × 80)

Table 2. Lymph nodes. Analysis of variance: weight of mediastinal lymph nodes (cubic root) and percentage of cortex of all lymph nodes (angular transformation)

	Degrees of freedom	Weight of mediastinal lymph nodes F	Mediastinal % Cortex F	Axillary % Cortex F
Dose I, II, III	2	11,982**	333,272***	298,469***
Periods $I_2+II_2+III_2$, $I_4+II_4+III_4$	1	26,909**	0,082 NS	2,202 NS
Doses × periods	2	6,222**	1,744 NS	0,022 NS
Total dosage in periods I_2, II_2, III_2, I_4, II_4, III_4	5	12,642**	134,642***	119,837***
Residual	54			
Mean		4,263	43,404	45,050
Standard deviation		0,380	2,085	2,173
Coefficient of variation		8,93%	4,80%	4,82%

NS = not significant, ** = significant, *** = highly significant

Quantitative Changes.. An attempt to quantify histological changes was carried out by measuring areas corresponding to defined lymphoreticular structures. Sections were projected with suitable magnification and planimetric measuring of the different areas was performed.

The relationship of the thymic cortical area to total tissue was calculated. In the same way, the mean area of lobules was calculated, by dividing the total area by the number of lobules. One section was used for each organ and each animal.

In lymph nodes the percentage of cortex and medulla was calculated. It was taken into account that the distribution of these two zones is not uniform in the different sections: serial sections (7 μ thick) spaced by 70 μ were examined. The mean value was used for calculations.

Statistical Analysis. This was performed by comparing the means, using the techniques of variance analysis and Tukey's test (Gomes, 1973).

Results

Weight. No significant difference was found among the body weight means of the six groups.

Thymus (Table 1). A significant increase of organ weight and lobular area was found in all treated animals, as compared with the control groups, but there were no significant differences among them. The percentages of cortical area and lymphoblasts also increased in the treated groups, but were proportional to dosage and time of treatment. Treated animals showed widening of cortex (Fig. 1 B). Imprints did not reveal any morphological abnormality of the cells. *Lymph Nodes* (Table 2). A significant increase of weight was found in the mediastinal nodes of animals treated for 4 months. This increase was the same for

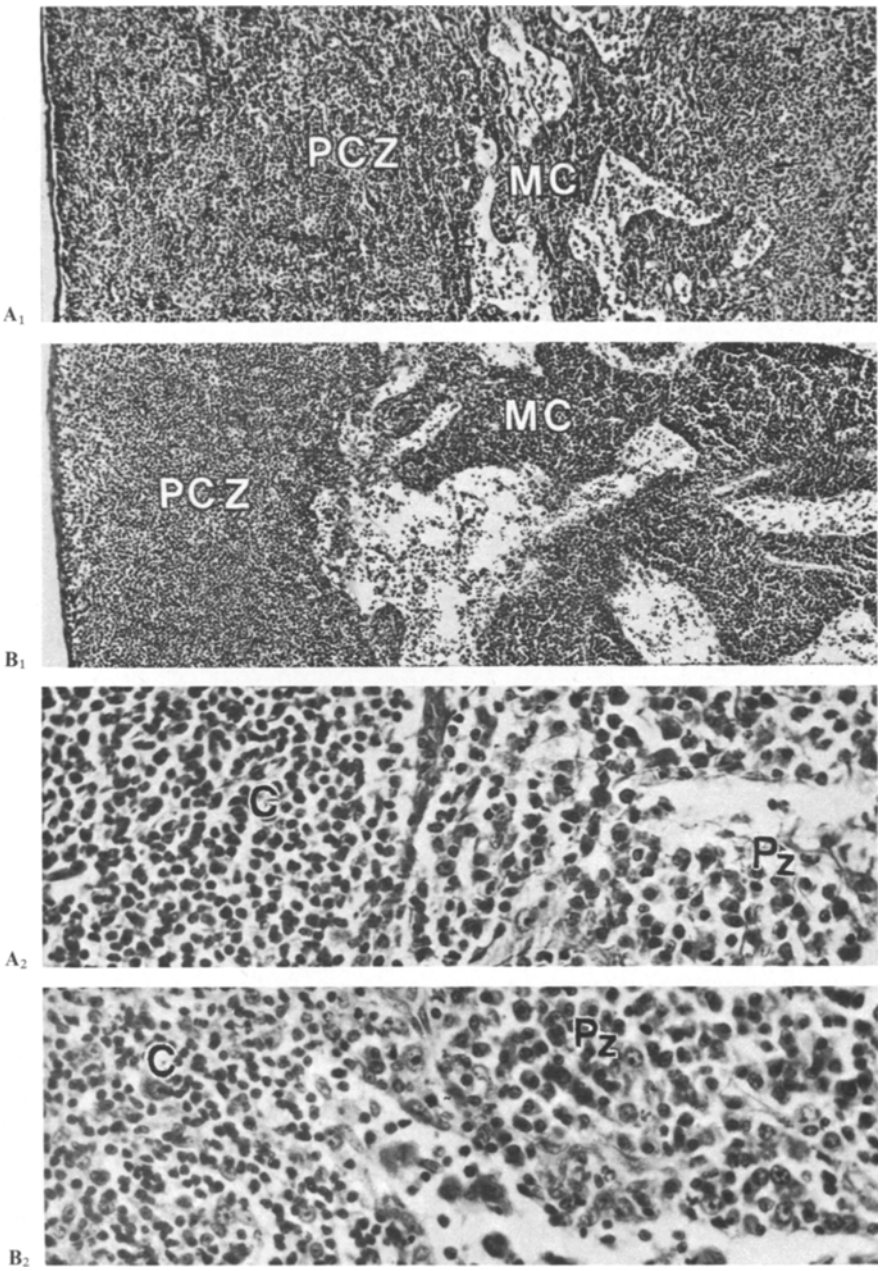

Fig. 2. Axillary lymph node. $\mathbf{A}_1$, $\mathbf{A}_2$ group III_4 (control), $\mathbf{B}_1$, $\mathbf{B}_2$ group II_4 (treated). Treated animal presents reduction of paracortical zone (*PCZ*) and hypertrophy of medular cords (*MC*) with increase of number of plasma cells (*Pz*). (H × E, A_1, $B_1 \times 80$; A_2, $B_2 \times 300$)

both dose schedules. The percentage of cortex of all nodes decreased equally in the four treated groups when compared with controls. This decrease depends on depletion of the paracortical zone (Fig. $2B_1$) which is narrowed and hypocellular. The follicles are prominent. The medullary zone is very large and presents intense hyperplasia of plasma cells at the medullary cords (Fig. $2B_2$), shown well by methyl green-pyronine stain. Reticular cells show a conspicuous and well-impregnated cytoplasm; the medullary sinuses are very prominent (Fig. $2B_1$). There are no cellular morphological abnormalities.

Discussion

The changes observed in the thymus suggest that increase in weight is due to the growth of cortex. Since the lobular area keeps constant in spite of progressive increases of cortex, there must be a gradual decrease of medulla. These findings, together with increase in the percentage of lymphoblasts present, suggest a maturation block in the thymus, since cell differentiation proceeds from the periphery to the center of the lobule (Sainte-Marie et al., 1958; Hwang et al., 1974). The mechanisms responsible for cell differentiation in the thymus are not well known. A humoral factor, thymosin, seems to promote maturation of thymic cells, and to influence the functional capacity of T lymphocytes (White et al., 1970). Our results suggest that the thymic changes could be produced by a drug effect which antagonizes the action of these factors.

De Srulijez (1963), studying rats injected with mesantoin, referred only to lymphoid hypertrophy; no comment relevant to our observation was made. Krüger (1970) treated mice with DPH with doses similar to those used by us, and did not find any noteworthy change. With very high doses he observed that the thymic hypertrophy found by us is accompanied by a striking hypoplasia of the paracortical zone of nodes. This zone is considered as thymus-dependent (Parrot et al., 1971; Müller-Hermelink, 1974). Thus in both cases, there is presumably depressed thymic function.

The hypoplasia of the paracortical zone found in all lymph nodes is not described in the literature on the hydantoins. However, Lennert (1961) and Holland (1965), reporting clinical cases, as well as Krüger (1970) in his experimental work, referred to the fact that hyperplasia of the lymph node medulla can reach the capsule indicating depletion of the paracortical zone.

Plasma cell hyperplasia in medullary cords—another prominent feature in our animals— is widely described, both in case reports (Saltzstein et al., 1959; Lennert, 1961) and experimental studies (De Srulijez et al., 1963; Krüger, 1970). The same is true of reticulum cell hyperplasia (Kaslaris, 1953; Salzstein et al., 1959; Lennert, 1961; Krüger 1970). Areas of necrosis or fibrosis were not present in our sections.

The histologic changes found in lymph nodes, showing depletion of T cell populations, together with increase in number of plasma cells, suggest a functional disturbance of the lymphoreticular system, implying a less effective control of the immunological response. Depletion of the paracortical zone is in keeping with depressed cellular immunity observed in hydantoin-treated patients (Grob et al., 1972; Sorrel, 1975). However Levo et al. (1975) found a normal response

to sheep red cell sensitization. They conclude that the drug would depress antibody production and maintain thymus-dependent function. Sensitization by sheep red cells depends on B and T cells (Mitchell et al., 1968) and depressed PHA lymphocyte response has been repeatedly shown in DPH-treated patients (Grob et al., 1972, Sorrel 1975). Dyminsky et al. (1974) describe two functional populations of T cells: one that responds to concanavalin A and alloantigens, and another that responds to PHA and is involved in the sensitization to sheep red cells. Therefore, DPH would inhibit a selective population of T cells that responds to PHA and has a helper function. Another relevant finding is the frequent appearance of antinuclear antibodies in these patients (Alarcon-Segovia et al., 1972).

In our material we did not find atypical cells or morphological features that would suggest lymphoma. Such features were found only by Krüger et al. (1972) in mice treated with DPH for long periods of time. In man, DPH has been associated with the appearance of lymphomas, especially in long-term DPH-treated patients (Hyman et al., 1966; Iashima, 1974). The incidence of lymphomas in hydantoin-treated patients is greater than the overall incidence of that disease in the same population (Anthony, 1968; Charlton et al., 1971; Li et al., 1975). This might be due to the chronic stimulation of lymphoreticular tissue by DPH (Gams et al., 1968), to a direct carcinogenic action of the drug (Hyman et al., 1966), or to a modification of susceptibility of the lymphoid tissue to an oncogenic stimulus (Aisenberg, 1973; Peckhman, 1974). All these hypotheses remain to be proved.

It appears to be clear that DPH induces a depression of cellular immunity which is in keeping with our own study. In fact Krüger (1970) found thymic atrophy using higher doses of the drug. On the other hand, there seems to exist a close relationship between immunodeficiency and the occurrence of lymphomas, especially Hodgkin's disease (Bobrove, 1975). Therefore DPH could be oncogenic, not by directly inducing cellular changes, but by modifying the immunological response of the host.

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