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EXPERIMENTAL PATHOLOGY OF DAIRY CALVES INGESTING ONE-THIRD THE DAILY REQUIREMENT OF CAROTENE

By

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Histological and biochemical alterations in severe bovine vitamin A deficiency have been well established. Lesions of marginally deficient animals are much less understood and the pathogenesis of total or sub-total deficiency is still a matter of speculation.

Better concepts of nutrition have abated much of the economic loss associated with animals having a severe vitamin A depletion. However, the marginal syndrome still occurs, both in dairy and feedlot cattle receiving a ration consisting of much concentrate and little roughage. The gross and histological changes associated with severe vitamin A deficiency have been reviewed (*Mills* 1967), and the pathology of calves on graded carotene intakes, from 10 to 60 μg per 0.45 kg of live-weight daily for 12 or 24 weeks has been described recently from this laboratory (*Nielsen et al.* 1966a, b). In the latter studies, a metaplasia scoring system was devised to correlate the degree of parotid metaplasia with graded intakes of carotene.

It is the purpose of this report to substantiate these findings in a larger group of calves on a fixed intake of 15 μg carotene which is approximately one-third of the daily minimal requirement of 48 μg per 0.45 kg of live-weight (*Eaton et al.* 1964a). This deficient intake was fed to 15 calves for 17 weeks; 15 calves receiving 150 μg of carotene per 0.45 kg of live-weight served as controls. Clinical, biochemical and morphological findings of the 2 groups were compared.

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MATERIALS AND METHODS

At approximately 35 days of age, each of 30 male Holstein-Friesian calves was fed a vitamin A depletion ration to provide a weight gain of 4.5 kg*) per week. One week following depletion of the blood plasma vitamin A levels to or below 12 µg/100 ml (53 ± 6 days of age) each calf's depletion ration was augmented with either 15 or 150 µg of carotene intake per 0.45 kg of live-weight per day. Hereafter, the low intake group will be referred to as deficient and the high intake group as controls. Details as to feeding schedules, biochemical and physiological measurements have been given by *Woelfel et al.* (1963).

All calves were fed the experimental diet for a period of 17 weeks after which euthanasia was performed. At necropsy, the following tissues were taken and fixed in 10 % neutral buffered formalin: eyes with optic tracts, pituitary, brain, thyroid, lung, spleen, liver, parotid gland and duct, pancreas with duct, testes and epididymis, urethra with seminal vesicles, vas deferens and prostate, penis, prepuce, kidney, bladder, skin and various levels of the alimentary tract.

Skin sections were taken from 4 body areas, the upper lip, scrotal and lumbar skin, and the eyelid to include the tarsal gland. Sections of rumen and reticulum were removed from the right anterior dorsal wall of the former and the most anterior aspect of the latter. The prepuce-penis was sectioned from the middle of glans penis since normally a marked hyperkeratinization may occur more distally. Following fixation, 6 sections were cut through the parotid duct system, 3 from the main duct (Stensen's duct) and 3 from the gland. To evaluate transition of the buccal

*) 0.45 kg = 1 American pound (1 lb.).

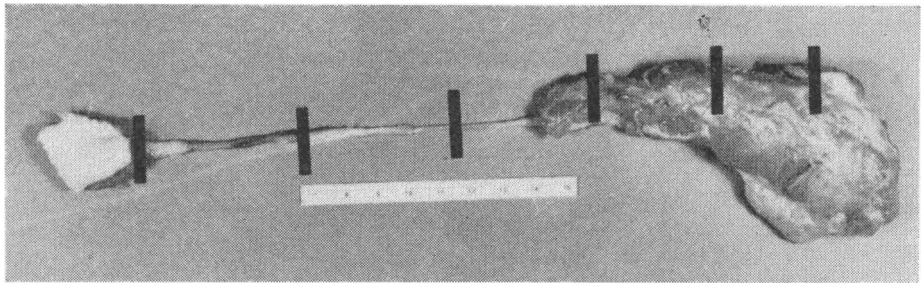


Figure 1. Bovine parotid salivary gland and duct, indicating the 6 sites examined histologically for evidence of squamous metaplasia. Measurement is in cm.

mucosa into the parotid duct, a longitudinal section was cut through the papilla salivalis; the remaining 5 levels were taken as cross-sections of the ductal system (Fig. 1). All tissues were embedded in paraffin, cut at 6 μ and stained with hematoxylin-eosin (H & E) and McManus' periodic acid-Schiff (PAS). Using a modified scoring system for parotid duct metaplasia with 6 levels instead of 8 (*Nielsen et al.* 1966a), values were assigned in an ascending manner from the papilla salivalis in the oral mucosa to a level through the parotid gland fundus, which included the parotid lymph node. Results were recorded as positive (+), suspicious (±) or negative (—). Metaplasia found at all 6 levels resulted in a score of 21 (1+2+3+4+5+6).

RESULTS

The mean plasma carotenoid and plasma vitamin A concentrations of the control calves were 5 times as high as the means of the deficient calves. Mean concentration of liver vitamin A and carotenoids of control calves was respectively, 100 times and 4 times that of the deficient calves (Tables 1 and 2).

Table 1. Biochemical measurements of 15 male Holstein calves receiving 15 μg of carotene per 0.45 kg live-weight daily for 17 weeks.

Calf accession no.	Plasma carotenoids μg/100 ml		Plasma vit. A μg/100 ml		Liver μg/100 g		Intraocular pressure, mm Hg	
	Terminal	Av. for period	Terminal	Av. for period	Vit. A	Carotenoids	Mano.	Tonom.
D1306	8	8	3.6	5.2	3.8	19	19.2	20.6
D1312	15	11	8.4	6.0	8.4	25	23.2	15.9
D1314	13	10	6.9	5.8	8.4	22	19.2	15.9
D1324	10	10	6.8	6.8	11.6	15	7.9	13.9
D1328	10	13	4.0	4.6	8.8	10	16.2	15.9
D1303	10	8	4.0	4.5	3.8	28	15.0	8.5
D1309	21	16	6.6	5.6	11.1	32	17.5	10.0
D1311	16	12	7.2	6.8	9.5	30	11.4	9.7
D1300	10	14	5.7	5.5	2.1	26	11.8	9.4
D1317	16	13	6.7	6.9	14.3	20	29.9	—
D1326	20	12	7.7	5.5	5.5	16	13.0	16.2
D1318	15	12	6.6	5.2	10.2	26	21.8	17.0
D1321	16	13	4.7	5.7	5.6	20	12.9	16.7
D1304	12	9	7.0	7.2	8.0	24	16.2	12.7
D1322	22	17	7.6	6.6	6.4	16	26.7	40.0
Average	$\bar{x}$=14	$\bar{x}$=12	$\bar{x}$=6.2	$\bar{x}$=5.8	$\bar{x}$=7.8	$\bar{x}$=22	$\bar{x}$=17.5	$\bar{x}$=15.9

Table 2. Biochemical measurements of 15 male Holstein calves receiving 150 μg of carotene per 0.45 kg live-weight daily for 17 weeks.

Calf accession no.	Plasma carotenoids μg/100 ml		Plasma vit. A μg/100 ml		Liver μg/100 g		Intraocular pressure, mm Hg	
	Terminal	Av. for period	Terminal	Av. for period	Vit. A	Carotenoids	Mano.	Tonom.
D1301	61	54	23.9	22.6	510.7	92	31.4	—
D1315	106	76	26.3	23.4	603.4	90	11.2	11.6
D1323	60	50	24.2	25.0	976.0	100	12.2	29.0
D1302	55	55	26.4	23.4	456.7	80	10.6	16.2
D1319	70	71	25.3	28.6	257.8	76	17.3	17.3
D1305	90	68	29.1	27.9	332.5	86	8.6	7.8
D1313	68	64	26.2	25.9	445.3	94	26.9	15.1
D1307	68	55	24.2	23.5	908.2	90	16.2	19.2
D1308	68	54	35.8	31.1	1059.0	100	21.9	—
D1310	80	68	33.6	31.1	758.6	96	15.6	15.4
D1316	71	56	29.1	25.8	534.2	74	8.4	10.4
D1327	99	71	39.2	29.8	490.2	68	19.0	17.0
D1320	117	90	26.2	23.6	1026.5	108	7.0	6.7
D1325	73	53	25.8	25.8	844.0	65	30.4	22.0
D1329	58	54	29.4	26.7	726.5	72	31.7	26.6
Average	$\bar{x}$=76	$\bar{x}$=63	$\bar{x}$=28.3	$\bar{x}$=26.3	$\bar{x}$=682.0	$\bar{x}$=86	$\bar{x}$=17.9	$\bar{x}$=16.5

Carotenoid and vitamin A concentrations of plasma and liver correlated well with the elevated CSFP, papilledema, parotid duct metaplasia, and presence of pituitary cysts (Table 3).

A slight decrease in ration consumption and smaller daily gains in live-weight as well as a slight incidence of diarrhoea were found in the deficient calves.

a. *Cerebrospinal Fluid Pressure (CSFP)*

In the deficient calves, 12 of the 15 had cisternal pressure above 200 mm of saline in the standing unanaesthetised position.

Table 3. Comparison of pathological and biochemical alterations in calves receiving 15 μg (deficient) or 150 μg (control) of carotene per 0.45 kg of live-weight per day.

Carotene intake (μg) 0.45 kg live weight/day	Vitamin A		Carotenoids		Metaplasia score	CSFP H_2O mm of saline	Papill-edema	Pituitary cysts
	Plasma μg/100 ml	Liver μg/100 g	Plasma μg/100 ml	Liver μg/100 g				
15	5.8	7.8	12.0	22.0	16.8	241.0	14/15	6/15
150	26.3	682.0	63.0	86.0	2.8	78.3	3/15	0/15

The remaining 3 had pressures of 140, 155, and 185 mm which represent the upper limit of pressures in normal calves taken in the same manner, which have a range 42 to 154, with an average 78 mm (*Calhoun et al.* 1965). An elevated CSFP was the first detectable, functional change in the deficient group.

The mean pressure for the deficient group was 241 mm, 3 times the average of the control calves (Tables 4 and 5).

b. *Eye*

Intraocular pressures (IOP), measured by manometer (direct) and electric tonometer (indirect) differed only little between the marginally deficient and control calves; as previously reported by *Woelfel et al.* (1964), the 2 methods of measurement did not correlate well and there was considerable calf-to-calf variation (coefficient of variation (CV) 36 % for tonometry and 42 % for manometry). The mean IOP's were in close agreement with previous values recorded for calves, 15.6 mm mercury (range 10—24) from birth to 3 months of age and 22.6 mm (range 13—30) from 3 to 18 months of age. Papilledema was detected clinically in some of the depleted calves. At necropsy, the optic papilla was swollen and edematous, appearing as a greyish protrusion 1.5 mm at its highest elevation and of varying extent,

Table 4. Pathology of 15 male Holstein-Friesian calves receiving 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks.

Calf no.	CSFP mm saline	Parotid duct levels 1 2 3 4 5 6	Metaplasia score	Papill-edema (mm)	Pituitary cysts (mm)*
D1306	260	+ + + + + +	21.0	8×5	10.0×9.0*
D1312	365	+ + + + + +	21.0	7×5	5.0×0.25
D1314	230	+ + + + + +	21.0	4×5	5.0×1.0
D1324	155	+ + + + + +	21.0	6×4	7.0×3.0*
D1328	255	+ + + + + +	21.0	5×4	5.0×0.25
D1303	220	+ + + + ± +	18.5	2×2	4.5×0.5
D1309	140	+ + + + ± +	18.5	5×3	7.5×0.75
D1311	205	+ + + + ± ±	15.5	7×5	6.0×1.0
D1300	270	+ + + + + —	15.0	8×5	9.0×2.5*
D1317	185	+ + + + + —	15.0	nil	9.0×1.0
D1326	265	+ ± + + ± ±	14.5	7×4	9.0×6.0*
D1318	308	+ + + ± ± ±	13.5	5×3	3.5×0.5
D1321	205	+ + + + — ±	13.0	9×7	9.0×0.5
D1304	295	+ + + + ± —	12.5	7×4	8.0×3.0*
D1322	260	+ + ± + ± —	11.0	6×5	6.0×2.0*

* denotes true cysts (larger than 2 mm in width).

Table 5. Pathology of 15 male Holstein-Friesian calves receiving 150 μg of carotene per 0.45 kg of live-weight daily for 17 weeks.

Calf no.	CSFP mm saline	Parotid duct levels 1 2 3 4 5 6	Metaplasia score	Papill-edema (mm)	Pituitary cysts (mm)
D1301	154	+ ± — + — —	6.0	nil	6.0×0.25
D1315	70	+ ± + — — —	5.0	nil	5.0×1.0
D1323	88	+ ± + — — —	5.0	nil	3.0×0.5
D1302	90	+ — ± ± — —	4.5	nil	4.5×0.5
D1319	75	+ + — — — —	3.0	nil	3.5×0.2
D1305	52	± ± ± — — —	3.0	4×2	7.0×1.0
D1313	42	± — — ± — —	2.5	nil	9.0×1.5
D1307	92	+ ± — — — —	2.0	nil	6.0×0.7
D1308	65	+ ± — — — —	2.0	nil	4.5×0.5
D1310	65	+ ± — — — —	2.0	nil	5.0×0.5
D1316	82	+ ± — — — —	2.0	5×2	6.0×0.5
D1327	122	+ ± — — — —	2.0	nil	4.5×0.5
D1320	50	± ± — — — —	1.5	4×2	6.0×1.0
D1325	78	+ — — — — —	1.0	nil	7.5×1.0
D1329	65	± — — — — —	0.5	nil	5.0×0.3

from 2.0 × 2.0 to 9.0 × 7.0 mm (Fig. 2). A slight central depression was noted in a few calves. Papilledema was absent in only 1 calf (D1317) in the deficient group. Stenosis of the optic foramen with resultant pinching of the enclosed optic nerve was not found. The degree of papilledema in an affected calf was of equal severity in both eyes. Moderate papilledema was found in 3 control calves (D1305, D1316, D1320) but was not associated with elevated CSFP.

In the normal retina, it was found upon histological examination that different layers were continuous into the optic papilla (Fig. 3a). In affected eyes, a swollen papilla was observed, protruding into the vitreous humor; interstitial edema separated nerve bundles of the optic tract and there was some lateral swelling, causing minute peripapillary detachments of the retina (Fig. 3b).

Edematous fluid infiltrated the converging optic nerve fibers for a considerable distance lateral to the optic disc. Other layers of the retina were affected, especially the outer and inner nuclear and plexiform layers. Nuclear layers had marked separation of cells. Folding of the retina was found in a few calves, associated with deposits of fibrin; this was interpreted as a consequence of retinal edema rather than faulty fixation techniques.

Layers of the edematous retina did not converge into the disc but gradually faded out toward the papilla. The disappearance of various layers was in accordance with their anatomical arrangement from interior to exterior, the ganglionic cell layer affected

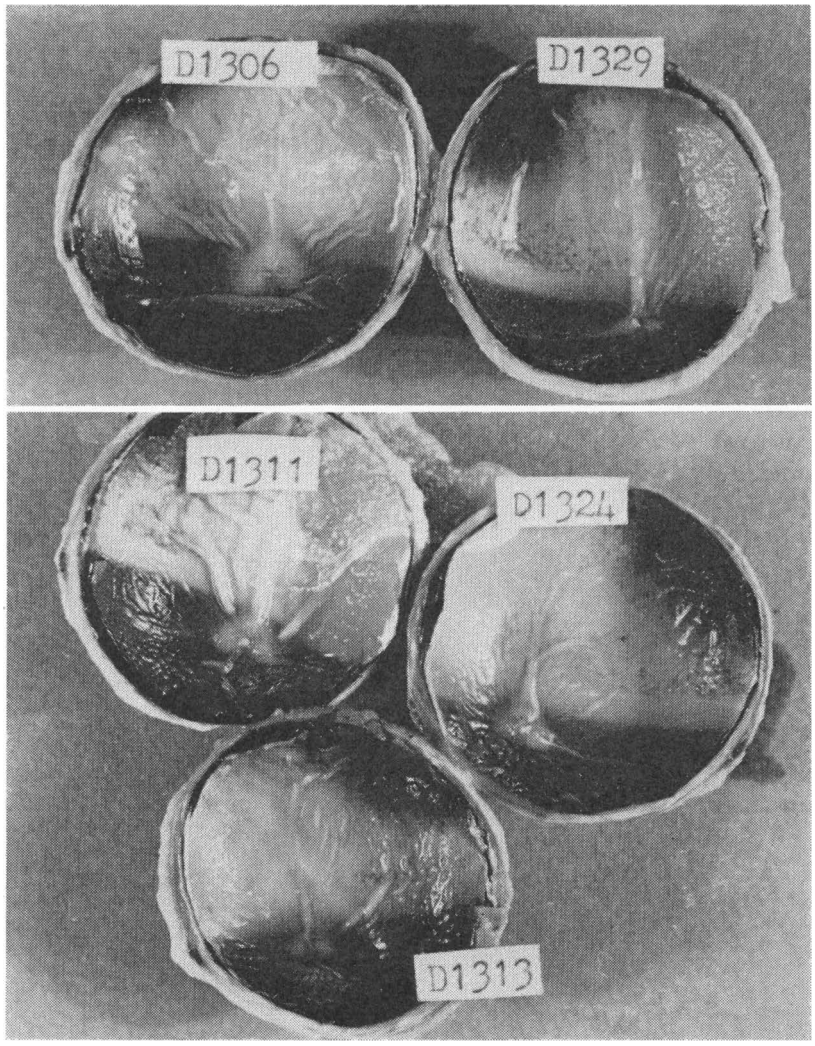
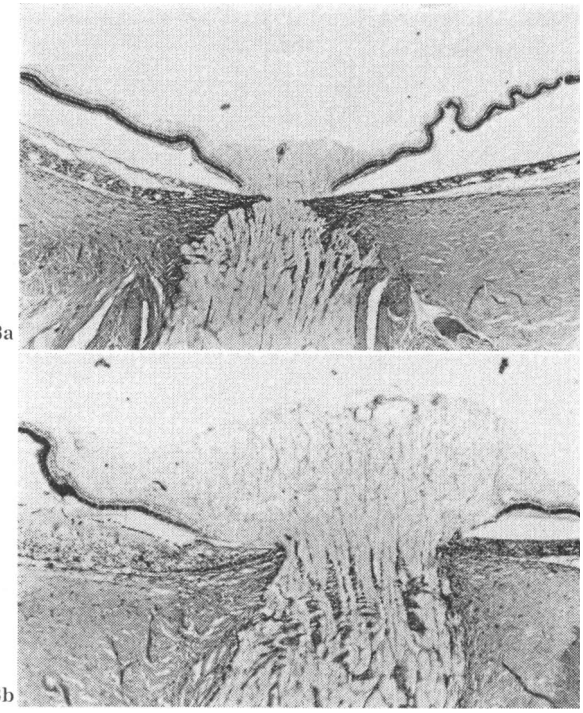


Figure 2. Fixed ocular fundi from 5 different calves demonstrating presence or absence of papilledema in vitamin A deficiency. Calves D1306, D1311, and D1324 received 15 μg of carotene per 0.45 kg of live-weight per day for 17 weeks. Calves D1313 and D1329 received 150 μg of carotene for an identical time interval. Papilledema is present in the deficient calves.



3a

3b

Figure 3a. Section through optic nerve, sclera, and optic papilla of a normal calf (D1313) that was fed 150 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. H & E stain; × 15.

Figure 3b. Section through same plane as Fig. 3a of a deficient calf (D1311) that was fed 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. The optic papilla and nerve are markedly swollen and the former protrudes into the posterior chamber of the eye. H & E stain; × 15.

most often. This finding was related to, first, the presence of, and second, the degree of papilledema since the fading of the different layers, although in the same sequence, was less marked in calves with less vitreous protrusion.

c. *Pituitary*

A narrow slit-like cleft occurs normally in the bovine pituitary gland, separating the pars distalis from the pars intermedia and pars nervosa (*Jubb & McEntee* 1955; *Trautman & Fiebiger* 1957). Dilatation of the residual hypophyseal cleft was considered present when it measured 2 mm or wider, regardless of length or volume. Six of the 15 deficient calves had enlarged clefts whereas

smaller slit-shaped spaces were found in other deficient calves (Table 4). The largest cyst (D1306) measured 1.25 ml in volume (Fig. 4). No true cysts were found in the control calves.

Histologically, the cleft was either void of stainable fluid content or had a homogeneous pink, proteinaceous material. Where there was distortion of the normal architecture due to the larger cysts (D1306, D1326), lining cells were flattened, particularly those proximal to the pars intermedia. The pars intermedia was separated widely from the pars distalis and, as a result of cystic pressure, cells of the pars distalis and pars intermedia lost their acinar orientation and became ovoid and flattened, especially around the terminal portions of the cleft.

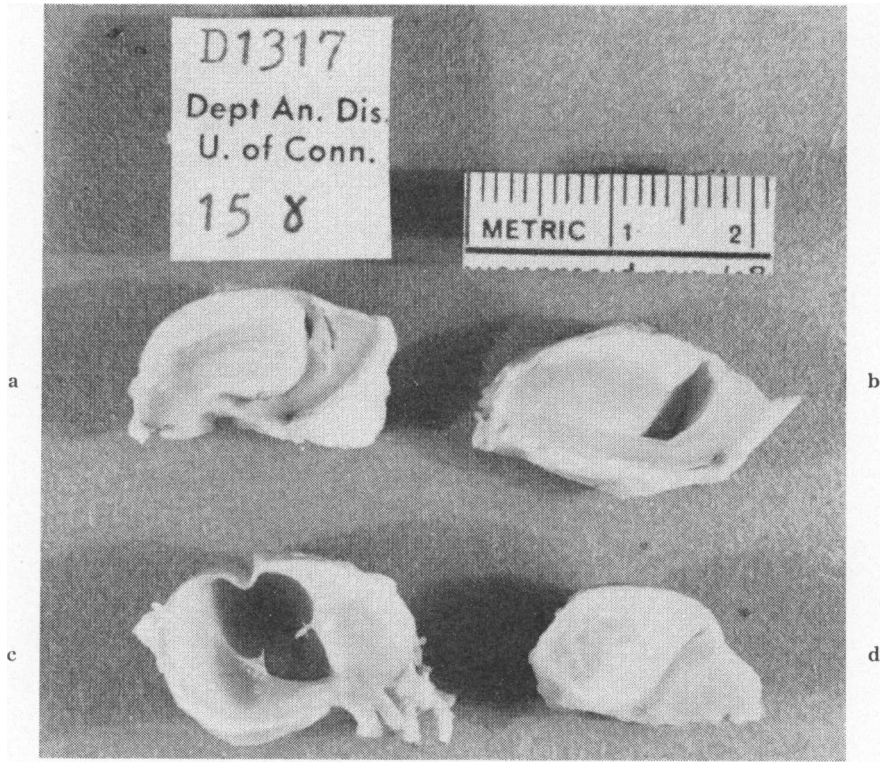


Figure 4 a, b, c, d. Sagittal sections of the pituitary gland. (a) Calf D1317, fed 15 μg of carotene per 0.45 kg of live-weight daily; the cleft measured 9.0 × 1.0 mm and was not considered a true hypophyseal cyst. (b) Calf D1324 and (c) calf D1306; both were fed 15 μg of carotene, the clefts measured 7.0 × 3.0 and 10.0 × 9.0 mm respectively, and were true hypophyseal cysts. (d) Calf D1316, was fed 150 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. The cleft measured 6.0 × 0.5 mm and is normal.

d. *Parotid gland*

Squamous metaplasia was found at all 6 levels of the parotid duct system in 5 of the 15 deficient calves and a metaplasia score of 21 was assigned (Table 4). Metaplasia was found sporadically throughout Stensen's duct in the remaining 10 calves in this group with squamous cells and dysplasia present in the entire perimeter of the main duct. The basal cell layer stained deeply basophilic, which disappeared towards the surface. The cells became eosinophilic and were replaced by flattened, keratinized

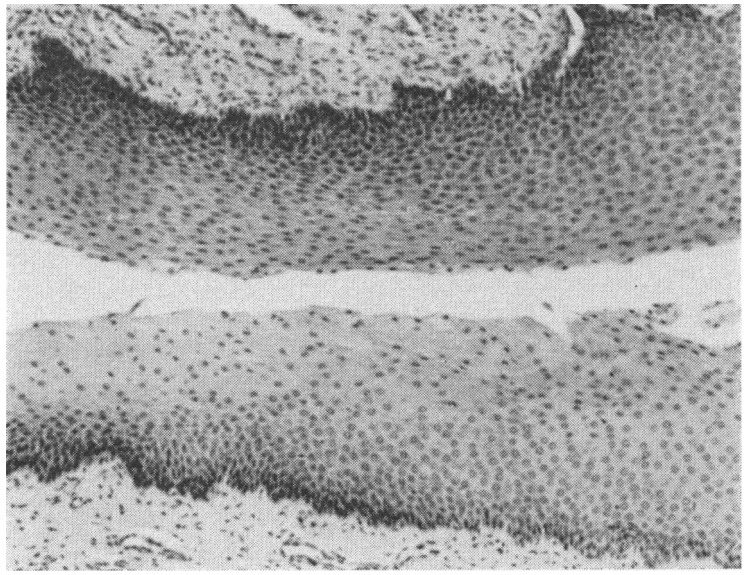


Figure 5. Squamous metaplasia in Stensen's duct of the parotid salivary gland at level 3 from a 6-month-old Holstein-Friesian calf (D1303) that was given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. There is complete loss of goblet cells and replacement by flattened squamous cells with keratinization at the mucosal surface. H & E stain; × 150.

cells at the surface (Fig. 5). Intercellular bridges were prominent in deeper layers. Metaplasia was advanced to such an extent that normal, pseudostratified columnar cells were not found in the main duct. Two deficient calves (D1312, D1314) had a sialoductitis, with a lymphocytic inflammatory reaction affecting both the mucosa and lamina propria (Fig. 6).

Squamous metaplasia affected the interlobular ducts at level 4 in the gland proper of all 15 calves receiving the carotene de-

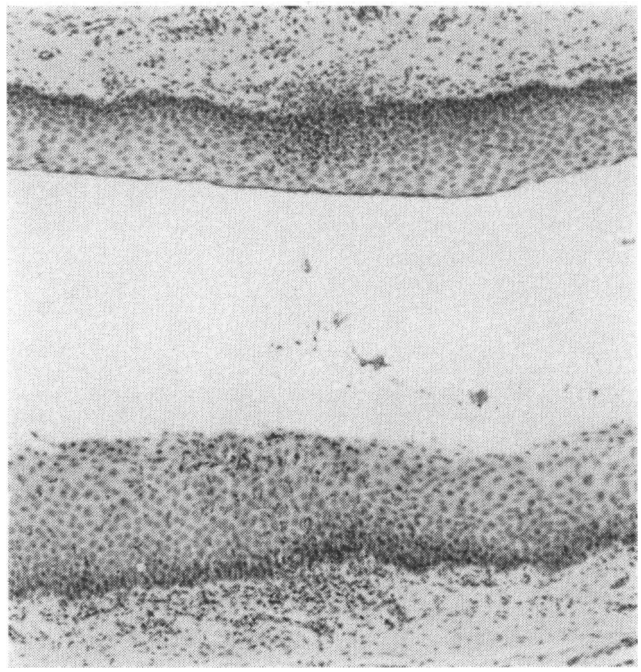


Figure 6. Squamous metaplasia and lymphocytic sialoductilitis in Stensen's duct (level 2) of deficient calf (D1312) given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. H & E stain; × 125.

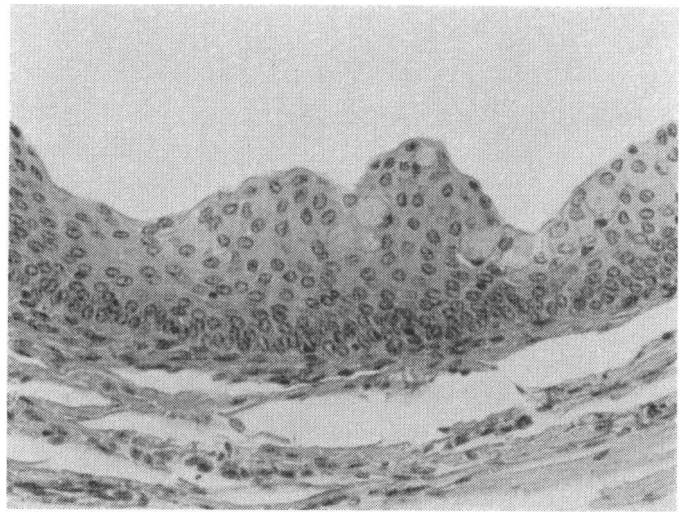


Figure 7. Section from level 4, calf D1303 given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. A few mucin-filled goblet cells remain in the uppermost part of the mucosa with squamous cells present beneath. H & E stain; × 250.

ficient ration. The entire perimeter of 1 or several lumina was involved, with complete loss of the mucin-staining cells found normally in interlobular ducts.

Epithelial dysplasia was found in 14 of 15 deficient calves at level 5 and in 11 animals at level 6 (Table 4). Metaplasia affected the entire perimeter of the interlobular ducts, with loss of mucin and PAS-positive material in the superficial layers of the mucosa. Squamous cells appeared beneath the sloughing secretory epithelium (Fig. 7).

When only a part of the ductal perimeter was affected, the section was recorded as moderate metaplasia (±), and such a duct usually had loss of undulations and mucin-staining cells with desquamation of the overlying secretory epithelium (Fig. 8). Commonly, a duct having moderate metaplasia was found adjacent to a normal duct of a smaller diameter (Fig. 9).

The PAS reaction was dramatic in demonstrating subtle changes. Goblet cells surrounding a normal duct were of a dark burgundy color and of equal distribution about the entire ductal perimeter.

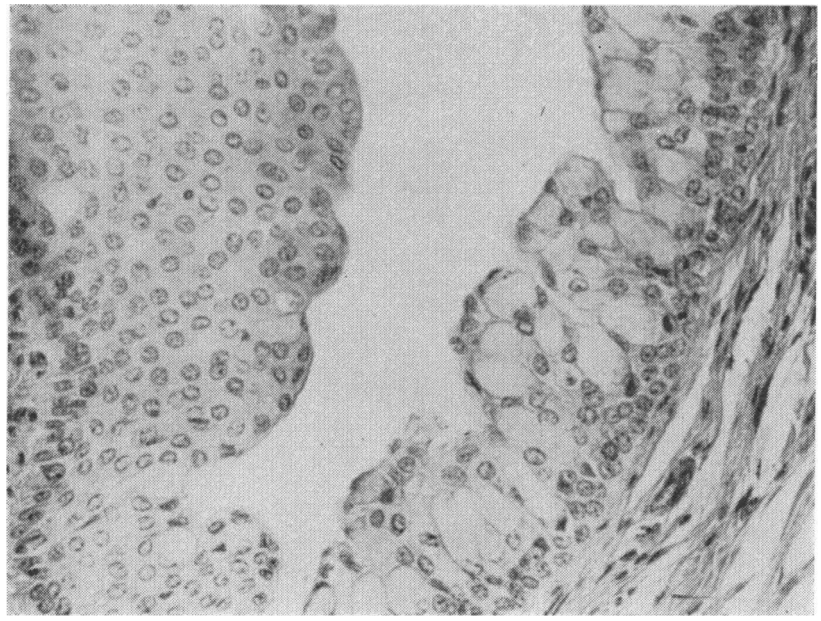


Figure 8. Level 5 of calf D1324, given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. Squamous metaplasia is present on the left with normal mucosa on the right. H & E stain; × 250.

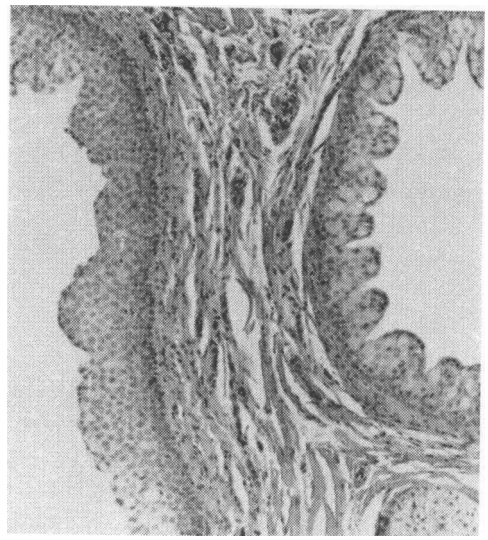


Figure 9. Level 6 of same calf as Fig. 8. Squamous metaplasia affects the mucosa of the interlobular duct on the left while the duct on the right is normal. H & E stain; × 100.

Calves receiving 150 μg carotene per 0.45 kg live-weight had occasional placques of metaplasia, which were restricted to Stensen's duct. The normal histology of level 1 was found consistently and only 1 of 15 calves had definite squamous metaplasia at level 2; 10 calves had very small keratin pearl formation and these were recorded as suggestive (±). Definite metaplasia was found in 2 calves at levels 3 (D1315, D1323; Table 5). At level 4, 1 calf (D1301) had positive (+) and 2 animals, moderate epithelial dysplasia (±). Normal interlobular ducts prevailed at levels 5 and 6 in the control calves.

Metaplasia scores for the deficient group varied from a low of 11 to the maximum, 21. The mean score was calculated to be 16.8; the median, 15.5. Metaplasia scores for the control group ranged from 0.5 to 6.0 with an average of 3.0; the median was 2.0.

e. *Lung*

Interstitial pneumonia was found in both deficient and control calves. There was moderate thickening of alveolar septums with proliferation of alveolar epithelial cells and histiocytic infiltrations. Very few polymorphonuclear leukocytes were found. Marked lymphoid peribronchiolitis involving both large and small

air passages was found in a few calves. One deficient calf (D1312) had a suppurative bronchopneumonia of lobular distribution; the inflammatory reaction consisted of marked congestion and edema, profuse polymorphonuclear infiltration and bronchial debris.

f. *Skin*

The 4 skin sections from each calf were examined for histological evidence of hyperkeratotic changes affecting the epidermis, sebaceous and sweat glands of hair follicles. The eyelid of 1 deficient calf (D1300) had pronounced keratin pearl formation in the hair follicles but the tarsal gland was not affected. Keratin pearl formation was found in hair follicles of the eyelid in other deficient calves (D1303, D1322, D1324). Hyperkeratotic changes were not found in other deficient or control calves.

g. *Rumen and reticulum*

Many deficient calves had marked hyperplasia of the stratum germinativum with invasion of the underlying lamina propria in ruminal sections. The keratin covering was uniformly thickened and deeply eosinophilic; desquamating keratin debris was common and an occasional keratin pearl was found (Fig. 10). Hyper-

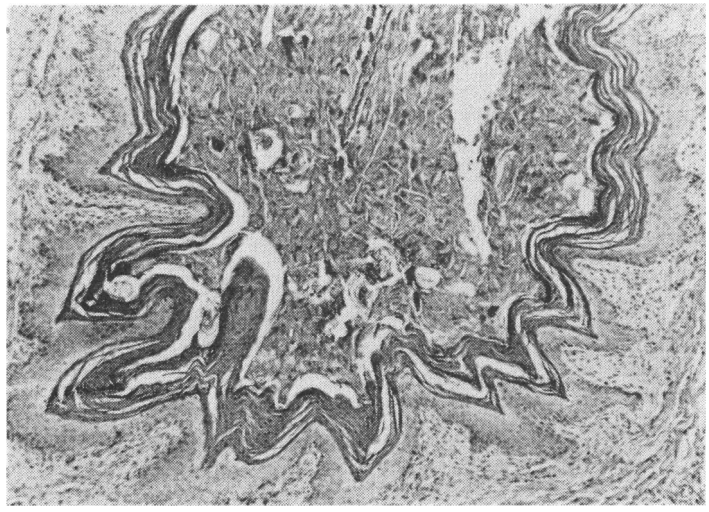


Figure 10. Ruminal mucosa from calf D1306, given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. Hyperkeratosis is present, with some hyperactivity of the stratum germinativum. H & E stain; × 75.

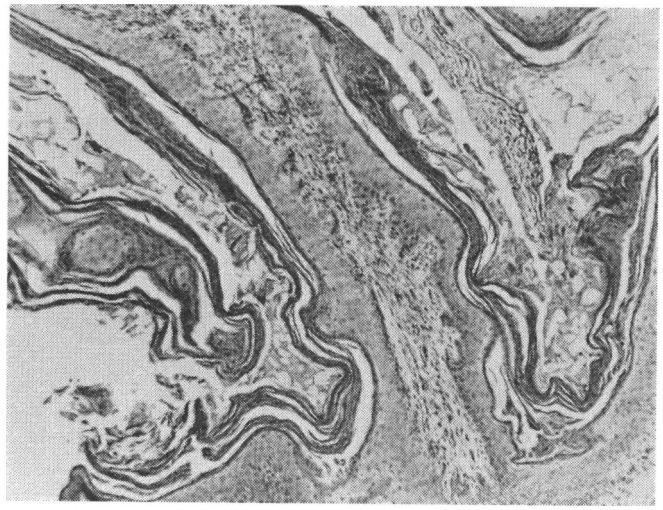


Figure 11. Villous fold of the reticulum from the same calf as Fig. 10. Hyperkeratinization is present. H & E stain; × 75.

keratinization and basal cell activity was less pronounced in ruminal sections of control calves.

Hyperplasia of the stratum germinativum was less pronounced in sections of reticulum but the degree of hyperkeratosis and amount of desquamating debris were often greater in deficient calves (Fig. 11). Lymphocytic foci and keratin pearls were present in both groups, but pronounced keratinization and acanthosis were associated with high metaplasia scores.

h. *Prepuce*

The penile-preputial fold had an increased keratinization and keratin pearl formation in most deficient calves. Cysts were visible grossly, measured up to 3 or 4 mm in diameter, and contents varied from mature keratin to keratin debris. The stratum germinativum was hyperactive, strongly basophilic and had palisading nuclei. Several large cysts were present in 4 of the 5 calves having a metaplasia score of 21 but is was difficult to relate size to metaplasia scores. Two deficient calves (D1311, D1326) had a lymphocytic balano-posthitis with infiltration of the lamina and purulent debris in the penile-preputial fold (Fig. 12). A few small cysts were found in control calves.

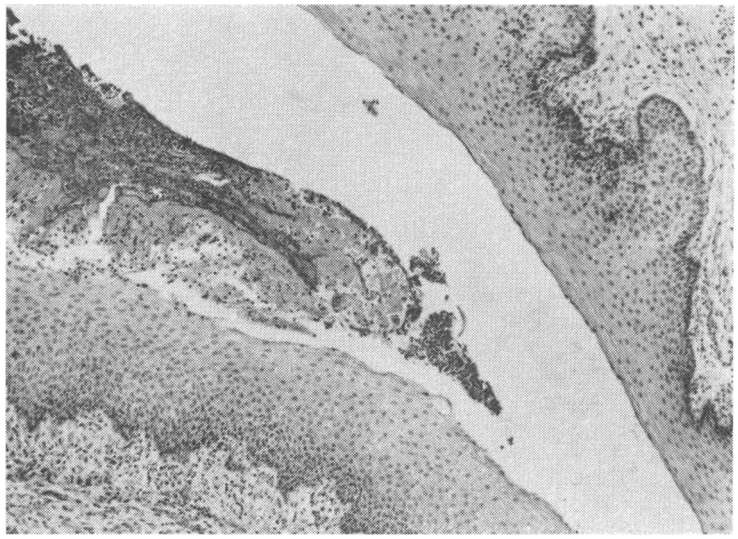


Figure 12. Section of junction of the penile and preputial folds of calf D1326, given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. There is a lymphocytic balano-posthitis and the penile-preputial fold contains purulent debris. H & E stain; × 100.

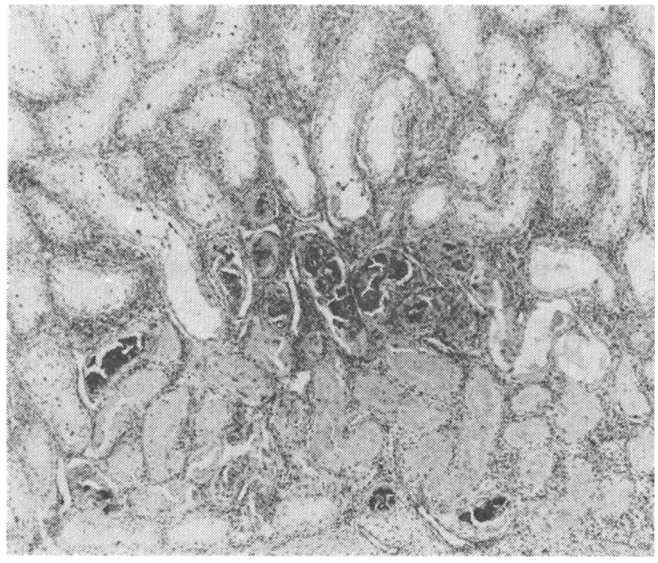


Figure 13. Testicle of calf D1314 given 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. The organ contained several focal areas of tubular necrosis and mineralization. H & E stain; × 75.

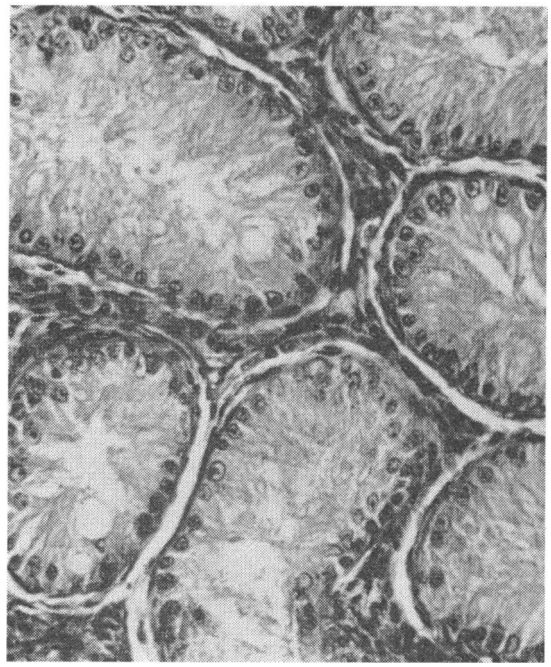


Figure 14. Seminiferous tubules of a calf (D1306) fed 15 μg of carotene per 0.45 kg of live-weight daily for 17 weeks. There is no evidence of spermatogenesis. This calf also had a true hypophyseal cyst. H & E stain; × 250.

i. *Testes*

Because the calves were approaching puberty at time of euthanasia, evaluation of testicular morphological alterations was equivocal. However, spermatozoa were rarely seen free in the tubular lumen and none was seen in the epididymis. Focal areas of tubular necrosis and mineralization, primarily restricted to the periphery of the organ, were found in 2 deficient calves (D1312, D1314; Fig. 13), and the testes from 2 calves (D1306, D1326) were markedly immature, the germinal cells present interpreted as primary spermatogonia (Fig. 14).

j. *Other organs*

Alterations were not observed in the pancreatic duct as it empties into the duodenum at the duct of Wirsung. The width of the adrenal cortex varied from 1.5 to 2.4 mm regardless of carotene intake for the 2 groups, with an average of 1.90 mm. However, the mean of the deficient group was greater than the control calves, 1.93 mm to 1.88 mm, respectively.

DISCUSSION

In a recent review on the minimum carotene requirements for Holstein male calves, an elevated CSFP was cited as the first clinically detectable sign, proportional to the degree of deficiency and occurring within the narrow range of daily intake of 30—33 μg of carotene per 0.45 kg of live-weight (*Eaton et al.* 1964a). More recently *Eaton et al.* (1964b) determined that above intakes of 48 μg of carotene per 0.45 kg of live-weight the CSFP remains normal, 75 mm of saline (99 % confidence limits, 36 to 157 mm); values approaching 200 mm or over are, respectively, markedly suggestive or indicative of hypovitaminosis A.

In 12 of the 15 calves CSFP's were elevated above 200 mm saline; other calves had pressures in the upper range of normal while the pressures of the entire control group were within normal limits. Metaplasia scores correlated well with elevated CSFP's and the 5 calves with metaplasia affecting the entire parotid duct system had a mean CSFP of 253 mm (Table 4). Worth noting is that 1 control calf (D1301) had a CSFP of 154 mm of saline (the upper limit of normal) and a metaplasia score of 6.0.

It has been assumed that all neurological manifestations of vitamin A deficiency were sequel to faulty osteogenesis, the consequence of which was constriction and herniation of central and peripheral nerves. However, in adult cattle, where skeletal growth was terminated, elevated CSFP still occurs and is associated with clinical papilledema, incoordination and syncope. It seems therefore likely that increased CSFP is due to faulty absorption and/or increased rate of formation of CSF. Recently, *Calhoun et al.* (1967) have demonstrated an impaired bulk absorption of CSF in experimental bovine vitamin A deficiency.

A daily carotene intake of 24 to 36 μg per 0.45 kg of live-weight is required to prevent the development of papilledema (*Eaton et al.* 1964a) which was detected in 14 of the 15 depleted calves. With 1 exception, the 7 calves having the highest CSFP's also had the greatest area of papilledema. Five of the 6 hypophyseal cysts were found in these same 7 calves. Metaplasia scores did not correlate to the 2 former parameters; the 5 calves (D1306, D1312, D1314, D1324, D1328) having metaplasia scores of 21 had CSFP's ranging from the highest to the second lowest; all had marked papilledema but only 2 of these animals (D1306, D1324) had pituitary cysts (Table 4). In fact, 4 of the 6 calves with pituitary cysts had metaplasia scores below the mean for the deficient

group. These findings support our previous conjecture that alterations in tissue fluids, notably CSF, may result from a different pathogenetic mechanism than does the transition to squamous metaplasia that occurs in epithelia (*Nielsen et al.* 1966b). The different deficiency intake (24 μg of carotene per 0.45 kg of live-weight required to prevent parotid duct metaplasia from that intake necessary to prevent papilledema and CSFP (24—36 μg of carotene) suggests that this may be the case. The obvious question then is why is the parotid duct system more prone to squamous transition than other mucosal surfaces, such as the urogenital system which does not undergo squamous metaplasia even after 24 weeks on a ration providing 10 μg of carotene per 0.45 kg of live-weight (*Nielsen et al.* 1966b).

In both natural and experimentally produced vitamin A deficiency, numerous ocular manifestations are described, including papilledema and blindness, the 2 changes most commonly reported. In severe deficiency, nyctalopia develops early, when daily carotene intake is below 11 μg per 0.45 kg of live-weight but the onset of total blindness is sudden and unexplained. Stenosis of the optic foraminae has been demonstrated in calves; others consider that blindness is due to degenerative optic or retinal changes resulting from papilledema. In this study, diffusing edema produced tapering of retinal layers near the optic papilla; onset of blindness may be the response to severe retinal edema with resultant failure of conversion of light into electrical energy.

Many severely deficient animals have an exophthalmos and some, a distinct coning protuberance of the cornea. Intraocular pressures of these animals have been recorded infrequently and published less so. These ocular changes are unexplained but assumed to be associated with an elevated CSFP and accompanied by an increased retinal venous pressure which, in turn, might affect intraocular pressure (*Davson* 1956). The deficient calves in this study had a mean CSFP 3 times the average of the control calves, but IOP values were not altered significantly. Measurements of totally depleted animals might be more revealing.

The development of true hypophyseal cysts has been documented in vitamin A deficiency (*Moore et al.* 1948); occurrence is limited to the young animal but cysts persist into adulthood. No evidence has been accumulated as to over-all effects of cystic distention on the pars distalis or pars intermedia. In vitamin A-deficient calves, there is a reduction in growth and, if immature,

spermatogenesis may not develop (*Hodgson et al.* 1946), suggesting subtle effects of pressure atrophy in some marginally deficient animals.

Of the 6 hypophyseal cysts found, 2 were quite large in area and volume (D1306, D1326); there was loss of acinar orientation and pressure necrosis of cells, particularly near the lumen of the cleft. Both calves had markedly elevated CSFP's and pronounced papilledema. In all 6 calves with true hypophyseal cysts, the arithmetic mean of the degree of papilledema and the CSFP was above the group mean for all the depleted calves (Table 6).

Table 6. Comparison of measurements of 6 calves with residual hypophyseal cysts with the remaining 9 calves in the deficient (15 μg) group and the control (150 μg) group.

(All values are arithemetic means).

	CSFP (mm)	Papilledema (mm^2)	Increase in wt. (kg/lb)	Inc. in withers ht. (cm/in)	Inc. in paunch girth (cm/in)	Inc. in heart girth (cm/in)	Ad. cort. width (mm)
6 calves with pituitary clefts — 15 μg group —	251	31.7	85/188	17.3/6.8	38.6/15.2	27.9/11.0	2.07
9 calves, 15 μg, no pituitary cleft	235	23.0	90/200	18.5/7.3	37.8/14.9	29.7/11.7	1.84
Entire 15 calves in 15 μg group	241	28.3	88/195	18.0/7.1	38.4/15.1	29.0/11.4	1.93
15 calves — 150 μg group —	78	1.7	92/204	18.5/7.3	39.1/15.4	29.0/12.4	1.88

Testicles from the 2 calves (D1306, D1326) had no evidence of morphological development. The other 4 calves had little or no suggestion of spermatogenesis while the remaining 9 calves of the deficient group had a moderate degree of spermatogenesis.

Effects of hypovitaminosis A on growth rate are manifested when the daily deficient ration is between 11 and 33 μg of carotene per 0.45 kg of live-weight (*Eaton et al.* 1964a). The average increase in weight, increase in height at withers, and increase in heart girth of the 6 animals having true pituitary cysts was less than the arithmetric mean for the entire 15 deficient calves and these same 6 calves caused the greatest deviation of the average measurements of the deficient calves from the control group (Table 6). The corresponding values for the remaining 9 deficient

calves approached or were equal to the means of the control group. Comparison of averages for CSFP and papilledema of the 6 calves with hypophyseal cysts to the entire deficient group averages or these same 6 calves to the averages of the remaining 9 calves in the deficient group indicated marked differences in various measurements of the former group (with hypophyseal cysts) to the other animals, whether deficient or controls. The average width of the adrenal cortex of the deficient calves was 1.93 mm, compared to 1.88 mm for the control group. Average thickness of adrenal cortex of the 6 calves with hypophyseal cysts was 2.07 mm, markedly greater than the average for the 9 remaining deficient calves or the control group.

The foregoing does not imply a cause and effect relationship of the cystic pituitary to other pathological and biochemical lesions. All changes, including the hypophyseal cysts, may well be varied manifestations of the deficiency, pressure effects of the cyst not a direct cause of other changes.

Marked differences in plasma and hepatic carotenoid and vitamin A levels were found between the deficient and control calves (Tables 1 and 2). Group averages of the deficient calves indicated a state of marked hypovitaminosis A. The 5 calves with metaplasia scores of 21 had plasma and liver carotenoid and vitamin A concentration below the means for the deficient group (Table 1). Averages of the biochemical measurements of the 8 deficient calves with a metaplasia score below the mean (16.8) of the deficient group were equal to or higher than the averages for the entire deficient group. Plasma and liver vitamin A and carotenoid levels thus correlated very well with the metaplasia scores but do not predict necessarily the presence of elevated CSFP, papilledema, or hypophyseal cysts.

Differences in concentration between deficient and control calves, or liver vitamin A (100 times less) and liver carotenoids (4 times less) are in accordance with previous findings that hepatic vitamin A levels are very sensitive to changes in carotene and/or vitamin A intake (*Teichman et al.* 1957).

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SUMMARY

At 53 days of age, 30 male Holstein-Friesian calves were divided into 2 equal groups and fed for a 17 week period a ration containing either 15 μg (deficient) or 150 μg (control) of carotene per 0.45 kg of live-weight per day.

Twelve of the 15 deficient calves had an elevated cerebrospinal fluid pressure (CSFP), above 200 mm of saline. The mean CSFP of the deficient group was 3 times that of the control group. Fourteen of the 15 deficient calves had varying degrees of papilledema and 6 had hypophyseal cysts. The main parotid duct (Stensen's duct) was affected by squamous metaplasia, in all the deficient calves.

The mean plasma carotenoid and vitamin A concentrations for the control calves were 5 times higher than the deficient animals. Liver carotenoid and vitamin A concentrations were markedly lower in the deficient calves and correlated well with elevated CSFP, papilledema and parotid duct metaplasia.

ZUSAMMENFASSUNG

Experimentelle Untersuchungen über pathologisch-anatomische Veränderungen bei Kälbern mit einer täglichen Karotinzufuhr von einem Drittel des Bedarfs.

Dreissig schwarzbunte Bullkälber (Holsteinisch-friesische), alle 53 Tage alt, wurden in zwei Gruppen mit je 15 Kälbern geteilt. In der einen Gruppe (der Versuchsgruppe) bekamen die Tiere 33 und in der anderen Gruppe (der Kontrollgruppe) 333 μg Karotin pro kg Lebendgewicht pro Tag in einer Periode, die sich über 17 Wochen erstreckte.

Bei 12 von den Tieren in der Versuchsgruppe stieg der Druck der Cerebrospinalflüssigkeit auf 200 mm Wassersäule. Der Mitteldruck der ganzen Gruppe war um dreimal höher als in der Kontrollgruppe. Die Plasmakonzentration von Karotin und Vitamin A war um fünfmal höher in der Kontrollgruppe. Bei 14 der Tiere in der Versuchsgruppe wurde ein Ödem in der optischen Papille nachgewiesen, 6 Tiere hatten Zysten in der Hypophyse und bei allen 15 wurde eine Verhornung des Epithels im Ductus parotideus festgestellt.

SAMMENDRAG

Eksperimentelle undersøgelser over patologisk anatomiske forandringer hos kalve med en daglig karotintilførsel på en trediedel af behovet.

Tredive sortbrogede tyrekalve (Holstein-Friesian), der alle var 53 dage gamle, blev delt i 2 grupper med 15 i hver. I den ene gruppe (forsøgsgruppen) tilførtes dyrene 33 og i den anden gruppe (kontrolgruppen) 333 μg karotin pr. kg legemsvægt pr. dag i en periode af 17 uger.

Hos 12 af dyrene i forsøgsgruppen steg cerebrospinalvæsketrykket til 200 mm vandsøjle, og middeltrykket for hele gruppen var 3 gange højere end i kontrolgruppen. Plasmakoncentrationen af karotin og vitamin A var 5 gange højere i kontrolgruppen. Hos 14 af dyrene i forsøgsgruppen påvistes ødem i den optiske papil, 6 havde cyster i hypofysen, og hos dem alle 15 fandtes forhorning af epithelet i ductus parotideus.

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