PATHOGENESIS OF THYMIC CHANGES IN NZB MICE WITH HEMOLYTIC ANEMIA*, ‡

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PLATES 57 TO 63

(Received for publication, June 14, 1965)

NZB/Bl mice spontaneously develop a severe hemolytic anemia associated with the presence of circulating antibody which reacts with the red cells (1). Although difficult to breed, these mice offer a valuable laboratory model for the study of hemolytic anemia and the associated disturbed immune mechanisms. Young mice of this inbred strain are normal in health and appearance. The first signs of disease develop by 5 months, when a progressive anemia and positive Coombs' test are noted (2). With the anemia, histologic alterations have been described in the thymuses and kidneys, and a lupus-like condition of the skin has been noted (2), associated with an antinuclear factor (3). Aspects of the natural history of this disease have been summarized recently (4–7). Burnet and Holmes (7) noted lymphoid follicles and distended lymphatic vessels in the thymuses of affected mice, and suggested that these germinal centers, "represent areas of proliferation of abnormal clones of lymphoid cells that are resistant to normal control processes" (7). In addition, the thymuses of NZB mice are unique in having been described as having no Hassall's corpuscles (7). As part of our studies on thymic function, and on the relation of the thymuses to leukemia (8, 9), we examined the thymuses of NZB mice of varying ages to determine the nature and pathogenesis of these changes. The following report is a description of these changes and an interpretation of their significance.

Materials and Methods

Animals.—Strain NZB/Bl mice were received from Dr. Marianne Bielschowsky in the 57th filial generation. From this nucleus a breeding colony was established in our laboratory. Standard inbreeding methods were observed and strict brother-sister matings were used. The animals were housed under usual laboratory conditions and fed commercial pelleted mouse food, supplemented by greens and cheese. At weaning, males not needed for breeding were placed in ample sized cages and overcrowding was avoided. By this means fighting was reduced without introducing the factor of castration into the experiment. A frequent cause of

* This study was supported by grants (CA 06947–02, and CA 06711–03) from the National Cancer Institute, National Institutes of Health, United States Public Health Service.

‡ A portion of this study was presented before the American Society for Experimental Pathology on April 10, 1965.

litter loss was failure of the mother to nurse, followed by cannibalism. Pregnant females were separately caged, provided with fresh greens, cheese, nesting materials, and left completely undisturbed until the litter was a week of age. This technique reduced neonatal losses considerably. Non-inbred Swiss albino ICR mice were obtained from our breeding colony.

Protocol.—NZB/Bl mice were selected to provide specimens of varying ages from 20 through 520 days. When available, males and females were matched in numbers for sacrifice. Swiss albino mice of varying ages were selected for sacrifice at the same time. A specimen of peripheral blood for the Coombs' test was obtained either by laceration of the retro-orbital plexus or by aortic aspiration. In selected cases enumeration of the peripheral cells was performed on this specimen. The animals were sacrificed with ether, weighed, and dissected. The left and right axillary nodes, left thymus, right thymus, and spleen were weighed separately on paper tares before fixation with other vital organs and sternum. Following fixation in a solution of 1000 cc of 80 per cent ethanol, 100 cc of 40 per cent formaldehyde, and 50 cc of glacial acetic acid for 24 hours, the tissues were washed in 80 per cent ethanol for an hour, and trimmed. The sternum was decalcified by continuous agitation in a solution consisting of equal parts of 50 per cent formic acid and 20 per cent sodium citrate for 48 hours. The tissues were then dehydrated in graded alcohols, and cleared overnight in cedarwood oil. They were then rinsed in xylene and embedded in paraplast. Step serial or semiserial sections were cut at 4 μ and stained with a hematoxylin-eosin preparation, supplemented in selected cases with a periodic acid-Schiff stain.

Coombs' Test.—A direct Coombs' test was performed following the method outlined by Long, Holmes, and Burnet (4). Rabbit anti-mouse globulin serum was purchased from Microbiological Associates, Bethesda, Maryland.

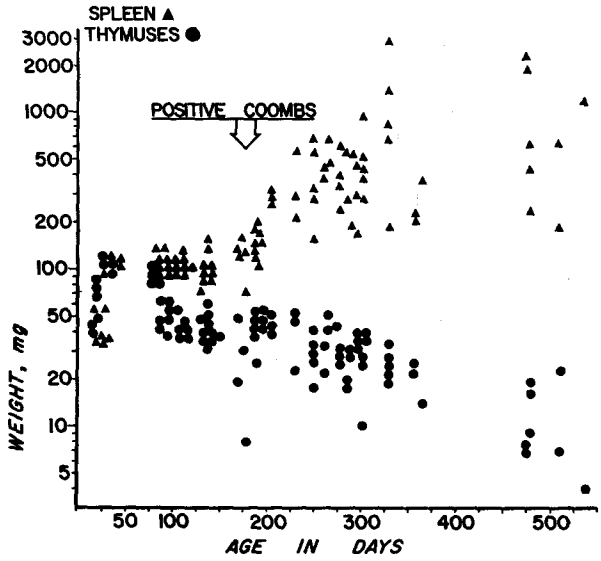
RESULTS

Complete histologic and serologic data were available from 99 NZB mice between 20 to 520 days of age. Complete histologic studies of normal Swiss mice were made from 86 Swiss mice killed at various ages from 1 through 350 days of age. Coombs testing was performed on all NZB mice and 50 of the 86 Swiss mice.

Text-fig. 1 records the weights of the spleen and the combined weight of the two thymuses of NZB mice at the time of sacrifice. It may be seen that at 20 days the spleen weighed somewhat less than the combined weight of the two thymuses, and about the same as the thymuses by day 30 to 35. Thereafter, the splenic weight remained constant until approximately 200 days, when it showed a sustained increase in weight to levels as high as 2500 mg. The onset of splenomegaly at 200 days corresponded to the development of the positive direct Coombs' test. Most NZB mice killed before 200 days had a negative direct Coombs' reaction (1/5 dilution) whereas most mice killed thereafter had a positive reaction. None of 50 normal Swiss mice killed at this age or thereafter had a positive Coombs' reaction.

The right thymus and the left thymus were weighed and sectioned separately. Because no unilateral changes in weight or histology were observed, the combined weight of the two thymuses of an animal was charted, rather than the weights of individual thymuses. It may be observed that the initial increase in thymic weight paralleled that of the spleen until about 80 days, after which the thymuses begin to decrease in size and weight. This senescent atrophy of

the thymuses was a slow, continuous process, so that by 520 days the thymuses weighed 10 to 20 mg combined, compared to their maximum weight of 100 to 200 mg. It is of interest to note that, in contrast to the spleen, no change in thymic weight occurred at the time of Coombs' test became positive. The slow decrease in thymic weight observed in the NZB mice was also observed in normal Swiss mice of comparable age. Data for these normal mice are not plotted, but the pattern of their weight decline with age was identical to that of the NZB mice, ranging from 80 to 120 mg at 30 days, to 10 to 20 mg at 300 days. The spleens of normal, Swiss mice rarely weighed above 150 mg at any age.



TEXT-FIG. 1. The combined weight of the two thymuses (circle), and the weight of the spleen (triangle) of NZB/Bl mice.

Normal Thymic Anatomy.—Before describing the histopathology of the NZB thymuses, a brief description of the pertinent normal anatomical features may be of value.

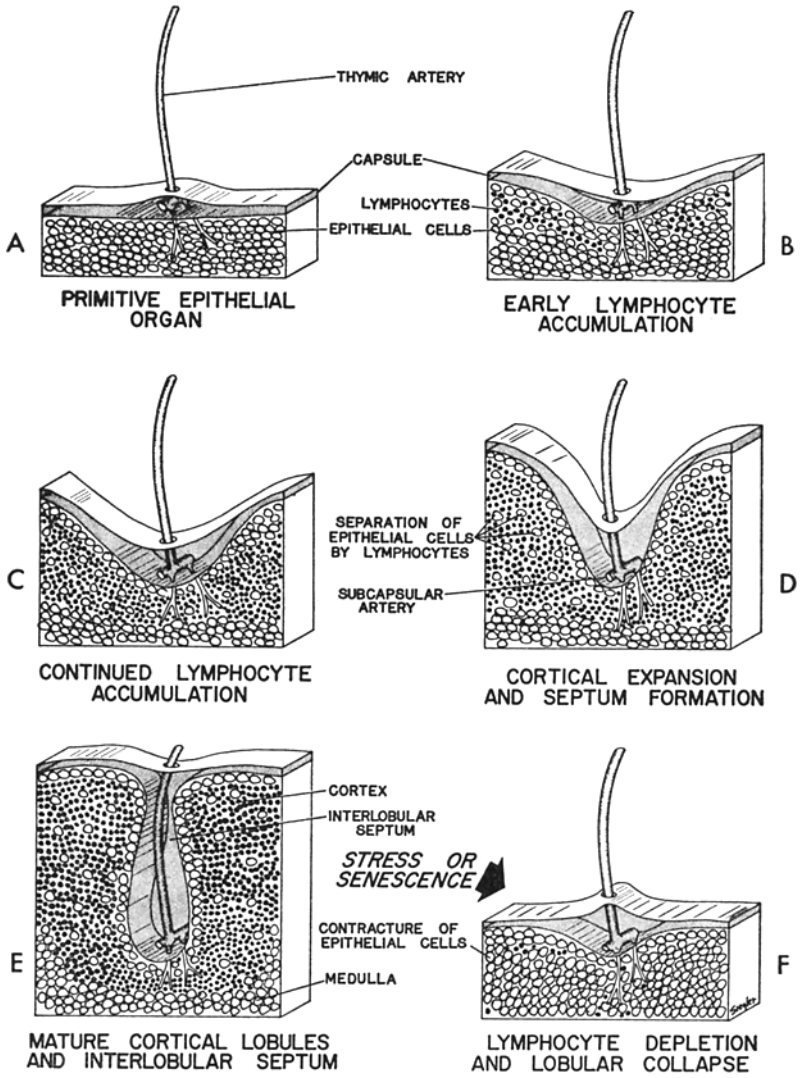
As the thymuses develop, a capsule forms around each thymus by condensation of contiguous mesenchyme (Text-fig. 2 A). This mesenchymal tissue carries the nutrient vessels, the lymphatic channels described by Sainte-Marie and LeBlond (10), and vasomotor nerve twigs. At the time of lymphocyte formation within the thymuses, the cortical epithelial cells separate from each other, producing local bulges in the hitherto smooth circumference of the cortex (Text-fig. 2, B and C). The depression in the capsular surface between these bulges remains filled with mesenchymal connective tissue. The effect of this bulging is

to enormously increase the volume of cortical mass relative to the inner medullary region (Text-fig. 2 D). With maturation of the thymuses, the cortical bulges swell as they fill with lymphocytes, compressing the mesenchymal connective tissue between adjacent cortical bulges. The connective tissue which is thus included between adjacent cortical bulges forms a connective tissue *septum.* The formation of many such septae partially subdivides each thymus into *lobules* (Text-fig. 2 E). The lobules produced by this process are labile anatomical features, however, and retain their shape only as long as they are filled with lymphocytes. Following lymphocyte depletion these lobules collapse as their constituent epithelial cells draw together (Text-fig. 2 F). With reconstitution of the thymic lymphocytes, these lobules reexpand again to their earlier size. By this process of lobule formation large masses of mesenchymal tissue become included within the physical confines of the thymuses. It is in this interlobular mesenchymal connective tissue, which embryologically is non-thymic, that the significant changes in the NZB mice occur. Fig. 1 illustrates a low power view of a left thymus from a young NZB mouse, to show a small interlobular connective tissue septum, at the bottom of which are several vessels. Fig. 2 is a medium power view of a venule in the cortex of a thymus from an 83-day-old NZB mouse, to illustrate the normal appearance of the perivascular connective tissue compartment, before the follicles develop.

Changes in NZB Mice.—In NZB mice, from about the age of 200 days and irregularly thereafter, at a time when the thymuses had already lost over half their weight (Text-fig. 1), small focal aggregates of lymphocytes, plasma cells, and reticulum cells could be found in the perivascular connective tissue between the lobules (Figs. 3 to 6). At the time these early cellular infiltrates were first noted, the adjacent thymic lymphocytes and epithelial cells showed no changes. With increasing age, the number and size of such perivascular lymphoid structures increased. As these aggregates enlarged, circumferential organization became apparent, and a follicular pattern emerged (Figs. 7 and 8).

The young aggregates and follicles contained numerous small lymphocytes, plasma cells, reticular cells, pigment-laden macrophages, and mast cells (Figs. 3, 5, and 6). As the follicles enlarged and organized, sheets of pale-staining, large, reticulum cells came to occupy the centers of these follicles (Figs. 7, 8, 11, and 12), producing the familiar "germinal" or secondary centers. Fragments of lymphocyte nuclei could often be observed within the cytoplasm of the reticulum cells of these follicles, a finding so characteristic of germinal centers. Variable numbers of lymphocytes were present at the periphery of the lymphoid follicles, but it was not histologically apparent whether they derived from that follicle or from elsewhere.

Those follicles which developed in relation to the centrally-located medullary vessels often tended to be more highly organized than those which developed near the capsule. The perivascular follicles at the capsule often followed



TEXT-FIG. 2. Wedge sections, to show progressive normal development of thymic lobules. The primitive epithelial thymus is presented in A. In B, lymphocytes have begun to accumulate in the interstices between the thymic epithelial cells, thereby swelling the cortical tissue. With continued lymphocyte accumulation (C to D), the cortical regions expand peripherally, enclosing connective tissue between the interlobular spaces. In E, the fully-expanded cortex has formed lobules, which enclose between them a connective tissue *septum*. The lobules thus formed by the expansile cortical tissue collapse following lymphocyte depletion, in F.

the vessels outside the capsule for varying distances, producing a local bulging of the cortical surface.

With increasing age, the thymuses became very small (Text-fig. 1), and the relative area involved by the follicles appeared to increase (Fig. 11). In these senescent thymuses, the area occupied by the follicles often appeared large in comparison to that occupied by cortex and medulla. Consideration of the *absolute* weights of these specimens, however, (Text-fig. 1) indicated that this increase was relative, and was due in large part to a decrease in cortex and medulla rather than solely to an increase in the volume of tissue occupied by the follicles. No differences were noted between the sexes with regard to the basic morphology of the follicles, although characteristically the male thymuses were smaller and the follicles were not as well developed.

Of special interest was the observation that the venules which were found in the central portions of the thymic lymphoid follicles had large, plump, endothelial cells, and strongly resembled the postcapillary venules described by Gowans (11). These postcapillarly venules were present in the follicles of both NZB and Swiss mice. A high power view of a typical postcapillary venule from the center of a thymic follicle in a Swiss mouse is illustrated in Fig. 19.

A characteristic type of erythrophagic macrophage was present in large numbers in thymuses from anemic NZB mice. These cells, 15 to 20 μ in diameter, were filled with brightly eosinophilic material and resembled Russell's bodies. In favorable specimens, variable sized red cells and red cell fragments could be recognized in these cells. Thymuses which contained these cells in large numbers also had increased numbers of periodic-acid–Schiff positive (PAS) liopchrome cells, both in the cortex, and in the perivascular connective tissue spaces. The erythrophagic macrophages were not limited to the thymuses, but were found in most other organs of anemic NZB mice.

The Hassall's Corpuscles.—The Hassall's corpuscles of young NZB mice were normal in numbers and appearance. All stages in their development, from small, individual epithelial cells with cytoplasmic fibrillization and vacuolization (Figs. 13 and 14); intermediate sized complexes with central cavities (Figs. 15 and 16); and large, cystic structures (Figs. 17 and 18) could be found. Direct *intrusion* into the central cavity by epithelial cells and lymphocytes was frequently observed. An example of an epithelial cell in the process of *intrusion* is illustrated in Fig. 16. Many of the central cavities of the Hassall's corpuscles contained partially autolysed cell debris from intruded cells (Figs. 16 to 18). Comparison of the Hassall's corpuscles of the young NZB mice with those of Swiss mice of the same age revealed no differences in number, size, or appearance. The intrathymic portions of the old thymico-pharyngeal ducts could often be recognized in the cephalad portions of each thymus as an imperfectly formed epithelial

tube with a rudimentary central cavity, and intrathymic parathyroid glands[1] were often present (12).

In thymuses of all mice 200 days of age or more, the number of small Hassall's corpuscles was greatly diminished. Large cysts, frequently partially lined by secretory and ciliated epithelium, were common, however. By serial section, these cysts could be traced in depth, and it was observed that whereas, in some cases, adjacent cysts opened into a common central cavity, in most cases they did not. Most of these cysts contained considerable numbers of lymphocytes, lipochrome (PAS) cells, and cell debris. The remnants of the old thymico-pharyngeal duct were often dilated in both thymuses, and frequently contained large numbers of lymphocytes and cell debris in inspissated eosinophilic fluid. Occurring at the cephalad portion of the capsule, and often bulging well outside the capsule of that thymus, these distinctive cystic structures occurred in both NZB and in Swiss mice.

Related Changes in Other Organs in NZB Mice

Perivascular Infiltrations and Follicle Formation.—At the time that the Coombs' test became positive and the spleen began to enlarge in NZB mice (200 days), a widespread proliferation of plasma cells, lymphocytes, and mesenchymal cells occurred in the perivascular connective tissue spaces of many organs. The lungs and kidneys were especially heavily involved. Fig. 9 illustrates some of the proliferative lesions of the perivascular connective tissue spaces of the lungs. Fig. 10 shows comparable lesions in the kidneys. Particularly noteworthy was the presence of large numbers of characteristic erythrophages in these focal perivascular collections of cells. Most animals which had follicles identifiable in the thymuses also had perivascular lesions of comparable morphology in the lungs and kidneys.

The spleen and lymph nodes, which normally do contain lymphoid follicles, enlarged markedly at the same time that lymphoid structures were appearing in organs where they normally are not found (lungs, kidneys, and thymuses). Histologically, much of the increase in splenic weight could be observed to be due to hypertrophy of the existing lymphoid follicles. The splenic weight increase, quantitated in Text-fig. 1, may therefore be used as a rough index of the degree of lymphoid follicle formation and enlargement. When one compared the degree of lymphoid hypertrophy in the spleen, partially reflected by the 20- to 30-fold increase in splenic mass, to the degree of lymphoid hypertrophy in the thymuses, reflected by the absolute *decrease* in thymic weight, it may be seen that the thymic contribution of lymphoid follicles in this disease is minimal.

Some NZB mice had thymuses which showed dilatation of the perivascular lymphatic spaces filled with lymphocytes. In these same mice numerous com-

[1] We are indebted to Dr. William E. Ehrich of the University of Pennsylvania for this interpretation.

parable structures were found in other organs in analogous locations, particularly in the perivascular spaces of the spleen, kidney (Fig. 10, arrow), lung, and lymph nodes.

In NZB mice over 300 days of age changes related to the hemolytic anemia were common.

Intravascular Agglutination.—In several mice red cell agglutination could be observed *intravascularly* through the transparent walls of peritoneal vessels. In one instance the severed vessels leaked completely agglutinated blood cells of distinctive appearance. This intravascular agglutination has been noted to occur in high incidence in old NZB mice by Dr. Bielschowsky.[2]

Biliary Changes.—Small and large friable pigmented stones were common in the gall bladders of older mice, associated with varying degrees of cholecystitis, observed histologically. Intrahepatic biliary stasis was evidenced by bile pigment sludge in distended bile ductules.

Erythrophagia and Erythrocytosis.—The marked increase in splenic weight noted after 200 days was due in part to a pronounced increase in the red pulp. Erythroblasts were present in large numbers in the actively proliferating red pulp tissues, and in the sternal marrow. Foci of extramedullary hematopoiesis were common in lymph nodes and on occasion in the liver. In the lymph nodes and spleen many erythrophagic cells were noted. They contained from 1 to 4 red cells in various stages of decomposition. The deeply eosinophilic homogeneous staining of the red cell breakdown product, and the relatively small amount of hemosiderin present compared to the severe degree of anemia, suggested the possibility that the hemoglobin decomposition might follow an abnormal route.

Other Changes.—Renal amyloid-like glomerular lesions, and chronic ulcerations of the skin associated with mast cell infiltrations were frequently observed. Their appearance conformed to previously published descriptions (2).

Thymic Changes in Swiss Mice

The weight of the thymuses of Swiss mice showed the same rapid increase early in life, and the same long, slow senescent decrease, observed for the NZB mice (Text-fig. 1). The histologic alterations of the thymuses of these mice showed a sequence similar to those of the NZB mice. Young Swiss mice had normal numbers of Hassall's corpuscles of all stages. In animals over 200 days of age, few small Hassall's corpuscles were present, but numerous large, cystic forms were observed. In many instances some of the epithelial cells lining these cysts had developed cilia, while others showed cytoplasmic granulations suggestive of serous secretion. All the smaller corpuscles contained partially autolysed cells and cell debris, whereas the larger corpuscles or cysts contained intact lymphocytes, lipochrome (PAS) cells, and occasionally, neutrophiles.

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[2] Personal communication, M. Bielschowsky.

Thymuses of Swiss mice under 200 days of age rarely contained lymphoid follicles. After this time, however, focal collections of perivascular lymphocytes and mesenchymal cells were present and fully developed follicles could be recognized by 220 to 250 days. Figs. 21 to 24 illustrate the low power appearance of characteristic follicles in normal Swiss mice. After 300 days of age they could be recognized in the majority of mice examined, and many had germinal centers. As illustrated in Table I, 70 per cent of normal Swiss mice over 200 days of age have thymuses which contain lymphoid follicles and distended lymphatic spaces. This may be compared to 87 per cent of the anemic NZB mice which have similar thymic lesions at comparable age. While the lymphoid follicles in the thymuses of Swiss mice appeared to be larger and more numerous with age (Figs. 21 and 22), this increase may be more apparent than real. As the thymuses became smaller with age, the lymphocytes of both cortex and medulla became less numerous, bringing these follicular structures into stronger

TABLE I

Presence of Lymphoid Follicles in the Thymuses of Swiss and NZB Mice over 200 Days Old

	Swiss	NZB
Total No. of mice (over 200 days old)	77	48
No. with follicles	54 (70 per cent)	42 (87 per cent)
No. Coombs' tested	50	36
No. positive	0	34 (94 per cent)

contrast with the surrounding tissues. In many cases the follicles occupied an estimated 60 per cent of the volume of that thymus (Figs. 21 and 22). It must be remembered, however, that these senescent thymuses were quite small relative to young thymuses, and ranged in weight from 5 to 20 mg. Chromolipid or PAS cells, recently noted to be autofluorescent (13), were numerous in the thymuses of old Swiss mice, but only the thymuses of NZB mice contained many erythrophages or numerous mast cells.

In the thymuses of old NZB and Swiss mice (over 300 days), large numbers of perivascular lymphocyte cisterns were observed (Fig. 20). These structures were identical in form and appearance in NZB and Swiss mice. On occasion, lymphocyte cisterns were also present in the spleen or lymph nodes of aged Swiss mice. In a few instances focal perivascular lymphoid aggregates in the lungs and kidneys were present. The intrathymic follicles in the Swiss mice contained specialized venules similar in appearance to postcapillary venules, described above for the NZB follicles. An example of these structures is illustrated in Fig. 19.

DISCUSSION

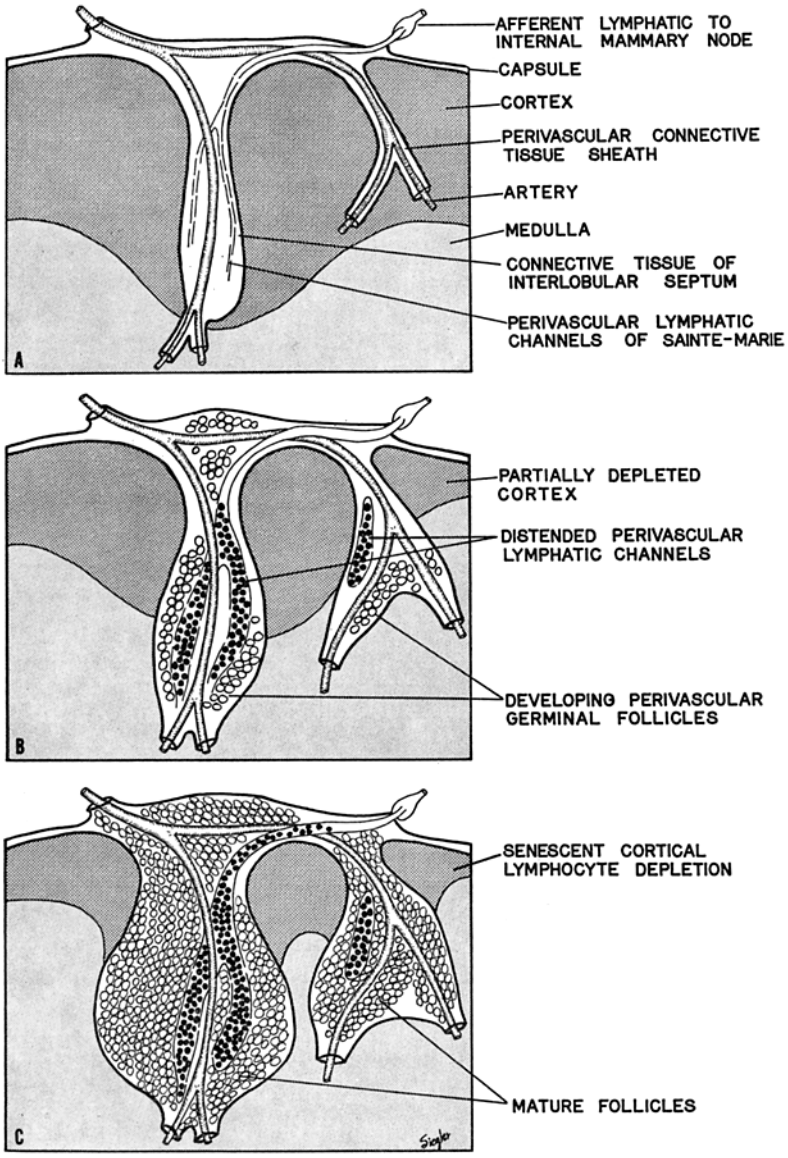
The present study confirms earlier reports that NZB mice develop characteristic structures in their thymuses which resemble lymphoid nodules or follicles (5, 7). Pending further study, the exact designation of these follicle-like structures should remain open. Favoring their identity as lymphoid follicles is their circumferential orientation (Figs. 7 and 11); the presence in their centers of large, pale-staining reticulum cells (Figs. 8 and 12); and the presence of postcapillary venules (Fig. 19). Opposing this is the relative absence of mitotic activity in the central region of the follicle, and the lack of sharply defined borders. Our use of the term follicle or nodule means only a dense mass of lymphatic tissue showing definite spatial orientation.

Our interpretation of the *significance* of these intrathymic structures relative to the autoimmune anemia of these mice differs from that advanced by Burnet, who suggested that their presence in the thymuses indicated that they played a causal role in the hemolytic anemia (7). In the present study it was demonstrated that lymphoid follicles developed also in other organs of NZB mice (Figs. 9 and 10), and that they could be found in the thymuses of most aging, *normal* Swiss mice (Figs. 21 to 24). Based on the widespread occurance of these lymphoid nodules or follicles in the perivascular connective tissue of most organs in NZB mice, it was concluded that the thymic follicles in NZB mice did not constitute unique or primary changes of the thymuses.

In Swiss mice the Coombs' test was invariably negative, despite the presence in the thymuses of these mice of many large lymphoid follicles (Figs. 21 to 24). Thus, the presence of lymphoid follicles in the perivascular connective tissue of the thymuses was not necessarily associated with a positive Coombs' test. Furthermore, since they occur in apparently normal mice, they need not even constitute evidence of specific pathology. The development of these structures may be regarded as an exaggeration of normally existing lymphoid aggregates about the intrathymic vessels, the existence of which may be obscured in younger animals by the presence of contiguous lymphocytes. Slight enlargement of these structures, associated with senescent decrease in thymic mass, brings them into prominent view by 300 days in normal mice. Where they are conspicuously enlarged, as part of a generalized enlargement of lymphoid structures in NZB mice, they become visible by 200 days.

The formation of these lymphoid follicle-like structures in the thymuses, while not specific for NZB mice with hemolytic anemia, as previously reported (5), are nevertheless in themselves of interest. In Text-fig. 3 a possible sequence of events leading to the formation of these structures is diagrammatically indicated.

The presence of postcapillary venules in the central portions of these thymic follicles (Fig. 19) is of interest, because of the studies by Gowans (11) which indicate that systemically circulating lymphocytes leave the blood vessels by



TEXT-FIG. 3. Suggested pathogenesis of the thymic lymphoid follicles of NZB/Bl mice. Note that as the follicle enlarges, the relative and absolute volume of the thymic cortex decreases, so that the follicle comes to lie almost entirely in the medulla with age.

way of these specialized vessels. The finding of these vessels in these thymic follicles in NZB and Swiss mice suggests that the lymphocytes present within the thymic follicles may have been derived from systemically circulating lymphocytes rather than from local origin.

Hassall's Corpuscles.—The thymuses of all animals contain unique epithelial structures called Hassall's corpuscles. These corpuscles are found only in the thymuses, and are so characteristic in appearance that they are used by many to identify thymic tissue. At present, their function is unknown, although it has been suggested that they play a role in intrathymic cell physiology, perhaps that of cell removal by digestion (8). An interesting histochemical study pointing in this direction has recently appeared (14). NZB mice have been emphatically characterized as being free of Hassall's corpuscles in a recent study which noted that the medullary epithelial cells were "often arranged in little groups but never producing anything resembling Hassall's corpuscles" (7). In the present study we were not able to confirm this observation, but instead found normal numbers of Hassall's corpuscles in all stages of development (Figs. 13 to 18).

There is at present no unanimous agreement about the origin of these structures. While most morphologists have derived these epithelial structures from thymic epithelial cells originally associated with the thymico-pharyngeal ducts (see reference 8), they have recently been declared to originate from blood vessels by Mackay *et al.* (15). These workers postulated that the Hassall's corpuscles derived from short capillary segments isolated from the circulation by focal closure of the lumen. They explained their predominant location in the medulla of the thymus by stating that the blood flow was more sluggish there than in the active cortex. In the present study, all stages in the development of these epithelial structures from single epithelial cells to large, multicellular complexes were observed (Figs. 13 to 18). The relation of these developing structures to adjacent blood vessels was followed in serial sections, but in no instance was direct continuity observed. These data support the view that Hassall's corpuscles originate from epithelial cells rather than from blood vessels.

SUMMARY

Lymphoid follicles evolve in the perivascular connective tissue of many organs, including the thymuses, in NZB/Bl mice with hemolytic anemia. In previously published studies, these *thymic follicles* have been held to be causal in the autoimmune genesis of the hemolytic anemia. The present study contradicts this interpretation by demonstrating: (*a*) lymphoid follicles develop in the perivascular connective tissue of *many* organs in NZB mice, and are not restricted to the thymuses; and (*b*) thymic lymphoid follicles develop in aged Swiss mice without hemolytic anemia.

Contrary to previous reports, the thymuses of NZB mice contain normal

numbers of Hassall's corpuscles, which develop from preexisting thymic epithelial cells, and not from blood vessels.

The author is grateful to Dr. Marvin A. Rich, Albert Einstein Medical Center, Philadelphia, for encouragement, stimulating guidance, and much help with the manuscript. Thanks are due to Dr. Marianne Bielschowsky, of the University of Otago, New Zealand, for her kindness in making these mice available, and Dr. William E. Ehrich, of the University of Pennsylvania, for informative discussion of this work. The author is very pleased to acknowledge with thanks the skilled technical assistance of Mrs. Wilhelmina Harrell, and Mrs. Shirley Siegel.

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EXPLANATION OF PLATES

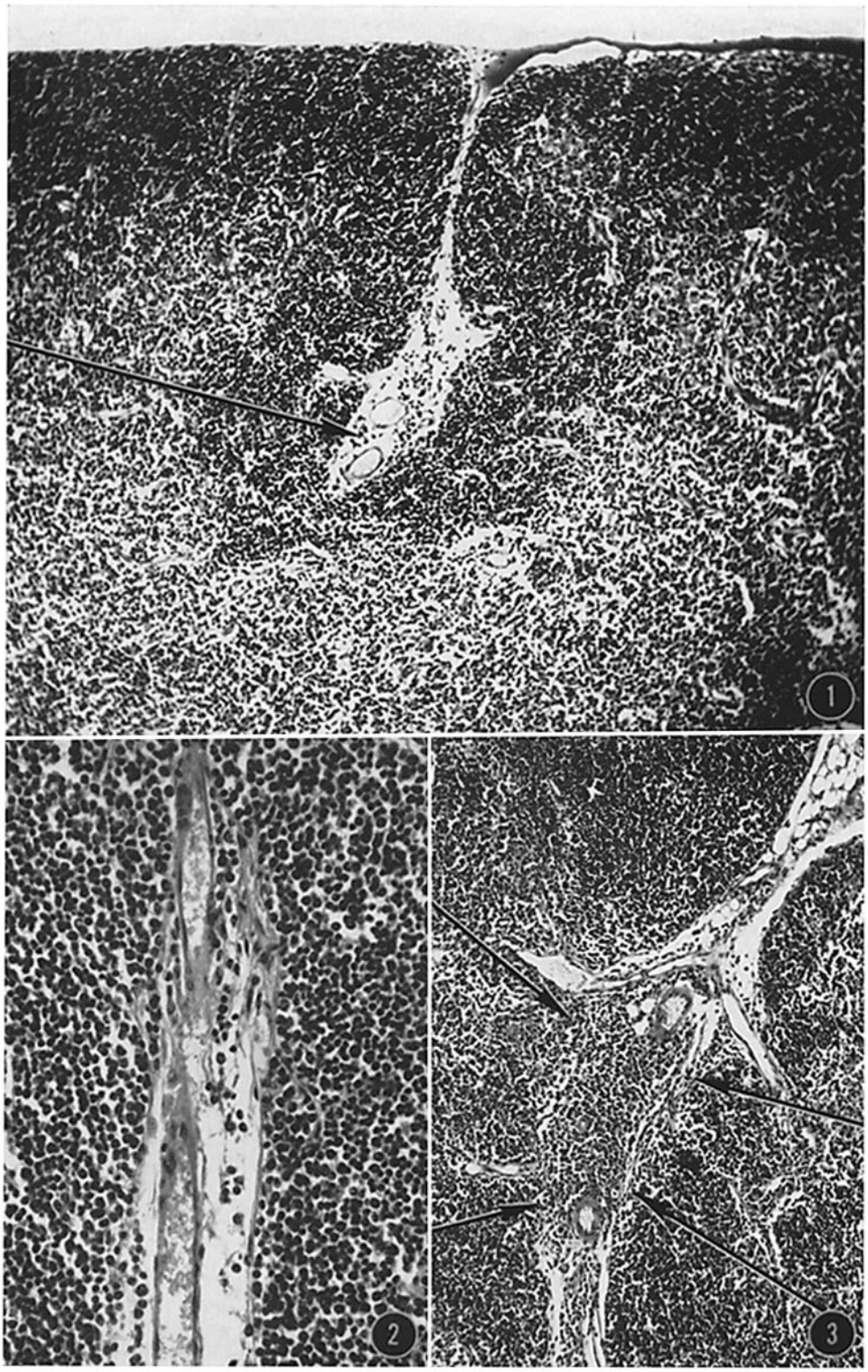
Unless otherwise noted, all staining is with hematoxylin and eosin.

PLATE 57

FIG. 1. Low power view of normal thymic cortex, to illustrate two small lobules separated from each other by an interlobular connective tissue septum. At the bottom of the septum lie the nutrient vessels (arrow). 25 mg left thymus, 3-week-old NZB mouse. × 100.

FIG. 2. Medium power view of penetrating vessel illustrating the normal perivascular connective tissue compartment which surrounds the vessel. 59 mg left thymus, 83-day-old NZB mouse. × 200.

FIG. 3. Low power view of very early aggregation of cells in the perivascular connective tissue space (arrows) in an NZB mouse. While not visible at this magnification, these cells consist of lymphocytes, plasma cells, eosinophils, pigment-containing macrophages, and mast cells. 14 mg left thymus, 187-day-old NZB mouse. × 50.



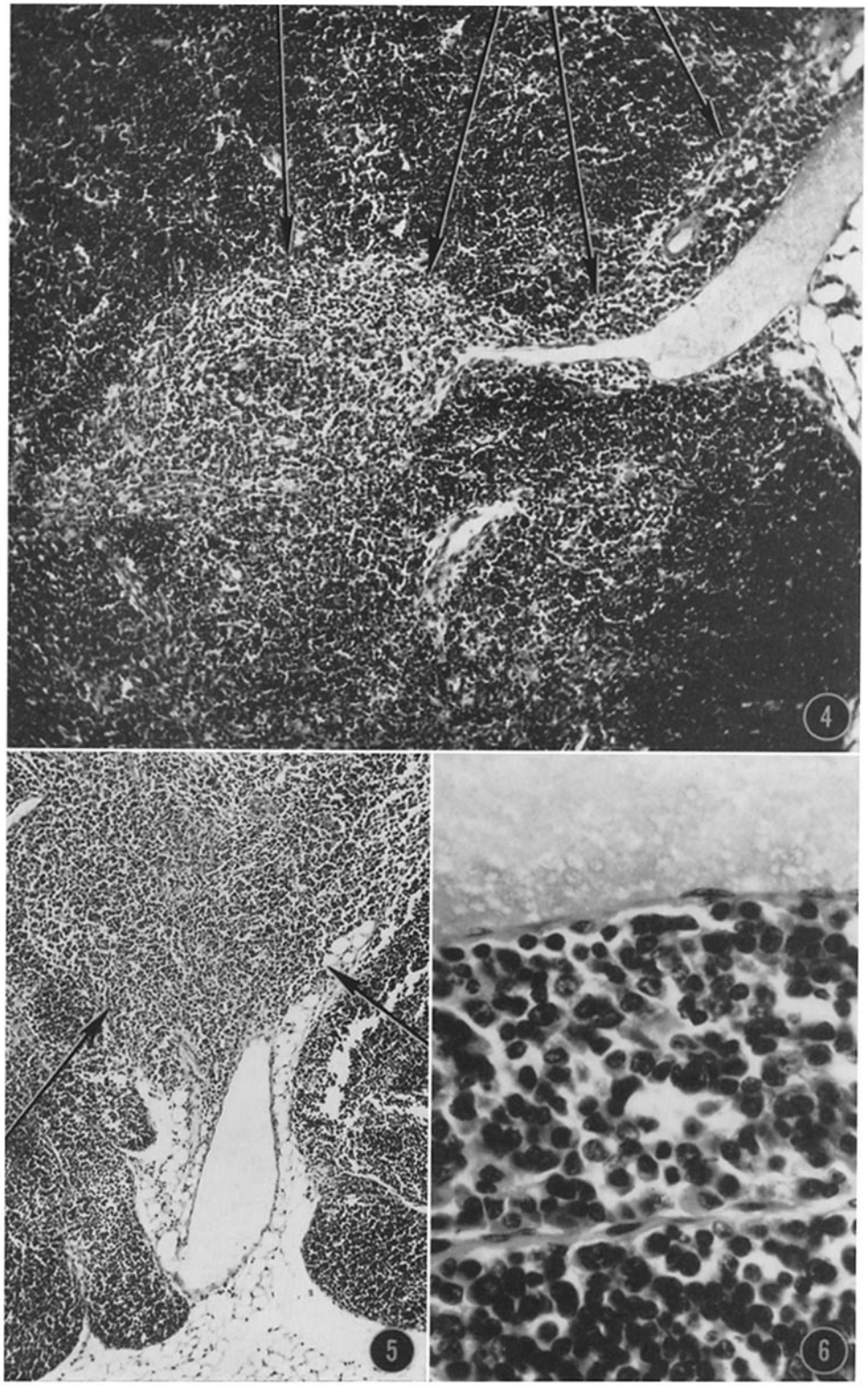
(Siegler: Pathogenesis of thymic changes in NZB mice)

PLATE 58

FIG. 4. Early organization of perivascular aggregate into small follicle. Note the extension of the cellular aggregate in the perivascular compartment (arrows) (see Fig. 6). 20 mg right thymus, 331-day-old NZB mouse. × 100.

FIG. 5. Massive cellular aggregate in perivascular space near cortex. Lobules of cortex are present at both sides of a penetrating venule, at bottom of photo. The upper portion of photo shows the greatly expanded connective tissue compartment, filled with lymphocytes and plasma cells (between arrows). 16 mg left thymus, 329-day-old NZB mouse. × 50.

FIG. 6. High power view of cellular aggregate under upper arrow in Fig. 4. Cells consist of lymphocytes and plasma cells. Top of photo is vein lumen, middle area is perivascular aggregate, and lower portion is thymic cortex. Note sharp separation of the cortex from the perivascular aggregate. × 400.



(Siegler: Pathogenesis of thymic changes in NZB mice)

PLATE 59

FIG. 7. Organization of small follicle. Central cells are large, pale-staining reticulum cells, circumferentially oriented to produce the familiar "germinal" or secondary center (see Fig. 8). 20 mg left thymus, 191-day-old NZB mouse. × 100.

FIG. 8. High power view of reticulum cells from center of follicle in Fig. 7. × 400.

FIG. 9. Lymphocyte aggregation in perivascular and peribronchial connective tissue of lung of mouse with similar aggregates in thymuses. See also Fig. 10. 253-day-old NZB mouse. × 50.

FIG. 10. Lymphocyte aggregate and distended lymphatic channel (arrow) in perivascular connective tissue of kidney from same animal as Fig. 9. × 50.

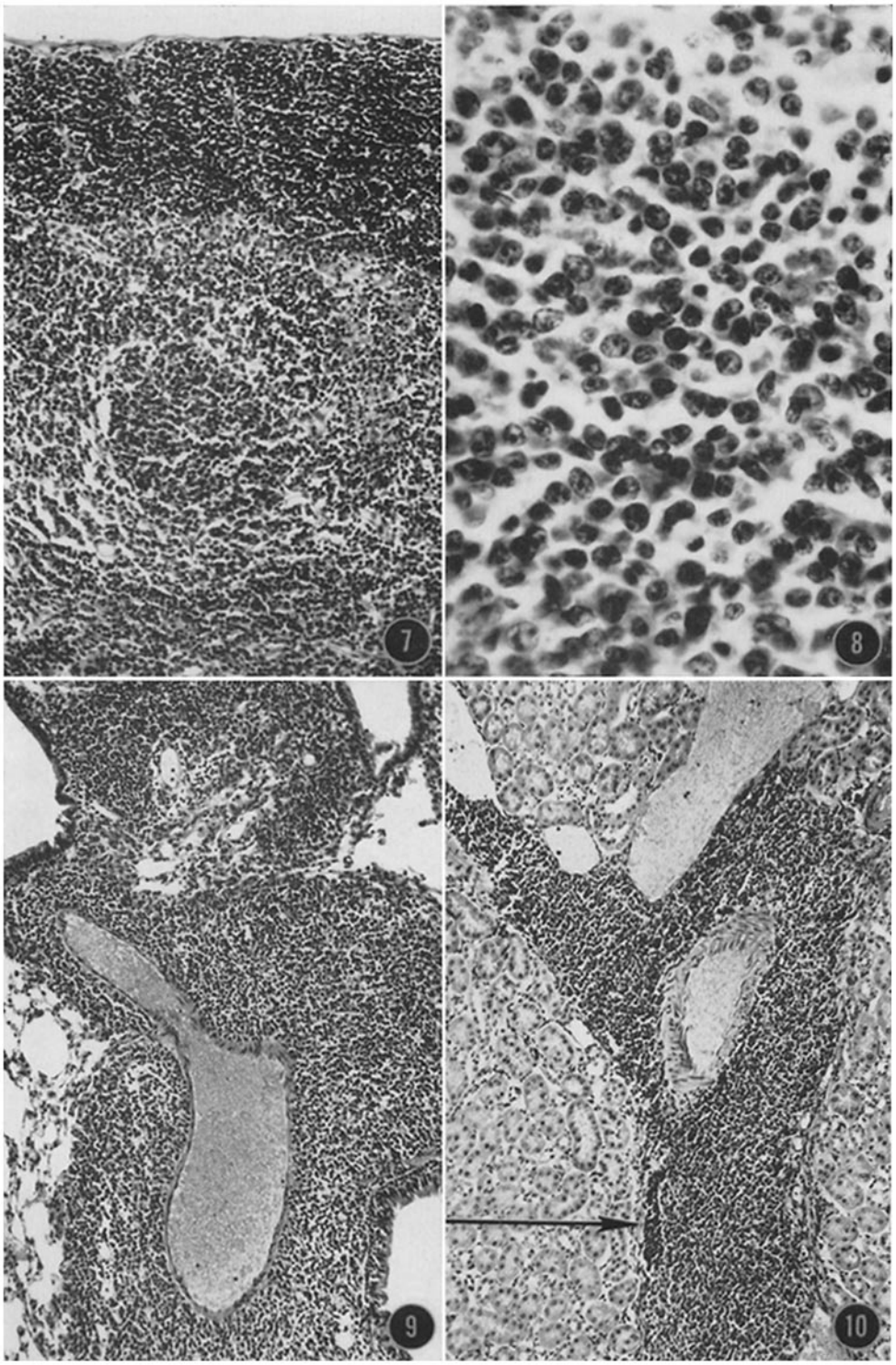


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PLATE 60

FIG. 11. Coalescence of two lymphoid follicles with early secondary centers. The overlying cortex, while intact, is reduced in thickness. See Fig. 12. 24 mg right thymus, 231-day-old NZB mouse. × 100.

FIG. 12. High power view of lymphoid follicle (right) and adjacent cortex (left) from Fig. 11. Follicular cells include lymphocytes, plasma cells, macrophages, and reticulum cells. Note sharp demarcation of follicle from cortex. × 400.

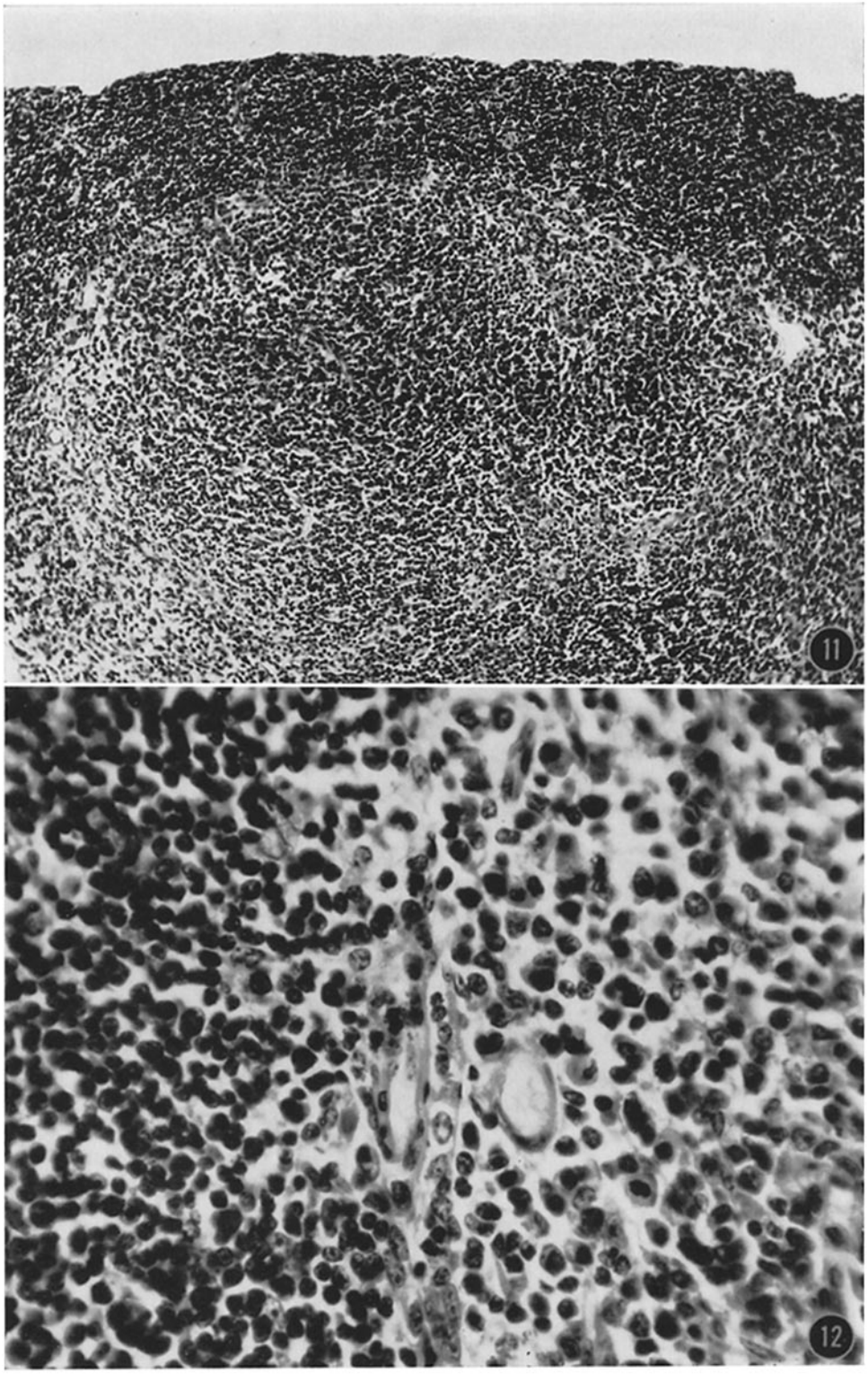

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PLATE 61

FIGS. 13 to 18 are from the thymuses of NZB mice between 30 to 60 days of age.

FIG. 13. Single epithelial cell with fibrillization and vacuolization of cytoplasm and marked nuclear alteration. × 2000.

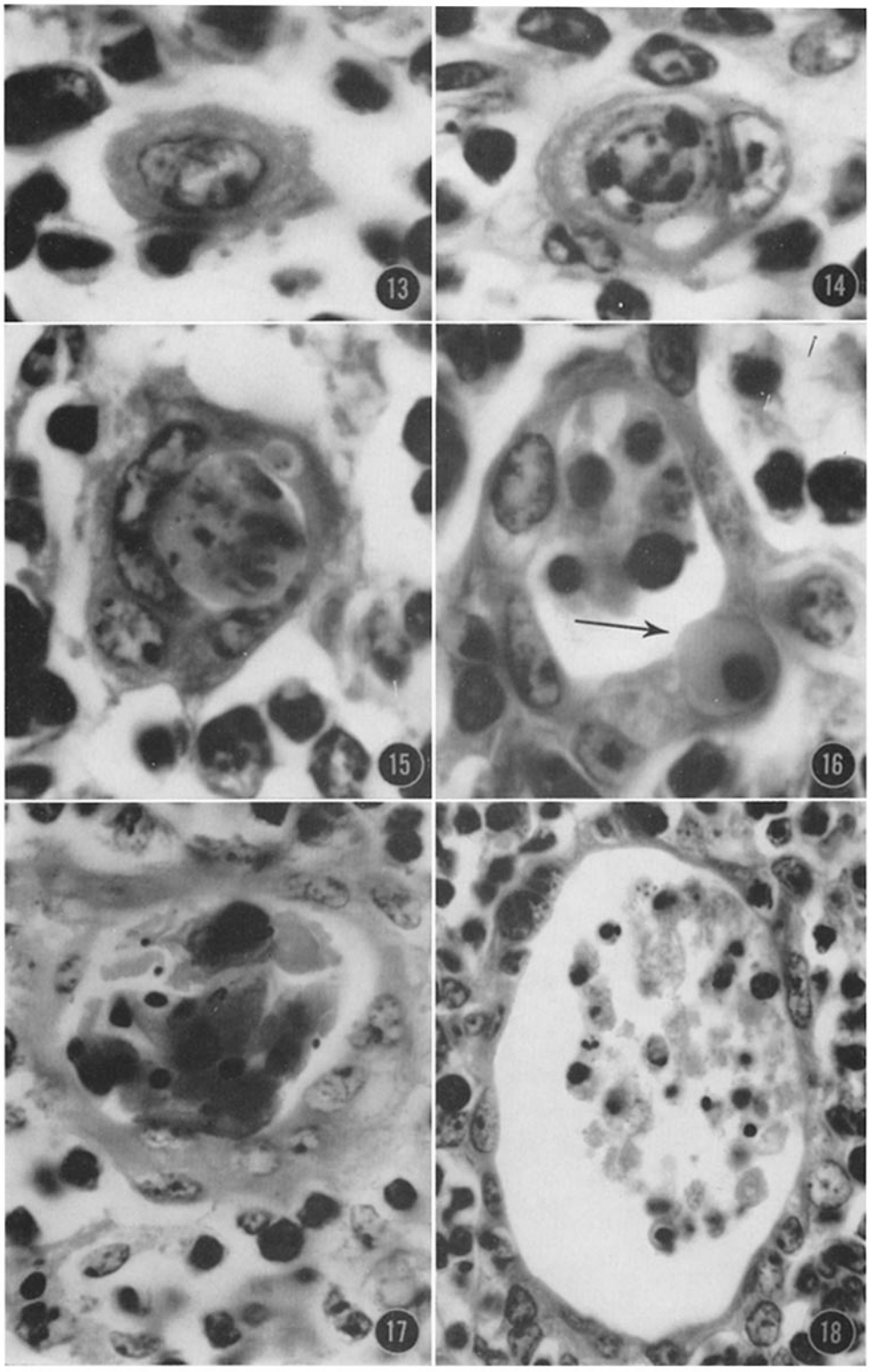
FIG. 14. Concentric lamination produced by three epithelial cells. Central cell shows fragmentation of nuclear chromatin and nuclear vacuolization. Second epithelial cell, at 3 o'clock, is peripherally applied around the first cell. Third epithelial cell (nucleus slightly out of plane of focus) at 7 o'clock showing peripheral orientation of cytoplasm. × 2000.

FIG. 15. Early central cavity formed by enlargement of corpuscle. Nuclei of five epithelial cells visible in syncytial wall. Small vacuole in corpuscle wall at 2 o'clock contains cell debris. Central cavity filled with eosinophilic cell debris. × 2000.

FIG. 16. *Intrusion* of a lining epithelial cell into central lumen (arrow). × 2000.

FIG. 17. Fully developed corpuscle now measures over 100 μ, in diameter. Central cavity filled with partially autolysed epithelial cell debris and lymphocyte nuclei fragments. × 1000.

FIG. 18. Almost complete autolysis of intruded cells in mature cyst. × 1000.

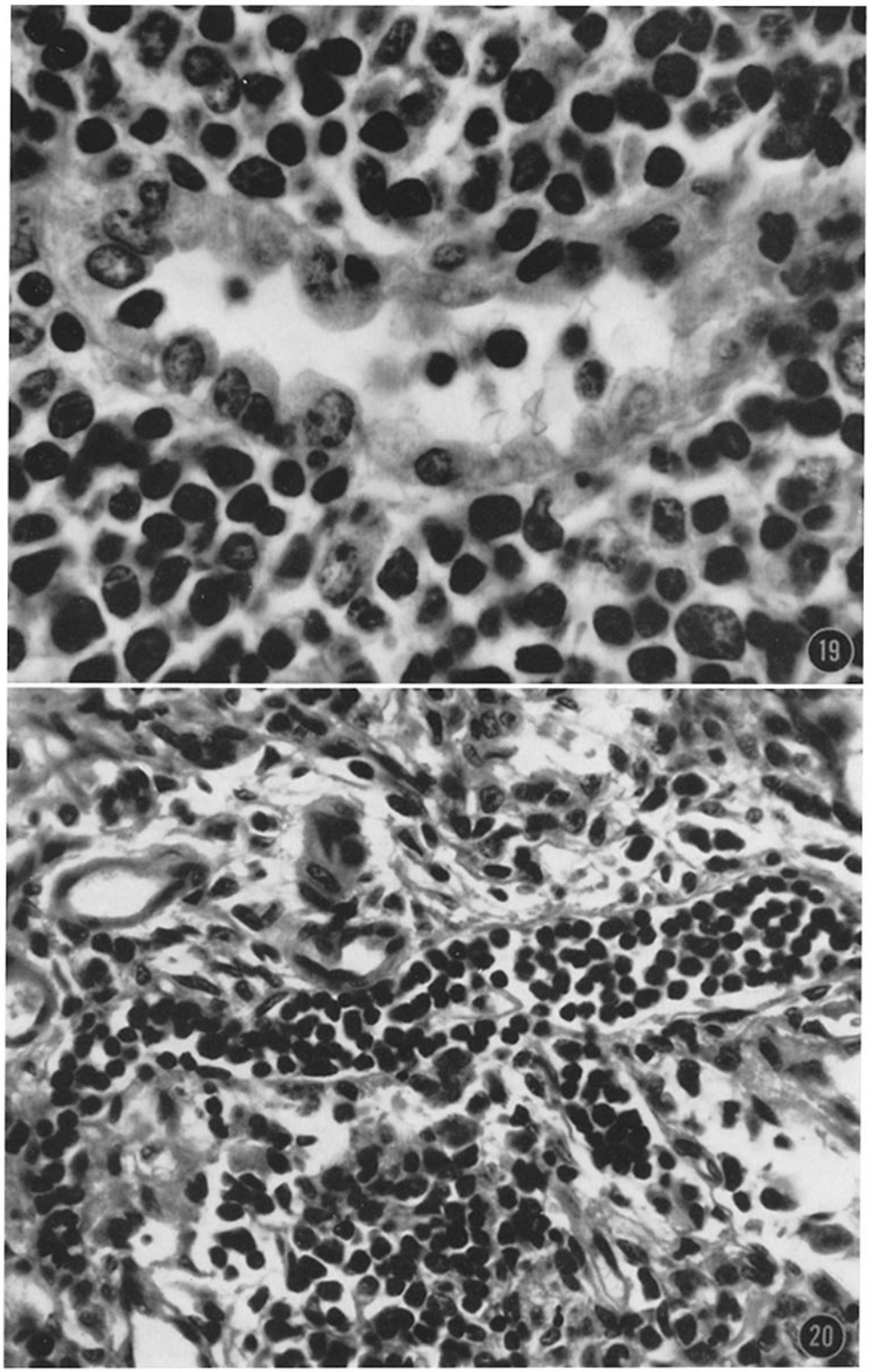


(Siegler: Pathogenesis of thymic changes in NZB mice)

PLATE 62

FIG. 19. Postcapillary venule, located in central region of lymphoid follicle in thymic perivascular connective tissue space. These structures were present in most thymic follicles in both NZB and Swiss mice. 45 mg right thymus, 215-day-old Swiss mouse. × 1000.

FIG. 20. Characteristic perivascular thymic lymphatic channel distended with lymphocytes. These structures were present in most NZB and Swiss mice over 200 days of age. 12 mg right thymus, 329-day-old NZB mouse. × 400.



(Siegler: Pathogenesis of thymic changes in NZB mice)

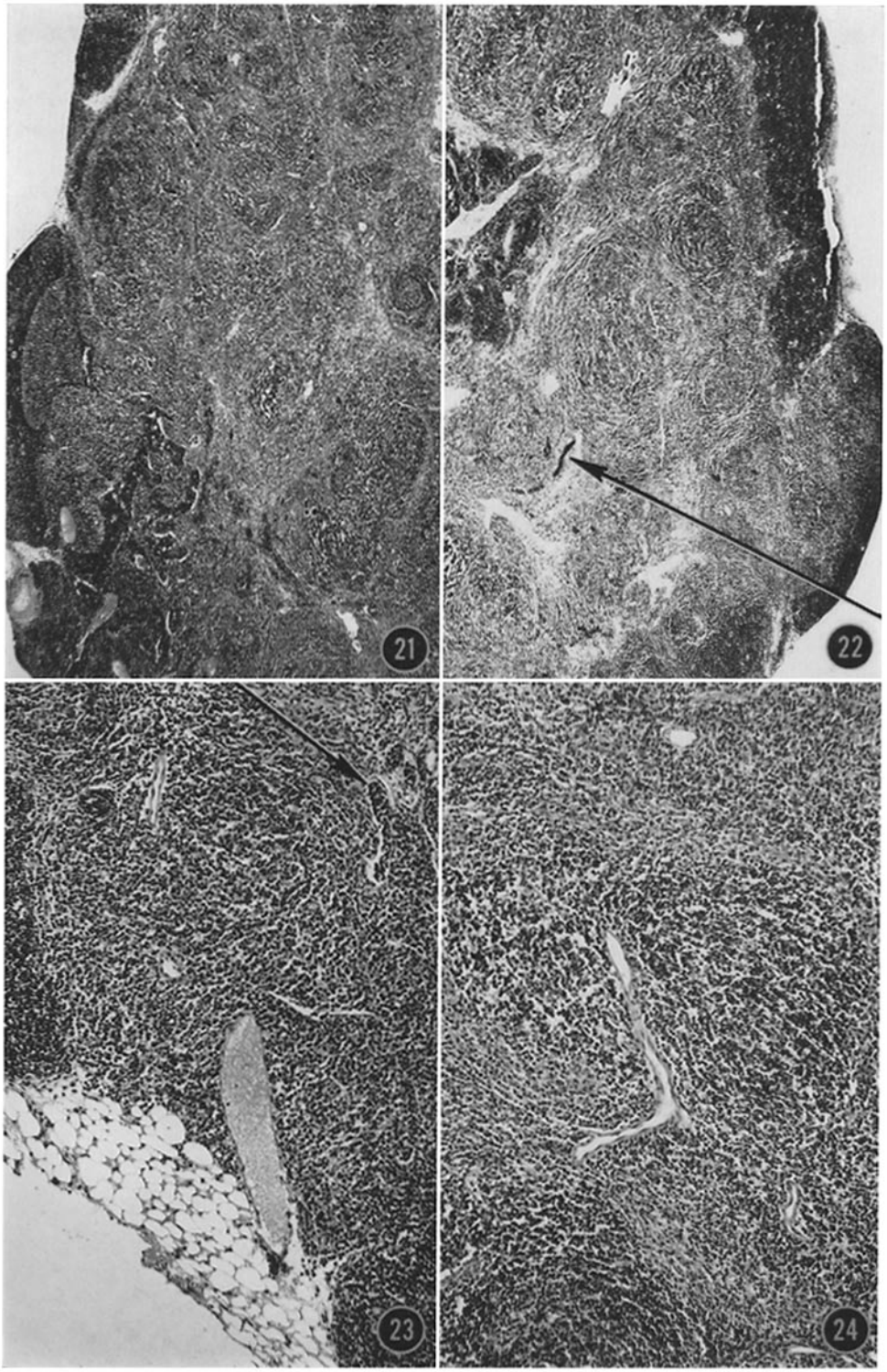
PLATE 63

FIG. 21. Extensive coalescence of numerous lymphoid follicles, most of which have secondary centers, in a Swiss mouse with negative Coombs' test and free of apparent disease. 32 mg right thymus, 220-day-old Swiss mouse. × 50.

FIG. 22. Extensive replacement of central portion of a left thymus by lymphoid follicles with secondary or "germinal" centers. This Swiss mouse was free of apparent disease and had a negative Coombs' test. Arrow indicates distended lymphatic channels. 38 mg left thymus, 222-day-old Swiss mouse. × 50.

FIG. 23. Perivascular lymphoid aggregates, enveloping a venule leaving right thymus. Arrow indicates distended lymphoid channels. This Swiss mouse was free of apparent disease and had negative Coombs' test. 26 mg right thymus, 215-day-old Swiss mouse. × 100.

FIG. 24. Extensive perivascular follicle formation disrupting normal thymic architecture. This Swiss mouse had no apparent disease and a negative Coombs' test. 22 mg left thymus, 215-day-old Swiss mouse. 100 ×.



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