Thymic Calcification in the Neonatal Rat.* (29920)

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Selye and Padmanabhan(1) described selective calcification of the thymus in young adult rats following sensitization with dihydrotachysterol, a calcifying agent, and challenge with triamcinolone, a thymolytic glucocorticoid. Since the thymus has greater functional importance in the neonate than in the adult, we have extended this technique to produce thymic calcification in neonatal rats.

Methods. First pregnancy Sprague-Dawley rats (Holtzman Farms) of the same age and initial body weight (± 10 g) were received on the 15th day of gestation. This allowed approximately 7 days of adjustment to our environment before delivery. Only 4-day-old neonates with average body weight of 9 g (range 8-10 g) were used. The entire neonatal population was pooled at the beginning of each experiment and 4 males and 4 females were selected randomly for each mother. Mothers and litters were housed in single cages in an air conditioned room at 75°F. Mother rats were maintained on Purina Laboratory Chow and tap water. They were allowed to construct nests of shredded newspaper on the surface of ¼″ wire mesh inserts on the floors of suspended cages.

Infant rats were sensitized on the 4th day postpartum either directly *per os* with dihydrotachysterol† (DHT) in 0.05 ml corn oil, or indirectly by nursing from mothers that had received various doses of DHT in 0.5 ml corn oil *per os*. Four-day-old neonates were used because variability of results and mortality were high at earlier times. Stomach tube for neonates was a PE 10 polyethylene tube attached to a blunted #27 × ½″ needle on a 1 ml Luer-lok syringe, while for mothers it was a female urethral catheter (#8 French) attached to a 1 ml syringe. Regardless of the method of DHT sensitization, neonates were challenged subcutaneously with 0.24 mg triamcinolone (Triam) in 0.1 ml water on the fifth day postpartum. Survivors were sacrificed with chloroform on 7th day following sensitization (11th day postpartum). Organs of mothers and neonates were studied for gross pathology. Calcification of thymus, heart, and kidney was graded on an arbitrary scale: 0 = none, 1 = just visible, 2 = moderate, 3 = maximal. For histological verification, tissues were fixed in alcohol-formol (4 parts absolute alcohol, 1 part 10% formalin) for subsequent embedding in paraffin and staining with Von Kossa technique.

Results. Highly selective thymic calcification was produced by direct sensitization and challenge of neonatal rats (Table I, .03 mg DHT group). The calcified thymus was white, hard and brittle (Fig. 1). Calcification was often so intense that the cortex was obliterated or unrecognizable (Fig. 1). The medulla had only slight calcification, in walls of blood vessels. Other lymphoid tissues were not involved. At autopsy (11th day postpartum), surviving neonates were emaciated, weak and runted. No attempt has yet been made to assess viability beyond the 11th day.

Thymic calcification was produced in neonates that were challenged with Triam after they had been sensitized indirectly with DHT by allowing them to nurse from DHT treated mothers (Table II). Indirect DHT sensitization through mother's milk was as effective as direct sensitization. However, the exact dose of DHT that reached the neonate through mother's milk was unknown. Although this dose of DHT could not be estimated, it could be controlled by varying the time that the neonates were exposed to the DHT sensitized mother. Neonates were permitted to nurse from DHT sensitized mothers for varying periods before they were fostered to normal untreated mothers (Table III). Nursing from DHT sensitized mothers during the 24-hour period within which the mother received DHT was sufficient to produce thymic calcification in the neonates.

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† Calcamin ®, Dr. A. Wander; S. A. Bern, Switzerland.

This was true whether the neonates were fostered to normal untreated mothers immediately after the 24-hour nursing period or at a later time. In fact, thymic calcification did not occur if the neonatal exposure to DHT sensitized mothers was delayed until

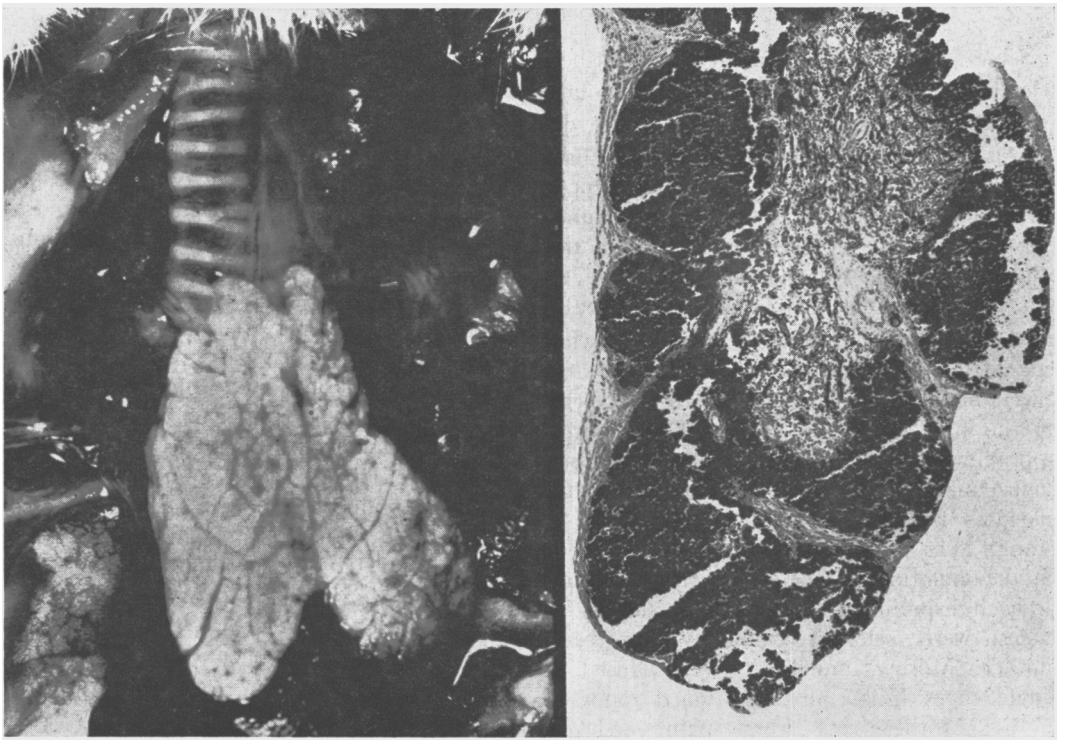

FIG. 1. Thymic calcification in a neonatal rat treated with DHT and Triam. A (left): Gross. Thymus is white due to extensive calcium deposition. B (right): Microscopic. Calcification is predominantly in cortex, but there is some calcium in walls of blood vessels of medulla. (Von Kossa, × 150).

TABLE I. Thymic Calcification Produced by DHT (to Neonates) + Triamcinolone (to Neonates).

DHT to neonates 4th day postpartum	Triam to neonates 5th day postpartum	Mean gross calcification (scale 0-3)			Mortality %
		Thymus	Heart	Kidney	
.0075 mg	.24 mg	0	0	0	0
.015	.24	.7	.5	.3	19
.03	.24	2.0	.5	.2	40
.06	.24	1.0	2.3	1.0	92

Each line represents 3 litters, 8 neonates per litter.

TABLE II. Thymic Calcification Produced by DHT (to Mother) + Triam (to Neonates).

DHT to mothers 4th day postpartum	Triam to neonates 5th day postpartum	Mean gross calcification (scale 0-3)			Mortality %
		Thymus	Heart	Kidney	
.06 mg	.24	0	.5	.4	0
.5	.24	.3	1.0	.8	8
1.0	.24	2.2	1.0	.3	15
2.0	.24	1.8	1.5	2.0	50

Each line represents 3 litters, 8 neonates per litter.

after the first 24 hours of maternal sensitization (Table IV).

Administration of both DHT and Triam to the postpartum mother failed to cause thymic calcification in nursing neonates. Also, administration of both agents during gestation did not produce thymic calcification in the prenatal rat. The latter failure may be due to the placental barrier and/or immaturity of the fetal thymus.

Discussion. Calcification of the adult thymus by the technique of Selye and Padmanabhan is so severe that functional capacity may be impaired. However, methods for evaluation of thymic function in the adult are difficult. In the neonate, thymic function is of greater importance for the economy of the organism, and loss of function (as following surgical thymectomy) has profound effects that are easily evaluated(2). Therefore, the accomplishment of thymic calcification in the neonate offers possibilities for further study of thymic function.

Sensitization of the neonate by DHT was accomplished either directly by gavaging the neonate or indirectly through mother's milk. It is not certain whether the active component in mother's milk was DHT itself, calcium, or a hypothetical calcifiable substrate. It is known that the blood calcium of normal adult female rats reaches its peak 48 hours after administration of 1 mg DHT (3). If these data can be applied to lactating mothers, and if blood calcium levels are reflected in the milk, then the neonates would receive the largest amount of calcium 48 hours after maternal DHT sensitization. However, fostering untreated litters to mothers 48 hours after the latter were sensitized with DHT failed to prepare neonates for thymic calcification (Table IV). Thus, it seems unlikely that calcium was the active component in milk.

Summary. Thymic calcification in neonatal rats was produced by challenging neonates directly with a glucocorticoid (Triam-

TABLE III. Thymic Calcification Produced by DHT (to Mother) + Triam (to Neonates Fostered from DHT Mothers to Normal Mothers).

Days postpartum						
DHT* to mothers	Triam† to neonates	Foster to normal mothers	Mean gross calcification (scale 0-3) Thymus	Heart	Kidney	Mortality %
(day)	(day)	(day)				
4	5	5	1.6	1.0	.4	36
4	5	6	1.4	1.3	.4	13
4	5	7	3.0	3.0	0	36
4	5	8	1.8	1.4	0	36
4	5	9	2.2	1.1	0	0

* DHT (1 mg) in 0.5 ml corn oil *per os* to mothers.
† Triam (0.24 mg) in 0.1 ml H_2O subcutaneously to neonates. Each line represents 1 litter of 8 neonates.

TABLE IV. Thymic Calcification Produced by DHT (to Mothers) + Triam (to Neonates Fostered from Normal Mothers to DHT Mothers).

Days postpartum						
DHT* to mothers	Foster from normal to DHT mother	Triam† to neonates	Mean gross calcification (scale 0-3) Thymus	Heart	Kidney	Mortality %
(day)	(day)	(day)				
4	4	5	1.4	.4	0	19
3	4	5	0	0	0	0
2	4	5	0	0	0	0
1	4	5	0	0	0	0

* DHT (1 mg) in 0.5 ml corn oil *per os* to mothers. The original litters of DHT treated mothers in last 3 groups (not shown in this Table) died despite being fostered to normal mothers on fourth day postpartum.
† Triam (.24 mg) in 0.1 ml H_2O subcutaneously to neonates. Each line represents 2 litters, 8 neonates per litter.

cinolone) after sensitization with dihydrotachysterol (DHT) either directly by gavage or indirectly by nursing from DHT treated mothers. Milk from DHT treated mothers was capable of sensitizing neonates for thymic calcification only if nursing was permitted within the 24-hour period that the maternal DHT sensitization occurred.

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On the Nature of Tolerance of Endotoxin.* (29921)

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Tolerance of endotoxin has been found to be related to the pinocytic and phagocytic capacity of the reticulo-endothelial system (1-4). Recently the plasma of the tolerant animal has been found to contain a transferable factor that suppresses pyrogenicity and increases the rate of phagocytosis in the recipient(5-7). Such data, though doubtless of considerable significance, do not sufficiently explain what it is that constitutes increased tolerance.

We have reported the presence in normal spleen of a protein with an esterase-like behavior(8) which is capable of rapidly degrading endotoxin(9), as indicated by tests: *a,* for toxicity of endotoxin for the 10-day-old chick embryo, and for the pertussis-treated mouse(10), and *b,* for the capacity of endotoxin to fix complement on exposure to specific antibody.† That this protein can alter the endotoxin molecule was also demonstrated by its ability to block 2 phage receptor sites on the endotoxin molecule,‡ and by production of multiple precipitation bands, instead of a single one, on Ouchterlony plates. The validity of these tests for assessing the presence or absence of potency in this protein fraction of spleen was affirmed by the finding that the results by more than one of the above 5 methods on any given test sample, though not quantitatively comparable, have nearly always been in the same direction.

The endotoxin-detoxifying protein appears to be an important feature of the host's defense mechanisms for the following reasons:

Normal rabbits, after a brief exposure to traumatic shock, lose their defense against bacterial endotoxin, so that they succumb to as little as several gammas instead of several milligrams of a potent endotoxin(12). In such rabbits the detoxifying fraction of spleen, which is always potent in normal rabbits, has lost its potency.

A degree and duration of traumatic shock that is usually lethal in normal rabbits is not lethal in rabbits with increased tolerance of endotoxin(13). This increased resistance to traumatic shock disappears when tolerance of endotoxin disappears.

Such data led to the study reported below, which was undertaken to determine how far

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‡ We are indebted to Drs. Gabriel Michael and Fred Rosen, Harvard Medical School, for this unpublished observation.