Morphologic Alterations in the Trachea and the Salivary Gland Following the Induction of Rapid Synchronous Vitamin A Deficiency in Rats

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The use of the synchronous induction method enables both assessment of the sequence and reliability of the appearance of morphologic signs of vitamin A deficiency, and their accurate correlation with biochemical and physiologic abnormalities. In the trachea, hyperplasia of basal epithelial cells was observed by Day 4 (T_4) following the withdrawal of retinoic acid from retinoate-cycled, stringently deficient rats. Keratinization was observed by Day 6, the upper part of the trachea showing the highest incidence of keratinization. All such metaplastic changes originated in the narrow strip of tissue directly cojoining the esophagus. In the submaxillary glands, atrophy of the acini, an increase in interlobular spaces, and fibrosis and dilatation of the ducts was observed by Day 10. In more advanced stages of deficiency (T_{14}-T_{18}), cyst formation associated with suppuration and extensive cell atrophy was observed. Morphologic changes were less marked in the sublingual glands, although mucin levels were noticeably depressed by Day 12 of deficiency. Following the oral dosing of deficient animals (T_{12}) with 350 μg retinyl palmitate, all such changes were reversed within 6 days in the trachea and within 10 days in the submaxillary and sublingual glands. Similar patterns were observed whether animals were force-fed or were fed *ad libitum*. Apart, therefore, from cause-effect considerations *per se*, morphologic changes are also potentially valuable reference indicators of deficiency, particularly in time course studies, or where force-feeding attenuates other signs of deficiency. (Am J Pathol 1980, 98:717-732)

KERATINIZATION OR ATROPHY of epithelial tissue is one of the more characteristic features of vitamin A deficiency.[1,2] For instance, in the trachea,[3,4] salivary glands,[5,6] bladder,[7] eye lens,[8] and vagina[9] epithelial cells are replaced by keratinized, or cornified, cells. In rat testis epithelium [10] or intestine,[11] germinal epithelial or mucus-secreting cells atrophy without undergoing squamous metaplasia. Conversely, keratinized epithelium undergoes mucous metaplasia with a corresponding increase in the number of columnar or cuboidal cells following treatment with vitamin A.[12,13] Roles for vitamin A in glycoprotein synthesis,[14] cell division in the basal lamina,[15] and differentiation [16] have been proposed. No one theory has proven entirely satisfactory. Analysis of the role of vitamin A in epithelial systems, moreover, has often been complicated by secondary considerations, including age, duration of deficiency, inanition, and infection.

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In chronic vitamin A deficiency, for instance, the keratinization of respiratory epithelial tissues is much reduced in the absence of concomitant infection.[17] Thus, the extent to which a given pathologic lesion is a primary or secondary consequence of deficiency has often been difficult, if not impossible, to determine.

To overcome these and a number of other problems in studies of the nonvisual function of vitamin A, we have developed an animal rearing system [18] enabling the rapid and essentially synchronous induction of vitamin A deficiency. Such a system has several theoretical and practical advantages over conventional means of inducing deficiency.[18,19] For instance, the ability to control the onset of deficiency precisely enables one to determine the sequence and reliability of appearance of the many and diverse signs of deficiency.[19] Once this sequence has been adequately defined, the temporal relationships in any given pathologic sequence may be more easily related to biochemical or physiologic correlates of deficiency.

We report here our studies of morphologic changes in the trachea and salivary glands of synchronously vitamin A-deficient rats and the effects of differences in the feeding regime. Our studies of intestinal goblet cell metabolism are the subject of a separate report.[20]

Materials and Methods

Synchronously vitamin A-deficient rats (190–210 g) were obtained as earlier described.[18–20] Briefly, weanling male rats of a Fischer–Wistar cross-strain were fed a stock semipurified vitamin A-free diet *ad libitum* for approximately 3 weeks until early weight plateau (60–70 g). Thereafter, they were fed the stock diet first supplemented with and then lacking in 2 μg retinoic acid per gram diet in repeating 18-day:10-day cycles. A minimum of three such supplementation:deprivation cycles subsequent to primary growth plateau were used to promote a systemic rather than peripheral vitamin A deficiency restricted to the more rapidly dividing tissues of the body. Animals selected as A^+ controls received a total of 500 μg retinyl palmitate in 0.4 ml safflower oil by stomach tube in split doses two days (T_{-2}) and one day (T_{-1}) prior to the ultimate withdrawal of retinoic acid (T_0). To make them vitamin A-deficient (A^-), we gave the animals safflower oil only. Finally all rats were given, by stomach tube, 10 μg retinoic acid in 0.25 ml safflower oil at day zero (T_0) in order to overcome any differences in endogenous retinoate levels due to prior differences in the meal-eating patterns of vitamin A-deficient and control groups and to synchronize still further the ultimate excretion of retinoic acid and the onset of deficiency. Following the withdrawal of retinoic acid, animals were fed a vitamin A-free diet *ad libitum* or were tube-fed 5 g of a vitamin A-free diet twice daily (9 AM and 5 PM). In such experiments micropulverized casein and sucrose in place of starch were used to facilitate mixing and feeding.

We reared conventionally vitamin A-deficient rats (80–90 g) by feeding weanling male rats stock vitamin A-deficient diet. They were considered vitamin A-deficient when 1) the net weight gain on 4 successive days was less than 1 g and 2) they failed to gain weight on 2 of these days.[21]

Control pellet-fed rats (200–250 g) were fed a commercial pelleted a vitamin A-sufficient diet [18] *ad libitum*.

Animals were killed with an overdose of anesthetic ether. The trachea, submaxillary, and sublingual glands were removed as quickly as possible and fixed either in acetic acid,

alcohol, formalin, or calcium acetate–formalin fixative.[22] The latter fixative has the advantage of preserving the convoluted granules of the submaxillary gland.[23,24] After embedding in Paraplast, 5-μ sections were cut and stained with hematoxylin and eosin (H&E) or, where neutral mucopolysaccharides were of interest, periodic acid–Schiff (PAS) reagent. When we determined the degree of keratinization in the upper, central, and lower regions of the trachea, two sections from the middle of each segment were scored for ciliated and columnar, pleomorphic cuboidal, and squamous cells throughout the entire circumference of the trachea according to the classification of Crocker and Sanders.[25] This usually entailed the examination of 20–30 fields, depending on the diameter of the trachea. Both for tracheal and salivary gland sections, we recorded the data without prior knowledge of the nutritional status of the animals.

The vitamin A status and secondary feeding regimen of all animals used in the present study are summarized in Table 1.

Results

Trachea

The tracheal epithelium of control vitamin A-sufficient rats (Groups 1, 2, and 3) was of a simple columnar type. A typical tracheal segment distal to the tracheal-esophageal junction in 18% casein force-fed retinyl palmitate control rats is shown in Figure 1A. In synchronously vitamin A-

Table 1—Summary of Vitamin A Status and Feeding Regimes, Including Number of Animals Used

	Code	Treatment	Number of rats
Group 1	A^+ pellet *ad lib*	Vitamin A-sufficient rats fed a commercial pelleted diet *ad libitum*.	15
Group 2	RA *ad lib*	Retinoate-cycled control animals, 10 or more days after resupplementation with 2 μg retinoic acid per gram diet, fed *ad libitum*.	15
Group 3	A^+ ff	Retinyl palmitate control rats force-fed 5 g diet twice daily following the ultimate withdrawal of retinoic acid.	44
Group 4	A^+ starved	Retinyl palmitate control rats given drinking water only following the ultimate withdrawal of retinoic acid.	4
Group 5	A^- ff	Vitamin A-deficient rats force-fed 5 g diet twice daily following the ultimate withdrawal of retinoic acid.	71
Group 6	A^- *ad lib*	Vitamin A-deficient rats fed *ad libitum* following the ultimate withdrawal of retinoic acid.	48
Group 7	A^- conv. *ad lib*	Vitamin A-deficient rats reared conventionally and fed *ad libitum* throughout.	17

deficient animals, hyperplasia in the basal region of the tracheal epithelia was observed within 4 days of the withdrawal of retinoic acid. Metaplastic changeover of ciliated and columnar cells to keratinized cells was observed at Day 6 following withdrawal of retinoic acid regardless of secondary dietary considerations. In all instances initial foci of metaplastic changes were located in the region where the esophagus and the trachea merged. The onset of keratinization in the tracheal esophageal region is shown in Figure1B.

Metaplastic changes were always more marked in the upper than in the central or lower regions of the trachea (Table 2). Thus, in determining the percentage of squamous cells as a function of time in the trachea as a whole, duplicate sections from the upper, central, and lower regions of the surface epithelial layer of the trachea were scored; then squamous cell values as a percentage of total epithelial cell numbers in each of the tracheal regions were averaged and pooled to provide a mean value for each rat.

The effect of vitamin A status on the overall incidence of tracheal keratinization at various times after the withdrawal of retinoic acid is shown in Table 3. For simplicity, the data has been broken into four groups: animals in which keratinization was never observed and animals exhibiting degrees of keratinization from 1% to 10%, 11% to 50%, and 51% to 100%. From such studies it was found 1) that tracheal metaplasia from ciliated

Table 2—Percentage Distribution of Surface Epithelial Cell Types in the Upper, Central, and Lower Regions of the Trachea in Synchronously (T_{10}) Vitamin A-Deficient Force-Fed Rats and Synchronously (T_{10}) Vitamin A-Deficient Rats Fed *Ad Libitum*

Tracheal region*	Force-fed (n = 6)	Fed *ad libitum* (n = 3)
Upper		
Ciliated and columnar	10 ± 7†	25 ± 16
Pleomorphic cuboidal	16 ± 7	13 ± 9
Squamous	74 ± 11	62 ± 24
Central		
Ciliated and columnar	18 ± 8	39 ± 24
Pleomorphic cuboidal	21 ± 8	10 ± 3
Squamous	61 ± 15	51 ± 24
Lower		
Ciliated and columnar	59 ± 8	69 ± 15
Pleomorphic cuboidal	8 ± 3	11 ± 6
Squamous	33 ± 9	19 ± 9

* Trachea were cut into three approximately equal lengths. Cell types were scored in duplicate sections cut from the middle of each region. Group mean values for individual regions were calculated using averaged values for individual animals.

† Values are means ± SEM to the nearest whole number.

and columnar cells to squamous and keratinized cells was never observed in vitamin A-sufficient animals (Groups 1–3), 2) that the percentage of the incidence of squamous cells increased with the duration of vitamin A deficiency, and 3) that vitamin A-deficient animals showed a comparable incidence of squamous metaplasia regardless of feeding regimen.

In experiments to determine the rate of recovery of vitamin A-deficient animals after dosing with retinyl palmitate, eighteen rats were force-fed vitamin A-deficient diet until T_{12} following retinoate withdrawal. They were then given a single dose of 350 μg retinyl palmitate by stomach tube. Thereafter, pairs of animals were killed on even-numbered days and single animals on odd-numbered days to monitor recovery from deficiency. Ciliated and columnar cells reappeared by Day 6 following retinyl palmitate treatment. By Day 8 the trachea was completely back to normal, including the upper and most severely keratinized section.

Salivary Glands

The wet weight (g/100 g body weight) of the submaxillary and sublingual glands in control animals was unchanged even when animals were starved. In contrast, the salivary glands of deficient animals exhibited

Table 3—Effect of Feeding Regimen and Duration of Vitamin A Deficiency on the Incidence of Squamous Metaplasia in Rat Trachea

Code*	Experiment	Days after retinoic acid withdrawal	Number of rats	Number of rats with squamous epithelial cells in the range† (%)‡			
				0%	1–10%	11–50%	51–100%
A^+ ff	1	8–22	10	100	0	0	0
A^- ff	1	6–10	41	10 (24)	14 (34)	9 (22)	8 (20)
	2	8–15	5	0	3 (60)	0	2 (40)
	3	12–21	6	0	0	2 (33)	4 (67)
A^- *ad lib*	1	10–12	8	0	3 (38)	1 (12)	4 (50)
	2	12–34	5	0	0	0	5 (100)
A^- conv. *ad lib*	1	10–23§	17	0	4 (24)	11 (65)	2 (11)

* See Table 1 for details of the various groups.

† Precentage squamous cell values for individual animals were calculated by taking the mean of averaged values for the upper, central, and lower tracheal regions (see Table 2).

‡ Percentage values are given in parentheses.

§ Days after onset of vitamin A deficiency according to the criteria of Bliss and Roels.[21]

marked changes in wet weight (Table 4) and morphology in the late stages of deficiency, regardless of secondary nutritional status.

Submaxillary Glands

In describing the normal gland and changes in structure following withdrawal of retinoic acid, the terminology suggested by Jacoby and Leeson [26] has been used. The acinar cells of vitamin A-sufficient pellet-fed rats (Group 1), retinoic acid-supplemented rats (Group 2), and retinyl palmitate-dosed control animals (Group 3), were usually triangular and contained dense eosinophilia (Figure 2A). The intercalated ducts connecting the different acini of individual lobules were lined with small cuboidal cells containing prominent nuclei. The convoluted granular tubules were of a characteristic urn shape and contained tall columnar cells with nuclei near the base of the cell. The intralobular striated ducts were lined with columnar cells with more or less centrally placed nuclei. The appearance of dense granules in the convoluted granular tubules depended on the method of fixation. After acid–alcohol fixation, relatively few granules were observed. After calcium acetate–formalin fixation, however, and in agreement with earlier reports,[23,24] these granules were prominent and easily seen.

Synchronously vitamin A-deficient animals showed only slight morphologic alterations 8 days following the withdrawal of retinoic acid. By 12 days, however, extensive disorganization of the submaxillary gland was evident. The earliest and most striking change was atrophy of acinar cells 10 days after the withdrawal of retinoic acid (Figure 2B). Histologically,

Table 4—Effect of Vitamin A Deficiency and Feeding Regimen on Salivary Gland Wet Weight

Code*	Days after retinoic acid (RA) withdrawal	Number of glands	Salivary gland wet weight (g/100 g body weight)	
			Submaxillary	Sublingual
RA *ad lib*	−†	8	67.3 ± 2.4‡	16.1 ± 0.8
A^+ ff	22	6	67.9 ± 3.7	15.9 ± 0.5
A^- ff	8–12	8	64.7 ± 3.2	16.0 ± 0.9
A^- *ad lib*	10–15	12	78.9 ± 8.4§	28.4 ± 3.4 ‖
	12–37	24	105.2 ± 10.3 ‖	29.5 ± 3.5 ‖
A^+ starved	6–8	8	66.4 ± 2.8	16.4 ± 0.9

* See Table 1 for details of the various groups.

† Cycled animals resupplemented with 2 μg retinoic acid per gram diet for 10 or more days following a standard 10-day deprivation phase.

‡ Values are means ± SEM.

§ $P < 0.05$, compared with RA *ad lib* group.

‖ $P < 0.01$, compared with RA *ad lib* group.

the nuclei of such cells appeared more closely packed together due to a reduction in the volume of cell cytoplasm. Interlobular partitions were widened due to water imbibition. In edematous glands (Table 4), fibroblast cells were observed between the stromata. The convoluted granular tubules were generally atrophied and markedly diminished. At about this time, too, the striated duct cell types became more prominent due to atrophy of the acinar cells. As deficiency progressed (T_{12}–T_{37}), excretory ducts were invaded by neutrophils. Only after 3 weeks, in the few animals that survived that long, was keratinization of the ducts observed. Thus, keratinization of salivary gland tissues in rats is a less valuable guide to the onset or degree of vitamin A deficiency than is keratinization of tracheal epithelium.

The changes observed in conventionally reared vitamin A-deficient animals (Group 7) were essentially the same as in synchronously deficient animals. Similarly, morphologic changes in the submaxillary gland in *ad libitum* and force-fed vitamin A-deficient rats were comparable (Table 5). As with the trachea, the duration of deficiency had the single most marked morphologic effect on the gland. For instance, excretory duct keratinization was observed only in acutely vitamin A-deficient animals (Table 5: A^- ff, Experiment 3; A^- *ad lib*, Experiment 2; and A^- conv. *ad lib*) but not in mildly deficient groups (A^- ff, Experiment 1; A^- *ad lib*, Experiment 1), regardless of the feeding regime.

In recovery experiments similar to or overlapping the tracheal experiments, the dosing of T_{12} deficient animals with retinyl palmitate resulted in the full recovery of submaxillary gland tissue within 10 days.

Sublingual Glands

Companion studies of the sublingual glands in vitamin A-sufficient pellet-fed rats (Group 1), retinoic acid-supplemented animals (Group 2), and retinyl palmitate-dosed animals (Group 3) revealed mainly mucous acini, intercalated ducts, and striated ducts. Most strikingly, the mucous acini contained an intensely PAS-positive foam-like material (Figure 3A). In deficient animals, the morphologic characteristics of the sublingual glands and the degree of PAS-staining in the mucous acinar region were essentially unchanged during the first week of retinoic acid withdrawal. At about the time marked morphologic changes in the submaxillary gland were first observed (T_{12}), PAS-negative vacuoles were also observed in the sublingual gland of deficient animals, whether the animals were force-fed or were fed *ad libitum* (Figure 3B). Dilatation of the striated ducts of the sublingual glands, however, was less evident than in the submaxillary gland. Again, conventionally reared vitamin A-deficient rats showed mor-

Table 5—Morphologic Alterations in Submaxillary Glands in Vitamin A-Deficient Rats as a Function of Time After Onset of Deficiency and Feeding Regimen

Code*	Experiment	Days after retinoic acid withdrawal	Number of rats	Number of rats exhibiting (%)†				
				Edematous glands	Abnormal acinar cells	Abnormal striated ducts	Cyst formation	Excretory duct keratinization
A^+ ff	1	8–22	10	0	0	0	0	0
A^- ff	1	8–10	34	13 (38)†	9 (26)	7 (21)	2 (6)	0
	2	8–15	5	3 (60)	4 (80)	4 (80)	3 (60)	0
	3	12–21	6	2 (33)	5 (83)	5 (83)	4 (67)	2 (33)
A^- *ad lib*	1	10–12	8	4 (50)	6 (75)	6 (75)	5 (63)	0
	2	12–37	9	4 (44)	9 (100)	9 (100)	8 (89)	6 (67)
A^- conv. *ad lib*	1	10–22‡	17	15 (88)	15 (88)	13 (76)	15 (88)	8 (47)

* See Table 1 for details of the various groups.
† Percentage values are given in parentheses.
‡ Days after onset of vitamin A deficiency according to the criteria of Bliss and Roels.[21]

phologic changes similar to those observed in rapidly induced vitamin A-deficient rats, ie, edema, dilatation of the ducts, reduced mucin production, vacuole formation in the mucous acini, and widening of the interlobular stroma.

In recovery experiments, the administration of retinyl palmitate by stomach tube resulted in the recovery of the sublingual glands within 12 days. The most striking sign of recovery was the reappearance of PAS-positive foam-like material in the mucous acini.

Discussion

In the past, data on the effects of vitamin A deficiency on pathologic signs in different tissues have been drawn variously from long-term chronically vitamin A-deficient animals fed *ad libitum*,[15] weanling animals fed *ad libitum* at the primary weight plateau or early decline phase of deficiency,[1] animals from both of these categories but pair-fed,[6,14] germ-free animals,[27,28] and children suffering from concomitant protein-calorie malnutrition.[29,30] To provide more readily controlled experimental conditions, we have recently systematized the use of retinoic acid in studies of the nonvisual function of vitamin A. With the exception of vision and reproduction, retinoic acid has the same growth-promoting activity and tissue functions as retinol but cannot be converted to retinol or stored in the body.[31,32] Withdrawal of retinoic acid from adequately cycled, stringently vitamin A-deficient animals leads to the induction of a rapid, systemic, and essentially synchronous vitamin A deficiency.[18-20] Due to the short time course of such experiments, nutritional imbalances immediately prior to or following the withdrawal of retinoic acid may be overcome by the force-feeding of deficient and control animals. Additionally, one may determine whether a given sign of deficiency is accentuated by force-feeding, as is the case with altered urinary taurine levels,[33] is unaffected, or is minimized by force-feeding, as with the decrease in body weight following the onset of deficiency.[19] Thus, one may determine not only the sequence of appearance of the many and diverse signs of vitamin A deficiency, but also whether a given metabolic or pathologic lesion is independent of or is compounded by secondary inanition or metabolic inefficiency.

In the trachea, metaplastic changes were evident as early as Day 4 following the withdrawal of retinoic acid. Keratinization proceeded sharply, starting on Day 6 of deficiency. All such changes originated in the narrow strip of tissue directly cojoining the esophagus. There was also a higher incidence of metaplasia in the upper region of the trachea. Similar results were obtained whether cycled animals were fed *ad libitum* or were force-

fed, or whether conventionally reared animals were fed *ad libitum*. Although there was a tendency for greater keratinization in force-fed synchronously deficient animals, these differences were not statistically significant. DeLuca[34] also observed foci of keratinized cells in the trachea of vitamin A-deficient hamsters, but these were randomly distributed.

The single most important factor determining the degree of tracheal keratinization was the duration of vitamin A deficiency. Qualitatively, the changes we observed were the same as those described by Harris et al,[3] Wong and Buck,[4] and Wolbach and Howe.[5] Following the dosing of vitamin A-deficient animals (T_{12}) with retinyl palmitate, recovery similarly took 6 days. The regeneration of tracheal epithelium following shallow curettage in vitamin A-deficient rats similarly takes 6–8 days.[35] Moreover, the time to onset of tracheal keratinization in synchronously deficient animals paralleled closely the transition time from the growth plateau or early decline growth phase (D_E^-) to the late decline phase (D_L^-) of deficiency,[19] the onset of hypertauremia,[33] and sharply increased sleeping or paralysis times in animals given daily doses of barbiturates or narcotics.[36] These 6-day induction–recovery periods may well reflect intrinsic rates of turnover or replacement of intracellular moieties requiring vitamin A for their synthesis or functional integrity. Infection, by further stressing conventionally reared animals, may indeed hasten the onset of deficiency,[17] but where animals are already stringently vitamin A-deficient, such external factors appear less important. Definitive evaluation of this point would require the use of germ-free animals.

In contrast with tracheal keratinization, the morphologic appearance of the salivary glands of vitamin A-deficient and control animals was unaltered 6 days following the withdrawal of retinoic acid. In particular, the dense granules in the convoluted granular tubules of the submaxillary gland were still amply evident after calcium acetate-formalin fixation. By Day 10 of deficiency (T_{10}), however, atrophy of the acinar cells and convoluted granular tubules was observed. Castration of mice similarly results in atrophy of the convoluted granular tubules of the submaxillary gland, changes which are reversed by testosterone injection.[37] In vitamin A deficiency, the synthesis of a number of steroid hormones is decreased, due seemingly to depressed testicular $\Delta^5,3\beta$-hydroxysteroid dehydrogenase and Δ^4-5,3-oxosteroid isomerase activities.[38] It might be asked, therefore, whether decreased testosterone levels are similarly responsible for the changes we have observed. This seems unlikely, however, since the convoluted granular tubules in the salivary glands of retinoic-acid-cycled animals (Group 2) were normal, despite the fact that the testes of such animals are severely atrophied.[10] Thus, atrophy and degranulation of the convoluted granular tubules in submaxillary glands in vitamin A defi-

ciency are more likely a direct result of deficiency rather than an indirect effect mediated through hormonal imbalance.

In comparison, the changes observed in the sublingual glands of synchronously deficient animals were restricted mainly to disappearance of mucin and mild distension of the ducts 12 or more days after the withdrawal of retinoic acid. In that vitamin A has been implicated in the synthesis of specific glycoprotein(s),[14] one might reasonably expect greater morphologic alterations in the sublingual gland, which synthesizes primarily mucin, than in the submaxillary gland. Others, however, have similarly observed that the sublingual gland is less markedly affected in vitamin A deficiency both in rats[6] and in hamsters.[39]

Overall, the degrees of alteration in the submaxillary and sublingual glands of rats that were force-fed, fed *ad libitum*, and conventionally vitamin A-deficient were similar. The only minor difference noted was that force-feeding (T_{8-12}) decreased water imbibition, compared with *ad libitum* feeding (T_{10-15}) (Table 4). As in the trachea, therefore, duration of deficiency was the single most important determinant of morphologic change (Table 5). The extensive infiltration of lymphocytes and plasma cells into the ducts of the salivary glands we observed in the late stages of deficiency has also been reported for a child suffering from severe vitamin A deficiency.[40] The similarity in the pathologic changes resulting in salivary gland cyst formation suggests a common etiology.

In terms of mechanism, the lack of vitamin A induced marked but quite different effects in specific cells of sensitive organs: a proliferation of basal cells at specific foci in the trachea, which leads to squamous metaplasia, between Days 4 and 6, a degeneration of secretory acinar cells of the submaxillary gland at Day 10, and a reduction of mucin formation in acinar cells of the sublingual gland at Day 12. In the latter two tissues, keratinization of duct cells, duct blockage, and cyst formation were late sequelae of vitamin A deficiency. Whether such changes in cell type reflect a direction action of vitamin A on specific processes of differentiation or the response of cells to a modified biochemical milieu is not known. Seemingly, atrophy and keratinization of epithelial tissues are not primary signs of deficiency as earlier claimed by Wolbach,[41] in that they are preceded by a host of physiologic and metabolic defects including impaired growth and appetite,[19] altered nitrogen and amino acid metabolism,[42] and abnormal taurine metabolism.[33] Whether any of these changes are causally related to epithelial keratinization remains to be seen. For instance, dietary taurine exacerbates the keratinization of epithelial tissues observed in psoriasis,[43] a condition that responds favorably to vitamin A and a number of synthetic retinoids.[44]

Morphologic changes are nonetheless "absolute" correlates of defi-

ciency in the sense that they are independent of general nutritional status. Thus, the force-feeding of animals was without discernible effect on any of the changes noted here. The same was true of the decrease in intestinal goblet cell numbers.[20] As such, morphologic changes are potentially useful reference indicators of deficiency where dietary interactions are of concern. For instance, in metabolic studies in force-fed vitamin A-deficient rats the increased nutrient input reverses the decrease in body weight observed in conventionally fed animals.[19] Without some absolute criterion of deficiency in such situations, it is difficult to determine whether animals are genuinely vitamin A-deficient.

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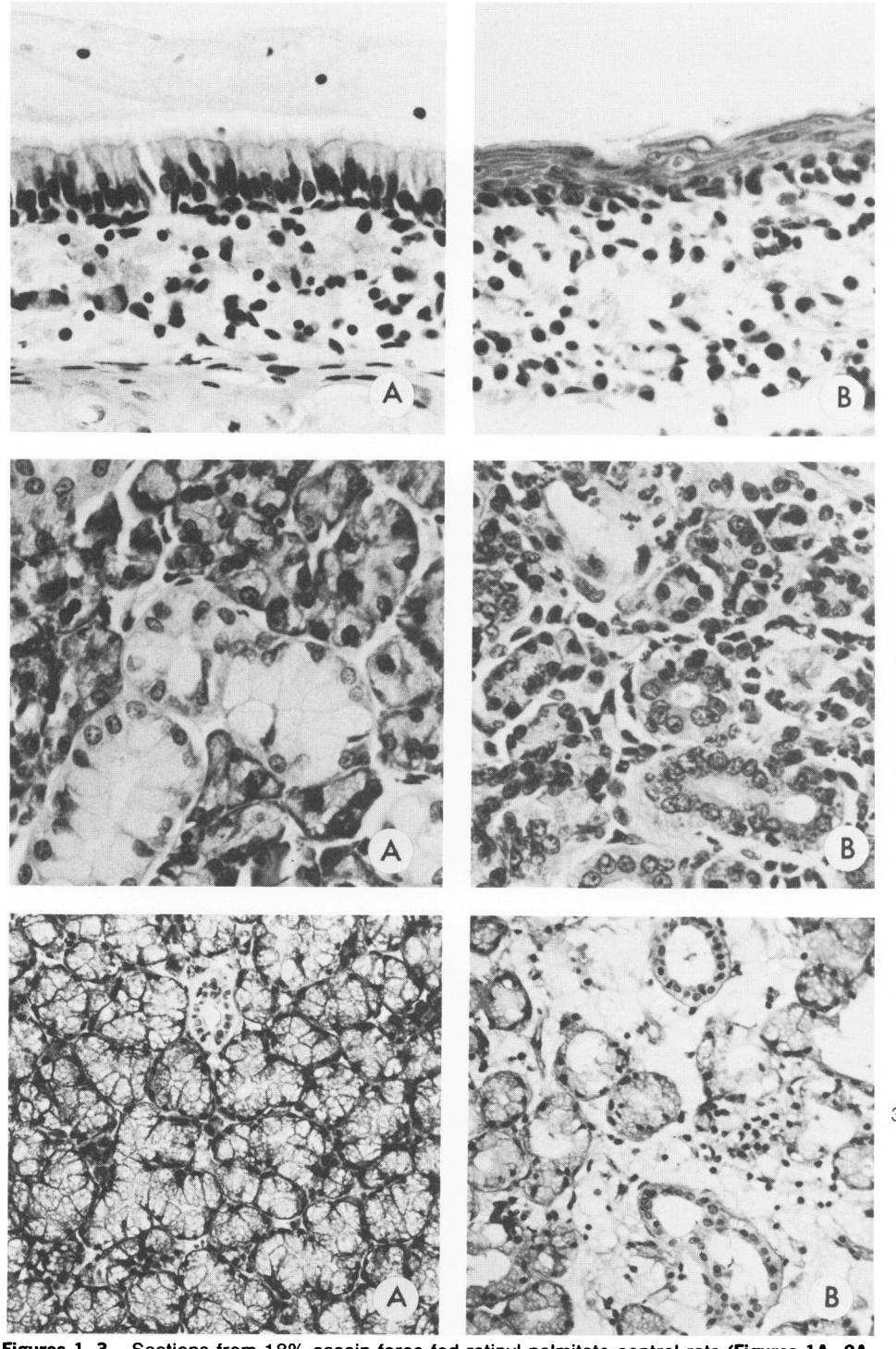

Figures 1–3—Sections from 18% casein force-fed retinyl palmitate control rats (**Figures 1A, 2A, and 3A**) and vitamin A-deficient rats (**Figures 1B, 2B, and 3B**) showing the trachea at Day 6 (**Figures 1A and 1B,** H & E, × 400), submaxillary gland at Day 10 (**Figures 2A and 2B,** H & E, ×400) and sublingual gland at Day 12 (**Figures 3A and 3B,** H & E, × 200) following the ultimate withdrawal of retinoic acid.

[*End of Article*]