Studies on the immunological recovery of neonatally thymectomized mice
by James Thomas Hunter

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
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Abstract:
A time-course study was made on the immunological responsiveness of Swiss Manor mice which had been thymectomized at birth. The production of hemolysins and hemagglutinins, tested 5 and 10 days after stimulation with sheep erythrocytes, was found to be depressed in mice immunized 30, 40, or 50 days after thymectomy. No age dependant increase in the ability to produce hemagglutinins or hemolysins was found as no titers above the subnormal levels generated by the 30 day old mice were found in either the 40 or 50 day old mice. The ability of spleen cells obtained from the neonatally thymectomized mice to induce graft-versus-host disease in newborn A/jax mice was found to be severIy impaired when 20 x 10 6 cells from 30-70 day old animals were used or when 40 x 10 6 cells from 30, 40, or 50 day old animals were used. However, substantial recovery of the ability to induce graft-versus-host disease was observed when 40 x 10 6 cells from 60 or 70 day old donors were given newborn A/jax mice. The ability of the thymectomized mice to reject skin homografts from A/jax donors was found to be normal at 30 days.

The mice used in these experiments suffered from a high incidence of post thymectomy wasting (33/. mortality). When spleen cells obtained from the thymectomized mice were used to induce graft-versus-host disease in A/jax recipients a high incidence of early death (prior to 6 days post-injection) was encountered in the recipient animals. The early deaths could be prevented by incubating the donor cells in media containing low levels of antibiotic prior to injection into the A/jax recipients. An infectious agent was postulated and subsequently Streptococcus faecalis was isolated from the spleens of two Swiss Manor mice undergoing post-thymectomy wasting disease.

Peripheral studies were conducted in which Swiss Manor mice were splenectomized within 24 hours of birth. Two out of fourteen of the animals were observed to die of a wasting like syndrome at 18 days of age.

The data are interpreted as supporting the concept that neonatalIy thymectomized mice tend to recover immunological competence with increasing age. Further, the data indicate that enteric bacteria may be associated with post-thymectomy wasting and that the spleen may be of some importance in the development or maintenance of the immunologic responsiveness of newborn mice.

204

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Approved:

Head, Major Department

Chairman, Examining Committee

Dean, Graduate Division

MONTANA STATE UNIVERSITY
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TABLE OF CONTENTS

Page

INTRODUCTION 1

MATERIALS AND METHODS 14

Experimental Animals 14

Surgical Procedures 14

Anesthesia 14

Thymectomy 15

Splenectomy 16

Skin Grafting 17

Production of GVH Disease 18

Criteria of GVH Disease 19

Immunization with Sheep Erythrocytes 20

Antibody Titrations 20

Bacteriological Methods 21

Histological Procedures 22

RESULTS 24

Time-Course Studies on the Ability of Spleen Cells from Neonatally Thymectomized Mice to Induce Graft-Versus-Host Disease 24

Time-Course Studies on the Ability of Neonatally Thymectomized Mice to Produce Hemolysins in Response to Immunization with Sheep Erythrocytes 26

Time-Course Studies on the Ability of Neonatally Thymectomized Mice to Produce Hemagglutinins in Response to Immunization with Sheep Erythrocytes 26

The Ability to Reject Homografts in Neonatally Thymectomized Mice 31

Attempts to Isolate Bacteria from the Spleens of Neonatally Thymectomized Mice 32

Incidence and Description of Wasting Disease in Swiss Manor Mice Thymectomized at Birth 32

Miscellaneous Studies 33

DISCUSSION 35

SUMMARY 41

LITERATURE CITED 43

LIST OF TABLES

Page

Table I. Mortality in A/jax mice caused by graft-versus-host disease induced by injection of spleen cells from neonatally thymectomized or sham-thymectomized Swiss Manor mice 25

Table II. Hemolysin response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls at 5 days 27

Table III. Hemolysin response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls at 10 days 28

Table IV. Hemagglutination response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls at 5 days 29

Table V. Hemagglutination response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls at 10 days. 30

Table VI. Skin homograft rejection in neonatally thymectomized Swiss Manor mice and in sham-thymectomized or partially thymectomized littermate controls. 31

ABSTRACT

A time-course study was made on the immunological responsiveness of Swiss Manor mice which had been thymectomized at birth. The production of hemolysins and hemagglutinins, tested 5 and 10 days after stimulation with sheep erythrocytes, was found to be depressed in mice immunized 30, 40, or 50 days after thymectomy. No age dependant increase in the ability to produce hemagglutinins or hemolysins was found as no titers above the subnormal levels generated by the 30 day old mice were found in either the 40 or 50 day old mice. The ability of spleen cells obtained from the neonatally thymectomized mice to induce graft-versus-host disease in newborn A/jax mice was found to be severly impaired when 20×10^6 cells from 30-70 day old animals were used or when 40×10^6 cells from 30, 40, or 50 day old animals were used. However, substantial recovery of the ability to induce graft-versus-host disease was observed when 40×10^6 cells from 60 or 70 day old donors were given newborn A/jax mice. The ability of the thymectomized mice to reject skin homografts from A/jax donors was found to be normal at 30 days.

The mice used in these experiments suffered from a high incidence of post thymectomy wasting (33% mortality). When spleen cells obtained from the thymectomized mice were used to induce graft-versus-host disease in A/jax recipients a high incidence of early death (prior to 6 days post-injection) was encountered in the recipient animals. The early deaths could be prevented by incubating the donor cells in media containing low levels of antibiotic prior to injection into the A/jax recipients. An infectious agent was postulated and subsequently Streptococcus faecalis was isolated from the spleens of two Swiss Manor mice undergoing post-thymectomy wasting disease.

Peripheral studies were conducted in which Swiss Manor mice were splenectomized within 24 hours of birth. Two out of fourteen of the animals were observed to die of a wasting like syndrome at 18 days of age.

The data are interpreted as supporting the concept that neonatally thymectomized mice tend to recover immunological competence with increasing age. Further, the data indicate that enteric bacteria may be associated with post-thymectomy wasting and that the spleen may be of some importance in the development or maintenance of the immunologic responsiveness of newborn mice.

INTRODUCTION

The thymus is a comparatively large lymphoid organ located in most animals in the ventral anterior mediastinum of the chest in close association with the pericardium and great veins at the anterior aspect of the heart. Prior to 1961, the role played by the thymus was uncertain, although the work of numerous early investigators, most notably Beard (1900) and Hammer (1921), had implicated the thymus as being important in the physiology of the lymphoid system. In 1961 a dramatic change in the impetus of research on the thymus was initiated by the independent demonstration by Miller (1961, 1962a) and Good's group (Martinez et al., 1962; Archer et al., 1962) of the profound immunological effects of neonatal thymectomy. The work of Miller demonstrated that thymectomy of mice in the immediate neonatal period was associated with severe lymphopenia and an impaired ability to reject homografts in the adult animal (Miller, 1961, 1962a). Simultaneous work by Good's group demonstrated that skin homograft survival in neonatally thymectomized mice was prolonged in certain donor host combinations (Martinez et al., 1962) and that the ability of neonatally thymectomized rabbits to respond to stimulation with soluble antigens was markedly impaired (Archer et al., 1962). Experiments dealing with thymic extirpation had been carried out prior to the work of Miller and Good's group, but these investigations were conducted in mature animals and the results were equivocal. Such experiments are typified by those reported by Fichtelius, Laurell, and Phillipsson (1961) using adult male guinea pigs. Since the first discoveries on the effects of neonatal thymectomy an enormous volume of

research has been done and the thymus has come to occupy a central role in immunobiological thought particularly in regard to the ontogeny of the immune system.

The impairment of the adult immune system brought about by perinatal thymectomy of various facets is now well documented. A number of studies exemplified by the experiments of Good et al. (1962) and those of Waksman et al. (1962) have established that thymectomy of newborn animals is related to a diminished lymphocyte population in the adult animal. Generally, the level of humoral antibody response in adult neonatally thymectomized animals is greatly depressed. Subnormal levels of antibody have been reported to be produced in response to challenge with many antigens such as sheep erythrocytes (Fahey, Barth and Law, 1965), Salmonella typhi H, O and Vi antigens (Humphrey, Parrott and East, 1964) and bovine serum albumin (Arhason et al., 1964). However, the situation is not clearly defined as normal or near normal levels of response to other antigens such as ferritin, hemocyanin (Fahey, Barth and Law, 1965) and tetanus toxoid (Hess, Cottier and Stoner, 1963) have been reported. Such discrepancies may be due to a multiplicity of variables such as sex variations (Balner and Dersjant, 1966) or to time elapsed between immunization and serum collection (Sinclair, 1965). There is also conflicting evidence concerning the ability of neonatally thymectomized animals to mount a primary and a secondary immune response. One worker has reported a depressed primary response and a normal secondary response (Sinclair, 1967a, b) while another group has reported a normal primary

response and a depressed secondary response (Hess, Cottier and Stoner, 1963). Again, the discrepancy may be due to a wide number of uncontrolled variables. Numerous reports indicate that neonatal thymectomy impairs cell-mediated immune reactions. In neonatally thymectomized mice there is, generally, marked impairment of ability to reject homografts and in some cases heterografts (Dalmasso, Martinez and Good, 1962a; Miller, 1962b; Miller, Marshall and White, 1962). The same situation has been reported in other species such as rats (Arnason, Jankovic and Waksman, 1964) and hamsters (Sherman, Adner and Dameshek, 1964). Various reports such as that of Martinez, Dalmasso and Good (1962a) and that of Perri et al. (1963) indicate that allogenic tumor grafts are accepted by thymectomized animals. Neonatal thymectomy has been reported to diminish the effects of hypersensitivity reactions such as those associated with systemic lymphocytic choriomeningitis infection as reported by Rowe, Black and Levey (1963). Cells from the lymphoid organs of neonatally thymectomized mice were found to be less capable of eliciting graft-versus-host reactions than were similar numbers of cells from normal mice (Dalmasso, Martinez and Good, 1962b; Miller, Marshall and White, 1962). Still further evidence for the long range immunosuppressive effects of neonatal thymectomy can be inferred from the development of a wasting disease following neonatal thymectomy. Mice thymectomized at birth may develop a syndrome in latter life characterized by lethargy, ruffled fur, hunched posture, weight loss, diarrhea and, ultimately, death (Miller, 1962b, 1964). Wasting, however, does not occur in all

all strains of mice and onset may begin anywhere from 4 weeks to 4 months after thymectomy depending on the strain of mouse involved (Parrott, 1962). The pathogenesis of the post thymectomy wasting syndrome is not definitely established, but there is a preponderance of evidence favoring an infectious etiology (Azar, 1962; McIntire, Sell and Miller, 1964).

Simple thymectomy of adult animals has not been associated with immediate immunological defects (Harris, Rhoads and Stoakes, 1948; Fichtelius, Laurell, and Phillipsson, 1961). However, when antigenic stimulation was delayed until several months post-thymectomy deficient antibody responses were detected. Taylor (1965) reported depressed responses to bovine serum albumin immunization and Metcalf (1965) reported lowered responses to immunization with sheep erythrocytes following thymectomy of adult mice. The capacity of spleen cells from thymectomized adult mice to induce graft-versus-host reactions degenerated gradually over a period of time (Miller, 1965). Another line of evidence indicates that the thymus can function to restore the immunological competence of adult animals whose immune mechanism has been impaired or destroyed. Numerous studies typified by the work of Globerson and Feldman (1964), Miller, Doak and Cross (1963) and Jeejeebhoy (1965) indicate that the recovery of the ability to respond to various antigens following total-body irradiation is impaired or completely suppressed by thymectomy in adult animals. Thymectomy of mature animals made tolerant to bovine gamma-globulin at birth has been reported to delay the abrogation of the tolerant state (Claman and Talmage, 1963; Hunter and Jutila,

1964). From the evidence accumulated by experiments dealing with the effects of neonatal and adult thymectomy it appears that the thymus functions to generate or assists in generating the immune system during the perinatal period and that it also functions to maintain or regenerate the immune system throughout the life of the animal. How the thymus accomplishes this function is still a matter of controversy and investigation.

A number of approaches have been used in attempts to elucidate thymic function. They include either grafting of thymus tissue, implantation of thymus tissue in diffusion chambers, injection of cell suspensions or injection of thymus extracts into thymectomized mice. Also, embryological studies, and studies involving the use of chromosomal markers or radioactive labeling have served to identify cellular components of the thymus which participate in the generation of the immune response. Various studies have established that lymphopenia and loss of immunological functions following neonatal thymectomy can be precluded by the grafting of either syngeneic or allogeneic thymic tissue into thymectomized animals (Miller, 1961, 1962b; Dalmasso et al., 1963). The same results have been reported in the case of adult thymectomized irradiated mice (Miller et al., 1964; Leuchars, Cross and Dukor, 1965). A number of workers such as Dukor et al. (1965) have found that the implanted thymuses are composed of donor type cells initially but that gradually these cells are replaced by host lymphocytes and in the case of homografts and graft is rejected by the host. These results have

been interpreted as indicating a directive influence by the thymus upon the lymphoid precursors of the host as opposed to a repopulation of the host by precursor cells arising in the grafted thymus (Miller and Osoba, 1967). This interpretation is supported by the observation that diffusion chambers containing thymic tissue restore the immunological competence of neonatally thymectomized animals, (Levey, Trainin and Law, 1963; Levey et al. 1963; Osoba and Miller, 1963; Aisenberg and Wilkes, 1965). Similar results have been reported using thymus tissue enclosed in diffusion chambers to restore competence to thymectomized irradiated adult mice (Miller et al., 1964). However, the restoration of lymphocyte levels by implantation of thymus tissue in diffusion chambers has not been a consistent finding (Levey, Trainin and Law, 1963). The diffusion chamber experiments suggest that the thymus elaborates a humoral factor or factors which act on lymphoid precursor cells causing them to become immunologically competent. Several investigators have questioned the validity of results obtained with diffusion chambers by indicating that the chambers may have leaked, that the experiments were inadequately controlled, and that the porosity of the diffusion bariers used was large enough to allow the passage of cells (Auerbach, 1964; Weissman, 1967). It is possible to reconstitute the immune mechanism of neonatally thymectomized mice by injecting lymph node cells or spleen cells obtained from normal mice (Dalmasso et al., 1963; Cross, Leuchars and Miller, 1964). Cells from neonatally thymectomized mice or bone marrow or liver cells from normal mice failed to protect

neonatally thymectomized recipients against immunological defects (Cross, Leuchars and Miller, 1964). These results suggest that replenishing the supply of immunologically competent cells in a thymectomized animal will protect that animal. However, Trainin, Law and Levey (1965) have reported a 20% incidence of death in animals protected with spleen or lymph node cells when the animals were 3 to 7 months old. This would seem to indicate that cells alone are not sufficient to protect thymectomized animals over extended time periods. Although the previously mentioned experiments utilizing thymus grafts, diffusion chambers and cell suspensions have been construed as indicating a noncellular thymus factor capable of restoring immunological reactivity in thymectomized animals (Miller and Osoba, 1967), attempts to isolate and demonstrate the effects of a thymus humoral factor have been inconclusive. Metcalf's lymphopoiesis stimulating factor (Metcalf, 1956) is often referenced by thymus researchers but attempts to restore immunological competence to thymectomized animals utilizing thymus extracts obtained according to the procedure of Metcalf have proved unfruitful (Dalmasso et al., 1963; Miller, 1964). Other workers have reported isolating thymus humoral factors which stimulate the lymphoid system (Comsa and Bezssonoff, 1958; Jankovic, Isakovic and Horvat, 1965; Comsa, 1966; Brunkhorst and Herranen, 1967), but definite proof of the existence of an effective thymus humoral factor produced under physiological conditions is still lacking (Miller and Osoba, 1967).

Embryologically the thymus arises as an outgrowth of the third and fourth branchial pouches (Bloom and Fawcell, 1964). The thymus is, therefore, of epithelial origin. However, considerable doubt remains as to the origin of the lymphoid cells found in the thymus. The thymus. The experiments of Auerbach (1960, 1961) strongly suggest that the thymic lymphoid cells arise directly from the epithelial elements of the thymus with mesenchymal tissue exerting a morphogenetic effect. Auerbach's experiments with mice are supported by those of Ackerman and Knouff (1964, 1965) whose results suggest that the thymic lymphocyte of embryonic chicken and hamster have an epithelial origin. On the other hand, the work of Smith (1965) has indicated that lymphoblasts may already have migrated to the epithelium of the branchial pouches prior to the outgrowth of the pouches to form the thymus. Although the transformation of thymic epithelial cells to lymphocytes remains an open question it is well established that, ontogenetically, the thymus is the first lymphoid organ to develop in the embryo (Archer et al., 1964; Good and Papermaster, 1964). It has been postulated that lymphocytes formed in the thymus during embryogenesis are seeded to the peripheral lymphoid tissues (Auerbach, 1961). If the thymic epithelium does give rise to lymphoid cells during embryonic development this situation must be modified to some extent soon after birth. In this regard, studies carried out using chromosomes markers in conjunction with grafted thymuses (Harris et al., 1964), with bone marrow grafts (Tyan and Cole, 1965), and with parabiosis (Harris and Ford, 1964; Brumby and Metcalf,

1967) have convincingly demonstrated an afferent flow of cells to the thymus from myeloid tissues. However, there is also in the adult animal a low rate of efferent flow of cells from the thymus to peripheral lymphoid tissues as indicated by radio-labelling studies (Nossal, 1964a, 1964b; Weissman, 1967) and studies with chromosome markers (Harris et al., 1964; Harris and Ford, 1964). The low rate of efferent flow of lymphocytes from the thymus is puzzling in view of the high mitotic activity found in the thymus which is 4 to 6 times that found in the lymph nodes or spleen in the case of young mature animals (Andreasen and Christensen, 1949). Thus, it seems that many lymphocytes flowing into the thymus or being born there must die locally in a short time. This led Burnet (1962) to postulate that the thymic epithelium may give rise to progenitors of immunologically competent cells and may "censor" those cells or aberrant cells coming in from the "peripheral" lymphoid organs destroying those cells capable of reacting against "self" antigens.

The concept that the thymus may "censor" self reacting clones of cells has led to experiments dealing with the role of the thymus in controlling immunological tolerance. Several series of investigations have been carried out (Isakovic, Smith and Waksman, 1965; Tolluett and Waksman, 1966) but there is not sufficient evidence to support the thesis that tolerance is induced by interaction of antigens with the thymus (Miller and Osoba, 1967).

The characteristics of the thymus thus far mentioned suggest that it is a "central" lymphoid organ controlling the development of cells found in the "peripheral" lymphoid structures. However, investigations have been conducted which indicate that the thymus may not be the only "central" lymphoid organ participating in the ontogeny and maintainence of the lymphoid system. Work conducted as early as 1956 has indicated that the bursa of Fabricius in fowl is an organ as important or nearly as important as the thymus in the ontogeny of the immune system (Glick, Chang and Jaap, 1956). Investigations carried out using rabbits have shown that the cecum may be analogous to the bursa of Fabricius (Archer, Sutherland and Good, 1963; Archer, Papermaster and Good, 1964). Recent work in mice has intimated that there may be a second central lymphoid organ in those animals (Dukor, Dietrich and Rosenthal, 1966; Rogister and Lejeune, 1964; Rogister, 1965; Sinclair and Millican, 1967; Takeya and Nomoto, 1967a, b). The bursa of Fabricius is a lymphoid organ in fowl which develops from an outpouching of the hind gut and bears some histological similarity to the thymus. The experiments of Glick, Chang and Jaap (1956) and Chang, Rheins and Winter (1957) showed that its removal early in life resulted in marked diminution of antibody response to soluble antigens. Subsequent work wherein hormonally bursectomized chickens were used demonstrated that there was a dissociation of immunologic responsiveness in the chicken. Bursectomy did not inhibit skin homograft rejection while it did inhibit response to soluble antigens and reduced delayed hypersensitivity reactions. Hormonally induced

atrophy of the thymus cortex and bursa inhibited response to soluble antigens, mitigated delayed hypersensitivity reactions and delayed the rejection of skin homografts, but the ability of blood cells to produce lesions on chorio-allantoic-membranes was still present (Warner, Szenberg and Burnet, 1962; Warner and Szenberg, 1964). Surgical removal of the bursa, or thymus or both in young chickens followed by sub-lethal irradiation allowed the recognition of two morphologically distinct cell systems in the peripheral lymphoid system. The thymus evidently governs cellular immunity by seeding small lymphocytes to the circulation whereas the bursa governs the production of immunoglobulins by seeding large lymphocytes to the circulation (Cooper et al., 1966). Studies conducted with rabbits have shown that the appendix of the animal may be analogous to the bursa in fowl. Archer, Sutherland and Good (1963) reported that rabbits responded atypically to neonatal thymectomy in that recovery of the immune system following neonatal thymectomy was not as great as nor as general as the impairment following neonatal thymectomy of mice, and that by 14 weeks of age the rabbits appeared to have recovered normal levels of lymphocytes in the peripheral blood. Of the lymphoid organs they examined only the development of the appendix was not hindered by neonatal thymectomy. Further studies by this group (Sutherland, Archer and Good, 1964) showed that removal of both the thymus and the appendix from neonatal rabbits severely impaired the ability of the animals to respond to stimulation with soluble antigens and to reject homografts. Further,

they found no apparent recovery of the peripheral lymphocyte level by 14 weeks of age. Recent work has indicated that mice may recover immunologic responsiveness spontaneously over a period of time following neonatal thymectomy. Rogister and Lejeune (1964) reported an increase in ability to respond with hemagglutinins to sheep red cell immunization in neonatally thymectomized mice when tested at 60 days whereas they failed to report 30 days post thymectomy. Rogister (1965) latter extended these findings and reported an increased ability to reject homografts as a function of time post thymectomy as well as increased numbers of peripheral lymphocytes which reached near normal levels by 150 days post thymectomy. Extensive studies by Dukor, Dietrich and Rosenthal (1966) indicated that by 9 - 13 weeks post thymectomy colony bred Swiss mice recovered their ability to form hemagglutinins and hemolysins following injection of sheep erythrocytes. They also reported that antibody-plaque-forming ability had increased over a 13 week interval post thymectomy, but they found no increase in the numbers of peripheral lymphocytes. On the other hand, they reported no spontaneous reconstitution in the case of neonatally thymectomized highly inbred CBA mice. Their work is substantiated to some extent by that of Sinclair and Millican (1967) who reported a delayed development of the ability to respond with hemolysins to immunization with sheep erythrocytes in neonatally thymectomized, colony bred Swiss mice during a post immunization time-course study involving 10 days to 6 months. Takeya and Nomota (1967a) have reported increasing ability to respond

with hemolysins in neonatally thymectomized inbred mice of the SL strain following stimulation with sheep red cells over a period of 120 days post thymectomy. They also reported decreasing survival times for homografts and increasing numbers of antibody plaque forming cells over the course of the observation period although the mice had not reached control levels by the end of the experimental period. Of possible significance is a report by Kalpaktosoglou, Yunis and Good (1967) which indicated that neonatal splenectomy depresses the lymphocyte levels of C3H mice and results in a considerable incidence of wasting disease. They suggested that the spleen may be important in the ontogenetic development of hematopoietic tissue in mice.

The work presented in the following pages consists of a study of the immunological recovery of Swiss Manor mice thymectomized at birth. This study was carried out in order to confirm and extend the reports on the immunological recovery of neonatally thymectomized mice. Further, the work was undertaken to provide data for comparison with a separate study being conducted in this laboratory dealing with the immunological recovery of Swiss Manor mice whose immune system was suppressed at birth by treatment with cortisol acetate.

MATERIALS AND METHODS

Experimental Animals

The non-inbred Swiss Manor mice used in this study were originally obtained from the germ-free stock of Manor Farms, Staatsburg, New York in 1964. A conventionally reared and colony bred stock of these mice has since been maintained in the Department of Botany and Microbiology, Montana State University.

The inbred A/jax mice used in these experiments were originally obtained from the Jackson Memorial Laboratories, Bar Harbor, Maine in 1964 and have since been maintained here with frequent brother-sister matings.

All stock animals were fed Purina Laboratory Chow while breeding mice were maintained on Purina Mouse Breeder Chow. Experimental animals were fed Purina Mouse Breeder Chow supplemented with Quaker Rolled Oats and water ad libitum.

Surgical Procedures

Anesthesia

New born mice were anesthetized by a cooling technique modified from the procedure described by East and Parrott (East and Parrott, 1962). Newborn mice were gently separated from their mother and placed three at a time into a 50 ml. plastic beaker. The beaker containing the mice was then placed into the freezing compartment of a domestic refrigerator and held at -10° C for 8 minutes. At the end of 8 minutes the mice had ceased to breathe, did not respond to tactile stimuli and had lost all

pink color. The mice were then considered fully anesthetized and were operated on within the next 8 minutes. Animals were revived by gentle handling and warming under a lamp.

Sodium pentobarbital (Nembutal-Abbott Laboratories) was used for anesthesia of adult mice. Mice to be anesthetized were weighed to the nearest tenth of a gram and were injected intraperitoneally with .02 ml of a 1:10 aqueous dilution of pentobarbital per gram of body weight. Recovery of anesthetized animals was facilitated by warming them under a lamp.

Thymectomy

Thymectomy of new born mice was performed using an amalgamation of techniques modified from those described by several groups of investigators (Gross, 1959; Miller, 1960; East and Parrott, 1962). Newborn Swiss Manor mice (less than 24 hours old) were anesthetized by the cooling technique above described and were taped to the surface of a chilled glass operating platform with Scotch tape. Taping was done so that the mouse was positioned on its back with its head sharply extended toward the operator. Extension of the head was necessary in order to draw the thymus forward in the mediastinal cavity.

A sternal splitting incision was made utilizing a pair of angle point irridectomy scissors one point of which was inserted at the manubrial notch and pushed, with the point held high, to the level of the fourth rib. The sternum was then split with a single stroke of the

scissors and the incision was spred with a pair of curved fine point forceps to expose the thymus. The thymus was removed by gentle aspiration at its anterior apices with a pasteur pipette attached with flexible rubber tubing to a vacuum pump. The incision was closed with one to two interupted 4-0 silk autures placed through the skin with no attempt being made to approximate the edges of the sternum. A drop of collodion was applied to the wound in order to seal it, prevent pneumothorax, and to reduce maternal cannibalism. The young were returned to their mother upon recovery from the effects of anesthesia. Sham thymectomy was performed by duplicating all operative procedures but no suction was used and the thymus was left in place.

Initially, immediate operative mortality was extreme but with practice mortality was reduced to less than 5%. However, maternal cannibalism was high with approximately 25% of all operated litters being lost.

Completeness of thymectomy was determined by sacrificing the mice at the beginning or end of experimental procedures and checking the mediastinal cavity macroscopically for evidence of thymic tissue. Suspicious tissue was removed for histological examination. Animals presenting thymus remnants were used as partially thymectomized controls.

Splenectomy

Newborn Swiss Manor mice (less than 24 hours old) were anesthetized by the cooling technique and were taped to a chilled glass operating

platform in such a way as to expose the left lateral surface of the animal. A transverse incision was made over the region of the stomach with irridectomy scissors. The stomach was extruded through the wound and turned slightly to expose the spleen which was adherent to the dorsal surface of the stomach. The spleen was then removed by aspiration with a pasteur pipette attached to a vacuum pump with flexable rubber tubing. Following removal of the spleen, the stomach was gently pushed back into the abdominal cavity and the incision was closed with three to four interupted 4-0 silk sutures. A drop of collodion was applied to the wond to help prevent maternal cannibalism and the young were returned to their mother. Sham splenectomy was performed by duplicating all operative procedures except that no suction was used and the spleen was left intact and in situ.

Immediate operative mortality was nil and mortality due to maternal cannibalism was less than 20%.

Completeness of splenectomy was determined by sacrificing the mice at the end of the experimental period and examining the abdominal cavity for the presence of splenic tissue. Animals having splenic remnants were discarded from the experiment.

Skin grafting

Skin grafting of adult Swiss Manor mice was performed using a modification of the technique developed in E. J. Eichwald's laboratory (Eichwald, 1967). To prepare the graft beds, mice were first anesthetized

with pentobarbital and then pinned to an operating board in such a fashion as to expose and stretch the left dorsolateral skin of the midsection. The area was wetted with 70% ethanol and shaved. A square section approximately one centimeter on a side was then removed using a razor blade. Into the graft bed was placed a close fitting graft or autologous or allogeneic tail skin. The mouse was subsequently removed from the board and a strip of transparent polyester plastic approximately 8 x 2 cm was wrapped tightly around the animal's midsection and secured with heat resistant Scotch tape. This dressing permitted visulization of the graft, prevented scratching of the graft by the animal or its cage mates and held the graft securely in place without the need of sutures. The dressing was changed on the 3rd and 6th day post-graft and removed on the 9th day post-graft.

Grafts were prepared from the tail skin of anesthetized or sacrificed donor animals. The tail was amputated with a razor blade and the skin was split down the midline of the tail. The skin was then peeled free and cut into appropriately sized grafts. The proximal portion of the tail was not used as it was usually severely traumatized during the stripping procedure. Grafts were routinely placed on recipients within 15 minutes of removal from the donor and were kept moist on saline soaked gauze prior to use.

Graft rejection was evaluated by macroscopic examination of the graft daily after the 9th day post-grafting. Rejection was considered to have occurred when the graft had become brown and dry.

Production of Graft versus Host Disease

Thymectomized or sham-thymectomized Swiss Manor mice 30 to 70 days of age served as spleen cell donors. Following sacrifice by cervical dislocation the spleen was aseptically removed from each donor and placed in a small sterile petri dish (60 mm in diameter) containing 1 ml of sterile phosphate buffered saline pH 7.2 (PBS). The spleen was then gently ground apart using a sterile plunger from a 10 ml syringe. Coarse debris was removed from the splenic preparation and a 1:1,000 dilution in 4% acetic acid was made from an aliquot of the preparation. The nucleated cells were counted with a standard hemocytometer and the cell concentration of the splenic suspension was adjusted to contain 20×10^6 or 40×10^6 nucleated cells per .1 ml. To the preparation were added 500 units of dihydro-streptomycin sulfate (Nutritional Biochemicals Corporation) and 1,000 units of penicillin "G" (Nutritional Biochemicals Corporation) per 1 ml. The preparation with the antibiotics was incubated at 37° C for 30 minutes prior to being injected into mice.

A dose of either 20×10^6 or 40×10^6 spleen cells from neonatally thymectomized or sham thymectomized Swiss Manor mice was injected intraperitoneally into neonatal A/jax mice within 24 hours of birth. The dose was administered with a 1 ml tuberculin syringe fitted with a 25 gauge needle through the thigh muscle into the peritoneal cavity in order to minimize leakage. Mice which leaked excessively were discarded from the experiment.

Criteria of Graft versus Host Disease

The criteria used for judging graft versus host disease in mice injected with allogeneic spleen cells within 24 hours of birth were failure to gain weight normally and ultimately death which commonly occurred within 21 days post-injection. Mice dying before day 6 post-injection were considered to be victims of injection trauma or maternal cannabilism.

Other symptoms noted during the course of graft versus host disease were diarrhea, a hunched posture, high-stepping gait and alopecia.

Immunization with Sheep Erythrocytes

Sterile sheep blood preserved in Alsever's solution was thrice washed with saline prior to injection. A dose of 0.1 ml of a 10% solution of packed erythrocytes in saline was injected by the intraperitoneal route into 30, 50, and 70 day old Swiss Manor mice which had been thymectomized, sham-thymectomized or partially thymectomized at birth. The mice were bled from the tail vein 5 and 10 days post-injection. Ten drops of blood were collected in 0.5 ml of saline giving a serum dilution of approximately 1:5. The blood was allowed to clot and the sera were collected following low speed centrifugation. The sera were stored at 5° C and hemolysin and agglutinin titers for sheep erythrocytes were determined within two weeks of the bleeding date.

Antibody Titration

Endogenous complement was inactivated by incubating the sera at 56° C for 30 minutes. The sera were then serially diluted 1:1 starting with the 1:5 dilution and ending at a dilution of 1:2560. To each tube was added 0.1 ml of thrice washed 1% sheep erythrocytes in saline and the tubes were incubated at 37° C for 30 minutes. Hemagglutination was read following low speed centrifugation of the reaction tubes. A titer of 0 was arbitrarily assigned to sera producing no detectable hemagglutination at a dilution of 1:5. Hemagglutination patterns were scored 4+ to 1+ and final titer was expressed as the serum dilution in the last tube showing 1+ hemagglutination.

Hemolysin titrations were carried out in the same reaction system as that employed on agglutination titrations. Following reading of the hemagglutination patterns one drop of guinea pig complement (Difco) diluted 1:5 in saline was added to each tube. The tubes were agitated and incubated at 37° C for 30 minutes. The sera were held at 10° C overnight and the titers were read the following morning. A limiting dilution giving complete hemolysis was considered the titer of the serum. An arbitrary value of 0 was assigned to those sera having a titer of 1:10 or less.

Bacteriologic Methods

Attempts were made to isolate microorganisms from the spleens of thymectomized or sham-thymectomized 30 day old Swiss Manor mice. Mice

were killed by cervical dislocation and their spleens were aseptically removed. The spleens were placed into small petri dishes containing 1 ml of sterile saline and ground apart with a sterile plunger from a 10 ml syringe. Freshly prepared blood agar plates, 5% sheep blood in blood agar base (Difco), and thioglycolate (BBL) were inoculated with 0.3 ml each of the aseptically prepared spleen suspension from each mouse. One series of blood agar plates was incubated anaerobically and the other aerobically. All incubations were carried out at 37° C and media was held for at least 10 days at the temperature before considered negative for growth.

In six cases, the spleen suspension was freeze-thawed twice using a bath of acetone and dry ice before being inoculated into the media. This was done in order to release possible intracellular organisms. Organisms isolated from splenic tissues were characterized by standard bacteriological procedures.

Histological Procedures

Suspected thymic remnants were removed from the mediastinal cavity of supposedly thymectomized mice for histological examination. Immediately after removal from the animal the tissue samples were placed in buffered (pH 7.6) formalin and held there until processed for sectioning. The tissues were processed according to standard histological procedures. Dehydration and clearing was carried out in a series of alcohols and toluene and infiltration was with paraffin at 52° C. Sections

were cut at 10 micra and stained with a standard hematoxylin and eosin procedure.

RESULTS

Time-Course Studies on the Ability of Spleen Cells from Neonatally Thymectomized Mice to Induce Graft-versus-Host Disease

Time-course studies on the ability of spleen cells from neonatally thymectomized Swiss Manor mice to ellicit fatal graft-versus-host (GVH) disease in newborn A/jax mice were carried out according to the procedure previously described (Materials and Methods). The data presented in Table I shows that a recovery of the ability of spleen cells from neonatally thymectomized Swiss Manor mice to ellicit fatal GVH disease in newborn A/jax recipients was not detected when a dose of 20×10^6 cells from 30 to 70 day old donors was used. The over-all mortality in the above group was 9% whereas the mortality in the control mice which received 20×10^6 spleen cells from 30 to 70 day old neonatally sham-thymectomized Swiss Manor donors was 78%. On the assumption that 20×10^6 cells was too small a dose to allow detection of recovery, the number of cells injected was increased to 40×10^6 cells. When this dose was used a partial recovery of the ability of cells from 60 and 70 day old donors was detected (Table I). The degree of mortality among A/jax mice receiving 40×10^6 spleen cells from neonatally thymectomized Swiss mice in the 60 and 70 day old groups was 83% and 67% respectively, as compared to 100% mortality, regardless of age, when spleen cells from neonatally sham-thymectomized donors were used (Table I).

Table I. Mortality in A/jax mice caused by graft-versus-host disease induced by injection of spleen cells from neonatally thymectomized or sham-thymectomized Swiss Manor mice.

Number of spleen cells injected per mouse	Days post-thymectomy	Thymectomized Mice		Sham-operated Mice	
		No. Dying / No. Injected	% Mortality	No. Dying / No. Injected	% Mortality
20×10^6	30	2/10	20%	7/8	88%
	40	0/5	0%	0/2	0%
	50	0/5	0%	4/4	100%
	60	1/6	17%	3/4	75%
	70	1/5	20%	4/5	80%
40×10^6	30	1/10	10%	7/7	100%
	40	1/8	13%	4/4	100%
	50	1/5	20%	4/4	100%
	60	5/6	83%	9/10	90%
	70	8/12	67%	7/7	100%

Time-Course Studies on the Ability of Neonatally Thymectomized Mice to Produce Hemolysins in Response to Immunization with Sheep Erythrocytes

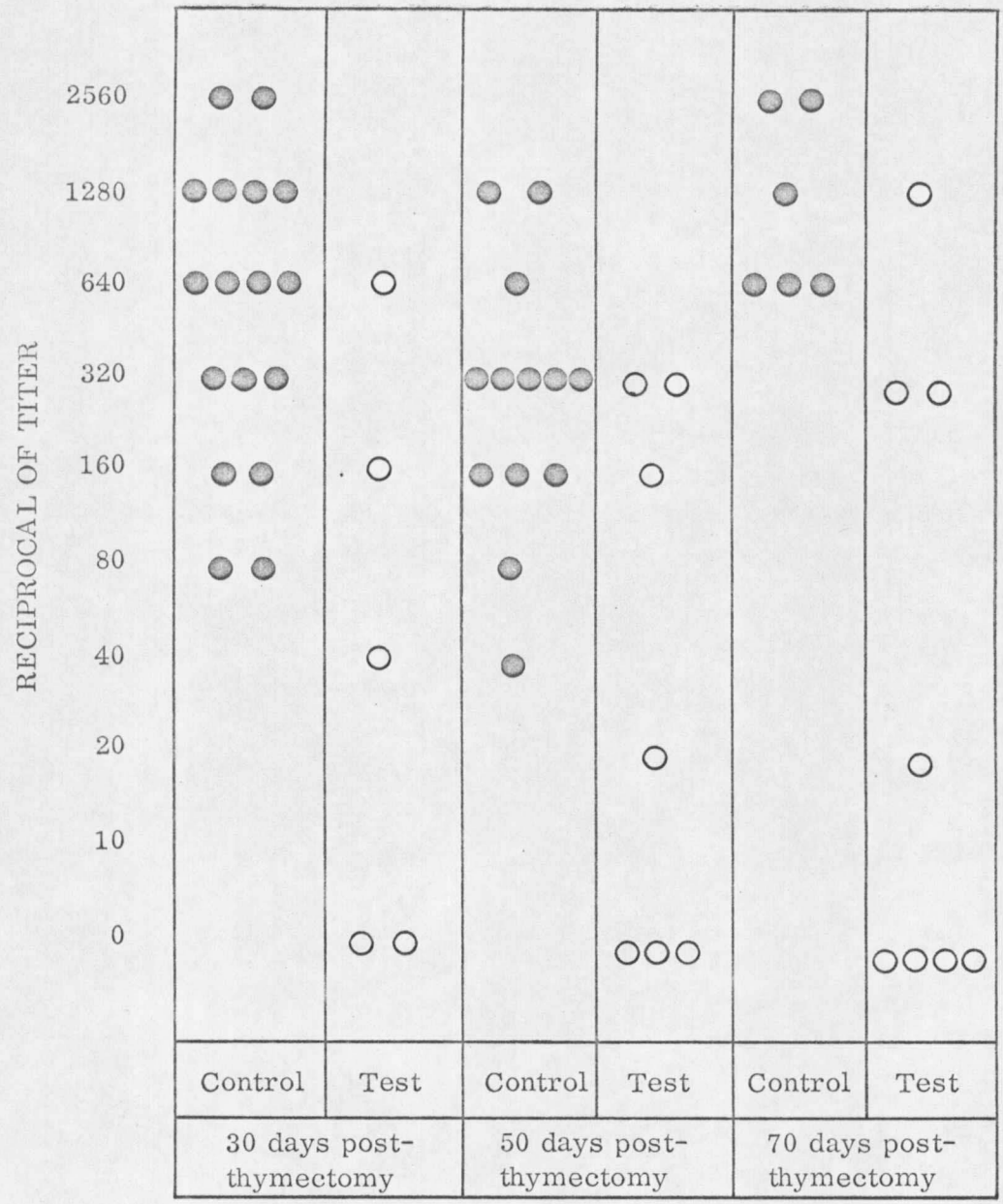
The ability of neonatally thymectomized Swiss Manor mice 30, 50, and 70 days old to produce hemolysins in response to injection of sheep erythrocytes was determined from sera obtained 5 and 10 days after immunization. The data presented in Table II indicated that the hemolysin response of neonatally thymectomized Swiss Manor mice 5 days after immunization was subnormal at each of the time intervals studied and that no age dependent change in the ability to respond had occurred. The data from hemolysin titrations conducted on sera obtained 10 days after immunization is presented in Table III. These data indicated that the response of the neonatally thymectomized mice was not greatly different from that of the control mice and that no apparent age dependent change in the ability to respond had occurred. When the data presented in Tables I and II were compared they indicated that the control animals had gone through a normal regression in hemolysin responsiveness from day 5 to day 10. In the case of the neonatally thymectomized test animals the regression was not marked.

Time-Course Studies on the Ability of Neonatally Thymectomized Mice to Produce Hemmagglutinin in Response to Immunization with Sheep Erythrocytes

The data shown in Tables IV and V indicated that the ability of neonatally thymectomized Swiss Manor mice to respond with hemagglutinins after immunization with sheep erythrocytes was severely impaired both

Table II. Hemolysin response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls. Serum was collected 5 days after immunization.

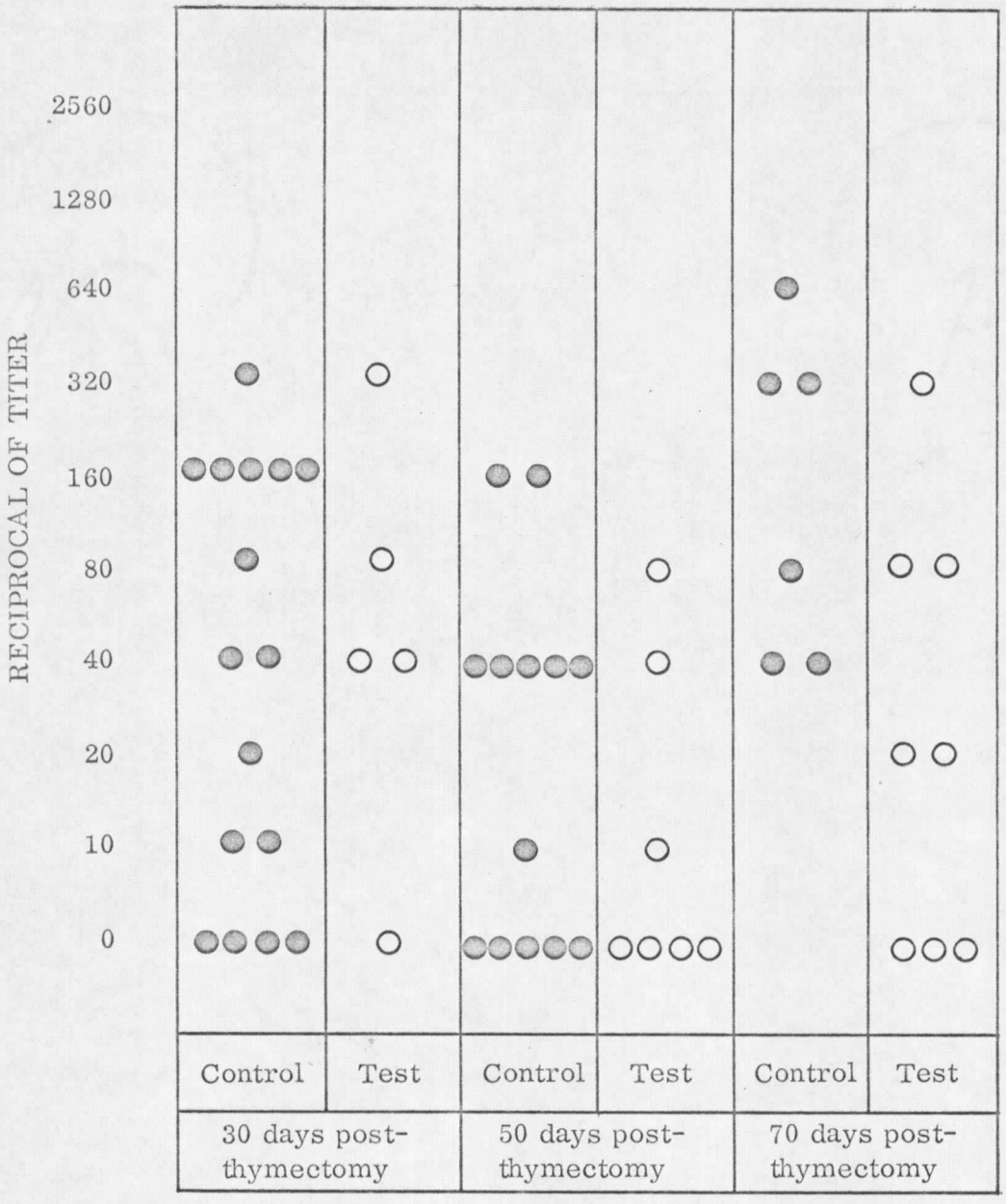
HEMOLYSIN RESPONSE AT DAY FIVE



Test = thymectomized mice.
Control = sham-operated or partially thymectomized mice.
Each circle represents the titer of the serum from a single mouse.

Table III. Hemolysin response to sheet erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls. Serum was collected 10 days after immunization.

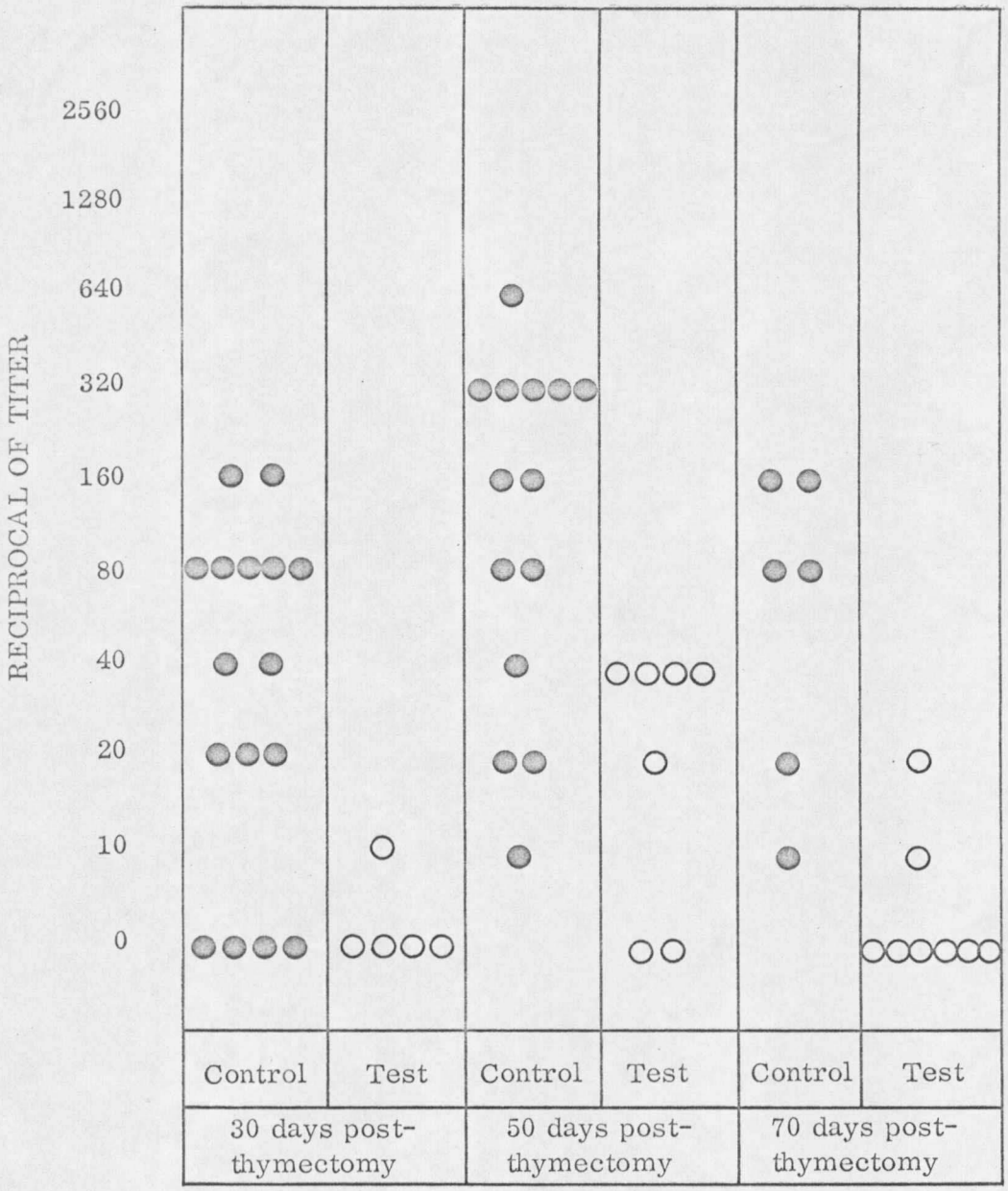
HEMOLYSIN RESPONSE AT DAY TEN



Test = thymectomized mice.
Control = sham-operated or partially thymectomized mice.
Each circle represents the titer of the serum from a single mouse.

Table IV. Hemagglutination response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls. Serum was collected 5 days after injection.

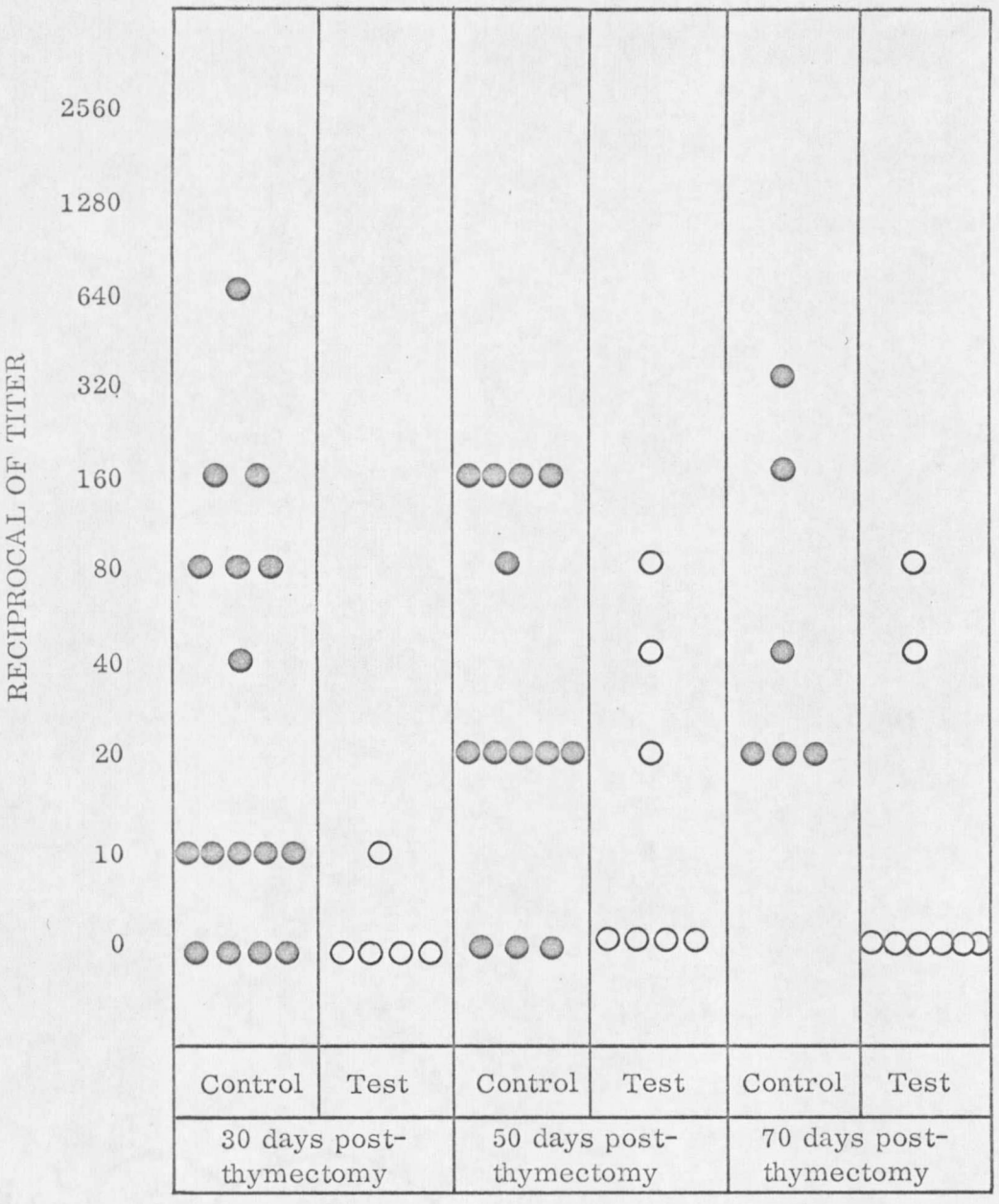
HEMAGGLUTINATION RESPONSE AT DAY FIVE



Test = thymectomized mice.
Control = sham-operated or partially thymectomized mice.
Each circle represents the titer of the serum from a single mouse.

Table V. Hemagglutination response to sheep erythrocytes in neonatally thymectomized Swiss Manor mice and in littermate controls. Serum was collected 10 days after injection.

HEMAGGLUTINATION RESPONSE AT DAY TEN



Test = thymectomized mice
Control = sham-operated or partially thyectomized mice.
Each circle represents the titer of the serum from a single mouse.

5 and 10 days after immunization and that little or no recovery had occurred at any of the three time intervals tested.

The Ability to Reject Homografts in Neonatally Thymectomized Mice

In view of the apparent recovery of GVH disease eliciting capacity on the part of spleen cells from 60 to 70 day old neonatally thymectomized Swiss Manor mice as opposed to their failure to recover responsiveness to sheep erythrocyte immunization, studies were undertaken to determine the homograft rejection capacities of such mice. Swiss Manor mice 30 days of age which had been thymectomized, sham-thymectomized or partially thymectomized at birth were grafted with A/jax tail skin. The normal homograft rejection time was found to be approximately 14 days and normal rejection times were seen in 4 out of 5 neonatally thymectomized mice (Table VI). The one animal which showed a prolonged rejection was undergoing post-thymectomy wasting and died with the graft intact.

Table VI. Skin homograft rejection in neonatally thymectomized Swiss Manor mice and in neonatally thymectomized or partially thymectomized littermate controls. Mice were 30 days old at the time of grafting.

Nature of Group	Number of Mice	Day of Rejection (Post-Graft)
Neonatally Thymectomized	5	10, 14, 14, 15, 23
Sham-Thymectomized or Partially Thymectomized	15	9, 12, 13, 14, 14, 14, 14, 14, 14, 14, 14, 14, 15, 15, 17

Attempts to Isolate Bacteria from the Spleens of Neonatally Thymectomized Mice

Large numbers of early deaths (before day 6 post-injection) were encountered during preliminary attempts to produce GVH disease in newborn A/jax mice with non-antibiotic treated spleen cells from neonatally thymectomized Swiss Manor mice. When cells from neonatally sham-thymectomized mice were used there was a paucity of early death. This led to the postulation of an infectious agent present in the spleens of neonatally thymectomized mice. Attempts were made to isolate bacteria from the spleens of 30 day old mice which had been thymectomized at birth. The spleens from 5 thymectomized and 5 sham-operated mice were cultured and of these, three of the spleen cell preparations from thymectomized mice and three from sham-thymectomized mice were freeze-thawed prior to culturing. None of the sham-thymectomized animals yielded positive cultures but Streptococcus faecalis var. zymogenes was isolated from the spleens of two thymectomized animals. It was noted that both of the mice from which bacteria were isolated were exhibiting symptoms of wasting whereas those exhibiting no symptoms yielded negative cultures.

Incidence and Description of Wasting Disease in Swiss Manor Mice Thymectomized at Birth

During the course of the experiments previously described numerous neonatally thymectomized Swiss Manor mice were observed to waste and die between the ages of 30 to 60 days. The symptoms observed were

general deterioration in appearance with hunched posture, unsteady gait, loss of weight, diarrhea and death within about 7 days from onset of symptoms. The observation of wasting was unexpected as other workers previously reported that outbred mice were very refractive to post-thymectomy wasting or that they did not waste (Parrott, 1962; Dukor, Dietrich and Rosenthal, 1966). It was, therefore, of interest to determine the incidence of post-thymectomy wasting encountered in the Swiss Manor mice maintained by our laboratory. The observations on 6 litters of mice revealed incidence of 33% mortality due to the wasting syndrome described above. The disease was found to occur between 30 and 60 days of age with a mean age at time of death of 51 days. In addition to the occurrence of post-thymectomy wasting 11% of the mice thymectomized at birth developed apparent bacterial infections prior to 30 days of age with large quantities of pus being found in their mediastinal cavities.

Miscellaneous Studies

A very recent report indicated that splenectomy of newborn C3H mice resulted in a high incidence of wasting. The investigators suggested that the spleen might be important in the ontogenetic development of the immune system (Kalpaktsoglou, Yunis and Good, 1967). If this were so and if the spleen were also important in the ontogeny of the immune system of Swiss Manor mice, then perhaps such a second "central" lymphoid organ would account for the recovery of the GVH disease inducing abilities

of spleens from neonatally thymectomized mice. Therefore, splenectomies were performed on newborn Swiss Manor mice and the mice were observed for the ensuing 90 days for symptoms of wasting. Of the 14 splenectomized mice used in the experiment two were observed to precipitously lose weight and die on the 18th day post-operation. The mice exhibited some of the typical signs of wasting such as a degeneration in general appearance and high-stepping gait but no diarrhea was observed and death was sudden. Three other splenectomized mice were observed to exhibit slight weight gains during the period from 17-20 days post-splenectomy, however, this was also true in the case of one of the 10 sham-splenectomized control mice.

DISCUSSION

Previous workers have reported the delayed recovery of various facets of the immune response in mice thymectomized at birth (Rogister and Lejeune, 1964; Rogister, 1965; Dukor, Dietrich and Rosenthal, 1966; Takeya and Nomoto, 1967; Sinclair and Millican, 1967). The work reported here can be interpreted as supporting their finding in that the GVH disease inducing capacities of spleen cells from neonatally thymectomized mice of the Swiss Manor strain appeared to recover considerably between 60 to 70 days post-thymectomy.

While the recovery of GVH disease eliciting ability was demonstrated, no increase in the ability to give more than minimal hemagglutinin and hemolysin responses was demonstrated during the 70 day experimental period. This was in conflict with the work of other investigators who have reported substantial recovery of the hemagglutinin response by 60 days post-thymectomy in A.SW mice (Rogister and Lejeune, 1964) and who have reported considerable recovery of the hemolysin response by 59 days post-thymectomy in outbred Swiss mice (Sinclair and Millican, 1967), by 45 days post-thymectomy in SL mice (Takeya and Nomota, 1967) and by 63 days in colony bred Swiss mice (Dukor, Dietrich and Rosenthal, 1966). The answer to the discrepancy may lie in a number of reasons. Possibly, a longer period of observation in these studies may have led to the detection of a recovery of hemolysin and hemagglutinin responses in Swiss Manor mice thymectomized at birth. This possibility is somewhat supported by the findings of Jutila and Hunter (1967) in Swiss Manor mice whose immune system had been suppressed with cortisol acetate treatment at birth.

They found that GVH disease eliciting abilities and homograft reactivity recovered first and that recovery of hemagglutinin and hemolysin responses occurred latter. Another explanation of the conflicting data may be the reported post-immunization delay in the response of thymectomized mice to immunization. Sinclair (1965) reported that neonatally thymectomized mice maximally responded with 19S antibody 10 days after immunization as opposed to maximal 19S antibody response at 5 days in normal mice. Recently Takeya and Nomota (1967b) indicating that the 7S response in neonatally thymectomized mice may behave somewhat similarly in that there is a delayed interval between the appearance of 19S and 7S antibody which becomes shorter with age. The 5 and 10 day post-immunization bleeding times used in this investigation may not have coincided with the delayed maximal 19S and 7S responses of the thymectomized Swiss Manor mice. Another possible explanation is that there is no recovery in the hemagglutinin or hemlysin responses of Swiss Manor mice thymectomized at birth.

The neonatally thymectomized mice used in this investigation rejected skin-homografts normally by 30 days of age. Other workers have reported substantial recovery of homograft rejection capacity in neonatally thymectomized mice by 45-60 days in A.SW mice. (Rogister, 1965) and by 60 days in SL mice (Takeya and Nomoto, 1967). The observation that homograft reactivity had recovered by 30 days in Swiss Manor mice or that it was never inhibited while the GVH disease eliciting capacity of spleen cells from mice of the same strain was inhibited up to 60 days

may be explained by the greater sensitivity of skin grafting as a test of immunological capacity.

The mechanism responsible for the recovery of the immune system following thymectomy of mice at birth remains undetermined. True self-reconstitution would indicate the presence of a second "central" lymphoid organ in the mouse somewhat analogous to the bursa of chickens or the appendix of rabbits. This organ in mice could not be exactly analogous to the bursa of chickens or appendix of rabbits as there does not seem to be any dissociation of immunological responsiveness in thymectomized mice as was found in thymectomized chickens (Warner, Szenberg and Burnet, 1962; Warner and Szenberg, 1964) and thymectomized rabbits (Archer, Sutherland and Good, 1963; Sutherland, Archer and Good, 1964). There is very little direct evidence for the existence of a second "central" lymphoid organ in the mouse although Weissman (1967) indicated that his experiments with total body x-irradiation and shielding of various lymphoid organs implicated the Peyer's patches as being possible secondary "central" lymphoid organs in mice. The work of Good's group wherein wasting was induced by neonatal extirpation of the spleen in C3H mice was interpreted by the group as indicating the spleen to be an organ important to the ontogeny of the immune system (Kalpaktosoglou, Yunis and Good, 1967). Work reported in this thesis indicated that neonatal splenectomy induced a small incidence of wasting in Swiss Manor mice. However, the early death of splenectomized mice as opposed to the delayed wasting of thymectomized mice indicated that the effects of splenectomy

might be due to removal of large numbers of competent cells during a critical period in the life of mice and not due to some "central" failure of immune ontogeny. Further studies are necessary to elucidate the pathogenesis of post-splenectomy wasting and to reveal the role of the spleen in the ontogeny of the immune system if, indeed, it has any role. Another explanation of the immunological recovery of neonatally thymectomized mice might be the delayed maturation and multiplication of cells formed by the thymus prior to its removal. Presumably the delay might be due, at least in part, to the lack of the long postulated thymus humoral factor. Undetected ectopic thymic tissue could account for the recovery of immunological competence in mice thymectomized at birth. The presence of such tissue in association with the thyroid glands has been reported by one group to occur with varying degrees of frequency depending on the mouse strain (Law et al., 1964). This possibility does not seem very likely as an explanation for the results presented in this thesis as mice having demonstrable thymic fragments of thymic tissue in their mediastinal cavities responded normally to antigenic stimulation at 30 days of age and one would expect that mice having ectopic thymic tissue would do the same. No such early recovery was detected on the part of mice not possesing thymus fragments. Still another explanation for the recovery of neonatally thymectomized mice might be the selective pressure caused by post-thymectomized infections and wasting. In the experiments reported herein wasting killed 33% of the neonatally thymectomized animals prior to 60 days and early infections killed an additional 10%. Such a death

rate may have selected for the hardier animals and given an apparent recovery in immunological capacity. However, Dukor, Dietrich and Rosenthal reported that the mice they used did not waste following neonatal thymectomy and that the mice did recover immunological competence. The incidence of early bacterial infections was not reported by these workers.

The role of bacteria in the pathogenesis of post-thymectomy wasting is fairly well established as the germ-free state protects mice from the effects of post-thymectomy wasting (McIntire, Sell and Miller, 1964). It has also been reported that the effects of thymectomy in depressing the immune system are not as severe in neonatally thymectomized mice in the germ-free state as they are in conventionally reared mice (Bealmer and Wilson, 1966; Miller et al., 1967). That the normal flora of the mouse may play a role in the pathogenesis of other forms of wasting disease is indicated by the work of Jutilá and Reed (1967) who isolated organisms forming part of the normal flora from the blood and organs of mice undergoing wasting induced by the neonatal injection of cortisol acetate. The work reported in this thesis indicated that the normal flora might play a role in post-thymectomy wasting as well since Streptococcus faecalis var. zymogenes was isolated from the spleens of two wasting animals. Thus it appears that studies on the immunological recovery of mice thymectomized at birth should be carried out in the germ free state to exclude the influence of pathogens or organisms of the normal flora on the mice.

If neonatally thymectomized mice do recover immunological competence the recovery must be transient as Taylor (1965) and Metcalf (1965) have reported a gradual decline in the immunological capacities of mice thymectomized during adult life.

SUMMARY

Mice of the Swiss Manor strain were thymectomized at birth and various facets of their immunological competence were tested during a 30 to 70 day interval post-thymectomy. The ability of the mice to reject skin homografts from A/jax donors was found to be normal at 30 days of age. The ability of spleen cells form the thymectomized mice to induce GVH disease in A/jax recipients was severely impaired at 30 to 50 days post thymectomy, but a substantial recovery of GVH eliciting activity was found to have occurred at 60 to 70 days post-thymectomy. Production of hemolysins and hemagglutinins in response to immunization with sheep erythrocytes was found to be depressed when the mice were tested at 30, 50 and 70 days post-thymectomy and no age dependent tendency towards normalization of the response was detected.

The mice suffered a high incidence of post-thymectomy wasting disease and Streptococcus faecalis was isolated from the spleens of wasting animals.

A miscellaneous study was carried out in which newborn Swiss Manor mice were splenectomized and observed for 90 days. Two out of fourteen of the splenectomized mice were observed to die of a wasting like syndrome.

It is concluded that the data tend to support the concept that (1) thymectomized mice recover some degree of immunological competence with increasing time following thymectomy, (2) bacteria may be associated with post-thymectomy wasting, and (3) the spleen may be important to the development or maintainence of the immunologic responsiveness of newborn

mice.

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