Lymphocyte Depression Induced in Chickens on Diets Deficient in Vitamin A and Other Components

Betsy G. Bang, BA, Frederik B. Bang, MD and Marie A. Foard, BS

Chickens maintained from time of hatching to age of 30 days on a diet lacking vitamin A, and on 2 diets lacking vitamin A and other nutritional components showed depletion of lymphocyte and plasma cell populations in nasal, paranasal and bursal lymphoepithelial tissues. Effects were significant with all diets but most severe with the most deprived diet. Infection of these birds with Newcastle disease virus showed further depletion of plasma cells, subnormal inflammatory response, keratinization of bursal epithelia and postsloughing metaplasia of nasal mucociliated epithelia. The bursae of infected chickens on the diet lacking only vitamin A were completely devoid of lymphocytes 6 days after virus inoculation. Infected chickens on the most deprived diet showed atrophy of areas of intranasal epithelia which indicated failure of basal cells to synthesize replacement cells (Am J Pathol 68:147–162, 1972).

SINCE SPICER [1] noted in 1892 that several childhood infectious diseases precipitated acute keratomalacia, many interactions between malnutrition and atrophy of tissus, and between malnutrition and disease have been reported in humans and experimental animals.[2–4] Recently the effects of protein calorie malnutrition (PCM) on depressing the cell-mediated immune (CMI) response in children have been highlighted, especially by Smythe *et al.*[5] Over the past three years we have observed effects of diets lacking either vitamin A alone or vitamin A and other dietary components on lymphoid and epithelial tissues in growing chickens. This report will summarize histologic findings on lymphoepithelial tissues associated with the upper respiratory tract and the bursa of Fabricius in chicks maintained on deprived diets from the time of hatching to 1 month of age.[6] Some chickens in each group were inoculated with the mesogenic strain of Newcastle disease virus (NDV) after 21 days on the respective diet; effects of the virus are compared with effects on birds maintained on normal mash and on a vitamin A-deficient diet supplemented with vitamin A palmitate.

From the Department of Pathobiology, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Md.

Accepted for publication Feb 23, 1972.

Address reprint requests to Mrs. Frederik Bang, Department of Pathobiology, Johns Hopkins University, School of Hygiene and Public Health, 615 N Wolfe St, Baltimore, Md 21205.

Chickens have no cervical lymph nodes [7] nor do they normally have lymphoid nodules in the nasal submucosa, as do Japanese quails.[8] In healthy young stock chickens, however, there are 5 consistent loci of aggregate lymphoid cell expression associated with the oculonasal organ system:[8] in the fauces, lacrimal ducts, lateral nasal gland ducts and the nictitating-membrane glands of Harder and their ducts. Foci in fauces, lacrimal and Harderian ducts are invasive small-lymphocyte aggregations which often have germinal centers; those in nasal gland ducts are populations of plasmacytes which intrude among the basal and epithelial cells lining the ducts, and those in the Harderian glands are heavy concentrations of circulating plasma cells in the interlobular spaces. It was previously noted that: a) while the invasive foci meet Lucas' definition of abnormal or "ectopic" reactive sites,[9] the distinction between normal and abnormal may be academic in areas in which cells are constantly exposed to a sheet of mucus containing infectious agents; b) nasal gland ducts were possible shedding sites for plasmacytes or their products; and c) the heavy concentrations of plasma cells in the Harderian gland suggested a lymphocytopoietic function for this organ.[8] This latter possibility has since been explored by Glick,[10] who found that when sheep red blood cells were dropped onto the chick eyeball, plaque-forming cells were produced in the gland of Harder. He also found that 2 successive injections of cyclophosphamide into hatchling chicks apparently eliminated plasma cells from the Harderians within 16 days.[11] Leslie *et al*[12] have concluded that chickens have a secretory immunologic system wherein the secretory Ig is of the same class as the predominant serum Ig.

Another key lymphoid system in young chickens is the bursa of Fabricius, an organ essential for development of circulating antibody-producing cells and possibly for other aspects of the immune response. The discovery of Glick *et al* [13] of the role of the bursa of Fabricius in antibody production set off a variety of experiments in many laboratories; effects of bursectomy in chickens of various ages, with or without concomitant thymectomy or splenectomy, have been studied after subsequent challenge with various antigens. Results have not yet been sorted out in a way in which "the" function of the bursa at a given age in growing chicks can be fully specified.

Materials and Methods

Commercially obtained White Leghorn chickens were maintained from the time of hatching until the termination of the experiment on each of the following diets (25 to 35 chicks group):

Diet I: Purina Chow's normal chick mash diet, "Growena";
Diet II: Diet III (below) supplemented daily with 3.2 mg of vitamin A palmitate kg diet, in a sucrose base;*
Diet III: A specially formulated* vitamin-A-deprived diet for chickens,†

	g kg
Soy assay protein	300.00
L-Cystine	3.00
DL-Methionine	2.00
Salt Mix Fox-Briggs ‡	60.00
Cottonseed oil	40.00
Choline chloride	1.00
Corn starch	593.323
Sodium selenite (Na_2SeO_3)	0.002043
DL-α-Tocopherol acetate (1000 U g)	0.200
Santoquin (Exthoxyquin)	0.040
Thiamine HCl	0.008
Riboflavin	0.008
Calcium pantothenate	0.020
Nicotinic acid	0.100
Pyridoxine HCl	0.008
Biotin	0.0003
Folic acid	0.003
Vitamin B_{12} in mannitol (0.1%)	0.020
Vitamin D_3 trituration (3000 U g)	0.2667
Menadione	0.001

Diet IV: Nutritional Biochemical Chick Basal Ration, with cod liver oil USP 1.0% *deleted* to provide a vitamin A-deficient diet;
Diet V: Nutritional Biochemicals Vitamin A (Rat) Test Diet.

---

* We are indebted to Dr. J. G. Bieri of the Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, for his advice in the composition of Diet III and the regime of supplement in Diet II.

† General Biochemicals Diet TD–71122
‡ General Biochemicals Catalogue No. 170740

After 21 days on the respective diets, 8 to 15 chickens in each diet group except Diet IV were inoculated intranasally with 1 drop nostril of undiluted allantoic fluid stocks of the mesogenic (B) strain of Newcastle disease virus in which virus titers were between $10^{7.5}$ and $10^{9.0}$. Normally fed, deprived, uninfected and NDV-infected chickens (2 per category when possible) were sacrificed at intervals of 7 hours approximately 3, 5, 7, 9 and 12 days after the time of inoculation. Blocks of nasal fossa including the area of the upper skull from the center of the nostril to the posterior limit of the eye, and the entire bursa of Fabricius from at least one of each pair were fixed in neutral formalin. Nasal blocks were decalcified in commercial "Decal." Four areas of nasal fossa selected to include the anterior mucocutaneous junctions, the lateral nasal gland and its ducts, the central portion of the maxillary turbinate and central portion of the nasolacrimal duct and the central portion of the gland of Harder were sectioned. Four to six sections area were stained with hematoxylin and

eosin, 2 to 4 area with alcian blue (1%)–periodic acid-Schiff (AB-PAS) stain. Methods of virus assay have been previously described.[14]

Results

This report is primarily concerned with the effects of the deficient diets on depression of lymphoid cell systems; however, the effects on epithelial cells lining the nasal fossa and bursa may be directly relevant and will be briefly noted.

A summary of the test diets follows for ready reference: Diet I: normal mash, Diet II: Diet III supplemented daily with vitamin A palmitate, Diet III: special chicken diet with complete nutrients lacking vitamin A, Diet IV: commercial chicken basal ration lacking vitamin A, Diet V: commercial vitamin-A-deprived diet for rats.

Epithelial Cells

After 25 days of vitamin A deprivation the mucociliated cells contiguous with mucocutaneous junctions of chickens on the 3 vitamin-A-deprived diets (III, IV, V) began to show keratinizing metaplasia which progressed posteriorly with time; by 30 days portions of some lacrimal ducts and lateral nasal gland ducts of chicks on Diets IV and V were keratinized (Table 1). Cell numbers in both ciliated and mucous components of the mucosa and in the mucous acini were reduced by about 50%, so that all mucosae were reduced in height. Mucus was conspicuously retained in the more anteriorly positioned acini and the surface sheet of mucus was thicker than normal.

The epithelial cells lining the lumen of the bursa of Fabricius were also affected. In normally fed birds this epithelium is a single row of regularly aligned secretory cells which stain faintly alcian blue, plus a less regular row of basal cells; there is a very fine sheet of alcian blue-positive surface secretion. After 30 days on Diet III, surface epithelia were disorganized, appeared pseudostratified and occasionally cystic; the tips of the rugae were metaplastic and heavily seeded with heterophils. On Diets IV and V the normally smooth surfaces of the rugae were indented and occasionally acinar; the cells were pseudostratified due to compression and some showed squamous metaplasia and heterophilia. On Diet IV the tips of many rugae were keratinized. In chickens on all three vitamin-A-deprived diets these bursal epithelial cells contained brilliant alcian blue-positive secretory material in the entire cytoplasm; there were quantities of alcian blue positive surface secretion and exudate in the lumen. There was no mucous metaplasia of the surface epithelial tuft cells at any stage of the diets.

Table 1—Effects of Each Diet on Oculonasal Epithelial and Lymphoid Cells

Diet	No. chicks	Days on diet	Degree of keratinization of mucociliated epithelial anterior fossa	Degree of plasmacyte abnormality, lateral nasal gland ducts	Degree of infiltration small lymphocytes in lacrimal ducts	Degree of infiltration small lymphocytes in tonsil area	Relative No. plasma cells in intralobular spaces in glands of Harder
I	1	24	0	0	+	+	+++
	1	26	0	0	++ GC	++ GC	+++
	1	31	0	0	+++	+++	+++
II	1	22	0	+	0	+ GC	+++
	1	24	0	+	++ GC	++ GC	+++
	1	26	0	0	+	++	+++
	1	29	0	±	++ GC	++ GC	+++
III	1	18	0	++	0	0	+
	1	21	+	++	0	+ GC	+
	1	24	+	+	±	+ GC	++
	1	29	+	+	0	0	++
IV	1	18	0	0	±	+	++
	2	25	+	+ M	M ++ GC	+ GC	+
	3	30	++	++ K	+ GC	+ GC	0
V	2	18	0	0	0	0	+
	2	25	++	+ M, K	±	+	+
	2	30	++	++ M, K	0 K	+	0

GC = germinal centers; M = metaplasia; K = keratinization

Lymphoid Cell Systems

Nasal and paranasal lymphocyte populations

Lacrimal Ducts and Fauces. There was significant depression in the amount of small lymphocyte infiltration on Diets III and IV; however, small germinal centers formed at both sites. In chickens on Diet V the infiltrations were essentially eliminated from lacrimal ducts and sharply reduced in fauces; germinal centers were not found. The effects are summarized in Table 1. Because of the volume of sectioning required for histologic review of the Harderian gland ducts, these were not included in the present survey.

Lateral Nasal Gland Duct. Populations of plasmacytes were also reduced after 30 days on all three vitamin-A-deprived diets. (Plasmacytes normally found associated with this duct epithelium are somewhat smaller than those found in the glands of Harder.) In the vitamin-A-deprived chickens nearly all of the cells found along the basal cell area were strongly eosinophilic agranular cells with dark compressed excentric nuclei; it could not be determined, when using the light microscope, whether they were abnormal plasmacytes or another cell type. As shown in Table 1, these changes were initially significant in Diet III but less significant at later stages; they were progressive over time in Diets IV and V. Heterophils were common in the loose areolar tissues adjacent to the ducts and were occasionally found in the duct walls.

The Harderian gland population of plasma cells showed the most remarkable depression in vitamin-A-deficient birds whose diets also lacked other components. In chickens on Diets IV and V, the rich populations of large clear plasma cells found in the Harderian gland of chickens on normal diets (Figures 1, 2) were apparently entirely eliminated at 30 days (Figure 3). Chickens on Diet III showed similar changes at 18 and 21 days, but at 25 and 30 days there were modest numbers of normal looking plasma cells in the intralobular spaces.

Thus it appears that each of the vitamin-A-deprived diets depressed or partly eliminated the infiltration of small lymphocytes into submucosal areas of the nasal fossa at sites most directly exposed to injurious particles carried in the mucous blanket (fauces and lacrimal ducts). Normal populations of plasma cells in the nictitating-membrane gland of Harder were reduced in number or were eliminated in all three deficient diets, but recovered partly in Diet III. Since the secretory flow from this gland is from gland body to eyeball via a long duct, airborne stimuli impinging on the eye must elicit response

from the gland via information from circulating cells; it will be remembered that the long gland duct is lined with villi heavily infiltrated with small lymphocytes and germinal centers. Plasmacytes associated with the lateral nasal gland duct epithelia were reduced in number and were morphologically abnormal in all diets. Effects were less severe in birds on Diet III, and these seemed to improve in some respects with time. Depletion of lymphocytes was most severe in the most deprived diet, and the capacity to form germinal centers in normal conditions may have been abolished.

Bursa of Fabricius Lymphocyte Populations

While Diet II seemed to support normal development of epithelial tissues in the nasal fossa, it was evidently less adequate for fully normal development of the bursa. In contrast to Diet I at 30 days (Figure 4), there was less uniform development of follicles; follicles were less mature, very few were polygonal, and there was some subepithelial fibrosis and heterophilia.

Diet III produced follicles which were quite irregular in development (Figure 5), showed marked interfollicular fibrosis and degrees of fibrosis (rarely, granulocytosis) in the medullae; cortical development was uneven, and there were several sites in which cortical lymphocytes were replaced peripherally by heterophils. Changes in lining epithelial cells have been described above.

Diet IV produced widely spaced follicles which lacked small lymphocytes in the medullae. These latter consisted of loose networks of pale-nucleated epithelial cells which stained positively for alcian blue, a peripheral scatter of rounded dark nuclear remnants and a distinct margin of cuboidal epithelial cells adjacent to the often discontinuous vascular circlet of the cortex. The cortex varied in relative width and also contained numbers of nuclear remnants.

Diet V follicles were atrophic, entirely lacked the vascular circlet and lacked a cortex; medullae consisted of degenerate and degenerating cell nuclei in a disintegrating matrix of pale epithelial cells (Figure 6). Basal cells in surface epithelia, in the necks of tuft-cell areas and surrounding the medullae had swollen nuclei, were usually three or more layers thick and were disorganized in the medullae. There was marked interfollicular fibrosis and/or degeneration (Figure 7).

To summarize the bursal effects: Diet II caused moderate alteration (delay?) in bursal follicular development; Diet III induced retarded development or premature involution, irregular maturation of follicles and degrees of focal heterophilia. Diets IV and V seemed to have

induced premature atrophy of lymphoid elements; Diet V seemed to have induced the most rapid attrition of cortical lymphocytes.

Effects of NDV Infection on Diet-Deprived Chickens

Nasal Fossa

Results of viral assays during the course of infection in chickens on Diets I and V have been reported.[15] For the present report selected results are summarized in order to compare results in uninfected and infected chickens on deprived diets. Chickens infected after 3 weeks on normal mash showed an initially strong submucosal inflammatory reaction and mild sloughing typical of response of nasal mucociliated areas to this strain of NDV. In addition there was a sharp initial drop in numbers of plasma cells from the Harderian gland; this was not fully restored until 10 days.

Chickens on Diet III (vitamin-A-deprived only) after infection showed delayed and depressed inflammatory response and increased keratinization of anterior nasal mucosae, persistent sloughing and/or squamous metaplasia of conchal mucociliated mucosae, abnormal plasmacytes in and occasional keratinization of lateral nasal gland ducts and *depletion* of plasma cells from the gland of Harder.

On Diet V, infected birds showed focalized *atrophy* in several epithelial cell areas in which there were no visible basal cells present to provide for synthesis of replacement cells. There was some inflammatory cell response in both lacrimal ducts and fauces, but in only 1 of 6 birds were there questionable small germinal centers. The Harderian plasma cells, depleted in uninfected chicks at the time of infection, remained absent.

Thus, in contrast to normally fed pairs, infected diet-deprived chickens showed increased oculonasal epithelial metaplasia or (Diet V) atrophy; inflammatory responses were depressed; and plasmacyte populations were either further altered morphologically, replaced by other types of cells or eliminated.

Bursa of Fabricius

Single specimens of bursae from chickens on Diets I and II at 4 intervals from 1 to 10 days after NDV infection showed no difference from bursae of uninfected birds at the same intervals. Those on Diet III after infection showed a rapid attrition of lymphocytes, atrophy of the vascular system, involution and progressive surface epithelial metaplasia, including marked keratinization. This effect was evident 3 days after NDV inoculation, was advanced by 5 days and by 6

days was indistinguishable from the situation seen in Figures 6 and 7, in comparison with the uninfected vitamin-A-deficient bursa the same age (Figure 4). Bursae of infected chickens on Diet V, already deprived of lymphocytes, showed significant advance over uninfected birds in bursal fibrosis, more epithelial metaplasia and greater basal cell proliferation, but no evident keratinization.

Discussion

With the wisdom of hindsight one can find clues in the literature on malnutrition and infection that the cell-mediated immune system is affected by malnutrition. Jackson [2] referred to several reports on intensive "migration or atrophy" of lymphocytes in the thymus and lymph nodes of starving or malnourished humans and experimental animals; Vint [15] in 1937 recognized that there was severe atrophy of the thymus in children who died of kwashiorkor; and Smythe and Campbell in 1959 noted that the bacteremia of kwashiorkor suggested a defect in the immune mechanism of the gut.[16] After Oomen [17] postulated that infection precipitated acute clinical hypo-A in persons with a latent deficiency, there were a number of accounts of precipitating effects of infections on clinical signs of vitamin A deficiency. These have been reviewed in Scrimshaw *et al*,[3] in DeSilva and Baptist,[4] and in a recent Ciba symposium on malnutrition and infection.[18] There has also been a highly pertinent observation by McLaren in 1963 that death rates were 65% higher in children who had PCM and showed xerophthalmia than in those who had PCM and no xerophthalmia.[19]

Yet it is Smythe *et al* [5] who have clearly highlighted the fact that the cell-mediated immune (CMI) response is profoundly depressed by PCM. McFarlane [20] has added that the degree of immunosuppression may depend on the degree of malnutrition plus the amount of concomitant infection.

Smythe's findings are germane to our present studies, which were prompted by observations that chickens which had been vitamin A-deprived in order to test the effects of hypo-A on the course of NDV infection [21] showed marked depletion of lymphoid tissues,[22] that 100 times more virus was recovered from throat swabs of vitamin A-deprived than from normally fed NDV-infected birds,[14] and that vitamin-A-deprived chicks were susceptible to influenza virus while normally fed were not.[14] Together these strongly suggested morphologic and functional suppression of lymphocytes in diet-deprived chickens. Since the diets used in these original experiments (Diets IV, V) lacked not only vitamin A but apparently other components

necessary for optimal growth of chickens, a diet containing full nutritional requirements but specifically lacking vitamin A (Diet III) was added to the experimental design of the present series. Results with this diet were still significant but were less severe (except in the case of the bursa of infected birds). Which of the specific factors lacking in Diets IV and V were responsible for the more intensive lymphoid depression remains to be systematically investigated.

The chicken model may be an excellent analogue in which to study effects of specific dietary deficiencies on particular lymphoid cell systems involved in immune responses, since thymus-mediated and bursa-mediated cell systems are so clearly defined. It is obviously necessary in future studies with this model to use precisely designed diets; to include histologic data on thymus, spleen and cecal tonsils; to carry out tests for delayed hypersensitivity, lymphocyte transformation and antibody components; and to have parallel data on body and lymphoid organ weights in noninfected and infected deprived and control chicks.

The present study has suggested that in lymphoepithelial systems the primary lesion induced by vitamin A-deprivation in epithelial cells may interfere with normal information to the lymphocyte-line cells associated with them, especially in the presence of infection. This in turn, especially when concomitant with degrees of other nutritional deficiencies, could influence the differentiation of cells in the lymphocyte line. For example, in birds on Diet III incursions of heterophils into the cortex of the bursa and into the gland of Harder displaced or replaced lymphocytes; it is noteworthy that in pipping and newly hatched chicks the Harderians are filled with heterophils yet within one or two weeks after hatching these are replaced by a population of plasma cells.[8] The study also suggested that immune tolerance might be induced by infection in diet-deprived animals, since during early stages of postembryonic life the CMI response is presumably depressed; thus a gradual recovery of function of these cells might occur after the immune tolerant state had been established.

It is clear that there is a relationship between protein deficiency and vitamin A deficiency, but the manner in which they interact is not clear, nor are effects of these deficiencies on immune-cell mechanisms and mucous membranes.[23] As Follis [24] pointed out, the pathology of different deficiency states is traditionally expressed as the effect of a specific deficiency on the total growth of the organism. It remains to be seen whether the lymphoid regression described in this report, and the lymphoid regression described in the cited reports on human

and experimental malnutrition and starvation, hinge on one or more specific dietary deficiencies, or on particular combinations of deficiencies. Axelrod's continuing studies [25,26] of specific states of vitamin deficiency have established the role of particular vitamins—especially in the vitamin B complex—in several aspects of immune responses.

A final important question is raised by the fact that the deficient diets, as well as infection, induce premature atrophy of the bursa. Since bursal involution normally begins at about 4½ months of age,[27] the possibility that the effects of malnutrition may be at least partly mediated through the hormonal system in these 1-month-old birds should not be overlooked.

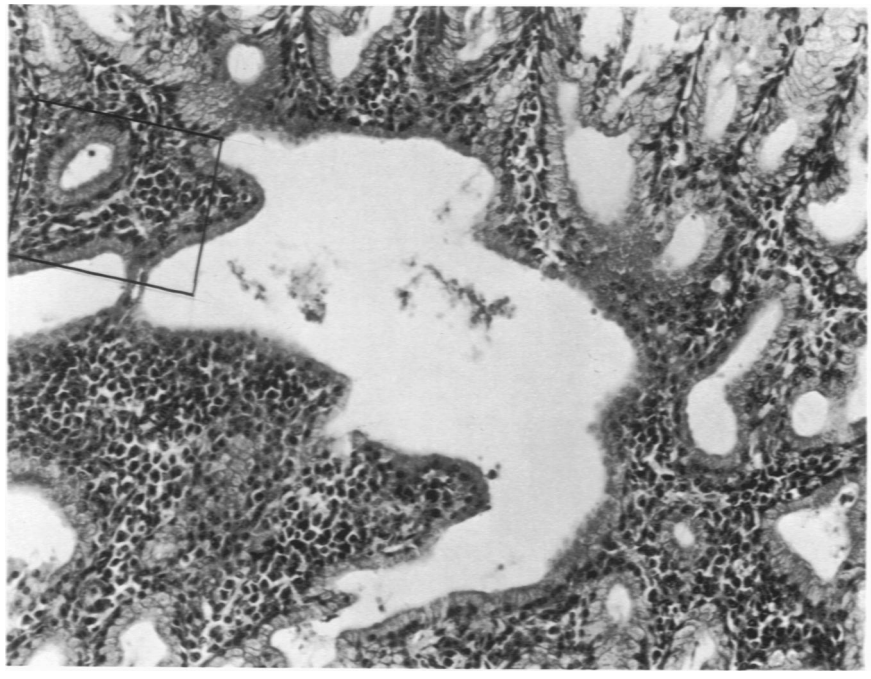
References

1. Spicer H: Keratomalacia in young children. Lancet 2:1387–1388, 1892
2. Jackson CM: The Effects of Inanition and Malnutrition on Growth and Development. Philadelphia, P. Blackiston's Son & Co, 1925
3. Scrimshaw NS, Taylor CE, Gordon JE: Interactions of nutrition and infection. World Health Organization Monograph Series 57. Geneva, WHO, 1968
4. DeSilva CC, Baptist NG: Tropical Nutritional Disorders of Infants and Children. Springfield, Illinois, Charles C Thomas, Publisher, 1969
5. Smythe PM, Brereton-Stiles CG, Grace HJ, Mafoyane A, Schonland M, Coovadia HM, Loening WEK, Parent MA, Vos GH: Thymolymphatic deficiency and depression of cell-mediated immunity in protein-calorie malnutrition. Lancet 2:939–944, 1971
6. Jolly J: La bourse de Fabricius et les organes lympho-épitheliaux. Arch Anat Microsc Morphol Exp 16:363–547, 1915
7. Fürther H: Beiträge zur Kenntnis der Vögel-lymphknoten. Jena Z Naturwiss 50:359–410, 1913
8. Bang BG, Bang FB: Localized lymphoid tissues and plasma cells in paraocular and paranasal organ systems in chickens. Am J Pathol 53:735–751, 1968
9. Lucas AM, Oakberg EF: Lymphoid tissue and its relation to so-called normal lymphoid foci and to lymphomatosis. II. Quantitative analysis of lymphoid areas in the pancreas of laboratory and farm chickens. Am J Pathol 26:75–111, 1950
10. Glick B, Sato K, Mueller AP: The contribution of antibody producing cells by the chicken lacrimal gland, gland of Harder, spleen, cecal tonsil and accessory spleen. Fed Proc 30(2):291, 1971
11. Glick B: Morphological changes in humoral immunity in cyclophosphamide-treated chicks. Transplantation 11:433–439, 1971
12. Leslie GA, Wilson HR, Clem LW: Studies on the secretory immunologic system of fowl. I. Presence of immunoglobulins in chicken secretions. J Immunol 106:1441–1446, 1970
13. Glick B, Chang TS, Jaap RG: The bursa of Fabricius and antibody production. Poult Sci 35:224–225, 1956

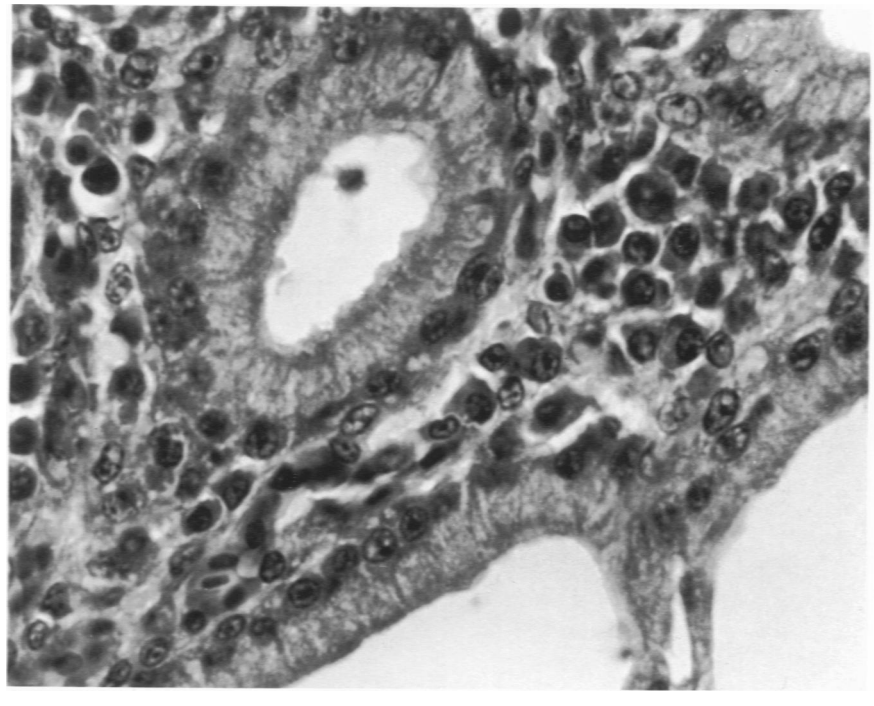
14. Bang FB, Foard MA: The effect of acute vitamin A deficiency on the susceptibility of chicks to Newcastle disease and influenza virus. J Hopkins Med J 129:100–109, 1971
15. Vint FW: Post-mortem findings in natives in Kenya. East Afr Med J 13:332–340, 1937
16. Smythe PM, Campbell JAH: The significance of the bacteraemia of kwashiorkor. S Afr Med J 33:777, 1959
17. Oomen HACP: Clinical experience on hypovitaminosis A. Nutritional disease: Proceedings of the Conference on Beriberi, Endemic Goiter and Hypovitaminosis A. Princeton, New Jersey, June 1–5, 1958
18. Ciba Foundation Study Group No. 31: Nutrition and Infection. Edited by GEW Wolstenholme and M O'Connor. Boston, Little, Brown and Co, 1967
19. McLaren DS: Xerophthalmia: a neglected problem. Nutr Rev 22:289–291, 1964
20. McFarlane H: Cell-mediated immunity in protein-calorie malnutrition. Lancet 2:1146–1147, 1971
21. Bang BG, Bang FB: Replacement of virus-destroyed epithelium by keratinized squamous cells in vitamin A-deprived chickens. J Exp Biol Med 132:50–54, 1969
22. Bang FB, Foard M, Bang BG: Histology of vitamin A deficiency in chicks and susceptibility to influenza virus. Fed Proc 30:1224, 1971
23. DeLuca L, Wolf G: Vitamin A and protein synthesis in mucous membranes. International Symposium on the Metabolic Function of Vitamin A. Am J Clin Nutr, 1969
24. Follis RH: Deficiency Disease. Springfield, Illinois, Charles C. Thomas, Publisher, 1958
25. Axelrod AE: Nutrition in relation to acquired immunity. Modern Nutrition in Health and Disease. Fourth edition edited by MG Wohl and RS Goodhart. Philadelphia, Lea and Febiger, 1968
26. Axelrod AE: Immune processes in vitamin deficiency states. Am J Clin Nutr 24:265–271, 1971
27. Jolly J: L'involution physiologique de la bourse de Fabricius et ses relations avec l'apparition de la maturité sexuelle. C R Soc Biol (Paris) 75:638–640, 1913

Addendum

After submitting this paper, we found a recently published report which not only confirms our original observations[8] of early heterophil and late plasma cell populations in the gland, but also contains important quantitative data and growth curves: Wright PAL, Burns RB, Rothwell B, Mackenzie GM: The Harderian gland of the domestic fowl. J Anat 110:307–315, 1972



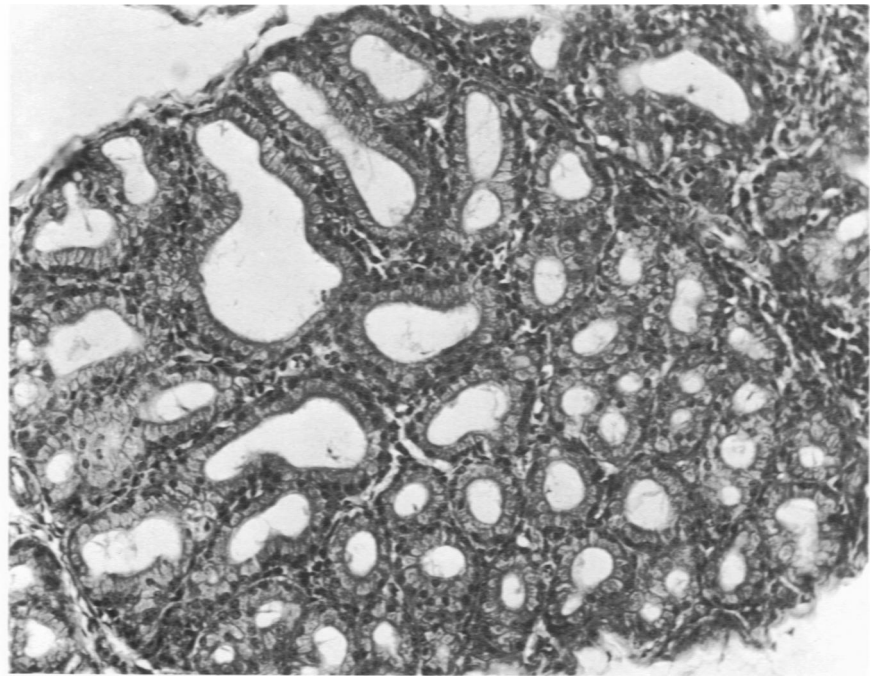
1



2

Fig 1—Portion of follicle of gland of Harder of normally fed 1-month-old chicken showing populations of plasma cells in the interfolicular spaces (H&E, × 250). **Fig 2**—Area in Fig 1 enlarged to show plasma cells (H&E, × 1000).

3



4

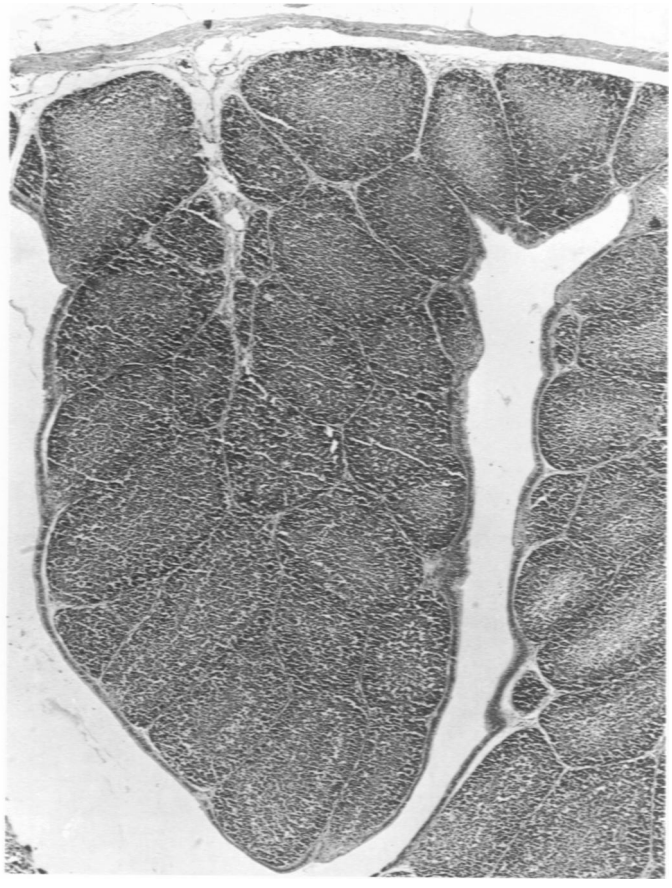


Fig 3—Gland of Harder of 1 month old chicken on Diet V since hatching, showing total lack of plasma cells (H&E, × 250). **Fig 4**—Rugal fold of bursa of normally fed 1-month-old chicken: regular, polygonal mature follicles; normal epithelium (H&E, × 45).

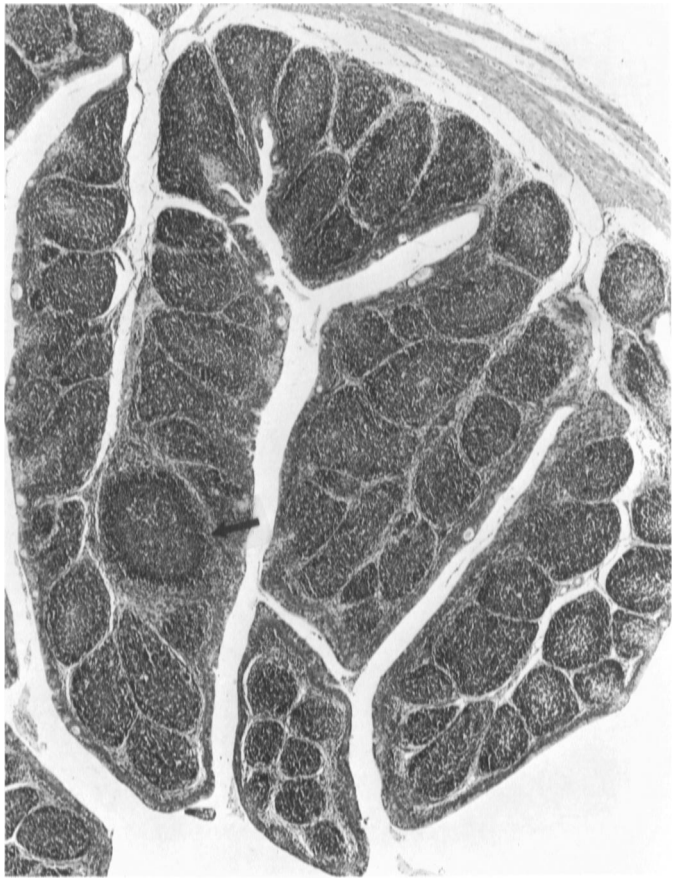


Fig 5—Comparable area of bursa of chicken on vitamin A-deprived Diet III: irregular follicular development, focal heterophil invasion (*arrow*) and disturbed epithelium (H&E, × 45).

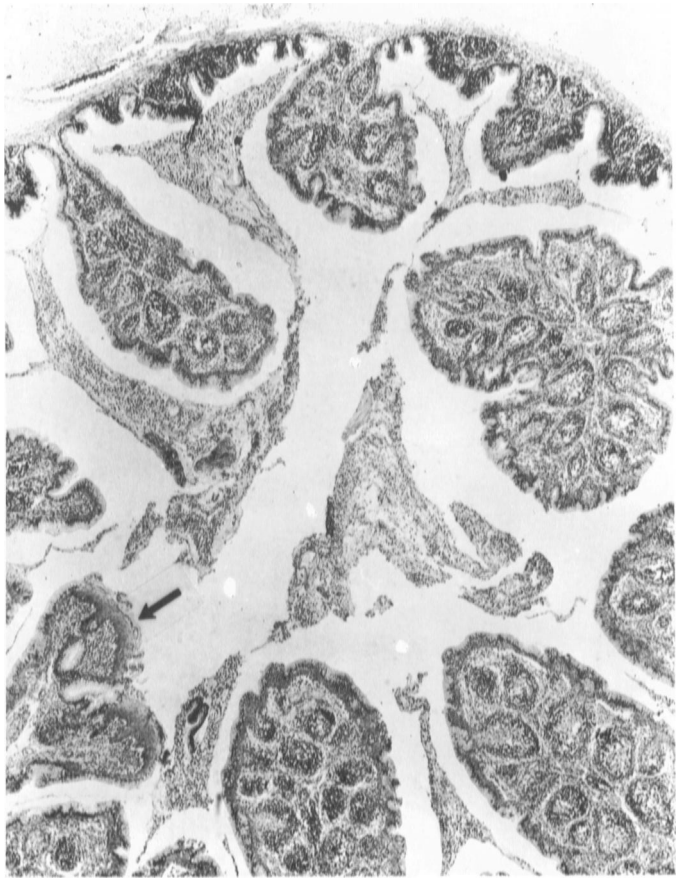


Fig 6—Comparable area in chicken on Diet V, lacking vitamin A and other components: atrophy of follicles, mucous and keratinizing (*arrow*) epithelial metaplasia, exudate in lumen (H&E, × 45).

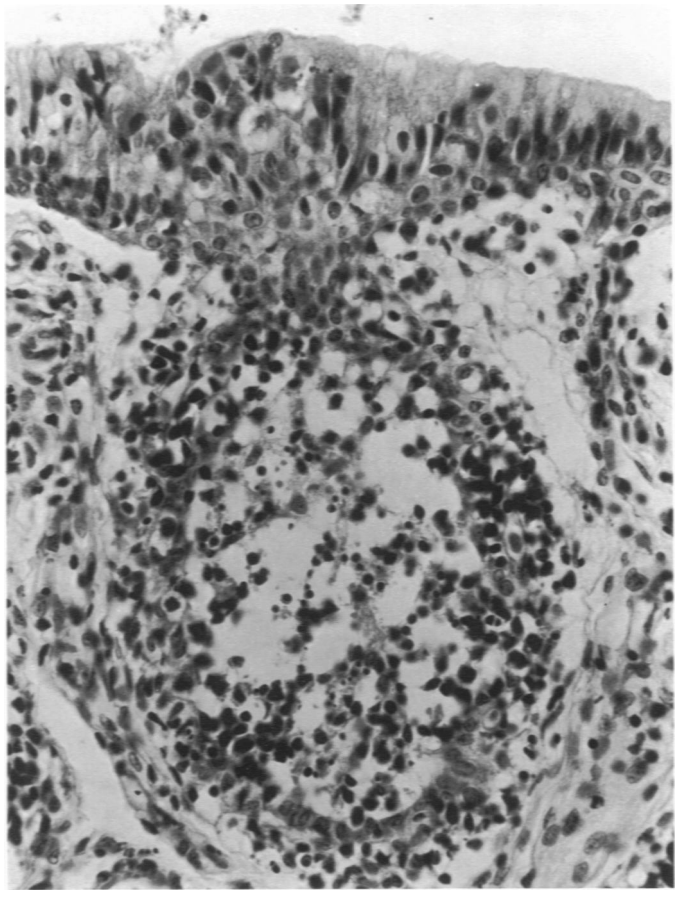


Fig 7—Enlarged follicle from Fig 6: loss of all normal lymphocytes and of the vascular circlet; disintegrating cell nuclei; basal cell hyperplasia (H&E, × 500).