THE INFLUENCE OF CERTAIN BACTERIAL ANTIGENS ON THE MORPHOGENESIS OF MOUSE THYMUS AND PERIPHERAL LYMPHOID TISSUE

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Shirley Mae Selden

1966

ABSTRACT

THE INFLUENCE OF CERTAIN BACTERIAL ANTIGENS ON
THE MORPHOGENESIS OF MOUSE THYMUS AND
PERIPHERAL LYMPHOID TISSUE

by Shirley Mae Selden

Bacterins containing 10^{10} or 2×10^{10} organisms per milliliter were prepared from Staphylococcus aureus, Lactobacillus casei, and Lactobacillus acidophilus. Newborn mice were repeatedly given intraperitoneal injections of specific bacterins and littermate controls were given 0.85% NaCl solution. In one part of the study dams were given bacterin injections. Some newborn mice in following litters were given repeated injections of the same bacterin, saline solution, or sterile skim milk. The mice were observed for growth retardation, and the lymphoid tissues from test animals and controls were studied and compared.

Five runts were produced and a slight to moderate growth retardation occurred frequently. The normal development of the peripheral lymphoid tissues did not appear to be altered by the bacterin injections. Lesions produced in this experiment included thickened edematous splenic and renal capsules containing aggregations of lymphocytes and plasma cells. Foci of lymphocytes and/or plasma cells, increased amounts of connective tissue, and atrophy of acinar cells were present in the pancreas of many of the mice that were given bacterin injections.

THE INFLUENCE OF CERTAIN BACTERIAL ANTIGENS ON THE MORPHOGENESIS OF MOUSE THYMUS AND PERIPHERAL LYMPHOID TISSUE

By

Shirley Mae Selden

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Pathology

1966

ACKNOWLEDGEMENTS

The writer wishes to express sincere appreciation to Dr. D. A. Schmidt, major professor, for guidance, encouragement, and special assistance during the performance of the experiment and the preparation of this thesis. It is a pleasure to thank Dr. G. L. Waxler, Dr. S. D. Sleight, and Dr. C. C. Morrill for their constructive criticisms and guidance in writing this thesis.

The writer is grateful for the assistance of Mrs. M. J. Long, Mrs. B. Bradley, and Mr. R. A. Brooks, for helpful suggestions made by Dr. W. E. Giddens and Dr. M. A. Richardson, and for the processing of the large number of tissues by Mrs. M. Sunderlin and the other histology technicians.

The assistance of Mrs. M. Bennett, Dr. O. Kaufmann, Dr. G. Jaggard, and Dr. C. K. Whitehair in obtaining materials and equipment and the advice of Dr. J. Gill on the statistical analysis are appreciated.

The writer is grateful for the financial assistance of a Michigan State University Graduate Office Scholarship and expresses appreciation to those who made it available.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

In recent years investigators have introduced the concept of 2 lymphoid systems (Dalmasso et al., 1963; Sutherland et al., 1965). These are the central and the peripheral lymphoid tissues. The thymus is the prototype of the central lymphoid tissue, to which category the bursa of Fabricius of the chicken and the appendix of the rabbit also belong. The spleen and lymph nodes are the characteristic peripheral lymphoid tissues. They are dependent on central lymphoid tissue for the development of normal immunologic capacity in late embryonic and early postnatal life, but are more autonomous in the mature organism. Research (Cooper et al., 1966) also indicates that the thymus and bursa of Fabricius in the chicken are responsible for the development of 2 different morphologic and functional cell lines in the peripheral lymphoid system.

The immunologic mechanisms involving the thymus and peripheral lymphoid tissues are of particular interest because of the part they may play in autoimmunity and connective tissue diseases. Systemic lupus erythematosus is the human disease considered most typically an autoimmune disease (Dameshek et al., 1961; Waksman, 1962). The idea has been proposed that lactobacilli and a cross-reacting antibody may be involved in the cause of systemic lupus erythematosus (Stevens, 1964).

Growth retardation with reported peripheral lymphoid depletion has been produced in mice by injections of sterile bacterins. Ekstedt and Nishimura (1964) used bacterins prepared with a variant of the Smith strain of Staphylococcus aureus and a strain (D 24) of Group A, Type 30 Streptococcus.

In the work to be reported here newborn mice were repeatedly injected with bacterins prepared from Staphylococcus aureus, Lactobacillus casei, and Lactobacillus acidophilus. The mice were observed for growth retardation, and lymphoid tissues from test animals and littermate controls that were given saline solution injections were studied and compared at various ages. Lung, heart, liver, pancreas, adrenal, and kidney sections were also examined for changes which might indicate a reaction of the immunologic system. In additional studies dams were injected with a specific bacterin. Some newborn mice in following litters were injected with the same bacterin, saline solution, or sterile skim milk. Tissues from mice that were not given any injections were also examined.

The object of this study was to produce runting and lesions similar to those found in autoimmune processes. The lactobacilli were used because of the possible connection between lactobacilli and systemic lupus erythematosus in young women. The Staphylococcus aureus was used in an attempt to repeat the runting reported by Ekstedt and Nishimura (1964).

REVIEW OF LITERATURE

Early anatomists studied the structure of the thymus. In 1779, William Hewson told of the lymphocyte distributing function of the thymus and gave a preview of the concept of 2 different levels of functioning lymphocytes (Dameshek, 1963). Beard (1899) described the transformation of epithelial cells in the thymus into lymphocytes in the smooth skate (Raia batis) and stated that this was the first source of lymphocytes within the body. Good et al. (1964) attributed the background for much of the recent research on this subject to the writings of J. August Hammer.

Despite the fact that much early work was done on the thymus, students were taught for many years that it is a lymphoid organ with unknown function. In recent years, much has been learned about the role the thymus plays in immunobiology. Current research is concerned mainly with functions in relation to lymphopoiesis, leukemogenesis, and immunologic mechanisms (Smith, 1964). As the role of the central and peripheral lymphoid systems becomes better understood, a close and careful look at the cells that compose these tissues during their development and life history and the effects of different conditions on these cells becomes essential.

Development of the thymus

There are species differences in the development and structure of the thymus and peripheral lymphoid tissues. Mice are less mature immunologically at birth than are rabbits and dogs and are more ideal for the study of the

development of the thymus and other lymphoid tissues (Good et al., 1962). Much of the description here will be confined to the mouse.

In mammals, the thymus develops from the 3rd and 4th pharyngeal pouches in early embryonic life (Good et al., 1962). Embryonic thymus of the mouse becomes grossly visible at dissection 11 days after fertilization. At this time it is composed of vesicular epithelial cells surrounded by a capsule of mesenchyme (Auerbach, 1964). Twelve days after fertilization the lobes are separated from each other by a considerable distance and are predominantly epithelial (Auerbach, 1960). The mesenchymal layer is only 1 to 3 cells thick, and the total population of the thymus is about 4000 cells. Mesenchyme and epithelium are distinct prior to the 13th day; after this they become difficult to separate (Auerbach, 1964). The rudiment at this time does not appear lymphoidal and has not reached its definitive position (Auerbach, 1961).

During the next 4 days the lobes grow, become lymphoidal in nature, and migrate posteromedially (Auerbach, 1960, 1961). The total lymphoid cell population increases rapidly between the 16th and 18th days of embryonic life. This is followed by a period of stability and then a period of less rapid increase. A shift of cell types from large to small lymphocytes is seen in size-distribution studies of the thymus in the 3-day period before birth (Auerbach, 1964). Histologically the thymus of the newborn mouse shows development of the cortex and medulla, and is composed of small lymphocytes (thymocytes) and epithelial stromal cells (Good et al., 1962).

The experimental approach to the analysis of mammalian lymphoid development has become practical in recent years because of the development of precise procedures for cell separation and the improvement of techniques for transplantation and tissue culture (Auerbach, 1964). Some of the

recent work lends support to ideas that were put forth many years ago. In an experimental study of the origin of cell types in the thymus, Auerbach (1960) demonstrated that the epithelial cells of the thymus of the mouse embryo differentiate into lymphocytes. He has also demonstrated that this lymphopoiesis is dependent upon an interacting mesenchymal factor which will function across a 20-micron millipore filter barrier. These experiments were carried out using techniques of transplantation to the anterior chamber of isologous adult mouse eyes, chick chorioallantoic membrane, and tissue culture. By using a combination of mouse and chick thymic cells, which can be readily distinguished from each other, Auerbach (1964) has shown that mesenchyme provides an initial inductive stimulus to lymphocyte formation and the stromal elements of the developing structure, whereas the epithelium is the source of the lymphoid cells of the developing thymus.

In the mouse the thymus is regularly the only organ with true lymphocytes at the time of birth (Good et al., 1962). The growth of the thymus is most rapid during the first 14 postnatal days on the basis of relative weight, and during the first 35 days on the basis of absolute weight (Smith, 1964).

There are some species differences in the thymus. The thymus of the mouse is not divided into lobules as in most other species, but fibrous septa occur in thymuses grafted subcutaneously in mice. Thymic lobules in various species appear uniform in size, especially with regard to the width of the lymphoid cortex (Metcalf, 1964). In the guinea pig the thymus is located in the neck. In man it is predominantly intrathoracic but may extend upward into the lower parts of the neck. In the opossum, portions are located in both sites, and in hoofed animals there are separate cervical and thoracic thymuses (Arnason et al., 1962; Metcalf, 1964). In the mouse,

Hassall's corpuscles are usually comprised of only 3 or 4 cells and a central cavity is not conspicuous (Siegler, 1964). There are several opinions about the origin of Hassall's corpuscles (Hammar, 1921; Smith, 1964). The weight of the human thymus increases from birth to puberty (Hammar, 1921).

Involution of the thymus

At puberty the mass of the thymic parenchyma begins a gradual decrease. This process has been termed age involution or physiologic involution (Hammar, 1921; Fisher, 1964). Age involution in the mouse begins at approximately 6 weeks (Metcalf, 1964). A variety of infections, malnutrition, traumatic experiences, Roentgen-ray treatment, or pregnancy will cause another form of thymic involution referred to as accidental involution. Adrenocortical hormones cause a marked involution (Hammar, 1921; Fisher, 1964). Hammar (1921) states that the thymus is never found in the normal condition in subjects who have died of disease.

Age involution involves a reduction in the parenchyma of the organ and a replacement of interstitial connective tissue by adipose cells. Mitotic division of lymphocytes continues and the parenchyma remains as a system of cords gradually growing smaller with age. The occurrence of mitoses, the presence of Hassall's corpuscles and the power to react by accidental involution remain (Hammar, 1921). The thymus probably never completely disappears.

Throughout life the size and activity of the thymus are influenced by complex interacting endocrine factors. Testosterone and estrogen are thymolytic and thyroidectomy hastens involution. Hyperthyroidism is associated with enlargement of the thymus. After adrenalectomy and in Addison's disease the thymus becomes hyperplastic. In castrated animals the thymus remains large and involution is not marked (Arnason et al., 1962).

Development of the peripheral lymphoid system

The spleen in the newborn mouse lacks development of the red and white pulp and lymphocytes and plasma cells are virtually lacking (Good et al., 1962). From birth until 3 weeks, it is composed mainly of myelopoietic, erythropoietic, and reticular cells (Parrott et al., 1966). No lymphoid tissue is found in the intestine at birth. The peripheral lymphoid structure of mice develops gradually. Its development in the spleen, intestine and lymph nodes begins during the first week of life and continues for the next month (Good et al., 1962).

Although the thymus of the newborn mouse has the normal adult type of architecture, some extrinsic antigenic stimulus appears to be necessary for the normal development of other lymphoid organs. In germfree animals the lymph nodes remain rudimentary (Good et al., 1962).

Function of the central lymphoid system

Several hypotheses have been made regarding the function of the thymus. It is the producer of a large number of lymphocytes (Miller, 1961; Good et al., 1962; Metcalf, 1964; Sainte-Marie and Leblond, 1964).

It is likely that thymectomy in mice and rats interferes with the development of the peripheral lymphoid tissues beyond the stage of maturation reached at the time the surgical procedure is carried out (Dalmasso et al., 1963). The bursa of Fabricius in the chicken also plays a key role in the development of the immunologic system (Good et al., 1962). The function of the thymus in initiating immunogenesis is not necessarily restricted to early life. The thymus appears to be essential for complete recovery of immune mechanisms following sublethal irradiation in the adult mouse (Miller, 1962).

Burnet (1962), in an extension of the clonal theory of immunity, proposed that the thymus be regarded as the chief "first level" immunologic organ, and that the spleen, lymph nodes, bone marrow and local lymphoid cell aggregations be regarded as "second level" immunologic organs. He hypothesized that the thymus, and other central lymphoid tissues, were the sites of origin of cells concerned with the recognition of foreign chemical patterns, and that the thymus releases "uncommitted" lymphoid cells, which are distributed to second-level lymphoid organs. In the second-level sites, on appropriate stimulation, these cells give rise to descendants which are immunologically "committed".

Several investigators give support to the idea that lymphocytes from the thymus are distributed to peripheral lymphoid tissues (Good et al., 1962; Arnason et al., 1962; Parrott, 1966). Autoradiographic studies were made by injecting tritiated adenosine labeled cells from the thymus and spleen into neonatally thymectomized mice. Specific thymus-dependent areas in the spleen and lymph nodes were demonstrated. These areas in the spleen were located within the follicles immediately surrounding the central arterioles. The thymus-dependent areas were depleted of small lymphocytes, and large, pale-stained, nucleated reticular cells were more prominent. In the lymph nodes of these mice some lymphoid follicles developed in the outer cortex, but the mid and deep cortex, except for a narrow band adjoining the medulla, was depleted of lymphocytes. This work was interpreted to indicate that the thymus contributes cells directly to a "mobilizable pool" of lymphocytes, and that another system primarily responsible for the production of the plasma cell series probably exists (Parrott et al., 1966).

In the chicken the thymus-dependent system consists mainly of small lymphocytes and is responsible for delayed hypersensitivity and homograft rejection. The bursa-dependent system is composed of plasma cells and the larger lymphocytes seen in germinal centers. Functionally it is the immunoglobulin producing system. The thymus-dependent system appears to be the same in mammals and birds. Tonsils and other lymphoepithelial tissue of the gastrointestinal tract in mammals may serve the same functions as the bursa of Fabricius (Cooper et al., 1966).

Other workers, using lymphocytes labeled with tritiated thymidine and autoradiographic analysis of their migration in mice, concluded that the majority of thymic lymphocytes do not leave the thymus (Matsuyama et al., 1966). The functions of the production of a hormone or hormones which stimulate lymphopoiesis (Metcalf, 1956; Auerbach, 1964), and normal function of lymphoid tissue (Good et al., 1962) are frequently attributed to the thymus. Osoba (1965a, 1965b) has shown that neonatal thymic tissue enclosed in a millipore diffusion chamber, and humoral substances which have crossed the placental membranes, are capable of restoring the immunologic responses of a significant number of neonatally thymectomized mice to normal. Claims of anti-cancer properties, growth-promoting, and growth-retarding effects, not widely substantiated, have also been made (Osoba, 1965b).

Models used to study autoimmune processes

Animal diseases which produce conditions with similarities to human autoimmune diseases include Aleutian disease of mink (Leader, 1964) and the Coombs-positive, hemolytic anemia of New Zealand mice (Burnet and Holmes, 1964).

A deficiency of small lymphocytes throughout the body has been the most frequently reported finding in laboratory models used to produce conditions with similarities to autoimmune processes (Siskind and Thomas, 1959; Waksman et al., 1962; Ekstedt and Nishimura, 1964; Miller, 1965; Stastny et al., 1965). Other findings were not always in agreement.

"Runt disease" results from the injection of immunologically competent cells into newborn animals. Most workers have considered it to be an immunologic reaction of the grafted cells against a tolerant host (Siskind and Thomas, 1959; MacKay and Burnet, 1963). "Runt disease" may also be due to an increased adrenocortical hormone level resulting from a graft-host interaction (Ekstedt and Nishimura, 1964). Brooke (1964) reported isolating Salmonella typhimurium var. copenhagen from the spleens of runts. Active and passive immunization with this strain of S. typhimurium did protect against runting and salmonellosis, but treatment of the infected spleen cells with sound waves, heat, and x-irradiation did not reduce the incidence of runting.

The clinical signs reported for "runt disease" have included illness after an initial period, failure to grow at a normal rate, diarrhea, and death by 4 weeks of age. Coombs-positive anemia, splenomegaly, hepatomegaly with focal coagulative necrosis, and atrophic changes of other lymphoid tissues were sometimes noted in the runted mice (Siskind and Thomas, 1959). Other workers have reported leukopenia, abnormal plasma electrophoretic patterns, thrombocytopenia, negative lupus erythematosus cell preparations and alopecia. The mice reportedly became hunched, developed ruffled fur, and were hypothermic before they died (Oliner et al., 1961). Runted mice were also tolerant of skin grafts from the original cell donor's strain (Siskind and Thomas, 1959).

"Homologous disease" is produced in tolerant adult recipients by the administration of homologous lymphoid cells. The reported changes in adult rats involved the lymphoid and hematopoietic tissues, skin, joints, and heart. The lymph nodes were characterized by fewer and smaller follicles and some replacement with plasmacytoid cells and histiocytes. There was also an increased prominence of sinusoidal architecture. The thymus medulla sometimes had an increase in vacuolated, eosinophilic, reticular cells, and the cortex was involuted in rats dying between 24 and 27 days after 3 injections of homologous lymphoid cells. Loss of lymphocytes from the follicles and a relative increase in reticular cells was noted in the spleen. There was an associated increase in plasmacytoid cells and hemosiderin deposits, but a loss of megakaryocytes. Fibrosis occurred in more advanced cases. The skin lesions of "homologous disease" grossly and histologically resembled autografts undergoing rejection in these rats. A migratory and transient arthritis with a mononuclear reaction occurred. Histologic changes in the heart involved the valves, myocardium, and epicardium. These lesions were composed of focal edema, mononuclear inflammation, and serous atrophy of fat. Vasculitis and arteritis with fibrinoid change were seen occasionally (Stastny et al., 1963, 1965).

"Wasting disease" develops in some neonatally thymectomized mice, rats, and hamsters and is similar to "runt disease". This wasting syndrome does not occur in germfree animals and appears to depend upon the presence of a microbial flora (Cooper et al., 1966). Serum antibody response to some antigens is depressed following neonatal thymectomy and there is permanent impairment of the ability to reject skin from donors of foreign strains. Lymphoid cells from neonatally thymectomized mice appear to be less able to induce a graft-versus-host reaction in appropriate recipients

than similar cells from normal donors (Miller, 1965; Parrott and East, 1964; Sherman and Dameshek, 1964). The principal histologic finding in the lymphoid tissues of neonatally thymectomized animals is a depletion of small lymphocytes from their usual position in the spleen and lymph nodes (Waksman et al., 1962). The age at which thymectomy must be performed in order to cause this wasting syndrome varies with different species. Thymectomized or bursectomized chickens in which the peripheral lymphoid tissue has been destroyed by Roentgen-ray treatment provide other useful models (Cooper et al., 1966).

Ekstedt and Nishimura (1964) observed runting in neonatal mice while studying the immunologic reactions to species of staphylococci and streptococci. Repeated intraperitoneal injections of sterile suspensions of the Smith strain of S. aureus and a strain (D 24) of Group A, Type 30 Streptococcus were made. They reported that, in representative members of the treated groups, there was loss of delineation of cortex and medulla in the thymus and fewer lymphoid follicles in the spleen. The follicles present were poorly developed. Few cells resembling mature lymphocytes were present in the spleen. They were not able to identify lymph nodes, but found Peyer's patches in some of the runted mice. No abnormality in serum electrophoretic patterns was seen. Circulating antibody could not be detected in runted animals during the period of treatment with bacterins. Precipitating antibody could be detected in surviving animals 2 weeks after termination of the injections. Following termination of treatment the runted animals gained weight and appeared healthy. Germfree mice were more resistant to this runting than conventional animals. Runting was produced in neonatal germfree mice treated with a S. aureus bacterin to which a staphylococcal antiserum prepared in rabbits was added. These studies

suggested that small amounts of antibody might be required to complex with the injected antigen to initiate the reaction.

Stevens (1964) suggested that systemic lupus erythematosus and other autoimmune conditions might be caused by cross-reactions between antibodies to bacterial polysaccharides and similar chemical groupings of polysaccharides in body membranes. The antibodies found in systemic lupus erythematosus reacted with DNA of mammalian, fish, and bacterial origin. This disease is usually confined to women of child-bearing age, at which time large numbers of lactobacilli are present in the normal flora of the vagina. Most experimental models which have been considered similar to systemic lupus erythematosus do not show the elevation in gamma-globulin found in this condition, and it has been suggested that they are mediated by lymphoid cells rather than humoral antibody.

MATERIALS AND METHODS

Mice

Ten pairs of Swiss Webster mice were obtained from Spartan Research Animals, Incorporated, Haslett, Michigan. The mice were housed as monogamous pairs. The female was allowed to give birth in the presence of the male, and sometimes mating occurred at postpartum estrus. They were given water ad libitum and fed a standard mouse and rat ration.[1]

Newborn mice were cared for by the dam. Mice in excess of 9 to 11 per litter were killed to provide conditions favorable to uniform growth rates. The mice were sexed and moved to separate cages when they were 3 weeks old.

A total of 161 young mice was used in this study. Bacterin injections were given to 75, saline solution injections were given to 47, and milk injections were given to 3. The normal development of the lymphoid tissues at various ages was studied in 36 mice that were not given injections.

Individual mice in each litter were identified by the removal of different toes. During the early part of the experiment an attempt was made to identify the mice with various stains, but this method did not prove satisfactory. The dye could not be seen clearly on the hairless mice, and the mother often removed the stain.

---

[1]Purina Laboratory Chow, Ralston Purina Co., St. Louis, Mo.

Preparation of inoculums

A hemolytic strain of Staphylococcus aureus was obtained from Mrs. Marian Bennett, Veterinary Clinic, Michigan State University. A culture of Lactobacillus casei was obtained from Dr. Oliver Kaufmann, Department of Microbiology and Public Health, Michigan State University, and one of Lactobacillus acidophilus from the Dairy Laboratory, Philadelphia, Pennsylvania. The latter was a commercial preparation supplied in vials.[1] One vial was added to 1 quart of autoclaved skim milk and incubated at 37 C for 24 hours. Subcultures were then made in brain-heart infusion broth.[2] The lactobacilli cultures were maintained on B_{12} assay medium[2] and the S. aureus culture on nutrient agar.[3] These were maintained at 4 C and subcultured at biweekly intervals.

Bacteria used in preparing bacterins were propagated in brain-heart infusion broth at 37 C for 18 to 24 hours. The cultures were centrifuged and the organisms washed once in 0.85% NaCl solution. The washed bacteria were autoclaved at 115 C, 15 pounds pressure, for 15 to 20 minutes.

The bacteria were counted by the Wright method (Carpenter, 1965), and the stock suspensions diluted with sterile 0.85% NaCl solution to contain 10^{10} organisms per milliliter. Bacterins subsequently prepared were standardized by visual comparison of the turbidity with the previously standardized suspensions. The bacterins were prepared in sterile vacuum tubes prepared for blood collection.[4] In the latter part of the experiment the concentration

---

[1]Flav-o-lac Flakes, Dairy Laboratories, Philadelphia, Pa.

[2]Difco Laboratories, Detroit 1, Mich.

[3]Eugonagar, Baltimore Biological Laboratory, Baltimore, Md.

[4]Vacutainer, Becton, Dickinson and Co., Rutherford, N.J.

of the L. acidophilus bacterin was increased to 2 x 10^{10} organisms per milliliter by the removal, after sedimentation, of 1/2 of the total volume. Each tube of bacterin was stored at 4 C and tested for sterility at the time it was used.

Milk was reconstituted from a dehydrated preparation[1] according to the instructions on the package. The milk was distributed in small tubes and autoclaved at 115 C, 15 pounds pressure, for 15 to 20 minutes. Each tube of milk was stored at 4 C and tested for sterility at the time it was used.

Mouse inoculations and observations

Group I. Mice were injected intraperitoneally with a specific bacterin or 0.85% NaCl solution as soon after birth as possible and thereafter on alternate days. The number of mice in each litter and the amount and type of injection given are listed (TABLE 1). All mice given injections were weighed every other day for the first 3 weeks and at less frequent intervals after this. The mice were observed daily for normal activity and signs of growth retardation.

Group II. Three dams were each given 2 intraperitoneal injections, 4 or 5 days apart, of 0.5 ml. of bacterin. One was given the S. aureus bacterin, the 2nd the L. casei bacterin, and the 3rd the L. acidophilus bacterin. Subsequent litters born to these mice and the injections they received are indicated (TABLE 2). Observations made of these injected mice were the same as in the previous group.

---

[1]Carnation Instant Nonfat Dry Milk, Carnation Co., Los Angeles, Calif.

TABLE 1. Inoculation data for mice in Group I.

Litter number	Number of Mice*	Number of mice and inoculum: 10^{10} organisms /ml. bacterin**			2×10^{10} organisms /ml. bacterin***		
		S. aureus	L. casei	L. acidophilus	L. acidophilus	0.85% NaCl** solution	None
1	7	3	2	---	---	2	---
4	7	1	3	---	---	3	---
5	9	4	3	---	---	2	---
6	5	2	2	---	---	1	---
8	6	2	2	---	---	2	---
9	7	---	3	---	---	---	4#
10##	6	2	2	---	---	2	---
2-1	4	---	---	2	---	2	---
2-3	10	---	---	---	4	4	2
2-5	7	---	---	---	3	4	---
2-7	9	---	---	---	5	4	---
2-9	8	---	---	---	5	3	---
2-10	7	---	---	---	3	4	---
3-1	4	---	---	---	2	2	---
Total	96	14	17	2	22	35	6

*Does not include mice that died during first 3 days or those removed to limit size of litter.

**Intraperitoneal injections of 0.1 ml. amounts were given on alternate days.

***Mice were given 0.05 ml. until 1-2 weeks old and 0.1 ml. thereafter.

#Injections were given on the 1st day, but the stain was removed by the dam and identification was not recognizable.

##All mice in this litter were ill with an unidentified infection.

TABLE 2. Inoculation data for mice in Group II.

Inoculum given dam*		Litters born following inoculation of dam			Number of mice and inoculum**					
					10^{10} organisms /ml. bacterin		2×10^{10} organisms /ml. bacterin			
10^{10} organisms /ml. bacterin	Dates	Litter number	Date of birth	Number*** of mice	*S. aureus*	*L. casei*	*L. acidophilus*	0.85% NaCl solution	Skim milk	None
S. aureus	2/21/66 2/25/66	2-8	3/21/66	6	5	---	---	1	---	---
L. casei	2/13/66	2-2	2/22/66	7	---	4	---	3	---	---
	2/18/66	3-2	3/21/66	11	---	6	---	5	---	---
L. acidophilus	2/23/66	2-6	3/19/66	5	---	---	2	3	---	---
	2/28/66	3-6	4/15/66	8	---	---	3	---	3	2
Total				37	5	10	5	12	3	2

*Dams were given 0.5 ml. intraperitoneal injections.

**Mice in litter 2-6 were given 0.05 ml. intraperitoneal injections on alternate days until they were 2 weeks old and then 0.1 ml. on alternate days until killed. All others were given 0.1 ml. intraperitoneal injections on alternate days until killed.

***Does not include mice that died during first 3 days or those removed to limit size of litter.

Hematologic technique

Packed cell volumes, total leukocyte counts and differential leukocyte counts were performed on tail blood from a group of test mice and controls. Absolute lymphocyte values were determined using the total leukocyte count and the relative number of lymphocytes. Bone marrow imprints were stained with Wright's stain followed by Giemsa stain.

Histologic technique

Mice were killed with diethyl ether at various ages. The thymus, spleen, cervical and axillary lymph nodes, Peyer's patches, lung, heart, liver, pancreas, adrenals, and kidneys were fixed in 10% buffered formalin and saved for histologic examination.

Tissues were embedded in paraffin. The processing time for dehydration, clearing, and infiltration was shortened to 20 minutes in each solution in an attempt to prevent excessive hardening of the tissues. Sections were cut at 5 to 6 microns and stained with hematoxylin and eosin.

Imprints of thymus, spleen and axillary lymph nodes were made. These imprints were stained with a modified Shorr's stain and with Wright's stain followed by Giemsa stain. Imprints were air dried before staining by the Wright-Giemsa technique. Imprints for the Shorr's stain were fixed while wet and stored in an alcohol-ether solution until stained. The modification of Shorr's stain used in this experiment may be found in the appendix.

The spleen, Peyer's patches, and lymph nodes were examined for depletion of small lymphocytes and the presence or absence of germinal centers. The point sampling method was adapted to count the number of small

lymphocytes and larger cells in the area immediately surrounding the central arterioles in the splenic corpuscles (Erănkő, 1955; Weibel, 1963). The cells were counted in those splenic corpuscles in which the central arteriole seen in the section was near the center of the follicle. A grid with 64 intersections was placed in the eyepiece of the microscope. The central arteriole was centered and the number of intersection points falling on various cell types was recorded (Figure 1). The oil immersion objective was used. Intersection points were counted as falling on small lymphocytes, reticular cells, granulocytes, and interstitial spaces. Large lymphocytes, endothelial cells, and early granulocytes were not differentiated and were counted as reticular cells in this examination.

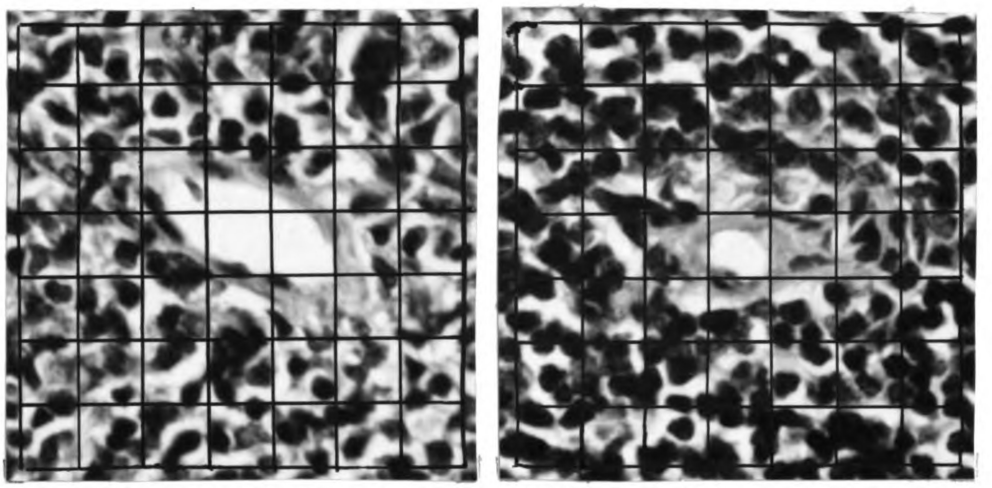

Figure 1. Grid superimposed over splenic corpuscles illustrating method used for point sampling counts. Test mouse that was given L. casei bacterin injections (left) and control that was given saline solution injections (right). Hematoxylin and eosin. 750X.

S	S	R	R	S	R	R	L
L	L	S	R	S	R	R	L
R	R	R	R	S	L	L	R
L	R	S	S	S	R	R	R
R	S	S	R	R	R	R	R
R	R	S	R	S	R	L	L
S	R	R	S	S	S	S	R
R	S	R	S	R	S	L	L

S	R	L	R	S	L	L	L
S	L	S	L	R	R	R	S
R	S	R	S	R	R	L	S
L	R	L	R	R	S	L	S
L	L	R	R	R	R	L	S
L	L	L	R	S	S	L	R
L	L	L	L	S	R	R	L
S	L	L	L	L	L	L	S

Intersection points counted as falling on lymphocytes (L), reticular cells (R), interstitial spaces (S) or granulocytes (G).

RESULTS

Growth retardation

The number of mice in which there was no growth retardation, slight to moderate growth retardation, and marked retardation are listed (TABLES 3 and 4). Those having slight to moderate growth retardation generally gained steadily each day but less than their littermate controls. Mice with marked growth retardation gained at a much slower rate and will be referred to as runts.

A runt that was given the L. acidophilus bacterin and a littermate control given saline solution injections are shown (Figure 2). This photograph was taken on the 12th day of age. At this time the runt weighed 2.49 Gm. and the littermate control 7.50 Gm. The runt appeared very active and the dam was observed nursing this mouse several times. It did not assume the hunched position described by other workers (Oliner et al., 1961) as one of the characteristics of "runt disease". This mouse and the littermate control were killed on the 21st day of age. The runt gained weight steadily for the first 17 days but lost 0.38 Gm. in the 4 days before it was killed.

Another runt that died at the age of 10 days is shown (Figure 3). This runt had also been given the L. acidophilus bacterin and the littermate control pictured with it, saline solution. This runt had made no significant weight gain since birth and remained hairless.

Five runts were produced in this experiment. Three runts occurred in Group I; 1 was given the L. casei bacterin and 2 the L. acidophilus bacterin. Two runts occurred in Group II; 1 was given the L. casei bacterin and the

TABLE 3. Summary of histologic findings and observations on growth rate in mice of Group I.

Inoculum	Total number of mice	Growth retardation: Marked	Growth retardation: Slight to moderate	Growth retardation: None	Thymus: Loss of delineation between cortex and medulla	Spleen: Cells*	Spleen: Thickened, edematous capsule	Lymph nodes: Fibroblasts	Lymph nodes: Plasma cells	Lymph nodes: Germinal centers present	Intestine: Cross section of parasite	Intestine: Germinal centers present	Intestine: Peyer's patches present	Lung: Cells**	Lung: Congestion	Lung: Emphysema	Lung: Atelectasis	Heart: Endocarditis	Liver: Other lesions***	Liver: Hematopoieses present	Liver: Myelopoietic cells around vessels	Pancreas: Cells#	Pancreas: Inclusion-like bodies	Pancreas: Atrophy of acinar cells	Pancreas: Increased C.T.	Kidney: Thickened, edematous capsule
Number of Mice																										
10^{10} organisms/ml. bacterin																										
S. aureus	14	0	5	9	0	3	5	1	0	5	3	8	11	4	7	1	3	0	3	2	5	6	5	4	3	1
L. casei	17	1	12	4	3	4	6	0	0	2	1	9	10	3	11	2	9	1	5	2	4	4	12	1	10	1
L. acidophilus	2	1	0	1	0	1	2	0	1	1	1	1	1	0	1	0	1	0	1	2	0	2	1	1	1	1
2 x 10^{10} organisms/ml. bacterin																										
L. acidophilus	22	1	15	6	0	14	22	5	3	12	8	14	19	10	17	2	6	0	14	1	5	21	8	13	21	11
0.85% NaCl solution	35	0	5	30	4	0	1	9	2	22	13	29	33	2	20	1	5	0	7	3	3	1	18	0	0	0
None	6	0	0	6	0	0	0	1	0	2	3	5	5	0	3	1	2	1	0	2	0	0	2	0	2	0

*Predominantly lymphocytes and plasma cells in thickened capsule.

**Usually focal areas with lymphocytes, occasionally diffuse scattering of neutrophils.

***Predominantly granulomatous reactions or surface lesions with increased amount of connective tissue with lymphocytes and plasma cells.

#Predominantly focal areas with lymphocytes and plasma cells, occasionally few eosinophils and neutrophils.

TABLE 4. Summary of histologic findings and observations on growth rate in mice of Group II.

Inoculum	Number of Mice																									
		Growth retardation			Thymus	Spleen		Lymph nodes			Intestine			Lung				Heart	Liver			Pancreas				Kidney
	Total number of mice	Marked	Slight to moderate	None	Loss of delineation between cortex and medulla	Cells*	Thickened, edematous capsule	Fibroblasts	Plasma cells	Germinal centers present	Cross section of parasite	Germinal centers present	Peyer's patches present	Cells**	Congestion	Emphysema	Atelectasis	Endocarditis	Other lesions***	Hematopoieses present	Myelopoietic cells around vessels	Cells#	Inclusion-like bodies	Atrophy of acinar cells	Increased C. T.	Thickened, edematous capsule
Dam previously inoculated with *L. casei* bacterin																										
L. casei 10^{10} organisms/ ml. bacterin	10	1	7	2	2	5	8	3	0	7	1	7	8	3	8	1	6	0	4	2	1	8	4	6	8	3
0.85% NaCl solution	8	0	1	7	1	0	0	2	0	5	1	5	7	0	5	1	3	0	1	0	1	1	4	0	0	0
Dam previously inoculated with *S. aureus* bacterin																										
S. aureus 10^{10} organisms/ ml. bacterin	5	1	0	4	0	0	3	0	0	2	2	2	5	1	0	0	0	0	4	0	0	3	2	0	4	0
0.85% NaCl solution	1	0	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0

TABLE 4--continued

Inoculum	Number of Mice																																							
	Total number of mice	Growth retardation			Thymus	Spleen		Lymph nodes			Intestine			Lung				Heart	Liver			Pancreas				Kidney	
		Marked	Slight to moderate	None	Loss of delineation between cortex and medulla	Cells*	Thickened, edematous capsule	Fibroblasts	Plasma cells	Germinal centers present	Cross section of parasite	Germinal centers present	Peyer's patches present	Cells**	Congestion	Emphysema	Atelectasis	Endocarditis	Other lesions***	Hematopoieses present	Myelopoietic cells around vessels	Cells#	Inclusion-like bodies	Atrophy of acinar cells	Increased C. T.	Thickened, edematous capsule
Dam previously inoculated with L. acidophilus bacterin																																							
L. acidophilus 2×10^{10} organisms/ml. bacterin	5	0	3	2	0	3	4	1	1	1	2	4	5	1	5	0	2	1	3	0	2	5	2	3	5	4
0.85% NaCl solution	3	0	0	3	0	0	0	0	2	1	1	2	3	0	2	0	0	0	0	1	0	0	0	0	0	0
Skim milk	3	0	0	3	0	3	2	1	0	3	1	3	3	0	3	1	1	0	0	0	1	3	3	0	3	0
None	2	0	0	2	0	0	0	0	0	1	0	2	2	0	1	1	1	0	0	0	0	2	2	0	0	0

*Predominantly lymphocytes and plasma cells in thickened capsule.

**Usually focal areas with lymphocytes, occasionally diffuse scattering of neutrophils.

***Predominantly granulomatous reactions or surface lesions with increased amount of connective tissue with lymphocytes and plasma cells.

#Predominantly focal areas with lymphocytes and plasma cells, occasionally few eosinophils and neutrophils.

Figure 2. A 12-day-old runt that was given L. acidophilus bacterin injections (bottom) and littermate control. Notice lack of hair development and difference in size.

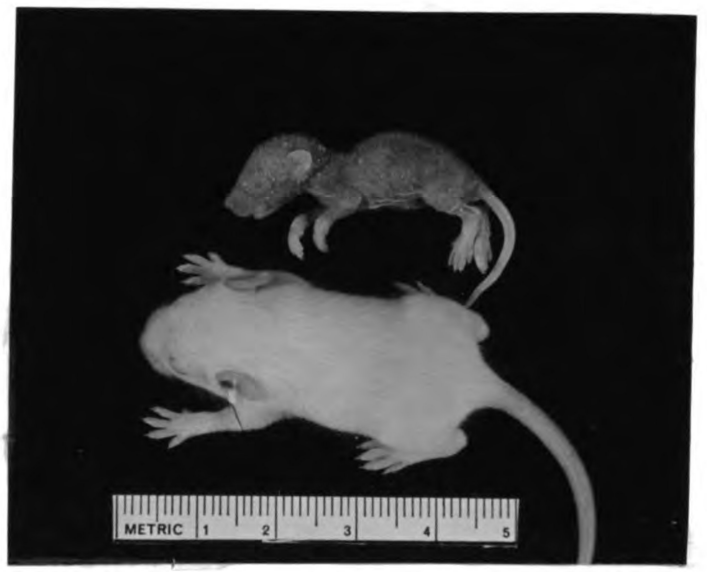

Figure 3. A mouse that was given L. acidophilus bacterin injections (top) and littermate control. The former died at the age of 10 days, remained hairless, with no significant weight gain.

other the S. aureus bacterin. Mice with growth retardation during the first 4 weeks later gained at a rapid rate and became normal in appearance, although the bacterin injections were continued.

During the experiment several mice became ill between the ages of 2 and 4 weeks. The test mice were affected more often than the controls, but both mice injected with saline and those without injections became sick and some died. The sick mice frequently assumed a hunched position and lost weight. Some had signs of diarrhea. Bacteriologic cultures and gross and microscopic examinations of tissues did not reveal the cause of the illness. Because there was weight loss and periods of no weight gain with this illness, mice that were affected are included with those showing slight to moderate growth retardation (TABLES 3 and 4).

Hematologic findings

The mean packed cell volumes, total leukocyte counts and absolute lymphocyte counts, and the ranges obtained for each, in mice that were given specific injections are listed (TABLES 5 and 6). The number of mice on which the blood tests were performed and their ages are also given in the tables. Lymphopenia was not found, and anemia was indicated in only 2 mice. No abnormalities were seen on examination of the bone marrow imprints.

Gross and histologic findings

The number of mice with different histologic findings for each type of injection are also listed (TABLES 3 and 4). The histologic findings in each organ will be discussed separately.

Thymus. No significant gross findings were present in the thymuses of mice killed if growth retardation was the only sign of ill health. In mice that died as the result of an unidentified infection, the thymuses were usually smaller than normal. The normal delineation between cortex

TABLE 5. Hematologic findings in mice of Group I.

Number of mice	Age (wk.)	Inoculum	Packed cell volume (%) Mean	Packed cell volume (%) Range	Leukocyte count (10^3/cu. mm.) Mean	Leukocyte count (10^3/cu. mm.) Range	Lymphocyte count (10^3/cu. mm.) Mean	Lymphocyte count (10^3/cu. mm.) Range
1	3	L. acidophilus*	---	---	9.00	---	6.20	---
1	3	0.85% NaCl	---	---	7.55	---	6.50	---
3	5	L. acidophilus**	37.0	33-42	12.70	10.00-14.20	9.80	6.50-11.60
8	5	L. casei*	44.7	40-49	11.25	9.45-15.65	7.10	3.70-12.20
2	5	S. aureus*	46.0	45-47	8.60	---	7.40	---
11	5	0.85% NaCl	44.9	38-52	8.50	5.00-12.85	5.85	2.45- 8.75
1	6	L. acidophilus**	45.0	---	11.90	---	8.55	---
4	6	S. aureus	44.4	41-48	15.65	8.65-20.40	11.00	5.80-13.70
4	6	0.85% NaCl	47.2	43-49	15.30	11.80-20.90	12.40	8.75-16.70
1	6	None	50.0	---	14.50	---	12.20	---
2	7	L. casei*	43.5	38-49	7.25	7.15- 7.30	4.20	---
3	7	S. aureus*	46.0	43-49	13.95	13.65-14.50	7.55	6.15-10.00
2	7	0.85% NaCl	46.5	46-47	7.70	6.95- 8.40	6.15	5.50- 6.80
5	8	L. acidophilus*	40.8	37-48	14.15	12.50-15.85	10.80	7.90-13.65
6	8	0.85% NaCl	45.3	39-48	16.40	11.90-20.35	14.00	8.35-17.10
5	10	L. acidophilus*	44.6	42-48	16.10	13.50-18.40	13.25	11.30-15.65
3	10	0.85% NaCl	47.3	46-48	13.75	12.00-14.45	10.65	10.40-10.90
1	12	S. aureus*	48.0	---	14.55	---	12.50	---
1	12	0.85% NaCl	50.0	---	18.15	---	17.05	---

*Bacterin containing 10^{10} organisms/ml.

**Bacterin containing 2 x 10^{10} organisms/ml.

TABLE 6. Hematologic findings in mice of Group II.

Number of mice	Age (wk.)	Inoculum	Packed cell volume (%) Mean	Packed cell volume (%) Range	Leukocyte count (10^3/cu. mm.) Mean	Leukocyte count (10^3/cu. mm.) Range	Lymphocyte count (10^3/cu. mm.) Mean	Lymphocyte count (10^3/cu. mm.) Range
Dam previously inoculated with *L. casei* bacterin								
2	3	*L. casei**	40.0	39-41	3.25	2.50- 4.00	1.75	1.35- 2.10
2	3	0.85% NaCl	40.5	39-42	4.40	3.80- 5.00	2.60	2.30- 2.85
3	9	*L. casei**	43.6	39-47	17.95	13.10-21.80	15.35	11.65-18.95
2	9	0.85% NaCl	48.5	48-49	14.30	13.10-15.50	12.40	10.75-13.65
3	13	*L. casei**	49.0	48-50	13.75	12.20-16.10	11.10	9.75-13.05
2	13	0.85% NaCl	50.0	50-50	11.20	9.80-12.60	9.05	8.15- 9.95
Dam previously inoculated with *S. aureus* bacterin								
5	9	*S. aureus**	48.8	48-50	13.15	9.80-16.80	9.45	7.95-10.40
1	9	0.85% NaCl	49.0	---	13.20	---	10.95	---
Dam previously inoculated with *L. acidophilus* bacterin								
1	3	*L. acidophilus***	28.0	---	7.70	---	6.00	---
2	3	Skim milk	35.5	35-36	12.35	8.70-16.00	10.00	7.15-12.80
1	3	None	35.0	---	7.20	---	6.00	---
1	5	*L. acidophilus***	35.0	---	9.50	---	5.80	---
1	5	Skim milk	35.0	---	12.60	---	10.35	---
1	6	*L. acidophilus***	38.0	---	9.65	---	6.55	---
1	6	None	42.0	---	13.30	---	11.20	---
2	10	*L. acidophilus***	46.5	45-48	17.25	15.60-18.90	12.15	11.35-12.95
2	10	0.85% NaCl	48.0	47-49	10.85	7.80-13.90	8.60	6.80-10.40

*Bacterin containing 10^{10} organisms/ml.

**Bacterin containing 2×10^{10} organisms/ml.

and medulla, present at the time of birth and thereafter, was not seen in 9 test mice and in 1 saline control (Figure 4). All the mice with this loss of normal architecture had signs of illness several days before death or before being killed. The thymus of the runt (Figure 2) killed when growth retardation was the only visible effect of the bacterin injections retained the normal delineation between cortex and medulla (Figure 5), as did all other mice with growth retardation that were killed before other signs of illness were seen. Each normal appearing thymus was composed of a cortex with tightly packed small lymphocytes and a medulla composed of epithelial and stromal cells, few lymphocytes, and small Hassall's corpuscles. Cilia-lined cysts, sometimes seen in the thymus of the mouse (Smith, 1964), were present in 2 mice. A few adipose cells were sometimes seen in sections from mice 12 weeks old, but involution was not marked in any of the thymuses examined.

Cellular detail was better in imprints than in sections. The cells seen in the imprints were mainly small and medium size lymphocytes (Figure 6). No differences between the thymic cells of control and test mice were seen.

Spleen. The spleen in the newborn mouse was composed mainly of erythropoietic and myelopoietic cells (Figure 7) with a connective tissue capsule. Splenic corpuscles, composed of pale-staining reticulum cells, were first seen in a normal mouse at the age of 6 days. One section from a normal 10-day-old mouse contained small darkly-stained lymphocytes in the splenic corpuscles.

Flat, grayish-white areas on the capsule of the spleen were seen by gross examination in 14 test animals. Thickening of the capsule and edema,

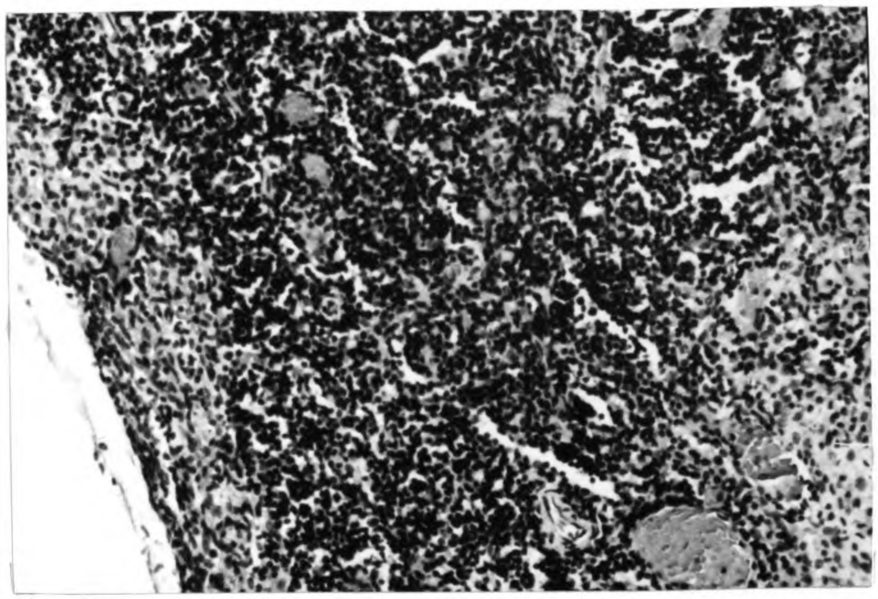

Figure 4. Thymus from 3½-week-old mouse that was given L. casei bacterin injections but died of an unrelated infection. Loss of normal delineation between cortex and medulla with congestion. Hematoxylin and eosin. 188X.

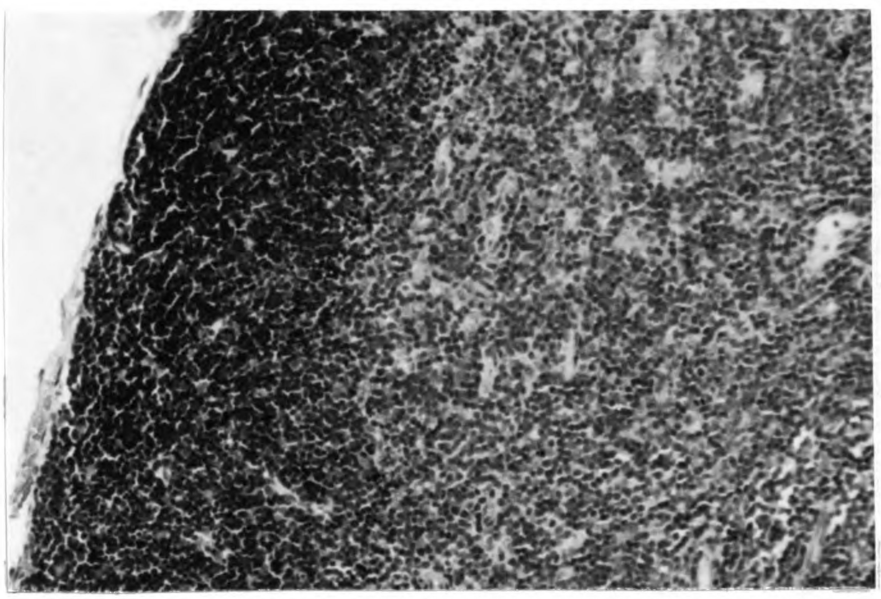

Figure 5. Thymus from runt seen in Figure 2, that was killed at the age of 3 weeks while growth retardation was the only sign of illness. Normal delineation between cortex and medulla. Hematoxylin and eosin. 188X.

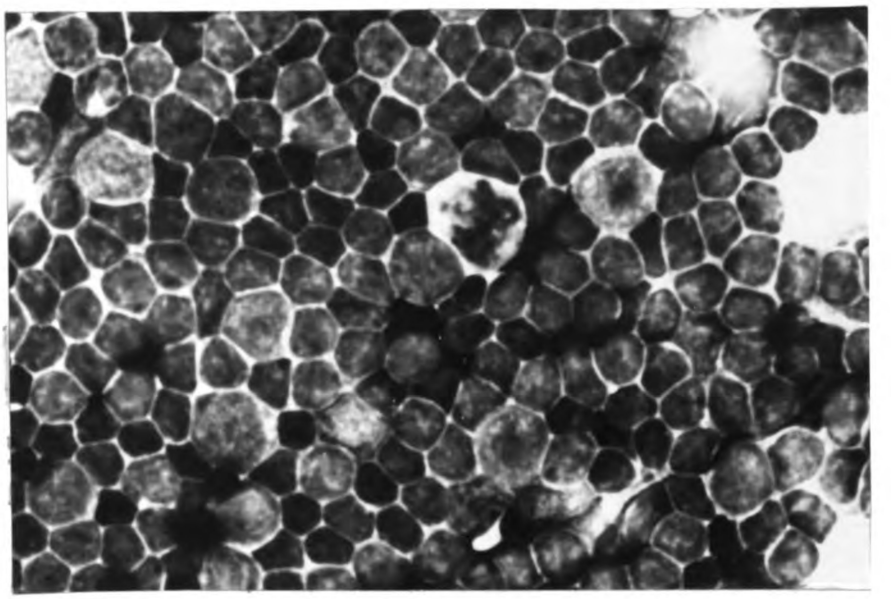

Figure 6. Imprint of thymus from normal mouse on day of birth. Predominant cells are small and medium size lymphocytes. Wright-Giemsa. 750X.

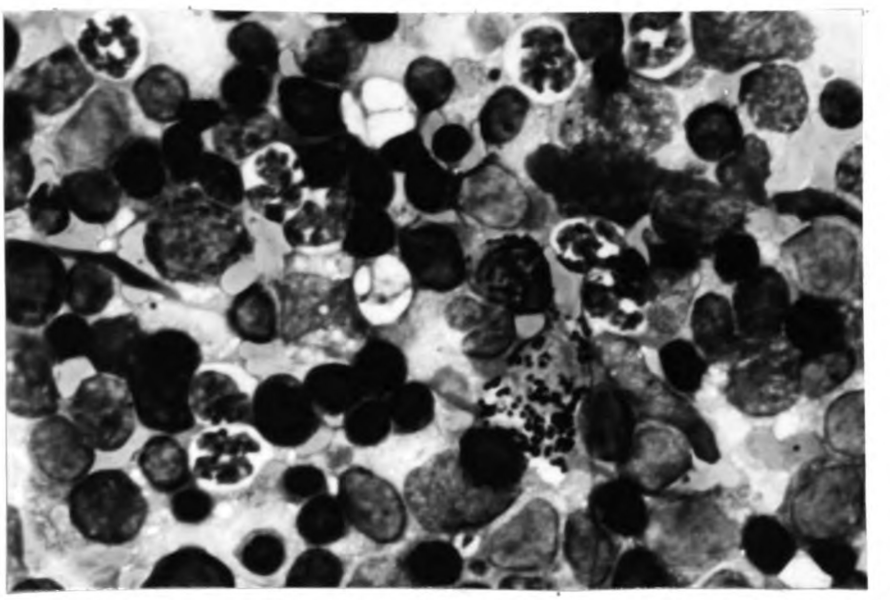

Figure 7. Imprint of spleen from normal mouse on day of birth. Predominant cells are erythropoietic and myelopoietic. Wright-Giemsa. 750X.

noted microscopically, were present in the spleens of 50 test animals (35 out of 55 in Group I and 15 out of 27 in Group II). Many of the thickened capsules contained lymphocytes and plasma cells. Aggregations of lymphocytes were sometimes found surrounding blood vessels (Figure 8). Neutrophils, eosinophils, and macrophages were also present in some instances. This lesion was more frequent in the mice injected with the L. acidophilus bacterin than in those given other injections.

The mean numbers of lymphocytes, interstitial spaces, and reticular cells obtained from splenic corpuscles using the point sampling technique in mice of Groups I and II are listed (TABLES 7 and 8). The number of lymphocytes counted was lower in the test mice than in saline controls, and the number of reticular cells was higher.

Dunnett's t-test (Dunnett, 1955) was used to test the significance of the mean numbers of lymphocytes in the mice that were given the various bacterins in Group I as compared to controls that were given saline solution. The results of this analysis are given (TABLE 9). The decreased number of lymphocytes was significant at the 1% level in mice that were given the L. acidophilus bacterins.

An F test for non-orthogonal comparisons (Ostle, 1963) was used to test the significance of the mean numbers of lymphocytes obtained from splenic corpuscles in Group II. The results of this analysis are listed (TABLE 10). The decreased number of lymphocytes was significant at the 1% level in mice that were given the S. aureus bacterin versus the saline controls, when the dam was given previous injections of the S. aureus bacterin. The decreased number of lymphocytes was significant at the 5% level in mice that were given the L. acidophilus bacterin versus the saline controls, when the dam was given previous injections of the L. acidophilus

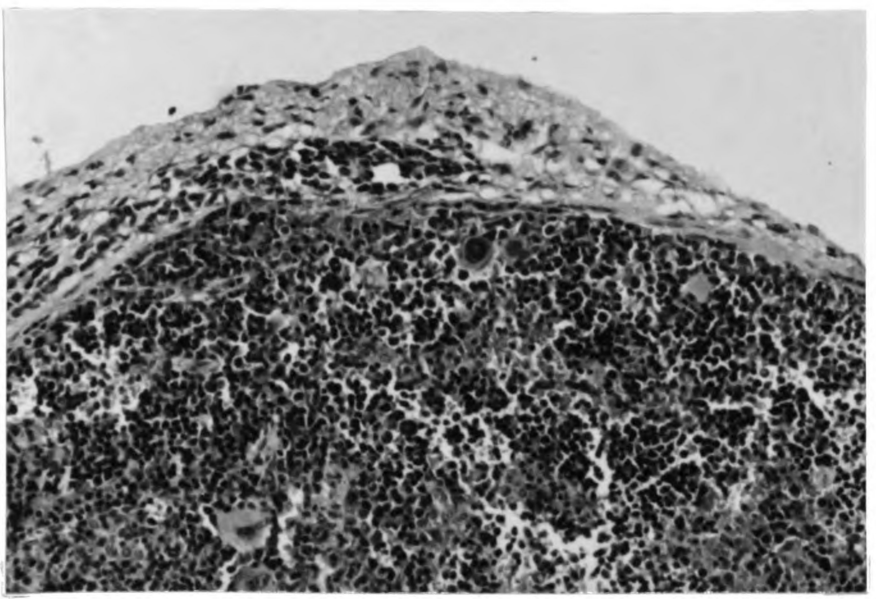

Figure 8. Spleen from 9-week-old mouse that was given L. acidophilus bacterin injections. Notice thickened edematous capsule containing lymphocytes and plasma cells surrounding blood vessel. Hematoxylin and eosin. 188X.

TABLE 7. Mean values obtained by point sampling counts of splenic corpuscles in mice of Group I.

	0.85% NaCl solution	Inoculum 10^{10} organisms /ml. bacterins			$2x10^{10}$ organisms /ml. bacterin
		S. aureus	L. casei	L. acidophilus	L. acidophilus
Number of mice	33	12	17	2	21
Mean number of lymphocytes	21.8	17.2	19.1	8.5	16.1
Mean number of interstitial spaces	16.7	14.7	14.0	10.0	15.8
Mean number of reticular cells	24.9	31.8	30.2	44.5	32.3

TABLE 8. Mean values obtained by point sampling counts of splenic corpuscles in mice of Group II.

	Dam inoculated with *S. aureus* bacterin		Dam inoculated with *L. casei* bacterin		Dam inoculated with *L. acidophilus* bacterin			
	Inoculum		Inoculum		Inoculum			
	Saline	10^{10} organisms/ml. bacterin *S. aureus*	Saline	10^{10} organisms/ml. bacterin *L. casei*	0.85% NaCl	Milk	None	2×10^{10} organisms/ml. bacterin *L. acidophilus*
Number of mice	1	5	8	9	3	3	2	5
Mean number of lymphocytes	31.0	16.0	24.7	23.3	29.3	22.3	18.0	20.8
Mean number of interstitial spaces	18.0	15.6	18.0	13.5	17.6	11.3	14.5	17.6
Mean number of reticular cells	15.0	32.2	20.8	23.4	16.7	30.0	31.5	25.4

TABLE 9. Results of Dunnett's t-test comparing mean numbers of lymphocytes in splenic corpuscles in mice of Group I.

Inoculum	t
Saline versus 10^{10} organisms/ml. *L. acidophilus* bacterin	3.13*
Saline versus 2×10^{10} organisms/ml. *L. acidophilus* bacterin	3.80*
Saline versus 10^{10} organisms/ml. *L. casei* bacterin	1.69
Saline versus 10^{10} organisms/ml. *S. aureus* bacterin	2.16

* Significant at the 1% level.

TABLE 10. Results of F test comparing mean numbers of lymphocytes in splenic corpuscles in mice of Group II.

Bacterin dam received / Inoculum test mice received	F
S. aureus / saline versus *S. aureus* / *S. aureus*	8.52*
L. casei / saline versus *L. casei* / *L. casei*	0.38
L. acidophilus / saline versus *L. acidophilus* / *L. acidophilus*	6.20**
S. aureus / *S. aureus* versus *L. casei* / *L. casei* + *L. acidophilus* / *L. acidophilus*	6.92**
L. casei / *L. casei* versus *L. acidophilus* / *L. acidophilus*	0.94
L. acidophilus / saline versus *L. acidophilus* / milk + *L. acidophilus* / *L. acidophilus*	1.42
L. acidophilus / milk versus *L. acidophilus* / *L. acidophilus*	0.20

*Significant at the 1% level.

**Significant at the 5% level.

bacterin; and in mice that were given the S. aureus bacterin versus the total of those given the L. casei and L. acidophilus bacterins, when the dams of each group were given the corresponding bacterins.

Spleen imprints of control mice given saline solution contained areas composed primarily of lymphocytes (Figure 9), whereas some imprints from the mice injected with the different bacterins contained few or none of these areas. Erythropoietic and myelopoietic cells were the predominant types present in the spleen imprints of these test mice (Figure 10).

Lymph nodes. Cervical and axillary lymph nodes could usually be found by gross examination in mice 3 weeks of age or older. The youngest age at which a cervical lymph node was found was 6 days. Enlarged edematous lymph nodes were occasionally present. The presence of germinal centers was variable. Germinal centers were observed in 63% of the control mice in Group I and in 58% of the controls in Group II. They were seen in 35% and 50% of the test mice in Groups I and II, respectively. Plasma cells were prominent in only a few, and areas with many fibroblasts were found in some lymph nodes of both test and control animals. No abnormalities were seen in lymph node imprints.

Intestine. An aggregation of large, pale-staining mononuclear cells was present in the lamina propria of 1 mouse on the day of birth (Figure 11). This mouse had not been given any injections. Follicles containing some reticulum cells and some lymphocytes were seen in the intestine of a 3-day-old mouse. Lymphoid nodules were regularly observed in normal mice 10 days of age or older. Peyer's patches were usually visible at gross examination of mice 3 weeks of age or older and were found in both test and control animals. The numbers of test and control mice in which

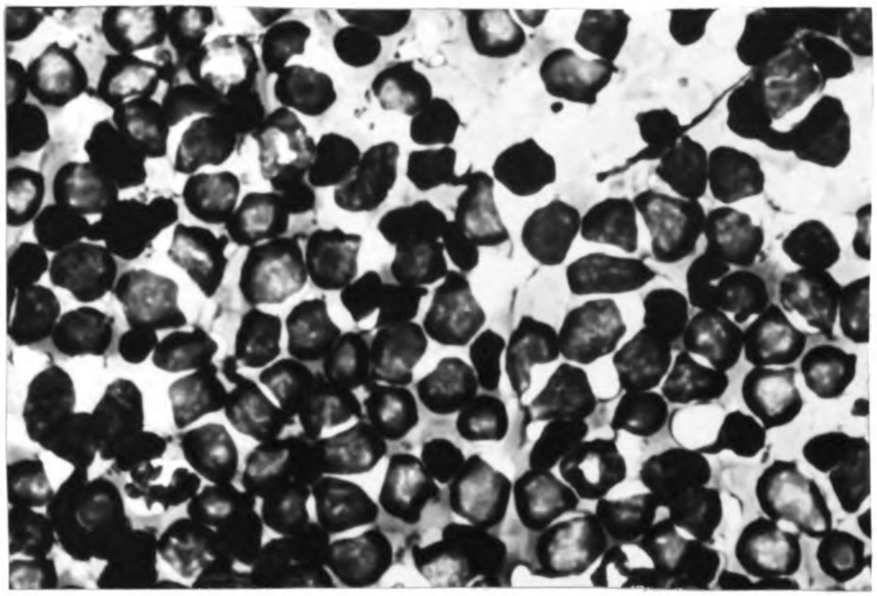

Figure 9. Imprint of spleen from 9-week-old control mouse that was given saline solution injections. Area composed predominantly of lymphocytes. Wright-Giemsa. 750X.

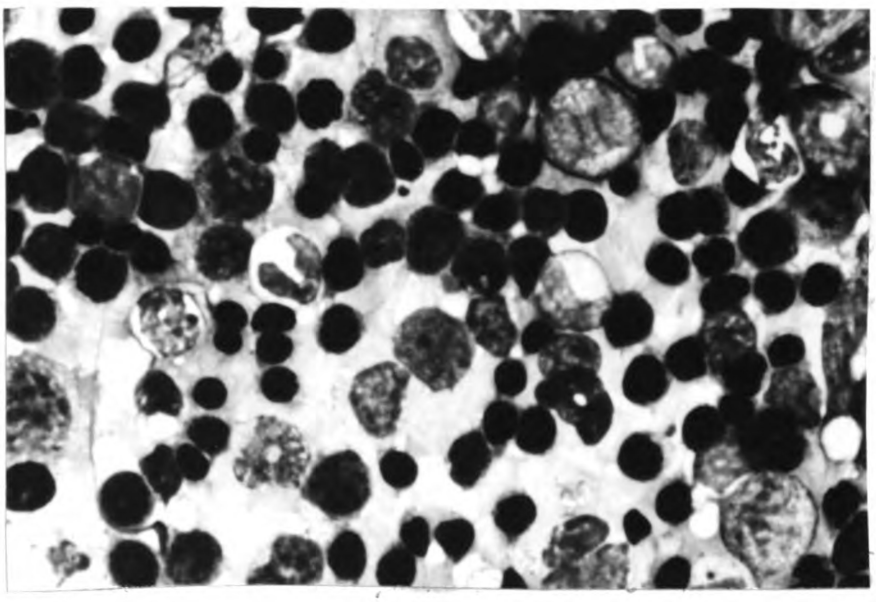

Figure 10. Imprint of spleen from 9-week-old mouse that was given L. acidophilus bacterin injections. The predominant cell types are erythropoietic and myelopoietic with only occasional lymphocytes present. Wright-Giemsa. 750X.

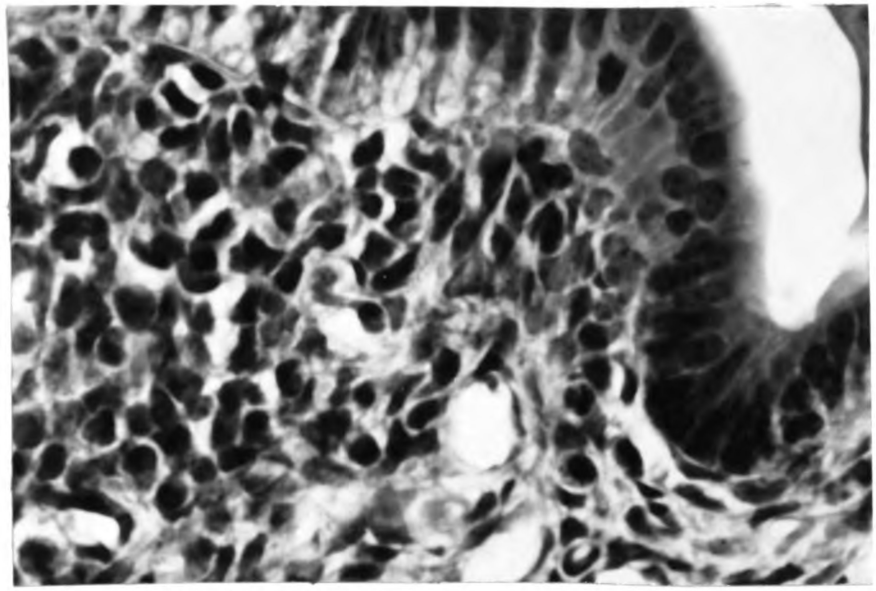

Figure 11. Aggregation of mononuclear cells in the lamina propria of the intestine of a normal mouse on the day of birth. Hematoxylin and eosin. 750X.

Peyer's patches and germinal centers were present are listed (TABLES 3 and 4).

Cross sections of parasites were found in the lumens of 27% of all mice. These were interpreted to be Aspicularis tetraptera.

Lung. Congestion of varying degrees, usually slight, was seen in large numbers of both test and control mice. The diethyl ether used for killing the mice may have caused this congestion. Congestion and atelectasis was marked in mice that were sick and died during the experiment, but it did not appear extensive enough to have been the cause of death. Some of the lungs contained focal aggregations of neutrophils and others lymphoid follicles. Slight emphysema was also present in a few instances.

Heart. In 1 test mouse and 2 controls a mild endocarditis and slight mononuclear infiltration were noted.

Liver. Grossly visible surface lesions were seen on the liver in 8 mice. Seven of these were test mice and 1 was a saline control. These lesions appeared as raised white areas.

Histologic lesions were more frequent in test mice, but were also found in control mice. In Group I, histologic lesions were present in 42% of the test mice and 20% of the controls; and in Group II they were present in 55% of the test mice and 6% of the controls. These lesions were of 2 types. One was a granulomatous type reaction with macrophages and giant cells which, in a few instances, surrounded calcified or amorphous material. The other type of lesion found on the surface of the liver was composed of an increased amount of connective tissue, lymphoid cells, plasma cells, a few neutrophils and eosinophils.

Necrosis was not prominent in the liver. Pleomorphic nuclei were a common finding, and binucleated hepatic cells were occasionally seen.

Hematopoietic cells were found in the livers of normal mice until the age of 3 weeks. After this age their presence was variable. Hematopoietic cells were seen in the livers of some test mice 12 weeks old. The incidence of hematopoietic cells in the livers of control mice was lower than that in the test mice (TABLES 3 and 4).

Pancreas. Several histologic changes were seen in the pancreas. Increased loose areolar connective tissue surrounding lobules of acinar cells was present in 63% of the test mice in Group I and 85% of the test mice in Group II. The incidence of this finding was also higher in those mice given the L. acidophilus bacterins (95% in Group I and 100% in Group II). There was an apparent relationship between atrophy of the acinar cells and increased connective tissue. Atrophy of the acinar cells was prominent in 64% of the test animals in Group I which had increased connective tissue and in 52% of those in Group II. Focal aggregations of lymphocytes and plasma cells were a common finding (Figure 12). There were also areas in which lymphocytes were diffusely scattered among the acinar cells. A few neutrophils and eosinophils were seen in some sections. In some areas the atrophied acinar cells appeared to be replaced by adipose tissue. In 1 mouse, aggregations of lymphoid and plasma cells surrounded some of the islets; however, this was an exception, and the islets generally did not appear to be involved. Large cells with eccentric nuclei and globules of eosinophilic material were present in 1 section. These were thought to be plasma cells with Russell bodies (Figure 13).

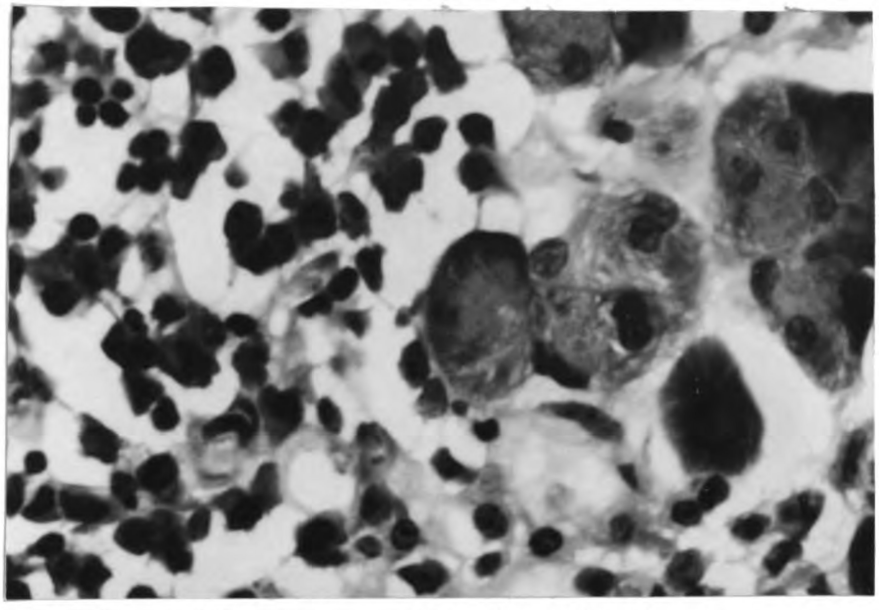

Figure 12. Pancreas from 9-week-old mouse that was given L. acidophilus bacterin injections. Notice focal collection of plasma cells and lymphocytes. Acinar cells appear atrophic (compare with Figure 14). Hematoxylin and eosin. 750X.

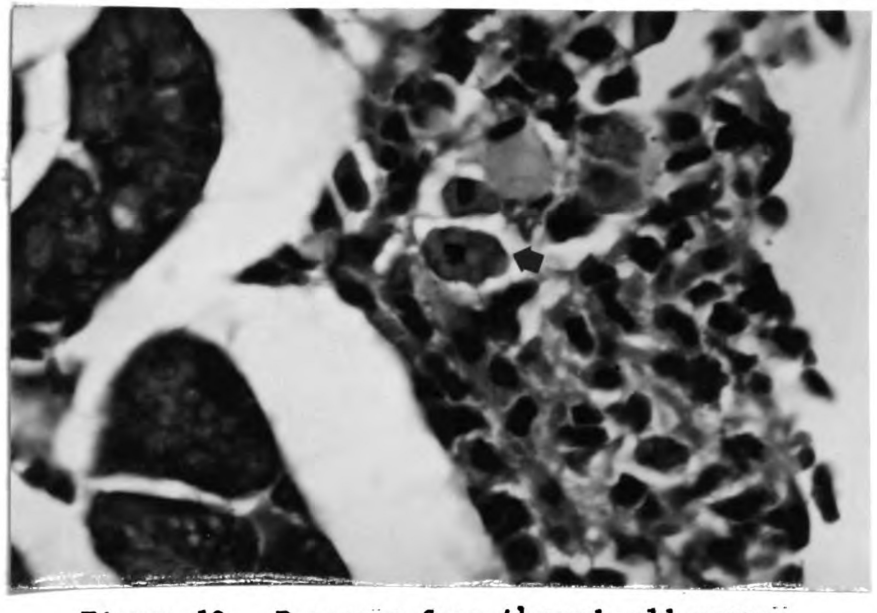

Figure 13. Pancreas from 4½-week-old mouse that was given L. acidophilus bacterin injections. Notice lymphocytes and plasma cells. Large cells with eccentric nuclei (arrow) contain globules of eosinophilic staining material and are plasma cells with Russell bodies. Hematoxylin and eosin. 750X.

Some increased connective tissue was also present in the mice that were given injections of sterile milk. The dam had been given injections of the L. acidophilus bacterin previous to the birth of this litter. Increased connective tissue was not noted in the pancreatic tissues of any control mice given saline solution; however, a small amount was found in 2 mice, 7 and 12½ weeks of age, which were given 1 injection on the day of birth and none thereafter. The identification of the 1st injection is unknown.[1]

Adipose cells and a few lymphocytes were seen in the pancreatic tissue of some of the control mice given saline solution. A lymphoid follicle was found in the pancreas of a 5-day-old mouse that was given 1 injection[1] on the day of birth and in a 3½-week-old mouse that had not been given any injections. The latter had appeared sick for several days.

Prominent inclusion-like bodies were seen in the nuclei of acinar cells of both test and control animals (Figure 14). These inclusion-like bodies were noted in 47% of the test animals and 49% of the controls in Group I and 40% and 53%, respectively, in Group II. They were also found in mice that were not given any injections.

Adrenal. Sections of adrenal glands were not adequate to confirm cortical hyperplasia. Accessory adrenals were found in 3 mice. Neutrophils were seen in the medulla of 1 adrenal gland.

Kidney. Slight congestion and proliferation of reticuloendothelial cells were present in both control and test mice. A thickening of the capsule was present in this organ in some test mice. This was visible at

---

[1]The stain used to identify the mouse was removed by the dam.

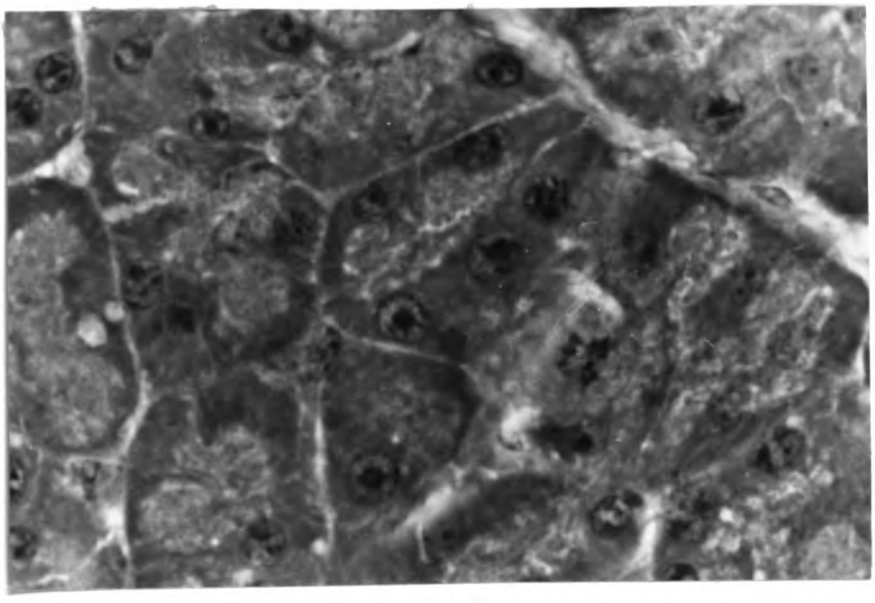

Figure 14. Acinar cells of pancreas with prominent intranuclear inclusion-like bodies (arrow). Hematoxylin and eosin. 750X.

gross examination in 9 of the test mice, and always appeared more prominent on the left kidney than on the right. Microscopically lymphocytes, plasma cells and occasionally neutrophils and macrophages were seen in the thickened capsules. This lesion resembled those seen in the capsule of the spleen.

Nitrogen content of inoculums

It was desirable to know if the milk used for injection contained more or less nitrogen than the bacterins. Results of semi-micro Kjeldahl nitrogen determinations[1] indicated that the 2×10^{10} organisms per milliliter *L. acidophilus* bacterin contained 0.04% N, the 10^{10} organisms per milliliter *L. casei* and *S. aureus* bacterins each contained 0.01% N, and the milk contained 0.60% N.

[1]The semi-micro Kjeldahl nitrogen determinations were made by Mrs. Betty Bradley, Department of Animal Husbandry, Michigan State University.

DISCUSSION

Marked growth retardation was seen in a few instances following repeated intraperitoneal injections of S. aureus, L. casei, or L. acidophilus bacterin, and a slight to moderate growth retardation occurred frequently. The results do not indicate that the incidence of growth retardation was increased in those litters in which the dam was given previous injections of the specific bacterins.

The results of this experiment do not agree with those previously reported in which marked depletion of lymphoid tissues was the predominant finding in runted mice (Ekstedt and Nishimura, 1964). The thymuses of severely or moderately runted mice in the work reported here did not have a loss of delineation between cortex and medulla unless the mice appeared to be ill from some infectious process. Loss of delineation between cortex and medulla, along with decreased size, was the main change reported by Ekstedt and Nishimura (1964) in the thymuses of the runts they produced with bacterin injections.

Other workers have used the number of splenic corpuscles or their size as criteria for denoting lymphoid depletion in the spleen (Ekstedt and Nishimura, 1964; Osoba, 1965). In the work reported here it was observed that the number of splenic corpuscles seen in a longitudinal section and their diameter depended on where the section was cut. In cross section the mouse spleen is triangular in shape. A section cut longitudinally through one of the apices would have a single bead-like strand of splenic corpuscles. A section cut longitudinally through the thickest part would

have from 2 to 4 rows of splenic corpuscles. The number and size of splenic corpuscles seen in runted mice did not appear to be markedly decreased.

A more exact method for evaluation of lymphoid depletion was desired so the point sampling method was used. The use of this method demonstrated that the mean lymphocyte counts of splenic corpuscles for the test mice were lower than for the control mice and, correspondingly, the number of reticular cells in the same area was higher in the test mice. The number of interstitial spaces was variable. The difference in the number of lymphocytes was statistically significant in Group I in mice that received the L. acidophilus bacterins but not in those that received the L. casei or the S. aureus bacterins. In Group II the difference was most significant between the saline controls and test mice that had received S. aureus bacterin following previous injection of the dam with S. aureus bacterin. This finding might indicate that the dam produced antibodies to the S. aureus and these antibodies were passed through the placenta, so that they later complexed with the antigen in the neonatal mice and participated in initiating this reaction in the spleen. Although these findings indicate that there could be a significant decrease in the number of lymphocytes present in the splenic corpuscles, they are not conclusive. A relative difference in the distribution of the cells in the areas counted could result from a swelling of the white pulp, with edema around the central arteriole. This is one of the changes which occur in the spleen following the intravenous administration of large amounts of antigenic materials (Congdon, 1964; Cosgrove and Upton, 1965). Examination of the lymph nodes and Peyer's patches indicated that the bacterin injections did not delay or prevent their normal development.

Lymphopenia was not noted, and evidence of anemia was present in only 2 instances. The leukocyte counts seemed to vary with age, and a more

complete study of normals would be necessary to evaluate this finding. Peripheral blood obtained from the tail vein contains a higher number of white blood cells (mean 21,510) than ventricle blood (mean 3,717), and there is also some variation among strains (Fekete, 1941).

It is possible that the amount of material remaining in the peritoneal cavity of the mouse on initial injection might influence the final outcome in this experiment. Difficulty with leakage of the injected material from the site of injection was experienced with mice less than 1 week old. In an attempt to prevent this a smaller volume of inoculum was used. The concentration of the L. acidophilus bacterin was increased so that the number of organisms previously used was contained in a smaller volume. With this change in technique a severely runted mouse resulted (Figure 3) and led to the use of the increased concentration of L. acidophilus.

The use of milk injections in some controls was introduced to determine if the growth retardation produced was the specific result of a reaction to bacterial substance or if it would result with any injected protein. The small number of mice injected with milk, and the fact that the dam had received previous injections of L. acidophilus bacterin prevented any valid conclusions being drawn from this part of the experiment.

The increased growth and edema of connective tissue and the large numbers of plasma cells and lymphocytes present in sections of pancreas and on the surface of the spleen and kidneys are of particular interest. The high incidence of these lesions in the mice receiving the bacterin injections suggests that they are a manifestation of some reaction to the injected substance.

The exact nature of this reaction in the pancreas is open to speculation. The presence of lymphocytes and plasma cells is suggestive of a

delayed hypersensitivity reaction (Waksman, 1959). Several mechanisms appear possible under the conditions of this experiment. The first is that an immune reaction may occur against antigenic components of organisms when they are introduced into a site where they normally do not occur, or against organisms found in the normal flora under adverse conditions. If there is a similarity between the injected material and some tissue substance, a cross-reaction might occur which could lead to this type of lesion in the pancreas. Assuming that an antibody formed against the injected bacterins could cross-react with cell membranes in the pancreas, a progressive interaction of antibody and tissue substance could occur. It is also possible that a small amount of cross-reacting antibody might become attached to the acinar cells and alter the cells enough that they could become antigenic. Many bacterial cell walls, including lactobacilli and streptococci groups A-F, contain N-acetyl-glucosamine. Mammalian membranes contain hyaluronic acid, which is composed of N-acetyl-glucosamine and glucuronic acid. Thus, it is possible that a similarity between the sequence of N-acetyl-glucosamine end-groups in mammalian membranes and bacterial cell walls is the basis for an immune reaction resulting in a cross-reacting antibody (Stevens, 1964).

Other possible causes for the pancreatic lesions in the experiment might be related to traumatic injury resulting from repeated injections, or injection of the bacterin into the pancreas itself. Traumatic injury might result in the release of tissue substances normally not in contact with the immune apparatus of the body. The released substances might attract antibody-forming cells and the bacterin might serve as an adjuvant. The possibility of the bacterin being injected directly into the pancreas appears remote, but if this did occur, a local inflammatory reaction could follow. Another possibility is that a localized peritonitis may have

developed as a result of the multiple injections; however, if this were true these lesions would have occurred in the saline controls also. This was not observed.

The facts that the first injections were given at an age when the mouse is presumed immunologically tolerant and that lactobacilli are notably poor antigenic substances may detract from the idea that this is an immune reaction. However, immune tolerance is a variable finding, and it may be that "cytophilic" antibodies are produced in response to lactobacilli, and suitable procedures for identifying them have not yet been introduced. The tissue reactions reported in this experiment suggest that further work including serum protein, immunologic, and histochemical studies might be of interest.

The mode in which the pancreatic lesions might lead to growth retardation is also open to consideration. Atrophy of acinar cells in this experiment might in some way interfere with the exocrine secretions of the pancreas which include amylase, lipase, and trypsin. In the adult mouse this may not cause any notable ill effects, but in a rapidly developing young mouse, it may interfere with the processes of digestion or absorption to such an extent as to cause growth retardation. Hallman and Jordan (1965) studied the effects of pancreatic resection in mice at the age of 4 weeks. They found no demonstrable change in growth rate, absorption of fat, nitrogen or calcium. In the present work the growth retardation was produced at an earlier age.

Although the islets of Langerhans did not appear to be affected, there could be alterations involving insulin production which would also cause a delayed development. Other mechanisms which could be considered include the possible effects that the bacterin injections might have on humoral substances produced by the thymus and the effects of stress on adrenocortical hormones and the pituitary.

Senile and lipomatous atrophy of the pancreas reportedly occur in mice (Tuffery, 1963), and it is of interest to note human conditions which have similarities to the lesions of this experiment. Polyarteritis nodosa has been reported to result in an infiltration of eosinophils, lymphocytes, and plasma cells around the involved vessels in the pancreas (Anderson, 1964). Arterioles and capillaries are involved in systemic lupus erythematosus, and there may be secondary exudation of inflammatory cells, composed primarily of lymphocytes and plasma cells. Lesions identical to those in polyarteritis nodosa have been found in serum sickness and hypersensitiveness to sulfonamides (Boyd, 1961). No fibrinoid changes were noted in the sections of pancreas, spleen or kidney in this experiment, but the increased connective tissue contained many small vessels, and foci of lymphoid cells frequently appeared to be clustered around them.

In human pancreatitis, predisposing factors such as cholelithiasis and alcoholism are not implicated in 25 to 30% of the cases. Histologic changes include an intense lymphoid infiltration in the early phases followed by progressive fibrosis and destruction of the acini (MacKay and Burnet, 1963). Pancreas-specific isoantibodies have been demonstrated by gel diffusion and tanned cell hemagglutination techniques, but autoantibodies have not been demonstrated. Pancreas-specific antibodies have also been demonstrated in cases of carcinoma and cystic fibrosis of the pancreas and in diabetes mellitus (MacKay and Burnet, 1963; Humphrey and White, 1964). Pancreas-specific isoantibodies have been produced experimentally in rabbits and rhesus monkeys, and the isoantigens in rabbit pancreas have been located in the cytoplasm of the apex of the acinar cells using fluorescent antibody techniques (Metzgar, 1964).

It was unfortunate that infection and illness occurred among the mice used in this experiment. However, this did indicate 3 important facts: (1) mice injected with bacterins were more susceptible to infection than the saline controls; (2) increased amount and edema of the connective tissue and lymphocyte and plasma cell foci in the pancreas did not occur in control mice which had signs of illness; and (3) loss of delineation between the cortex and medulla did not occur in the thymuses of severely runted mice without other signs of illness but did occur in control mice that appeared ill.

The effects that the Aspicularis tetraptera infestation may have had in the experimental results cannot be measured. The significance of the intranuclear inclusion-like bodies in the acinar cells of the pancreas is not known. Intracytoplasmic inclusion bodies are known to occur in the acinar cells of the pancreas and other epithelial cells in infectious ectromelia (Dingle, 1941). These coincidental findings do point out the value of using specific-pathogen-free or germfree animals in long-term investigations and those involving reactions of the immune system.

SUMMARY AND CONCLUSIONS

Newborn mice were repeatedly given intraperitoneal injections of bacterins prepared from Staphylococcus aureus, Lactobacillus casei, and Lactobacillus acidophilus. In one part of the study dams were injected with a specific bacterin. Some newborn mice in following litters were given repeated injections of the same bacterin, saline solution, or sterile skim milk. Runting was seen in a few instances and a slight to moderate growth retardation occurred frequently. The bacterin injections did not appear to delay or prevent the normal development of the peripheral lymphoid tissues and the thymus was not morphologically affected. An apparent quantitative decrease in the number of lymphocytes surrounding the central arterioles of splenic corpuscles was demonstrated. Lesions produced in this experiment included thickened edematous connective tissue in renal and splenic capsules, with some aggregations of lymphocytes and plasma cells. Large numbers of plasma cells and lymphocytes, increased amounts of connective tissue, and atrophy of acinar cells were present in sections of pancreas of many test animals. The pancreatic changes may have resulted from some form of an immune reaction and may be the mechanism causing the growth retardation.

APPENDIX

Modification of Shorr's Stain for Imprints

Fixation: Imprints should be fixed while wet in a 50/50 alcohol-ether solution. The imprints should be fixed for at least 15 minutes but can be stored in the fixing solution until a time convenient for staining.

Staining procedure:

1. Pass imprints through absolute and 95% alcohols to distilled water.
2. Stain in Harris's hematoxylin for 30 seconds.
3. Rinse in water to blue.
4. Place imprints in Shorr's staining solution for 1 minute.
5. Wash in 95% alcohol.
6. Rinse several times in absolute alcohol, clear in xylene, and mount in Permount.

Results: This is a good stain to demonstrate nuclear detail. Chromatin masses stain blue, cytoplasm a light green, and erythrocytes orange red.

Solutions: (Armed Forces Institute of Pathology, 1960)

Shorr's Staining Solution

Alcohol, 50%	100.000 ml.
Biebrich scarlet	0.500 gm.
Orange G	0.250 gm.
Fast green, FCF	0.075 gm.
Phosphotungstic acid	0.500 gm.
Phosphomolybdic acid	0.500 gm.
Glacial acetic acid	1.000 ml.

Harris's Hematoxylin

Hematoxylin crystals	5.0 gm.
Alcohol, 95%	50.0 ml.
Ammonium or potassium alum	100.0 gm.
Distilled water	1000.0 ml.
Mercuric oxide	2.5 gm.

REFERENCES

Anderson, W. A. D. 1964. Synopsis of Pathology, 6th ed., C.V. Mosby Co., St. Louis. 258-259.

Armed Forces Institute of Pathology. 1960. Manual of Histologic and Special Staining Technics, 2nd ed., McGraw & Hill Book Co., Inc., New York. 26, 197.

Arnason, B. C., Jankovic, B. D. and Waksman, B. H. 1962. A survey of the thymus and its relations to lymphocytic and immune reactions (a review). Blood, 20: 617-628.

Auerbach, R. 1960. Morphogenetic interactions in the development of the mouse thymus gland. Devel. Biol., 2: 271-284.

Auerbach, R. 1961. Experimental analysis of the origin of cell types in the development of the mouse thymus. Devel. Biol., 3: 336-354.

Auerbach, R. 1964. Experimental analysis of mouse thymus and spleen morphogenesis, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 95-113.

Beard, J. 1899. The true function of the thymus. Lancet, i: 144-146.

Boyd, W. 1961. A Textbook of Pathology, 7th ed., Lea & Febiger, Philadelphia. 523-524.

Brooke, M. S. 1964. Experimental runt disease in mice caused by Salmonella typhimurium var. copenhagen. J. Exp. Med., 120: 375-386.

Burnet, F. M. 1962. The immunological significance of the thymus: an extension of the clonal selection theory of immunity. Aust. Annals of Med., II: 79-91.

Burnet, F. M. and Holmes, M. C. 1964. Thymic lesions associated with autoimmune disease in mice of strain NZB, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 656-667.

Carpenter, P. L. 1965. Immunology and Serology, 2nd ed., W. B. Saunders, Philadelphia. 408-409.

Congdon, C. C. 1964. The early histologic effects of antigenic stimulation. Arch. Path., 78: 83-96.

Cooper, M. D., Peterson, R. D. A., South, M. A. and Good, R. A. 1966. The functions of the thymus system and the bursa system in the chicken. J. Exp. Med., 123: 75-105.

Cosgrove, G. E. and Upton, A. C. 1965. Pathology of the reticuloendothelial system, in The Pathology of Laboratory Animals, edited by W. E. Ribelin and J. R. McCoy, C. C. Thomas, Springfield, Ill. 21-41.

Dalmasso, A. P., Martenez, C., Sjodin, K., and Good, R. A. 1963. Studies on the role of the thymus in immunobiology. J. Exp. Med., 118: 1089-1107.

Dameshek, W., Schwartz, R. and Olines, H. 1961. Current concept of autoimmunization: an interpretive review. Blood, 17: 775-783.

Dameshek, W. 1963. W. William Hewson, thymicologist; father of hematology? Blood, 21: 513-516.

Dingle, J. H. 1941. Infectious diseases of mice, in Biology of the Laboratory Mouse, edited by G. D. Snell, Dover Publications, Inc., New York. 434-438.

Dunnett, C. W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Amer. Statist. Assoc., 50: 1096-1121.

Ekstedt, R. D. and Nishimura, E. T. 1964. Runt disease induced in neonatal mice by sterile bacterial vaccines. J. Exp. Med., 120: 795-804.

Eränkö, O. 1955. Quantitative Methods in Histology and Microscopic Histochemistry, Little, Brown & Co., Boston. 63-70.

Fekete, E. 1941. Histology, in Biology of the Laboratory Mouse, edited by G. D. Snell, Dover Publications, Inc., New York. 92-94.

Fisher, E. R. 1964. Pathology of the thymus and its relation to human disease, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 676-729.

Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O., Pierce, J. and Papermaster, B. W. 1962. The role of the thymus in development of immunologic capacity in rabbits and mice. J. Exp. Med., 116: 773-795.

Good, R. A., Martinez, C. and Gabrielsen, A. E. 1964. Clinical considerations of the thymus in immunobiology, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 3-48.

Hallman, G. L. and Jordan, G. L. 1965. Subtotal pancreatectomy and growth of young mice. J.A.M.A., 191: 233-234.

Hammar, J. A. 1921. The new views as to the morphology of the thymus gland and their bearing on the problem of the function of the thymus. Endocrinology, 5: 543-573, 731-760.

Humphrey, J. H. and White, R. G. 1964. Immunology for Students of Medicine, F. A. Davis Co., Philadelphia. 442.

Leader, R. W. 1964. Lower animals, spontaneous disease, and man. Arch. Path., 78: 390-404.

MacKay, I. R. and Burnet, F. M. 1963. Autoimmune Diseases, C. C. Thomas, Springfield, Ill. 239-240.

Matsuyama, M., Wiadrowski, M. N., and Metcalf, D. 1966. Autoradiographic analysis of lymphopoiesis and lymphocyte migration in mice bearing multiple thymus grafts. J. Exp. Med., 123: 559-575.

Metcalf, D. 1956. The thymic origin of the plasma lymphocytosis stimulating factor. Brit. J. Cancer, 10: 442-457.

Metcalf, D. 1964. The thymus and lymphopoiesis, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 150-182.

Metzgar, R. S. 1964. Immunologic studies of pancreas-specific isoantigens. J. Immunology, 93: 176-182.

Miller, J. F. A. P. 1961. Immunological function of the thymus. Lancet, II: 748-749.

Miller, J. F. A. P. 1962. Immunological significance of the thymus of the adult mouse. Nature, 195: 1318-1319.

Miller, J. F. A. P. 1965. Immunity and the thymus. Lancet, II: 43-45.

Oliner, H., Schwartz, R. and Dameshek, W. 1961. Studies in experimental auto-immunization. I. Clinical and laboratory features of auto-immunization (runt disease) in the mouse. Blood, 17: 20-44.

Osoba, D. 1965. Immune reactivity in mice thymectomized soon after birth: normal response after pregnancy. Science, 147: 298-299.

Osoba, D. 1965. The effects of thymus and other lymphoid organs enclosed in millipore diffusion chambers on neonatally thymectomized mice. J. Exp. Med., 122: 633-650.

Ostle, B. 1963. Statistics in Research, 2nd ed., Iowa State University Press, Ames, Iowa. 306-309.

Parrott, D. and East, J. 1964. Studies on a fatal wasting syndrome of mice thymectomized at birth, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 523-541.

Parrott, D., DeSousa, M. A. B. and East, J. 1966. Thymus dependent areas in the lymphoid organs of neonatally thymectomized mice. J. Exp. Med., 123: 191-203.

Sainte-Marie, G. and Leblond, C. P. 1964. Thymus-cell population dynamics, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 207-235.

Sherman, J. D. and Dameshek, W. 1964. Post-thymectomy wasting disease of the golden hamster, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 542-550.

Siegler, R. 1964. The morphology of the thymuses and their relation to leukemia, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 623-655.

Siskind, G. W. and Thomas, L. 1959. Studies on the runting syndrome in newborn mice. J. Exp. Med., 110: 511-523.

Smith, C. 1964. The microscopic anatomy of the thymus, in The Thymus in Immunobiology, edited by R. Good and A. Gabrielsen, Harper & Row, New York. 71-84.

Stastny, P., Stembridge, V. A., and Ziff, M. 1963. Homologous disease in the adult rat, a model for autoimmune disease. I. General features and cutaneous lesions. J. Exp. Med., 118: 635-648.

Stastny, P., Stembridge, V. A., Vischer, T. and Ziff, M. 1965. Homologous disease in the adult rat, a model for autoimmune disease. II. Findings in the joints, heart, and other tissues. J. Exp. Med., 122: 681-692.

Stevens, K. M. 1964. The aetiology of systemic lupus erythematosus. Lancet, II: 506-508.

Sutherland, D. E. R., Archer, O., Peterson, R. D. A., Eckert, E., and Good, R. A. 1965. Development of 'autoimmune processes' in rabbits after neonatal removal of central lymphoid tissue. Lancet, I: 131-133.

Tuffery, A. A. and Innes, J. R. M. 1963. Diseases of laboratory mice and rats, in Animals for Research, edited by W. Lane-Petter, Academic Press, Inc., New York. 95-96.

Waksman, B. H. 1959. A comparative histopathological study of delayed hypersensitive reactions, in Cellular Aspects of Immunity, edited by G. E. W. Wolstenholme and M. O'Connor (Ciba Foundation Symposium), Little, Brown & Co., Boston. 280-329.

Waksman, B. H. 1962. Autoimmunization and lesions of auto-immunity. Medicine, 41: 93-141.

Waksman, B. H., Arnason, B. G. and Jankovic, B. D. 1962. Role of the thymus in immune reaction in rats. III. Changes in the lymphoid organs of thymectomized rats. J. Exp. Med., 116: 186-205.

Weibel, E. R. 1963. Morphometry of the Human Lung, Academic Press, Inc., New York. 19-27.

VITA

The author was born April 12, 1931, in Grand Blanc, Michigan. Elementary education was obtained at the South Mundy School, and she graduated from Linden High School, Linden, Michigan, in 1949. She was employed in a Flint physician's office from 1950 to 1953, and in the laboratory of the former Goodrich General Hospital, Goodrich, Michigan, from 1953 to 1956. She received an A.S. degree from Flint Junior College, Flint, Michigan, in 1956, and a B.S. degree in Medical Technology from Wayne State University, Detroit, Michigan, in 1958. Upon completion of 12 months of clinical training at Detroit Receiving Hospital, Detroit, Michigan, the author wrote and passed the national examination of the Registry of Medical Technologists, American Society of Clinical Pathologists, in July of 1958. She was employed as a medical technologist at McLaren General Hospital, Flint, Michigan, from July, 1958, until June, 1962, and as chief medical technologist at Clinton Memorial Hospital, St. Johns, Michigan, from June, 1962, until November, 1965. She began graduate study at Michigan State University on a part-time basis in September, 1962, and as a full-time student in November, 1965. She is a member of the American Society of Medical Technologists, the Michigan Society of Medical Technologists, and the Michigan Association of Blood Banks.