is based on the observation that the casein diet is low in inorganic sulfate and that an alternate pathway for taurine synthesis in the liver of chick and rat, and possibly the cat, involves the fixation of inorganic sulfate with serine. Again the liver decarboxylase could be limiting in the conversion of cysteic acid to taurine.

The reason for disruption of outer segment structure and photoreceptor cell death in the taurine-deficient cat is not known. Young has shown that taurine-H3 is initially concentrated in the pigment epithelium and then distributed throughout the photoreceptor cells of rats and frogs. On the other hand, Ehinger has observed that the pattern of distribution of taurine-H3 follows that of the Müller cell in the rabbit retina. Studies with labeled taurine are in progress in the taurine-deficient cat to learn more about the pathogenesis of this photoreceptor cell degeneration.

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Key words: retinal degeneration, taurine, electroretinogram, retina, cat, diet, casein, amino acids.

REFERENCES


Retinal degeneration in cats fed casein. II. Supplementation with methionine, cysteine, or taurine. ELIOT L. BERSON, K. C. HAYES, ARNOLD R. RABIN, SUSAN Y. SCHMIDT, AND GAIL WATSON.

All cats fed a taurine-free casein diet for 23 weeks have shown a nondetectable electro-
study, the casein diet was supplemented with either taurine or taurine precursors (methionine or cysteine) for 23 weeks to see if retinal function would be preserved. Cats fed the casein diet supplemented with methionine or cysteine showed ERC's reduced in amplitude and delayed in implicit time and had plasma and retinal taurine levels that were well below normal by 23 weeks. Only those cats given taurine in the diet (i.e., those fed chow or casein supplemented with taurine) retained normal ERC function and normal plasma and retinal taurine concentrations. These findings establish a role for taurine in maintaining normal retinal function in the cat.

Taurine, an aminosulfonic acid (Fig. 1), has been considered a nonessential component in the diet since synthesis has been shown to occur in the liver of all vertebrate species studied. The carbon skeleton and amino group are derived from either cysteine or serine, while the sulfur is supplied by either methionine or inorganic sulfate. Nevertheless, a selective plasma and retinal taurine deficiency was observed in cats fed a casein diet which contained normal amounts of serine and methionine. Progressive photoreceptor cell malfunction and then photoreceptor cell death developed by 23 weeks in association with this taurine deficiency.

The cat has a low level of liver decarboxylase so that synthesis of taurine from either cysteine sulfonic acid (a metabolite of cysteine) or cysteic acid (a metabolite of cysteine or serine) would be expected to proceed at a slow rate. An alternate pathway for taurine synthesis is conversion of cysteine to cysteamine and then oxidation to hypotaurine and taurine; this pathway does not require liver decarboxylase. These considerations raised the possibility that endogenous synthesis of taurine could be increased by supplementing the casein diet with cysteine or its precursor, methionine.

In the present study, the casein diet was supplemented with either methionine, cysteine, or taurine to see if cats fed these diets retained normal retinal function as measured by the electroretinogram (ERG). Cats fed the casein diet alone (which contains no taurine) or a commercial laboratory chow (which contains taurine) were followed simultaneously as controls. Plasma and retinal concentrations of methionine, cysteine, and taurine were evaluated after cats were fed the respective diets for 23 weeks.

Methods. Twenty-five domestic cats (1 to 2 kilograms) were fed either laboratory chow (eight animals), the casein diet alone (four animals), or the casein diet supplemented with either methionine (three animals), cysteine (three animals), or taurine (seven animals) for 23 weeks. The casein diet contained 0.5 Gm. methionine, 0.1 Gm. cysteine (half-cystine), and no taurine per 100 Gm. of diet, whereas chow contained 0.4, 0.8, and 0.1 Gm. of each of these amino compounds. In the present study, the casein diet was supplemented with equimolar amounts of either methionine (1.0 Gm. per 100 Gm.), cysteine (0.8 Gm. per 100 Gm.) or taurine (0.8 Gm. per 100 Gm.). Animals were housed individually. Food intake was monitored daily, and body weight was recorded weekly to be certain that the animals were eating the respective diets.

Fundus examinations were performed at 0, 5, 10, 15, 19, and 23 weeks. At the same intervals, full-field ERG testing was done as previously described. Particular attention was given to cone ERG's obtained in response to either a 40 cps. flickering white light stimulus or to single flashes of white light in the presence of a steady full-field white background light sufficient to eliminate the rod contribution to the ERG. Cone ERG implicit time was defined as the time interval between stimulus onset and the major cornea-positive peak of the response. After 23 weeks of diet, amino acid analyses were performed on deproteinized plasma or retina with a Beckman Model 121 automatic amino acid analyzer as described previously.

Results. Fig. 2, A, B, and C illustrate representative ERG's from cats fed chow, the casein diet alone, and the casein diet supplemented with methionine, cysteine, or taurine. The responses of all cats fed casein alone became reduced in amplitude below the normal range by 10 weeks and were nondetectable by 23 weeks. Cats fed casein supplemented with taurine retained a normal ERC for the 23 weeks of this study. Those fed casein supplemented with either methionine or cysteine showed a gradual reduction in ERG amplitude; by 23 weeks ERC amplitudes were reduced about 75 per cent below normal. ERC cone b-wave implicit times were delayed outside the normal range at 10 weeks in cats fed casein alone, while normal cone b-wave implicit times were retained throughout this study in cats fed casein supplemented with taurine. In cats fed casein supplemented with methionine or cysteine, cone b-wave implicit times were minimally, if at all, delayed after 15 weeks but were clearly delayed by 23 weeks.

Table I shows that after 23 weeks of diet the...
Fig. 2, A, B, and C. Representative full-field ERG responses from cats fed chow, casein diet alone, or a casein diet supplemented with either methionine, cysteine or taurine, respectively, for 10, 15, and 23 weeks. Responses were obtained to single flashes of white light (30 foot-lamberts) in the dark adapted state (left column), to 40 cps. flickering white light stimuli (middle column), and to single flashes of white light in the presence of full-field white background (right column). Responses in middle and right columns represent computer summation of 256 and 128 sweeps, respectively. Normal range (mean ± S.D.) for response amplitudes from cats fed chow were 400 nV ± 65 nV (left column), 8.2 nV ± 1.9 nV (middle column), and 13.0 nV ± 3.0 nV (right column). Normal range (mean ± S.D.) for cone b-wave implicit times from cats fed chow was 22.0 msec. ± 1.5 msec. Calibration symbols (lower right corner of each set of tracings) represent vertically 50 µV for the left column, 2 µV for the middle column, and 4 µV for the right column, and horizontally 20 msec. for all tracings. Stimulus onset is a vertical hatched line for left and right columns and the vertical shock artifact for the middle column. Cone implicit times are designated by horizontal arrows in the middle column.

Fig. 2, C. For legend, see Fig. 2.

After 23 weeks, the fundus appearance of cats fed the casein diet supplemented with taurine had retinal and plasma taurine levels identical to those of the normal cats fed chow. Cats fed casein supplemented with methionine or cysteine had higher retinal taurine concentrations than cats fed the casein diet alone, but these concentrations were only 50 per cent of normal. Plasma levels of taurine were reduced to less than 5 per cent of normal in both the methionine and cysteine supplemented cats even though plasma cysteine levels were higher than normal in both groups.

Discussion. This study has demonstrated a role for taurine in maintaining normal retinal function in the cat. Abnormal ERG responses were recorded from all cats with taurine deficiency. This taurine deficiency was produced by feeding cats a taurine-free casein diet or a taurine-free casein diet supplemented with methionine or cysteine. Those cats that were fed chow (which contains taurine) or the casein diet supplemented with taurine had normal taurine levels in plasma and retina and had normal ERG's.

These observations raise the possibility that exogenous taurine is required to maintain normal retinal function in this species. Retinal taurine deficiency developed in casein-fed cats deprived of exogenous taurine even though taurine precursors, methionine and cysteine, were normal in
Fig. 3. Representative fundus photographs of the posterior pole of cats fed chow, the casein diet alone, or the casein diet supplemented with either methionine, cysteine, or taurine for 23 weeks. Fundus was normal only in the cats fed chow or casein plus taurine. A highly reflective white zone in the center of the area centralis was conspicuous in cats fed casein or casein plus methionine and was seen to a lesser extent in the cats fed casein plus cysteine. Fundus appearance was the same in both eyes of each cat.
Table I. Sulfur containing compounds in the retina and plasma of cats after 23 weeks of specified diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>RETINA (nmoles/mg. dry weight)</th>
<th>PLASMA (nmoles/ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>Taurine</td>
</tr>
<tr>
<td>Chow</td>
<td>21</td>
<td>166 ± 7</td>
</tr>
<tr>
<td>Casein</td>
<td>10</td>
<td>40 ± 7</td>
</tr>
<tr>
<td>Casein + taurine</td>
<td>9</td>
<td>163 ± 13</td>
</tr>
<tr>
<td>Casein + methionine</td>
<td>8</td>
<td>71 ± 10</td>
</tr>
<tr>
<td>Casein + cysteine</td>
<td>6</td>
<td>83 ± 14</td>
</tr>
</tbody>
</table>

The values represent the mean ± S.E.M.

the plasma of these animals. Retinal taurine deficiency also developed in cats fed the taurine-free casein diet supplemented with methionine or cysteine even though cysteine and/or methionine were well above normal in plasma. It is unlikely that casein had a direct toxic effect on the photoreceptor cells because varying concentrations of casein in the diet have been associated with the same retinal degeneration; and because supplementation of the casein diet with taurine prevented the development of the degeneration.

The mechanism by which taurine deficiency developed in the casein-fed cat remains to be defined. One possibility is that limited endogenous synthesis of taurine occurred in the cat due to the known low level of liver decarboxylase in this species. Similarly, man has a low level of decarboxylase activity in the liver, and normal human subjects fed methionine or cysteine showed no significant increase in plasma taurine even when plasma levels of methionine or cysteine were at least 10 times above normal. A second possibility is that casein or a component of casein has some direct effect on taurine uptake. This could be resolved by feeding cats synthetic diets differing only in taurine content.

Cats fed the casein diet supplemented with either methionine or cysteine had higher than normal plasma cysteine concentrations and retinal concentrations of taurine that were approximately twice the level of that measured in cats fed casein alone. Several explanations for these observations are possible. First, liver decarboxylase induction in the presence of cysteine or cysteine metabolites may have occurred such that more taurine was synthesized in the liver and actively taken up by the retina. Second, in situ synthesis of taurine, which has been observed in brain homogenates, may have occurred in the retina, particularly when taurine precursors were in high concentrations in the plasma. A third alternative to explain increased taurine synthesis is that the higher than normal plasma levels of cysteine led to increased formation of cysteamine and then oxidation of cysteamine to hypotaurine and taurine.

The role of taurine in normal retinal function is not known. Increased retinal uptake of $^{35}$S-labeled taurine in the dark and increased release of taurine from the retina as a consequence of light exposure have been demonstrated in the chick. In vitro studies of the retina have shown that uptake of taurine is inhibited by iodoacetate, ouabain, or omission of sodium from the medium, while release of taurine following light exposure appears to depend on the presence of calcium in the medium. In dog heart it has been postulated that isethionic acid, a metabolite of taurine, may bind potassium ions, but conversion of taurine to isethionic acid, known to occur in dog heart and cat liver, has not been studied in the retina.

It is interesting that delays in cone ERG b-wave implicit time seen in the taurine-deficient cat also are present in the early stages of practically all types of hereditary retinitis pigmentosa in man and that taurine has been found in high concentrations in the normal human retina. The role of taurine, if any, in the pathogenesis of human retinal degenerations remains to be defined.

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Key words: taurine, methionine, cysteine, diet, casein, electroretinogram, retina, cat, retinitis pigmentosa.

REFERENCES


Pattern reversal visually evoked potentials in infants. SAMUEL SOKOL AND VELMA DOBSON.

Visually evoked potentials (VEP) were recorded from infants two to six months of age using a checkerboard pattern reversal stimulus. By six months, infants produced the largest amplitude VEP to checks subtending visual angles of 7.5 or 15 minutes of arc, as do adults with 20/20 acuity.

This finding indicates that by six months an infant's sensory capacity for a visual acuity of 20/20 is established.

When visually evoked potentials (VEP) are recorded from adults using checkerboard pattern stimuli, there is a close correlation between the relative amplitude of the pattern VEP to different check sizes and subjectively determined visual acuity. The largest amplitude VEP in an adult with 20/20 acuity is found with checks subtending 10 to 20 minutes of arc; as the size of the checks is increased or decreased the VEP amplitude is attenuated. In addition, the peak of the adult VEP amplitude-check size function shifts to larger check sizes as the subjects' acuity is degraded with opthalmic lenses of increasing power. This link between the pattern VEP and subjective acuity in adults can be used to measure infant acuity since there is no accurate way to measure their acuity subjectively. In the present experiment, we have recorded the VEP from infants with a checkerboard pattern reversal stimulus using checks which subtended visual angles of 7.5, 15, and 30 minutes of arc and then compared these data to that obtained from adults with 20/20 acuity. Using checks of less than 30' will more readily assure that the VEP is contrast specific and is elicited from central regions of the retina. In addition, a pattern reversal stimulus maintains a constant state of luminous flux which minimizes luminance contributions to the VEP.

Methods. The VEP was recorded from 15 infants between the ages of two and six months. A single electrode was placed over the inion along the midline and referenced to the right ear. The mother held the infant 75 cm. from the checkerboard stimulus, which subtended a visual angle of 12°. The checkerboard patterns were produced by polaroid vectograph prints and pattern reversal was obtained by rotating a sheet of polaroid between the light source and each of the vectographs. The luminance of the stimulus field was 75 ft. Lamberts and the contrast of the checks was 0.75. One experimenter remained in the shielded room with the mother and infant and had a remote control switch to operate a computer of average transients. When the experimenter was satisfied that the infant was quiet and fixating, the averaging was initiated. The experimenter's criterion for starting the averaging was that the reflection of the stimulus field be in the center of the pupil. Since the total mean luminance of the pattern reversal field remains constant, the experimenter sees a continuous reflection from the cornea. This allows for a more reliable judgment of fixation. While we cannot be absolutely certain that our criterion for fixation ensures that the infants had the pattern stimuli in focus on the retina it is clear.