the reduced accumulation of radioactivity after simultaneous injection of tritiated TRH and cold TRH. Therefore, future studies on the feed-back mechanism in the hypothalamo-hypophyseo-thyroid axis should consider the existence of such a peripheral effect. The decrease of radioactivity by simultaneous injection of cold TRH is also shown for the pituitary and hypothalamus. For the pituitary, the prime site of action of TRH, this effect is expected. For the hypothalamus some reservations on the significance of these data are justified, because the dpm are always below the counts in the plasma. However, in view of the observation of TRH-sensible neurons in the hypothalamus[10], a hypothalamic effect may also be considered.

Résumé. La TSH-releasing hormone (TRH) tritiée a été produite. La substance radioactive est accumulée non seulement dans l'hypophyse et le rein, mais également dans la thyroïde après injection par voie endocarotidienne. La signification physiologique de l'action directe de la TRH sur la thyroïde est discutée.

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Supression of Fetal Hematopoeitic Development by Prednisone Treatment of Pregnant Rats

Some authors have reported stimulation of the bone marrow in adult animals following cortisone treatment[1–4]. Other investigators have found that cortisone depresses, in different animal species, the maturation and proliferation of hematopoietic cells[5–8]. Inhibition of nucleic acid synthesis in the bone marrow by cortisone has also been observed[9], and BETZ[10] reported that cortisone impeded recovery of rat hematopoietic organs after X-irradiation.

We found previously that daily prednisone administration produced, at certain doses, bone marrow aplasia in rats previously irradiated with subletal doses of ^{60}CO[11]. In this work we have studied the influence of 16β-methyl prednisone administration to pregnant rats on the development of hematopoietic organs in their offspring.

24 inbred pregnant hooded rats were used. 12 animals were treated daily during the last week of pregnancy by s.c. injection with a suspension of 16β-methyl prednisone (Deltisona B, 8 mg, Lepetit) at a dose of 2 mg/kg/day (4 rats) and 4 mg/kg/day (8 rats). The rest of the animals were used as controls. Duration of pregnancy was checked in every case by daily inspection of vaginal contents, for cycle stage and presence of spermatozoids. As first day of pregnancy we took the last day in estrous with spermatozoids present followed by a prolonged diestrus and detectable fetuses. In total 181 animals were killed within the first 24 h of life. Body, thymus and spleen weights were taken. Spleen, thymus, liver and the right femur were embedded in 10% formol (the bone was also decalcified in 6% nitric acid), imbedded in paraffin, serial sections at 6 μm prepared and then stained with hematoxylineosin.

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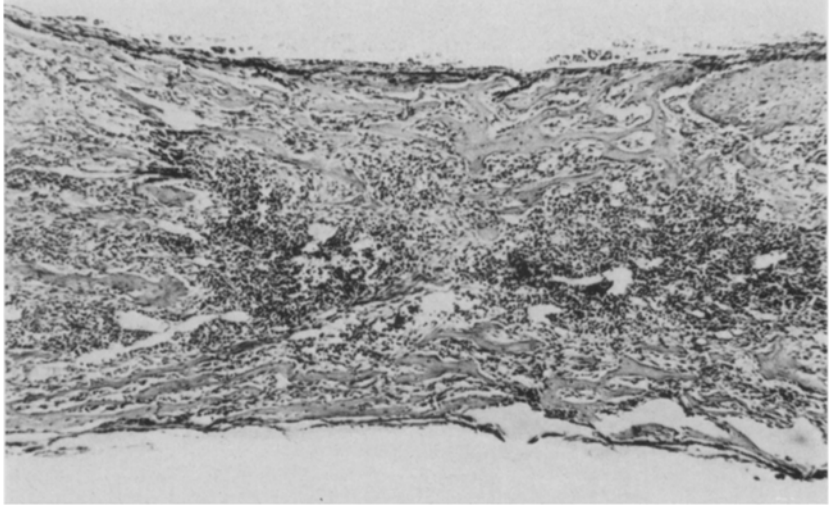

Fig. 1. Femur section of newborn rat born from untreated mother.

Reduction of body weight, absolute and relative thymus and spleen weights in newborn rats of mothers treated during pregnancy with 16β-methyl prednisone

Steroid doses	No. of mothers	No. of offsprings	Weight			Relative weight[b]	
			Offsprings (g)	Thymus (mg)	Spleen (mg)	Thymus	Spleen
2mg/kg/day	4	31	5.53 ± 0.14[a] ($p < 0.05$)[c]	4.63 ± 0.26 ($p < 0.001$)	12.24 ± 0.27 ($p < 0.05$)	0.83 ± 0.035 ($p < 0.001$)	2.21 ± 0.05 ($p > 0.1$)
4 mg/kg/day	8	58	4.75 ± 0.12 ($p < 0.001$)	2.82 ± 0.21 ($p < 0.001$)	8.02 ± 0.31 ($p < 0.001$)	0.52 ± 0.029 ($p < 0.001$)	1.68 ± 0.04 ($p < 0.001$)
Control	12	92	6.19 ± 0.14	8.72 ± 0.14	14.84 ± 0.28	1.41 ± 0.024	2.41 ± 0.04

[a] Mean $\pm$ standard error. [b] Organ weight/body weight. [c] Student's t-test.

The femur bones of 73 newborns, after heparinization before sacrifice, were used for the quantitative study of bone marrow. The bones were carefully dissected, weighed and then longitudinally incised with a scalpel. The bone marrow was removed by means of a blunt instrument. The manipulations were performed with the bone immersed in 0.5 ml of heparinized Ringer solution. Once dissection was completed, the bone was discarded and 0.5 ml of a 6% acetic acid solution added and the cells counted in a Neubauer chamber. The values were expressed as number of nucleated cells per femur.

The mothers were killed within 24 h post-partum, and the bone marrow cellularity was studied following the FRUHMAN and GORDON[4] technique. Each animal was heparinized, bled and 20 mm^3 of the bone marrow from the left femur were obtained. The sample was suspended in 2 ml of homologous serum and the cells counted. The values were expressed as number of nucleated cells per mm^3. The right femur was processed for histological studies in the same way as described for the newborns.

Duration of pregnancy was normal, except in 3 rats treated with 4 mg/kg/day of 16β-methyl prednisone; in these it was prolonged and dead fetuses were delivered. These mothers and fetuses were discarded from the experiments. The newborn rats from mothers treated with 16β-methyl prednisone showed a statistically significant decrease in body weight and thymus and spleen weight, with the sole exception of the relative spleen weight of litters born from mothers submitted to 2 mg/kg/day of 16β-methyl prednisone which showed no changes (Table).

Histologically, the thymus showed marked depopulation of lymphoid cells with pyknosis and degenerative changes in the epithelial structures. Marked depopulation of hematopoietic cells was noted in the spleen, liver and bone marrow (femur). Figures 1 and 2 show the decreased cell population in the bone marrow. The missing cells in the bone marrow belonged to the myeloid, erythroblastoid and lymphoid lines. Juvenile reticular cells were observed in the spleen. The number of hematopoietic cells in the bone marrow from the left femur is shown in Figure 3. The results are expressed as number of cells per femur, number of cells per femur/body weight and number of cells per femur/femur weight. A statistically significant decrease was observed in the number of nucleated bone marrow cells in litters of mothers submitted to 4 mg/kg/day of 16β-methyl prednisone. This decrease was independent of the way data are expressed. As can be seen in Figure 3, the decrease in cell number cannot be ascribed to the decrease in femur weight.

The number of nucleated cells per mm^3 bone marrow was determined in mothers sacrificed within 24 h post-partum, and no differences were found between animals treated with 16β-methyl prednisone and controls (Figure 4).

The administration of 16β-methyl prednisone to pregnant rats induced a marked decrease in absolute and relative thymus and spleen weights of the litter, and a

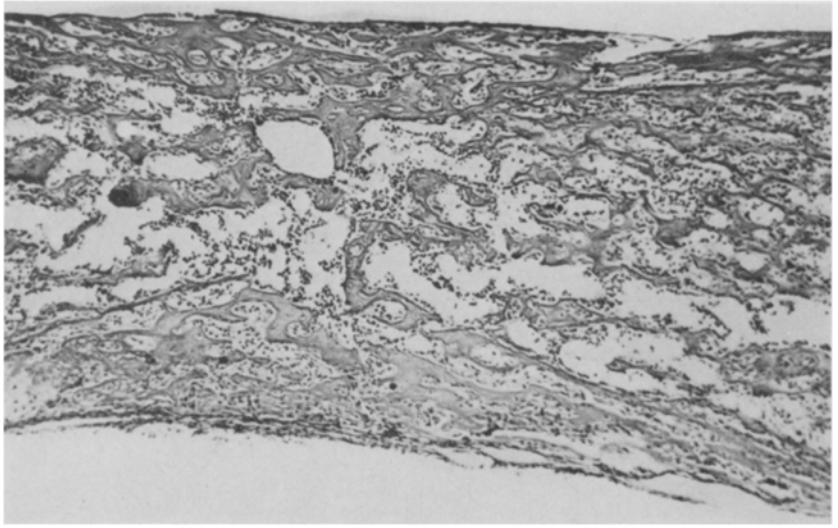

Fig. 2. Femur section of newborn rat from mother treated during pregnancy with 4 mg/kg/day of 16β-methyl prednisone.

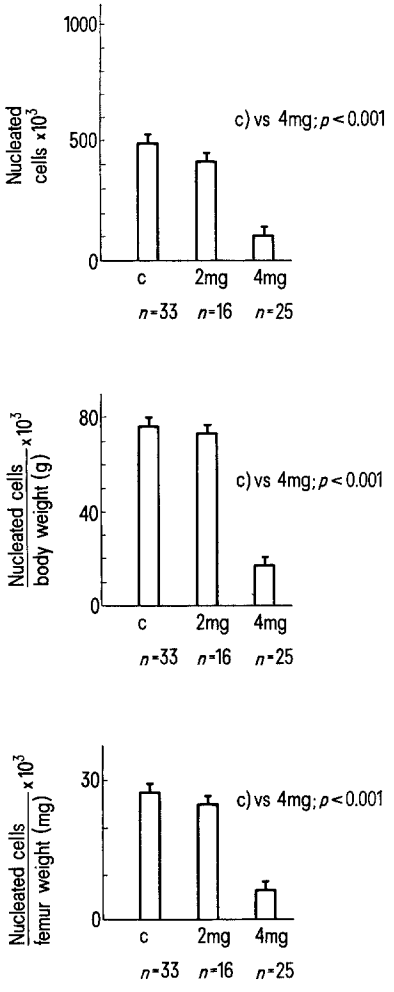

Fig. 3. Nucleated hematopoietic cells per femur of litter from rats treated with 16β-methyl prednisone (4 mg/kg/day) during pregnancy. The results are expressed as number of cells per femur, number of cells per femur/body weight and number of cells per femur/femur weight. C) controls. Experimental: Litters from mothers treated with 2 mg/kg/day or 4 mg/kg/day of 16β-methyl prednisone.

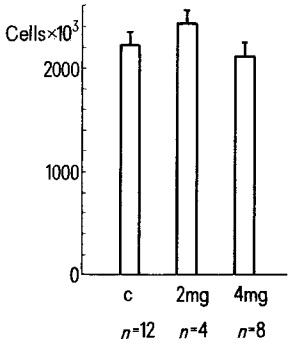

Fig. 4. Nucleated hematopoietic cells per mm³ from rats treated with 16β-methyl prednisone during pregnancy. C) control. Experimental: Mothers treated with 2 mg/kg/day and 4 mg/kg/day of 16β-methyl prednisone.

decrease in thymic lymphoid cells and splenic hematopoietic cells, thus confirming previous results[12]. Both quantitative and morphological studies of the newborn bone marrow showed that the treatment with 16β-methyl prednisone induced, at the dose range used, an inhibition in the development of the hematopoietic cells belonging to myeloid, erythroblastic and lymphoid lines. The doses used did not produce hematopoietic inhibition in the mothers.

It could be speculated that the different effects of the applied corticosteroid on fetal and maternal hematopoietic cells could be due to a higher concentration of the exogenous hormone in fetal blood. Against this possibility must be considered the fact that bi-directional movement of 21 C steroids has been found in the placenta[13], so that fetal and maternal blood corticosteroid levels should be in equilibrium. Furthermore, we have found previously[11] that administration of twice the maximum dose of 16β-methyl prednisone reported here, and given over a longer time periode to adult rats, does not induce gross changes in the bone marrow cell population. The hematopoietic depression in newborn animals could be a consequence of the inhibition in the nucleic acid and protein synthesis induced by corticoids, and it could also be related to the hypothesis, put forward by several authors, that the lymphocyte would play an important role in hematopoiesis[14–16]. Lymphoid tissue is poorly developed in the newborn rat and the corticosteroid could have lead to the observed hematopoietic depression by favoring this condition[12]. This interpretation is in line with a previous report[11] in which it was found that doses of corticosteroids that did not produce changes in bone marrow cells of normal rats, impeded the hematopoietic recovery of previously irradiated animals. These experiments suggest that a significant depression of hematopoietic function following 16β-methyl prednisone can be achieved when the lymphoid tissue is in a critical 'physiological' (birth) or artificially induced condition (X-irradiation).

Resumen. Se estudia en este trabajo los efectos sobre el desarrollo de los organos hematopoyeticos de la rata recien nacida, producidos por la administracion de 16β-methyl prednisone a la madre durante la ultima semana de gestacion. Evidencias cuantitativas muestran un marcado impedimento en al desarrollo hematopoyetico en la medula osea de los animales recien nacidos, asi como desminucion en el peso y celularidad del timo y bazo. En contraste con estos resultados, con las dosis del corticosteroide utilizadas, la medula osea de la madre no evidencia cambios cuantitativos en el numero de celulas hematopoyeticas.

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