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Effect of Dilantin in Mice

I. Changes in the Lymphoreticular Tissue after Acute Exposure

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Dilantinwirkung auf Mäuse

I. Veränderungen im lymphoretikulären Gewebe nach akuter Einwirkung

Zusammenfassung. Dilantinbehandlung von Mäusen führt zu einer Herabsetzung der Blutbildung, Zerfall von Lymphocyten und zur Vermehrung pyroninophiler Reticulumzellen in Lymphknoten. Die Wirkungsweise wird als direkte Folge eines toxischen Effektes vermutet oder/und als Folge einer Arzneimittel-Überempfindlichkeit. Tumorentwicklung wurde nicht beobachtet.

Summary. Dilantin treatment in mice is followed by a depression of hemopoiesis, destruction of lymphocytes, and a proliferation of pyroninophilic reticulum cells in lymph nodes. The mode of action is felt to be a direct toxic effect of the drug as well as an induction of a state of hypersensitivity. Tumor development was not observed.

Hydantoin derivatives are widely used for the treatment of epilepsy, and lymphadenopathy with a morphologic resemblance to Hodgkin's disease is one of the several well documented effects of this drug in human pathology (Chiari, 1951; Saltzstein, 1959). Since laboratory mice of several inbred strains are susceptible to the development of induced or spontaneous lymphoreticular neoplasms, they were chosen as an experimental model for studies of the effects of hydantoin on the lymphoreticular system. Only brief and incomplete reports of the effect of this drug in mice were found in two earlier publications and these give no detailed description of changes in the lymphoid tissues (Gruhzit, 1939; Staple, 1954). The present report presents the results of a short-term study in mice where special attention was given to the morphology of the lymphoreticular organs.

Materials and Methods

Female C57 Bl mice 2—3 months old from the Animal Production Branch of the National Institutes of Health were caged together in groups of 6 and divided into 8 experimental groups (see Table 1).

A liquid diet was used in this experiment because it was the most satisfactory method of incorporating the drug in the food, and delivering a steady, fixed dosage. The diet consisted of Metrecal (Dutch Chocolate flavor) as available for humans. Its basic ingredients are concentrated sweet skim milk, milk protein concentrate, sugar, partially hydrogenated soy oil, and cocoa. The liquid is further enriched by vitamins and trace metals. Metrecal contains 7.4% protein, 2.1% fat, 11.6% carbohydrate, and gives 900 Calories per 8 U.S. fluid ounces (= 236.58 ml).

Feeding of mice with Metrecal according to a method of Dunn (1968) excluded the addition of regular mouse foods, but tap water was given ad libitum. Mice of groups IV and V

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Table 1. *Experimental groups*

Group number	Number of mice	Strain of mice	Food	Treatment	Dose[a] (mg/100 gm)
I	12	C57Bl	Metrecal	Dilantin	0.06
II	12	C57Bl	Metrecal	Dilantin	0.6
III	12	C57Bl	Metrecal	Dilantin	6.0
IV	12	C57Bl	Metrecal	Dilantin	25.0
V	12	C57Bl	Metrecal	Dilantin	50.0
VI	12	C57Bl	Metrecal	—	—
VII	12	C57Bl	Metrecal	(Starvation)	—
VIII	12	C57Bl	Purina lab chow	—	—

[a] Dose added to Metrecal per 100 g mouse.

received tap water only during weekends to stimulate Metrecal consumption. Mice of group VIII used as controls were fed Purina Lab Chow pellets and tap water ad libitum.

Metrecal was given daily in amounts of 80 ml per cage of 6 mice according to the estimated food consumption per mouse. Dilantin and folic acid were added just before feeding. The dilantin preparation used was the solution provided for intravenous injection (Dilantin Steri-Vials, Parke & Davis; 100 mg/2 ml). The undiluted preparation was used for groups III, IV, and V, while a 1:10 dilution in distilled water was used for groups I and II. Since Metrecal contains no folic acid, this was added to assure an adequate amount.

Commercial folic acid solution (Folvite Lederle, 15 mg/ml) was diluted 1:10 in distilled water, and 0.4 ml of this solution added to each 80 ml of Metrecal. This way every mouse was supplied with an average dose of somewhat below 0.1 mg folic acid per day.

Complete *autopsies* were done after two months of treatment in mice of groups I, II, III, VI, and VIII. Group IV, V, and VII mice were autopsied after 14—16 days of treatment. Among the lymphoreticular tissues investigated histologically were thymus, spleen, Peyer's patches, and the following lymph nodes: inguinal-, axillary-, brachial-, mesenteric-, parapancreatic-, pararenal-, tracheo-bronchial, and cervical nodes. The tissues were fixed routinely in Helly's solution, and for certain stains in neutral 4% formalin or Carnoy's solution. All sections were stained with hematoxylin and eosin; selected tissues in addition were stained with PAS, methylgreen pyronine, Wilder's reticulin reaction, iron reaction with Prussian blue, methenamine silver, van Gieson, Masson's trichrome, Brown-Brenn for bacteria, and Kenyoun's acid fast stain.

The *quantitation* of the lymph node response was done by counting pyroninophilic reticulum cells in a comparable reference area. This area was obtained by superimposing a large composite square consisting of 25 smaller squares in such a way that a cross-section of a postcapillary venule was located in the center of this large square. To find this area, the square was moved from a lymph node follicle to its periphery, and from there to the nearest cross-section of a postcapillary venule. Only pyroninophilic reticulum cells each with a large prominent nucleolus were counted at 400 times magnification. The "cell response" was recorded therefore as the number of pyroninophilic reticulum cells per unit area in a perifollicular area.

Cell Measurements. 100 pyroninophilic reticulum cells from the perifollicular area were measured for the width of the cell diameter, the nuclear diameter, and the nucleolar diameter from one each of the following mice: untreated C57Bl, Metrecal-fed C57Bl, Dilantin-treated C57Bl of group III (medium dose group), and tubercle bacteria sensitized C3H mice. Two measurements at a 90° angle were taken. Of each group two mesenteric-, two salivary gland-, two inguinal-, and two axillary lymph nodes were investigated per mouse. All 6 mice per experimental group were investigated this way. Measurements were performed with a Zeiss eye piece micrometer after calibration. For comparison of lymphoid tissue reactions 6 C3H mice in addition were sensitized with an antigen to give rise to a delayed-type of immune response. Sensitization of C3H mice was done with two subcutaneous injections of 0.1 mg H37Ra tubercle bacteria in 0.1 ml incomplete Freund's adjuvant at one weeks interval. Mice were

skin-tested with PPD (purified protein derivative) 14 days later, and only lymph nodes of mice exhibiting a positive delayed-type reaction were taken for cell measurements.

Dilantin Sensitization. 12 C3H/HeN female mice two months of age and 8 C57Bl female mice of the same age were sensitized by Dilantin administration to their shaved skin of the back. All C57Bl mice and six of the C3H mice received Dilantin suspended in acetone (100 mg in 10 ml), while the remaining six C3H mice received Dilantin suspended in turpentine (100 mg in 10 ml). Of these solutions two drops were used at each time. The C57Bl mice received altogether 4 treatments at one weeks interval; the C3H mice 11 treatments at three day intervals except a ten-day interval between the tenth and eleventh dropping. At the last day 0.01 ml Dilantin suspension in saline was injected intracutaneously into the foot pad. Injection of saline alone served as control. Forty eight hours later, the mice were sacrificed and treated skin and foot pad tissue as well as regional lymph nodes were taken for histology.

Results

1. Metrecal Consumption

Metrecal was well accepted by all mice as replacement for the usual food pellets. The daily intake per mouse was 12.3—12.5 ml, when mice were caged in groups of six. Decreasing the number of mice per cage led to an increase in Metrecal consumption per mouse.

2. Dilantin Dosage

Estimation of the Dilantin dose administered in Metrecal to mice was quite accurate when calculated on the basis of Metrecal consumption. However, the dose in the high Dilantin-dose groups (IV and V) had to be re-calculated downward, since the intake of Metrecal was greatly reduced (see Table 2).

Table 2. *Metrecal consumption and dilantin dose*

Group number	Metrecal intake per mouse per day (ml)	Dilantin dose as added[a] (mg)	Dilantin dose actually taken-up[a] (mg)	Dilantin dose total per mouse (mg)
I	12.6	0.06	0.06	4.55
II	12.6	0.6	0.6	45.44
III	11.8	6.0	5.8	415.7
IV	5.0	25.0	23.5	273.9
V	4.3	50.0	36.1	342.5

[a] Per 100 g mouse.

3. Clinical Findings

Mice on the low and medium-dose Dilantin, and mice on Metrecal diet alone behaved like normal untreated mice. Mice with reduced food intake (low Metrecal group VII, high Dilantin groups IV and V) weighed between 10 and 16 g as compared with 21 and 23 g in normal mice and mice of groups I, II, III, and VI. The activity of starved mice was increased and they were more irritable. Seizures were observed several times from no more stimulation than the usual noise during cleaning of cages. Starved mice on high doses of Dilantin (Groups IV and V) became more sluggish towards the end of the experiment and many mice appeared to be moribund but on mechanical stimulation they exhibited slow atactic movements and abnormal posture. It became most obvious in an abnormal

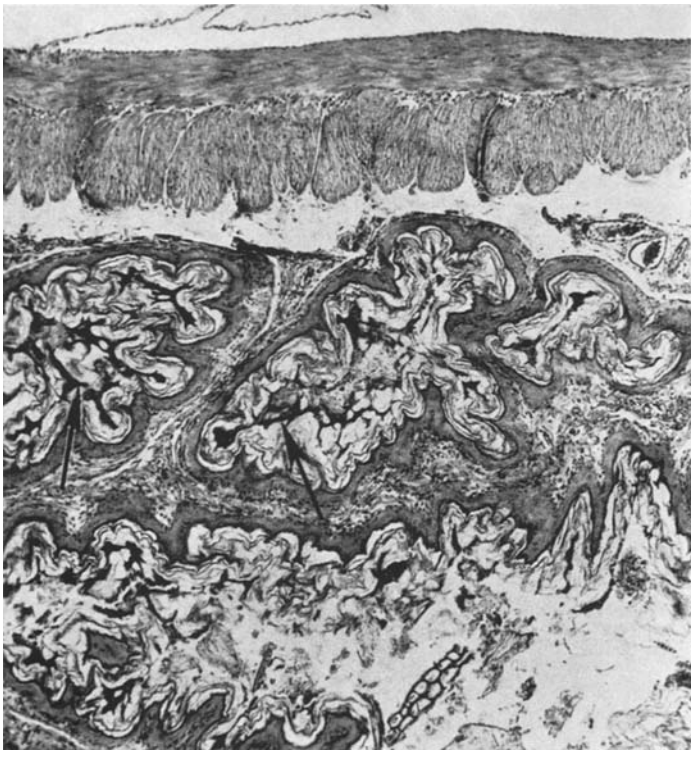

Fig. 1. Four months old female C57Bl mouse, since two months on Metrecal diet. Note the many lamellae of keratin and black accumulations of bacteria in the forestomach (arrow). (H. and E., 65×)

position of head and tail. The head often was kept turned to one side and the tail stiffly was held to one side or upwards. Such mice were unable to feed from drinking bottles; they were killed and autopsied therefore. Two mice of the highest Dilantin dose group (V) died after 14 days of treatment.

4. Morphology

The observations on the treated mice were always compared with the group of untreated C57Bl mice (VIII). This group is not described separately.

Group VI (Metrecal only). When folic acid was added to Metrecal, no changes in the lymphoreticular tissues were seen. The most prominent difference of Metrecal fed mice as compared to pellet-fed mice consisted in a hyperkeratinization of varying degree in the epithelium of the forestomach, and masses of bacteria in the lumen (Fig. 1). No acid-fast bacteria or fungi were found in special stained sections. Peyer's patches in Metrecal-fed mice showed somewhat more frequent secondary follicles, but were not striking otherwise.

Groups I—III (low and medium Dilantin dose). Macroscopically, the lymph nodes and Peyer's patches were somewhat enlarged but not striking otherwise. The spleen of medium dose Dilantin mice (group III) was also somewhat enlarged,

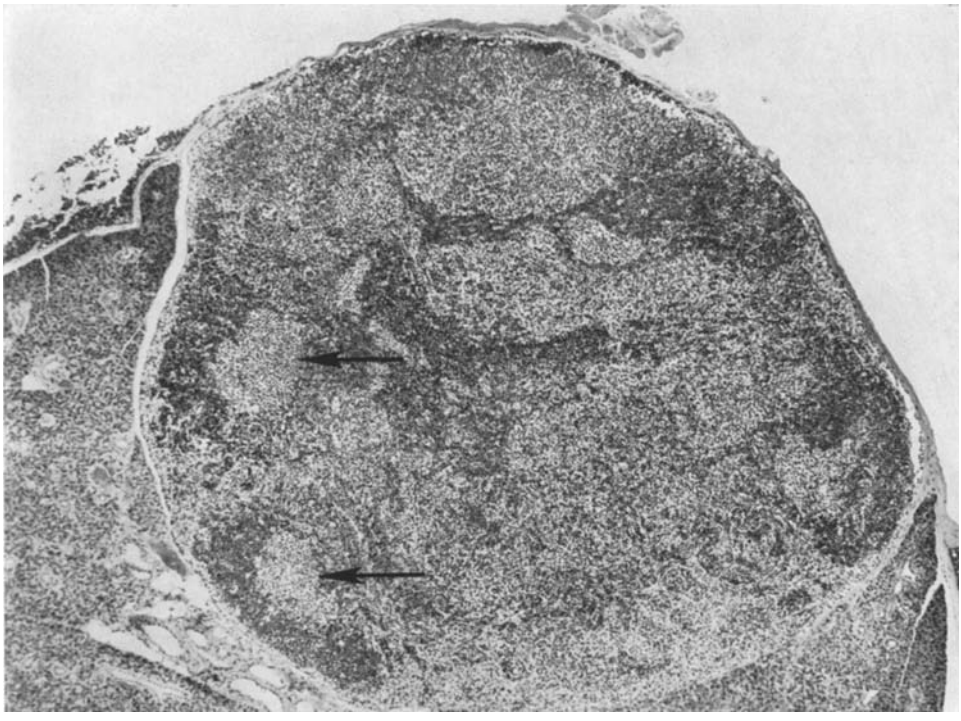

Fig. 2. Four months old female C57 B1 mouse, since two months on Metrecal-Dilantin diet (group III). Note deranged structure of salivary gland lymph node. Two remaining follicles (arrow) consist only of pale cells. Irregular increase in large cells partly arranged in large foci without respect to the pre-existing lymph node structure. (H. and E., 65×)

but otherwise normal. The color of the organ was dark purple and the consistency regular. The thymuses of all groups appeared normal.

The types of changes observed histologically in the lymphoreticular tissue were similar in all three groups; they differed only in degree. The *lymph nodes* showed prominent secondary follicles containing many macrophages loaded with nuclear debris. In some groups of lymph nodes (cervical nodes) the secondary follicles appeared to be in direct contact with medullary cords. These were densely filled with large immature looking plasma cells and pyroninophilic reticulum cells. In the perifollicular area were many pyroninophilic reticulum cells of varying sizes and shapes with large plump nucleoli. Few binucleated reticulum cells, an occasional mononuclear giant cell, and large cells in mitosis were also seen in this area (Figs. 2, 3 and 4).

Measurements of pyroninophilic reticulum cells in the perifollicular area indicated a greater variation in cell size, and more relatively large cells, when compared with the cells in the same areas in untreated and Metrecal fed mice (see Fig. 5). Also nuclei and nucleoli were in general larger than in untreated mice. The number of blastic reticulum cells with more than one nucleolus was also increased. This was similar to the observations made in antigen-stimulated mice.

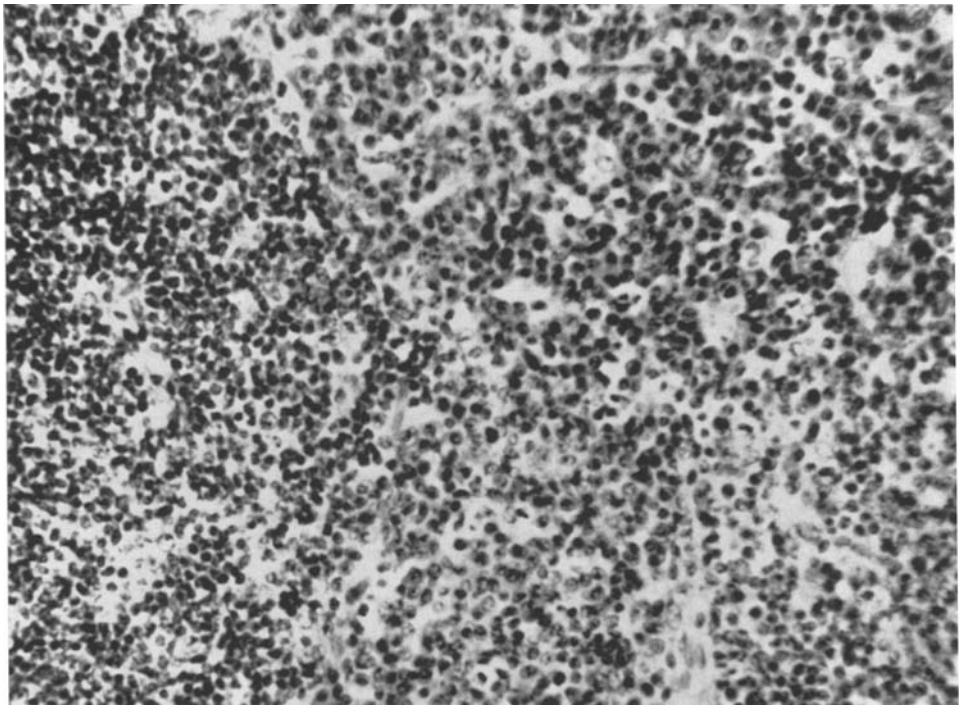

Fig. 3. Perifollicular area of lymph node of Fig. 2 at higher magnification. Note decrease in small lymphocytes and increase in reticulum cells and histiocytes. (H. and E., 340×)

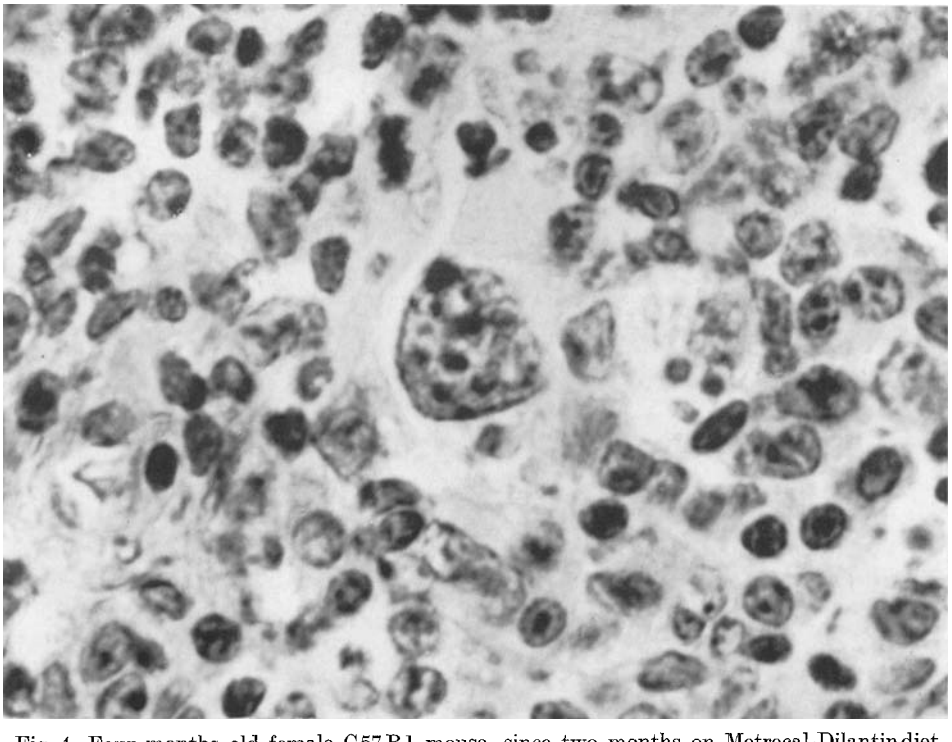

Fig. 4. Four months old female C57B1 mouse, since two months on Metrecal-Dilantin diet (group IV). Detail of cell population in perifollicular area with giant cell, probably of histiocytic origin. (H. and E., 1,340×)

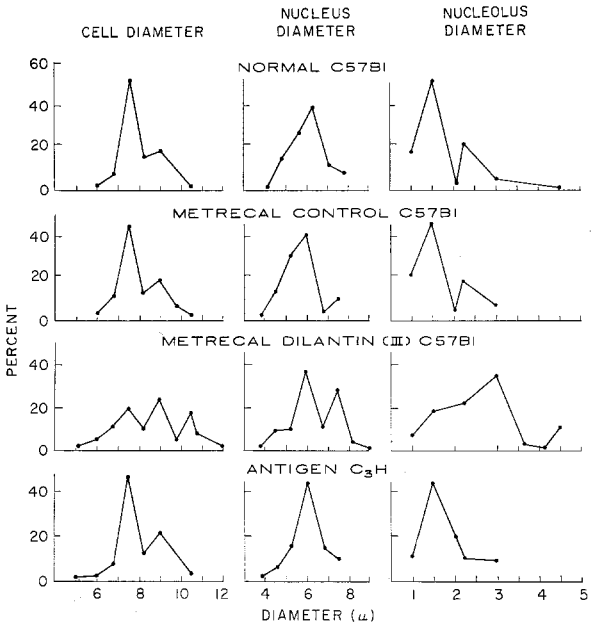

Fig. 5. Cell measurements

The endothelium of the postcapillary venules formed a monolayer of cuboidal cells which were faintly pyroninophilic. A few small lymphocytes were found between these cells. The endothelial cells of the peripheral sinuses were often swollen and appeared more numerous; many cells were pyroninophilic and similar to activated reticulum cells i.e. blastic pyroninophilic reticulum cells.

The *thymus* in these mice did not differ from the thymus of normal untreated C57Bl mice of the same age. A broad cortex contained many small thymocytes, and several larger lymphoblast-like cells with variable degree of pyroninophilia were seen beneath the capsule. The medulla was composed of a loose network of reticulum cells that appeared inactive, and a few small thymocytes were seen among them. Single epithelial cells and small aggregated foci of similar cells were found regularly.

The *spleen* in group I mice (low Dilantin dose) was not obviously changed. The red pulp of the spleens from mice on the semi-low and medium Dilantin dose (groups II and III) was hyperemic and otherwise less cellular. The paratrabecular cell foci especially were smaller, and immature progenitor cells of the erythrocytic and granulocytic series were obviously decreased in number. Fewer megakaryocytes were seen, and counts made of ten high-power fields (objective 40×, wide angle eye piece 10×) showed only 6—7 megakaryocytes in medium-dose Dilantin mice (group III) as compared with 11—13 megakaryocytes in the red pulp of untreated mice. The outer zone of the splenic follicles consisting of pale reticulum cells, appeared broader than normal, but otherwise the follicles were unchanged. A few pyroninophilic secondary follicles were present.

Table 3. *Number of blast cells per unit perifollicular area*[a]

Group number	Mesenteric	Lymph nodes		Renal	Parapancreal	Cardial
		peripheral[b]	salivary gland			
I	13.8	8.6	16.8	6.0	8.0	4.4
II	14.5	10.4	27.3	7.0	8.7	4.8
III	12.8	8.8	16.5	8.3	17.2	8.2
IV	26.8	13.3	21.7	15.3	18.8	10.5
V	23.3	15.4	22.7	17.0	19.0	11.2
VI	8.0	5.5	7.0	4.3	7.4	4.0
VII	6.6	4.5	4.0	1.8	—	3.8
VIII	7.0	6.5	8.0	2.5	6.5	4.5

[a] Average from 5—10 counted areas.
[b] Inguinal and axillary nodes.

The *Peyer's patches* of mice on low- and medium-low Dilantin treatment (groups I and II) looked the same as in Metrecal-fed mice: 2—4 well defined secondary follicles composed of a pale and a basophilic part were seen. They contained several phagocytes with nuclear debris. The secondary follicles were surrounded by small lymphocytes; only occasional blastic reticulum cells appeared in the perifollicular area. Postcapillary venules showed a flat endothelium that appeared inactive. Mitoses were rare in the secondary follicles. Mice on a medium level Dilantin dosage showed an increase in debris-laden follicular macrophages. Mitoses were found more frequently in the secondary follicles of these mice. Pyroninophilic reticulum cells also were increased extrafollicularly and tended to accumulate in the subepithelial, i.e. the area of the Peyer's patches next to the intestinal lumen.

Groups IV and V (high Dilantin dose). The histology of the *lymph nodes* did not differ qualitatively from that described in the low- and medium Dilantin groups. However, the extent of the response was obviously greater: The secondary follicles were abundant and often appeared to be disrupted, and in advanced lesions the secondary follicles consisted mainly or exclusively of pale cells. Many large follicular phagocytes containing nuclear debris and PAS-positive cytoplasmic droplets were seen within secondary follicles, and in nearly every area of the lymph nodes as well. In the perifollicular area, the number of pyroninophilic reticulum cells was in excess of the number seen in mice on the lower-dose Dilantin (see Table 3). The variation in the size of these cells also appeared to be increased, with a relative increase in the number of large cells. A few giant cells were observed in the perifollicular area (Fig. 4). The postcapillary venules showed a monolayer of large cuboidal epithelial cells with varying degrees of pyroninophilia. The endothelial cells of the peripheral sinuses were swollen, pale pyroninophilic, and often formed solid sheets of cells. Three mice showed multiple foci of epithelioid cells throughout their mesenteric lymph node. This lesion resembled histologically with what was described as the Potter lesion or reticulum cell neoplasm type C (Dunn, 1954). This lesion also was seen in two instances in Metrecal fed mice. Abundant masses of immature large plasma cells and pyroninophilic reticulum cells populated

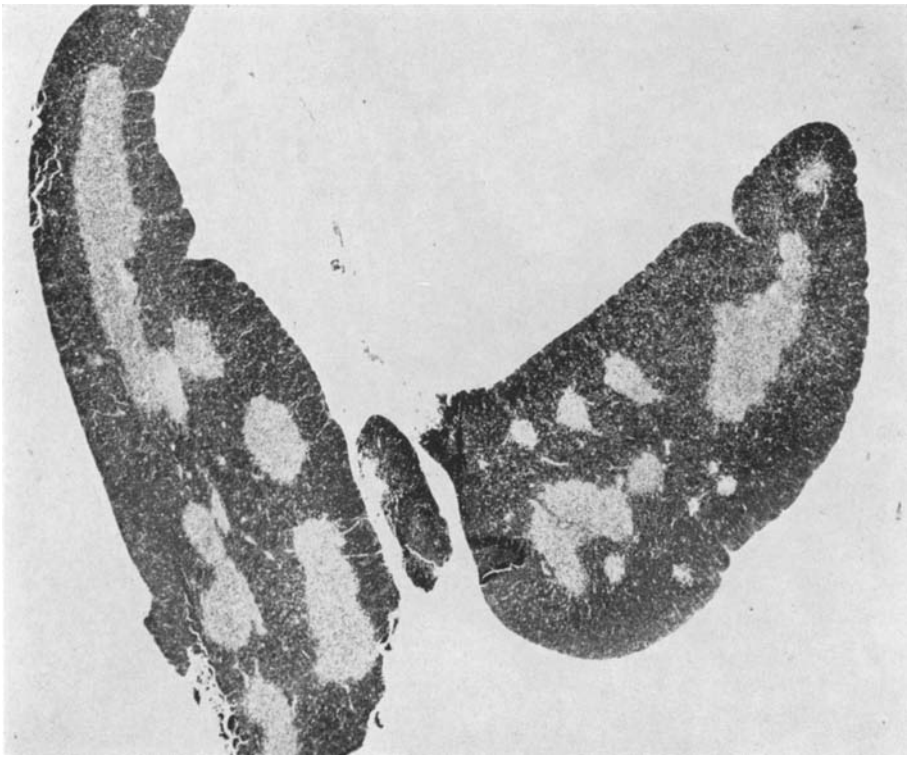

Fig. 6. Untreated four months old female C57Bl mouse kept on Purina lab chow diet. Normal size and appearance of the thymus. (H. and E., 22×)

the medullary cords; among these many debris laden phagocytes were seen similar to follicular macrophages.

The *thymuses* in these mice were severely atrophic. The cortex appeared almost completely depleted of small thymocytes, and a web of pale reticulum cells and foci and sheets of epithelial cells became visible. Many Hassal's corpuscles were seen, and many of them showed central lumina filled with cell debris and granulocytes. The thymic medulla contained more thymocytes than the cortex so that a picture was seen which has been described as "cortical inversion" (Figs. 6—9).

The *spleen* of high-dose Dilantin mice also showed an increased atrophy of the red pulp. The number of megakaryocytes and hematopoietic cells of the erythrocytic and granulocytic series was further reduced as compared to low-dose Dilantin groups. Only an occasional pyroninophilic stem cell was seen in the peritrabecular area. Some spleens exhibited small foci of hemorrhage. The splenic trabeculae and the capsule appeared thicker than normal. The follicles also were small and consisted mainly of small lymphocytes. These were less compact than normal indicating a loss of cells. Between the follicular lymphocytes many phagocytes filled with nuclear debris ("tingible bodies") were seen. The outer follicular zone of pale reticulum cells was smaller than in low- and medium-dose Dilantin mice. Occasional foci of pyroninophilic reticulum cells appeared within the follicles

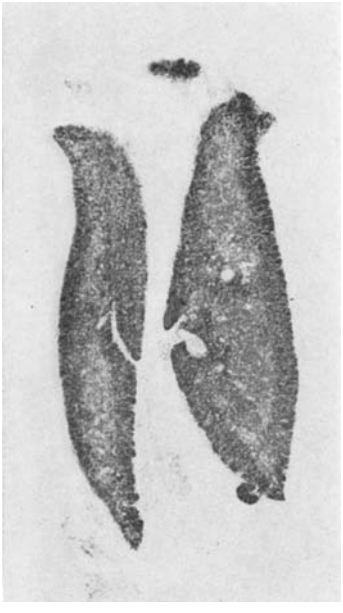

Fig. 7

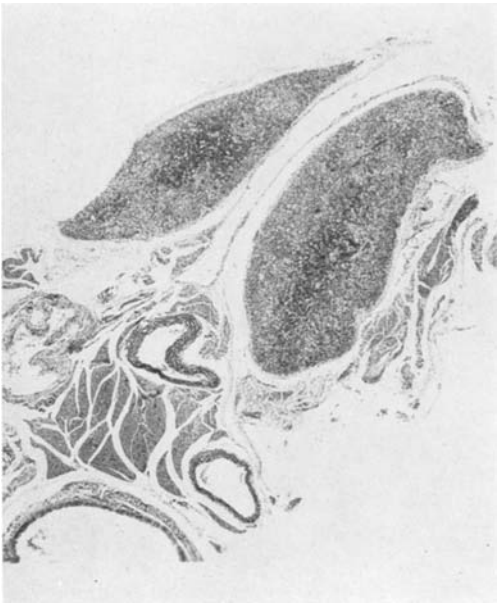

Fig. 8

Fig. 7. 10 weeks old starved female C57Bl mouse, since two weeks on low Metrecal diet. Severe homologous atrophy of the thymus. (H. and E., 22×)

Fig. 8. 10 weeks old starved female C57Bl mouse, since two weeks on Metrecal and high dose Dilantin diet. Severe atrophy of the thymus with signs of cortical inversion. (H. and E., 22×)

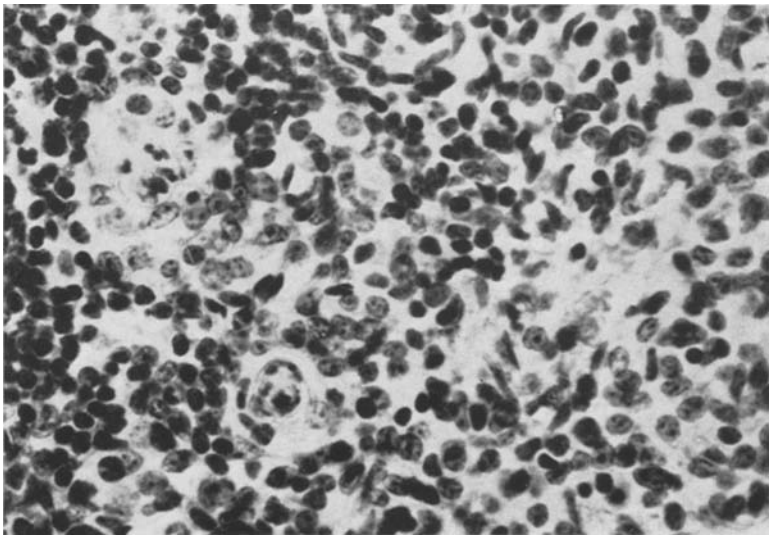

Fig. 9. Higher magnification of corticomedullary junction of thymus from Fig. 7. Apparent decrease of small lymphocytes in cortex, two Hassal's corpuscles at junction, more small lymphocytes in medulla. (H. and E., 675×)

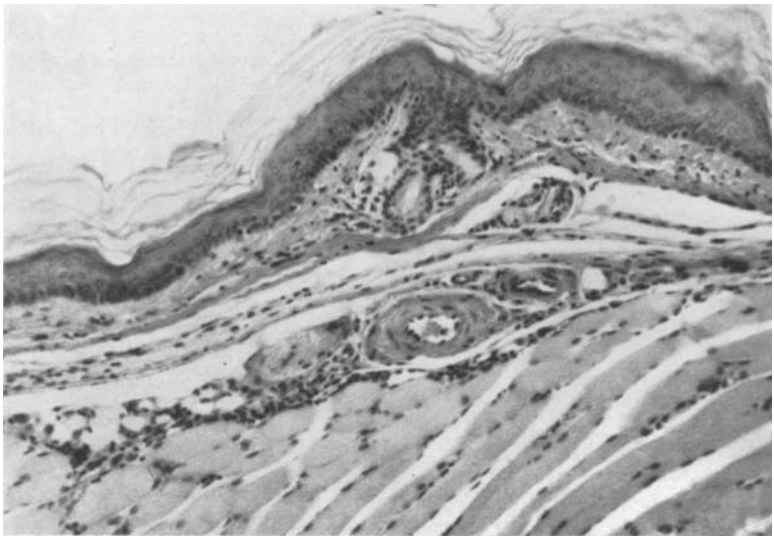

Fig. 10. Female C57B1 mouse sensitized by cutaneous dropping of Dilantin in aceton suspension. Foot pad tissue 48 hours after intracutaneous Dilantin test injection. Note perivascular lympho-histiocytic infiltrates. (H. and E., 150×)

and close to them. The cells of these foci were quite large with an abundance of cytoplasm and reached sizes up to 12.6 μ cytoplasmic diameter (nuclear diameter 9.5 μ, nucleolar diameter 3.2 μ). They sometimes resembled epithelioid cells.

The *Peyer's patches* in mice with high doses of Dilantin showed many secondary follicles that were often quite dissociated and occupied nearly the whole Peyer's patch. Many debris-loaded follicular macrophages were present through the tissue. Large irregular foci of pyroninophilic reticulum cells occupied the area of the Peyer's patch nearest the intestinal epithelium. The general picture of the Peyer's patches in these mice resembled that in medium-dose Dilantin mice, but the extent of the changes was more striking.

One mouse of the high-dose Dilantin mice (group V) showed foci of pyroninophilic reticulum cells in the bone marrow.

Group VII (Metrecal starvation mice). Starvation in Metrecal fed mice resulted in a homologous atrophy of lymphoreticular tissues, i.e. all structural elements appeared to be equally reduced in size and number. The cortical inversion seen in the thymus in mice with high-dose Dilantin treatment was not observed (Fig. 9). An exception to the generalized atrophy was seen in the Peyer's patches where 1—2 secondary follicles could still be found and also several pyroninophilic reticulum cells in the perifollicular area. These changes, however, did not reach the extent seen in Metrecal-fed Dilantin mice.

Observations on sections stained by silver of lymphoreticular tissues in any group failed to show destruction or a change in the basic structure. No infection by fungi or acid-fast bacteria was demonstrable by histologic methods, and no evidence for a generalized bacterial infection was obtained.

5. Dilantin Sensitization

Two out of 8 C57Bl mice showed slight perivascular lympho-histiocytic infiltrates in the foot pad tissue when challenged with Dilantin suspension (Fig. 10). No reaction was seen in the control mice. The regional (abdominal wall-) lymph nodes showed several pyroninophilic reticulum cells in the perifollicular area in all 8 mice.

The C3H mice sensitized by Dilantin in acetone solution showed slight perivascular lympho-histiocytic infiltrates in the foot pad tissue of one out of 6 mice, while 4 mice had increased numbers of pyroninophilic reticulum cells in the perifollicular area of the draining lymph nodes. The mice treated with Dilantin suspended in turpentine showed comparable perivascular infiltrates in the foot pad tissue of 2 out of 6 mice, and reticulum cell activation in the perifollicular area in all 6 mice. The skin at the site where Dilantin was dropped exhibited chronic inflammatory changes with hyperkeratosis, ulceration and lympho-granulocytic infiltrates accompanied by fibroplasia.

Discussion

The *doses of Dilantin* administered to mice were chosen to be comparable to dose levels used in patients with epilepsy. The dose calculation was done on a body weight basis, and the dose of Dilantin given to mice of group II may be compared to a medium dose used for human treatment. However, it is not possible to determine dose effects from one species to another on a body weight basis alone. Therefore, a range of doses had to be tried, and the Dilantin dose in group I was one-tenth of that of group II, and in group III ten times the dose of group II. The high doses given in groups IV and V were to obtain information on an acute toxic dose. The dose calculation was based on a report of Gruhzit (1939) who stated that mice tolerate a fifth of the maximum dose in rats (which is 2,200 mg/kg), which would be about 44 mg/100 g mouse. The mice of groups IV and V in this experiment with a reduced uptake of Metrecal did not reach this dose. This was probably because of the bitter and soapy taste of a high concentration of Dilantin, and a toxic effect of Dilantin and loss of appetite could also be factors. Whatever the reasons and probably intensified by starvation, the Dilantin doses as used in groups IV and V appeared to be close to the upper limit for oral administration of Dilantin to mice, since at this dose level mice did not survive for more than 16 days.

A state of hypersensitivity induced by Dilantin is indicated by these studies. The morphology of lymph nodes in Dilantin treated mice was consistent with changes reported to occur in antibody formation (Ringertz, 1950; Movat, 1965). The increase in size of secondary follicles and the increase in number of immature plasma cells in the medullary cords indicate an activation of the follicle-plasma cell-system, while the activation and proliferation of reticulum cells in the perifollicular area indicate an activation of the thymus-dependent perifollicular system. Perivascular lympho-histiocytic infiltrates in foot pad tissue of some of the Dilantin sensitized mice as well as the morphology of draining lymph nodes

provided further support for the ability of Dilantin to elicit hypersensitivity reactions. Skin rash, anemia, and pancytopenia in human patients with Dilantin treatment, therefore, may well result from drug hypersensitivity.

There appears to be a direct relationship between Dilantin concentration and lymph node response. Mesenteric-, parapancreatic-, and cervical lymph nodes draining areas of resorption and secretion of the drug showed the most pronounced changes. It is of interest that in rats and cats salivary glands concentrated Dilantin (Babcock, 1964), which may explain the intense effect of this drug on draining cervical lymph nodes.

The association of hyperplasia of pyroninophilic reticulum cells in lymph nodes with Dilantin treatment is clearly evident, but an increase in the bacterial flora in the intestine of Metrecal fed mice may have added to the intensity of the morphological changes. The bacterial alteration, however, cannot explain the Dilantin dose-dependent increase in pyroninophilic reticulum cells. Additional antigenic stimulation can arise from the products of cell destruction, for instance from lymphocytic breakdown. This reaction, also, may be directly related to the Dilantin treatment as will be discussed below. In a similar fashion, antibody formation against host tissue components was found after administration of hepatotoxic substances (Sargent, 1966).

A direct *toxic effect* of Dilantin is also apparent besides induction of hypersensitivity. Numerous germinal center macrophages laden with nuclear debris (tingible bodies) were observed in lymph nodes concomitant with decreasing numbers of small lymphocytes in the perifollicular area; and many Hassal's corpuscles in the thymus were filled with chromatin debris and appeared to be undergoing atrophy. These observations are indications of a greatly increased lymphocytolysis (Blau, 1968). Despite some morphological resemblance of these results with those obtained in lymphoid tissues after glucocorticoid treatment (Dustmann, 1968) the thymus in Dilantin treated mice did not respond to the same extent (Lundin, 1966, 1969). Also, the effect on lymphocytes of Dilantin was apparently not mediated by glucocorticoids as with other drugs that produce similar effects in lymph nodes (salicylic acid and phenacetin, Uher, 1961), because Dilantin decreased ACTH secretion and glucocorticoid synthesis (Bürger, 1959), and the adrenal glands in mice showed weight loss and atrophy accordingly (Staple, 1954).

The toxic effect of Dilantin apparently is not limited to cells of the lymphocytic series, but includes also erythrocytic and granulocytic cell types. A depression of hemopoiesis in spleen and bone marrow (as will be reported extensively later) is supporting this impression. Anemia and granulocytopenia as observed in human patients with Dilantin treatment, therefore, may be a consequence of direct toxicity of the drug for hemopoietic tissues besides its action through hyersensitivity.

Tumor development following Dilantin therapy as described in human pathology was not observed in mice in this short-term experiment. The morphology of lymph nodes draining areas of resorption and secretion of Dilantin, however, showed often changes that exceeded quantitatively the response to known

antigens; the cytomorphology of pyroninophilic reticulum cells showed a greater variability reflecting a highly increased cell proliferation (Lennert, 1961). In high Dilantin dose groups, the activation of reticulum cells in the perifollicular area was most striking while the secondary follicles were usually smaller than in the low Dilantin groups and often did not show the basophilic area. Coincident with this, plasma cells remained more immature but were numerous. This change, however, has to be confirmed by further studies, and its meaning is not clear so far. The reticulum cell lesion type C as seen in Dilantin mice does not exceed the spontaneous occurrence of this lesion, and its neoplastic nature still needs confirmation.

Cortical inversion of the thymus as seen in starved high-dose Dilantin mice was reported to precede leukemia development in AKR mice, and has been regarded as a preleukemic change (Metcalf, 1966). However, no conclusion about the possible preleukemic significance of this thymic alteration can be drawn from our experiment so far, since all the mice showing cortical inversion were severely sick and had to be killed so the further course of the lesion could not be followed. An experiment now in progress where chronic Dilantin administration of medium doses is given to mice may help in understanding this thymic lesion.

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