Beta-Carotene Content of Certain Organs from Two Patients Receiving High Doses of Beta-Carotene

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We measured carotene in the livers of two patients receiving beta-carotene therapy for photosensitivity. In both patients, the amount of carotene was within reported ranges for patients on normal diets; no abnormal amounts of beta-carotene had accumulated in the livers after oral administration of large amounts of beta-carotene. Light-microscopic examination of the liver (specimen obtained at cholecystectomy) of one patient with erythropoietic protoporphyria and cholestasis showed findings characteristic of these conditions. Electron-microscopically, semicrystalline inclusions were seen in the mitochondria of the hepatocytes; such inclusions have been reported in several conditions, including erythropoietic protoporphyria and porphyria cutanea tarda, and in control subjects. Microscopic examination of the liver of the other patient, who had Rothmund-Thomson syndrome and died of acute bacterial meningitis, revealed no abnormalities. The cerebrum of the latter patient contained 13 μg of carotene per kilogram (wet weight) of brain. No previous report of analysis of human brain for carotenoids could be found.

Additional Keyphrases: brain, liver • erythropoietic protoporphyria • therapy for photosensitivity • Rothmund-Thomson syndrome • vitamin A • semicrystalline inclusions in hepatocytic mitochondria

Treatment with high doses of crystalline beta-carotene is currently being used for amelioration of photosensitivity in erythropoietic protoporphyria and certain photosensitivity disorders (1, 2). To date, no reports have appeared of toxicity attributable to beta-carotene in the treated patients, nor has a search of the literature revealed any reports of toxicity from crystalline carotene. It is nevertheless necessary to continue to observe patients receiving high doses of beta-carotene for possible toxicity. It is also relevant to examine for carotene content the organs of any such patients who may come to autopsy or operation. Opportunities arose to study the carotenoid content of the liver of two patients taking beta-carotene, and also to study the brain of one of these.

Materials and Methods

Patients. Patient A, a 4-year-old girl with Rothmund-Thomson syndrome, had been taking beta-carotene (30 mg/day) to alleviate the photosensitivity characteristic of this disease. This patient died suddenly of acute bacterial meningitis. At postmortem examination, the findings in the brain and meninges were consistent with meningitis. Also present was minimal evidence of a coagulopathy similar to that seen in fulminant meningitis, i.e., focal unilateral adrenal hemorrhage, and pulmonary capillary thrombosis. Gross and microscopic examination of the liver revealed no significant pathological changes. The last blood carotenoid value was 2.5 mg/liter.

Patient B, a 22-year-old female with erythropoietic protoporphyria (free erythrocyte protoporphyrin, 17 mg/liter), had been taking beta-carotene (180 mg/day) for about a year to alleviate the photosensitivity associated with the disease. Because of a history of several attacks of right upper quadrant and mid-epigastric pain after fatty meals, she underwent a cholecystectomy. Specimens of liver and gall bladder, and of gall-bladder contents, were obtained and analyzed for carotene content. At operation the liver was found to be grossly normal; the gall bladder contained rather thick, viscid bile, but no stones. The lining of the gall bladder was characteristic of cholestasis on gross examination, and this was confirmed on microscopic examination. The patient's blood carotenoid concentration on the day before surgery was 8.64 mg/liter.

The patient reported that she had received steroids (cortisone) for the treatment of severe photosensitivity reactions, most recently in 1974, before starting beta-carotene therapy. She also stated that she had never taken any oral contraceptives.

Electron microscopic studies were done on the liver biopsy specimen of patient B. After fixation in glutaraldehyde (50 ml/liter, pH 7.4) for 60 min, the specimen was postfixed with 10 g/liter osmium tetroxide and then dehydrated and embedded in EPON 812. After toluidine blue staining, 1-μm sections were studies by light microscopy and ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Philips 300 electron microscope.

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Table 1. Carotene and Vitamin A in the Liver

<table>
<thead>
<tr>
<th>Source (ref.)</th>
<th>Carotene, mg/kg wet wt</th>
<th>Vitamin A, int. kilounits/kg wet wt</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Ralli et al. (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>diabetic patients</td>
<td>26.30</td>
<td>1.20–96.80</td>
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<tr>
<td>nondiabetic patients</td>
<td>10.08</td>
<td>1.50–37.50</td>
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<tr>
<td>Dzialoszynski and Tomaszewski (5)</td>
<td>5.40</td>
<td>0–14.00</td>
</tr>
<tr>
<td>Jensen and With (6)</td>
<td>20.42</td>
<td>1.12–42.59</td>
</tr>
<tr>
<td>Ralli et al. (7)</td>
<td>14.40</td>
<td>3.30–39.00</td>
</tr>
<tr>
<td>Blankenhorn (8)</td>
<td>7.64</td>
<td>2.31–27.30</td>
</tr>
<tr>
<td>Hoppner et al. (9)</td>
<td></td>
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</tr>
<tr>
<td>males</td>
<td>8.40</td>
<td>0–35.50</td>
</tr>
<tr>
<td>females</td>
<td>11.20</td>
<td>0–43.40</td>
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<td>Present report</td>
<td></td>
<td></td>
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<tr>
<td>Patient A</td>
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<td></td>
</tr>
<tr>
<td>Patient B</td>
<td>11.92</td>
<td></td>
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<tr>
<td>Patient with ASHD</td>
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</table>

Results and Discussion

Table 1 gives the amount of carotene and vitamin A found in the livers of the two patients under study, and in the liver of an elderly patient who died of arteriosclerotic heart disease. Also listed are the liver carotenoid and vitamin A concentrations reported by several investigators (4–9). This includes a study done on diabetics (4), whose carotenoid values are often increased because of their increased intake of carotenoid-containing vegetables and fruits. There was no abnormally high accumulation of beta-carotene in the livers of the two patients taking carotene. Although the values are higher than those of the patient not taking carotene, the values fall within the ranges reported by other workers (Table 1). The lack of abnormal accumulation of beta-carotene in the liver has been seen in animal studies (10). The vitamin A concentrations of the patients taking carotene, although higher than those of the patient dying of arteriosclerosis, were still within the ranges reported by others for hepatic vitamin A content (Table 1).

Two reports were found of necropsies with microscopic examination of the liver (11, 12) and a third report of a liver biopsy (13) from patients with diabetes and carotenemia owing to overingestion of vegetables. Two of the reports noted the presence of yellow granules in some hepatic cells. None of the cases reported had any alteration in the normal anatomy of the liver, although in one case the presence of fat vacuoles in some cells was noted (12). In the third case no remarkable amount of pigment was seen, but fat globules were said to be present in about 80% of the cells (13).

Routine light-microscopic findings in patient B demonstrated normal overall hepatic architecture with intrahepatic and canicular cholestasis. Red fluorescent deposits were seen by fluorescence microscopy. The Kupffer cells contained strongly periodic acid-Schiff positive cytoplasm resistant to diastase treatment. Doubly refractile material was present in occasional Kupffer cells. These findings have been previously reported in patients with erythropoietic protoporphyria (14, 15). Routine microscopic studies of patient A revealed no abnormality. In neither patient did we see the fatty changes and the marked pigmentation reported in the diabetic patients.

Electron microscopic observations on the liver of patient B revealed the presence of semicrystalline inclusions of stacked laminar character in most of the mitochondria (exemplified in Figure 1). These were often multiple and were found in all of 55 hepatocytes examined. Crystalline inclusions have previously been seen in the hepatocyte cytoplasm of patients with erythropoietic protoporphyria (14, 15). Recently, Wolff et al. (16) have reported crystalline inclusions in the mitochondria of the hepatocytes of one of two patients with this disorder whom they studied. Hepatocyte mitochondrial crystalline inclusions have been reported...
in other conditions, including porphyria cutanea tarda, patients taking steroids, and control subjects (17–19). The significance of these inclusions remains unknown.

We found no detectable carotenoids in the gall-bladder wall or in the gall-bladder contents removed at operation. Only traces of carotenoids were found in the biliary drainage (180 μg/liter) and duodenal drainage (80 μg/liter) taken the day before operation.

We found no report of the quantitative determination of carotenoid pigments in human brain in the literature. Analysis for carotene content of the cerebral tissue of patient A revealed a content of 13 μg/kg wet weight of brain. The cerebrum of the patient who died of arteriosclerosis contained 27 μg of carotene/kg wet weight of brain. The greater content of carotenoids in the brain of the older patient seems to be consistent with the observations of Karnaukhov et al. (20) of higher carotenoid content in the brains of older animals as compared to younger ones, but this is, of course, only a single observation.

Our data suggest that, as in animal studies, abnormal amounts of beta-carotene and vitamin A do not accumulate in the liver as a result of the ingestion of crystalline beta-carotene. This may also be true for brain, although more studies are needed before this conclusion is certain.

Although two patients is a small number to study, such patients with photosensitivity do not come to operation or autopsy with great frequency. For this reason we wish to report the data we have obtained to date, and to urge other investigators to analyze for carotenoid content the organs of those patients receiving carotenoid therapy who may come to operation or autopsy. This is of special interest in patients with erythropoietic protoporphyria, as there are reports that suggest an increased incidence of gall bladder and hepatic dysfunction in occasional patients with this disease [for review, see (21)].

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References