PREVENTION OF WASTING DISEASE BY PREGNANCY ASSOCIATED WITH HYPERTROPHY OF THE FETAL THYMUS*

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The thymus gland has been established as essential for normal immunologic development. Although its physiologic role is not entirely understood, one clearly demonstrable effect of neonatal thymectomy is a marked decrease in immunologic responsiveness (1). Another consequence of early ablation of the thymus is growth retardation or "wasting disease" (2, 3). Restoration of growth and immunologic responsiveness in the neonatally thymectomized animal can be accomplished by implantation of intact thymus tissue (4), injection of thymus or spleen cell suspensions (5), implantation of intact thymus, spleen, or lymph node tissue in Millipore chambers (6, 7), maintenance of the animals in a germ-free state (8, 9), or by allowing female mice to become pregnant (10). During pregnancy, a factor or factors originating in the fetus are presumed to restore the thymectomized mother to normal. Thymic hyperplasia has been reported in the offspring of mice thymectomized immediately prior to breeding (11).

This paper reports experiments designed to evaluate the effect of neonatal thymectomy on fertility in the female C3H/HeJ mouse and the effect of neonatal maternal thymectomy on fetal development.

Materials and Methods

Male and female C3H/HeJ Bar Harbor strain mice were obtained from Jackson Memorial Laboratory (Bar Harbor, Maine) 20 female breeders that would not destroy their operated young were selected. Each female selected was paired with a known fertile male and the pair placed in separate cages. The colony was kept in an isolated room and no new breeding stock introduced after the experiment was begun. Animal room temperature was maintained at approximately 27°C. Purina chow and tap water were offered ad lib.

The offspring of these breeding pairs were thymectomized within the first 16 hr after birth. A modification of the technique described by Sjodin et al. (12) was followed using ether anesthesia and removal of the thymus under a microscope by dissection. Alternate members of each litter were either sham-operated or thymectomized.

All animals were weaned at 3 wk of age and placed in individual cages. The females in

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groups to be bred (groups IV and VI below) were placed with known fertile males at 4 wk of age; they remained with the male for 7 days. The male was then removed and the female observed for 14 additional days. If pregnancy occurred, it was allowed to proceed to term. The newborns were dissected within 12 hr after birth, and the organs placed on moist filter paper in covered Petri dishes and weighed within 30 min using a Mettler microbalance. If pregnancy did not occur, a different fertile male was reintroduced and the observation cycle repeated. Breeding cycles were repeated until three consecutive pregnancies had occured for each female. The breeder males were rotated randomly among the females in the sham-operated and thymectomy groups. Adequacy of thymectomy was evaluated by gross and microscopic examination of the mediastinum at death or sacrifice. If thymic remnants were found, that animal was excluded from the analysis.

Data analyses were performed with a 1401 IBM computer. Statistical comparisons were made using Student's t test. All data are expressed as the mean and standard error of the mean.

Experimental groups: 267 mice were placed in six groups:

Group I, 50 males, sham operation.

Group II, 57 males, neonatal thymectomy.

Group III, 34 females, sham operation, virgin controls.

Group IV, 37 females, sham operation, placed with known fertile males at 4 wk of age.

Group V, 44 females, neonatal thymectomy, virgin controls.

Group VI, 45 females, neonatal thymectomy, placed with known fertile males at 4 wk of age.

A total of 269 newborn animals of group IV females and 192 newborns of group VI females were dissected (to obtain organ weights) and examined for gross anomalies.

RESULTS

Effect of Neonatal Thymectomy on Fertility in the Female.—Of the 37 sham-operated females that were mated with known fertile males, 32 subsequently became pregnant. 26 pregnancies occurred in the 45 thymectomized females that were mated randomly with the same group of known fertile males. The 58% fertility in the group of thymectomized animals is significantly less than the 86% in the sham-operated group ($P < 0.01$).

Effect of Pregnancy on Growth.—Animals in each group were weighed weekly. Mean weights at 4, 8, 12, and 16 wk are shown in Table I. From these data, it is apparent that body weight is similar in surviving animals of the several groups at the time intervals shown. However, the number of animals surviving after the first month decreases rapidly in thymectomized groups II and V, but not in group VI. The mean age at death of the animals in groups II and V was 12 wk, while the mean age at death for animals in group VI was 27 wk. Thus, the body weight data reflect only those animals that were surviving at the time listed.

The effect of pregnancy on the growth and mortality of the thymectomized female mouse is graphically depicted in Figs. 1 and 2. These data represent the growth curves from the first 12 animals studied in groups V and VI. All thymectomized pregnant animals grew normally except two which wasted and died by 22 wk (Fig. 1). In contrast, in the virgin control group, 8 of 12 thymectomized animals were dead by 13 wk and all except one had developed wasting disease by 24 wk.

Effect of Pregnancy on Mortality.—Mortality data for all groups are shown in

Table II. The observation period was limited to 32 wk because mortality is difficult to assess in the female thymectomized animal after this time due to death from breast tumors. None of the sham-operated males were dead at 32 wk, but 57% of the thymectomized males were dead at 16 wk and 68% by 32

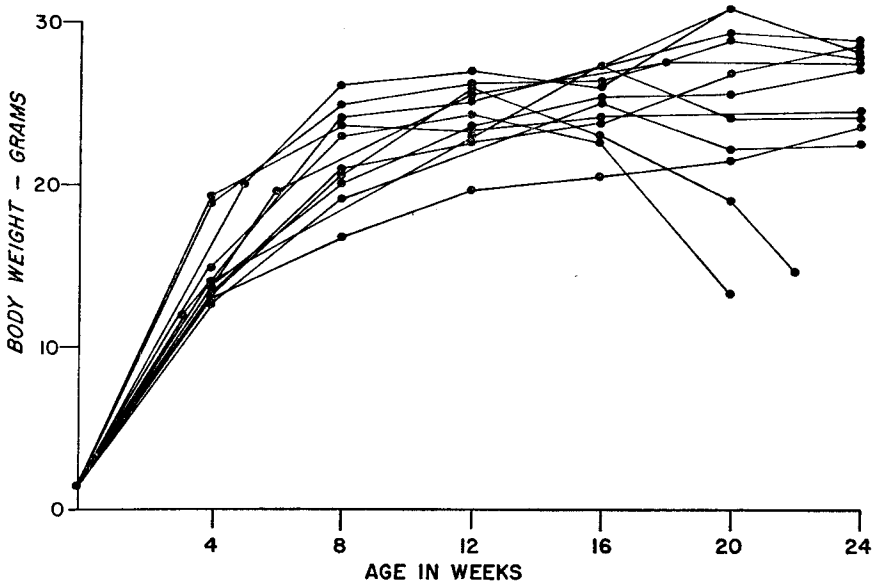

FIG. 1. Body weight curves for first 12 animals in group VI (thymectomized-pregnant).

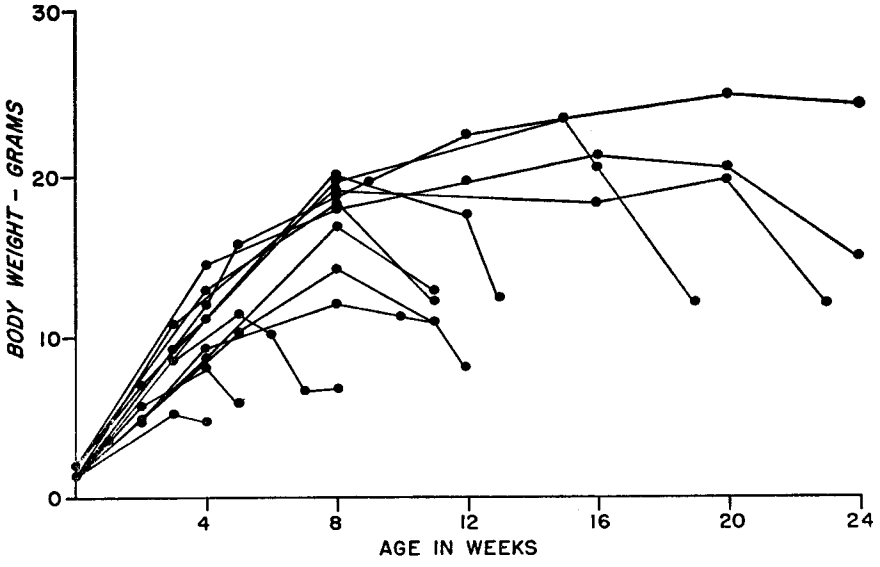

FIG. 2. Body weight curves for first 12 animals in group V (thymectomized-virgin control).

wk. In group V (thymectomized virgin controls) 63% of the animals were dead at 16 wk and 86% by 32 wk. The 68% mortality in the thymectomized male animals (group II) is significantly less than the 86% mortality in the females

TABLE I

Mean Body Weight of the Experimental Groups

Age	Group I ♂ Sham-operated		Group II ♂ Thymecto-mized		Group III ♀ Sham-operated		Group IV ♀ Sham-operated		Group V ♀ Thymecto-mized-virgin		Group VI ♀ Thymecto-mized-pregnant	
	Total no.‡	Body wt*	Total No.	Body wt	Total No.	Body wt	Total No.	Body wt	Total No.	Body wt	Total No.	Body wt
wk		*g*		*g*		*g*		*g*		*g*		*g*
4	50	13.1 (0.39)§	57	12.0 (0.49)	34	13.2 (0.42)	37	13.9 (0.45)	44	10.9 (0.39)	26	14.0 (0.25)
8	50	22.1 (0.33)	52	17.6 (0.47)	34	18.4 (0.34)	34	21.4 (0.57)	42	14.8 (0.65)	26	22.1 (0.36)
12	50	24.9 (0.24)	26	22.4 (0.69)	32	22.6 (0.54)	34	24.2 (0.53)	17	17.9 (1.1)	26	24.6 (0.47)
16	50	26.0 (0.24)	24	22.1 (0.91)	32	21.2 (0.39)	34	24.8 (0.59)	16	21.1 (0.91)	22	23.9 (0.63)

* Mean body weight for surviving animals (to age 16 wk).

‡ Animals surviving.

§ Standard error of means (SEM).

TABLE II

Per Cent Mortality for All Groups

Group	Total No.	No. dead at	
		16 wk	32 wk
I Male—sham operation	50	0 (0%)	0 (0%)
II Male—thymectomy	57	33 (57%)	39 (68%)
III Female—sham, virgin control	34	2 (5%)	4 (11%)
IV Female—sham, pregnant	32	0 (0%)	7 (21%)
V Female—thymectomy, virgin control	44	28 (63%)	38 (86%)
VI Female—thymectomy, pregnant	26	5 (19%)	10 (38%)

Mortality in experimental groups at 16 and 32 wk of age.

(group V, $P < 0.05$). In the thymectomized animals that became pregnant (group VI) the mortality of 38% is significantly less than the 86% mortality in the thymectomized nonpregnant animals (group V, $P < 0.001$).

The age at death (or sacrifice because of the presence of large tumors) is shown in Table III. These data were compiled after 52 wk of observation. The 39 deaths in the male (group II) and the 38 deaths in the female (group V) thy-

mectomized animals were all spontaneous; none were sacrificed because of tumors. 7 of the 15 females in group IV and 10 of the 15 thymectomized females in groups VI died spontaneously. The remaining animals were sacrificed because of breast tumors. The mean age of spontaneous death in the thymectomized male and female groups was approximately 100 days, while the mean age in the sham-pregnant group and the thymectomized pregnant group at the time of death was approximately 200 days. These data support the conclusion from the data in Table II that pregnancy in the thymectomized female animals provides significant protection against death from wasting disease.

Effect of Maternal Thymectomy on Fetal Development.—The litter size, body weight, and organ weights of newborn mice are shown in Table IV. The newborns from the first 20 pregnancies of the sham-operated group and the first 14

TABLE III

Mean Age at Death for All Groups

Group	Total No.	Dead*	Days‡	SEM
I Male—sham operation	50	0	No deaths	
II Male—thymectomy	57	39	91	6
III Female—sham, virgin control	34	4	110	—
IV Female—sham, pregnant	32	15	200	17
V Female—thymectomy, virgin control	44	38	101	8
VI Female—thymectomy, pregnant	26	15	190	19

* Animals dying or sacrificed after 52 wk observation.

‡ Mean age (± SEM) at death in days for animals dying or sacrificed because of tumors after 52 wk observation.

in the thymectomized group were dissected within 16 hr of birth. Newborns from litters of the second and third pregnancies were also dissected and weighed. The last line lists the weight of the dam at the time of pairing with the known fertile male; there was no significant differences between the body weights of the mothers at the time of pairing. Mean litter sizes varied between 5.9 and 8.8 with no significant differences between sham-operated and thymectomized groups. Mean body weights of the newborn mice were almost identical in all groups. The mean weight of the gastrointestinal tract with its contents increased with each pregnancy for the sham-operated groups. The weight of the GI tract, in thymectomized animals, was higher during the first and second pregnancies than during the third pregnancy. Accurate interpretation of these data is not possible since no attempt was made to empty the GI tract prior to weighing. The mean kidney, liver, heart, and spleen weights for the various groups did not vary significantly.

The only striking difference in the development of the fetuses was the en-

largement of the fetal thymus gland in newborns from thymectomized females. The mean weights of the thymus glands from the newborn for the three litters are shown in Table V. In animals from first and second litters of thymectomized females, the mean thymus weight of 4.2 mg is significantly larger than the 3.6

TABLE IV

Organ Weights of Offspring Born of Sham-Operated and Thymectomized Females

Pregnancy	Sham-operated litters			Thymectomy litters		
	1st	2nd	3rd	1st	2nd	3rd
No. of offspring	143	67	59	90	53	49
No. of litters	20	8	10	14	6	7
Litter size	7.1	8.3	5.9	6.5	8.8	7.0
	(0.12)*	(0.17)	(0.21)	(0.10)	(0.21)	(0.41)
Body weight, *g*	1.4	1.4	1.4	1.4	1.4	1.5
	(0.0083)	(0.012)	(0.025)	(0.019)	(0.029)	(0.028)
GI weight, *mg*	136.9	144.6	154	146.4	155	144.4
	(2.4)	(5.1)	(5.9)	(4.1)	(6.7)	(6.6)
Liver weight, *mg*	74.4	74.4	74.3	78.4	74.5	85.3
	(1.4)	(1.8)	(2.5)	(2.5)	(2.5)	(2.5)
Kidney weight, *mg*	15.8	14.3	15.0	15.6	15.9	17.9
	(0.57)	(0.32)	(0.55)	(0.42)	(1.1)	(1.2)
Heart weight, *mg*	7.3	7.9	7.9	7.8	7.6	9.9
	(0.19)	(0.19)	(0.21)	(0.22)	(0.26)	(0.44)
Spleen weight, *mg*	1.6	1.5	2.0	1.8	1.9	2.3
	(0.07)	(0.10)	(0.17)	(0.08)	(0.15)	(0.20)
Weight of dam at pairing, *g*	19.2	23.1	26.3	19.5	24.8	26.1
	(0.20)	(0.28)	(0.43)	(0.20)	(0.36)	(0.23)

Data on offspring from the first, second, and third litters of sham-operated and thymectomized females.

* SEM.

TABLE V

Newborn Thymus Weight

	Litter 1		Litter 2		Litter 3	
	Total No.	Wt	Total No.	Wt	Total No.	Wt
		mg		*mg*		*mg*
Sham Operation	143	3.6	67	3.4	59	4.2
		(0.09)*		(0.12)		(0.26)
Thymectomy	107	4.2	53	4.2	49	4.8
		(0.13)		(0.23)		(0.26)
P value		<0.001		<0.001		>0.05

*SEM.

and 3.4 mean weights in the sham-operated animals. However, the thymus weight in third litter newborns of thymectomized females was not significantly larger than third litter newborns of sham-operated controls.

The mean weight of the thymus gland in the newborns from mothers that had been sham-operated increased significantly during the third pregnancy ($P < 0.01$). This was true when the mean weight of 4.2 mg was compared either to the mean of 3.6 mg in the first litter or the mean of 3.4 mg in the second litter.

Histological examination of the newborn thymus glands and GI tracts revealed no obvious differences between fetuses in the control or thymectomized groups in either the cortical medullary ratio of the thymus glands or the amount of lymphoid tissue in the gastrointestinal tracts.

DISCUSSION

Neonatal thymectomy significantly decreased fertility in the C3H/HeJ female mouse. The time of onset of wasting disease varies between 4 and 16 wk in various strains of mice studied (4). The C3H/HeJ mouse was selected for these studies because of the late onset of wasting disease, i.e., after 8 to 12 wk. This provided sufficient time to evaluate the effect of thymectomy on fertility since fertile females of this strain are all parous by 8 wk. The majority of the thymectomized female animals appeared to be in good condition at 8 wk of age in this study. Therefore, their infertility could not be attributed to either inanition or age and appears to be related only to the absence of the thymus gland. Comsa found that thymectomy in the male guinea pig produced a degenerative change in the testes (13). This finding may be pertinent to the present experiments; however, we did not examine the ovaries of our females, histologically.

Our studies demonstrate a significant protection by pregnancy for the neonatally thymectomized animal against wasting disease and death. However, mice died with typical wasting disease after having been pregnant one, two, or even three times. In the thymectomized pregnant females (group VI), 26 animals became pregnant one time, 19 became pregnant twice, and 16 became pregnant three times. Of the 10 animals that died spontaneously, 9 had been pregnant more than one time. 5 of these 9 had 2 litters and 4 had 3 litters prior to death. This suggests that the protective factor provided by pregnancy may not persist indefinitely since 9 or the 10 animals dying spontaneously were pregnant more than one time.

We have done preliminary studies using both soluble and particulate antigens as a measure of immunologic competence. These data suggest that immunologic responsiveness in neonatally thymectomized animals does fall off as a function of time following pregnancy (15). However, it is not known whether wasting disease will occur when the animals are immunologically competent. Our data suggest that immunologic responsiveness completely disappeared prior to wasting in those animals which waste after having been pregnant.

The protective effect of pregnancy on neonatally thymectomized female mice observed in the present studies is in agreement with the data reported by Osoba (10). He studied 17 thymectomized CBA female mice which were bred to T6 males, 9 of which became pregnant and had 1 or 2 litters by 13 to 17 wk of age. Immunologic competence in these females after pregnancy appeared to be intact. They had the ability to reject skin homografts and develop hemagglutinins to sheep red blood cells. He found that the postpartum CBA females would not accept skin grafts from T6 males and concluded that immunologically competent cells had not crossed the placenta; if intact lymphocytes had crossed from the CBA/T6 hybrid fetuses, the CBA mother should have been tolerant to T6 antigens. One question which was not clarified was whether or not pregnancy imparts permanent immunologic competence to the thymectomized female. All nine of the neonatally thymectomized pregnant females Osoba reported were normal after 17 wk of observation. Our data suggest that pregnancy may not offer permanent protection against wasting disease.

If a thymectomized female becomes pregnant, there appears to be no adverse effects on the pregnancy per se. The females go to term, and fetal death during pregnancy did not occur more frequently in the thymectomized animals than the sham-operated controls. The litter sizes were comparable and body weights identical. Since the GI tract weight is heavier in the offspring of the thymectomized mothers, one might speculate that there is hypertrophy or hyperplasia of GI lymphoid tissue; however, we did not weigh the appendix separately and made no attempt to empty the gastrointestinal tract.

The observation that the weight of the lymphoreticular system increases with parity has also been made by Albert and coworkers in C3H/Sp mice (16). They found the thymus and spleen of offspring (studied at age 7 wk) increased with increasing parity of the mother.

The hypertrophy of the fetal thymus found in offspring from thymectomized mothers reported in this paper would appear to be significant in two respects: First, the fetal thymus hypertrophies in response to a deficiency of thymus gland factors in the mother. Secondly, if one can equate hypertrophy with increased secretion, the fetal thymus must have secreted a sufficient quantity of active principal to influence maternal growth and immunologic competence. Metcalf has reported isolating a humoral lymphocytosis stimulating factor (LSF) from the thymus and established that LSF acted on target cells distant from the site of secretion, circulated in the plasma, showed feedback control of the secretion by the thymus, and was not species specific (17, 18). Our data on thymic hypertrophy in the fetus of a thymectomized mother supports his concept. However, Metcalf has subsequently reported that the size of the thymus gland is autonomously controlled and not responsive to feedback control. Grafts of up to 20 thymuses did not alter the size of the host thymus gland (19). Metcalf's data seem quite conclusive in this regard and we cannot reconcile our data with this finding unless the fetal thymus has not attained the degree of autonomy characteristic of the adult gland.

The available evidence would appear to leave little doubt that the thymus functions in a manner similar to a classical endocrine gland. It is not clear whether the thymus secretes more than one factor, one of which alters the metabolism of tissues other than the lymphoreticular system. Loutit has suggested that lymphocytes,

per se, carry both trophic and immunologic substances (20). The possibility exists that the thymus secretes a factor which stimulates the production of trophic as well as immunologic factors by the target lymphocytes.

Szent Györgyi's experiments demonstrated both a growth-promoting and growth-retarding substance in calf thymus (14). However, Szent Györgyi's growth-promoting factor did not prevent wasting in the thymectomized mouse (21). Metabolic effects of thymus extracts have been demonstrated in the guinea pig by Comsa (22, 23). His preparation, purified to a single peak by Sephadex chromatography and by preliminary analysis, was a glycopeptide (24, 25). Recent studies by White and coworkers also provide strong evidence for a thymic factor that stimulates nucleic acid and protein synthesis in lymphocytes incubated, in vitro (26–28). There are other reported active cell free thymic preparations which alter metabolic activity as well as immunologic responsiveness. De Somer cultured fragments of calf thymus in Hanks' solution and found activity in the medium which would stimulate lymphocyte production and prevent wasting disease (29). Using lipid solvents, extracts of thymus tissue have shown peculiar metabolic activity. The insulin-like activity in one preparation is striking (30). A lipid fraction from thymuses has also been reported to increase lymphocyte count, increase body weight, and return skin reactivity for bovine serum albumin to normal when injected into neonatally thymectomized rats (31).

Since there is a multitude of described active factors, one is tempted to speculate that the thymus secretes more than one active hormone. However, it is difficult at the present time to decide whether these many activities are specific substances or only artifacts of the preparation or assay procedures.

SUMMARY

Neonatally thymectomized female mice were studied and compared to appropriate controls. Neonatal thymectomy appears to decrease fertility significantly in the female C3H/HeJ mouse. However, if a thymectomized female mouse becomes pregnant, pregnancy offers significant protection against wasting disease and death. The maternal thymus appears to play no significant role in the development of the fetus as measured by gross examination and organ weights. Offspring of thymectomized females had comparable body and organ weights when compared to offspring from sham-operated females, except for the thymus gland and gastrointestinal tract. The absence of the maternal thymus gland did stimulate a significant increase in the gland weight of her offspring compared to sham-operated control female offspring. Parity, per se, in intact females also causes a significant increase in the weights of the thymus of newborn offspring.

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