EXPERIMENTAL AMYLOIDOSIS

AMYLOID INDUCTION WITH A SOLUBLE PROTEIN ANTIGEN IN INTACT, BURSECTOMIZED AND THYMECTOMIZED CHICKENS

ROBERT L. DRUET, M.D.,* AND DAVID T. JANIGAN, M.D.†
From the Department of Pathology and Oncology, University of Kansas Medical Center, Kansas City, Kansas

Various theories as to the role of plasma cells or their precursors in amyloidogenesis have been proposed.[1] Some believe that these cells either elaborate circulating immunoglobulins or glycoproteins which, alone or in combination with other factors, subsequently polymerize or precipitate in interstitial spaces to form amyloid, or that they elaborate amyloid directly and, therefore, represent the final cellular source of amyloid. On the other hand, there is growing evidence that amyloid is elaborated by reticuloendothelial cells, but whether or not plasma cells participate, in some way, has not been established.

In chickens, the development of plasma cells and nodular lymphoid tissue in the spleen can be markedly impaired[2-7] by surgical ablation of the bursa of Fabricius in newly hatched chicks. If sublethal body x-irradiation is applied to the chicks following bursectomy, the effect on these cells and nodules is more consistent and complete, with relatively little effect on the development of other lymphoid cells.[8] At maturity, chicks which have had bursectomy show a marked impairment of specific humoral antibody response[2-4,7-11]; some exhibit a complete absence of serum immunoglobulins.[8,11] There is no impairment, however, of delayed hypersensitivity or homograft (allograft) rejection responses. The latter responses, on the other hand, can be decreased or inhibited by neonatal thymectomy[12,13] which causes depletion of small lymphocytes, with little or no detrimental effect on plasma cell formation. These experimental observations led to the concept that the immune system of the chicken is composed of two definable components: (a) a thymic-dependent one, in which the thymus is responsible for the development of small lymphocytes which mediate delayed hypersensitivity and allograft rejection responses; this component is possibly involved in antigen recognition as

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* Present address: Laboratory of the Scientific Director, Armed Forces Institute of Pathology, Washington, D. C.
† Present address: Department of Pathology, Dalhousie University Medical School, Halifax, Nova Scotia, Canada.

well[11]; (b) a bursal-dependent component, in which the bursa of Fabricius is responsible for the development of plasma cells, pyroninophilic nodular lymphoid tissue and immunoglobulin formation against antigens.[2,7,8,11] Implicit in this concept is the condition that the two types of cells do not transform into one another, so that ablation of one type selectively decreases its respective immune function without impairing that of the other. Some believe, however, that plasma cells may arise from small lymphocytes,[14,15] and that the bursa of Fabricius may control this transformation through the medium of a humoral factor.[4] Even if this is true, neonatal bursectomy still results in a marked decrease in the number of plasma cells and the amount of nodular lymphoid tissue.

The basic experimental hypothesis tested in this paper is: if plasma cells and their precursors, pyroninophilic cells, play a crucial role in amyloidogenesis, then chickens, which had had bursectomy during the neonatal period and had been given sublethal amounts of x-irradiation, should show a marked resistance to the induction of amyloid by injections of a soluble protein which is amyloidogenic in intact chickens.

Our previous studies in mice showed a significant and inverse relationship between the depletion of small lymphocytes and the rates of amyloid induction following injection of soluble antigens, i.e., the shorter the induction time, the greater the depletion.[16,17] The similarity of the lymphoid changes to those found in various experimental states of lymphocyte depletion and immunologic tolerance[18] in association with amyloidosis was also emphasized.[17] Because of these observations and since the thymus is important for small lymphocyte development, chicks were subjected to thymectomy in the neonatal period and their susceptibility to amyloidosis was compared to that of intact chickens and others with bursectomy.

Material and Methods

Experimental Subjects. Inbred white Leghorn, single-comb chicks, line 151, 2 to 3 days old, were obtained from the United States Department of Agriculture, Regional Poultry Research Laboratory, East Lansing, Mich. This line has a very low natural occurrence of spontaneous lymphocytic neoplasm[19] and specific isolation techniques were therefore not employed. All birds were fed Ralston Purina® Startina Pellets and allowed water *ad libitum.*

Operative Procedures. Bursectomy and thymectomy were performed on the second to fourth day after hatching as described by Janković and Isaković.[5] Chickens operated upon were given 500 to 520 r total body irradiation within 24 hours after the surgical procedure(s); conditions of irradiation: 250 kv, 15 ma, 0.25 mm copper filter; skin distance 50 cm, at a dose in air of 73.5 r per minute). Intact (unoperated) chicks were not given irradiation. The experimental design and the number of animals used in each procedure are summarized in Table I.

Protein Antigen. Sulfanil azo-casein was synthesized as described previously.[16] It was dissolved in 0.01M $NaHCO_3$ to a concentration of 10 gm (w/v) per cent.

Experimental Groups. At 6 weeks of age, intact chickens and those operated upon

TABLE I

POSTOPERATIVE MORTALITY *

Procedure †	Deaths ‡	Alive at 6 weeks
Bursectomy (120)	97	23
Thymectomy (26)	10	16
Bursectomy and thymectomy (10)	9	1
No operation (41)	0	41

* Subjects were newly hatched chicks and were also subjected to sublethal whole-body x-irradiation.

† Number of chicks in parenthesis.

‡ Within first 11 postoperative days.

were divided into the groups listed in Table II. Each group was given daily subcutaneous injections of either the azo-casein solution, or of 0.01M $NaHCO_3$, both in 3 ml quantities.

The chickens were killed at weekly intervals (Table II). The operative sites of surgically treated chickens were closely examined for remnants of either the thymus or bursa, and the areolar neck tissue, including the carotid sheaths, and the cloacal bed of the bursa were removed for histologic examination. All tissues were fixed in 4 per cent formaldehyde in phosphate buffer pH 7.4 for 24 hours. Spleen, thymus, bursa, gastrointestinal tract, liver, pancreas, kidneys, adrenal, lung, heart, pectoral muscle and sternal bone marrow were sampled. After fixation the tissues were dehydrated, embedded in paraffin and sectioned at 5 to 6 μ. All were stained with Congo red [20] and examined by ordinary and polarizing microscopy. Selected sections were stained with hematoxylin and eosin, methyl green–pyronine, toluidine blue, crystal violet,[21] thioflavine T,[22] periodic acid-Schiff (PAS)[23] and the indole method for tryptophane.[24]

Spleen blocks from one of each group of chickens were fixed in cold (0 to 2° C) 6.25 per cent glutaraldehyde in 0.1M phosphate buffer for 16 hours. After washing in buffer for 24 hours, the tissues were postfixed in osmium for electron microscopy.[25]

Histologic changes in lymphoid tissues were numerically graded on a subjective basis, as described previously,[17] under two categories: (a) depletion of small lymphocytes: 0, no recognizable depletion; 1, slight, being just recognizable; 2, moderate, depletion obvious; 3, advanced, with only few lymphocytes remaining; 4, virtually complete, with difficulty finding lymphocytes; (b) changes in pyroninophilic cells: 0, no recognizable changes; +1 or −1, slight increase or decrease in numbers; +2 or −2, moderate increase or decrease; +3 or −3, marked increase or decrease.

RESULTS

The operative and irradiation mortalities occurring within the first 11 postoperative days are summarized in Table I. These chicks showed either marked dilatation of the cloaca and intestine or radiation enterocolitis. Gross and histologic examination of the cloacal region in all bursectomized chickens, both those dying early or those surviving the entire experiment, revealed the complete absence of bursal tissue. In all chickens with thymectomy, however, one or two small thymic lobes were found. At no time, on the other hand, did these remnants weigh more than 100 mg in aggregate in any one chicken. The thymus weight in normal chickens at 6 weeks of age varies between 1.9 and 3.3 gm.[13]

TABLE II

INCIDENCE OF AMYLOIDOSIS

Group *		Material injected	Days of injection †	Amount injected (gm)	Number surviving	Incidence of amyloidosis				
						Spleen	Liver	Kidney	Intestine	Heart
No operation	(4)	$NaHCO_3$	43	—	4	0/4	0/4	0/4	0/4	0/4
"	(2)	$NaHCO_3$	51	—	2	0/2	0/2	0/2	0/2	0/2
No operation	(4)	Azo-casein	14	4.2	4	0/4	0/4	0/4	0/4	0/4
"	(4)	"	21	6.3	4	0/4	0/4	0/4	0/4	0/4
"	(5)	"	35	10.5	5	5/5	4/5	3/5	4/5	0/5
"	(6)	"	42	12.6	6	6/6	5/6	2/6	2/6	0/6
"	(6)	"	42 ‡	5.7	6	6/6	2/6	1/6	1/6	0/6
"	(9)	"	47–49	14.7	9	9/9	8/9	6/9	6/9	0/9
"	(1)	"	56	16.8	1	1/1	0/1	1/1	0/1	
Bursectomy	(3)	$NaHCO_3$	51	—	1	0/1	0/1	0/1	0/1	0/1
"	(20)	Azo-casein	37–56	10.5–16.8	3	3/3	3/3	3/3	3/3	2/3
Thymectomy	(3)	$NaHCO_3$	49–51	—	3	0/3	0/3	0/3	0/3	0/3
"	(13)	Azo-casein	49–51	14.7–15.8	8	8/8	7/8	7/8	4/8	5/8
Thymectomy and bursectomy	(1)	Azo-casein	49	14.7	1	1/1	1/1	1/1	1/1	0/1

* Number of chickens in parenthesis.

† All chickens killed 24 hours after last injection.

‡ Injected 3 days per week.

Histologic Features of Avian Lymphoid Tissues

Although described previously,[5,7] the normal histologic appearance of chicken lymphoid tissues is reviewed to facilitate descriptions of the results.

Spleen. The white pulp of the spleen is composed of two distinct parts, both of which are intimately related to the arterial vessels of the spleen (Figs. 1a and b).

The first component is associated with small arteries and their small arteriolar branches. The arteries are surrounded by a cuff of small lymphocytes (Fig. 2) which extends to and along the small arterioles (Fig. 3). At this level, however, a different population of cells is interposed between the cuff and the vessels. These cells form a sheath ("Schweigger-Seidel sheaths"[5]) around the arterioles, and consist predominantly of reticuloendothelial cells with scattered clusters of small lymphocytes and lymphoblasts (Figs. 1, 2 and 3). The small lymphocytes appear in the neonatal period, and their development can be markedly impaired, although not completely eliminated, by neonatal thymectomy. This component has been called the "thymic-dependent" peripheral lymphoid tissue.[8,11]

The second component of the white pulp consists of sharply circumscribed round or oval-shaped nodules or follicles[8] and is called the "nodular lymphoid tissue".[5,6] These nodules, which are always adjacent to small arteries (Figs. 1a and 1b), are composed of large and medium size lymphocytes, pyroninophilic cells, lymphoblasts and occasional reticuloendothelial cells. They are surrounded by a thin "membrane"(Fig. 1b). The nodules are not fully developed until 4 to 5 weeks of age.[8] Neonatal bursectomy markedly impairs their development; accordingly, these elements are the "bursal-dependent" lymphoid tissue.[8]

The remainder of the spleen consists of red pulp containing small and scattered numbers of plasma and pyroninophilic cells and narrow blood-filled sinusoids, as well as the terminal portions of the Schweigger-Seidel sheaths.

Bursa of Fabricius. The bursa is an oval-shaped mass with a convoluted central cavity that is continuous with the cloaca (Figs. 6 and 7). It is composed of closely packed follicles, each with a cortex and medulla. The cortex contains abundant small lymphocytes with a few admixed undifferentiated cells and macrophages (Fig. 7). The medulla is separated from the cortex by a thin fibrous membrane (Fig. 7). The outer medullary areas are composed of blast-like cells showing varying degrees of lymphocytic maturation; the central areas contain small lymphocytes, predominantly.

Neonatal thymectomy results in numerical depletion of the small lymphocytes in the bursal follicles; in over one third of follicles they do not appear at all.[5] In this respect, the small lymphocytes are analogous to the thymic-dependent lymphocytes in the spleen.

Thymus. The chicken thymus is composed of 6 to 8 lobes disposed on each side of the neck in close proximity to the carotid sheaths. Each lobe consists of a thick cortex of predominantly small lymphocytes and a medulla containing reticular cells and Hassall's corpuscles (Fig. 10).

There are no lymph nodes in the chicken.

Amyloid Induction

Intact Chickens. None of the intact birds given injections of $NaHCO_3$ developed amyloidosis (Table II). All birds receiving 35 consecutive daily injections of azo-casein (a total of 10.5 gm) developed moderately severe splenic amyloidosis. The incidence of amyloidosis in the liver, kidneys and intestine in this group is shown in Table II. No other organ, including the thymus and bursa, was involved by amyloid.

With longer consecutive injections of azo-casein, i.e., 42, 49 and 56 days, the severity of amyloidosis increased. With the exception of the spleen, there was slight variation in the incidence of organ involvement (Table II).

One group receiving azo-casein on a 3-day per week injection basis for 6 weeks showed amyloidosis. The severity was, however, less than that seen in the chickens receiving 35 consecutive daily injections.

Bursectomized Chickens. Of the 20 chickens with bursectomy which received azo-casein injections beginning at 6 weeks of age, 17 died after receiving only from 1 to 11 injections (Table II). None of these showed amyloidosis. The 3 chickens surviving a course of injections comparable to that used in intact chickens, however, developed amyloidosis (Table II). The severity, staining features and histologic distribution of amyloidosis, as well as the incidence and types of organ involvement, were identical to that found in intact chickens. In addition, very slight amyloidosis of the myocardium was found in this group. The one chicken which had had both bursectomy and thymectomy, and had received azo-casein injections, also developed amyloidosis with similar features (Table II).

Only 1 out of 3 chickens subjected to bursectomy survived a full course of $NaHCO_3$ injections. No amyloidosis was found in any of the 3 (Table II).

Thymectomized Chickens. As shown in Table II, 13 chickens subjected to thymectomy at 6 weeks of age were given azo-casein injections. Six died after a few injections and did not have amyloidosis. Those chick-

ens receiving injections for 42 days or longer all developed amyloidosis (Table II). In this group, the severity of amyloidosis was slightly greater than that seen in intact and bursectomized chickens; otherwise all other features were identical.

Two thymectomized chickens given $NaHCO_3$ injections for the same number of days failed to develop amyloidosis.

Amyloidosis in Chickens

Amyloid Staining Features. The amyloid had a more distinct fiber nature than that seen in mammalian species but showed typical tinctorial features[1,16,17]: staining with Congo red, birefringence and dichroism in polarized light, binding of thioflavine T, alcohol-labile metachromasia with toluidine blue and crystal violet, and a positive staining reaction for tryptophane. The ultrastructural features of the amyloid fibrils were comparable to those seen in mammals.[25]

Histologic Sites of Amyloid Deposition. The localization of amyloid deposits in the liver (Figs. 12 and 13), kidney and intestine was virtually identical to that reported in mammalian species,[1,16,17] i.e., in hepatic sinusoids associated with prominent lining cells (Figs. 12 and 13), in the mesangial regions of renal glomeruli, in subendothelial locations in capillaries, and around lacteals in the mucosa of gastrointestinal tract. That portion of the cloacal mucosa lining the bursa of Fabricius was free of amyloid deposits. Amyloid in the liver was occasionally found in and around portal veins in association with giant cells, described later, with or without concomitant sinusoidal amyloidosis.

Splenic amyloid was first detected in the Schweigger-Seidel sheaths, intimately associated with the large reticuloendothelial cell population (Fig. 4). With prolonged azo-casein injections the amount of amyloid in and around these sheaths increased and expanded into the red pulp in an irregular manner (Fig. 5). The bursal-dependent nodules were uninvolved in the early phases of amyloid formation, although small deposits developed here after amyloidosis was marked in other locations.

Large multinucleated giant cells with abundant cytoplasm containing or merging with amyloid deposits were found in the spleen and liver (Figs. 12 and 14). These cells were present regardless of the severity of amyloidosis; they were, however, not seen in all deposits.

Histologic Lymphoid Changes

Intact Chickens. After azo-casein injections, there was considerable but variable depletion of small lymphocytes in the thymic lobes (Table III) (Fig. 11), and in the thymic-dependent white pulp of the spleen, i.e., small lymphocyte cuffs around small arteries and Schweigger-Seidel

sheaths (Fig. 5). In contrast, the splenic nodular components increased very slightly in size due to hyperplasia of pyroninophilic cells (Table III). As emphasized above, however, early or small amyloid deposits were not related to these nodules.

The small cortical lymphocytes in about one-half of the follicles of the bursa were moderately depleted. In a few of these, the cortex was reduced to a thin and sometimes discontinuous rim of undifferentiated or reticular cells. Depletion of small lymphocytes in the medulla was also noted and only variable numbers of reticular and occasional pyroninophilic cells remained.

The histologic features of the lymphoid organs in intact chickens given $NaHCO_3$ injections did not differ significantly from the normal.

Bursectomized Chickens. After azo-casein injections, depletion of thymic and splenic lymphocytes was similar to that seen in similarly treated intact chickens. Lymphocyte depletion did not occur after injection of $NaHCO_3$.

In those chickens subjected to bursectomy which were either given injections with $NaHCO_3$ or in those which died after only a few injections of azo-casein, the nodules of the spleen (the bursal-dependent component) were variably but definitely reduced with respect to both number and size; in one chicken no nodules were found. Concomitantly, no mature plasma cells were found and pyroninophilic cell hyperplasia did not occur. In the chickens which had bursectomy and survived the full number of azo-casein injections and developed amyloidosis, this reduction of nodules persisted. In two chickens, none could be found, and few pyroninophilic cells and no plasma cells were observed. As in intact chickens, early splenic amyloid deposits were not associated with these pyroninophilic cells or with those nodules which persisted.

Thymectomized Chickens. After azo-casein injections, chickens subjected to thymectomy showed severe depletion of small lymphocytes (Table III) in the spleen. In the bursa approximately one-half of the follicles exhibited severe depletion of small lymphocytes (Figs. 8 and 9). Lymphocyte depletion in this group was much greater than that seen in similarly treated intact chickens; the depletion in the intact group was, however, slightly greater than that found in thymectomized birds given $NaHCO_3$ injections.

Very slight pyroninophilic hyperplasia in the splenic nodules was found in the thymectomized chickens after azo-casein injections but not after $NaHCO_3$. Again early splenic amyloid deposits were not found in the nodules.

The changes in the spleen in the one chicken, with both thymectomy and bursectomy and azo-casein injections, were a composite of those found in chickens subjected to only one of these procedures (Table III).

TABLE III

CHANGES * IN LYMPHOID TISSUES

Group †		Material injected ‡	Small lymphocyte depletion *			Pyroninophilic cell changes (spleen and/or bursa)
			Thymus	Bursa	Spleen	
No Operation	(6)	$NaHCO_3$ (43–51)	0	0	0	0
"	(8)	Azo-casein (14–21)	0	0	0	0 to +1
"	(27)	" (35–56)	1–3	1–3	2	0 to +1
Bursectomy	(3)	$NaHCO_3$ (51)	0	0	0	—3
"	(20)	Azo-casein (37–56)	2	—	2	—3
Thymectomy	(3)	$NaHCO_3$ (49–51)	—	1	1	0
"	(13)	Azo-casein (49–51)	—	4	2	+1
Thymectomy and bursectomy	(1)	Azo-casein (49)	—	—	2	—2

* Grading of changes, see text.
† Number of chickens in parenthesis.
‡ Days of injection in parenthesis.

Discussion

Different investigators[2-11] have shown that removal of the bursa of Fabricius from newly hatched chicks results in severe depletion of splenic plasma and pyroninophilic cells, variable depletion of splenic nodules, and functionally, in decreased antibody formation. Total absence of plasma cells, of the nodular lymphoid tissue and agammaglobulinemia have been reported when bursectomy is combined with sublethal radiation.[8] The studies reported in the present paper showed that similarly treated chicks, when given azo-casein injections developed generalized amyloidosis. In these birds the conditions of induction and the severity of the amyloidosis was virtually identical to that found in intact chickens.

In our hands, bursectomy and irradiation did not completely prevent the appearance of pyroninophilic cells as reported by others[8] using the same procedure and strain of chicks. That our surgical procedure was adequate, however, was attested by the following points: (a) careful gross and microscopic examination of the cloacal region failed to reveal any bursal remnants; (b) splenic lymphoid nodules, whose development is dependent upon the bursa, were clearly decreased and were completely absent in three chickens; (c) no mature plasma cells were ever found in any bursectomized chicken, and the numbers of splenic pyroninophilic cells were distinctly and significantly smaller than those seen in intact chickens, whether or not both groups were given injections of azo-casein or $NaHCO_3$; (d) finally, as shown first by Glick,[9] bursectomized chickens show a marked susceptibility to antigenic challenge. The staggering mortality occurring after injection of azo-casein (Table II) was, therefore, expected and inherent in the logistics of the experimental method. Thus, although the number of bursectomized chickens surviving the entire experiment was small, they still showed both gross and histologic evidence of adequate bursectomy.

The results indicated that amyloidosis could be induced with equal ease in both intact and bursectomized chickens, despite the total absence of mature plasma cells and a severe deficiency of pyroninophilic cells and bursa-dependent lymphoid nodules in the latter. These cellular and nodular changes following bursectomy were identical to those reported by others.[3-7] When the functional consequences of this procedure, reported by these authors, are considered, i.e., markedly diminished capacity to produce immunoglobulins, the present results strongly support the possibility that plasma cells or their precursors may not play a crucial role in the amyloidogenesis[1] and that they are not the final cellular pathway of amyloid elaboration. In studies with mice, the spatial separation of early splenic amyloid deposits from pyroninophilic cells was em-

phasized.[16,17] In the chicken spleen this spatial separation was even more striking. Amyloid deposition started in or among the reticuloendothelial cells of the Schweigger-Seidel sheaths which are, topographically, well separated from the bursal-dependent nodules of pyroninophilic cells. Indeed, the sheath location of amyloid deposits was consistent with the many suggestions (reviewed in Reference 1) that amyloid is elaborated by reticuloendothelial cells.

Neonatal thymectomy in certain mammalian species [18] and in chickens results in depletion or absence of small lymphocytes of lymphoid organs.[2,5,6] Functionally, the capacity to exhibit delayed hypersensitivity and allograft rejection responses is impaired but not the ability to form plasma cells and specific antibody to strong antigens.[3,7,8,11-13] This apparent paradox has been explained reasonably on a tentative basis by the two-component concept of the immune system wherein the thymus is responsible for small lymphocyte development and for the two immune reactions which are impaired when the thymus is removed. The bursa of Fabricius or "bursa-like" tissue in mammals,[11] on the other hand, is responsible for plasma cell and antibody responses.[2,7,8,11] It has also been suggested that in the intact animal the thymus may exert some control, of a negative feedback inhibition type,[11] over plasma cell and immunoglobulin production since both may increase dramatically in thymectomized animals, sometimes in association with "autoimmune" phenomena. Since amyloidosis has also been found in thymectomized mammals, the possibility that amyloid formation might be related to such overactivity of the immunoglobulin system has been implied or raised.[11,26,27] The results in bursectomized chickens reported here, however, do not support this possibility. The severity of amyloidosis in the thymectomized chickens injected with azo-casein, however, was greatest among all the groups, thus confirming previous observations of a relationship between lymphocyte depletion and amyloidosis.[16,17] The present results, therefore, emphasize that some explanation for this relationship, as well as that of amyloidogenesis to antigenic challenge which does not involve the immunoglobulin-synthesizing system, must be sought. Previous work [16,17] suggests that this explanation may be found in possible reticuloendothelial cell alterations associated with changes in the function of the thymic-dependent lymphoid tissue.

Summary

The role of plasma cells or their precursors in the pathogenesis of amyloidosis was evaluated in chickens, a species in which the development of these cells can be markedly and selectively impaired by neonatal surgical ablation of the bursa of Fabricius.

Inbred, I51 white Leghorn chicks were subjected to either bursec-

tomy, thymectomy or a combination of both, followed by sublethal total body x-irradiation, within the first 4 days of life. At 6 weeks of age, groups of intact (without operation) and surgically treated chickens were given daily injections of either $NaHCO_3$ or an azo-casein solution for periods up to 8 weeks. The following were found at necropsy in these animals:

Amyloidosis was induced in intact chickens after 35 consecutive daily injections of azo-casein. The amyloid showed typical staining features and was found only in the spleen, liver, kidney and gastrointestinal tract. In the spleen early amyloid deposits were associated with reticuloendothelial cells in locations (Schweigger-Seidel sheaths) that were spatially separated from slightly hyperplastic foci of pyroninophilic and plasma cells of the nodular lymphoid component of the spleen. Amyloid in other organs was related to cells lining sinusoids or capillaries. Depletion of small lymphocytes in the thymus and bursa accompanied these changes.

Bursectomy in the neonatal period resulted in a marked decrease in both pyroninophilic cells and lymphoid nodules in the spleen and an absence of plasma cells. This persisted even after azo-casein injections. The decrease, however, had no significant effect on either the rate of amyloid induction by azo-casein or the resulting organ and histologic distribution of amyloid deposits as compared to the intact chicken. Significant depletion of small lymphocytes in the thymus and spleen was observed in the chickens subjected to bursectomy after azo-casein injections.

Chickens subjected to thymectomy and given injections of azo-casein developed amyloidosis of a slightly greater severity following a period of injection comparable to that used in intact chickens and those which had had bursectomy. This group exhibited the greatest degree of lymphocyte depletion in the spleen but changes in pyroninophilic cells and the nodular lymphoid tissue were comparable to that found in intact chickens given similar injections.

The results strongly support previous suggestions that plasma cells and their precursors may not play a crucial role in amyloidogenesis and that these cells are not the final common pathway of amyloid formation.

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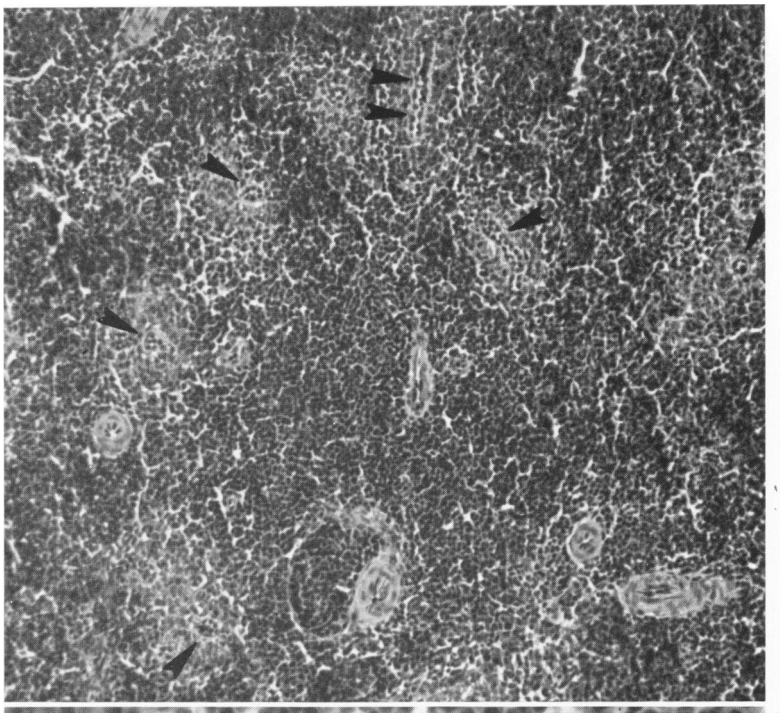
LEGENDS FOR FIGURES

All photomicrographs were prepared from paraffin sections stained with hematoxylin and eosin.

FIG. 1a. Normal chicken spleen. Pale cellular areas correspond to Schweigger-Seidel sheaths. A small arteriole, cut in cross-section or obliquely, with prominent endothelial cells can be seen in the centers of some sheaths (points). Medium and small arteries are shown in cross section; adjacent to the artery near the bottom-center is a splenic nodule, shown at higher magnification in Figure 1b. Other arteries are surrounded by non-nodular cuffs of cells, predominantly lymphocytes, which merge with the red pulp. × 210.

FIG. 1b. Spleen as in Figure 1a. A splenic nodule is situated adjacent to an artery and is separated from adjacent cells by a fibrous membrane. The cells in the nodule are pyroninophilic. Methyl green–pyronine staining. × 630.



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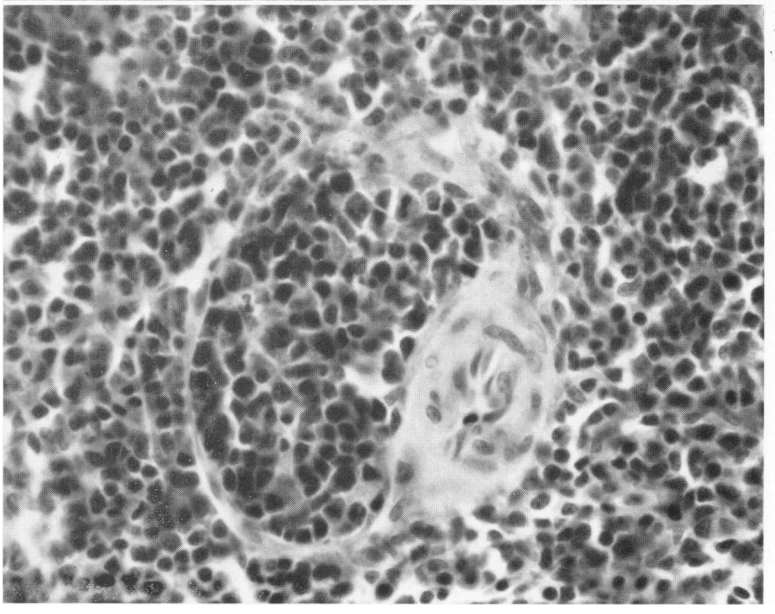

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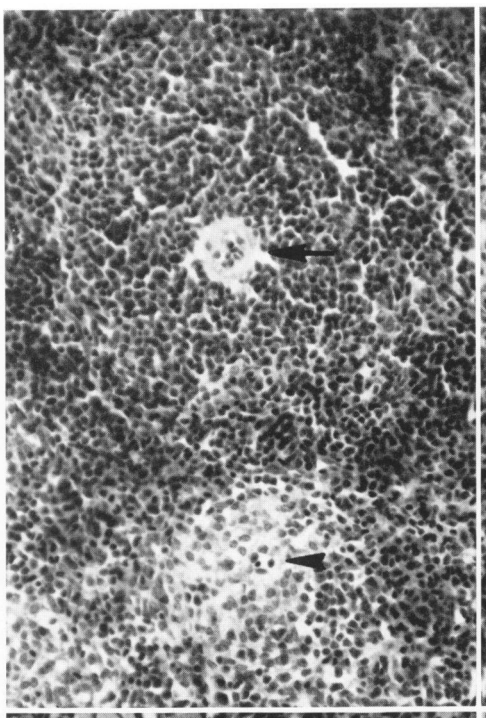
FIG. 2. Normal chicken spleen. A cuff of small lymphocytes surrounds an artery (arrow). The pale staining cellular area represents a Schweigger-Seidel sheath around a small arteriole (point). × 250.

FIG. 3. Normal chicken spleen. Details of Schweigger-Seidel sheaths are shown. Each is composed of a central arteriole (points) which is surrounded by prominent reticuloendothelial cells. In turn these are surrounded by small lymphocytes, some of which are intermixed with the sheath cells. The small artery, left and below center (arrow), is surrounded by a cuff of lymphocytes. × 250.

FIG. 4. Spleen, chicken subjected to thymectomy and given injections of azo-casein for 49 days. Early amyloid deposits appear in a Schweigger-Seidel sheath; the amyloid merges with the arteriole. Sheath cells persist in some areas and are lost in others. A multinucleated giant cell is evident in the peri-arteriolar amyloid deposit. × 325.

FIG. 5. Spleen, intact chicken receiving azo-casein for 8 weeks. Massive deposits of amyloid extend from around arterioles of Schweigger-Seidel sheaths (arrows) to, and replace much of, the red pulp. There is depletion of small lymphocytes about the arteries (compare to Figures 1 and 2). × 130.



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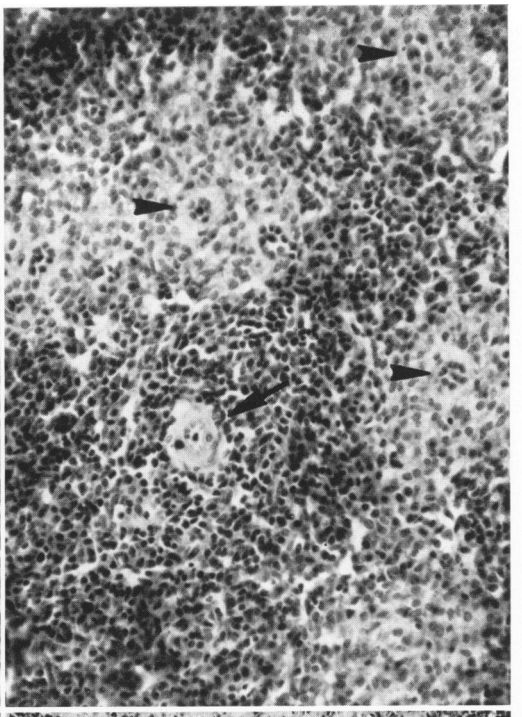

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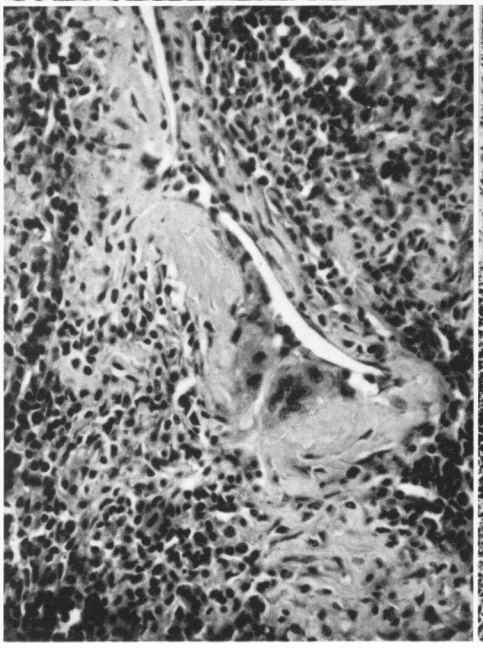

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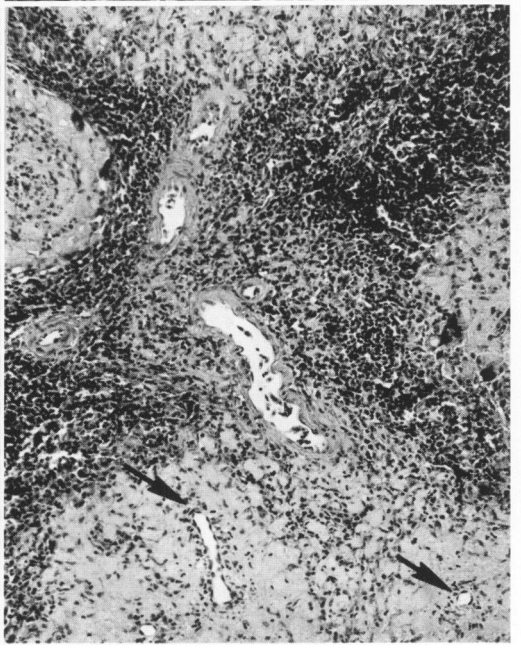

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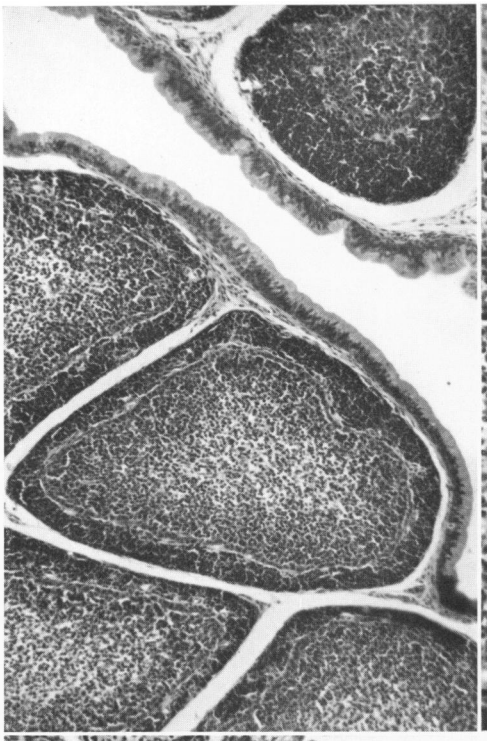
FIG. 6. Bursa of Fabricius, normal chicken. Distinct follicles composed of a prominent cortex of lymphocytes and a paler, less cellular medulla are evident. The cloacal epithelium forms the bursal lining. × 130.

FIG. 7. As in Figure 6. Lymphocytes in the cortex are separated from the medulla by a thin fibro-cellular membrane. Some small lymphocytes appear in central medulla. × 275.

FIG. 8. Bursa of Fabricius, chicken subjected to thymectomy and receiving azocasein for 49 days. There is marked depletion of small lymphocytes of the cortex and medulla. A few follicles are still normal in appearance. × 130.

FIG. 9. A higher magnification of Figure 8. × 325.



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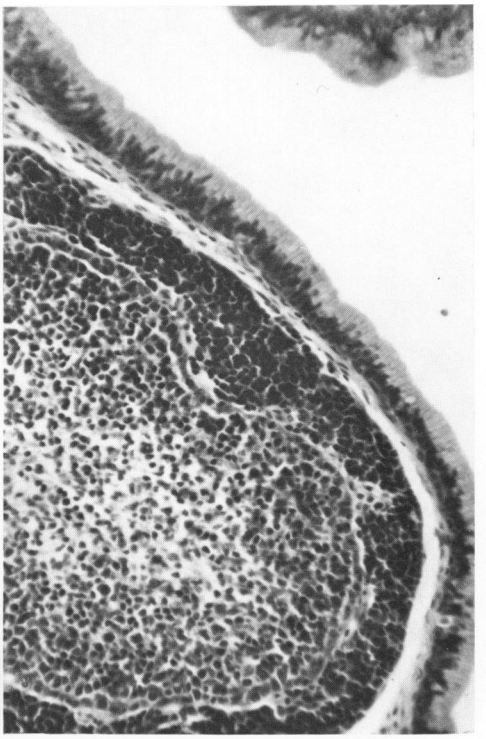

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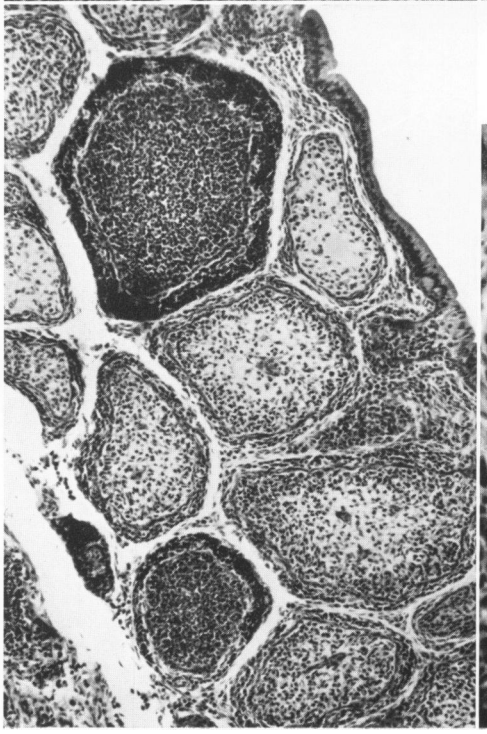

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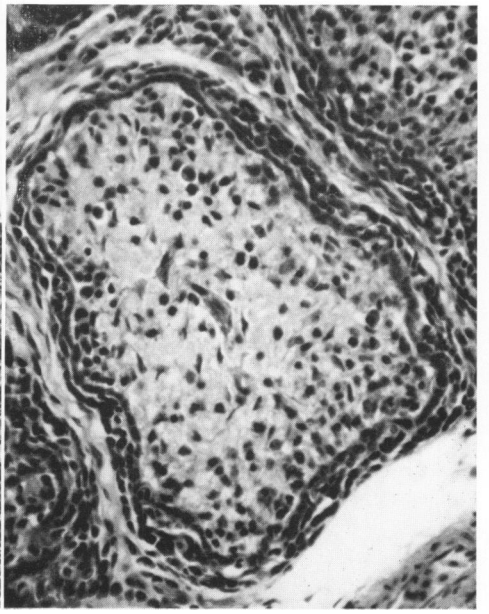

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FIG. 10. Normal chicken thymus. Part of two thymic lobes are shown. The cellular cortex is composed primarily of small lymphocytes. The medulla is pale and less cellular and contains prominent Hassall's corpuscles. × 130.

FIG. 11. Thymus, intact chicken given injections of azo-casein for 56 days. The depletion of small lymphocytes is so severe that the normal cortico-medullary boundary is absent. × 130.

FIG. 12. Liver, intact chicken given azo-casein for 49 days. Pale-staining amyloid deposits are demonstrated in sinusoids. × 325.
Inset. Minimal sinusoid amyloid deposit is associated with a giant cell. × 325.

FIG. 13. Liver, as in Figure 12. Amyloid is associated with sinusoidal reticuloendothelial cells (points). × 820.

FIG. 14. Spleen, chicken subjected to thymectomy and given azo-casein for 49 days. Giant cells surround large amyloid deposits in a Schweigger-Seidel sheath. The sheath arteriole is still visible (arrow). × 325.

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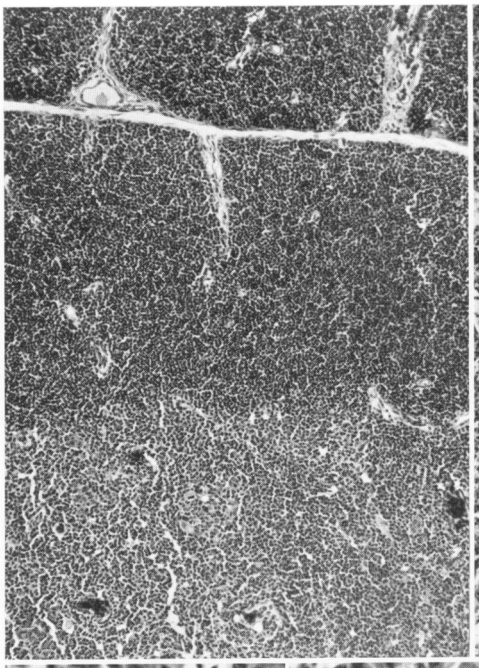

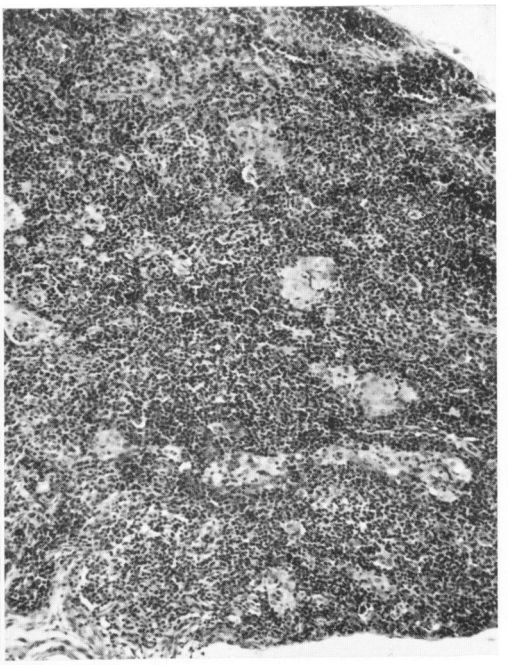

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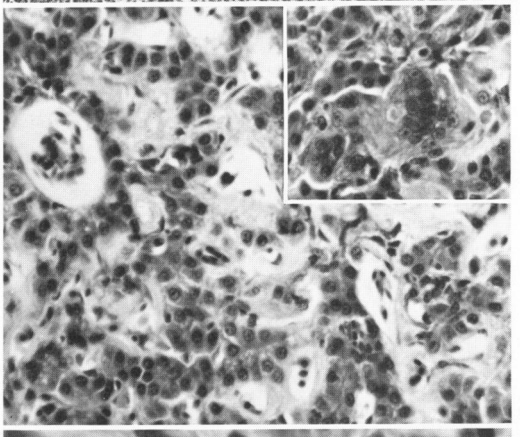

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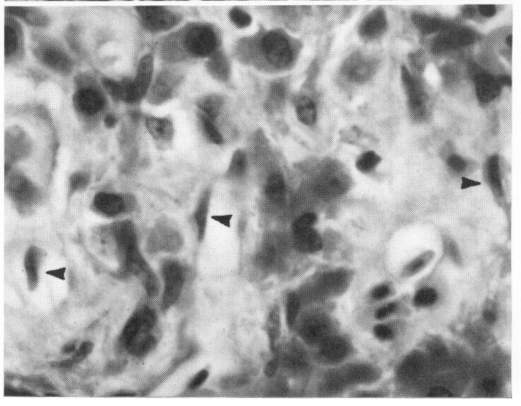

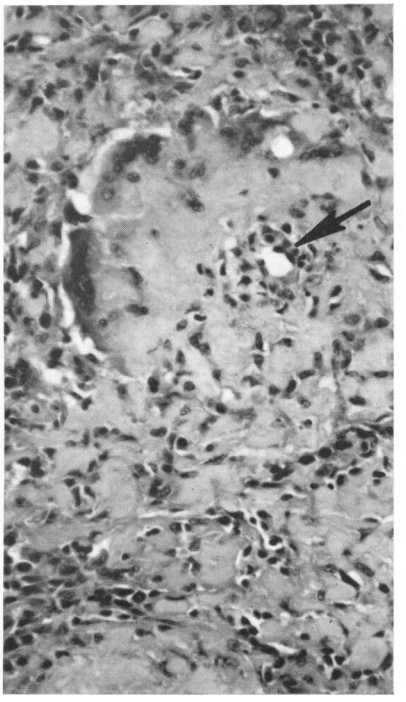

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