Current Concepts of the Immunological Function of the Thymus

J. F. A. P. MILLER AND DAVID OSOBA

The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; and The Ontario Cancer Institute, Toronto, Canada

I. INTRODUCTION

Two centuries ago, Hewson noted that the thymus gland was filled with cells resembling those found in lymph, lymph nodes, and blood, and diminished in size with age. He concluded that "the thymus exists during the early periods of life when these particles seem to be the most wanted" (176). At the turn of the century, an important role for the thymus in the physiology of the lymphoid system was revealed by the experiments of Beard, who suggested that the thymus produced the leucocytes that migrate out to create "new centres for growth, for increase and useful work for themselves and for the body" (54). Hammar, also in the early part of this century, performed some basic fundamental work on the embryology and microanatomy of the thymus and considered that the thymus had "antitoxic func-
The purpose of this article is to review a large body of information obtained in the present decade that has established that the thymus plays an essential role in the development of a normally functioning immune system.

The structure of the thymus has been studied in detail by both light and electron microscopy (66, 110, 207, 418, 701). However, these studies have not revealed the functional significance of the distinction between the thymus cortex and medulla, nor has any function been firmly established for those structures unique to the thymus, the Hassall’s corpuscles. The thymus is part of the lymphoid system but differs from other lymphoid organs not only in its structure but also in its mode of origin (457), in the reaction of its cells to physical, chemical (147, 666), and antigenic stimuli (section II, 5 and 6), and in their proliferative behavior (section II, 3). In addition the effects of thymectomy are clearly different from those occurring after resection of other lymphoid organs (section III). In these respects, the thymus resembles the bursa of Fabricius, a lymphoid organ of birds, situated near the cloaca. The mammalian equivalent of this organ has not yet been unequivocally identified. The special characteristics of the thymus and bursa and the anatomical and functional defects that follow their removal have suggested that these organs are “primary” or “central” lymphoid organs that control the development of cells found in “secondary” or “peripheral” lymphoid structures, such as lymph nodes and spleen.

This article does not attempt to review the many nonimmunological functions of the thymus that have been suggested at one time or another. The possibility of a trophic function has been indicated by our failure to account for the incredibly vast numbers of short-lived lymphocytes being produced in the thymus (418). A hint of a detoxification function comes from experimental and clinical observations relating to the disease myasthenia gravis (70, 228, 574). A function in granulopoiesis (607, 608) and erythropoiesis (13, 14) has been suggested by morphological observations, experimental observations (454), and by the clinical association of thymoma and erythroid aplasia (583).

A section on the relationship of the thymus to disease is included because it illustrates how the malfunction of one organ, or of a system dependent on that organ, can lead to widespread disorders of immune function and even lymphoreticular malignancy.

II. LYMPHOPOIESIS

1. Lymphocyte Compartments

Round cells with a high nucleocytoplasmic ratio and very scanty cytoplasm, measuring 5–8 μ in diameter in smears, qualify morphologically for the name “small lymphocyte.” They are the major cellular constituents of lymphoid tissues. They are widely distributed throughout the body but are found particularly in
lymph nodes, in the gut-associated lymphoid tissues (such as the tonsils, adenoids, appendix, and Peyer's patches), spleen, thymus, and bone marrow. They comprise a large percentage of the leukocytes normally found in blood and peritoneal fluid and 95% of the lymphocytes found in the major lymph channels. There is now abundant experimental evidence to indicate that small lymphocytes as defined by size and morphology are a heterogeneous collection of cells with different origins, functions, fates, and life spans. For convenience, small lymphocytes can be arbitrarily grouped into various compartments, bone marrow, circulating pool and thymus, but it must be borne in mind that a functional heterogeneity exists even in the cells within any one compartment.

A) Bone marrow. In some species, notably the mouse, rat, and guinea pig, lymphocytes form a considerable proportion of the cells in bone marrow. Autoradiographic data (501) indicate that in the guinea pig the majority of marrow lymphocytes are formed in situ, the entire marrow small lymphocyte population being renewed in 3 days or less. From these data it can be calculated that in a standard 400-g guinea pig the marrow produces daily about 1000 million lymphocytes. Lymphopoiesis in the marrow thus occurs on a much more extensive scale than had hitherto been considered. Significant numbers of marrow small lymphocytes find their way into the blood stream. Electron micrographs (292) show that they migrate out of the marrow into the blood stream by passing between cells of the sinusoidal endothelium (not through the cells as is the case in the postcapillary venule of the lymph nodes).

The marrow contains cells capable of repopulating the thymus, spleen, and lymph nodes, as well as the myeloid centers, of lethally irradiated animals (189-192). In serial transplantation experiments, where bone marrow was given to lethally irradiated mice, the proliferative capacity of the injected cells was correlated with the number of marrow lymphocytes (133). A relationship could be established between the marrow small lymphocytes and erythropoiesis though not with granulopoiesis. Other experiments (682, 683) showed that the bone marrow was an important source of "small round cells" that emerged from the blood in sites of acute inflammation and gave rise to macrophages at these sites. Radioactively labeled monocytes were also found in the blood of rats that had received injections of labeled bone marrow. These experiments hint at the possibility that bone marrow small lymphocytes may function as hemopoietic stem cells, as originally postulated by Maximow (396) and upheld by Yoffey (718). Since, however, hemopoietic stem cells form only about 0.1% of the nucleated cells in bone marrow, any attempt to correlate stem-cell capacity with a particular morphological cell type must remain inferential until methods become available to increase considerably the proportion of stem cells in a given population.

B) Circulating pool. In contrast to the situation in bone marrow, none of the lymphocytes found in lymph nodes and lymphatic channels can function as general hemopoietic stem cells. They cannot repopulate the hemopoietic tissues of irradiated animals (215). Most small lymphocytes in thoracic duct lymph are neither newly formed cells nor end cells but recirculating cells (247). After entering the blood stream from the thoracic duct, these cells migrate through the endothelial
cells of the postcapillary venules to enter lymph nodes, traverse the lymphocytic fields to leave via the efferent lymphatics eventually to reach the thoracic duct and hence the blood. They pass through the periarteriolar lymphocyte sheaths of the spleen but do not normally pass through the thymus. For convenience, they may be referred to as cells of the circulating pool. The majority (95%) of these cells have a long life span, of the order of months in rodents (366) and years in man (84). Their source of origin has not been unequivocally demonstrated and is discussed more appropriately below. They are capable of proliferating to give rise to new lymphocytes and other types of lymphoid cells in response to antigenic stimulation. As will be seen in section II, 5, their function is in the initiation and induction of immunological reactions.

C) Thymus. The lymphocytes within the thymus exhibit certain characteristic features that set them apart from lymphocytes elsewhere. The mitotic indices of thymic lymphoid cells are much higher than those of other lymphoid cells (section II, 3). There are quantitative differences in the response of lymphocytes in the thymus and lymphocytes elsewhere to hormonal and nutritional influences (147, 148, 316) and to ionizing radiations and chemicals (666). In contrast to circulating pool lymphocytes, the majority of thymus lymphocytes have a short life span (section II, 4) and are not responsive to antigenic stimuli (section II, 5 and 6). In addition, thymus lymphocytes possess distinct antigens (76, 535, 546-548, 581) and differ from other lymphocytes in their susceptibility to certain antisera (579), being particularly susceptible to guinea pig serum (545) and to natural antibodies present in the sera of certain strains of mice (580).

2. Origin of Thymus Lymphocytes

The origin of the lymphocytes present in the thymus has been the subject of much discussion and enquiry. Theoretically, the precursor of the thymus lymphocyte could be a cell type resident within the thymus itself or one immigrating from without.

The thymus anlage arises in the epithelium of the ventral region of the third and fourth branchial pouches. The idea that the thymus lymphocytes originate from epithelial elements has arisen from morphological studies of the developing thymus rudiment. The early morphologists (53, 54, 157, 395, 628) maintained that the small thymic cells arose directly from the 'epithelial reticulum and had the potentiality of reversion to their original epithelial form. More recently, light- and electron-microscopic studies of the sequential cytological changes in the embryonic thymus of the chicken (3), hamster (4, 697), and human (399) have suggested that lymphoblasts develop by the gradual proliferation and transformation of the "undifferentiated" epithelial cells comprising the primordial thymus. As no stable cell markers were available in these studies, the conclusions were based solely on a dynamic interpretation of a series of static morphological pictures, which constitutes wholly inadequate evidence for the transformation of one cell type into another. A more dynamic approach to the problem was demonstrated by Auerbach (35, 36,
38, 39), who used a combination of tissue culture and transplantation methods. Mesenchyme was separated from the epithelial thymus rudiment of 12-day mouse embryo by trypsin. Experiments employing combinations of chick and mouse mesenchyme and epithelial tissue, and grafts to the chorioallantoic membrane and into the anterior chamber of the eye as well as in vitro explantation, established 1) that mesenchyme was responsible for the initial inductive stimulus to morphogenesis and provided the stromal elements of the developing thymus, and 2) that the epithelium was the source of the lymphoid cells. These experiments suggest that the thymic lymphoid cells arise directly from the epithelial cells of the rudiment in vitro. Auerbach’s evidence, however, does not exclude the possibility that lymphoid precursor stem cells or some type of lymphocyte had already penetrated into the thymus epithelial rudiment. In fact, recent studies in the mouse (617) support observations made by the earlier histologists in the rabbit, guinea pig, rat, mouse, and cat (262, 396, 397) that “basophilic lymphoblasts” have already migrated into the endodermal epithelium of the branchial pouches from which the epithelial rudiment of the thymus is derived. The transformation of thymus epithelial cells to lymphocytes thus still remains an open question.

There is evidence from three main experimental situations that the thymus sequesters from the circulation lymphoid precursor cells that behave in the thymus as typical thymus lymphocytes: experiments with thymus grafts (154, 189, 253, 266, 430, 439, 441, 446, 448, 452, 465, 466, 487), radiation chimeras (43, 190, 191, 192, 212, 433), and parabionts (189, 267, 426). In all these experiments, use was made of a chromosome marker or a radioactive label to demonstrate the entry of cells into the thymus. Chromosome marker studies have shown that in the chicken, too, the lymphoid cells of the bursa of Fabricius are the progeny of some precursor cells immigrating via the blood stream (302, 477, 478).

The regeneration of thymus tissue under the skin or under the renal capsule has been studied histologically and cytologically. For the first 24–48 hr after grafting the implant consisted of a central necrotic core and a peripheral rim of viable tissue containing both epithelial and lymphoid elements. The central necrosis was probably the result of inadequate blood supply to the implant in the early hours after grafting. The viable rim always contained lymphoid cells that had survived the initial transplantation injury. During the next two days, mitotic activity became evident in the lymphoid rim and by 5–6 days the typical structure of the thymus cortex had been restored. By day 6, normal thymus architecture with characteristic corticomedullary lobules had become evident. The extent of host contribution to the regeneration of thymus grafts was determined by employing hosts whose cells could be recognized at metaphase by the presence of a marker chromosome (T6). For about the first 2 weeks, the cells dividing in the graft were entirely derived from the donor cells surviving in the peripheral rim of the original implant, there being no dividing host elements in the graft. During the 3rd week, when no histological changes could be detected, host cells were found dividing in the graft, and by 21 days and after, the dividing donor cell population had entirely been replaced by host cells (154). Reimplantation of a graft repopulated by host cells into a new host resulted in the eventual replacement of the original
host's cells by those of the new, second host (266). When the donor cells were rendered incapable of proliferation by pretreatment with radiation in vitro, there was no initial regenerative phase, and only host cells participated in the total regenerative process (154). However, if the host, too, had been irradiated, the preirradiated implant remained an epithelial structure and was never lymphoidal (452). All these findings are consistent with the notion that some, or most, lymphoid cells are not derived directly from epithelial cells but are the progeny of cells entering the grafts from the circulation. Since donor-type lymphoid cells disappeared from the implant one must presume that the thymus has only a relatively small, limited, stem-cell compartment and that the precursors of thymus lymphocytes have a capacity for proliferation and maturation but exhaust themselves during the process of differentiation. Their capacity for self-renewal must thus be limited.

Mice can be protected against the lethal effects of high doses of ionizing radiations by an injection of hemopoietic cells (such as marrow, fetal liver, or spleen). It has been clearly established in such experiments that both the hemopoietic and lymphatic tissues (including the thymus) of the irradiated host are repopulated by donor cells (190, 212). By means of chromosome marker techniques, it was found that marrow cells injected into an irradiated host proliferated initially in both marrow and thymus whereas injected thymus and lymph node cells proliferated only in lymph nodes. The cells proliferating in thymus had all the characteristics of thymic cells. Furthermore, the cells bearing the marker of the injected thymus and lymph node cells completely disappeared after a period of time and were replaced by cells bearing the marrow marker, which became present throughout (192). The cells from the thymus and nodes, therefore, selectively settled in lymphoid tissue to be replaced eventually by cells originally derived from the marrow. These experiments show that the irradiated thymus will receive cells from the circulation and suggest that it commits such cells to differentiation along lymphoid pathways. When the appearance of donor labeled or marked cells was followed temporarily in a radiation chimera, the bone marrow, spleen, and lymph nodes were the first organs to sequester a large number of donor cells. The thymus acquired significant numbers of donor cells at a much later time (43, 212). Furthermore, the few cells that were sequestered during the early postirradiation period did not appear to be actively dividing (43). Similar observations were made (154) during the earliest regenerative phase in irradiated thymus grafts. The cells that eventually proliferate in the thymus of a lethally irradiated animal or in an irradiated thymus graft may thus be initially sequestered by the bone marrow, spleen, or lymph nodes of the host, and only later migrate to the thymus.

In the parabiosis experiment (267) two pairs of normal 1-month-old mice were joined together. One member of each pair was from the CBA/H strain, while the other member was CBA/H-T6T6. Thus, the cells of the latter could be distinguished in mitosis by the presence of two small T6 marker chromosomes, but the two strains of mice were syngeneic with respect to their histocompatibility antigens. After 4-5 weeks of parabiosis, it was found that cells had been exchanged between the two partners: the exchange was greatest in spleen and lymph nodes (50%), less in the thymus, and least in the bone marrow. Although the thymus of parabiotic
mice cannot be regarded as strictly normal, owing to the stress of the surgical procedure and of the subsequent restraint associated with parabiosis, these experiments nevertheless hint at the possibility that cells from the circulation may enter into the pool of mitotic cells in the intact thymus.

Nothing is known concerning the identity of the cell or cell types that migrated to the thymus under the above experimental conditions. One of the sources of the immigrating cell is likely to be the hematopoietic tissue of the bone marrow, spleen, and fetal liver, since the thymus of lethally irradiated animals was repopulated by cells derived from such tissues. It is very unlikely that cells found in the lymph nodes or thoracic duct lymph normally migrate into the thymus. In the radiation experiments referred to above, lymph node cells were never seen to proliferate in the thymus (192). Radioactively labeled small lymphocytes from the thoracic duct injected into normal unirradiated adult rats homed to the lymph nodes, the Peyer's patches, and white pulp of the spleen, but not to the thymus (247). Only a few labeled large lymphocytes were seen in this organ. When large numbers of labeled cells, obtained from the lymph nodes draining a foreign skin graft, were injected into syngeneic mice, a small number appeared in the thymus of both neonatal and adult mouse recipients. No evidence, however, was found to suggest that proliferation of these cells occurred in the thymus (207).

3. Proliferation of Thymus Lymphocytes

The thymus is the organ with the highest rate of production of small lymphocytes. Morphological examination of the thymus cortex and of smears of cortical thymus lymphoid cells has shown that thymic cell mitotic indices (mitotic counts per unit area of tissue or per total number of primitive lymphoid cells) are about 10 times higher than those of subcutaneous lymph nodes and 5 times higher than those of mesenteric lymph nodes and Peyer's patches in the rat (19, 58, 326–329, 355, 568, 570), mouse (415, 419, 428, 491), and man (419). The mitotic activity of thymic lymphoid cells is not constant throughout life: it is highest in the neonatal period, falls slightly during the first few weeks of life, and undergoes a more pronounced fall during the period of age involution. Thereafter it remains relatively constant at a level still exceeding that in other lymphoid tissues (19, 419, 491). Another index of cellular proliferation is the rate of DNA turnover. This was found to be 2–5 times higher in the thymus than in the lymph nodes (20, 21, 173, 584). From data on mitotic indices and counts of lymphocytes of various sizes, it was concluded that the small lymphocyte in the thymus cortex must have been derived from the division of larger cells giving rise to progressively smaller cells (40, 355, 568, 570, 571). This agreed with previous morphological observations made on embryonic and adult thymuses (54, 158).

The proliferative pattern of thymus lymphoid cells has been studied in rats (127, 173, 483, 584) and mice (75, 394, 424, 431) injected with the radioactive precursor, tritiated thymidine. Only short-term studies with the isotope were meaningful since the label released from the breakdown of labeled cells can be reutilized,
becoming incorporated into other previously unlabeled cells (431, 470). Immediately after a single pulse of tritiated thymidine many large and medium lymphocytes, but only a few small lymphocytes, were labeled; later, more and more of the labeled cells were small lymphocytes (83, 127, 172, 424, 431, 483). These findings support the conclusion made from morphological studies that at least some small thymus lymphocytes arise from the division of larger lymphoid cells.

The accumulation of labeled small lymphocytes occurred at a greater rate in the thymus than in the lymph nodes, blood, and even bone marrow (424, 501). The increase in percentage of labeled small lymphocytes occurred in a linear fashion when plotted arithmetically against time. Since 50% of the small lymphocytes were labeled by 1.5–2 days, the total population of small lymphocytes in the thymus cortex must be replaced every 3–4 days (74, 424). Since only 95% of the population was labeled by 4 days, about 5% may have a longer life span. Since the size of the adult thymus remains relatively constant over long periods of time, the rapid renewal of the small lymphocyte population must be balanced by an equally rapid loss through either cell death or cell migration. Either or both of these two events do not occur on a random basis, since it has been shown that the total level of tritiated thymidine in the thymus, measured radiochemically (127) or by grain counts (424), remained constant during the first 3–4 days after labeling and began to fall thereafter. Newly formed thymus small lymphocytes are therefore not lost on a random basis but migrate or die at the end of their 3- to 4-day intrathymic life span.

The labeling experiments have enabled calculations to be made for the cell cycles of the primitive thymus lymphoid cells. Large lymphocytes divided every 6.8 hr and medium lymphocytes every 8.2 hr (424, 431). These cell cycle times are very rapid but no more rapid than those of lymphoid cells dividing in the germinal centers of the lymph nodes and spleen of antigenically stimulated rats (187) and mice (264). The lower rate of production of small lymphocytes in the lymph nodes as compared with that in the thymus must therefore result from factors other than those related to the proliferative rates of the primitive lymphoid cells. It is likely to result from 1) the existence in the nodes of a smaller percentage of primitive lymphoid cells (0.5% large and 2.6% small lymphocytes in the nodes as compared with 1.4% large and 7.2% medium lymphocytes in the thymus) (424) and 2) the production from some of these lymph node cells of cells other than small lymphocytes (e.g. plasma cells) and of nonviable cells (187).

In contrast to the intense proliferative activity of cortical lymphocytes, the lymphoid cells in the thymus medulla exhibit little or no mitotic activity (418, 419). There are certain features, in addition to mitotic activity, that distinguish lymphocytes in the cortex from those in the medulla. The lymphocytes in the medulla are few and more closely resemble lymphocytes in peripheral lymphoid organs than cortical lymphocytes. Thus medullary lymphocytes have in common with many peripheral lymphocytes a richer supply of mitochondria, a few sacs of rough and smooth endoplasmic reticulum, and a well-developed nucleolus. Cortical lymphocytes lack these features (484). The small lymphocytes in the cortex are more radiosensitive than the medullary lymphocytes, the LD70 being 46 r for cortical
lymphocytes and 180% for medullary ones, a fourfold difference (667). A similar resistance of medullary lymphocytes has been reported after cortisone injection (300). Autoradiographic studies have indicated that the small proportion of long-lived thymus small lymphocytes may be located in the medulla (394). Work on the chicken thymus (see section II, 5) has suggested that cortical lymphocytes are devoid of immunological competence whereas medullary lymphocytes can apparently be stimulated to perform immunological reactions (692). It is possible that the medullary lymphocytes form part of the recirculating pool of long-lived small lymphocytes; if this is so, however, it is difficult to explain the failure of any of the “circulating pool” small lymphocytes to home to the thymus (247). The different properties of cortical and medullary lymphocytes suggest either that these two cell types belong to two distinct races of cells or that the medullary lymphocytes are a more mature form of their cortical precursors.

Various models of thymus lymphopoiesis have been proposed (355, 424, 568, 569, 571). Eight successive generations of lymphocytes were found to account for the observed proportions of large, medium, and small lymphocytes in the thymus. From studies summarized in section II, 3, it is now known that there is a continuous immigration of cells into the thymus. A large part (75%) of the population of primitive cells in the thymus is being replaced by immigrant stem cells or lymphoid precursor cells at the slow rate of about 1% per day. Up to 25% of the primitive thymus lymphoid cells are thought to be replaced by cells of intrinsic origin—either the epithelial cells, as suggested by studies with embryonic thymus organ cultures (36), or by dedifferentiation of thymus small lymphocytes, as suggested by studies with tritiated thymidine (127). Some small lymphocytes in the thymus are capable of mitotic activity since 2% of these cells directly incorporate tritiated thymidine (424). Whether these are thymus small lymphocytes (i.e. cells originating from thymus medium lymphocytes) or small cells immigrating from the circulation (and in the process of replacing the population of large cells) is not known. However, the inability of injected thymus cells to repopulate the lymphocyte-depleted thymus of irradiated animals (192) suggests that the small thymus cells are incapable of dedifferentiating in the thymus to reenter a proliferative cycle.

4. Fate of Thymus Lymphocytes

Theoretically, lymphocytes produced in the thymus can die locally without ever entering the circulation or migrating elsewhere, or they can be exported from the organ.

The possibility that lymphocytes emigrate from the thymus was suggested by morphological studies in the rat and guinea pig (55, 340, 355, 570). Diapedesis of lymphocytes across perivascular channels, venules, and capillaries has been claimed (355, 570) and an increased content of lymphocytes has been found in the blood leaving the thymus (168, 170). More direct evidence for the emigration of thymus lymphocytes comes from studies in which thymus cells were radioactively labeled (485, 494, 766). In one such study tritiated thymidine was injected either intra-
arterially or subcapsularly into the thymus of newborn and of 6- to 8-week-old guinea pigs. Amounts sufficient to produce heavy labeling of some thymus lymphoid cells, but not enough to label significantly other cells in the body, were used. The guinea pigs were killed at intervals ranging from 1 hr to 7 days after injection. Examination of the lymphoid organs showed that some thymus-derived medium or small lymphocytes had migrated to the mesenteric lymph nodes, spleen, and intestinal lymphoid tissues. Seeding to popliteal lymph nodes or bone marrow was rare. No evidence was obtained to suggest that the tritiated thymidine-labeled thymus-derived lymphoid cell could transform to plasma cells in the antigenically stimulated popliteal lymph node or to macrophages in the peritoneal cavity (494). The rate of seeding of cells from the newborn thymus was much higher than from the young adult thymus (494, 706). These results were extended to the chicken in relation to the cells of the bursa of Fabricius (716).

In another study (394, 424) multiple thymus implants were grafted to a recipient mouse that was injected with tritiated thymidine 1 month later. The labeling patterns were found to be the same in the thymus implants as in the normal thymus of the host animal. By 3 days, up to 90% of the lymphoid cells in the host thymus and in the grafts were labeled in contrast to only 10% in the lymph nodes. Since 95% of thymus small lymphocytes are regenerated every 3-4 days (vide supra) a great majority of lymphocytes must either die or leave the thymus grafts to make way for the new generation of cells. Assuming 3 million thymic lymphoid cells per milligram of thymus tissue (40), 0.6% mitosis in thymus-graft lymphoid cells (429) and a 35-min period for the mitotic process (491), it can be calculated that approximately 1 million lymphoid cells were produced per day by 1 mg of thymus tissue. Mice bearing 24 thymus grafts carried approximately 800 mg of thymus tissue, which represented an approximate eightfold increase in the calculated daily production of lymphocytes for control mice of the same age (417, 491). Yet the lymphoid population of the peripheral blood, spleen, nodes, and intestinal tract of mice grafted with as many as 24 thymus implants was not increased and showed little deviation from normal. There was no correlation between the total weight of the thymus grafts and the weight of the lymph nodes in individual mice. The host's own thymus and lymph nodes showed normal morphology and mitotic activity. Lymphoid infiltration was not observed in liver, kidney, lung, and bowel (417) and, in the labeling experiments, there was no significant increase in the percentage of labeled lymphocytes in the blood, lymph nodes, and other tissues for a period of up to 14 days after initial labeling (394, 424). These results imply that only very few of the cells produced in the thymus graft and host thymus could have emigrated. This may be the case in adult mice with their fully developed lymphoid system. It would be interesting to know whether a significant cellular contribution from the thymus could be detected in similar experiments involving not normal adult mice, but either neonatal mice or adult mice that had been subjected to procedures depleting their lymphocyte populations.

By using chromosome markers (T6) and thymus grafts it has been shown that some lymphocytes of graft origin appeared in the lymph nodes and spleen (154, 266, 439, 441, 446, 455, 466) and that their numbers increased considerably after
antigenic stimulation (158, 311, 455, 456). The functional significance of this increase is not clear. The studies with thymus grafts may not give a picture strictly representative of what normally takes place with the thymus in situ; as a result of grafting, tissue disruption occurs and graft-derived lymphocytes could escape in large numbers to seed the host tissues.

If the majority of the thymus lymphocytes do not emigrate, their rapid production must be balanced by their extensive destruction. Under normal conditions, a few small lymphocytes show evidence of degeneration in the thymus (419). The process seems to start during mitosis, particularly at anaphase and telophase (158, 311, 570). Pyknotic nuclei with a diameter less than 4.6 μ are found in the cortex (570). Hence there is obvious morphological evidence that some small lymphocytes die in the cortex even in normal animals not subjected to stress but their numbers are not large. This may be because lymphocytes disintegrate rather than become pyknotic.

It seems from the above experiments that only a few of the cells produced in the thymus of the adult rodent (either host thymus or established thymus grafts) could have seeded the host tissues and hence that the vast majority of thymus small lymphocytes are destroyed within the organ after a short life span of 3 days. This raises major questions such as what are the functions of these short-lived cells and what is the purpose of most of the intense thymus lymphopoiesis? These questions still remain unanswered. It has been suggested that short-lived thymus lymphocytes may act as trephocytes, giving up their DNA and perhaps other nutrients to newly formed cells. However, all attempts to establish a trophic function for thymus lymphocytes have been unsuccessful (418). Another suggestion is that the dying lymphocytes may represent self-reactive cells that have been destroyed by some censorship function of the thymus (87, 685; section VI, 2).

The few cells that migrate out of the thymus may conceivably be those with a long life span and may represent an important contribution to the pool of small lymphocytes outside the thymus (706). This contribution may be even more important in early life, when the lymphoid system is developing, or in adult life when the animal is recovering from a lymphopenic state produced by stress, irradiation, lymphocyte drainage, or other experimental manipulation. The function of the long-lived thymus lymphocytes that are exported may be similar to that of the cells in the circulating pool, although no direct evidence to support this has been produced.

5. Regulation of Lymphopoiesis

Lymphopoiesis can be thymic or extrathymic. The factors that determine thymic lymphopoiesis can be extrinsic and intrinsic. Among the extrinsic factors are those determining the availability of normal stem cells or lymphoid-precursor cells that can migrate to the thymus and hormonal agents that influence the activities of lymphoid cells.

Agents that interfere with the availability of stem cells or with their capacity
for self-renewal or differentiation adversely affect thymus lymphopoiesis. For instance, when preirradiated thymus tissue (thymus tissue depleted of lymphocytes in vitro and thus consisting only of the epithelial cytoreticulum) was grafted to marrow-injected, lethally irradiated mice, lymphoid cells appeared in the implant between 4 and 8 days and cell division occurred at 8 days: the dividing cells were of marrow-donor type. If, however, no marrow was supplied to the irradiated host, no lymphoid cells appeared in the implant, which remained wholly epithelial until death of the host (154, 452). Normal thymus tissue implanted into sublethally irradiated hosts did not grow as well as in normal hosts or irradiated hosts supplied with normal bone marrow (97). The availability of a certain pool of marrow stem cells, undamaged by ionizing irradiation is thus a prerequisite for normal thymus lymphopoiesis. It is conceivable that other agents (such as carcinogenic chemicals, hormones, and viruses) that damage the marrow and certain myeloid diseases also interfere with the stem-cell pool and hence with thymus lymphopoiesis. The involution of the thymus that takes place in aged animals does not seem to result from a deficiency of stem cells (422).

The most important extrinsic factors opposing thymus lymphopoiesis are the lymphocytolytic adrenal corticosteroids. Adrenalectomy (146–148, 300, 387, 575, 578) or suboptimal functioning of the adrenal gland (413) is associated with an increase in thymus size and lymphocyte mitotic activity. Stress procedures such as restraint, overcrowding, fighting, and infections cause thymus atrophy presumably by stimulating the production of adrenal corticosteroids (105, 147, 601). The gonadal steroids, testosterone and estrogens, can produce acute thymus involution and inhibit thymus lymphopoiesis by a direct action on thymus lymphocytes: their effect does not appear to be adrenal mediated since it occurred in adrenalectomized as well as in normal animals (576, 595). The thymus involution produced by cortical and gonadal steroids does not result from massive cell exportation from the thymus, but from lymphocytolysis (300). None of these hormonal influences altered the time of onset of age involution of the thymus (147).

Some hormonal influences, such as growth hormone (147, 285) and thyroxine (260, 261), have been associated with an increase in thymus size. Their effect on thymic cell proliferation has not been well documented, although in the case of thyroxine, evidence has been given for an increase in mitotic indices of thymus cortical lymphoid cells in the guinea pig (166, 169). Most of the experimental evidence, however, indicates that the influence stimulating the proliferation of thymus lymphoid cells is intrinsic to the thymus itself. This evidence comes mostly from the growth behavior of thymus grafts, which (as indicated in section II, 2) are chimeric organs, the epithelial elements being donor in origin and the lymphoid elements being derived mostly by the continuous immigration of host cells.

Thymus tissue can be grafted equally well to normal and thymectomized animals of the same inbred strain. The rate of replacement of the donor lymphocytes in the graft by host cells was found to be the same in normal and thymectomized hosts (154). The proliferative rate of thymus graft lymphoid cells was the same as that of the normal thymus in situ (394, 418, 419) and was identical whether the implants were in normal or thymectomized hosts (154, 419, 527) or in hosts bear-
ing as many as 23 other thymus implants (394, 417). The growth capacity of individual thymus grafts was thus not affected by the presence or absence of the host’s own thymus nor by the presence of other thymus implants. In addition, partial thymectomy was not associated with regeneration of the fragment that had a mitotic index identical to that of the normal whole thymus. Resection of other lymphoid tissues, such as spleen and lymph nodes, did not alter the proliferative rate of thymic lymphoid cells nor the growth of thymus grafts (419). On the other hand, spleen grafts in syngeneic splenectomized hosts were found to grow to a larger size and to contain a higher proportion of lymphoid tissue than similar grafts in sham-operated hosts (416). Further, in mice grafted with multiple spleen grafts, an additional competitive inhibition of spleen graft growth occurred: the average size attained by each graft decreased and the total spleen graft mass reached a plateau that approximated the weight of the normal mouse spleen (420). The fact that multiple thymus grafts had absolutely no effect on the growth capacity of individual thymus grafts, or of the host’s own thymus, strongly suggests that neither the grafted thymus nor the normal thymus is subject to thymus-specific feedback inhibition of proliferative activity (420). For instance, increasing the mass of thymus tissue did not seem to activate production of thymolytic agents or thymus cell mitotic inhibitors such as cortisone. This is in striking contrast to the homeostatic regulatory mechanisms that exist in some systems, notably in the endocrine system with its well-known negative feedback mechanisms.

When a newborn thymus was grafted, the implant showed, in addition to repopulation by host cells, a true growth for 5–6 weeks, i.e. until it reached the age at which normal thymus age involution takes place (419). The younger the thymus donor, the larger the size attained by the graft. Preinvolutorial thymuses grew to a larger size than postinvolutorial thymuses in recipients of the same age. In contrast, the age of the host had no effect on the growth capacity of the graft. Thus grafts increased in weight in hosts whose own thymuses were either increasing in weight, stable in weight, or rapidly losing weight during the same observation period. Further, 1-week-old grafts in 6-month-old hosts gained weight, and then lost weight in the same time interval as thymus weight gain and involution took place in the intact animal. The onset of age involution thus appears to be determined by factors intrinsic to the thymus itself. Since the supply of immigrant stem cells does not, under normal circumstances, become limiting with advancing age (422), it would appear that the factors determining the onset of age involution must be linked to the epithelial cytoreticulum.

Further evidence that the proliferative stimulus to thymic lymphoid cells is dependent on the epithelial cytoreticulum comes from experiments in which (parental type) AKR and C3H thymus tissue (which exhibit different lymphoid cell mitotic indices) were grafted into (AKR × C3H)F1 hosts (415, 429, 498). The lymphoid cell population in both types of grafts was replaced by F1 host cells (498), yet the proliferative rate of lymphoid cells in the AKR graft was higher than that in the C3H graft (415, 429). The different rates of proliferation of the F1 lymphoid cells must thus have been due to different proliferative stimuli emanating from the surviving parental epithelial cytoreticulum of the grafts.
The fact that there is no compensatory regeneration of a thymus fragment after subtotal thymectomy nor an increase or decrease in the mitotic activity of the fragment suggests that the proliferative stimulus may be associated with the presence of cell types widely distributed in the epithelial cytotreticulum. It has been claimed that medullary tissue was present in all successful thymus grafts, that the percentage of this tissue in all types of grafts was constant, and that cortical fragments lacking this tissue failed to grow on grafting (417). This led to the suggestion that the growth of thymus grafts and the size attained by them were dependent on the amount of surviving medullary tissue. However, in serial section of implants made during the first 2 days after grafting no surviving medullary tissue could be identified in the central necrotic core and only cortical tissue was apparent in the surviving peripheral rim (154). It is still possible that the medulla may exert some control over lymphopoiesis, the medullary epithelial cells in thymus grafts being regenerated from the epithelial cells surviving in the peripheral rim.

An association between mitotic lymphocytes and specialized "PAS cells" widely scattered throughout the cortex has been described (418, 419, 427). These cells are large phagocytic cells that stain with the periodic acid-Schiff reagent. They were seen frequently surrounded by tightly packed lymphocytes, one or more of which were in mitosis. Furthermore, when mitotic activity was increased, as in preleukemic thymuses, thymuses regenerating after cortisone-induced atrophy, or thymuses in adrenalectomized animals, the association between "PAS cells" and mitotic lymphocytes became more evident (300, 419). This association suggested that some cell contact mechanism might trigger off mitosis in neighboring lymphocytes. It is known, however, that in any rapidly proliferating tissue a number of the cells produced are defective: macrophages must then move in to engulf the defective cells, the fragments of which form PAS-positive inclusions. The macrophage may thus become automatically surrounded by one or more cells in mitosis. In hemolytic anemia marrow, a characteristic finding is the presence of macrophages containing phagocytosed (PAS-positive) material surrounded by proliferating erythroblasts (703). The PAS cells of the thymus may thus be simply engaged in phagocytosing defective mitotic lymphoid cells. If they do play a role in lymphopoiesis it is likely to be one in which the breakdown products of lymphocytes are made available for reutilization by other lymphoid cells (665).

The most outstanding feature of thymus lymphopoiesis is that, unlike lymphocyte production in lymph nodes and spleen, it is quite independent of antigenic stimulation. In the fetus, which is relatively shielded from antigens, the mitotic rate of lymphoid cells is very high in the thymus and low or nonexistent in other lymphoid tissues (419). The growth and lymphoid cell mitotic activity of the thymus are similar in germfree mice as in conventional mice (176, 711). By contrast, extrathymic lymphopoiesis in germfree mice is markedly decreased in comparison with conventional mice. An analogous situation has been described for both the thymus and the bursa of Fabricius in germfree chickens (656). The mitotic activity of lymphoid cells in the thymus is not increased in response to the parenteral injection of antigens (414). It has been well established that one of the effects of anti-
genic stimulation is to increase the total cell population in the lymph nodes and spleen. A plasma cellular reaction is induced and follicular activity (secondary nodule or germinal center formation) eventually appears, with the result that the stimulated node becomes actively lymphopoietic. Active lymphopoiesis in lymph nodes thus apparently occurs only after antigenic stimulation (453). Proliferation of lymphocytes in the absence of such stimulation would thus be primarily a function of the thymus.

In conclusion, it can be said that thymic lymphopoiesis is not affected by antigenic stimulation, resection of other lymphoid organs, partial thymectomy, or the presence of thymus grafts, single or multiple. The thymus is thus a composite of a multitude of autonomous subunits, each of which is independent of the other and not subject to external feedback mechanisms regulating its size. The primitive lymphoid cells in each subunit are stimulated to produce a large, though limited, number of smaller lymphocytes by repeated cell divisions associated with progressive differentiation. Their behavior is not in any way dependent on antigenic influences. More work is required to establish the exact cellular composition of each subunit, to characterize the cells responsible for providing the proliferative stimulus, to define the nature of this stimulus, and to delineate the internal feedback mechanisms that limit the size of the subunit and regulate the extent of lymphocyte differentiation.

6. Evidence Linking Some Lymphocytes to Immunity

There is evidence from phylogenetic and ontogenetic studies and from various types of experimental procedures to link at least one class of lymphocytes with immunological processes. Such processes appear to be limited to vertebrates. The hagfish, the most primitive representative of the living vertebrates, exhibits none of the characteristic vertebrate immune responses and has no organized lymphoid tissue and no recognizable thymus tissue. The more highly developed lampreys have a thymus primordium, consisting of foci of lymphoid cells in the epithelium of the second to fifth pharyngeal pouches, a few clusters of lymphoid cells in the spleen, and large, medium, and small lymphocytes in the blood; they can produce immunoglobulins and small amounts of antibody and they can develop delayed hypersensitivity and reject skin homografts (181, 509). In all the more highly developed fishes and higher vertebrates, the thymus differentiates from a primary epithelial rudiment to a lymphoid structure, the spleen shows organized lymphoid nodules, and all types of immune responses can be effected. These phylogenetic studies suggest that the development of the immune mechanism was dependent on the evolution of the thymus and organized lymphoid structures (236, 239).

The time at which a developing vertebrate can first react immunologically coincides with the period at which lymphocytes can first be identified in the circulation. For example, tadpoles develop the capacity for rejecting skin grafts at the very time (40–50 days posthatching) when small lymphocytes make their appearance (121) and the immature opossum in the pouch can produce antibody as soon
as, but not before, lymphocytes can be seen in the blood (315, 677). The development of the capacity to effect immune reactions thus coincides, both in phylogeny and ontogeny, with the appearance of circulating lymphocytes and organized lymphoid structures.

In order to evoke an immune response, an antigen has to be recognized. Recognition is likely to occur at some critical site within or on the cell. A number of studies (79, 594) suggest that lymphocytes of the circulating pool may bear "recognition units." These may be in the form of a "sentinel antibody" located on the cell membrane, the antibody belonging to a fraction of the immunoglobulin that has the special properties of a high degree of specificity.

A variety of procedures is available to deplete animals of lymphocytes (248). Included among these are thymectomy (vide infra), draining away lymph for several days by a thoracic duct fistula (401), exposure to ionizing irradiation (648), and the administration of chemical agents or hormones (e.g. cortisone) (147) that damage or kill lymphocytes. Such lymphocyte-depleted animals have an impaired capacity to undertake primary immune responses—both reactions against grafts and humoral antibody responses. Immunological capability can be restored to depleted animals by injecting lymphoid cells and by inocula consisting almost exclusively of small lymphocytes (248, 249). The fact that normal lymphoid cells can correct immunological defects provides only circumstantial evidence for the complicity of lymphocytes as immunologically competent cells, i.e. cells capable of initiating immune reactions. The lymphocyte-depleted animal may have lost its power to respond not through the loss of immunologically competent cells, but through the loss of cells serving some nonspecific trophic or other ancillary function in the immune response. There is, however, direct evidence that the immunologically competent cells in the thoracic duct lymph and blood of rodents, which can initiate transplantation immune reactions such as graft-versus-host reactions and skin homograft rejection, are the "circulating pool" small lymphocytes. Radioautographic studies have shown that under the impact of foreign histocompatibility antigens, a fraction of the cells present in inocula rich in small lymphocytes enlarged to pyroninophilic cells; these subsequently divided to produce a progeny of lymphoid cells of progressively decreasing size (193, 245, 246, 401). The sequence small lymphocyte → large pyroninophilic cell → large and medium lymphoid cells → small lymphocytes suggests the existence of two functional classes of small lymphocytes: the former are uncommitted, immunologically competent cells capable of acting as "recognition agents" and of initiating a response; the latter are committed, immunologically activated effector cells possessing the machinery for carrying out a specific type of response.

It has not been shown unequivocally that the circulating pool small lymphocytes are the precursors of antibody-producing plasma cells but the indirect evidence for this is quite strong (193, 249). Vinblastine, a mitotic poison that exerts its killing effect at mitosis, is a potent inhibitor of the primary immune response when given after, but not before, antigen (640). This indicates that cells can respond to antigen, or "antigen-sensitive" cells, are nonproliferating and may thus belong to the class of circulating pool small lymphocytes.
By contrast, there is no evidence that the small lymphocytes produced in the
marrow are immunologically competent cells: populations of marrow cells con-
taining small lymphocytes are deficient in cells capable of initiating graft-versus-
host reactions (63, 64) and of restoring immunological capability to immunologi-
cally crippled animals (130, 462).

The apparent nonparticipation of the thymus in immune reactions has been
the subject of numerous reports. Under normal circumstances, cells in the second-
ary lymphoid organs (e.g. lymph nodes and spleen) respond to antigenic stimula-
tion by proliferation associated with progressive differentiation to antibody-
producing plasma cells; immunoglobulins are produced and germinal center
reaction occurs in the lymphoid follicles. Although a few plasma cells (567) and,
much more rarely, a few germinal centers (338, 497, 615) have been found in the
thymus parenchyma, immunization did not, in experimental animals, lead to plas-
macytopoiesis and germinal center formation in the normal thymus (33, 41, 174, 656,
657). The cellular localization of immunoglobulin synthesis has been revealed by
immunofluorescent techniques. Cells synthesizing IgG, IgM, and IgA were plasma
cells and lymphoid cells in the germinal centers (e.g. 406). Cells in the thymus
synthesized only IgG and IgA. The plasma cells and medium-sized lymphocytes
responsible for this synthesis were localized predominantly in the perivascular
interstitial connective tissue and occasionally in the medulla near blood vessels.
It was apparent that these cells did not originate in the thymus but were trapped
from the circulation (205). Thymus tissue obtained from an immunized animal
and maintained in vitro did not elaborate gamma globulin in contrast to lymph
nodes from the same animal (655, 657). Immunization of mice with sheep erythro-
cytes did not lead to the production, in the thymus, of hemolysin-producing cells
(plaque-forming cells) (197, 455). However, a single dose of Salmonella enteritides
somatic antigen given intravenously did lead to the appearance of antibody-
producing cells in the thymus (345). Much larger numbers of such cells were found
in the spleens of these animals and even in the blood. As this antigen caused some
disorganization of thymus structure and marked thymus atrophy, it is quite con-
ceivable that the antibody-producing cells found in the thymus had migrated there
from other sites where they had been produced.

One report has claimed that subcutaneous autografts of thymus tissue im-
planted in thymectomized rats produced large numbers of plasma cells and germi-
nal center-like structures after immunization of the rats with diphtheria toxoid
(636). These experiments could not be repeated by others (425) nor could plasma
cells and germinal centers be found in thymus grafts of immunized thymectomized
mice (456).

Injection of antigens directly into the thymus has resulted in the production
of germinal centers, plasma cells, and immunoglobulins within the organ (388, 600).
When bovine serum albumin (BSA) and complete Freund's adjuvant were injected
into the thymus, plasma cells appeared generally in areas adjacent to the site of
the antigen and adjuvant deposit, and adjacent to blood vessels. No germinal
center formation occurred. Serum antibody titers to BSA, in those rats that re-
ceived injections of BSA directly into the thymus, were not detectable or very low;
in contrast, high titers were found in animals in which BSA had been injected into a lymph node or footpad. The injection of spinal cord plus adjuvant into the thymus failed to evoke a cellular reaction and failed to produce experimental allergic encephalitis in contrast to the regular production of the disease by footpad or lymph node injections (69). Marshall and White (388), using guinea pigs, found that if one thymic lobe was mechanically injured prior to injection of an antigen (Streptococcus pneumoniae type III capsular polysaccharide) at a distant site, germinal center formation and plasmacytopoiesis occurred. With the aid of immunofluorescent techniques the polysaccharide antigen could be seen in thymic tissue adjacent to the injured area, whereas in the uninjured lobe specific fluorescence was present mainly in the lumen of blood vessels and only very slight specific fluorescence was found in the cells of the cortex. On the basis of these findings, they suggested that the failure of the intact thymus to react to circulating antigen might be due to a barrier between the blood and thymus lymphocytes that prevented the entry of antigen into the thymus. Their experiments did not, however, exclude the possibility that reactive cells from the circulation could have accumulated in the injured area and initiated the reactions there.

The capillaries and small vessels in the thymus are lined by a relatively thick and unfenestrated endothelium and surrounded by a perivascular cuff consisting of extracellular connective tissue that forms a complete or incomplete concentric ring around the vessel. This is in turn bordered by a single layer of thymic epithelial cells or their processes resting on a basement membrane. Beyond this lie the intercellular spaces of the thymic parenchyma occupied by lymphocytes. These cells are therefore separated from the blood vessels and perivascular cuff by a barrier of epithelial cells and basement membrane (110, 701). Short discontinuities, however, do exist in the epithelial layer. The functional as well as anatomical integrity of the barrier has been questioned since it has been demonstrated that Thorotrast, a colloidal material, trypan blue, bovine albumin, India ink, ferritin, colloidal gold, and 125I-human albumin may pass from the blood to the cells of the thymic parenchyma. 125I-human albumin also entered the lymphoid cells (111). Furthermore, bovine albumin, injected intramediastinally in rats, could later be identified within the thymus (566).

The presence of large numbers of reticuloendothelial cells (histiocytes and macrophages) in the normal thymus (110, 337, 701), the uptake by thymus macrophages of particulate materials (254) and the rapid phagocytosis of pyknotic thymocytes after injection of adrenotrophic hormone or cortisone (300, 707) or exposure to ionizing radiations (268) indicate that a phagocytic mechanism can operate efficiently in thymus tissue. It is unlikely, therefore, that defects in phagocytic segregation of antigenic matter can account for the immunological inertia of the intact thymus.

It is possible that thymic lymphoid cells received such a strong proliferative stimulus that the response to antigenic stimulation by differentiation into antibody-secreting plasma cells becomes impossible within the microenvironment of the thymus cytoreticulum. Alternatively, it is possible that there is in the thymus a high concentration of a factor that suppresses the inductive phase of primary
immune responses. If either of these possibilities were true, one would expect that once thymus lymphocytes are taken out of the environment of the cytoreticulum they would be capable of reacting immunologically.

A blastoid transformation can be induced in a small percentage of thymus lymphocytes by phytohemagglutinin in cultures (106, 698) or in mixed cultures containing genetically dissimilar cells (593). It is not known, however, to what extent an immunological reaction is involved in these in vitro systems.

In contrast to spleen cells, thymus lymphoid cells generally failed to produce a primary response on transfer to an appropriate recipient (286). Mouse thymus cells injected with rat erythrocytes into preirradiated mice produced much less antibody than a similar number of spleen cells (655). Thymus grafts or thymus cells were capable of producing a secondary response in transfer studies (144, 269, 630, 631) but again spleen cells responded more effectively (631).

The existence of antigen-sensitive cells in a given population can be tested by transplanting the cells into lethally irradiated recipients and testing their capacity to produce, in the spleen, clusters of hemolysin-producing cells (325). Using this technique, antigen-sensitive cells could not be detected in the adult thymus (324).

No completely satisfactory evidence for the successful passive transfer of tuberculin sensitivity with thymic lymphocytes has yet been produced. Some success was claimed in the passive transfer of tuberculin sensitivity with thymus cells (614) but the reactions appearing in the recipient animals were rather small and unconvincing. Mouse thymus and "attached lymph nodes" successfully transferred tuberculin sensitivity passively in the mouse (131), but whether this was due to the thymus cells or to the lymph node cells cannot be determined.

In rats (63) suspensions of thymus cells from adult donors, even in very high doses (up to 50 million/recipient), failed to induce a graft-versus-host reaction in foreign newborn hosts. In the mouse, thymus cells from newborn or adult donors, were capable of producing such a reaction (61, 62, 64, 114, 435) "though rather fitfully" (63), and much less effectively than mouse lymph node or spleen cells. For instance, the production of secondary disease in lethally irradiated mice required 30 times as many thymus lymphocytes as lymph node cells (684). In order to prevent the protective action of allogeneic bone marrow in irradiated mice, 2-4 times as many syngeneic thymus lymphocytes as syngeneic lymph node cells were required (56, 119, 684). Parental strain thymus cells could induce a graft-versus-host reaction after inoculation into sublethally irradiated adult hybrid mice, although much less effectively than similar dosages of spleen cells (115, 317). Cell suspensions from newborn thymuses were as effective as cells from adult thymuses in producing graft-versus-host disease; newborn spleen cells, on the other hand, were ineffective at the same dose levels (114, 621). This suggests that there are a few cells in the thymus capable of inducing graft-versus-host reactions but that there is no relative increase with age in the number of these cells.

The ability of chick thymus cell suspensions to induce pock formation on the chorioallantoic membrane of the chick embryo (another manifestation of the graft-versus-host reaction) seemed to vary directly with the size of the population
of lymphocytes in the medulla (692). It may be, therefore, that the few cells present in the medulla are immunologically competent and this would be consistent with the observations that the medulla lymphoid cells morphologically resemble peripheral lymphocytes more than cortical thymus lymphocytes (484). Whether the medullary lymphocytes are competent cells trapped from the circulation or whether they are derived from noncompetent precursors present in the cortex is unknown.

In other experiments (182, 390), dissociated thymus cells were found capable of initiating a graft-versus-host reaction in the F1 hybrid mice only when the cells were obtained from parental donors that had been preimmunized against the antigens of the other parental strain. This finding is analogous to the apparent ability of thymus cells to produce a secondary response in the transfer experiments cited above.

The above experiments show that thymus cells removed from an immunized animal are capable, on transfer, of exhibiting a secondary response after repeated antigenic challenge, although the magnitude of this response is lower than that given by transferred spleen cells. Since some lymphoid cells from regionally stimulated lymph nodes can enter the thymus (207) it is possible that these are the cells that mediate the secondary response on transfer. On the other hand, the response of transplanted thymus cells to a primary antigenic challenge is weak and inconsistent. Two alternative possibilities can account for these findings. 1) According to one, the thymus contains no immunologically competent cells, the weak reactivity of thymus cell suspensions being due to contaminating, nonthymic lymphoid cells. It is not inconceivable that small numbers of immunologically competent, circulating pool, small lymphocytes may be passing through the thymus. The interlobular spaces in which lie the thymic blood vessels contain many lymphocytes and are actually extrathymic in location (603). Furthermore, the small parathymic lymph nodes closely adherent to the thymus capsule may not have been excluded from the cell suspensions used in some of the experiments. 2) According to the other possibility, the thymus is generating immunologically competent cells, but only a few have reached, in the thymus, the maturity that would enable their interaction with antigen. Some thymus lymphocytes, presumably the long-lived variety, may require time to achieve immunological maturity. This notion obtains support from the observations that some of the cells from the spleen and lymph nodes of thymectomized, thymus-grafted animals that proliferated during the course of a graft-versus-host reaction had originally been derived from the thymus implant (649). It was not, however, proved that the proliferating thymus-derived cells actually initiated the reaction. Further experiments are thus required to clarify the situation.

III. EFFECTS OF THYMECTOMY

1. Lymphocyte Population

Thymectomy is associated with a diminished population of lymphocytes. This has been reported in the mouse (235, 412, 437, 458, 515, 517), rat (9, 32, 58,
Thymectomy of 2-month-old mice was followed by a slow progressive fall in circulating lymphocytes to a minimum of 30–40% below normal levels (412, 458, 488). This low value persisted during an observation period of 4 months. Lymph node and spleen weights fell by 25%. The lymphoid follicles were less tightly packed with lymphoid cells and the germinal centers less prominent. There was a decreased mitotic activity (−28%) in the germinal centers and a decreased cell content (−30%) in the areas of the follicles (412). Regeneration of the lymphoid system, which normally occurs after irradiation-induced atrophy, could be partially prevented by thymectomy in the adult mouse (37, 222, 458, 462). By contrast, erythropoiesis and myelopoiesis were stimulated in the spleen during its regeneration in the postirradiation period (222).

Thymectomy of neonatal mice was accompanied by a striking lymphocyte deficiency. In sham-thymectomized mice, the absolute lymphocyte count and lymphocyte:polymorphonuclear ratio rose progressively during the first 8 days of life to reach almost the normal adult ratio. In mice thymectomized at birth, there was no significant increase in this ratio. The total white cell count 4–6 weeks after birth was just over half that of sham-thymectomized littermate controls, the decrease in absolute white cell count being mostly due to a lymphopenia (437, 439, 440, 443) affecting particularly the small lymphocyte population (448). The lymphocyte counts fell to even lower levels in older mice reaching values as low as 500/mm³ or 90% below control levels during the 3rd and 4th months (443, 446). When the thoracic ducts of 6-week-old mice thymectomized at birth were cannulated, the output of lymphocytes during the first 24–48 hr of drainage was only 1–3% of that of normal control mice of the same age and strain. The total number of lymphocytes drained in 48 hr was almost 100 million per average control mouse and never more than 3–4 million per neonatally thymectomized mouse. There was therefore a severe reduction in the population of "circulating pool" small lymphocytes in neonatally thymectomized mice (468). A marked deficiency of lymphocytes was reported in the lymphocytic fields of the lymph nodes and periarteriolar lymphocyte sheaths of the spleen (235, 439, 440, 443, 446, 448, 502, 515, 517, 519). These depleted areas were termed "thymus-dependent areas" (519). Corresponding areas in the rat are known to be traversed by the circulating pool small lymphocytes on their way from blood to lymph (247). All the experimental evidence is thus in accordance with the conclusion that the circulating pool small lymphocytes are a class of cells whose development is dependent on a normally functioning thymus.

Lymphoid follicles and germinal centers, on the other hand, were present in young neonatally thymectomized mice (519) although they tended to become smaller and less active with age and were often absent in older mice (235, 443, 502, 519). Plasma cells were not deficient and accumulated in the thymus-dependent areas in mice older than 6 weeks (517, 519). It is thus possible that, in the mouse, follicle cell production, germinal center formation, and plasmacytosis are not thymus-dependent processes; this possibility receives support from experimental work performed in birds and described below.

60, 385, 513, 514, 549, 586–588, 615, 668), guinea pig (113, 117, 167, 520, 551, 717, 719), hamster (5, 598), rabbit (322, 489, 637), opossum (454), chicken (125, 126, 295, 303, 639), dog (333, 659), and human (313, 522).
In contrast to the lymphocyte deficiency, neonatally thymectomized mice often showed evidence of a general hyperplasia of reticuloendothelial elements (444, 461, 589). The cortex of the lymph nodes was extensively replaced by tissue consisting of large numbers of reticular cells, histiocytes, and macrophages (461). There was an obvious increase in the size and number of Kupffer cells and the phagocytic activity, as measured by the rate of clearance of colloidal carbon from the blood, was increased in many mice (461, 589). Extensive extramedullary hemopoiesis was evident in the spleen of some thymectomized mice and was mainly responsible for the enlargement of the organ noted in some cases (461).

When thymectomy was performed in 2-month-old rats, the lymphocyte content of the blood and thoracic duct lymph fell 65 days later to 60% of normal levels but no significant changes occurred in the lymphocyte population and mitotic activity in the lymph nodes, spleen, and Peyer’s patches (58). When thymectomy was performed at 6 days of age, the thoracic duct lymphocyte output at 2 months of age was 26% of that of sham-thymectomized controls and the blood lymphocyte levels and weight of the cervical and mesenteric lymph nodes were markedly reduced (586, 587). An even more pronounced fall in the cell population of the thoracic duct lymph occurred when rats were thymectomized at birth (552, 553), and there was a considerable depletion of small lymphocytes in the peripheral blood, white pulp of the spleen, and lymphocytic fields of the lymph nodes, but no deficiency of germinal centers and plasma cells (688). There was no evidence of any decrease in the number of bone marrow small lymphocytes in rats thymectomized either at 7 days of age (265) or as adults (58-60). An increase in the number of marrow lymphocytes was found when thymectomy was combined with extensive resection of the lymph nodes and spleen (60). It was concluded that the origin of the marrow small lymphocyte must be independent of the presence of the thymus.

In the newborn guinea pig, subtotal thymectomy was followed by a generalized but transitory hyperplasia of the lymph nodes and Peyer’s patches, but not of the spleen (260), and total thymectomy was followed by an absolute decrease in the number of lymphocytes in lymph nodes and spleen (167), blood and thoracic duct lymph (171). Lymphopenia was also a feature of guinea pigs thymectomized at a young age (116, 117). When thymectomy was performed in young adult guinea pigs the thoracic duct lymphocyte levels fell to about 50% of normal but no significant changes were noted in either the weights or histological appearance of the spleen and lymph nodes (113, 549, 551).

Striking atrophy of the lymph nodes, Peyer’s patches, and spleen and lymphopenia were observed in male hamsters thymectomized during the first 4 weeks of life (5, 598, 599). Germinal centers and plasma cells were also absent in these animals. Thus some of the morphological features found after neonatal thymectomy in the mouse were also found after thymectomy at a much later age in the hamster.

In the rabbit, thymectomy resulted in some lowering of the percentage of blood lymphocytes (486) but neonatal thymectomy and appendectomy produced an even more pronounced depression (637). Moreover, there was either a lack or an immature development of lymphoid follicles and an almost complete lack of small lymphocytes in the lymph nodes and spleen.
At birth, the newborn opossum is still virtually an embryo. The thymus is a well-developed epithelial structure and does not contain any lymphoid cells (71). For this reason the newborn opossum would seem to be an ideal experimental animal to study the effects of neonatal thymectomy on the subsequent development of the lymphoid organs. Such a study was attempted (454), but because even a slight amount of blood loss would be incompatible with life, results were obtained only in "embryos" that were thymectomized at 10-12 days and at 24 days of age. The thymectomized animals were sacrificed when they were 28 and 45 days old. In the thymectomized animals the small and medium lymphocytes were markedly reduced in number in the lymph nodes and entirely absent in the spleen. The presence or absence of lymphocytes in the blood could not be adequately assessed because of the presence of large numbers of smudge cells. It was concluded that thymectomy had either removed the source of origin of splenic lymphocytes or had delayed the appearance of these cells in the spleen. In addition to these changes, myeloid tissue not only persisted but was found greatly increased in amounts at a time when it would normally have decreased. Maturation and proliferation of this tissue were abnormal, characterized by maturation arrest and ineffective erythropoiesis.

Decreased numbers of circulating lymphocytes were reported in dogs thymectomized as puppies (between 3 and 6 weeks of age). Furthermore, a marked diminution of small lymphocytes was observed in the spleen and lymph nodes of these dogs (659). In human patients treated for myasthenia gravis by thymectomy a moderate degree of lymphopenia became evident (313, 522).

Complete removal of the thymus in neonatal chicks has proved difficult and often small remnants of thymus tissue have been left behind. When less than 0.1 g of thymus tissue remained, there was a deficiency of small lymphocytes in the blood and spleen but no change in the number of plasma cells in the spleen (295, 303, 693). In chickens thymectomized and irradiated posthatching, cell depletion was marked in the periarteriolar lymphocyte sheaths of the white pulp of the spleen but not in the sharply circumscribed lymphoid follicles. The cells lacking were small lymphocytes, not plasma cells. By contrast, chickens bursectomized and irradiated at hatching lacked follicles and plasma cells, but not small lymphocytes in the periarteriolar sheaths. It was concluded that the bursa-dependent lymphoid tissue included lymphoid follicles and plasma cells, whereas the thymus-dependent lymphoid tissue was to be found in the periarteriolar lymphocyte sheaths (125, 126). Support for the concept of a bursa-dependent lymphoid tissue came from the disease visceral lymphomatosis, a viral-induced lymphoma, which is first manifested by a marked follicular hyperplasia and can be prevented by bursectomy in the early incubation period (524, 528).

The above studies performed in several species have shown that the most significant change associated with thymectomy is a depletion in circulating pool small lymphocytes. Deficiency of these cells was marked in the blood, thoracic duct lymph, and in those areas in spleen and lymph nodes normally traversed by circulating pool small lymphocytes. When thymectomy was performed in the neonatal period the depletion was evident at an early age, there being no postnatal increase in the number of these cells. In adults, thymectomy was associated with
a gradual decrease in the number of these cells, lowest levels being reached after
a period of several months. It seems, therefore, that the thymus influences the
development of the circulating pool small lymphocytes—their origin in early life
and the maintenance of their numbers throughout life. On the other hand, the
development of cells of the lymphoid follicles and of plasma cells is not a process
dependent on the thymus, at least in the chicken. Thymectomy is associated
with other changes that may depend on the fact that neonatally thymectomized
mice are susceptible to infections (see below). Thus infiltration of certain sites by
inflammatory cells and hyperplasia of reticuloendothelial elements may mask the
deficiency of circulating pool lymphocytes in the lymphoid tissues and even lead
to an increase in the size and cellularity of these tissues. A further contribution
to such increase may be caused by the proliferation of immature erythroid and
myeloid elements that occurs in some thymectomized animals.

2. Immunological Capacity

Since circulating pool small lymphocytes are immunologically competent
cells it can be expected that thymectomized animals show defects in immunological
performance. This was found to be the case and in general it can be said that
thymectomy impairs cell-mediated immune reactions, such as transplantation
immune reactions and delayed hypersensitivity reactions, but only few of the
responses mediated by humoral antibody. Immunological defects were more
evident when thymectomy was performed at birth than in adult life and have
been reported in a number of species: mice, rats, hamsters, rabbits, quokkas,
chickens, and frogs.

A) Circulating antibody and immunoglobulin production after neonatal thymectomy.
Varying antibody responses have been encountered in thymectomized animals
depending on the species and strain of animal used, the nature of the antigen given,
the age at which thymectomy was performed, the age at which antigenic challenge
was made, and the time after challenge at which the response was tested. Most
investigators have employed vastly different experimental designs so that it is
difficult to present a comprehensive picture of the effects of thymectomy on anti-
body production.

Adult mice, rats, hamsters, or rabbits, thymectomized at birth, produced
subnormal levels of antibodies in response to a first challenge with the following
antigens: sheep erythrocytes (51, 175, 198, 293, 446, 455, 456, 502, 507, 589,
609, 639, 646); Salmonella typhi H, O, and Vi antigens (293, 443, 446, 463, 639);
influenza A virus (446, 463, 639); T2 coliphage (235, 510); diphtheria toxoid
(31), human gamma globulin (599), ovalbumin (31); and bovine serum albumin
(22, 31, 51, 235, 308, 530, 649). On the other hand, normal or near-normal
responses were obtained after a first challenge with tetanus toxoid (279, 279),
hemocyanin (175, 293), Pneumococcus type III capsular polysaccharide (175,
293), Salmonella flagellar antigen (530), ferritin (175), MS-2 bacteriophage (51),
and polyoma virus (467).

It is apparent from recent work that the development of the capacity to
mount an antibody response may be delayed after neonatal thymectomy. When neonatally thymectomized mice were challenged with sheep erythrocytes at any time during the first 2 weeks of life they failed to produce in their spleens antibody-plaque-forming cells and no hemolysins could be detected in their serum. However, when they received their first challenge of sheep erythrocytes after 2 weeks they were capable of producing plaque-forming cells and hemolysins although the magnitude of the response was less than that of control sham-thymectomized mice (153, 554, 611, 705). The thymectomized mice thus exhibited both a delay in the development of immunological capacity and a peak response of magnitude lower than that expected for control mice of the same age. Several possibilities may account for these effects. The development of the capacity to react to sheep erythrocytes may be partly dependent on a system other than that governed by the thymus. The depressed response may be due to the fact that the proliferation of antigen-sensitive cells occurs at a lower rate in the absence of the thymus. Alternatively, the maturation of these cells from lymphoid precursor cells, originally processed in the thymus before birth and presumably present in the circulation at the time of thymectomy, may occur more slowly in the absence of a humoral thymus factor.

Chronic thoracic duct drainage has been associated in rats (401) and mice (469) with a depressed ability to produce a primary immune response. Drainage of about 100 million lymphocytes from normal 5-week-old mice was associated with a reduced number of sheep erythrocyte antigen-sensitive cells in the spleen and lymph nodes. In neonatally thymectomized mice the average total number of cells that could be drained was 3–4 million. After such drainage there were few antigen-sensitive cells remaining in the spleen and lymph nodes. In 1 million circulating pool lymphocytes there were about 60 antigen-sensitive cells when the pool came from sham-operated control mice and only 2–3 when the population came from thymectomized mice (468). This reduced proportion, in the thymectomized mice, suggests that the few cells that are responsive have been diluted out by cells that are insensitive to sheep erythrocytes either because they are qualitatively deficient to respond or because they form part of a population of cells committed to some other antigen. Assuming from the above figures that the total mobilizable pool of circulating lymphocytes in the 5-week-old sham-thymectomized mouse is approximately 100 million cells and 3–4 million cells in the 5-week-old neonatally thymectomized mouse, there are thus in the former pool 6000 and in the latter only 6–12 antigen-sensitive cells that can react to sheep erythrocytes. This clearly indicates the magnitude of the deficiency that exists in the neonatally thymectomized mouse. If all the available antigen-sensitive cells are recruited after challenge with sheep erythrocytes, the total number of plaque-forming cells per spleen at the peak of the response can be expected to be $6 \times 10^4$ in control mice and 600–1200 in thymectomized mice [assuming that 1 antigen-sensitive cell gives rise in the spleen to about 100 plaque-forming cells (325)]. In fact, the peak number of plaque-forming cells was found to be about $3 \times 10^4$ in control mice and about 1000 in thymectomized mice (705). This indicates that only 6% of all the available antigen-sensitive cells were recruited in the
normal mouse whereas close to 100% of the available responsive cells are recruited in the thymectomized mouse after a primary challenge. The normal mouse has thus a very large reserve of antigen-sensitive cells that can presumably be drawn on, depending on the circumstances attending the immunization.

Conflicting evidence is available concerning the ability of neonatally thymectomized mice to mount a primary and a secondary immune response. According to one report (610) the primary response to sheep erythrocytes was depressed but the secondary response was nearly normal as regards both the types of antibodies produced and the magnitude of the response. According to other reports (51, 278, 279) the ability to perform an anamnestic response was more affected by neonatal thymectomy than the ability to respond to a primary stimulation. In studies of the response to tetanus toxoid, normal or near-normal responses were obtained in neonatally thymectomized pathogen-free Swiss mice when challenged as early as 4 weeks or as late as 15 weeks. Twenty percent of the animals showed severe depression of the secondary response and the majority failed to respond to a third stimulation (278, 279). In another study, neonatally thymectomized C57BL and BALB/c mice failed to respond to a first challenge of sheep erythrocytes and bovine serum albumin but produced essentially normal titers of antibody after immunization with the coliphage MS-2 at 1 day or at 5-6 weeks of age. When a second challenge with the phage was given 25 days after the first, the secondary response was delayed and reduced in magnitude below that of control mice (51). The mechanism of the reduced secondary response in these particular cases has not been worked out. It is not known, for instance, whether the pool of antigen-sensitive cells available for recruitment in the primary response was limited or not. Recruitment of a limited number of cells could still be associated with a near-normal primary response but only a reduced secondary response. Alternatively, the thymus may influence the proliferation and differentiation of "memory" cells, although no evidence for this has been given. On the contrary, studies on the proliferation of antigen-sensitive cells in thymectomized animals suggest that the thymus exerts its influence during the differentiation of antigen-sensitive cells from more primitive precursors rather than during the response of antigen-sensitive cells to antigen (see section IV, 1).

There are conflicting reports about the ability of neonatally thymectomized mice to form immunoglobulins. Humphrey et al. (293), using C3H/ Bi and (C57BL × C3H/ Bi)F1 mice, found a delay in the time at which neonatally thymectomized mice began to synthesize immunoglobulins as compared to controls, although eventually similar levels were reached. The level of IgA was actually higher in the thymectomized mice than in the controls. In addition, antibody responses to sheep erythrocytes (IgM and IgC) and to Salmonella typhi H and O antigens were depressed. On the other hand, Arnason et al. (31) found that BALB/c mice thymectomized on the 4th day or 21st day of life reached adult IgC and IgM levels at the same age as normal controls, but serum IgA levels remained subnormal. Antibody responses to bovine serum albumin, diphtheria toxoid, and ovalbumin (these antibodies commonly reside in the IgA fraction) were depressed, but responses to Salmonella typhi O antigen were normal.
The presence of subnormal IgA levels in thymectomized mice corresponded to the low IgA levels also found in neonatally thymectomized rats (29, 30). The conflict has been resolved by the demonstration that the class called IgA by Humphrey was not the same class as that called IgA by Arnason. Arnason's IgA was not tested for by Humphrey (Arnason, personal communication). Thus, it has been shown that one of the four main immunoglobulin classes in mice is depressed after neonatal thymectomy. In another study, it was shown that the immunoglobulins of thymectomized mice did not differ qualitatively from those of normal mice on the basis of isoantigens, antigenic determinants, skin-sensitizing ability, and metabolic behavior. The synthesis of the two types of IgG molecules (IgG2 and IgG1) was either normal or increased after neonatal thymectomy; the low levels sometimes obtained with these proteins were due to accelerated catabolism; the IgA serum proteins were increased irregularly and the IgM proteins were usually normal (175).

Massive thymus involution and peripheral blood lymphocyte depletion were produced in rats by the administration of prednisolone at birth (78). Recovery of the thymus mass and blood lymphocyte levels had occurred by 3 weeks but the antibody response to Salmonella typhi H antigens was still depressed at that age. Diminished levels of IgA were found as late as 10 weeks. These effects were considered to be mediated by the action of prednisolone on the neonatal thymus.

The quokka, Setonix brachyurus, is an Australian marsupial having an external thymus in the neck and an internal one in the thorax. After a 28-day gestation period, the embryo migrates to the pouch, where it becomes firmly attached to the teat. Between 90 and 130 days, the size of the external thymus greatly exceeds that of the internal. External thymectomy performed before 30 days of age was found to reduce blood lymphocyte levels and to have a significant inhibitory effect on antibody production to sheep erythrocytes. No studies of the effect of internal thymectomy have yet been reported (622).

Thymectomy in the early life of the frog had an effect on antibody-producing capacity. Larvae of the American bullfrog Rana catesbeiana were thymectomized or sham-operated at 60 to 200 days of age and challenged with goldfish serum proteins: the majority of the controls produced specific precipitating antibodies whereas nearly all the thymectomized larvae showed markedly weakened responses (123).

Neonatal thymectomy in chickens had no consistent effects on immunoglobulin and antibody production (250, 693, 695). By contrast, surgical removal of the bursa of Fabricius at hatching, or inhibition of its development in ovo (220, 432, 540), was associated with considerable impairment of the capacity to produce antibody responses (101–103, 126, 220, 221, 250, 295, 296, 432, 481, 482, 511, 529, 540, 641, 696). The immunological defects of bursaless chickens could be accentuated by sublethal irradiation (125, 126). All chickens without a bursa lacked IgG (96, 126, 500, 529, 696) and when bursectomy was followed by sublethal irradiation the birds also lacked IgM and antibody responses were completely absent even after repeated antigenic stimulation (125, 126). By contrast, nonbursectomized birds subjected to irradiation recovered immunological capacity.
In summary, the antibody responses of neonatally thymectomized animals are variably affected depending on the species, the time of challenge, the nature of the antigen used, and other factors pertaining to the immunization. In some systems the primary response to a number of antigens was depressed; in other systems, the primary response was normal or nearly normal but the secondary response was delayed and reduced in magnitude. Thymectomy at birth does not appear to have had very significant effects on immunoglobulin levels, nor on the type of immunoglobulin produced. In birds, neonatal thymectomy has not impaired antibody production whereas bursectomy has consistently led to a reduction in the antibody response. The possible equivalent of the bursa in mammals is referred to later.

B) Delayed hypersensitivity and homograft reactions. There is considerable evidence that neonatal thymectomy impairs or inhibits the development of the cellular systems responsible for cell-mediated immune reactions. In the mouse thymectomized at birth, there was a marked impairment of the capacity to reject skin homografts from donors of foreign strains, not only from those closely related immunogenetically to the host (i.e. identical at the H-2 locus) (235, 392, 437) but also from donors differing at the H-2 locus and, in some cases, also from other species (135, 225, 235, 439, 440, 443, 446, 448, 463). The grafts remained intact with luxuriant growth of hair until death of the animals, which usually occurred from wasting disease, as described later. The defects in homograft immunity were evident even when mice were grafted with skin as early as 3 days after birth (448). A stronger depression of homograft immunity in male mice thymectomized at birth was reported in one study (45). Thymectomy between the 3rd and 7th day of life was still associated with some impairment of homograft immunity but only when immunogenetic differences between donors and hosts were slight (i.e. when there were no differences at the H-2 locus). Female C57BL mice thymectomized at 2 weeks of age developed no immune response to syngeneic male skin grafts but could reject allogeneic skin (235, 439, 443). Delaying thymectomy beyond the 1st day of life reduced the severity of the immunological deficiency (136, 235, 450). However, some defect in the response to skin grafts from donors of the same H-2 locus was still detected in mice thymectomized at 35 days of age (135).

Grafts of allogeneic tumors, a mammary adenocarcinoma (391), a mouse leukemia (518), a rat leukemia (448), a mast-cell tumor (398), and a human carcinoma of the cervix (505) became established in mice thymectomized at birth. Similarly, allogeneic lymphoid cells injected into young F1 hybrid mice thymectomized at birth were not rejected and produced a graft-versus-host reaction (235, 390, 448). Thymectomy at birth protected against the lethal hypersensitivity reactions associated with systemic lymphocytic choriomeningitis virus infection (161, 289, 562) and the local reaction following footpad inoculation (479). Neither partial thymectomy nor splenectomy at birth was found to be associated with impairment of cell-mediated immune reactions (437, 443, 446).

Cells from the lymphoid organs of neonatally thymectomized mice were less capable of inducing graft-versus-host reactions after inoculation into appropriate recipients than similar dosages of cells from normal mice (134, 235, 446, 463).
From 0.5 to 1 million thoracic duct lymphocytes from normal 5- to 6-week-old mice uniformly produced signs of graft-versus-host reaction into appropriate neonatal F\textsubscript{1} mice; by contrast, no evidence of any reaction was obtained after the injection of up to 1 million thoracic duct lymphocytes from 5- to 6-week-old neonatally thymectomized mice (464).

The repeated administration of rabbit antimouse serum from birth onward resulted in a marked lymphocyte deficiency and an impairment of delayed sensitivity to bovine albumin and tuberculoprotein in mice that had not been surgically thymectomized (565). The mice treated with antiserum from birth had pathological and immunological characteristics very similar to those of neonatally thymectomized mice.

Neonatal thymectomy has also impaired cell-mediated immune responses in species other than mice. In rats thymectomized at birth, skin graft rejection times were prolonged (26-28, 186), the ability to resist a graft-versus-host attack by allogeneic lymphoid cells was abolished (11), the growth of allogeneic tumors was uninhibited (186, 475, 523), the ability to produce delayed hypersensitivity reactions to tuberculin and to bovine serum albumin was impaired (26-28, 298, 308, 407), and the development of autoallergic encephalomyelitis was suppressed (28, 336). About 50\% of rats failed to develop an Arthus reaction, but the development of adjuvant arthritis was not affected by neonatal thymectomy (28, 298). As few as 2 million thoracic duct lymphocytes from normal rats were capable of producing a graft-versus-host reaction in appropriate newborn recipients. By contrast, up to 10 times as many cells from neonatally thymectomized donors were without effect (553). All these results strongly suggest that lymphocytes of the type responsible for the production of delayed hypersensitivity and transplantation immunity may either be lacking altogether or, if not, may be qualitatively deficient.

The survival of mouse skin in hamsters thymectomized as late as 2 weeks after birth was 4 times as long as that noted in controls (599). Allogeneic skin homograft reactions in 3- to 4-month-old tadpoles were impaired by thymectomy at 18-64 days of age (122). Thymectomy in newly hatched chickens caused a decrease in the capacity of the birds to reject foreign tissue (34, 303, 305, 679, 680, 693) and to develop delayed hypersensitivity reactions as measured by the occurrence of the tuberculin reaction (304, 305, 306, 644), allergic encephalomyelitis (304, 305), and allergic thyroiditis (306). On the other hand, bursaless chickens with intact thymus could reject homografts (34, 250, 295, 296, 512, 643, 696) and produce delayed hypersensitivity reactions (305).

In contrast to the above species, mongrel puppies thymectomized at birth were fully capable of rejecting renal homografts (184). This suggests that in some species such as the dog, in which the state of lymphoid development is well advanced by birth (323), the cellular system responsible for homograft reactivity has already developed to a stage where it can function, at least for a time, independently of the thymus.

C) Immune responses after thymectomy in the adult. No significant differences in antibody titers were reported in animals thymectomized or sham-operated in adult life and challenged within a few weeks with either microbial antigens, serum
proteins, or sheep erythrocytes (179, 263, 270, 379). Furthermore, the mean survival times of allogenic skin grafts were almost the same in mice (449) or rats (277) thymectomized or sham-operated as adults. In the case of skin homografts in mice (449) and Salmonella typhi H antigen in guinea pigs (179), the thymectomized animals reacted as vigorously as the controls to a second challenge. In the case of MS-2 coliphage (51), however, mice thymectomized as adults exhibited a secondary response of lower magnitude than controls. It is thus not possible to generalize concerning the effect of adult thymectomy on the secondary response. Depending on the species used, the antigen given, and the time of second challenge, normal or impaired secondary responses might be observed.

Work in the last few years has indicated that the adult thymus continues to influence immunological capacity. When animals were thymectomized in adult life and subsequently exposed to a sublethal dose of total-body irradiation they exhibited no recovery or a partial and delayed recovery of their capacities to produce a primary immune response. Sham-thymectomized irradiated controls had fully recovered immunologically within 3-4 weeks after irradiation (130, 458). These effects were observed in mice challenged with sheep erythrocytes (442), skin homografts (225, 358, 442), allogenic lymphoid cells (180), or tumor homografts (222, 223), in rats given sheep erythrocytes (132) and foreign skin (309), and in hamsters grafted with allogenic skin (556). An even greater and longer lasting depression of the capacity to produce an immune response was evident when mice (130, 140, 225, 458, 462, 604, 670) or rats (9) were thymectomized in adult life, exposed to a potentially lethal dose of total body irradiation, and protected with hemopoietic tissue. Thus there was little response to sheep erythrocytes when mice were challenged as late as 100 days after thymectomy and irradiation (130, 458). The capacity of such mice to reject skin homografts had not recovered even when they were grafted 223 days after irradiation (140). The greatest degree of impairment of the homograft response occurred when donor and host were most closely related: non H-2 difference > H-2 difference > interspecies difference (140).

A combination of thymectomy and immunosuppressive treatment has been tried in man after renal transplants (625, 626). Four patients, reported alive after 2 years, had excellent renal function and were no longer on steroid therapy. These findings cannot be interpreted, as the degree of matching with the donors was excellent in all four cases.

Thymectomy of adult mice was not effective in impairing the immune responses to an antigen given prior to irradiation: thus, thymectomized irradiated mice could give an adequate antibody response to a further challenge of sheep erythrocytes when the first challenge had been given before irradiation. The response to an unrelated antigen was, however, depressed (129). Moreover, thymectomy in the adult was not effective in altering a state of homograft reactivity when sensitization had been induced prior to irradiation (140).

Thymectomized irradiated mice protected with fetal liver (155, 156) appeared to have defects greater than those restored with adult bone marrow (48) presumably because fetal liver lacks the few immunologically competent cells that may be present in the bone marrow obtained from adult animals. Furthermore, thymec-
tomized irradiated mice protected with bone marrow showed no alterations in immunoglobulin levels in contrast to mice restored with fetal liver; these exhibited a significant hypergammaglobulinemia, different classes of immunoglobulins being irregularly increased in different mice (52). The significance of such immunoglobulin abnormalities is unknown.

When mice previously subjected to a potentially lethal dose of total-body irradiation are injected with allogeneic bone marrow, some delayed deaths occur from the development of "secondary disease," a syndrome similar to those seen in graft-versus-host conditions. The effect of adult thymectomy on the incidence of secondary disease in allogeneic radiation chimeras has been investigated. In one study (226), it was reported that the occurrence of delayed deaths could not be prevented although a slight prolongation of the survival time had been achieved by thymectomy. In another study (537) prior thymectomy, in those strain combinations with a high incidence of severe and early secondary disease, was associated with a significant decrease in the incidence of the disease and a delay in its development. It was considered that a class of cells in the inoculum of allogeneic marrow might require the host thymus in order to gain immunological competence and react against the foreign histocompatibility antigens of the host; in the absence of the thymus no immunological maturity could be achieved and no graft-versus-host reaction effected. Other investigators, however, did observe the development of secondary disease in thymectomized irradiated mice receiving allogeneic bone marrow (604). They concluded, therefore, that bone marrow must contain either some cells capable of mounting graft-against-host reactions or capable of maturing, in the absence of the thymus, to immunologically competent cells. This may well be the case with adult bone marrow. In experiments employing fetal liver cells, it was clearly shown that passage of the cells through intact, nonthymectomized hosts was required for maturation of the cells to the stage where they could initiate graft-versus-host reactions; passage through thymectomized hosts was inadequate (668, 669, 671).

A combination of steroid therapy, thymectomy, and splenectomy was ineffective in suppressing homograft rejection and humoral antibody formation in adult mice but a slight delay in second-set graft rejection was reported (729). Thymectomy in adults did not potentiate the immunosuppressive action of melphalan (370) although an additive effect was reported with other drugs (152).

Antilymphocyte sera administered to adult mice have interfered with their capacity to reject skin homografts (309, 361, 473, 487). It is interesting to note that the immunosuppressive effect of antithymus serum was far greater than that of anti-lymph-node (486) or anti-thoracic-duct lymphocyte sera (46). The immunological impairment may thus be produced not only as a result of an action against circulating lymphocytes, (361), but also by the suppression of some specific thymus influence implicated in the maturation of new immunologically competent cells (see section IV, 3). Recovery from the state of immunological depression induced by antilymphocyte sera was delayed in adult thymectomized mice (474) and rats (310). One study, however, showed no such delay (361).

As a general conclusion it may be said that the adult thymus is still required
in order to re-establish immune mechanisms when the immune system has been damaged or destroyed, and 2) that cells originating from the hemopoietic tissue of marrow or fetal liver can repopulate the myeloid and erythroid compartments of lethally irradiated mice but cannot, in the absence of the thymus, reach immunological maturity. The existence of a few immunologically mature cells in adult bone marrow is apparent from some of the experimental results.

Adult thymectomy, by itself, has not been associated with immediate defects in immunological capacity. However, when antigenic challenge was delayed for months after thymectomy, deficient response to bovine serum albumin (651) and sheep erythrocytes (423, 450) became evident. Furthermore, lymphoid cells from such mice had a diminished capacity to produce graft-versus-host reactions (450). These results imply that the thymus even in adult life must continue to influence the development of an adequate population of long-lived immunologically competent cells—presumably the circulating pool small lymphocytes. Only when this pool has become depleted, as a result of the limited life span of its cells, do defects in immune capacity become apparent.

D) Dissociation of immunological responsiveness. As has been seen above, thymectomy has led to a reduction in the number of circulating pool small lymphocytes and to defects in immune performance, particularly in cell-mediated immune reactions. On the other hand, plasma cells, lymphoid follicles, and germinal centers are less affected, and inconsistent effects on immunoglobulin and antibody production have been obtained. These findings have suggested the existence of two systems: one, a thymus-dependent system, composed of the thymus and circulating small lymphocytes and associated with the expression of "cellular immunity" as in delayed hypersensitivity and transplantation immune reactions; the other, a thymus-independent system, composed of plasma cells and follicular lymphoid cells and associated with the production of immunoglobulins and humoral antibodies (125, 126, 641, 696). The most impressive evidence for the existence of this thymus-independent system has come from the results of bursectomy and thymectomy experiments in the chicken, the results of which were briefly summarized above.

The equivalent of the bursa in mammals has not yet been unequivocally identified. It was first thought that the appendix in the rabbit was the mammalian homologue of the bursa: its development has features similar to that of the bursa (23, 24, 196) and combined thymectomy and appendectomy led to a more striking defect in antibody response than thymectomy alone (637). Neonatal thymectomy and appendectomy, however, by no means inhibited the development of plasma cells and the synthesis of immunoglobulins (322, 658). Later, the tonsils were considered (525), and more recently the Peyer's patches in the rabbit were claimed to be analogues of the bursa. Some rabbits subjected to surgical removal of the appendix, sacculus rotundus, and Peyer's patches and to total-body irradiation produced less antibody in response to bovine serum albumin and TAB vaccine than rabbits subjected to irradiation but not to resection of these tissue. In electrophoretic analyses of the sera, the experimental rabbits lacked a migrating protein that was present in the controls (124). However, although the immunological defects produced in this way were more marked, they were by no means as striking
as those observed after hormonal bursectomy alone in the chicken. The evidence that the Peyer's patches are bursal equivalents in mammals is thus still far from conclusive.

3. Susceptibility to Infections

During the last 100 years several investigators have reported a cachetic or wasting disease after thymectomy in a variety of animal species including frogs (1), guinea pigs (117), dogs (50), and goats (333). It is difficult to evaluate whether this "thymic cachexia" was the result of poor laboratory factors or sepsis or whether it was really an effect of thymectomy. Other studies, on the other hand, seemed to indicate that thymectomy in early life did not, in animals that remained healthy, lead to any alteration in growth, weight gain, or maturation (18, 513, 514). Thymectomy after 3 weeks of age has been performed in many strains of mice in the last 20 years, particularly in work on experimental leukemia. These studies have revealed no differences in weight curves, breeding behavior, or susceptibility to common laboratory infections in thymectomized and control groups of mice (reviewed in ref. 436). There were no significant differences in longevity, except in high leukemic strains in which life was prolonged as a result of thymectomy preventing early death from leukemia (204).

Prenatal inhibition of bursal differentiation by testosterone in chicks was followed by the development of a condition characterized clinically by diarrhea, poor growth, and poor survival, and anatomically by deficiency of the lymphoid system (481).

Mice thymectomized at birth grew normally but, after weaning, suddenly developed a syndrome characterized by wasting, lethargy, ruffled fur, hunched posture, periorbital edema, diarrhea, and death within about a week (439, 440, 443, 444, 446, 448, 463, 502, 515-517). This syndrome closely resembled those seen in graft-versus-host conditions such as runt disease in neonatal mice inoculated at birth with allogeneic lymphoid cells, F1 hybrid disease in F1 hybrid mice injected with parental lymphoid cells, and secondary disease in radiation chimeras (439, 461, 463). The earlier in life thymectomy was performed, the higher the incidence of the syndrome (444, 448). Thymectomy after 1 week of age was no longer associated with any significant mortality from this syndrome. Some strains of mice developed signs of wasting as early as 4 weeks after birth and others (particularly hybrid and noninbred mice) as late as 4 months (444, 448, 515, 517). About 50% of wasted mice had small discrete necrotic patches in the liver: granulomatous foci with aggregates of polymorphonuclear leukocytes, macrophages, and giant cells (160, 444, 517). These lesions have been associated with the presence of a hepatotropic virus (160). Other features of wasted mice were the lack of subcutaneous fat; thinness of skin and intestinal walls; marked deficiency of small lymphocytes in blood, peritoneal fluid, and tissues; anemia; and proliferation of reticuloendothelial elements in the cortex of the lymph nodes. The progression of the disease could not be arrested by high-vitamin diets or therapeutic doses of antibiotics (448).

A temporary period of weight loss was observed in mice thymectomized and
irradiated in adult life but no significant mortality was reported. The loss of weight occurred 30–70 days after irradiation (130, 462).

A small percentage of rats thymectomized at birth developed a similar wasting syndrome (308). According to some investigators (598) thymectomy of male but not female hamsters, as late as 2–4 weeks of age, was associated with the development of an essentially similar syndrome. The characteristic features were weight loss, dorsal kyphosis, unkempt fur, lethargy, lymphopenia, diarrhea, cachexia, lymphoid atrophy, atrophy of soft bones, periorbital and facial edema, pancytopenia, epistaxis, ataxia, relative plasmocytopenia, and hypogammaglobulinemia. Other investigators, however, could find no sex difference after neonatal thymectomy in hamsters (142, 556).

The pathogenesis of the postthymectomy wasting disease is debatable. The fact that it is associated with lymphoid atrophy and immunological deficiencies suggests either an infectious process (446) or an etiology based on the postulated trophocytic function of the lymphocyte (369). The resemblances between graft-versus-host disease and the postthymectomy wasting syndrome have suggested that autoimmune processes may operate in mice after neonatal thymectomy (461, 686; section VI, 2). However, a lot of experimental evidence is in accordance with the hypothesis that the wasting syndrome is precipitated by infectious factors.

1) Wasting disease does not occur universally: it has not been reported in neonatally thymectomized mice in some laboratories (278). In neonatally thymectomized rats, a litter effect was noted: most litters escaped but in a few, every thymectomized rat succumbed, individual animals being affected seriatim (308). These observations suggest the operation of an infectious process.

2) Neonatally thymectomized mice and rats were found to be more susceptible than sham-operated controls to infection with hepatotrophic viruses (160), herpes simplex virus (480), pyogenic bacteria (42), Mycobacterium leprae (543), Candida albicans (574), and to the endotoxins of Escherichia coli and Salmonella typhosa (574). In addition it was shown that the onset of wasting could be accelerated in neonatally thymectomized mice by repeated injections of S. typhosa endotoxin (574). A state closely resembling wasting disease has been induced in non-thymectomized mice by the injection of sterile suspensions of bacteria (164). Bacteria and endotoxins may thus be a factor in the pathogenesis of wasting syndromes. One study, however, showed no effect of endotoxin on thymectomized rats (534).

3) Injection of hydrocortisone into newborn mice has resulted in the development of a syndrome very similar to the wasting syndrome of neonatally thymectomized mice (582). Wasting was prevented when these animals were given oxytetracycline (151). Similarly, antibiotic treatment reduced the incidence of sepsis and wasting in neonatally thymectomized rats (42). Infectious processes may thus contribute to the development of wasting.

4) Pathogen-free mice thymectomized at birth have not developed any evidence of wasting disease (278, 279, 402).

5) Germfree mice thymectomized at birth and maintained in a germfree environment did not develop wasting disease (402, 460, 712) and showed none of
the proliferative or destructive lesions of the type seen in conventional thymectomized mice (402). When mice were removed from the germfree environment at the age of 120 days and exposed to the conventional environment, wasting developed within 4–8 weeks, only in the thymectomized mice, not in the controls (712). Allogeneic skin graft survival was not prolonged in about 30% of mice thymectomized at birth and kept in the germfree state. In the rest, graft survival was prolonged but not as extensively as in thymectomized conventional controls. The response of thymectomized germfree mice to sheep erythrocyte lysates was, however, depressed to the same degree as that of conventional controls (460). There are several reasons that may account for the less severe immunological deficiency of thymectomized germfree mice. In the conventional state, a higher proportion of the available immunologically competent cells in an already limited pool may become committed to bacterial antigens. Moreover, since thymectomized mice are highly susceptible to endotoxins, toxic factors may further limit the number of functional cells available for transplantation reactions.

6) When mice were injected at birth with hydrocortisone, the incidence of death in the germfree mice was significantly lower than in the conventional mice. The mortality in germfree mice was increased by monocontamination with Escherichia coli (542).

7) Wasting syndromes did occur in the germfree environment when newborn mice were injected with lymphoid cells from a foreign strain of germfree mice (402). This suggests that the wasting seen in graft-versus-host reactions is associated with the damage to various body systems resulting from an immunological onslaught of the injected cells. By contrast, the postneonatal thymectomy wasting syndrome, which cannot occur in the germfree environment, must be precipitated by factors of infectious origin.

IV. RECONSTITUTION OF THYMECTOMIZED ANIMALS

A number of methods have been tested for their capacity to protect against the effects of neonatal thymectomy and adult thymectomy and irradiation. They include injections of suspensions of cells from lymph nodes, spleen, thoracic duct lymph, bone marrow and thymus, transplantation of thymus tissue, and injections of thymus extracts.

1. Lymphoid Cells

A suspension containing $5 \times 10^8$ lymph node cells was effective in giving protection when injected into recipients of the same strain within a week after neonatal thymectomy (136, 448). Wasting disease was prevented in 60–80% of injected neonatally thymectomized C57BL mice. The survivors were capable of rejecting allogeneic skin homografts (445, 447, 448). Lymph node cells also restored the capacity of neonatally thymectomized mice to produce hemolysins in response to sheep erythrocytes (663), of neonatally thymectomized rats to produce anti-
bodies and develop delayed skin reactivity to bovine serum albumin (299), and of adult thymectomized irradiated mice to reject allogeneic skin grafts (225).

Spleen cells, like lymph node cells, were also effective in preventing wasting disease and restoring immune mechanisms in mice thymectomized at birth (136, 159, 447, 448, 517, 663). The dose capable of giving protection was as low as 5 million cells (447). Spleen cells from normal mice, but not from neonatally thymectomized mice (130, 462), effectively restored the capacity of thymectomized irradiated mice to produce hemagglutinins to sheep erythrocytes and to reject skin homografts (130, 156, 462). Discriminant spleen assays performed in thymectomized mice reconstituted with spleen cells showed that the restoration of immunological capacity was attributable to host cells (136).

Of considerable interest was the finding that 20% of the neonatally thymectomized mice restored with either spleen or lymph node cells died between 3 and 7 months of age (663). They exhibited a precipitous decline in body weight and drop in blood lymphocyte levels before death. It is conceivable that the pool of cells supplied in the inocula may have become exhausted through the commitment of some of the cells and the death of others: the thymus would normally be required to continue to supply such a pool with new cells.

It is probable that the effective cells in the spleen and lymph node suspensions were the circulating pool small lymphocytes, which are known to contain immunologically competent cells (or antigen-sensitive cells). When neonatally thymectomized mice were injected at birth or at 10 days of age with pure suspensions of thoracic duct lymphocytes they became capable of reacting to sheep erythrocytes as effectively as normal mice. Thymectomized mice receiving an intravenous injection of $10 \times 10^6$ lymphocytes the day after thymectomy produced, in response to a challenge of sheep erythrocytes at 10 days of age, a far greater number of plaque-forming cells in their spleens than uninjected sham-thymectomized control mice. In addition, the number of entities, detectable as antigen-sensitive cells by the hemolytic focus assay method, present in the spleens of thymectomized mice inoculated with lymphocytes increased in response to antigenic stimulation at the same rate as that in the spleens of normal immunized controls (705). These results clearly show that antigen-sensitive cells can undergo a burst of proliferation and differentiation to antibody-producing cells in response to the antigen (sheep erythrocytes) and that they can do this as efficiently in thymectomized hosts as in sham-operated controls. Neonatal thymectomy thus does not impair the proliferation and differentiation of antigen-sensitive cells once they have been produced: it must therefore inhibit the production of antigen-sensitive cells from some more immature antigen-insensitive precursors.

In contrast to spleen cells, neither fetal liver cells (130, 156), nor bone marrow cells in doses of up to 40 million cells/mouse (130), enabled thymectomized irradiated mice to recover normal immune functions. The marrow cells in these experiments had 20% of cells classifiable on morphological grounds as small lymphocytes. Functionally, therefore, these cells must be different from those found in the spleen, lymph nodes, and thoracic duct lymph, i.e. from the cells of the circulating pool. Marrow cells also failed to protect against the effects of neonatal thymectomy in some experiments (447) but were successful in others (663).
The success of bone marrow cells in restoring immune functions in neonatally thymectomized mice is difficult to reconcile with the known immunological incompetence of marrow cells: their general incapacity to produce graft-versus-host and other immune reactions (64, 324) and their general failure to restore immune functions to thymectomized irradiated mice (section II, 2). Different strains of mice may possibly have had varying degrees of contamination of their marrow compartments by immunologically competent cells and even by immunologically activated cells (346, 654) coming from elsewhere. This may account for some of the discrepancies in the data reported from various laboratories.

Many investigators have found that intravenously injected thymus cells in doses of up to $20 \times 10^6$ cells/mouse are much less effective than even $5 \times 10^6$ spleen and lymph node cells in correcting the immunological defects of neonatally thymectomized mice (136, 159, 437, 439, 443, 447, 448, 493, 466, 516, 663). Only huge numbers of thymus cells (100–300 million cells) could restore immunological capacity (721) and even reverse wasting diseases (280). In these experiments, neonatal thymus cells were as effective as adult thymus cells and syngeneic as well as allogeneic cells protected. Graft-versus-host assay of spleen cells from treated thymectomized mice led to the conclusion that the restoration was dependent solely on the immunological capacity of the injected cells. The restoration of thymectomized mice by thymus cells in doses above 100 million cells/mouse suggests that there is in the thymus a minor population of cells already on the pathway of differentiation to immunologically competent cells. As discussed in section II, 5, it is not inconceivable that a small percentage of thymus lymphocytes—the long-lived variety—can, after undergoing a process of maturation, act as immunologically competent cells. They may indeed be the precursors of the circulating pool small lymphocytes.

2. Thymus Grafts

The lymphocyte deficiency and immunological defects of neonatally thymectomized mice (136, 159, 437, 439, 443, 446, 448) and adult thymectomized irradiated mice (154, 360, 462) have been corrected by grafting either syngeneic normal (159, 437, 439, 446, 448), syngeneic preirradiated (154, 452, 456), or allogeneic (136, 154, 439, 446) thymus tissue in a subcutaneous site (136, 154, 159, 439, 446) or under the renal capsule (154, 159, 360, 456, 462).

The regeneration of syngeneic normal thymus tissue was identical, both histologically and cytologically, in thymectomized mice and in normal or sham-operated controls (154). As described in section II, 2, the implant regained normal thymus architecture within a week. It was composed of donor-type cells during the first 10–15 days but gradual and complete replacement of the mitotic lymphoid cells by host-type cells occurred during the subsequent week. Neither thymectomy nor preirradiation of the host affected this regeneration pattern. Some evidence of restoration of immune capacity was obtained in neonatally thymectomized mice bearing for only 1 week normal syngeneic thymus implants (456). Whatever effect the thymus implant exerts must thus be relatively rapid.

In contrast to syngeneic grafts, allogeneic thymus tissue implanted into normal
hosts lost the lymphocytes surviving in the peripheral rim and failed to undergo lymphoid differentiation. Instead, foci of myeloid cells appeared and the graft was eventually rejected after having been infiltrated by typical small lymphocytes and histiocytes. Irradiation of the host only delayed, but did not prevent, rejection of the graft. A similar sequence of events occurred in allogeneic thymus tissue implanted into adult thymectomized, irradiated, and marrow-protected mice (154). The grafts failed to become lymphoidal, never showed the presence of proliferating marrow-donor-type cells, and were eventually rejected. The thymectomized irradiated mice bearing allogeneic thymus grafts showed partial restoration of their immunological capabilities and were able to reject skin of thymus-donor type with an accelerated second-set response (360). These results clearly bring out the following: 1) the immunologically competent cells enabling thymectomized irradiated mice bearing allogeneic thymus grafts to perform transplantation immune reactions must have been derived from precursor cells in the injected marrow and not from cells of the thymus implant, which was itself rejected; 2) only a very short period of time was required for triggering the maturation of marrow precursor cells toward immunological competence; 3) this maturation must have been induced by a humoral mechanism initially dependent on the existence of the surviving epithelial cytoreticulum of the thymus implant; and 4) such a humoral mechanism could not have been strain specific. Similar observations have been made in thymectomized irradiated mice implanted with allogeneic thymus tissue and tumor grafts: a C57BL thymus homograft reactivated the immune response of thymectomized irradiated C3H animals against a C57BL sarcoma (177). Similarly, when hamster or rat thymus was grafted into neonatally thymectomized mice, 60–70% of the mice became capable of rejecting allogeneic skin grafts and of producing normal hemolysin titers in response to sheep erythrocytes. The xenogeneic thymus grafts did not become lymphoidal: only a few epithelial cells were discernible at 30 days and eventually central necrosis and polymorphonuclear cell invasion took place and fibrosis followed (350). These experiments strongly suggest that the thymus exerts a directive influence on lymphoid precursor cells to trigger off their maturation to immunologically competent cells.

The reactivity of bursaless chickens to human erythrocytes could be restored by either syngeneic or allogeneic bursal grafts (296). The allogeneic grafts, however, were rejected, as the chickens had not been thymectomized. The fact that they had restored antibody-producing capacity suggests that the bursa must exert some humoral influence on the differentiation of antibody-producing cells from precursor cells derived from the host.

Allogeneic thymus tissue implanted into neonatally thymectomized mice behaved like syngeneic grafts in striking contrast to its behavior in adult thymectomized irradiated mice. Thus the implants did regain normal architecture by the proliferation of donor elements surviving in the peripheral rim. After 2 weeks, gradual replacement of the donor lymphoid cell population by incoming host cells took place as in syngeneic grafts. The allogeneic grafts were not rejected and their hosts generally developed normal immunological capacity. However, in many cases they were specifically immunologically tolerant of thymus-donor-type skin
It is possible that this tolerance had been acquired through the escape of thymus-donor-type lymphocytes during and after the grafting procedure.

Thymus tissue preirradiated in vitro was depleted of lymphocytes and consisted only of the epithelial cytoreticulum. Between 4 and 8 days small "lymphocyte-like" cells, with practically no cytoplasm and a nucleus containing an extremely dense chromatin structure, appeared first in capillaries and then scattered in the epithelial cytoreticulum (154). These small cells progressively increased in numbers between the 6th and 7th days appearing at random throughout the epithelial cytoreticulum. Under the light microscope, some of these cells, because of their condensed nuclear mass, might be interpreted as pyknotic cells (154). As viewed with the electron microscope, these cells resembled small lymphocytes in terms of their small size (6–7 μ in diameter) and the dense coarsely clumped nuclear chromatin. They differed from the small lymphocytes of mouse thymus by the presence of 1) relatively large, usually multiple, nucleoli; 2) a high concentration of cytoplasmic ribosomes, most of which were freely arranged as small polysomal aggregates, a few being arranged along short segments of endoplasmic reticulum; 3) numerous microvesicles; and 4) a relatively conspicuous cell center containing well-developed Golgi elements and centrosomes (67). These features tend to set this class of small lymphocyte apart from the typical thymus small lymphocytes, the majority of which lack nucleoli (484). As in allogeneic implants, foci of myeloid cells appeared in the irradiated implants but, in contrast to allogeneic grafts, large lymphocytes and dividing lymphoid cells of the host type were seen by 7–8 days. By day 15–20 normal thymus architecture was restored (154). The origin of the small "lymphocyte-like" cell was considered to be from the host rather than from the graft since these cells were totally absent in implants grafted to lethally irradiated hosts not injected with bone marrow (452). Evidence was obtained that hosts bearing preirradiated thymus implants developed some degree of immunological capacity even if the implants were allowed to reside for only a period of 1 week. Preirradiation of thymus tissue with doses of 2000 r appeared to have impaired its capacity to restore immunological function in neonatally thymectomized hosts. Preirradiation with 500 r did not have this effect (456).

The extent and nature of the contribution from the thymus graft to the host have been studied. By the use of chromosome markers, it was found that both host-type and donor-type cells were dividing in the lymphoid tissues of neonatally thymectomized hosts (189, 266, 439, 446, 448, 463, 465, 466), that usually the majority of these dividing cells were host type, but that the proportion of thymus-donor-type cells increased significantly after specific antigenic stimulation (456). In thymectomized irradiated mice protected with marked marrow and bearing marked thymus implants, both marrow-donor-type and thymus-donor-type cells were found dividing in the lymphoid tissue and again, the majority of dividing cells were of marrow-donor origin (154) but a sharp increase in the proportion of thymus-derived cells occurred for a short period of time after antigenic stimulation (138). The significance of this increased proliferation in response to antigenic stimulation is not known. By using a transfer system in vivo it was shown that although thymus-derived cells responded vigorously to antigenic stimulation by
mitosis, they were not capable of antibody production (139). The antibody-producing cells had the chromosome marker of the marrow donor, but the experimental design failed to establish whether these marrow-donor-type cells had or had not been derived from a population that had migrated through the thymus graft. In other experiments, it was shown that suspensions containing adult marrow and thymus cells were far more active in producing hemolysins against sheep erythrocytes when transferred to irradiated syngeneic recipients than could be accounted for by summating the activities of each cell population alone (107). It seems, therefore, that some type of interaction may take place between thymus-derived cells and bone marrow-derived cells, but the nature of this interaction is obscure.

Discriminant spleen assays on the spleens of mice thymectomized at birth and grafted with allogeneic thymus tissue showed that host cells, not thymus-donor-type cells, were in general responsible for immunological reactivity in the restored mice (136). These findings suggest that the thymus acts by exerting some directing influence on host lymphoid precursor cells. This is in agreement with the results obtained with allogeneic thymus grafts in adult thymectomized irradiated hosts and with syngeneic irradiated grafts where no thymus-donor-type cells remained and only host cells could participate in the immune response. However, it is not established whether host cells must first migrate into the thymus environment in order to be triggered off or whether they can mature elsewhere under the directive influence of the thymus. Evidence for the latter possibility has been obtained in experiments where the thymus was enclosed in cell-tight diffusion chambers, as will be seen below.

In summary, it is evident that full lymphocytic and immunological reconstitution of thymectomized mice occurs after syngeneic thymus grafting. Although thymus-derived cells may be found in the lymphoid tissues of the restored host, the evidence obtained from reconstitution experiments with allogeneic thymus grafts points to the likelihood that the specific function of the thymus is to exert a directing influence on precursor lymphoid cells in order to trigger off their differentiation and maturation to immunologically competent cells.

3. Thymus in Diffusion Chambers

In order to test whether a direct cellular transfer between thymus implant and thymectomized host was crucial for the restoration of immune capacity, embryonic, neonatal, or adult thymus tissue was placed in cell-tight Millipore diffusion chambers into the peritoneal cavity of young mice (362, 363, 465, 506, 507), rats (10, 372), hamsters (715), and rabbits (664) thymectomized at birth and into adult mice thymectomized and irradiated (47, 462). Immunological responses to foreign skin grafts and to sheep erythrocytes or human gamma globulin were restored in the majority of these animals. However, restoration of lymphocyte populations in the blood and lymphoid organs did not occur consistently. Levey et al. (362) and Trench et al. (664), using chambers made of Millipore material having a porosity of 0.45 μ, claimed that there was a restoration toward normal of blood lymphocytes, but Osoba (502) and Wong et al. (715), using chambers with 0.1 μ
porosity, did not observe any significant increase in the lymphocyte population of the blood and lymphoid organs. Barclay (47) also noted no change in lymphocyte levels of immunologically restored, adult thymectomized-irradiated mice bearing either neonatal or adult thymus in diffusion chambers. Other lymphoid organs, i.e. spleen and lymph nodes, enclosed within diffusion chambers did not restore immune reactivity. Allogeneic thymus was as effective as isogeneic thymus (502). Embryonic thymus, from 14-day-old embryos, was also effective (506, 507). Thus, embryonic, newborn, and adult thymus within diffusion chambers appeared to elaborate a specific substance, not present in spleen or lymph nodes, that restored immunological capacity in neonatally thymectomized and thymectomized-irradiated mice.

Histological examination of the thymic tissue in the diffusion chambers several weeks after implantation usually revealed only epithelial cells, fibroblasts, and varying amounts of fibrous tissue interspersed with areas of necrosis (362, 502, 506, 507). Lymphocytes in the thymus tissue degenerated within the first 2 weeks after implantation and were seldom found thereafter (504, 507, 704). Thus, it has been suggested that the epithelial cells of the thymus are responsible for elaborating the specific factor. This is supported by the finding that implantation of diffusion chambers containing thymic tissue devoid of lymphocytes has resulted in restoration of thymectomized hamsters (715). Cytological indications of secretory activity in thymus epithelial cells have been documented by electron-microscopic studies (112, 704).

The results of the diffusion chamber experiments have been interpreted as indicating that thymic tissue is capable of elaborating a humoral substance that acts on lymphoid precursor cells present in the tissues of neonatally thymectomized mice, to trigger off their differentiation and maturation to immunologically competent cells (447, 502, 506, 507).

Analogous experiments in bursectomized chickens bearing fragments of bursa in diffusion chambers have indicated that the bursa of Fabricius may also elaborate a humoral substance that acts on peripheral lymphoid cells (307, 632).

Supporting evidence that a humoral factor plays a role in the maturation of immunological faculty comes from experiments in which neonatally thymectomized female mice were allowed to become pregnant and to deliver a litter of babies (503). After delivery, the parous females showed a normal capacity to respond to allogeneic skin grafts and sheep erythrocytes. The induction of a "pseudopregnancy" (producing multiple corpora lutea in both ovaries that persist for 15-16 days) did not restore immune responses and these mice were still susceptible to wasting disease (504). Thus, it is unlikely that elevation of the hormones accompanying pregnancy was responsible for the restoration of immune reactivity in the pregnant mice. However, the possibility that a specific restorative factor was produced by the placenta cannot be excluded.

Two other possible mechanisms could explain the restorative effects of pregnancy. First, it is possible that fetal lymphoid cells may have traversed the placenta, seeded the maternal lymphoid tissues, and later were instrumental in initiating immunological responses. The experiment was therefore designed so that a search
could be made for this possibility. Neonatally thymectomized CBA female mice were mated with T6 males, the cells of which contain two small chromosomes easily identified during metaphase. After cytological analysis of more than 100 maternal lymphoid cells in metaphase, no cells bearing the marker could be found (504). Furthermore, the parous females rejected T6 skin grafts within a normal time interval, thus indicating that their tissues were not chimeric. Thus, the creation of a chimeric state in the maternal tissues by a transplacental migration of fetal cells to the mother seems highly unlikely.

The migration of maternal cells into the fetal thymus and subsequent return to the maternal tissues was excluded by cytological examination of the newborn thymus (504).

The second possible mechanism by which pregnancy may restore maternal immune responses is a humoral one, i.e. the transplacental passage of a humoral substance from the fetus to the mother. Arguing from the known restorative effect of embryonic thymus tissue enclosed in diffusion chambers, it seems likely that a humoral substance produced by the fetal thymuses in utero was responsible for restoring maternal immune reactivity in the pregnancy experiment. However, it must be emphasized that direct evidence of the operation of such a mechanism is still lacking.

4. Thymus Extracts

Although the diffusion chamber and pregnancy experiments indicate a specific humoral factor capable of restoring immunological reactivity in neonatally thymectomized animals, direct evidence that the thymus produces such a factor under physiological conditions is still lacking. Such evidence could be provided by experiments with thymic extracts that specifically restore immunological reactivity.

A number of claims have been made for the isolation of thymic extracts with a variety of actions. Only those claims that bear on the lymphopoietic and immunological roles of the thymus are considered here. Thymic extracts have been claimed to a) induce a lymphocytosis in the peripheral blood (73, 93, 410, 411, 490, 544, 620), b) increase the size and cellularity of lymph nodes (330, 331), c) increase the incorporation of 3H-thymidine into DNA synthesis in lymph nodes in vivo (330, 331), and d) increase the rate of DNA and RNA synthesis in suspensions of rabbit mesenteric lymph node cells in vitro (227).

When plasma from patients with chronic lymphocytic leukemia was injected intracerebrally into 1-day-old mice, a twofold increase in the number of lymphocytes in the blood was produced by the 5th or 6th day (408). Subsequently lymphocytosis-stimulating activity was found in the plasma of patients suffering from lymphosarcoma and myelofibrosis (409). The plasma of normal humans did not exhibit any lymphocytosis-stimulating activity but emulsions of normal human thymus as well as thymus from patients with the above diseases were active. Mouse thymus emulsions showed similar activity and it was concluded that in normal individuals the origin of the lymphocytosis-stimulating factor was the thymus (410). These findings were in accord with the work of Bomskov and
Sladovic (73) and of Rehn (544), who showed that oily extracts of thymus produced a lymphocytosis in rats, guinea pigs, pigeons, and man. Others (93, 490, 620) have also reported a lymphocytosis after injection of saline thymus extracts.

More recently Klein et al. (330, 331) reported an enhancement of incorporation of $^3$H-thymidine into DNA in lymph nodes of mice treated with calf, rat, or mouse thymus extracts. Further purification of material from calf thymus by Goldstein et al. (227) yielded a carbohydrate-containing protein that had the capacity to increase the incorporation of $^3$H-thymidine into DNA in suspensions of rabbit mesenteric lymph node cells in vitro, as well as retaining activity in vivo in mice. Calf serum also exhibited an enhancing effect in vitro, but bovine serum albumin, phytohemagglutinin, and an extract prepared according to a method described by Comsa and Bezsonoff (118) were inactive. In contrast to Metcalf's lymphocytosis-stimulating factor, the extract prepared by Goldstein et al. was heat stable and dialyzable.

Although the extract described by Goldstein et al. appears to have some effect on DNA synthesis, its biological activity and specificity are still obscure. It would be of interest to determine whether any of the extracts mentioned have any effect on the development of immunological reactivity. Dalmasso et al. (136) found a growth-promoting substance (Promine) (645) to be ineffective in preventing wasting disease or restoring immune responses after neonatal thymectomy. Except for one unconfirmed report (620) simple saline extracts of thymus also have not prevented the immunological deficiency seen in neonatally thymectomized mice (136, 448). Very recently, it has been claimed that repeated injections of a protein fraction derived from calf thymus into neonatally thymectomized and adult thymectomized irradiated mice restored immunological mechanisms (663a).

V. IMMUNOLOGICAL TOLERANCE

Acquired immunological tolerance has been defined as a specific central failure of the mechanism of immunological response brought about by antigenic exposure. Tolerance can be induced to cellular antigens as well as to protein antigens, even when these are highly immunogenic proteins such as bacterial antigens. Tolerance can be induced in newborn animals and in adult animals also, provided certain experimental conditions can be fulfilled. Recent work has revealed that there are two zones of antigen dosage capable of inducing tolerance or paralysis: subimmunogenic microgram doses and much higher "paralyzing" doses. Intermediate dosage levels result in immunity rather than tolerance (471, 479, 597). Other experiments have revealed that the immunogenic capacity of a protein can be abolished when material suited to phagocytosis is removed (149). It can thus be stated, as a general rule, that low doses of antigen will paralyze in circumstances when immunization can be avoided, and that high doses are required where this cannot be arranged (472). The current hypothesis to account for tolerance induction (472, 495) is that encounter between an immunologically competent cell and antigen free in the extravascular fluid leads to tolerance,
either by destruction of the antigen-reactive cell or by some alteration of the cell's genetic mechanism. Immunization will result when the antigen is sequestered by macrophages or specialized dendritic reticulum cells so that, on the one hand, free access of antigen to circulating immunologically competent cells is not permitted and, on the other hand, the antigen is subjected, within the macrophage, to some type of processing that renders it immunogenic.

Does the thymus play an active and specific role in the induction of tolerance? This is an important question, particularly in connection with the problem of tolerance to native antigens (tolerance to self) and the development of autoimmunity. Does native antigen act on precursor cells, during their intrathymic differentiation to immunologically competent cells, to eliminate from the population any self-reacting cells? Is the exclusion of a foreign antigen from the thymus necessary to ensure comprehensive immunological development? Is this exclusion effected by a blood-thymus barrier? Can tolerance to foreign antigens be induced whenever these penetrate the barrier? Experimental evidence has shown that the successful induction of actively acquired homograft tolerance has coincided with the achievement of thymic chimerism, suggesting that the presence of allogeneic cells within the thymus might be an essential prerequisite for the induction and maintenance of tolerance (208). In the case of noncellular antigens, complete tolerance was associated with the antigen reaching and being diffusely spread throughout all the lymphoid areas, including the thymus (496). This association between the presence of antigen or cells within the thymus and the existence of tolerance need not necessarily reflect a cause-and-effect relationship: it may be a chance accompaniment, an epiphenomenon simply reflecting the level of antigen distribution in the body as a whole. On the other hand, if antigen must be present within the thymus for complete tolerance to be achieved, the target cells for tolerance induction might be the few immunologically competent cells existing there. The presence of antigen within the thymus of tolerant animals does not necessarily prove that tolerance is induced by an effect of antigen on a population of immature lymphoid precursor cells.

Further experiments have been set up in mice and rats in an attempt to determine whether tolerance is a property associated with the thymus. In one series of experiments, it was reported that injection of allogeneic lymphoid cells into the thymus of irradiated mice resulted in specific homograft tolerance and that grafts of thymus from donors tolerant of allogeneic cells conferred specific homograft tolerance on newborn or irradiated recipients (662, 678). Since, however, such thymuses or thymus grafts were made up of a chimeric population of lymphoid cells, tolerance may well have been produced in the classic way as a result of the systemic dissemination of the allogeneic lymphoid cells into the newborn or irradiated adult hosts. In another series of experiments, thymectomized irradiated adult rats were grafted with thymus from syngeneic donors, which were tolerant of bovine gamma globulin, and were found to exhibit a specific unresponsiveness to the antigen for a period of 3–6 weeks (297). Furthermore, when the antigen was injected directly into the shielded thymus of irradiated rats, the animals were specifically tolerant 3 weeks later (624). Grafting of thymus
from normal adult donors treated with large doses of bovine serum albumin over the preceding 36 hr also conferred specific nonreactivity for 3–6 weeks in thymectomized irradiated recipients (618). The unresponsiveness in these experiments was associated with the delayed hypersensitivity response and the formation of mercaptoethanol-resistant antibody. The findings suggested the hypothesis that interaction of antigen with lymphocyte precursors in the thymus was the basis of the tolerance induced. According to this hypothesis, therefore, the target cell for tolerance induction must be the lymphoid precursor cell: contact of antigen with this cell, during its maturation within the thymus environment, would result in tolerance. Other findings are at variance with this conclusion. Specific immunological tolerance to defined antigens has been induced in adult thymectomized animals, indicating that the lymphoid system can be rendered tolerant independently or in the absence of the thymus (109, 180). Furthermore, neither the age of an animal nor procedures such as irradiation, cytotoxic drugs, or thymectomy were found to affect the rate of induction of tolerance (472). Susceptibility to tolerance induction thus does not seem to be related to the stage of maturation of the immunologically competent cells. In the experiments on the transfer of “tolerant” thymuses (297, 618), it was stated that the controls ruled out the possibility that free proteins transferred at the time of thymus implantation could have been responsible for tolerance induction in the recipients. This may be true, but the assumption is unwarranted unless further data are made available concerning the molar concentrations of antigen present in the recipient. The doses used in the controls (which showed no tolerance) were in the intermediate range of Mitchison (471, 472) and hence capable of immunizing. The antigen dose carried over by the thymus transplant was estimated to be of the order of micrograms, roughly within the range shown by Mitchison to be required for low-zone paralysis. Experiments with serum proteins have shown that the immunogenicity may be abolished when phagocytosable material is removed, for instance by centrifugation (149) or by in vivo passage (194). Intrathymic passage of serum proteins in the tolerant thymus transfer experiments may have achieved just such an effect. Phagocytosable material in the proteins might have been removed by the thymic macrophages, leaving nonimmunogenic antigen free to diffuse out into the general circulation. There is, therefore, no positive evidence to show that lymphoid precursor cells are the target cells for tolerance induction. There is, on the other hand, a lot of experimental evidence demonstrating that tolerance is a property of a population of “mature” immunologically competent small lymphocytes (249, 472).

Tolerance breaks presumably when new virgin immunologically competent cells are recruited and there is no antigen available to drive these new recruits toward tolerance. An increase in the number of potentially reactive cells occurs during the postirradiation recovery phase: in the absence of a continuing concentration of antigen the new cells react normally to the tolerogen when it is injected so that the state of tolerance is broken (381, 495, 629). There is evidence that the breakdown of tolerance occurs by means of a thymus-dependent mechanism that allows the development of new, uninhibited cells (722). Since the thymus
is essential for the recovery of immune functions after total-body irradiation (442, 458), it seems likely that it is also responsible for the breakdown of tolerance under these circumstances. This has, in fact, been found to be the case. The spontaneous disappearance of tolerance to bovine gamma globulin in adult mice could be hastened by 450 r whole-body irradiation only if the thymus was present (108). Other experiments clearly showed that thymectomy of adult animals tolerant to a specific antigen prevented the reappearance of reactivity to that antigen (109). In these studies, mice had been repeatedly injected with bovine gamma globulin from birth until the age of 5-10 weeks and then thymectomized or sham-operated. Spontaneous loss of tolerance was evident in control mice by 130–160 days after cessation of antigen: immune disappearance of bovine gamma globulin (labeled with radioactive iodine) could then be induced. The thymectomized mice, on the other hand, remained tolerant. Recovery of mice from paralysis to bovine serum albumin was also impaired by thymectomy (650). Inability to prolong tolerance by thymectomy was reported by Weigle (699). These experiments differed from the previous ones in that a different species, the rabbit, was used and in that the animals were thymectomized much later, at 3-4 months, and then tested for tolerance only 28 days after thymectomy. Such factors may account for the different results.

Further evidence that the thymus is involved in breaking tolerance comes from work on thymus grafts. Transplantation of C3H thymus tissue to C3H mice tolerant of CBA skin grafts accelerated the breakdown of tolerance (25). A similar effect was noted in chickens. Polyvalent tolerance, of varying degrees, to histocompatibility antigens can be induced in noninbred birds by the neonatal injection of cells pooled from a wide range of donors. The competence of blood lymphocytes to produce lesions on the chorioallantoic membrane returned much more quickly in birds rendered tolerant while embryos and grafted with thymus at 1 week posthatching than in nongrafted tolerant controls (642).

As a general conclusion it can be said that there is very good experimental evidence that the thymus, by directing the differentiation of new immunologically competent cells, plays a role in breaking down artificially induced immunological tolerance. There is not, on the other hand, sufficient experimental evidence to support the thesis that tolerance is induced by an action of antigen on immature lymphoid precursor cells differentiating within the thymus.

VI. RELATIONSHIP TO DISEASE

Both clinical and experimental material have established a relationship between the primary lymphoid organs—the thymus and bursa of Fabricius or its mammalian equivalent—the secondary lymphoid tissues, immunological deficiency states, and susceptibility to develop autoimmune and neoplastic disorders. This relationship is examined, taking into account findings from both animal experimentation and human disease. First, an account of the immunological deficiency syndromes in man and of their autoimmune concomitants is given.
Next, the association between thymic abnormalities, thymus deficiency, and autoimmune disorders is reviewed. Finally, the coexistence of malignancy with either immunological deficiency, autoimmunity, or both is discussed.

1. Immunological Deficiency Diseases

The clinical types and the pathogenesis of immunological deficiency diseases have been reviewed in some detail by Good (232, 237, 238, 525). Only the salient features of the main syndromes are given here primarily to bring out the evidence that in humans, as in rodents and birds, the proper functioning of the immune system depends on the normal development of the thymus and other primary lymphoid organs. The experimental work reviewed in section III, 2, has indicated that there are two types of specialized lymphoid tissues: 1) the thymus-dependent system consisting principally of the thymus and circulating pool lymphocytes that act as recognition agents and produce cell-mediated immune reactions such as delayed hypersensitivity and homograft immunity; 2) the bursa-dependent system consisting of the bursa (or its mammalian equivalent not yet unequivocally identified), lymphoid follicles, and plasma cells and responsible for the production of immunoglobulins and specific antibodies. Both the thymus and bursa are primary lymphoepithelial organs derived originally from ecto-endodermal junctions in association with the gut epithelium (457). They direct the differentiation of lymphoid precursor or stem cells into immunologically competent cells capable of reacting to antigen in an appropriate manner within the microenvironments of the secondary lymphoid organs—lymph nodes, spleen, etc. A block at any one of the several stages in the development of the thymus-dependent system, or the bursa-dependent system, can be expected to lead to an immunological deficiency syndrome. Furthermore, interference with homeostatic control or failure of the feedback mechanisms that regulate the immune response is likely to result in immunological disorders.

A) Reticular dysgenesia. A rare condition, termed reticular dysgenesia, has been described in infants. They survive only a few days, dying of overwhelming sepsis, and they show deficiencies in all the leukocytic elements: agranulocytosis and alymphocytosis (218, 673). The thymus is composed of only epithelial elements and the lymphoid tissues are devoid of lymphocytes. A possible developmental basis for this condition would be the failure of the differentiation of a multipotent stem cell.

B) Thymic alymphoplasia with agammaglobulinemia. Thymic alymphoplasia with agammaglobulinemia is a severe form of immunological deficiency occurring in early infancy and characterized by growth failure, severe bacterial, fungal, and viral infections, ulcerative colitis, malabsorption, and death, usually before 18 months, despite antibiotics, blood transfusion, and gamma globulin therapy (145, 195, 216, 219, 281, 283, 314, 661). It is familial in origin, affects both sexes and is thus probably inherited as an autosomal recessive (195, 281, 283, 661). There is evidence that a similar disease, which affects boys only, may be transmitted
as a sex-linked recessive trait in certain families (216, 558). The syndrome may thus be the result of several genotypic abnormalities. Immunological defects are extreme: serum gamma globulin levels are less than 25 mg/100 ml; there is a failure of the development of delayed hypersensitivity to 2,4-dinitrofluorobenzene, 2,4-dinitrochlorobenzene, and Candida albicans and a failure to reject skin grafts (217, 557). Fatal cases of vaccinia gangrenosa have been associated with this syndrome (16, 271, 708), suggesting that there is a failure to resist virus infections. The blood lymphocyte levels are low, though rarely less than 1000/mm³ and lymphocytes in cultures in vitro fail to react to phytohemagglutinin (282, 558). The lymphoid tissues are poorly developed, lymphocytes being absent from the spleen, lymph nodes, and lamina propria of the intestinal tract. Plasma cells and germinal centers are absent. The thymus is small, weighing from 1 mg to less than 3 g (the normal for that age being 12–25 g), and has sometimes not descended to its normal intrathoracic position (661). There is no corticomedullary pattern and thymus lymphocytes and Hassall’s corpuscles are absent. The thymus resembles an embryonic anlage soon after its origin from the branchial clefts. Attempts at treatment with thymus grafts have failed (217, 282, 557) although temporary improvement was obtained when both a thymus transplant and injection of maternal bone marrow cells were given (558). The histological and immunological features of this syndrome strongly suggest that there has been a primary developmental defect of the thymus and the thymus-dependent system. In addition, the immunoglobulin-producing system has failed to develop. The reason why the developmental defect involves both systems is unknown. Is there a defect in a precursor cell that is essential for the development of both systems? Is the development of the immunoglobulin-producing system dependent on the thymus? The latter possibility seems unlikely in view of the reports of the existence of syndromes in which thymus alymphoplasia is not accompanied by deficiencies in immunoglobulin production as described below.

C) Thymic alymphoplasia without agammaglobulinemia. There are syndromes in children characterized by thymic alymphoplasia with alymphocytosis but without agammaglobulinemia (16, 202, 213, 344, 356, 493). There is persistent lymphopenia, an epitheliod thymus devoid of lymphocytes and Hassall’s corpuscles, and lymphoid tissues depleted of small lymphocytes. These tissues, however, contain abundant pyroninophilic large lymphoid cells, germinal centers, and plasma cells. Peyer’s patches were reported to be normal in one child (393). The serum immunoglobulin levels are generally within normal limits. There is an inordinate susceptibility to infection with Candida albicans and Pseudomonas and progressive vaccinia may develop (16, 202). In one case studied extensively, normal or near-normal antibody responses were obtained on challenge with poliomyelitis and parainfluenza viruses but there was no delayed-type skin reaction to mumps, parainfluenza, and monilia antigens and a maternal skin graft failed to be rejected (202). The thymic alymphoplasia and absence of delayed hypersensitivity reactions in spite of a relatively normal immunoglobulin-synthesizing capacity suggest that there is a dissociation of immunological capabilities in man. This
syndrome is hence probably due to a defect in the development of the thymus and thymus-dependent system. On the other hand, the immunoglobulin-producing system apparently has developed normally.

D) Ataxia telangiectasia. Ataxia telangiectasia is an autosomal recessive disorder characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, and frequent sinopulmonary infections (72, 368). It becomes manifest in early childhood, death usually occurring before adolescence and resulting from pulmonary infections and, sometimes, lymphoreticular malignancies (507). Serum gamma globulin levels may be as low as 80 mg/100 ml, and there is an absence or deficiency of IgA, but normal or low IgG and normal IgM (57, 165, 183, 257, 386, 400, 720). Many patients with low immunoglobulin levels have had deficient antibody production to diphtheria, mumps, tetanus, typhoid, and bacteriophage antigens. Some have shown no capacity to develop delayed hypersensitivity to viral, bacterial, and fungal antigens and to 2,4-dinitrofluorobenzene, and first- and second-set skin homografts have survived for several weeks (527). Other patients have been able to develop delayed hypersensitivity reactions (183). Lymphopenia was occasionally recorded and the reaction of blood lymphocytes to phytohemagglutinin in cultures was impaired (357). Autopsy revealed absence of the thymus in some cases and poor development in others (72, 527). In these, the lymphocytes were sparsely scattered among the epithelial cytoreticulum, there was no organization into cortex and medulla, and no Hassall's corpuscles. Lymph nodes showed marked reticuloendothelial cell hyperplasia and, sometimes, a paucity of small lymphocytes. Plasma cells were lacking in a few cases but normal in others. This syndrome is therefore a specific dysimmunoglobulinemia in which IgA is deficient or absent. In addition, there may be a failure of development of the thymus and of the capacity to produce cell-mediated immune reactions.

E) Congenital agammaglobulinemia. The congenital form of agammaglobulinemia, first described by Bruton (82), is a sex-linked disorder affecting boys. It becomes manifest at 5-6 months of age when the transplacentally acquired maternal antibodies disappear from the circulation (80). It is characterized by recurrent scvcre infections usually with pyogenic bacteria, though sometimes with Pneumocystis carinii and hepatitis virus. There is a nonpersistent lymphopenia (242). The characteristic serum abnormality is a lack of all three types of immunoglobulins. There is an inability to produce detectable circulating antibodies to most antigens (243). There is a normal response to virus infectionse.g. after vaccination, delayed hypersensitivity can be developed and skin homografts can be rejected, although sometimes skin graft survival is prolonged (230, 234, 533). Small lymphocytes are present in lymph node and spleen but lymphoid follicles and plasma cells are deficient. Pharyngeal lymphoid tissue is diminished or absent and the appendiceal tissue lacks germinal centers and plasma cells. By contrast, the thymus is usually normal with small lymphocytes, Hassall's corpuscles, and corticomediullary organization (237). Some patients have developed acute lymphatic leukemia (508). The immune system of patients with congenital sex-linked agammaglobulinemia thus bears a close similarity to that of chickens bursectomized and irradiated at hatching: the bursa-dependent system is deficient, whereas
the thymus-dependent system appears normal. The condition in man may thus result from a defect in the formation of the immunoglobulin-producing system.

F) Primary adult “acquired” agammaglobulinemia. “Primary adult ‘acquired’ agammaglobulinemia” is a rare disease that involves adults of both sexes. It is now thought to be conditioned by an underlying genetic abnormality with a generally late expression (713). Thymoma has been reported in 17 patients (206, 209, 224, 240, 301, 343, 379, 389, 476, 531, 538, 560, 561, 676, 714), this constituting about 10% of cases—a highly significant association in such a rare disease.

The serum gamma globulin levels are low (10–200 mg/100 ml) and all types of immunoglobulins are generally deficient. There is a feeble or undetectable antibody response to challenge with most antigens. Plasma cells are absent or deficient in lymph nodes and bone marrow. Germinal centers have been reported lacking in lymph nodes although a few patients have exhibited hyperplasia of the follicles not unlike that seen in giant follicular lymphoblastoma. The thymoma has frequently been a spindle-celled epithelial tumor. The pathogenesis of the disease is obscure for several reasons. In all the cases reported, there is no clue as to whether any relationship exists between the thymoma and the agammaglobulinemia. The tumor may have appeared as early as 8 years before the onset of the immunological deficiency (206, 301, 343, 538) or concurrently (206, 389) or it may have been discovered after the clinical features of agammaglobulinemia (379). Extirpation of the tumor has failed to alter the immunoglobulin deficiency (379, 389). The thymus-dependent system is not intimately involved in immunoglobulin production as indicated by experimental work on birds and rodents and as suggested by other clinical syndromes discussed above. However, nothing is known concerning the interrelationship between the thymus-dependent system and the immunoglobulin-producing system—how they may be linked together and whether feedback mechanisms occur between them. Until this knowledge becomes available one can only surmise that an abnormality of homeostasis or of feedback mechanisms may be the basis of the association between thymoma and agammaglobulinemia.

G) Autoimmune concomitants of immunological deficiency states. Animals or patients with immunological defects often have associated autoimmune abnormalities: for example, Coombs-positive anemia in immunologically defective mice and rabbits thymectomized at birth (section vi, 2); autoimmune hemolytic anemia and rheumatoid disorders in patients with agammaglobulinemia (200, 201, 234, 237, 241, 592); Coombs positive hemolytic anemia and other autoimmune phenomena in animals and patients treated with irradiation and alkylating agents (15, 259, 364, 590); rheumatoid factors and “connective tissue disease” in patients undergoing immunosuppressive therapy for renal transplants (691). There is also an increasing incidence of autoantibodies with age (689), which contrasts with the decreasing capacity to react to new antigens (382, 383). Amyloidosis has been reported in mouse radiation chimeras with lymphoid aplasia (77), in mice and rabbits subjected to irradiation and thymectomy (222, 323, 638) or neonatal thymectomy alone (686), in a number of patients with agammaglobulinemia.
The reasons why autoimmunity should be associated with immunological deficiency states are not known. Susceptibility to infections is, however, increased in immunologically deficient animals. The possibility that viruses, mycoplasmas, or other infectious agents play a role in the pathogenesis of autoimmune disorders has been suggested before (367, 466). Potentiation of virus infections, when the thymus-dependent immune system is deficient, has been documented in this section and in section III, 3. Cellular damage occurs during the course of an infection, and the release of sequestered native antigen (700) or of native antigen altered by the infectious agent or sharing antigenic determinants with it (319) could trigger off an autoantibody response. Alternatively, a virus could induce a mutation in lymphoid cells, converting these to autoreactive cells. The possible induction of autoimmune disease by viruses or similar agents receives support from a number of animal experiments. Inoculation of tissue culture-grown cells, obtained from the mesenteric lymphoblastic tumor of a virus-induced leukemia, into neonatal mice caused lymphopenia, lymphoid aplasia, runting, and Coombs-positive hemolytic anemia (612). Transmission of a cell-free extract of a murine plasma cell leukemia induced a disease characterized by hyper gammaglobulinemia, plasma cell infiltration, amyloidosis, Coombs-positive hemolytic anemia, and runting (541). Aleutian mink disease is virus transmitted and has features suggestive of an autoimmune disorder (321, 354). Mice tolerant to lymphocytic choriomeningitis virus developed a syndrome similar to graft-versus-host disease whereas mice non-tolerant to the virus did not (288). The majority of mice of the Prince Henry strain inoculated with reo virus type 3 died from an acute disease characterized by steatorrhea, jaundice, incoordination, and runting. The few mice that survived this acute phase developed a chronic immunological disease with leucopenia, splenic atrophy, and runting. Inoculation of spleen cells from these mice into newborn mice of the same strain caused an acute syndrome very similar to that seen in graft-versus-host reactions (623). It is thus quite possible that viruses may transform lymphoid cells so that these may react against their hosts. Immunological deficiency would serve to potentiate this effect: on the one hand, susceptibility to virus infection would be increased; on the other hand, transformed cells might be altered antigenically and not eliminated by a deficient thymus-dependent immune system.

2. Autoimmunity

The establishment of an immunological function for the thymus and the finding of thymus lesions in autoimmune disease led Burnet (86, 87) to ascribe to the thymus a dual role: a generative function, the production of "clones with definable immunological functions," and a censorship function, the "elimination or inhibition of self-reactive clones" (87). He considered that "the mutant cells which give rise to the forbidden clones of autoimmune disease must frequently
have a thymic origin, which can be recognized by the occurrence in the thymic medulla of germinal centers and lymph follicles" (86).

A) Autoimmune disorders in animals. Mice of the strain NZB/Bl spontaneously develop a positive antiglobulin test from the age of 5 months and a severe hemolytic anemia (274). Burnet and Holmes (90) described germinal centers in the thymuses of affected mice and suggested that these centers "represent areas of proliferation of abnormal clones of lymphoid cells that are resistant to normal control processes." They also claimed that the thymuses had no Hassall's corpuscles. There was an increase in plasma cells and, in some cases, in mast cells (89). Other workers confirmed that lymph follicles or germinal centers were present in the thymus of these mice (162, 603) but showed that the development of these structures occurred in the perivascular connective tissue and was therefore extrathymic in location (603). They questioned the significance of the thymic germinal centers in relation to the etiology of the autoimmune hemolytic anemia. Thymic germinal centers were present in young, healthy, Coombs-negative animals as well as in older, sick, Coombs-positive mice (162). They were situated not only in the thymus but in other organs as well, e.g. in the kidneys and lungs (162, 603). They were also seen in the thymus of aged Swiss mice that had neither a positive antiglobulin test nor hemolytic anemia (603). Furthermore, lesions in other organs of NZB/Bl mice were found before the onset of the thymic lesions (685). Marked hyperplasia of lymphoid structures with an increase in plasma cells and reticuloendothelial cells characteristically occurred in these mice. Transplantable lymphocytic neoplasms of the thymus were noted in some mice (162, 404). The impression gained from these immunopathological studies was that the thymus lesions were by no means unique but part of a generalized lymphoreticular hyperplasia associated with immunological hypereactivity.

In contrast to the report of Burnet and Holmes (90), Hassall's corpuscles were identified in the thymuses of NZB mice (162, 603, 685). Changes in the large medullary epithelial cells, involved in the formation of Hassall's corpuscles, were described (685). Degenerative changes in these cells were present in NZB mice in the first few weeks after birth. In NZW and (NZW X NZB)F1 hybrids, an extensive hyperplasia of the large epithelial cells and Hassall's corpuscles was seen. Invasion of these epithelial structures by lymphoid cells was reported and disintegrated lymphoid cells and degenerate epithelial cells were observed. Depletion of epithelial cells occurred at 8 months to 1 year. The phenomenon of invasion of epithelial structures by lymphoid cells that "subsequently" disintegrate was interpreted as evidence in favor of Burnet's concept that the thymus eliminates forbidden clones (342, 685). Since 95% of thymus lymphocytes are short lived and die within the thymus (section 11, 3 and 4), it is likely that disintegrating lymphoid cells will be seen. Since macrophages are associated with Hassall's corpuscles (336), it is evident that disintegrating cells will be observed in these sites. It has, in fact, been suggested that these corpuscles have a phagocytic function (68).

If an autoimmune disorder is due to the development of "forbidden clones" in some part of the thymus, then thymectomy should be able to alleviate or cure the autoimmune condition. This was tested in NZB mice and in F1 hybrid mice,
derived from a cross between NZB and NZW, which develop a disorder after 6 months of age closely resembling lupus nephritis and characterized by a positive lupus erythematosus cell test (275, 290). Neonatal thymectomy in NZB mice did not prevent the onset of autoimmune hemolytic anemia, the time of onset being in fact earlier than in nonthymectomized mice. In F1 mice, neonatal thymectomy accelerated rather than prevented the disease, inducing it earlier and in a more acute and florid form. Grafting a normal thymus into an autoimmune strain failed to prevent the development of the disorder. This suggests that if "forbidden clones" are responsible for the initiation of autoimmune disease, they cannot develop only within the thymus after birth. It suggests, furthermore, that the thymus from normal mice failed to eliminate the "forbidden clones" postulated to arise in the autoimmune strains. Neonatally thymectomized nonautoimmune CBA mice, grafted with thymus tissue from a newborn mouse of an autoimmune strain, developed serological and histological evidence of autoimmune disease, some within 2 months. The grafted thymus could have exerted its "autoimmune" influence either by producing autoimmune clones, by failing to eliminate autoimmune clones of cells arising in the host, or simply by transmitting a virus. The existence of a virus in autoimmune strains has now been reported (405). The results of the neonatal thymectomy experiments are not in accordance with the hypothesis that forbidden clones are disseminated from the "autoimmune" thymus. The failure of a normal thymus graft to prevent the development of autoimmune disorders in thymectomized autoimmune strains is not in accordance with the suggestion that the thymus eliminates forbidden clones. It appears, therefore, that virus transmission may account for the results. In the autoimmune strains of mice, the changes developing in the thymus may simply represent part of a general lymphoreticular response to viral infection.

Autoallergic inflammation of an organ can be induced experimentally by immunization with extracts of the organ emulsified in Freund's adjuvant. Thymectomy has impaired or alleviated the evolution of these reactions. This has been shown in autoallergic aspermatogenesis in mice (681), in autoallergic encephalomyelitis in rats (28, 336), in autoallergic encephalomyelitis (304) and thyroiditis (306) in chickens, and in autoallergic thyroiditis (371) in guinea pigs. The allergic response in these disorders is mediated by immunologically competent cells, the development of which is thymus dependent.

The development of a Coombs-positive antiglobulin test, hemolytic anemia, and a reticuloendothelial hyperplasia with increase in plasma cells has been noted in some mic (nonautoimmune strains) subjected to neonatal thymectomy (461, 686) and in rabbits subjected to neonatal extirpation of the thymus and appendix (322, 638). Furthermore, thymectomized irradiated mice developed renal lesions (258, 259), similar to those seen in some autoimmune disorders (403). Some similarities between the postneonatal thymectomy syndrome and graft-versus-host disease in mice have been reported: hyperplasia of reticuloendothelial elements, (461, 589, 686), increased phagocytic activity (461, 589), extensive extramedullary hemopoiesis (461, 686), scattered necrotic foci (461, 686), liver damage (160, 686), trophic disturbances, wasting, diminished immunological competence,
lymphoid aplasia, and premature death (section III). In graft-versus-host disease, the pathological processes are initiated by immunologically competent cells with reactivity directed against the foreign histocompatibility antigens of their hosts. After neonatal thymectomy, it was postulated that antiself immune reactions developed due to the origin of forbidden clones and the failure of their elimination, which normally takes place in the thymus (685, 686). It was, however, demonstrated that germfree mice thymectomized at birth developed neither wasting disease nor any of the characteristic hyperplastic lesions seen in conventional mice (402, 460, 712; see section III, 3). This indicates that bacterial and viral infections may induce the lymphoreticular hyperplasia and necrotic lesions seen in conventional thymectomized mice. Such mice, for instance, are particularly susceptible to mouse hepatitis virus (160). As suggested previously, an autoimmune process in a thymectomized animal might not be related to the absence of a particular censorship function exercised by the thymus but might be “secondary to the failure of development of normal immune reactivity” (466). As mentioned in section VI, I, children with an inadequate thymus-dependent immune system are susceptible to virus infections and some have developed progressive vaccinia. Potentiation of virus infection by failure of the thymus-dependent immune system might account for some of the autoimmune manifestations occurring in rabbits and mice subjected to neonatal thymectomy.

In conclusion, it may be said that experimental work on mice has failed to substantiate the hypothesis of the origin of forbidden clones in the thymus and to provide evidence that the thymus eliminates such clones by means of some censorship activity. The hypothesis of intrathymic surveillance is not supported by the finding that abnormal mutant cells with chromosomal abnormalities tend to persist within the thymus (312, 596). The effects of thymectomy and thymus grafting in normal and autoimmune strains, and in conventional and germfree mice, are compatible with the hypothesis that, with a defective thymus-dependent immune system, there is an inordinate susceptibility to infection. Damage to cellular systems and lymphoreticular hyperplasia result from infection and lead to autoimmune disorders.

B) The thymus and autoimmunity in man. There is considerable clinical, histopathological, and serological evidence that myasthenia gravis is an autoimmune disease as originally postulated by Simpson (606). There is a higher incidence of other autoimmune disease in patients with myasthenia gravis than in control populations (606). There are lesions in the thymus that suggest the existence of an immune reaction (388) and bear close similarities to lesions present in the thyroid in autoimmune thyroiditis (619). Serum complement fluctuates with disease activity (492) and there are multiple autoantibodies produced, including antibodies to nuclei and to thyroglobulin (6, 210). In 30% of cases, there is a specific antibody that reacts with antigens present in the A bands of striated muscles and cells in the thymus medulla (211). These cells are in fact myoid cells with the structure and antigenicity of striated muscle cells (178, 276, 633). Their function is unknown. The autoantibody cross reactive with striated muscle and thymus myoid cells is highly specific for myasthenia gravis, being absent in sera of control
patients, patients with other muscular disorders or other autoimmune diseases. It was, however, found in 12 of 15 patients with thymomas not associated with clinically apparent myasthenia gravis (635). No binding of the autoantibody has been detected at the neuromuscular synapse (399)—the site of the functional derangement in the disease.

Abnormalities of the thymus in myasthenia gravis are well documented (98–100, 615). About 10–15% of myasthenia gravis patients, mostly those in the older age groups, have thymomas. These tumors are an admixture of large, pale epithelial cells with lymphocytes in varying proportions. They are rarely of the spindle cell variety. Thymus tissue adjacent to a thymoma often contains germinal centers (348, 373). About 95% of patients with thymomas have high titers of autoantibody to striated muscle and thymus myoid cells. Some of the large, pale epithelial cells of the tumor react with myasthenic serum, indicating that they must share antigens with thymus myoid cells and muscle A bands (634).

About 75% of myasthenia gravis patients, mostly those in the younger age group, have nontumorous thymuses. These are not enlarged, do not show cortical atrophy (229), but show in the medulla lymphocytic infiltration and germinal centers (98–100, 615). In a survey of 150 thymuses from people who died suddenly, germinal centers were found only in 14 and were few and small and not comparable to the large ones seen in myasthenic thymuses (615). General lymphoid hyperplasia is not a feature of patients with myasthenia gravis (615).

A number of infants born of mothers with myasthenia gravis develop a short-lasting muscular weakness termed neonatal myasthenia gravis (627). This suggests that a humoral substance that blocks neuromuscular transmission has crossed the placenta. This substance is not the autoantibody to thymus myoid cells since neonatal myasthenia has occurred in infants whose mothers did not have the antibody in their serum (627). The facts that this autoantibody a) is present in only 30% of cases, b) does not bind at the neuromuscular junction, and c) does not occur in cases of neonatal myasthenia suggest that its existence is an epiphenomenon and that it is not a necessary factor in the pathogenesis of the disease. Some other biological factor must be responsible. Attempts to demonstrate a neuromuscular blocking agent in the sera or thymuses of myasthenic patients have so far been unsuccessful.

Thymectomy has resulted in a marked increase in the remission rate in myasthenia gravis patients and often in a complete cure. In a review of about 400 patients (605), 60–70% of a group without thymomas had remissions after thymectomy in contrast to only 30% of a similar group of patients not subjected to thymectomy. Results of thymectomy in cases with thymoma were less favorable, but the survival of unoperated cases with tumors was poor. The overall favorable results obtained with thymectomy have suggested that the diseased thymus may produce a substance blocking neuromuscular transmission (228, 230).

An “autoimmune thymitis” was produced experimentally by immunizing guinea pigs with calf thymus or muscle in complete Freund's adjuvant (230, 291). The guinea pigs developed delayed hypersensitivity to muscle and thymus. Those immunized with thymus developed antibodies that reacted by immunofluorescence
with the cytoplasm of calf thymus lymphocytes and epithelial cells but not with thymus myoid cells or skeletal muscle. Those guinea pigs immunized with muscle produced antibodies to thymus myoid cells and to skeletal muscles, similar to the autoantibody of myasthenic serum. Using serial sections it was shown that the same myoid cell that reacted with myasthenic serum was stained with serum from muscle-immunized guinea pigs. About 50% of the immunized animals developed "thymitis," a lesion characterized by an accumulation of lymphocytes in the central medulla around the Hassall's corpuscles. About half of these animals developed a neostigmine-responsive neuromuscular block demonstrable by electromyography. Thymectomy performed immediately before immunization with thymus or muscle did not inhibit the delayed hypersensitivity reaction nor the production of circulating antibodies but prevented demonstrable neuromuscular block. The conclusion was made that an active autoimmune reaction occurring within the thymus caused damage as a result of which a humoral substance with a neuromuscular blocking action was released. The hypothesis was suggested that in human myasthenia gravis the basic lesion is an autoimmune thymitis—an infiltration of the thymus medulla with lymphocytes, plasma cells, and the production of germinal centers. The thymitis occurs as a result of the autoimmune reaction. In 10% of cases, the cause of this reaction is a thymoma. In the remainder, the cause is unknown, but the overlap with other autoimmune diseases suggests that there is a genetically determined disorder of immune tolerance. The damage produced in the thymus releases a humoral substance that causes the neuromuscular block of myasthenia gravis (228). This hypothesis has the merits of simplicity and of being backed up by experimental work in guinea pigs. It may not, however, account for those cases of myasthenia gravis that do not benefit from thymectomy.

Thymus lesions have been reported in systemic diseases other than myasthenia gravis and in some of these there have been associated autoimmune phenomena. In systemic lupus erythematosus (L.E.), the thymus has shown the following characteristics (92, 293, 294, 374, 375, 377): marked cortical atrophy, spindle epithelial cell aggregates in the medulla, increased cystic and scanty epithelial Hassall's corpuscles, increased plasma cells, and in some cases germinal centers in the medulla. There are reports of thymomas associated with systemic lupus (238, 347) and two cases in which systemic lupus developed after thymectomy for myasthenia gravis (12). In two cases of thymoma, one associated with myocarditis (293) and one with erythroid aplasia (341), L.E. cells were detected. Thymectomy was performed in three patients with systemic lupus: all remained well for a time postoperatively but this was attributable to continued prednisolone therapy (376, 378). One died 4 years later of severe bronchopneumonia and showed, at autopsy, gross lymphoid atrophy (373).

Thymomas have also been reported associated with other diseases in which autoimmune manifestations have been described: myositis, in at least 10 cases (e.g. 332), dermatomyositis (274, 364), hyperglobulinemic purpura, in which thymectomy induced a remission (65), rheumatoid arthritis, scleroderma, and Sjogren's disease (348). Proliferation of epithelial cells and the presence of germinal centers have also been observed in the thymic medulla of patients with rheumatoid arthritis (91, 214).
In about 90 reported cases of erythroid aplasia, 51 patients had thymomas, usually of the spindle cell variety (348, 563, 583). This is a most significant association but its nature is not clear. Extirpation of the thymoma cured the disease in only four cases. In a number of cases, erythroid aplasia actually developed several years after the excision of a thymic tumor. It is possible that the thymus exerts a regulatory role on erythropoiesis. Differentiating erythroid elements have been described in the thymus (13, 14) and in thymus grafts in mice (154), and thymectomy in embryonic opossums has virtually caused a maturation arrest of erythropoiesis (454). The possibility that autoimmune factors play a role in the pathogenesis of erythroid aplasia has been suggested (17). In a few patients, there has been an association with myasthenia gravis and hemolytic anemia. In other patients there have been positive antiglobulin tests, positive lupus erythematosus tests, and antinuclear factors (48a). Since hemolytic anemia is not found in most patients, an autoantibody would have to be directed either against the erythroblasts or their precursors, or against erythropoietin. Erythropoiesis has been reduced in mice by an antiserum produced against erythropoietin (585).

Autoimmunity is implicated in both thyrotoxicosis and Hashimoto’s thyroiditis (555). In thyrotoxicosis, the thymus is enlarged, possibly as a result of excess thyroid hormone that causes a generalized lymphoid hyperplasia (260, 261). Thymic biopsies have been performed in thyroid disease and germinal centers in the medulla were found in a number of patients. The presence of thymic lymphoid follicles was correlated with the presence of lymphoid follicles in the thyroid rather than with the existence of autoantibodies to the thyroid (256). The significance of these associations is unknown.

Thymectomy produced prompt remissions in two severe cases of autoimmune hemolytic anemia in children (320, 710). The thymuses showed cortical atrophy, conspicuous epithelial tissue, and striking accumulations of cells resembling plasma blasts in the medulla. The results of thymectomy suggest that this procedure may be beneficial for such severe cases in infancy. Generalizations should not, however, be made on the basis of two cases and particularly in view of the fact that extirpation of the thymus in neonatal mice (686) and of the thymus and appendix in rabbits (322) has sometimes been associated with the development of autoimmune hemolytic anemia. Furthermore, the long-term effects of thymectomy in infants are not known.

In summary and conclusion it can be said that lesions of the thymus, notably germinal centers and, less often, lymphoepithelial tumors, have been found in mice and in patients with autoimmune disorders. No experimental evidence has been obtained to show that these abnormal collections of cells have initiated the disorder. Germinal centers have also been found in other organs, in situations where they normally do not occur, e.g. in the salivary gland in Sjogren’s disease, in the skin in cutaneous lupus, in the thyroid in Hashimoto’s thyroiditis, in the knee joint in rheumatoid arthritis, in the liver in lupoid hepatitis, and in many organs in the New Zealand mice. Their appearance in the thymus might therefore indicate the existence of an immunological reaction directed against some thymus component that may share antigens with other target organs. Evidence for this has been obtained in the best documented case—myasthenia gravis. The thymus
germinal centers are thus more likely to represent the result of some immuno-
pathological reaction directed against the thymus rather than the anatomical
expression of an autoimmune process initiated from within the organ. The nature
of the relationship between thymomas and autoimmune disease is not clear. It is
unlikely to be a cause-and-effect relationship. Thymomas occur in other diseases,
such as Cushing’s disease (185). They may occur in patients who never show auto-
immune phenomena, they may precede autoimmune phenomena by years, and
their extirpation may fail to be associated with remissions. Thymectomy in auto-
immune disorders has had either beneficial or drastic effects, depending on the
system used. In most patients with myasthenia gravis, it has been beneficial pos-
sibly because the damaged thymus is the source of a neuromuscular blocking
agent. In experimental autoimmune encephalomyelitis, thyroiditis, and aspermat-
genesis, it has been beneficial because it has prevented the development of those
immunologically competent cells that mediate the autoimmune reactions. In
mice with genetically determined autoimmune disease, it has precipitated and
aggravated the condition, presumably because, in the absence of a thymus-depend-
cut immune system, potentiation of viral infection occurs (probably a virus verti-
cally transmitted in these strains), resulting in damage to cellular systems, lympho-
reticular hyperplasia, and autoimmune reactions. A similar possibility applies to
conventional mice (of nonautoimmune strains) and rabbits in which autoimmune
disorders have developed after neonatal thymectomy: potentiation of bacterial
and viral infections leading to cell damage, lymphoreticular hyperplasia, and auto-
immune phenomena. In two infants with autoimmune hemolytic anemia, thymec-
tomy has been beneficial, in contrast to what might have been expected from work
on the autoimmune strains of mice. It can thus be seen that one cannot generalize
concerning the effects of thymectomy in autoimmune disease: the result depends
on whether the mechanism involved in producing the damage is dependent on the
thymus or potentiated in its absence.

3. Neoplasia

Since normal animals in nature are not ordinarily called on to make a distinc-
tion between compatibility and incompatibility of one another’s tissues (except
perhaps in the special case of the engrafted fetus) it has been postulated (85, 653)
that the biological raison d’être of homograft immunity might be to provide a
“surveillance mechanism whose function is to recognize and eliminate cells which,
by somatic mutation or otherwise, have become foreign to the body” (88). This
suggests the possibility that, in order to achieve their effects, carcinogens must not
only cause an excessive production of abnormal cells but also override or inhibit
any resistance that would otherwise eliminate them. There is now adequate ex-
erimental evidence to suggest an association between depressed or inefficient
immunological reactivity and the development of tumors induced by viruses and
chemicals. Since the thymus plays a key role in the development of some immuno-
logical faculties, particularly those concerned with homograft immunity, it is
important to determine whether thymic deficiency or malfunctioning of the thy-
mus-dependent immune system may be associated with an increased incidence of malignancies.

A) Carcinogenesis in thymectomized animals. A number of studies indicate that neonatal thymectomy has rendered mice more susceptible to the carcinogenic activity of oncogenic viruses and certain chemical carcinogens. The subject has been reviewed elsewhere (349, 451) so only the salient findings are briefly mentioned here.

The polyoma virus induces multiple neoplasms in most strains of mice, provided that they are inoculated within the immediate postnatal period. Mice injected after 2 weeks of age usually do not develop neoplasms. The C57BL strain of mice is not susceptible to the oncogenic activity of the virus. Thymectomy in the postnatal period has influenced polyoma virus oncogenesis in the following ways: 1) It abolished genetically determined strain resistance to the virus. Thus it rendered the highly resistant C57BL mice susceptible to tumor induction (467). 2) It extended the period of susceptibility to the virus. Thus, C3H mice remained susceptible even when inoculated as late as 16 days of age, and C57BL mice lost their resistance even when they received the virus as late as 30 days of age (352). 3) It decreased the latent period of tumor growth. Tumors were usually clinically manifest at an earlier age in the thymectomized than in the control group of mice (467). 4) It increased the total incidence of tumors and the spectrum of tumor types (352, 384). Essentially similar results were obtained with polyoma virus in neonatally thymectomized rats (674, 675) and hamsters (141).

In some experimental systems, the latent period for the development of palpable sarcomata in mice injected with low doses of chemical carcinogens was shorter in neonatally thymectomized mice than in the controls (251). In other systems little or no effect of thymectomy was observed (44). Experiments with skin carcinogenesis have also indicated that the induction and progression of tumors may be enhanced by neonatal thymectomy (252, 459).

Tumors induced by oncogenic viruses and chemical carcinogens possess distinct cellular antigens that can evoke a homograft type of reaction (273, 498, 613). The growth of these antigens is thus facilitated by an inadequacy of transplantation immune reactions, as occurs after neonatal thymectomy. In the case of the polyoma virus, the results were clear-cut. In the case of chemical carcinogens, the carcinogen treatment itself depresses the immune system of the host (367, 536) so that tumors may arise as readily in the nonthymectomized as in the thymectomized animals unless critical doses of carcinogen are used.

In contrast to the enhancing effect of neonatal thymectomy on carcinogenesis, thymectomy prevents the development of thymic lymphomas in rodents. In these types of neoplasms, malignant cells are not suppressed within the environment of the thymus (312, 596), proliferate there first, and subsequently disseminate. The role of the thymus in leukemogenesis has been discussed elsewhere (436, 438, 451). It should be pointed out that reduced immunological capacity is evident during the incubation period of leukemias induced by Gross virus (143, 526), Moloney virus (128), Friend virus (572), and Rauscher virus (602). It is possible, therefore, that leukemogenic viruses affect the thymus of mice in such a way as to impair
thymus-dependent immune functions. This in turn would allow the proliferation of antigenically distinct clones of leukemic cells in the absence of an efficient immune opposition from the host.

B) Immunological deficiencies and lymphoreticular neoplasms. The simultaneous occurrence of immunological deficiencies and lymphoreticular malignancies in man has been described (577). As mentioned in section IV, I, children with ataxia telangiectasia (165, 527) and with the Bruton type of congenital agammaglobulinemia (508) have developed lymphomas. Patients with chronic lymphocytic leukemia, Hodgkin's disease, and multiple myeloma (49, 104, 434, 577) have exhibited immunological deficiencies. In chronic lymphocytic leukemia, there is hypogammaglobulinemia, severe impairment of antibody formation in both the primary and secondary response, impaired delayed hypersensitivity reactions to primary sensitization (e.g. with 2,4-dinitrochlorobenzene), impaired rejection of skin homografts, and impaired blastoid transformation of lymphocytes in cultures in the presence of phytohemagglutinin (434, 577). The immunological defect of early Hodgkin's disease is characterized by a depression of the delayed hypersensitivity response but antibody production is generally normal (7, 8) although hypogammaglobulinemia has been described in two cases (284). Patients with multiple myeloma have a deficiency of normal immunoglobulins, but they are usually able to respond to a secondary antigenic challenge and some can respond to a first by producing serum antibodies and reactions of the delayed type (577).

The possibility can thus be envisaged that in some lymphoreticular malignancies there is a failure of the proper functioning of the thymus or thymus-dependent immune system. It must be strongly emphasized, however, that with the exception of ataxia telangiectasia, no primary thymus abnormality has been detected in these cases of lymphocytic neoplasms. Nevertheless, there is a case for the reappraisal of thymus function in some of these patients, e.g. those with Hodgkin's disease. The association between immunological deficiency and lymphomas may occur in one of several ways. The malignant process may lead to the production of noncompetent cells that replace immunologically competent cells (137). This is likely to be the case in multiple myeloma. Immunological deficiency, on the other hand, may be produced through some other factor, and may, itself, facilitate the development of lymphomas by allowing antigenically distinct mutant cells to proliferate, as discussed above (653). A third possibility is that a defect at some stage in the development of the lymphoid system may lead to both immunological deficiency and lymphoreticular malignancy (525).

C) Escalation of autoimmune disease into neoplasia. There are several reports of patients with lymphomas and autoimmune disorders (137, 318), including autoimmune hemolytic anemia (559, 590), thrombocytopenic purpura (163), systemic lupus erythematosus (291), rheumatic disease (94, 95), dermatomyositis (709), and Sjogren's disease (647). The coexistence of autoimmune phenomena and lymphocytic neoplasms is also evident in animal models and experiments. About 20% of NZB mice that survived autoimmune hemolytic anemia developed transplantable lymphomas (162, 404). About 30% of F1 hybrid mice that survived a
graft-versus-host reaction, produced by transplantation of parental lymphoid cells, succumbed to lymphocytic neoplasms (591). About 50% of mice of a low-leukemic strain, injected at birth with spleen cells differing immunogenetically only at a weak histocompatibility locus, developed lymphomas (689). Multiple myeloma-like disease has been reported after aleutian disease in mink (532). Lymphoblastic tumors, closely resembling Burkitt's lymphoma, have developed in mice that survived the acute runting resulting from neonatal injection of syngeneic spleen cells obtained from donors with reovirus-induced chronic immunological disease (623).

The coexistence of autoimmune disorders and lymphoreticular malignancies incriminates a common factor in the pathogenesis of both phenomena—a genetic defect, a virus infection, or both. It was suggested by Tyler (672) that a defect due to the inactivation of genes determining histocompatibility antigens would result in the defective cells being stimulated by the antigens present in normal cells. Excessive proliferation, associated autoimmune phenomena, and escalation into neoplasia would result. Some support for this hypothesis has come from the experiments of Schwartz and Beldotti (591) and Walford (689). An alternative possibility is that a virus may transform cells, which then react immunologically against their hosts, proliferate excessively, and produce neoplasms. A viral basis for these phenomena is strongly supported by the experiments of Stanley and Walters (623).

As a general summary, it can be said that both experimental and clinical material have indicated that disturbances of the thymus or thymus-dependent immune system can be associated with disorders characterized by immunological defects, autoimmune manifestations, and/or neoplasms. After neonatal thymectomy in animals, immunological deficiencies occur as a primary consequence, upon which may be superimposed autoimmune phenomena and neoplastic disease. In mice with genetically determined autoimmune disease thymus lesions and lymphocytic neoplasms have developed. In nonautoimmune, low-leukemic strain of mice immunological disorders have been induced either by virus infection or by the injection of allogeneic lymphoid cells. Thymus lesions, autoimmune manifestations and even slight immunological defects (6) have been reported in patients with myasthenia gravis and "collagen disease." The coexistence of the three phenomena—immunological deficiency, autoimmunity, and neoplasia—has been well documented. It has occurred within the same patient or experimental animal and has even been reported with greater than expected frequency in certain families (200). One can only speculate to explain the basis for these associations (199). Although it is certain that in some cases thymic function may have been normal, the disorders being precipitated by factors acting at other levels, it is equally certain from the experimental and clinical material reviewed here that an inadequacy of the thymus or thymus-dependent immune system can precipitate all three phenomena. It therefore becomes imperative to look for disturbances of thymic function in such cases and to find measures that correct immunological deficiency so as to prevent escalation into autoimmune and neoplastic disease.
VII. GENERAL DISCUSSION

Lymphopoiesis and the maintenance of an adequate pool of immunologically competent cells are the hallmarks of thymus function. In spite of extensive research in the last decade, we do not really know how these two activities may be related, nor do we have the answer to many questions of fundamental importance in the physiology of the thymus.

Lymphocyte production in the thymus is intensive. The proliferative stimulus to thymus lymphoid cells is not an extrinsic influence, such as antigenic stimulation: it is intrinsic, being linked in some obscure way to the epithelial cytoreticulum. The stimulus is imparted mostly to incoming stem cells or lymphoid precursor cells that migrate into the thymus from elsewhere. We do not know the identity of these immigrant cells nor do we know whether there is one stem cell common to all the hemopoietic tissues or whether there are separate stem cells for the lymphoid and myeloid compartments. We do not know the nature of the proliferative stimulus in the thymus nor do we know what cells or cell complex in the epithelial cytoreticulum are responsible for its existence.

About 95% of the lymphoid cells produced in the thymus cortex die within the organ after a short intrathymic life span of 3-4 days. What, then, is the purpose of the intense thymic lymphopoiesis as a result of which an incredibly large number of short-lived cells are produced each day? It has been suggested that intensive lymphopoiesis provides a mechanism that generates, by random genetic variation at mitosis, lymphoid cells with different immunological reactivity patterns and that the extensive lymphocyte disintegration in the thymus reflects the operation of a mechanism that destroys autoallergic cells (86, 87). The Hassall's corpuscles, in which dead lymphocytes are often seen, have been earmarked as the organelles responsible for this function (342, 685). Are 95% of cells produced in the thymus autoaggressive cells? Are the Hassall's corpuscles the traps and graveyards for all these undesirable cells? If 95% of thymus lymphocytes are autoaggressive, it is difficult to explain the beneficial effects produced by 100-300 million thymus lymphocytes injected into syngeneic neonatally thymectomized mice (280, 721). The persistence of abnormal mutant cells within the thymus (312, 596), the development of lymphoid leukemic cells first within the thymus (451), the failure of normal thymus to prevent autoimmune disease in autoimmune strains of mice (275, 290) suggest further that immunological surveillance does not occur at an intrathymic level. It is more likely to be a function of the thymus-dependent immune system.

It is known that thymic lymphocytes are exquisitely sensitive to hormones mediating stress reactions (147) but the general significance of this effect in the economy of the lymphoid system is not clear. The trophic disturbances and wasting disease associated with states of lymphoid aplasia have suggested that lymphocytes may act as trephocytes, supplying building materials for the growth and differentiation of other tissue cells (369). However, all attempts to demonstrate a trophic function for thymus lymphocytes have failed (418). Furthermore, the fact that
germfree mice thymectomized at birth, in contrast to conventional mice, have not
developed wasting disease indicates that it is more likely that such disturbances are
linked to a failure of animals with lymphoid aplasia to deal adequately with bac-
terial or viral infections. The function of the short-lived thymus lymphocytes thus
still remains a mystery.

Immunological deficiency is a primary consequence of the absence of the
thymus. It depends on the failure to produce an adequate number of immunologi-
cally competent cells. It is primary because it is an immediate effect and not de-
pendent on other processes such as infection or autoimmunity. Thymectomized
germfree mice do show immunological impairment although the extent of this is
not as extreme as it is in thymectomized conventional mice. As discussed above,
the implications here are that bacterial contamination, toxins, and other factors in
the conventional environment act to reduce even further the number of antigen-
sensitive cells available in an already limited pool of immunologically competent
cells. It is likely that in the germfree state more cells would be available to deal
with the antigens introduced experimentally.

The immunological impairment after thymectomy is limited to certain types
of responses. There are other primary lymphoid organs, such as the bursa of
Fabricius in birds, that govern the development of different classes of lymphoid
cells—cells producing immunoglobulins and antibodies, in contrast to the thymus,
which is concerned with cells acting as “recognition agents” and “effector agents”
in transplantation immune reactions and delayed hypersensitivity. The mammal-
ian equivalent of the bursa is likely to be found among the gut-associated
lymphoid tissues, and the Peyer’s patches have been named as candidates although
the evidence for this is not conclusive. The intense plasmacytopoiesis reported in
some animals thymectomized at birth suggests that there may be a hyperplasia of
the immunoglobulin-producing system in the absence of the thymus-dependent
immune system. We do not, however, have any knowledge of the existence of
feedback mechanisms between the two systems.

There must be some relationship between the thymus and the pool of circulat-
ing small lymphocytes. When thymectomy is performed before an adequate pool
has been built up, for instance at birth, the number of cells in this pool does not
increase significantly and an impairment of those immunological reactions known
to be mediated by these cells is immediately evident. When thymectomy is per-
formed in adult life, once an adequate pool has been built up, no immunological
defects become evident until months later, presumably after the pool has become
depleted owing to the limited life span of some of its cells and the immunological
commitment of others. The exact nature of the relationship between the thymus
and the pool of immunologically competent, long-lived, circulating small lympho-
cytes is, however, unknown. Very few thymus lymphocytes, if any, are immuno-
logically competent, and very few are exported from the thymus. The few cells that
do emigrate, however, may represent an important contribution to the circulating
pool. The simplest relationship between the thymus and the pool would thus be one
in which lymphoid precursor cells of marrow origin are transformed in the thymus.
to lymphocytes, some of which migrate out and then mature further to become immunologically competent cells. There is some experimental evidence to support the concept that the maturation of thymus lymphocytes to immunologically competent cells may occur outside the thymus and may require a period of time. After the administration of prednisolone to newborn rats, immunological function took several weeks longer to recover than did lymphocyte levels (78). Wasting disease in neonatally thymectomized mice could be prevented by transfused spleen and lymph node cells, even if given at the onset of the wasting, but transfused thymus cells were effective only if given several weeks earlier (721). Lymph node cells restored immunological function in thymectomized mice immediately on transfer but transfused thymus cells could do so only if several weeks had elapsed between transfer and antigenic challenge (649).

The exact mechanism by which the thymus influences immunogenesis is, of course, unknown. It is unlikely, however, that the thymus exerts an effect on the population of immunologically competent cells once they have been produced. The normal response to antigen, of antigen-sensitive cells transfused into neonatally thymectomized mice, strongly suggests that antigen does not require the help of any thymus factor to cause proliferation and differentiation of antigen-sensitive cells (705). The long delay in the regeneration of the capacity to produce an immune response after irradiation-induced damage stands in sharp contrast to the speedy regeneration occurring in other cellular systems. Thus, for instance, an animal’s capacity to respond to sheep erythrocytes returns 3–4 weeks after doses of 500–700 r (648). In contrast, the hemopoietic colony forming ability lost after 400 rads is completely replaced within 7–8 days by extensive proliferation of the surviving cells (658). It is thus probable that antigen-sensitive cells do not proliferate in the absence of antigen. A stimulus to their proliferation is unlikely to be a simple reduction in their numbers. New antigen-sensitive cells must thus be the progeny of more primitive antigen-insensitive precursors, i.e. cells that can proliferate independently of antigenic stimulation. The nature of these precursors awaits identification. Possible candidates are the thymus lymphocytes, cells in the bone marrow, or both. Since thymus lymphopoiesis, unlike lymphopoiesis elsewhere, is quite independent of antigenic stimulation, and since the, thymus is required for the anatomical and functional regeneration of the immune system after damage by irradiation (458), it is reasonable to suggest that the precursors of antigen-sensitive cells may be found in the population of thymus lymphocytes. Recent experimental results on marrow-thymus cell interactions could, however, be interpreted in favor of the precursor cell being of marrow origin, the thymus cells having some auxiliary function in allowing the conversion: precursor cell → immunologically competent cell (107, 138, 139). Much more work is required to clarify this situation.

Various experimental systems indicate that there are thymus humoral factors stimulating lymphocytosis (411) and inducing the differentiation of immunologically competent cells (506). The lymphocytosis-stimulating factor (LSF) of Metcalf (411) may conceivably act to promote the proliferation of antigen-insensitive thymus lymphoid cells. It is unlikely that it promotes the proliferation of
immunologically competent cells outside the thymus since, as mentioned above, antigen-sensitive cells do not proliferate in the absence of antigen but can do so after antigenic stimulation even in the absence of the thymus (705). Experiments on the restoration of immunological capability in neonatally thymectomized mice by thymus enclosed in diffusion chambers have suggested that the thymus may produce a "competence-inducing factor" (CIF) (449) to allow the differentiation of antigen-insensitive precursors. In the normal animal, this step may be initiated within the thymus environment, but under certain experimental conditions cells outside the thymus may be influenced. It is conceivable that both LSF and CIF are one and the same factor, which could adequately be termed "lymphopoietin," and that its action is somewhat analogous to "erythropoietin" (81). Lymphoid precursor cells would be sensitive to thymus lymphopoietin, not to antigen. Under the influence of lymphopoietin, they would undergo proliferation associated with progressive differentiation to give rise to a progeny of cells, some of which become antigen-sensitive cells. The bulk of the antigen-sensitive cells so produced would form part of the circulating pool and would no longer be susceptible to thymus lymphopoietin. Cells in this pool are known to be noncycling cells (248, 640) but to respond to appropriate antigenic stimulation by the production of a) cells that undergo proliferation associated with progressive differentiation to antibody-producing cells and b) possibly more antigen-sensitive cells. Whether the latter are identical to the parent cell, whether they differ from the parent cell in their degree of commitment, and whether they are responsible for immunological memory are unknown. An assay system for the precursors of antigen-sensitive cells is urgently required to test the above hypothesis, to identify the nature of the precursor cells, to determine the role of the thymus in the conversion precursor cell → antigen-sensitive cell, and to characterize the noncellular thymus influences that may play a role in this conversion.

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