SOME BIOCHEMICAL CONSTITUENTS IN SERUM, CEREBROSPINAL FLUID, AND AQUEOUS HUMOR OF VITAMIN A DEFICIENT HOLSTEIN CALVES 1

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SUMMARY

Sixteen male Holstein calves previously raised to 63 days of age on a limited whole milk, limited starter, and ad libitum chopped alfalfa hay ration were fed a vitamin A depletion ration until their plasma vitamin A averaged 8.3 ± 2.2 γ per 100 ml. Each calf was then fed for a 16-wk. period one of four carotene intakes, 16 or 24 γ per pound of live weight per day, to provide marked increases in cerebrospinal fluid pressure, 32 γ, to provide slight elevation in pressure, or 40 γ, to provide no elevation. Terminal cerebrospinal fluid pressure decreased with increasing intakes of carotene, apparent intrascleral pressure exhibited no consistent change, and plasma and liver concentrations of carotenoids and vitamin A increased. Of the biochemical constituents studied, no trends with carotene intake were observed for serum, and only potassium of cerebrospinal fluid decreased slightly; whereas, for aqueous humor, sodium was higher in the calves fed the 16-, 24-, and 32-γ carotene intakes, potassium increased with an increase in carotene intake, chloride decreased, and osmotic pressure was greater in calves fed the 16-, 24-, and 32-γ carotene intakes than in those fed the 40-γ intake. These results do not directly explain the increased cerebrospinal fluid pressure or change in aqueous humor composition occurring in vitamin A deficiency. However, the data pertaining to cerebrospinal fluid suggest either an overproduction or underabsorption of fluid (without change in the concentration of the constituents in serum or cerebrospinal fluid), resulting in greater volume within the restricted subarachnoid space and ventricles of the central nervous system.

The specific metabolic function of vitamin A at the molecular level, aside from its role in vision, has so far eluded the efforts of many investigators, T. Moore (32). Although definite changes in certain epithelial tissues, bones, and teeth are observed in avitaminosis A, Follis (19), little is known as to the role of vitamin A in preventing or alleviating these changes. Since the initial studies of L. A. Moore and J. F. Sykes (30, 31), it has become increasingly evident that the first measurable change which occurs in experimentally produced vitamin A deficiency in the calf (33), chick (40), rabbit (28), and pig (38) is an increase in the cerebrospinal fluid pressure, rather than the classical changes cited above.

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Davson (10) has stated that both cerebrospinal fluid and aqueous humor are formed by mechanisms that have a great deal in common. He also gave evidence of the positive association between cerebrospinal fluid pressure and retinal venous pressure, and discussed the possible elevation of intracocular pressure due to increased venous retinal pressure. At the initiation of this project in the spring of 1958, no reports were found in the literature by the authors of intracocular pressure measurements on vitamin A deficient animals. However, such had been suggested by Moore and Sykes (30), as well as by McLaren (26). Subsequently, in a pilot study (12) at this station with four pairs of calves, the vitamin A deficient calves of each pair were observed to average 3.4 mm. of Hg greater in intracocular pressure, measured in the scleral region, than the vitamin A adequate calves.

The cause(s) of increased cerebrospinal fluid pressure in vitamin A deficient animals is not known. One possible explanation is that the increased pressure is due to faulty and excessive bone growth, particularly of the skull bones which results in constriction of and eventual severing of the optic nerve (6). However, this does not seem tenable, for in mature cows fed a ration deficient in vitamin A (29) papilledema is present, which is preceded or accompanied by increased cerebrospinal fluid pressure, but not blindness due to constriction of the optic nerve. Also, the rather rapid decrease in cerebrospinal fluid pressures in vitamin A deficient animals (21, 27, 38), upon subsequent administration of vitamin A, strongly suggests that bone growth per se is not a causative factor of the increased pressure. Another explanation is that the increased cerebrospinal fluid pressure is due to an overproduction of the fluid by the choroid plexus, Millen and Dickson (27), and/or an obstruction at the sites of absorption of the fluid, both causing greater volume of fluid in the relatively restricted subarachnoid space and ventricles of the central nervous system. However, little has been accomplished in vigorously testing these hypotheses, because data with respect to the measurement of formation and absorption of cerebrospinal fluid are extremely difficult to interpret (35, 41).

Overproduction and/or underabsorption of cerebrospinal fluid as discussed above does not necessitate a concomitant change(s) in the concentration of constituents of cerebrospinal fluid or in blood. If there is sufficient change in the concentration of the constituents of blood such that its osmotic pressure decreases, then it would be anticipated that this change would be accompanied by increased cerebrospinal fluid pressure, Davson (10), due to the transfer of water across the blood-cerebrospinal barrier. While the present authors were unable to find literature with respect to both blood and cerebrospinal fluid composition of vitamin A deficient animals, some reports have been made with respect to blood. Moore and Sykes (31) in 1941 stated that "colloidal osmotic pressure measurements of blood plasma and various blood and urine analysis have not shown any abnormality which could be related to the raised pressure." Madsen and Earle (25), in an extensive study of field and experimental cases of hypovitaminosis A of beef cattle, reported in 1947 decreases in plasma albumin concentration, increases in total plasma globulin due primarily to marked increases in plasma.
fibrinogen, and slight decreases in serum calcium and inorganic phosphorous. Calculations indicated that the decrease in the colloid osmotic pressure of the plasma due to the decrease in albumin was largely offset by the increase in the other protein fractions. More recently, Erwin et al. (16) found a decreased percentage of albumin in vitamin A deficiency of steers and in one pair of identical-twin Holstein heifers (17) which was largely alleviated upon administration of carotene in the first study and vitamin A in the second. In the aforementioned pilot study conducted at this station (12), slight increases in total serum protein attributable primarily to the gamma-globulin fraction, were observed in the deficient calves. Also, cerebrospinal fluid total protein increased, but inappreciable change occurred in concentrations of sodium, potassium, inorganic phosphorous and calcium and osmotic pressure as determined by cryoscopy.

The primary objective of the present study was to determine several biochemical constituents in serum, cerebrospinal fluid, and aqueous humor of Holstein calves fed graded levels of carotene so as to produce marked, slight, and no increases in cerebrospinal pressure; secondly, to determine the possible effects of varying carotene intakes on intraocular pressure and, finally, to ascertain possible association between physiological changes and changes in biochemical constituents.

EXPERIMENTAL PROCEDURE

Animals and feeding. Sixteen, one-day-old male Holstein calves obtained during the period August-October, 1958, from various state institution herds, were brought to the research barn and placed in individual tie stalls. Upon arrival each calf received orally 1–500 mg. of chlortetracycline (Lederle’s Aureomycin) and one gelatin capsule containing 100,000 U.S.P. units of vitamin A (Nopcey “250”, Type M containing 250,000 U.S.P. units per gram). If the calf had not nursed, it received 4.0 lb. of colostrum from a frozen colostrum bank for two successive feedings. Each calf was then raised to approximately its 63rd day of age on a limited whole milk, limited vitamin A depletion grain, and ad libitum chopped alfalfa hay ration as previously described (15), except that artificially dehydrated alfalfa was added to the depletion grain at the rate of 5 lb. to 100 lb. of depletion grain so as to provide sufficient dietary carotene.

On approximately the 64th day of age, each calf received a vitamin A depletion ration, two-thirds depletion grain and one-third dried beet pulp, such that an increase in live weight of 10 lb. per week would be anticipated. When the blood plasma vitamin A concentration, based on blood samples taken at successive seven-day periods, had decreased to equal to or less than 12.0 γ per 100 ml., each calf was fed, after the next seven-day period, one of four carotene intakes, 16, 24, 32, or 40 γ per pound of live weight per day from artificially dehydrated alfalfa meal. The 16- and 24-γ levels of carotene intake were chosen on the basis of previous data (33) to provide insufficient carotene resulting in increases in cerebrospinal fluid pressure, 32 γ to supply slightly above borderline intake resulting in inappreciable increases in cerebrospinal fluid pressure, and 40 γ to provide ample carotene intake. Assignment of the calves to their respective carotene intakes was random in blocks of four calves each, with the first four...
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3 to arrive at the research barn comprising the first block, and so forth. Average age at the beginning of the carotene supplementation period was 78 days, with a standard deviation of four. Upon completion of 16 consecutive seven-day periods of supplementation with carotene, each calf was slaughtered.

Procedures followed in calculation of alfalfa to be fed to furnish the indicated carotene intake, mixing of the alfalfa into the vitamin A depletion ration, and treatment for scour were identical to those previously reported (33). Average daily minimum and maximum temperatures during carotene supplementation were 56.1 and 62.6°F., with their respective standard errors 0.2 and 0.2. Average artificial light intensity to which the calves were exposed from 6 A.M. to 6 P.M. daily, during supplementation with carotene and measured approximately at a height of 4 ft. in the center of each stall, was 7.9 ± 0.7 foot-candles.

Observations. With the exception of the artificially dehydrated alfalfa meal, all feeds fed and refused were weighed to the nearest 0.1 lb. Artificially dehydrated alfalfa was weighed to the nearest 0.1 g. Live weights were taken at successive seven-day periods throughout the experiment and linear growth measurements at the beginning and termination of supplementation with carotene.

Thirty-milliliter blood samples were obtained by jugular puncture at successive seven-day periods during vitamin A depletion, the period immediately prior to carotene supplementation, and during 28-day periods thereafter for plasma carotenoid and vitamin A analyses. On the fourth day prior to slaughter, 60 ml. of blood was withdrawn for preparation of serum for subsequent analyses for protein and minerals and for measurement of osmotic pressure by cryoscopy.

Cerebrospinal fluid pressures, in millimeters of saline, were measured ten days prior to slaughter by puncture of the subarachnoid space through the dorsal opening in the atlanto-occipital articulation, with an 18-gauge, 90-mm. length (to the base of the hub) spinal (Quincke) stainless steel needle. The needle was connected to a saline (0.85 g. NaCl/100 ml.) manometer by polyethylene tubing, both 2.0-mm. bore, and the pressures measured according to procedures as outlined by Sykes and Moore (39). Three days prior to slaughter another cerebrospinal puncture was made and approximately 40 to 60 ml. of fluid was removed with a glass syringe for subsequent analyses.

Apparent intraocular pressures four days prior to carotene supplementation and five days prior to slaughter were recorded by restraining the calf on a table. The eyelids were then held open and a topical anaesthetic, approximately 1 ml. of a 1.0% solution of cocaine hydrochloride, applied directly on the eyeball. An electronic tonometer (Model OP-9037, V. Mueller and Co.) with a 5.5-gram plunger in the tonometer head was used to record the intraocular pressure. All readings were made in the scleral region adjacent to the cornea.

Electrocardiograms were taken the week prior to slaughter after clipping both forelegs and the left hind leg, above the knees and hock, respectively, and placing the calf in a wood metabolism crate. The three standard limb leads (14) were secured after electrode paste had been applied. The calf was allowed to remain in the crate, with electrodes attached, until calm, and the electrocardiogram was then recorded, using a Sanborn Viso-Cardiette, Model 51.
At slaughter, both eyes were immediately removed, taken to the laboratory, and the aqueous humor obtained by puncture of the cornea with a scalpel, and the fluid from both combined for immediate analyses for protein, minerals, and osmotic pressure measurements. The eyes were fixed in formalin and subsequently examined for the presence or absence of papillary edema. The liver was removed, ground, sampled, and the samples were held at 0 ° F. for subsequent carotenoid and vitamin A analyses.

**Analyses.** Samples of all feeds fed were analyzed for proximate constituents at 4-wk. periods by A.O.A.C. methods (4), Table 1. The artificially dehydrated alfalfa meal was analyzed for carotene periodically during the course of the experiment by the A.O.A.C. procedure (4), which employed extraction overnight at room temperature instead of Soxhlet extraction.

Carotenoids and vitamin A concentrations in the plasma were determined by the Kimble procedure (23) and in the liver by a modification of the Gallup-Hoefer method (8).

Protein fractionation of serum and cerebrospinal fluid was carried out with a Spinco (Model R, Series D) paper electrophoresis system, using a barbiturate buffer consisting of 2.76 g. diethylbarbituric acid, 15.45 g. sodium barbital, and 2.0 ml. "Sterox" per 1,000 ml. (13), pH 8.6, and ionic strength 0.075, and employing a current of 5 ma. for 16 hr., all procedures essentially as described by Block, Durrum, and Zweig (7). The rapid bromphenol blue procedure (7) was used in dyeing the proteins and a Spinco Analytrol (Model RB) was used in quantitation. Prior to protein fractionation of cerebrospinal fluid, between 15 and 30 ml. were dialyzed against polyvinylpyrrolidone (Plasdone, commercial, General Aniline and Film Corp., N. Y.) as described by Lemmen et al. (24). Upon completion of dialysis, the bottom portion of the dialysis sack was washed with 250 μl. of the barbiturate buffer and 12 to 20 μl. of this applied to the filter paper strips. The amount applied to the filter paper strips was determined by consideration of the total protein concentration of cerebrospinal fluid and of serum, so that approximately equivalent amounts of protein from both sources were used in their respective electrophoretic separations. Total protein concentration of serum, cerebrospinal fluid, and aqueous humor was determined by the biuret reaction, Gornall et al. (20).

The calcium content of serum and cerebrospinal fluid was determined by flame photometry (11), the inorganic phosphorous concentration of serum, cerebrospinal fluid, and aqueous humor determined by the Fiske and Subbarow method (18), and sodium and potassium contents of the same three body fluids by flame photometry (2). Chloride contents were determined colorimetrically by the procedures of Barney and Bertolacini (5).

Osmotic pressure of serum, cerebrospinal fluid, and aqueous humor was determined, using a Model D, Fiske Osmometer (3). By definition, one milliosmol is equivalent to a freezing point depression of 0.00186 ° C.

Statistical procedures as set forth by Cochran and Cox (9) and Snedecor (37) were used. The analysis of variance consisted of isolating variability due to blocks of calves, among carotene intakes and remainder (error). Prior to the
<table>
<thead>
<tr>
<th></th>
<th>Per cent dry matter</th>
<th>Crude protein</th>
<th>Ether extract</th>
<th>Crude fiber</th>
<th>N.F.E.</th>
<th>Ash</th>
<th>Carotene (mg/lb)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alfalfa hay chopped</strong></td>
<td>90.6% ± 1.0*</td>
<td>19.4% ± 1.8</td>
<td>2.6% ± 0.3</td>
<td>32.2% ± 2.5</td>
<td>38.1% ± 1.4</td>
<td>7.7% ± 0.7</td>
<td>3.7 mg/lb</td>
</tr>
<tr>
<td><strong>Depletion grain</strong></td>
<td>89.8% ± 0.7</td>
<td>19.7% ± 0.7</td>
<td>4.4% ± 0.2</td>
<td>7.5% ± 0.2</td>
<td>61.7% ± 0.7</td>
<td>6.7% ± 0.2</td>
<td>0.8 mg/lb</td>
</tr>
<tr>
<td><strong>Vitamin A depletion ration</strong></td>
<td>90.6% ± 0.5</td>
<td>16.2% ± 0.4</td>
<td>2.7% ± 0.3</td>
<td>19.7% ± 0.3</td>
<td>39.8% ± 0.4</td>
<td>12.2% ± 0.3</td>
<td>98.5 mg/lb</td>
</tr>
<tr>
<td><strong>Artificially dehydrated alfalfa meal</strong></td>
<td>90.8% ± 1.0</td>
<td>24.0% ± 0.5</td>
<td>4.3% ± 0.2</td>
<td>19.7% ± 0.3</td>
<td>39.8% ± 0.8</td>
<td>12.2% ± 0.1</td>
<td>98.5 mg/lb</td>
</tr>
</tbody>
</table>

* Standard error of mean.
start of the experiment, the among carotene intakes variability was further subdivided into two sets of single degree of freedom orthogonal contrasts as follows: linear, quadratic, and cubic trends of response on carotene intake, between the 40-\( \gamma \) intake and the 16- plus 24- plus 32-\( \gamma \) intakes, and the linear and quadratic responses on the 16-, 24-, and 32-\( \gamma \) carotene intakes. The reasoning which led to these comparisons was that a trend in the responses might exist across all carotene intakes or, possibly, exist only in the three lowest carotene intake responses in which slight to markedly increased cerebrospinal fluid pressures were anticipated, based on past experience (33). The transformation of the liver vitamin A concentrations to logarithms and the functional form of the responses of both plasma vitamin A and the logarithm of liver vitamin A concentrations on log carotene intake have been dealt with previously (34). A physical injury resulted in the inability of one calf, in the fourth block fed the 16-\( \gamma \) carotene intake, to adduct its left hind limb and subsequently caused atrophy of the muscles of the affected limb. Another calf, in the fourth block fed the 40-\( \gamma \) carotene intake, threw and injured itself while a cerebrospinal puncture was made. Subsequent punctures resulted in only cloudy cerebrospinal fluid being obtained. In the case of the first-mentioned calf, missing values were calculated (9) for all criteria and in the case of the second, cerebrospinal fluid and aqueous humor constituents criteria.

RESULTS

Feed consumption, growth, and health. In general, calves readily consumed their vitamin A depletion ration allowance, but there was a slight tendency for increased feed refusals with decreasing carotene intakes from the 40- to the 16-\( \gamma \) level. This was evidenced by a significant \( (P < 0.05) \) negative linear trend in the transformed percentage of per cent days consuming vitamin A depletion ration allowance values, as presented in Table 2.

Growth of the calves was inappreciably affected by carotene intake. The average values prior to carotene supplementation were for live weight 184 lb., height at withers 34.2 in., heart girth 36.7 in., and girth of paunch 43.5 in., with their respective standard deviations being 16, 0.7, 1.2, and 1.8. Increases during the 16-wk. carotene supplementation period with standard deviations were, respectively, live weight 227 and 8, height at withers 7.4 and 0.9, heart girth 12.3 and 0.9, and girth of paunch 15.6 and 2.3.

There were no particular health problems encountered during the course of the experiment, with the exception of the two calves previously mentioned in the last portion of the Experimental section. Per cent days free from scours during carotene supplementation averaged 99.7. These percentage values showed no trend with carotene intake which was confirmed upon analysis of variance of the per cent days free from scours after applying the arc sin transformation. Since the occurrence of scours was never accompanied by rectal temperatures equal to or greater than 103°F, no calves required treatment for scours.

Cerebrospinal fluid and intraocular (scleral) pressures, papillary edema, electrocardiograms, carotenoid and vitamin A concentrations. As the carotene intake was decreased from 40- to the 16-\( \gamma \) level, cerebrospinal fluid pressures increased,
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TABLE 2

Effect of carotene intake on feed consumption, cerebrospinal and intraocular (scleral) pressures, and carotenoid and vitamin A concentrations of Holstein male calves

<table>
<thead>
<tr>
<th>Carotene intake (g/lb live weight/day)</th>
<th>16</th>
<th>24</th>
<th>32</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. per cent days consuming ration allowance</td>
<td>97.9(83)</td>
<td>99.6(88)</td>
<td>100.0(90)</td>
<td>99.8(89)</td>
</tr>
<tr>
<td>Terminal cerebrospinal fluid pressure (mm saline)</td>
<td>172</td>
<td>175</td>
<td>131</td>
<td>44</td>
</tr>
<tr>
<td>Log</td>
<td>2.21</td>
<td>2.24</td>
<td>2.10</td>
<td>1.64</td>
</tr>
<tr>
<td>Apparent intraocular (scleral) pressure (mm Hg)</td>
<td>21.2</td>
<td>17.0</td>
<td>20.5</td>
<td>19.5</td>
</tr>
<tr>
<td>Initial</td>
<td>23.4</td>
<td>24.8</td>
<td>22.6</td>
<td>23.1</td>
</tr>
<tr>
<td>Terminal</td>
<td>22.6</td>
<td>23.1</td>
<td>23.0</td>
<td>23.5</td>
</tr>
<tr>
<td>Terminal papillary edema (No. of calves)</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Plasma carotenoids (g/100 ml.)</td>
<td>18</td>
<td>17</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Initial</td>
<td>18</td>
<td>24</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Terminal</td>
<td>18.0</td>
<td>8.1</td>
<td>11.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Plasma vitamin A (g/100 ml.)</td>
<td>10.5</td>
<td>7.6</td>
<td>8.1</td>
<td>7.0</td>
</tr>
<tr>
<td>Initial</td>
<td>18</td>
<td>24</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Terminal</td>
<td>18.0</td>
<td>8.1</td>
<td>11.4</td>
<td>13.4</td>
</tr>
<tr>
<td>Liver Weight (g.)</td>
<td>2,699</td>
<td>2,694</td>
<td>3,024</td>
<td>2,898</td>
</tr>
<tr>
<td>Carotenoids (g/100 g.)</td>
<td>21</td>
<td>37</td>
<td>41</td>
<td>46</td>
</tr>
<tr>
<td>Actual</td>
<td>1.36</td>
<td>1.56</td>
<td>1.61</td>
<td>1.65</td>
</tr>
<tr>
<td>Log</td>
<td>1.63</td>
<td>1.49</td>
<td>1.77</td>
<td>1.84</td>
</tr>
<tr>
<td>Vitamin A (g/100 g.)</td>
<td>11</td>
<td>84</td>
<td>65</td>
<td>77</td>
</tr>
<tr>
<td>Actual</td>
<td>1.05</td>
<td>1.49</td>
<td>1.77</td>
<td>1.84</td>
</tr>
<tr>
<td>Log</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values represent four calves per carotene intake group, except that the 16 γ group represents three calves, plus calculated missing value according to procedures for a randomized block design (9).

b Are sin √Y, where Y equals per cent days consuming vitamin A depletion ration allowance.

Table 2. Since the variances of the cerebrospinal fluid pressures for a particular carotene intake group increased as the magnitude of the average pressure for the group increased, these values were transformed to logarithms. When thus expressed, the log of the cerebrospinal fluid pressure in millimeters of saline decreased 1.271 ± 0.017 units per log of carotene intake per pound of live weight per day, the simple correlation, r, between these two variables being 0.86.

Apparent intraocular pressures, Table 2, measured in the scleral region, were slightly higher for those calves receiving the 16- to 32-γ carotene intakes than those receiving the 40-γ carotene intake, 23.6 versus 23.1 mm. Hg. However, the difference between the two was small compared with the standard deviations per calf (calf plus measurement variability), so that the difference was not statistically significant. The average apparent intraocular pressure increased by 4.0 mm. Hg (23.5 minus 19.5) from the start of carotene supplementation to its termination, 16 wk. later.
The volume of aqueous humor from both eyes averaged for all calves 2.7 ml., with a standard deviation of 0.1. Level of carotene intake had inappreciable effect on this criterion.

Some of the calves in each group fed either the 16-, 24-, or 32-γ carotene intake exhibited papillary edema at the termination of 16 wk. of carotene supplementation (Table 2); whereas, none occurred in the group fed the 40-γ carotene intake.

Electrocardiographic intervals, in seconds, using the data from Lead II tracings obtained during the second week from termination of carotene supplementation, were unaffected by carotene intake. The average values for the PR interval with its standard deviation were, respectively, 0.17 and 0.02, for the QRS interval 0.06 and 0.01, and for the QT interval 0.31 and 0.03. Similar values for the systolic index, the ratio of the QT intervals to the square root of the time in seconds for a complete cardiac cycle (1), were 0.40 and 0.03. Average heart rate in beats per minute during electrocardiographic measurements was 98 and its standard deviation ten, and this criterion was also unaffected by carotene intake.

As might have been expected, carotenoid and vitamin A concentration of both plasma and liver, Table 2, increased with carotene intake. Plasma vitamin A increased 14 ± 3 γ per 100 ml. for each log unit increase in carotene intake and, similarly, the log of the liver vitamin A increased 0.72 ± 0.15. The simple correlations between the respective response variables and log carotene intake were 0.91 and 0.41.

Serum constituents. The various biochemical constituents determined in serum, Table 3, exhibited no particular or consistent trends with change of carotene intake.

Cerebrospinal fluid constituents. Most of the biochemical constituents determined in cerebrospinal fluid showed little change with changes in carotene intake, Table 4. There was a tendency for those calves fed the lowest level of carotene, 16 γ, to have somewhat higher concentrations of total protein and albumin, which was also reflected in the distribution of the proteins (per cent values, Herdan's value as well as the albumin/globulin ratio). None of these differences, however, approached statistical significance. The only constituent to show a consistent change was potassium, which decreased only slightly in a linear manner (P < 0.05) with an increase of carotene intake.

It should be pointed out here that the errors expressed as standard deviation per calf (calf plus biochemical measurement variability) for cerebrospinal fluid were, in relation to their respective experiment means, of considerably greater magnitude than those for serum.

Aqueous humor constituents. In possible contrast to serum and cerebrospinal fluid, biochemical constituents of aqueous humor with the exception of total protein concentration and inorganic phosphorous, showed significant change with change in carotene intake, Table 5. Sodium concentration was on the average greater in those calves fed the 16-, 24-, and 32-γ carotene intakes than in those fed the 40-γ intake (P < 0.10). Potassium concentration was less on the 16-, 24-,
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TABLE 3
Effect of carotene intake on several biochemical constituents in serum of Holstein male calves

<table>
<thead>
<tr>
<th>Carotene intake (γ/lb live weight/day)</th>
<th>Standard deviation per calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>24</td>
</tr>
<tr>
<td>Protein distribution (av. %)</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>67.9</td>
</tr>
<tr>
<td>Alpha-globulin</td>
<td>7.0</td>
</tr>
<tr>
<td>Beta-globulin</td>
<td>11.4</td>
</tr>
<tr>
<td>Gamma-globulin</td>
<td>13.8</td>
</tr>
<tr>
<td>Herdan's value&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.40</td>
</tr>
<tr>
<td>Protein concentration (g/100 ml.)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7.52</td>
</tr>
<tr>
<td>Albumin</td>
<td>5.10</td>
</tr>
<tr>
<td>Alpha-globulin</td>
<td>0.53</td>
</tr>
<tr>
<td>Beta-globulin</td>
<td>0.85</td>
</tr>
<tr>
<td>Gamma-globulin</td>
<td>1.04</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>2.13</td>
</tr>
<tr>
<td>Calcium (mg/100 ml.)</td>
<td>10.6</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/100 ml.)</td>
<td>9.0</td>
</tr>
<tr>
<td>Sodium (mg/100 ml.)</td>
<td>342</td>
</tr>
<tr>
<td>Potassium (mg/100 ml.)</td>
<td>18.1</td>
</tr>
<tr>
<td>Chloride (mg/100 ml.)</td>
<td>377</td>
</tr>
<tr>
<td>Osmotic pressure</td>
<td></td>
</tr>
<tr>
<td>(milliosmols/kg of H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>300</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values represent four calves per carotene intake group, except that the 16 γ group represents three calves plus a calculated missing value, according to procedures for a randomized block design (9).

<sup>b</sup> "An index of the internal mobility of the mixture of proteins and of the preponderance of abnormal constituents in it" and "calculated as the negative average logarithmic probability of the serum constituents" (22).

and 32-γ carotene intake groups than in the 40-γ (P < 0.05) and, in addition, potassium showed a significant positive linear trend with increases in carotene intake (P < 0.05). Chloride concentration was higher in those calves on the three lowest carotene intakes than in those on the highest intake (P < 0.05), and chloride concentration decreased linearly with increases in carotene intake (P < 0.10). The changes in these constituents of aqueous humor were accompanied by a greater osmotic pressure of the calves fed the 16-, 24-, and 32-γ carotene intakes than those calves fed the 40-γ carotene intake (P < 0.10).

The errors, expressed as standard deviation per calf (calf plus biochemical measurement variability), for aqueous humor were, in relation to their respective experiment means, of approximately the same magnitude or slightly greater than those for cerebrospinal fluid.

DISCUSSION

The results of this limited study tentatively suggest that the increased cerebrospinal fluid pressure observed in hypovitaminotic A calves is due to an overproduction of cerebrospinal fluid, as suggested by Millen and Dickson (27), and/or an obstruction to the sites of absorption, resulting in greater volume of fluid contained within the relatively fixed subarachnoid space and ventricles of the central nervous system. Evidence for this was the lack of consistent...
### Table 4

Effect of carotene intake on several biochemical constituents in cerebrospinal fluid of Holstein male calves

<table>
<thead>
<tr>
<th>Carotene intake (γ/lb live weight/day)</th>
<th>Standard deviation per calf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Standard deviation per calf</td>
<td></td>
</tr>
<tr>
<td>Protein distribution (av. %)</td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>60.2</td>
</tr>
<tr>
<td>Alpha-globulin</td>
<td>13.9</td>
</tr>
<tr>
<td>Beta-globulin</td>
<td>13.4</td>
</tr>
<tr>
<td>Gamma-globulin</td>
<td>12.4</td>
</tr>
<tr>
<td>Herdan's value b</td>
<td>1.60</td>
</tr>
<tr>
<td>Protein concentration (mg/100 ml.)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
</tr>
<tr>
<td>Albumin</td>
<td>26</td>
</tr>
<tr>
<td>Alpha-globulin</td>
<td>6</td>
</tr>
<tr>
<td>Beta-globulin</td>
<td>6</td>
</tr>
<tr>
<td>Gamma-globulin</td>
<td>6</td>
</tr>
<tr>
<td>Albumin/globulin ratio</td>
<td>1.52</td>
</tr>
<tr>
<td>Calcium (mg/100 ml.)</td>
<td>5.4</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/100 ml.)</td>
<td>1.7</td>
</tr>
<tr>
<td>Sodium (mg/100 ml.)</td>
<td>341</td>
</tr>
<tr>
<td>Potassium (mg/100 ml.)</td>
<td>12.9</td>
</tr>
<tr>
<td>Chloride (mg/100 ml.)</td>
<td>422</td>
</tr>
<tr>
<td>Osmotic pressure (milliosmols/kg H₂O)</td>
<td>294</td>
</tr>
</tbody>
</table>

*Values represent four calves per carotene intake group, except that the 16 and 40 γ groups each represent three calves plus a calculated missing value, according to procedures for a randomized block design (9).*

---

change with carotene intake of the biochemical constituents determined for serum and almost similar findings for cerebrospinal fluid. The one exception for the latter was a slight decrease in potassium with an increase in carotene intake. Since neither serum nor cerebrospinal fluid exhibited appreciable change in hypovitaminosis A, it would seem likely that the tissues involved in the production and absorption of cerebrospinal fluid are probably involved. It is of

### Table 5

Effect of carotene intake on several biochemical constituents in aqueous humor of Holstein male calves

<table>
<thead>
<tr>
<th>Carotene intake (γ/lb live weight/day)</th>
<th>Standard deviation per calf</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
</tr>
<tr>
<td>Standard deviation per calf</td>
<td></td>
</tr>
<tr>
<td>Protein concentration (mg/100 ml.), total</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Inorganic phosphorus (mg/100 ml.)</td>
<td>4.0</td>
</tr>
<tr>
<td>Sodium (mg/100 ml.)</td>
<td>339</td>
</tr>
<tr>
<td>Potassium (mg/100 ml.)</td>
<td>17.1</td>
</tr>
<tr>
<td>Chloride (mg/100 ml.)</td>
<td>456</td>
</tr>
<tr>
<td>Osmotic pressure (milliosmols/kg H₂O)</td>
<td>306</td>
</tr>
</tbody>
</table>

*Values represent four calves per carotene intake group, except that the 16 and 40 γ groups each represent three calves plus a calculated missing value, according to procedures for a randomized block design (9).*
interest that Moore and Sykes (31) could find no pathological changes of either the choroid plexus, probable site of production (10), or arachnoid villi, one of the possible sites of absorption (10), in the young bovine fed a vitamin A deficient ration. If these negative pathological findings are confirmed by further research, then other approaches, such as measurement of the rate of formation or absorption (which are difficult to interpret) are needed to test the hypothesis of overproduction or underabsorption of cerebrospinal fluid in hypovitaminosis A.

The negative findings of this study with respect to serum may at first inspection appear to contradict those of Madsen and Earle (25), Erwin et al. (16, 17), and Dehority et al. (12). Madsen and Earle studied field cases or experimentally produced vitamin A deficiency of cattle of prolonged duration, usually a year or longer, contrasted to the 16-wk. study reported herein. Therefore, duration of feeding low levels of carotene intake could have accounted for the differences between the two studies. Erwin et al. fed essentially a carotene-free ration to steers for about a 120-day period (16) and to a set of identical-twin heifers for a 150-day period (17), in both cases until the animal exhibited vitamin A deficiency changes. Thus, these workers were probably dealing with terminal vitamin A deficiency in which changes such as anasarca, blindness, convulsions, muscular incoordination, etc. are characteristic. In contrast, in the study reported herein no such changes were noted. While the pilot study reported previously from this station, Dehority et al., was somewhat similar to the one reported herein, the calves consisted of two pairs of Guernsey and two pairs of Holstein males. Guernsey calves fed the 15-γ carotene intake did not consume their vitamin A depletion ration allowance 68% of the time during carotene supplementation and those fed the 60-γ carotene intake, 11%. Holsteins had no ration refusals. Thus, inanition and breed possibly affected the results, the former being of particular importance in differentiating specific and nonspecific deficiency changes, Follis (19). It is recognized that to adequately resolve differences among experiments such as cited above, the variables mentioned should preferably be studied in a single experiment.

The absence of any consistent trend in apparent intraocular (scleral) pressure with increasing carotene intake could have indicated that this criterion is not very sensitive as an indicator of hypovitaminosis A. A second consideration is that the measurement of apparent intraocular pressure in the scleral region, even though the sclera of domestic animals is less resistant to internal pressures than in man (36), may not reflect the pressure within the eye. Therefore, this measurement may not be comparable to those taken directly or indirectly by tonometry in the corneal region. The second consideration appears more likely.

The rather marked changes occurring in the concentration of some of the constituents of aqueous humor, in contrast to no measurable changes in the concentration of the constituents of serum and a change only in concentration of potassium of the cerebrospinal fluid constituents were of interest. These differences may be explained, in part, by considering the relative magnitude of dilution accompanying similar changes in the absolute amounts of constituents in these three fluids. Why changes in the osmotic pressure and in concentrations
of some of the constituents of aqueous humor were accompanied by no increase in intraocular pressure, as measured indirectly in the scleral region, requires confirmation either by direct manometry or tonometry in the corneal area.

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The authors are indebted to B. A. Donohue and T. Watts for meticulous care in the feeding of the experimental animals and for considerable patience and assistance in cerebrospinal fluid and intraocular pressure measurements, to Barbara Kurian and Mrs. Mae Miller for technical assistance, and to Dr. H. J. Fisher, Connecticut Agricultural Experiment Station, New Haven, for proximate analysis of the feeds fed. Mr. J. Rovies, Nopco Chemical Co., Harrison, N. J., kindly supplied the vitamin A supplement and Drs. W. P. Johnson and A. L. Shor, Agricultural Experiment Station, American Cyanamid Co., Princeton, N. J., the chlortetracycline (Aureomycin) oblets. Various staff members at this institution read the manuscript and made helpful suggestions during its preparation, for which we are most grateful. We especially appreciate the many stimulating discussions and suggestions of L. A. Moore and J. F. Sykes regarding hypovitaminosis A.

REFERENCES

BIOCHEMICAL STUDIES OF VITAMIN A DEFICIENT CALVES


