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Antibiotics and the Wasting Disease in Neonatally Thymectomized Rats

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ANTIBIOTICS AND THE WASTING DISEASE IN NEONATALLY THYMECTOMIZED RATS

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by

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INTRODUCTION

Within recent years a very intensive study has been made to determine the exact function of the thymus gland. Several investigators have observed that a certain percentage of thymectomized male rodents undergo a so-called "wasting disease" following neonatal thymectomy. It is our purpose to determine the nature of this condition.

Review of Literature

We will first consider the embryology and the anatomy of the thymus and its presumed physiological role in the immunologic system of the body.

The thymus gland develops in the following manner: Smith (1965) discusses the findings of Crisan in his description of the thymic anlage as two main parts; (1) a tail from which the main body of the thymus develops as an endodermal derivative of the third pouch, (2) a head partly endodermal from the lateral one-half of the dorsal diverticulum and partly ectodermal from the medial end of the cervical duct and the cervical vesicle.

The lymphocytes, according to Miller, (1964) arise by direct transformation of epithelial cells under the inductive stimulus of the mesenchyme and it would appear that the thymus is the original source of some of the lymphoid elements of the immunologic system. Bierring and Grunnet (1964) present evidence that bone marrow lymphocytes have

a different source of origin than the extramedullary lymphocytes since they increase in the bone marrow after thymectomy, lymphadenectomy, and subtotal splenectomy rather than decrease.

The spleen, lymph nodes and other lymphoid structures contain a small number of lymphocytes and stem cells when the animal is born but these cells do not contain the ability to react with antigens until they are activated by a hormone from the thymus gland. Neonatally thymectomized animals may have a high lymphocyte production but may be deficient in the immune response to a virus injection which indicates the possible existence of a thymic hormone which stimulates antibody production (Levey, 1964). Evidence for a lymphocytic stimulating factor is advanced by Bridges and Camblin (1964) in which they state that a cell-free thymus extract induced lymphocytosis in leukopenic rats. In an experiment performed by another group (Law, et al., 1964) thymectomized mice received implantations of thymus tissue in millipore chambers and the following results were noted: (a) there was no depletion of lymphocytes in the thymectomized mice, (b) the ability to reject skin grafts was unimpaired, (c) the animals were protected against lymphocytic chorio-meningitis virus. These results indicate the existence of a thymuc hormone which stimulates lymphocyte production and maintenance of the normal immunologic system. The establishment of the immunologic defense system and the production of mast cells appears to be the major function

of the thymus gland (Csaba, 1961). Ackerman and Knouff (1965) reported evidence of elaboration of cytoplasmic deposits of glycoprotein from the reticulo epithelial cells of the thymus during late embryonic development. They postulate that since these cells are in close association with the vascular channels of the medulla perhaps there is a carbohydrate-protein substance elaborated by these cells and exported into the blood stream.

The wasting disease is, according to Sherman (1963), a debilitating process characterized by weight loss, ruffled appearance, hunched posture, lethargy, alopecia, periorbital and facial edema, leukopenia, lymphopenia, thrombocytopenia, agammaglobulinemia and, terminally, profound lethargy, labored respiration and death. These conditions, purported to be caused by the deficiency of lymphocytes induced by removal of the thymus gland, occurred in 44 percent of thymectomized animals. Since it has been noted that the thymus is important in nucleoprotein metabolism, the same author has postulated that the thymus furnishes nucleic acid to the organism and that the wasting disease may be due to a deficiency of protein synthesis because of atrophic lymphoid tissue. Jankovic et al., (1962) who experimented with rats noted that the onset of wasting was as early as two weeks and as late as six weeks. One feature noted was that female hamsters did not develop the wasting disease. It was postulated that the female hamster might have ectopic thymic tissue. A more plausible explanation

is that of Sherman (1963) who believes that the presence of ovarian tissue and estrogen is protective of the lymphoid system and of immunologic competence. The female could be made to waste if treated with testosterone or bilateral oophorectomy.

Maldonado et al. (1964) describes the wasting disease produced by thymectomy in newborn mice and states that the earlier after birth the thymectomy is done the greater the incidence of the wasting syndrome.

Helyer (1963) stated that the wasting disease can be prevented by grafting neonatal thymus after thymectomy and that there is uncertainty as to the exact role the neonatal grafted thymus plays in the protection against the wasting disease. Miller (1963) reports that the wasting syndrome is probably associated with lymphoid dysplasia, but it is questionable whether or not it can be explained on a trophic function of the lymphocytes. He concluded that deficiencies found in thymectomized mice can be corrected either by injections of lymphocytes from normal mice or by effective thymus implants. Law et al. (1964) stated that subcutaneous grafting of syngenic thymus tissue during the first week of life or as late as the third or fourth week effectively prevented the development of the so-called wasting disease.

Schlesinger (1964) stated in his work that cortisol acetate injected into young mice induced a wasting syndrome similar to that observed in runt disease and in post-

thymectomy syndrome. (Runt disease, or graft vs. host disease, is a disease in which the animal fails to achieve normal growth and presents many of the same symptoms as does wasting disease. It is caused by either a reaction of the host animal to a tissue graft or to an injection of donor lymphocytes). He postulated that the lymphoid depletion results in widespread metabolic dysfunction leading to fatal wasting. He also observed that large doses of adrenal corticosteroids administered to the mice produced severe metabolic derangements associated with protein breakdown and increased gluconeogenesis which resulted in negative nitrogen balance, cessation of growth, muscle wasting and thinning of the skin. In this case he feels that the thymus may have some form of antagonistic action to adrenal corticosteroids which is lost on ablation of the thymus or negated by large doses of corticosteroids.

Miller (1964) has listed the effects of thymectomy as: severely diminished lymphocyte population in blood and tissues but not in bone marrow, reduction in capacity to produce antibody to some but not all antigens, delayed hypersensitivity, impaired ability to reject foreign skin grafts, and, in rats, absence or deficiency of an immunoglobulin designated IgX. The serum levels of 7S or 19S gamma globulins were normal, at least in mice. In speaking of the wasting syndrome Miller says, "The pathogenesis of this syndrome is still unclear". He further stated that

the defects of immunologic responsiveness in many strains of mice after thymectomy were evident before the clinical onset of the wasting syndrome. Miller also carried out an experiment where he injected five million cells of spleen or lymph node, but not thymus, into newly thymectomized mice, and these treated mice did not develop the wasting disease. He also stated that mice thymectomized at birth and grafted within a week with an intact thymus from a one day to two week old mouse donor did not suffer from the wasting disease and had normal populations of lymphocytes and could reject foreign skin grafts as did normal mice.

The major points of similarity between the neonatal thymectomy syndrome and the graft-versus-host diseases are as follows: hyperplasia of the reticulo-endothelial system, proliferation of reticulum cells, histiocytes, and Kupffer cells, increased phagocytic activity, extensive extramedullary hematopoiesis, scattered necrotic leucocytes, diminished immunologic competence, lymphoid aplasia, with wasting and death. Miller states that the wasting syndrome associated with thymectomy in the newborn has never been observed in inbred mice kept in the germ free state and postulated that an infectious process must play some role in the pathogenesis of the wasting syndrome.

Jankovic (1962) stated that rats thymectomized at birth developed normally but were subject to what he describes as intercurrent infections. In his work he found that about

25 percent of thymectomized litters of rats, after an initial period of normal growth, began to fail to gain weight for several days and then exhibited gradual but accelerating weight loss leading to cachexia and death in ten to twenty days. The onset occurred as early as twenty days and as late as sixty days in different litters. The rats became lethargic, pallid and flabby to the touch, with coarse, and matted hair.

The only serious study relative to the significance of the infectious process was made by Azar (1964) with thymectomized animals. A group of animals were thymectomized and received routine care. Another group of thymectomized rats were maintained on oxytetracycline HCL, 6 gm. per liter, first to the mothers and then to the weaned rats. The animals were sacrificed at 8-9 weeks of age and autopsied. He noted that two forms of sepsis were encountered in the untreated animals: (1) walled off abscesses in the anterior chest wall in the region of the thymus, indicating infection at the site of the surgical procedure. (2) bronchial and pulmonary inflammations, usually in the form of bronchiectasis with pus filling dilated bronchi, chronic peribronchitis, chronic pneumonia and lung abscesses. In the animals to which oxytetracycline had been administered, he noted significantly fewer wound infections. Bronchial and pneumonic lesions were encountered less frequently. In his work he considered an animal as wasted when his total body weight

was equal to or less than half the weight of healthy sham operated litter mates of the same sex and age. He noted the symptoms of the wasting disease as described earlier. The animals treated with oxytetracycline showed less tendency to wasting than the group which did not receive it. All of the untreated group which developed the wasting syndrome all showed evidence of infection in the form of bronchopulmonary inflammation or of abscesses at the site of the neonatal operation.

Lymphopenia was present in both medicated and non-medicated thymectomized groups. A third group of animals made susceptible to infection by administration of large doses of cortisone could be protected by antibiotic administration. Wasting was absent in this group. Azar (1964) postulated that the immunological deficiency of neonatally thymectomized rats is partly an expression of immunological pre-occupation with the infection, but noted also that the marked susceptibility of thymectomized rats to sepsis indicates some form of participation of the thymus in a natural or acquired resistance.

The adult rat has a range of 6,000 to 18,000 lymphocytes with an average of 9,000. Rats have 70 percent lymphocytes where humans have 70 percent neutrophils (Harris, 1949).

The present experiment was set up to study the results of the administration of the antibiotic, Chloramphenicol, to the mothers of the thymectomized and thymectomized-

adrenalectomized male animals during the late fetal and early post natal period. Growth rate curves, blood counts on medicated animals and histologic studies were the basis of this study.

METHODS

A total of 50 rats were used for the experiment. They were divided into six groups as follows: (Table 1)

1. Thymectomy only---nonmedicated 17 rats
2. Thymectomy only---medicated 12 rats
3. Thymectomy-adrenalectomy---nonmedicated 5 rats
4. Thymectomy-adrenalectomy---medicated 8 rats
5. Control---nonmedicated 4 rats
6. Control---medicated 4 rats

Male rats only were used in this experiment as it has been determined in the hamster, that the females do not contract the wasting disease (Sherman, 1963). Sherman (1963) suggests, as noted above, that ovarian tissue and estrogen is protective to the lymphoid apparatus and to the immunologic system.

The rats were equally divided between Sprague Dawley and Simonson breeds. Initially, approximately 100 rats from 12 litters were thymectomized and within a few days almost all these thymectomized animals with the controls had contracted an infection and died. The animals for the subsequent experiment were isolated from the remainder of the animals in the animal care shelter, and, as a precautionary measure, Chloramphenicol was administered in the drinking water (5.2 mg per 8 oz. water) to the pregnant females four days following the birth of the young, as well as two days prior to delivery.

TABLE NO. 1

50 MALE RATS

	LITTER	MEDICATED (chloramphenicol)	NON-MEDICATED	THYMUS-REGION (NOT VALID)
CONTROL	A		1	
	B		1	
	C		1	
	D		1	
	E	1		
	F	1		
	G	1		
	H	1		
	I			
	J			
THYMECTOMIZED	A			
	B			
	C		6	
	D			
	E			
	F	3		
	G	1		2
	H	6		
	I		7	
	J		4	
THYMECTOMIZED ADRENALECTOMIZED	A		1	
	B		1	1
	C		1	
	D		1	
	E			1
	F	3		1
	G			
	H	2		1
	I			
	J			
TOTAL		19	25	6

The young, therefore, received Chloramphenicol transplacentally and through the mother's milk. Hereinafter, the progeny will be referred to as the medicated and nonmedicated groups. It was noted that the number of deaths in the medicated groups was less than in the nonmedicated ones.

On the day of birth and after the first feeding, the female young were removed from the litter cages and sacrificed. The males of a particular litter were assigned to a particular group as indicated above, one male from each litter being kept for a control. The males selected for thymectomy were then placed in 100 ml glass beakers and placed in the refrigerator for 10 minutes at which time respiration had stopped, and the heart beat was reduced to a slow rate. The frozen rats were placed in the dorsal position on a towel-covered cardboard, and the legs and mouth were anchored by rubber bands and pins to the board. An incision was made from the sternal notch to the third or fourth rib in the midline. When the skin, sternum and strap muscles were separated, the thymus was made visible, lying on the trachea and extending caudalward to the base of the heart. A dissection microscope was used to insure complete removal of the thymus. Glass tubing was heated and drawn out to a small diameter in the form of micro pipettes. These micro pipettes were attached to a hose connected with a suction pump. The pressure in the

suction pump was set at 7-10 pounds of pressure because higher pressures than this tended to rupture the blood vessels. By the above methods the thymus gland was removed from the rat. Cotton swabs soaked in alcohol were used to wipe the wound clean. The wound was then stitched with 6-0 chromic suture and three sutures were placed to close the wound. The sternum and skin were closed together and Myciguent ointment was then spread over the wound, and the area was then sprayed with aeroplast spray on plastic bandage. Sterile surgical gloves were worn at all times during the surgical procedures and the handling of the rats in the cages. This routine was followed to cut down risk of infection and to keep as much human smell as possible from the baby rats to prevent cannibalism by the mothers when the babies were returned to the cages. The post-operative rat was then placed in a shoe box warmed with a 100 watt light bulb, usually held in a gloved hand for about five minutes till visible signs of recovery were noted. After about twenty minutes in the box, the thymectomized rat was then returned to its mother.

Additional precautions were employed to prevent cannibalism. Librium (10 mg per liter) was added to the drinking water of the pregnant rats about three days before birth and for about six days after birth.

As the freezing anesthesia takes about ten minutes, the rats were placed at five minute intervals in the

freezer and in this way the surgical procedure was timed at five minutes per thymectomy. The total time involved with one rat was approximately 40 minutes from the time of removal from the cage to the time of replacement with its mother, 10 minutes freezing, 5 minutes for the surgery, 5 minutes for warming and 20 minutes in the recovery box.

At approximately three weeks after thymectomy, the rats were weaned and some were selected for adrenalectomy. The adrenalectomy procedure was carried out as follows:

The rat selected for adrenalectomy was first weighed, and then his back was shaved with electric clippers. The rat was then anesthetized with ether and placed prone on the operating board. A dorsal midline incision was made from approximately the 10th thoracic to the third lumbar vertebra, the incision being long enough to allow spreading it to either side for removal of both adrenals. The incision was made through the skin, and the muscle over the kidney was spread apart exposing the kidney. The adrenal gland was brought into view by gently pulling up the surrounding fat at the pole of the kidney. The gland could be teased from the fat and adjacent fascia by means of forceps. The same procedure was performed on the opposite side. After the muscle was closed with loose suturing, the area was covered with myciguent ointment. The skin was closed by means of three wound clips, covered with myciguent ointment and sprayed with aeroplast bandage. After the rat was

sufficiently recovered he was returned to a separate cage. Salt and sugar were administered to his daily drinking water. Daily weighings were made on the adrenalectomized thymectomized rats.

When the rats were approximately three months old, white blood cell counts were made on all animals except Groups 1 and 3. When an animal died from wasting disease an autopsy was done and certain tissues were sent to pathology for sections.

At the end of the experiment, the rats were sacrificed and examined for remnants of thymus tissue, and tissue sections of heart, lungs, spleen, kidney, muscle, bone, lymph node, ileum, liver, testis, skin and pancreas were made for comparison studies. Tissue sections were also made of thymus gland from one adrenalectomized rat, one control animal and a residual thymus found in one of the thymectomized-only rats.

Records were not kept on the remaining living non-medicated thymectomized animals beyond the eighth week of life. At this time the average weight of the wasted animals of this group was approximately 120 grams while the average weight of the remainder of the group was 175 grams. A description of the appearance of the non-wasted nonmedicated thymectomized animals (rats) is readily available in current literature.

RESULTS

The tissue sections from thymectomized and thymectomized-adrenalectomized both medicated and nonmedicated group proved to be inconclusive with respect to evidence of hormonal deprivation. There were instances of slight margination of leukocytes and congested alveoli as well as focal atalectasis and over-expanded alveolar spaces in the lungs suggesting the presence of infection. Some hemorrhage was noted. These findings were noted only in the thymectomized and in thymectomized-adrenalectomized animals that had not been medicated. In one instance a walled-off abscess was noted in a wasting thymectomized rat. In another instance the spleen of a thymectomized wasted rat showed many neutrophils both in the spleen itself and in parasplenic tissue. The spleen in this instance was markedly congested. In another thymectomized-adrenalectomized animal the spleen contained large numbers of monocytes as well as many neutrophils. In the medicated animals the tissue sections appeared normal with no evidence of any type of tissue change or of infectious processes.

Of a total of 15 medicated thymectomized and medicated thymectomized-adrenalectomized rats, (Table 2) 3 developed the wasting disease and died. This gives 20 percent wasted of the total. Of the above, ten were thymectomized-only and 2 or 20 percent were wasted. Five were thymectomized-

TABLE NO. 2

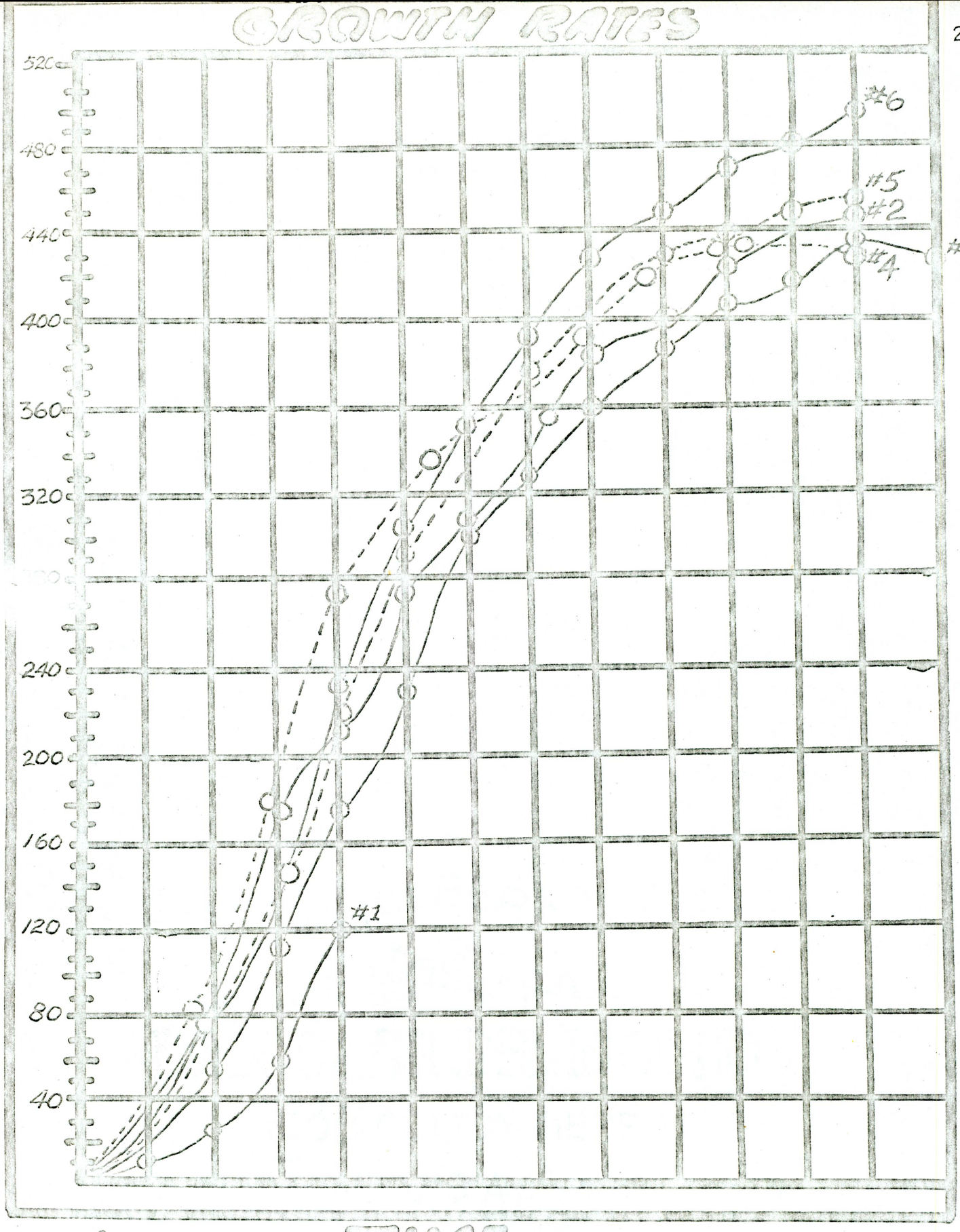
SURGERY		NO. OPERATIONS	WASTING DISEASE (W.D.)	PERCENTAGE (%)
MEDICATED	THYMECTOMIZED (M.T.)	10	2	20
	THYMECTOMIZED - ADRENALECTOMIZED (M.T-A.)	5	1	20
NON-MEDICATED	THYMECTOMIZED (N-M.T.)	17	7	40
	THYMECTOMIZED - ADRENALECTOMIZED (N-M.T-A.)	4	2	50

adrenalectomized, and 1 or 20 percent was wasted. Of 17 nonmedicated thymectomized rats, 7 or 40 percent developed wasting disease. Of 4 nonmedicated thymectomized-adrenalectomized, 2 or 50 percent were wasted.

As shown in the growth weight curve in Figure 1, the medicated control rats reached the highest weight (average weight 487 gms) in the 24 weeks of life. Their rate of growth exhibited a good sigmoid curve. The nonmedicated control rats reached an average weight of 448 gms. The medicated adrenalectomy-thymectomy rats showed a faster growth rate for the first eleven weeks of growth than the medicated controls, but after that time they slipped below both medicated and non-medicated controls to an average weight of 435 gms. The nonmedicated adrenalectomy-thymectomy rats showed a lower growth rate curve until the 24th week when their weight rose above the medicated thymectomized-adrenalectomized rats to 440 gms and then dropped in the 25th week to 427 gms. The medicated thymectomy rats showed a final average growth weight of 442 gms. The growth curve of the nonmedicated thymectomy rats that died of wasting during the eight weeks of life, was considerably below that of all the other rats. The growth curve of those that remained was the same as the thymectomized-adrenalectomized nonmedicated group up to the eighth week.

The white blood cell counts ranged from 8,800 for the medicated thymectomized animals to 18,000 for the

medicated controls (Figure 2). The average white blood count for the medicated control rats was 18,200. (Figures 3,4). The average count for adrenalectomized-thymectomized rats was 9,200. The average count for the thymectomized-only rats was 8,800. The average count for all medicated rats (thymectomized and thymectomized-adrenalectomized) showing thymus residue at sacrifice was 15,000. The count for the nonmedicated control rats was 12,000.



Legend:

1. non-medicated thymectomized ○
2. medicated-thymectomized ○
3. non-medicated thymectomized-adrenalectomized ○
4. medicated thymectomized-adrenalectomized ○
5. non-medicated controls ○
6. medicated controls ○

(FIGURE 1)

WHITE BLOOD CELL COUNT

CONTROLS

A. MEDICATED

1. 14,000
2. 19,800
3. 19,000
4. 20,000

AVERAGE: 18,200

B. NON-MEDICATED

1. 12,500
2. 11,800
3. 11,000
4. 12,700

AVERAGE: 12,000

EXPERIMENTAL

A. MEDICATED THYMECTOMY

1. 8,000
2. 10,200
3. 11,000
4. 8,600
5. 6,800
6. 8,000
7. 8,000
8. 9,600
9. 8,000

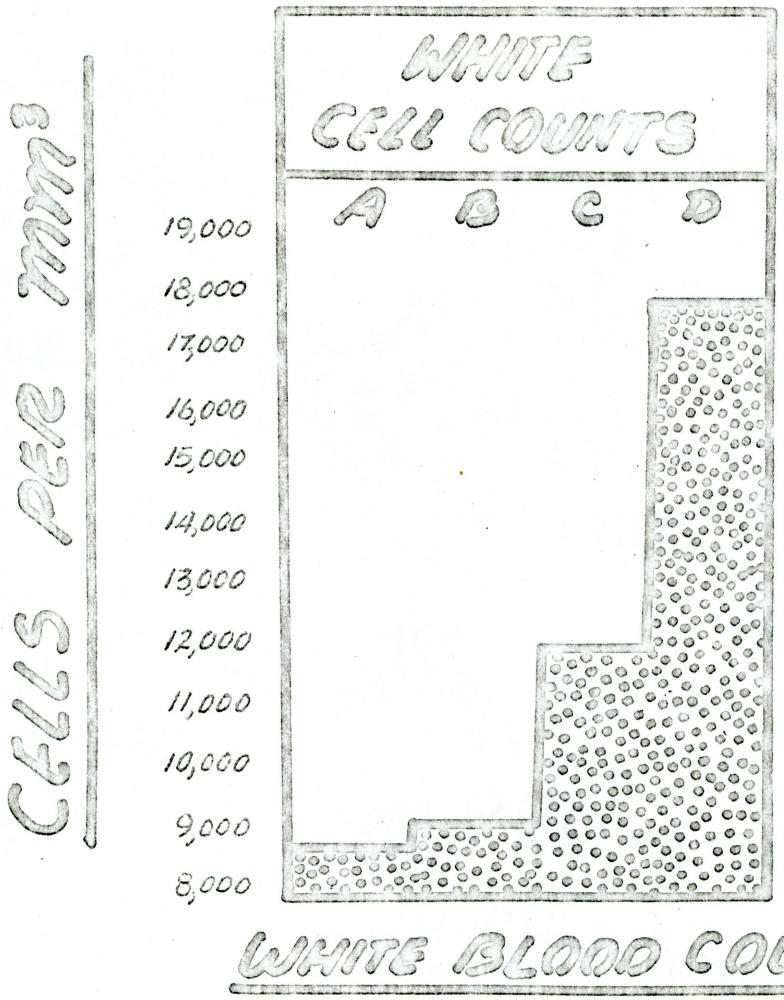
AVERAGE: 8,800

B. MEDICATED THYMECTOMY & ADRENALECTOMY

1. 11,000
2. 9,400
3. 6,600
4. 9,800

AVERAGE: 9,200

(FIGURE 2)



A	MEDICATED THYMECTOMIZED ONLY	8,800
B	MEDICATED THYMECTOMIZED ADRENALECTOMIZED	9,200
C	NON-MEDICATED CONTROL	12,000
D	MEDICATED CONTROL	18,000

(FIGURE 3)

DISCUSSION

The pathology tissue sections, although inconclusive, do indicate that some type of infectious process did take place in the wasted animals. It is known that bronchiectasis and wasting is a frequent conplication in vitamin A deficient rabbits. However, all animals in this experiment received the same diet which included an adequate amount of vitamin A. Vitamin A deficiency, then, would be ruled out in the case of the wasted animals in this project.

The growth curves showed that the medicated control animals, even though the medication had been given for approximately two days before birth and four days after, (in the first instance across the placental barrier and in the second instance in the mother's milk), and then stopped, seemed to thrive and grow better or at least as well as the nonmedicated controls. This suggests that if the body does not have to concern itself unduly with invasive organisms during the first few days of life it can have a more healthy growth and development. The medicated thymectomy rats showed a 20 percent wasting versus 40 percent for the non-medicated thymectomized rats. In addition, the weight and growth curve of the medicated-thymectomized rats indicates that the average weight is little different from that of the nonmedicated controls. A comparison of the medicated-thymectomy percentage of wasting and their average weight with the nonmedicated thymectomized rats which lagged far

behind in growth and weight until the eighth week in which they died of the wasting disease would indicate the efficacy of the medication in decreasing and possibly eliminating the wasting disease.

The medicated thymectomized-adrenalectomized rats showed in the early weeks a slightly higher growth rate curve than the nonmedicated animals similarly operated upon. At the end of the 24 weeks the two groups were almost equal in average weight. In contrast the other nonmedicated groups were indicating a downward trend in weight. It appears, then, that the medicated thymectomized-adrenalectomized animal has a faster rate of growth in terms of weight gain than even the medicated control animals during the early weeks of life. Water balance in these was an unknown factor.

The white count of the medicated thymectomized-adrenalectomized rats was only slightly higher than that of the medicated thymectomized animals. It is recalled that lymphocytes are preponderent in the white cell population of the rat. If there is any significance in these findings, it would indicate that the adrenocorticosteroids do suppress lymphoid tissue as stated by Schlesinger (1964). This is indirect evidence. The author was not able to adduce any direct evidence to indicate what effect the thymus has on adrenocorticosteroids. It was at first conjectured by the present author that the uninhibited corticosteroids might play a major role in causing the

wasting disease, but the same percentage of wasting occurred in the thymectomized-only rats as in the thymectomized-adrenalectomized animals. In view of Schlesinger's (1964) statement that thymus hormones depress adrenocorticoid hormonal production and since adrenal cortical hormones are stated to suppress lymphoid tissue we might also have expected a greater variation in the white blood counts of the two groups. On the basis of blood counts alone, it does appear that after thymectomy the uninhibited intact adrenal does depress the lymphoid tissues. It is the body's losing its ability to combat infection that results in the wasting syndrome, not a catabolic effect of the steroids themselves.

The ranges of the white blood counts are shown in figure 2. The white count (8,800) of the medicated thymectomized rats and the medicated thymectomized-adrenalectomized (9,200) rats, which was low compared to the others, would indicate that the thymus is essential for normal lymphocyte production. Since the rats were thymectomized on the day of birth, this would suggest that either the lymphocytes were seeded to the other lymphoid organs before birth or that lymphocytes originate in other tissue as well as thymus tissue. Levey (1964) points out as noted above, that even though an animal has been thymectomized at birth, it may maintain a high level of lymphocyte production but fails to show an immune response to virus injection. This further confirms the hypothesis that there

may be a thymic hormone that stimulates antibody production. The next question to arise would be the possibility of a thymotrophic hormone from the pituitary which might act as ACTH does in the absence of the adrenal cortex. This might explain the production of lymphocytes, albeit at a low level, in spite of removal of the thymus gland. The medicated thymectomized-adrenalectomized rats showed a slightly higher white count, still relatively low when compared to other groups, which might suggest that the absence of corticosteroids removes an inhibitor to lymphocyte production. The nonmedicated control rats had an average count of 12,000. The adult rat has a range of 6,000 to 18,000 leukocytes with an average of 9,000. Rats have 70 percent lymphocytes where humans have 70 percent neutrophils (Harris, 1949). The higher average in these rats is probably indicative of a higher incidence of infectious organisms present in the animal care shelter. The medicated rats in which thymus residue was found on sacrifice averaged a white count of 15,000. This indicates infectious processes, probably introduced at the time of surgery, were successfully warded off by the action of the hormone stimulus of the thymus remnant to increased lymphocyte production as well as the addition of the medication. The medicated control rats showed the highest average white count of all with a total of 18,000. The explanation for this difference is not readily apparent.

Agammaglobulinemia is certain to occur in neonatally thymectomized rats according to Levey (1964). It would appear that the administration of an antibiotic during late fetal and early postnatal period grants some degree of immunity to infection and to subsequently allow the animal to achieve near normal growth. Azar (1964) noted lymphopenia (see page 8) which could be relative if leukocytosis in the presence of an infection should occur as expected.

SUMMARY AND CONCLUSIONS

A total of 49 newborn rats were divided into the following groups:

1.	Thymectomy only---nonmedicated	17 rats
2.	Thymectomy only---medicated	12 rats
3.	Thymectomy-adrenalectomy---nonmedicated	5 rats
4.	Thymectomy-adrenalectomy---medicated	8 rats
5.	Control---nonmedicated	4 rats
6.	Control---medicated	4 rats

The thymectomy surgery was done on the day of birth. Before birth and for four days after, the mothers of groups 2, 4 and 6 were administered chloramphenicol in the drinking water. Growth rate and weight recordings were made of all progeny. The mothers of groups 1, 3 and 5 were not given any medication. Approximately two weeks after thymectomy the animals of groups 3 and 4 were adrenalectomized. White blood cell counts were taken on all progeny at the 12th week. At the end of the 22nd week the surviving animals were sacrificed and autopsied to determine the presence or absence of thymus and adrenal tissue.

The thymectomized nonmedicated progeny (group 1) showed a slower growth rate and weight gain curve and in the 8th week developed wasting disease and died. The medicated thymectomy progeny (group 2) exhibited a growth and weight curve similar to that of the nonmedicated control rats of group 5. The growth rates of the nonmedicated

adrenalectomy-thymectomy progeny (group 3) was apparently lower than that of the medicated adrenalectomized-thymectomized progeny (group 4) during the entire course of the experiment but the end weight of group 4 slipped slightly under that of group 3 (440 gms vs 448 gms). The nonmedicated control progeny (group 5) showed an end average weight of 448 gms as contrasted with the medicated control progeny of group 6 with 487 gms.

Growth rate curves would indicate that adrenalectomy when done in addition to thymectomy has no effect relative to the development of the wasting process whether the animal is medicated or nonmedicated as a comparison of the percentage of animals developing the wasting disease reveals that: 40 percent of the nonmedicated thymectomized-only rats (group 1) developed the wasting disease whereas only 20 percent of the medicated thymectomized-only rats (group 2) developed the wasting disease. Fifty percent of the non-medicated thymectomized-adrenalectomized rats (group 3) exhibited wasting, whereas 20 percent of the medicated thymectomized-adrenalectomized rats (group 4) were wasted. The percentage of animals of the medicated groups developing the wasting disease was half or less than that of the non-medicated animals of corresponding groups. (Table 2)

The white cell count of the medicated thymectomized and thymectomized-adrenalectomized animals was at the lower limits of normal and yet these animals exhibited growth

and weight curves similar to those of the control animals.

It is the conclusion of this writer that the wasting disease is, in all probability, an infectious process, either bacterial or viral, brought about by the lymphoid depletion of the neonatally thymectomized animals resulting in lowered resistance to infectious agents. The results of this experiment seem to be in agreement with the results of the work carried out by Azar (1964) as described earlier in this paper.

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ANTIBIOTICS AND THE WASTING DISEASE IN NEONATALLY THYMECTOMIZED RATS

An Abstract of

A Dissertation

Presented to

the Faculty of the Graduate School

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science

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by

Douglas M. Grignon

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ABSTRACT

The administration of the antibiotic, Chloramphenicol, to pregnant rats just prior to, and for four days after delivery, diminishes the incidence of the so-called "Wasting Disease" in neonatally thymectomized rats.

A total of 50 rats were divided into the following groups:

1.	Thymectomy only---nonmedicated	17 rats
2.	Thymectomy only---medicated	12 rats
3.	Thymectomy-adrenalectomy---nonmedicated	5 rats
4.	Thymectomy-adrenalectomy---medicated	8 rats
5.	Controls---nonmedicated	4 rats
6.	Controls---medicated	4 rats

Chloramphenicol was administered in the drinking water to the mothers of groups 2, 4 and 6 for about 2 days prior to delivery and 4 days after. On the day of birth or within three days after birth, the male progeny were thymectomized. Approximately 2 weeks after thymectomy adrenalectomies were carried out on some of the progeny (groups 3 and 4). Growth rate and weight records were kept of all groups. At the 12th week white blood cell counts were made on all groups except 1 and 3. At the end of the 24th week all survivors were autopsied for presence of thymus and adrenal tissue and those with evidence of such were excluded. Routine tissue sections were made of various organs of all

groups and search made for any evidence of hormonal deprivation or infection.

The medicated control animals exhibited a higher growth rate than all other groups. The medicated thymectomized rats showed an average weight just slightly under that of the nonmedicated controls. The thymectomized-adrenalectomized animals appeared to show the same average weight at the end of the experiment but the medicated group had a faster growth rate and weight gain during the early weeks of the experiment. The medicated animals of groups 2 and 4 showed a 20 percent wasting incidence while wasting occurred in 40 percent of group 1 and 50 percent in group 3.

The lowest white blood counts were 8,800 for the medicated thymectomized animals and 9,200 for the thymectomized-adrenalectomized animals, probably due to the lymphopenia reported by others. The white blood count of the nonmedicated control group was 12,000.

The administration of antibiotic to a pregnant rat just prior to and for a few days after birth of the young appears to lessen the incidence of the wasting disease in its progeny thymectomized within three days after birth leading to the conclusion that the wasting disease is primarily a pre-occupation of the immunologic system during the early weeks of life with infections acquired in the immediate post-operative period. These results corroborate the conclusions of Azar (1964) relative to the efficacy of

antibiotic administration in preventing the wasting disease, and also are in accord with the findings of Miller (1964) that germ free thymectomized mice do not evidence the wasting syndrome.