PAROTID GLAND LESIONS IN EXPERIMENTAL BOVINE
VITAMIN A DEFICIENCY

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The study was concerned with clinical, biochemical and pathologic manifestations of vitamin A deficiency in young dairy bulls which had been maintained on a diet low in carotene but otherwise of sufficient caloric value to permit normal growth. The experiments were terminated after an average period of 105 days, when convulsive symptoms were well established. The aim was to elucidate the basic lesions in rapidly developing, uncomplicated A-hypovitaminosis.

The pathology of bovine vitamin A deficiency of different intensity and duration has been studied by a number of workers, but the reports vary with respect to the significance and specificity of the lesions observed.

Ocular changes simulating infectious keratitis were found frequently by Hart and Guilbert (9) under natural conditions ascribed (10) to pinching of the optic nerve by a sphenoidal stenosis. Blindness without observable lesions was shown by Wetzel and Moore (22) to be due to edema of the optic papilla, resulting from increased cerebrospinal fluid pressure, according to Moore and Sykes (18).

The seminiferous tubules of young bulls were found, by Guilbert and Hart (7), to exhibit structural changes, a fact confirmed in detailed studies of Hodgson et al. (11), Erb et al. (5) and Bratton et al. (2).

Nephritic changes interpreted as parenchymatous nephritis were found to be associated with fatally terminating spontaneous cases in the experience of Hart (8). The corresponding experimental lesions were characterized by Langham et al. (13) as degenerative in the form of hydropic and necrobiotic alterations in the proximal portions of the nephron and as inflammatory in the form of cellular infiltrations and proliferations in the interstices. There was occasional metaplasia with rare hyperkeratinization of the transitional epithelium of the minor calices and the ureters. In a similar study Thorp et al. (21) confirmed these findings and reported only 2 of 25 animals as showing metaplasia in the calices.

The pituitary has been found to present cystic degeneration by Moore (16) or increased fluid between the anterior and posterior lobe by Sutton et al. (20). The latter authors also found an increase of "alpha" cells (acidophils) and believed the change to be similar to that in A-deficient rats, although their original studies on this species (19) showed an increase of "beta" cells (basophils). The cellular changes are interpreted as compensatory to the testicular degeneration, paralleling the so-called castration effect. On the basis of 10,000 slaughter-

Received for publication April 23, 1950.
ing house specimens, Madsen et al. (15) considered cystic pituitaries in young cattle as a pathologic expression of \( \Delta \) deficiency.

Anasarca or edema of subcutis and adjacent musculature has been described by Creech and Seibold (3) and was used as a criterion by Madsen and Earle (14) in characterizing "old corn" disease as vitamin \( \Delta \) deficiency.

Pneumonic lesions were frequently observed by Hart (8) in natural, fatal cases and occasionally under experimental conditions by Thorp et al. (21). The latter authors also reported mild hyperplastic lesions in the small intestine and necrobiotic changes of like intensity in the liver.

On the whole, it may be seen that the now universally recognized basic lesion of \( \Delta \)-hypovitaminosis, namely squamous metaplasia with varying degrees of hyperkeratinization (23), has been reported in the kidney only and even there as a distinctly minor alteration.

### TABLE 1

**Age, hemoglobin and carotene and vitamin \( \Delta \) liver storage of experimental animals**

<table>
<thead>
<tr>
<th>No.</th>
<th>Breed</th>
<th>Start</th>
<th>Finish</th>
<th>Difference</th>
<th>Start</th>
<th>Finish</th>
<th>Carotene</th>
<th>Vitamin</th>
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<tr>
<td></td>
<td></td>
<td>(g./100 ml.)</td>
<td>(g.)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>Jersey</td>
<td>339</td>
<td>403</td>
<td>64</td>
<td>11.5</td>
<td>11.5</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>Guernsey</td>
<td>53</td>
<td>134</td>
<td>81</td>
<td>8.5</td>
<td>8.4</td>
<td>0.1</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>Guernsey</td>
<td>210</td>
<td>312</td>
<td>102</td>
<td>8.5</td>
<td>10.5</td>
<td>0.3</td>
<td>0.1</td>
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<tr>
<td>4</td>
<td>Av.</td>
<td>201</td>
<td>283</td>
<td>82</td>
<td>9.5</td>
<td>10.1</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>5</td>
<td>Control Guernsey</td>
<td>208</td>
<td>310</td>
<td>102</td>
<td>9.4</td>
<td>10.7</td>
<td>0.4</td>
<td>25.7</td>
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<tr>
<td>6</td>
<td>Guernsey</td>
<td>240</td>
<td>375</td>
<td>135</td>
<td>10.0</td>
<td>11.4</td>
<td>0.8</td>
<td>8.2</td>
</tr>
<tr>
<td>7</td>
<td>Ayrshire</td>
<td>139</td>
<td>274</td>
<td>135</td>
<td>9.8</td>
<td>10.9</td>
<td>0.4</td>
<td>3.4</td>
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<tr>
<td>8</td>
<td>Holstein</td>
<td>148</td>
<td>231</td>
<td>183</td>
<td>10.4</td>
<td>10.1</td>
<td>0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>9</td>
<td>Holstein</td>
<td>176</td>
<td>293</td>
<td>118</td>
<td>10.1</td>
<td>10.8</td>
<td>0.5</td>
<td>3.9</td>
</tr>
<tr>
<td>10</td>
<td>Control</td>
<td>171</td>
<td>306</td>
<td>135</td>
<td>9.9</td>
<td>10.4</td>
<td>0.6</td>
<td>103.0</td>
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<tr>
<td>Av.</td>
<td>Deficient</td>
<td>188</td>
<td>288</td>
<td>100</td>
<td>9.7</td>
<td>10.7</td>
<td>0.5</td>
<td>2.0</td>
</tr>
<tr>
<td>Av.</td>
<td>Controls</td>
<td>189</td>
<td>308</td>
<td>119</td>
<td>9.7</td>
<td>10.6</td>
<td>0.5</td>
<td>64.4</td>
</tr>
<tr>
<td>Av.</td>
<td>Totals</td>
<td>189</td>
<td>293</td>
<td>105</td>
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</tr>
</tbody>
</table>

* Freemartin.

In a recent comprehensive treatise on the pathology of nutritional diseases, Follis (6) emphasized the importance of differentiating between specific and nonspecific damage due to deficiency of a single nutrient.

**MATERIALS AND METHODS**

The experiments were conducted on two groups of four bull calves each, except for one freemartin, representing four standard dairy breeds (one Jersey, four Guernseys, one Ayrshire, two Holsteins). In average terms, the first group, aged 204 days, was treated for 92 days during the summer of 1948, and the second group, aged 173 days, was treated for 126 days during the spring of 1949. The details are presented in table 1.
During treatment, each animal received a daily allowance of 4 lb. of grain mixture containing less than 350 μg of carotene per lb. and beet pulp ad libitum. One control calf in each group received a daily supplement of 100,000 I.U. vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. Clinical observations were made daily, and hemoglobin, plasma carotene and vitamin A content were determined weekly. Spinal fluid pressure readings according to Moore (17) and liver biopsies for histologic study were obtained approximately once per month. All of the animals were sacrificed when they showed daily convulsions, except for one which died on the 64th experimental day. The livers were frozen for later carotene and vitamin A determinations and the tissues subjected to thorough gross and microscopic examination.

**CLINICOPATHOLOGIC RESULTS**

Symptoms of spasmodic convulsions became manifest in the animal which later died, after about 45 days on experiment, in the others after about 75 days. The spasms increased in frequency until they occurred three to four times every day and were accentuated by sexual excitement. Bloat and diarrhea occurred occasionally. Some animals manifested impaired eyesight and exophthalmus.

The average clinicopathologic data for six treated and two control animals were as follows:

**Hemoglobin.** Expressed in grams per 100 ml. both the treated and control groups averaged 9.7 at the beginning of the experiment and 10.7 versus 10.6 at the end. There was no significant difference between groups, but all of the hemoglobin values increased slightly during the course of the experiments. The details are presented in table 1.

**Spinal fluid pressure.** Expressed in millimeters of water, the average values obtained in the first experiment were 307 in the treated group as against 322 in the control group at the start and 260 in the treated against 100 in the control group at the end. Later experiences showed that these values probably had been exaggerated by excitement. In the second experiment, the measurements averaged 107 for the treated against 91 for the control groups at the start and 190 versus 155 at the end.

The total averages were 207 for both the treated and control groups at the start and 225 for the treated versus 128 for the control groups at the end. There was a relative increase in spinal fluid pressure in the deficient group as compared with the control group, in accordance with the literature (18).

**Liver biopsies.** Narrow cylinders of hepatic tissue, obtained with an instrument designed for human prostatic biopsy, were fixed in Zenker's fluid, formol-saline and absolute alcohol, respectively. Special stains were applied to bring out cellular detail, neutral fat and glycogen. In general, both the deficient and the control groups failed to show any uniform structural changes or fatty meta-

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1 Grain mixture: Ground barley, 419.5; crimped oats, 500; wheat bran, 500; linseed oil meal (solvent process), 150; soybean oil meal (solvent process), 150; molasses, 200; 500-potency B-Y dried fermentation solubles, 40; steamed bone meal, 20; salt, 20; irradiated yeast (Standard Brand, type 9-F, 9,000 I.U. vitamin D per gram), 0.5; total, 2,000 lb.
morphosis. There was fair-to-good glycogen storage throughout the course of the experiments.

_Blood plasma carotene._ Expressed in micrograms per 100 ml. the treated groups averaged 184 and the controls 132 at the beginning and 8 _versus_ 9 at the end. Thus, there was no significant difference between groups. Both the deficient and the supplemented animals showed an approximately equal regression of plasma carotene under the conditions of these experiments. The details are presented in figure 1.

_Blood plasma vitamin A._ Expressed like carotene, the treated groups averaged 22.6 and the controls 22 at the start and 3.6 _versus_ 42.5 at the end. Thus,
the deficient animals exhibited a marked decrease and the supplemented animals a corresponding increase in plasma vitamin A levels (fig. 1).

**Final liver storage.** Expressed in micrograms per gram of liver, carotene in both the treated and control groups averaged 0.5 thereby failing to show differences due to treatment, in line with the corresponding plasma values.

Vitamin A, on the other hand, averaged 2.0 in the treated group, as against 64.4 in the control group. There was, therefore, a significantly higher storage in the supplemented groups in comparison with the deficient ones, as was to be
expected from the corresponding plasma values. The details are presented in table 1.

**PATHOLOGIC RESULTS**

On gross examination, animal no. 1 (table 1) which died presented significant hepatic changes in the form of multiple poppy-seed sized yellowish areas, which were interpreted as focal necrosis.

Histopathologically, animal no. 2 showed focal necrosis (fig. 2), portal cirrhosis (fig. 3) of the liver and early exudative pneumonia (fig. 4). Animal no. 6 showed focal interstitial nephritis. Brain sections often showed perivascular and perineuronal edema, so-called lamina cribosa. Because of their irregular occurrence, these lesions were considered as due to mild intercurrent diseases, not necessarily associated with treatment.

Microscopic changes of probable significance were found in the pituitary and the thyroid. The pituitary, especially the anterior lobe, has been stated in the literature to show both cystic (15) and cellular changes (20). In the present material, microcysts were found in both the treated and the control groups and, therefore, not accorded significance. The differential cellular picture, as presented in Bouin’s fixed Masson’s trichrome preparations, showed in the controls massive ribbon-like accumulations of acidophils in the periphery, leaving a narrow central area composed primarily of chromophobes and basophils (fig. 6). The principal differences in the treated animals were an apparent reduction of both chromatic cellular elements and a consequent predominance of chromophobes. In some sections from affected animals it was impossible to demonstrate any appreciable number of basophils (fig. 7). Although these numerical differences were based on estimates and not differential counts, they were contrary to expectations from the literature (19, 20) and suggested that this subject requires reinvestigation.

The thyroid of treated animals showed mild hyperplasia, while control animals presented more or less uniformly sized and well filled follicles lined by low cuboidal epithelium (fig. 8). Treated animals exhibited many small follicles with high cuboidal or nearly columnar epithelium which had a tendency to encroach upon the lumen (fig. 9). Other follicles varied widely in size and contained colloid with markedly scalloped margins. Hyperplasia of the thyroid

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**Fig. 2.** Liver of no. 2. Peripheral necrosis—Karyolysis of liver cells around interlobular vein.

**Fig. 3.** Liver of no. 2. Portal cirrhosis—Marked increase of connective tissues in portal island accompanied by proliferation of bile ducts.

**Fig. 4.** Lung of no. 2. Exudative pneumonia—Polynuclear and mononuclear cells in alveoli, bronchiol and alveolar ducts (high center).

**Fig. 5.** Kidney of no. 6. Interstitial nephritis—An atrophied glomerulus in low center surrounded by interstitial round cell infiltration.

**Fig. 6.** Anterior pituitary of no. 8. Normal—Massive cords of acidophiles (dark) separated by narrow cords of basophiles and chromophobes (light).

**Fig. 7.** Anterior pituitary of no. 5. Vitamin A deficiency—Broad bands of chromophobes (light) and islands of acidophiles (dark). Many microcysts.

All figures are photomicrographs of paraffin sections stained with hematoxylin-tricosin, 80×. The numbers refer to the experimental animals listed in table 1.
in vitamin A deficiency may be compensatory to increased stress upon this organ, which is known to have an important function in the conversion of carotene to vitamin A (4).

Specific microscopic changes were observed in the testes and the parotid gland. The testicular changes, which have been reported frequently in the literature (2, 5, 7, 11), consisted in the present material of various degrees of retardation in spermatogenesis. In the most advanced cases, the seminiferous epithelium in certain tubules was extremely cell-poor, with only a few Sertoli
cells near the basement membrane. In most instances spermatogenesis had not progressed beyond the spermatogonial stage. There were only isolated primary and secondary spermatocytes, but there was no evidence of any orderly progressive maturation. However, the abnormalities often were confined to certain selected tubuli with adjacent ones appearing almost normal (fig. 10).

The parotid gland, which, as far as the authors are aware, has not been mentioned in the literature, proved to be the only organ that regularly showed pathognomonic changes of vitamin A deficiency.

The parotid as the largest, chiefly serous salivary gland has a complex duct system which terminates in the oral cavity (Stenson’s duct) opposite the second upper molar. The serous alveoli drain into prominent intralobular and intercalated ducts which are lined by a single layer of columnar cells with centrally located nuclei. Where the ducts reach the interlobular connective tissue septa, the epithelium changes to a pseudostratified columnar epithelium with the nuclei in two or more layers (fig. 11) and maintains this architecture to its termination (1).

In vitamin A deficiency the specific changes were confined to the interlobular ducts of both small and large diameter. There the normally columnar epithelium in some of these ducts had changed to squamous epithelium (fig. 12) accompanied occasionally by hyperkeratinization. The pathologic epithelium was markedly hyperplastic and built up in irregular layers. The germinal layers were relatively rich in mitotic figures with the cytoplasm of some hypertrophied prickle cells occasionally containing round bodies, suggestive of dyskeratotic degeneration. The innermost surface cells not infrequently formed loops or bridges over vacuolar spaces (fig. 13) presumably containing retained secretion. Cross sections of affected interlobular ducts showed the narrowing effect of the pathologic process on the ductal patency and obviously suggested a pathogenetic relationship between stenosis of the parotid duct and vitamin A deficiency. On the whole, the lesions reflected the squamous metaplasia considered to be the basic lesion of vitamin A deficiency in mammals and birds.

Apparently the parotid gland in the bovine is one of the organs of predilec-

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**Fig. 8.** Thyroid gland of no. 4. Normal—Large, moderately filled follicles, lined by low cuboidal epithelium. Slightly hypoplastic state.

**Fig. 9.** Thyroid gland of no. 7. Vitamin A deficiency—Small follicles lined by high cuboidal to columnar epithelium, sometimes obliterating lumen. Colloid has scalloped margins and stains deeply. Hyperplastic state.

**Fig. 10.** Testis of no. 1. Vitamin A deficiency—Cessation of spermatogenesis in two lateral seminiferous tubules (uniformly gray), other tubules normal.

**Fig. 11.** Parotid gland of 6-week-old calf affected with pulmonary abscesses, caused by *Sphorophorus necrophorus*. Normal—Peripheral alveolar tissue; central H-like interlobular connective tissue with normal interlobular ducts lined by two-layered pseudostratified columnar epithelium.

**Fig. 12.** Parotid gland of no 1. Vitamin A deficiency—Interlobular connective tissue with large interlobular duct lined by irregularly built-up metaplastic squamous epithelium. Stratum corneum is nucleated (parakeratotic).

**Fig. 13.** Parotid gland of no. 7. Vitamin A deficiency—Alveolar tissue in upper third. Large interlobular duct in center with thickened wall and advanced squamous metaplasia of lining epithelium showing interepithelial bridges and microcysts.
tion for exhibiting specific lesions of vitamin A deficiency, in distinction from chickens where the corresponding locus seems to be in the mucocutaneous junction of the nasal septum (12).

SUMMARY

Two groups of four dairy bull calves, averaging 189 days in age were fed a grain mixture containing less than 350 γ per lb. of carotene and beet pulp *ad libitum* for about 105 days. One control calf in each group received a daily supplement of 100,000 I.U. of vitamin A from dogfish oil containing 25 per cent crude soybean lecithin. In weekly determinations, the hemoglobin values showed no significant changes for treated and supplemented animals, plasma carotene regressed in both groups, while plasma vitamin A was markedly higher in the supplemented animals. The same relationship was reflected in the final liver storage of carotene and vitamin A. Monthly readings of spinal fluid pressure indicated a relative rise in the treated animals, while simultaneous liver biopsies failed to manifest changes in glycogen and fat storage.

Pathologic studies of the animals killed after convulsions occurred daily failed to show consistent gross lesions. Irregularly occurring microscopic lesions of focal necrosis and/or cirrhosis in the liver, pneumonia and mild interstitial nephritis suggested intercurrent diseases. Consistent changes were found in the anterior pituitary showing a decrease in the chromatic cells and in the thyroid showing mild hyperplasia. The testes manifested retarded spermatogenesis in some seminiferous tubuli. The parotid gland showed a high incidence of specific squamous metaplasia in the interlobular ducts.

The parotid gland appears to be especially prone to exhibit specific histopathologic alterations of A-hypovitaminosis and is the only organ so far ascertained that lends itself to specific morphologic diagnosis of vitamin A deficiency in the ox.

REFERENCES


