The Role of Pyridoxine in the Metabolism of Polyunsaturated Fatty Acids in Rats*

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Isotopic data reported by Mead, Steinberg, and Howton in 1953 (1) and by Steinberg, Slaton, Howton, and Mead in 1956 (2) firmly established the incorporation of acetate and of linoleate, respectively, into arachidonic acid in the rat. Witten and Holman in 1952 (3) had suggested that pyridoxine stimulated this conversion. They had shown that rats deficient in essential fatty acids and in pyridoxine synthesized less arachidonic acid when given linoleate than did those deficient in essential fatty acids alone or the doubly deficient rats given pyridoxine.

The purpose of the present studies was to determine whether or not pyridoxine deficiency is specifically concerned in the conversion of linoleic to arachidonic acid and to identify the organs or tissues most susceptible to a deficiency. Our results indicate that pyridoxine is not concerned specifically with the conversion of linoleic to arachidonic acid but may affect in a general manner the metabolism of the saturated and monounsaturated as well as polyunsaturated acids.

EXPERIMENTAL PROCEDURE

Animal Experiments

Experiment 1—Weanling rats, 12 of each sex, born of Sprague-Dawley mothers fed laboratory chow, were fed a purified diet similar to Ration I described by Moneley, Tucker, and Darby (4) with the following modifications. Pyridoxine hydrochloride was omitted and Hubbell, Mendel, and Wakeman salt mixture (5) was used (2.9% by weight). This diet contained 25% casein and 20% Crisco (hydrogenated cottonseed oil) by weight and adequate quantities of known dietary essentials except pyridoxine. Analysis of the fat in the mixed diet was done by alkaline saponification and subsequent ultraviolet spectrophotometry of the ether-extracted fat. The complete diet contained 1.36% by weight of dienoic, 0.05% of trienoic, and no tetraenoic acid. Each of 6 male and 6 female rats received orally 20 µg per day of pyridoxine. These animals were pair-fed with pyridoxine-deficient partners of the same sex which were allowed to eat ad libitum. The rats were killed by decapitation when 3 months old. Selected organs were removed, weighed, sealed under N₂ and kept at -20° until analyzed. Carcasses (total body minus the internal organs and the head) were sealed and stored in a similar manner.

Experiment 2—Twenty-four male weanling Sprague-Dawley rats were fed the diet doubly deficient in pyridoxine and fat described by Witten and Holman (3), with the exception that it contained vitamin-free casein instead of egg albumin. After 6 weeks on this diet, the animals were caged individually and pair-fed randomly. All were given 100 mg per day of linoleic acid as either the methyl or ethyl ester. One of each pair received daily 1.0 mg of pyridoxine and was pair-fed with its pyridoxine-deficient partner which was allowed to eat ad libitum. After 3 weeks, only 7 deficient animals remained alive. Six of these and their pair-fed supplemented partners were killed and their tissues treated in the manner described under Experiment 1. Pyridoxine was added to the drinking water of the seventh deficient animal and it returned to full health in several weeks.

Experiment 3—Weanling Sprague-Dawley rats, 12 of each sex, were treated until 3 months of age in the same manner as those in Experiment 1. After a 16-hour fast, each rat was given by stomach tube 10 µg of pyridoxine-1-C¹⁴ (10 mg of pyridoxine in 0.5 ml of olive oil). Expired air was collected in NaOH traps, and the animals were killed after a 3- or 6-hour period. Tissues were removed and stored as described for Experiment 1.

Assays

The tissues were saponified in 10% ethanolic KOH by heating at 96° under an atmosphere of oxygen-free nitrogen. Hydroquinone was added to each sample to minimize oxidation of unsaturated fatty acids.

The nonsoap and unextracted materials were extracted with petroleum ether from an alkaline solution, and the total fatty acids were removed by extraction with petroleum ether of the acidified layer. Total fatty acids were determined by titration with 0.004 x ethanolic NaOH with metacresol purple indicator. Nitrogen was bubbled through each sample before and continuously throughout the titration.

Polyunsaturated fatty acids were estimated by ultraviolet spectrophotometry after alkaline isomerization according to the method of Holman and Hayes (6) with minor modifications. Extinction coefficients were determined with pure linoleic acid.


† Work done while a predoctoral fellow of the National Heart Institute, United States Public Health Service. The work reported in this paper was taken from a thesis submitted to the Faculty of the Graduate School, Vanderbilt University, in partial fulfillment of the requirements for the Degree of Doctor of Philosophy. Present address, Chemical Research Department, Atlas Powder Company, Wilmington, Delaware.
linolenic, and arachidonic acids. For pentanoic and hexanoic acids the extinction coefficients used were those given by Holman and Hayes (6).

Expired $^{14}C\text{O}_2$ was precipitated as BaCO$_3$, and its radioactivity was assayed in a windowless counter. Appropriate corrections were made for self-absorption.

$^{14}C$ activity of fatty acids was determined in a Packard Tri-Carb liquid scintillation spectrometer. Toluene was used as solvent and 2,5-diphenyloxazole as phosphor. Indicated corrections were made for quenching.

Fatty acids were converted by diazomethane to their methyl esters, and these were separated by gas-liquid chromatography on a Barber-Colman instrument equipped with a U-shaped glass column (8 feet x 4 mm I.D.) and with an argon ionization detector. The column packing was 20% by weight of diethylene glycol succinate polyester coated on 80 to 100 mesh acid-washed firebrick. Other conditions used were: flash heater temperature 300°, column temperature 197°, detector temperature 225°, argon flow rate 175 ml per minute, argon gas pressure 40 p.s.i.

Retention volumes for methyl linoleate and methyl arachidonate were determined with reference compounds obtained from The Hormel Foundation, Austin, Minnesota.

Collection of methyl linoleate and methyl arachidonate from hepatic fatty acid esters in adequate quantities for chemical and radioactivity analyses was accomplished by several repeated gas-liquid chromatographic runs of each sample. The eluent was allowed to flow through a defatted cotton plug in the end of a glass tube immersed in chilled acetone. Because liquid phase bleeding from the chromatographic column contaminated these collected fractions, the fractions were hydrolyzed in 10% ethanolic KOH by heating at 96° under nitrogen and extracted in the same manner as described for the fatty acids of the tissues analyzed. The total fatty acid concentration of each collected fraction was determined by titration and aliquots used for radioactivity assay. A portion of the collected fatty acid was methylated with diazomethane and the purity checked by gas-liquid chromatography under conditions described before.

### RESULTS

Results obtained with the experimental conditions described for Experiments 1 and 2 were similar and are illustrated in Table I by the polyene levels observed in the male animals of Experiment 1. The concentrations, expressed as milligrams per 100 mg of total fatty acids, in tissues of pyridoxine-deficient animals were as high or higher than those in tissues of their respective supplemented partners. In addition to the tissues listed in the table, samples of intestine, spleen, lung, serum, and brain were analyzed and similar results were obtained. Furthermore, similar patterns were observed in tissues from female rats. Since total fatty acid levels generally were higher in the pyridoxine-supplemented than in the deficient animals, greater absolute amounts of polyenes were present in the supplemented than in the deficient.

If the tetraenoic acid content of carcass was calculated as milligrams per g of defatted carcass, there was obtained a constant ratio which was not significantly different for supplemented and deficient animals (Table II). For the group receiving 100 mg of linoleic acid per day (Experiment 2), the average value in supplemented rats was 1.35 (S. E. = 0.03) and in deficient animals 1.30 (S. E. = 0.01). For those given the diet containing 20% fat (Experiment 1), the ratio was 1.44 for supplemented and 1.41 for deficient animals.

When linoleic acid-1-$^{14}C$ was administered by stomach tube to pyridoxine-deficient and supplemented rats, the amount absorbed (i.e. number of counts per minute administered minus the number of counts per minute in the feces plus gastrointestinal contents) in 6 hours varied greatly from one animal to another (from about 22 to 90% of the administered dose). In most of the paired animals a greater percentage of the administered dose was absorbed by the deficient animal. As much or more of the absorbed dose was oxidized to $^{14}C\text{O}_2$ by the deficient animal than by the supplemented animal in both 3- and 6-hour experiments.

#### Table I

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No. of animals</th>
<th>Pyridoxine supplement</th>
<th>Polyunsaturated fatty acids</th>
<th>Total fatty acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>mg/100 mg total fatty acids</td>
<td>mg/g wet weight of tissue</td>
</tr>
<tr>
<td>Liver</td>
<td>6</td>
<td>+</td>
<td>9.6 (0.3)*</td>
<td>16.1 (0.9)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>9.0 (0.8)</td>
<td>17.7 (1.3)</td>
</tr>
<tr>
<td>Testes</td>
<td>6</td>
<td>+</td>
<td>1.9 (0.4)</td>
<td>15.7 (1.7)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>1.8 (0.6)</td>
<td>17.8 (0.3)</td>
</tr>
<tr>
<td>Heart</td>
<td>6</td>
<td>+</td>
<td>8.5 (0.4)</td>
<td>19.6 (0.9)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>9.9 (1.0)</td>
<td>18.8 (0.6)</td>
</tr>
<tr>
<td>Kidney</td>
<td>6</td>
<td>+</td>
<td>8.3 (0.8)</td>
<td>24.9 (1.1)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>-</td>
<td>8.7 (0.4)</td>
<td>27.6 (1.0)</td>
</tr>
<tr>
<td>Muscle</td>
<td>3</td>
<td>+</td>
<td>31.7 (7.8)</td>
<td>11.5 (0.1)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>-</td>
<td>18.1 (2.2)</td>
<td>13.5 (0.6)</td>
</tr>
</tbody>
</table>

* Number in parentheses is standard error of the mean.

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2 Obtained from the Hormel Foundation, Austin, Minnesota.

3 Standard error of the mean.
The data for the 6-hour experiments are shown in Table III. If the data are expressed as % of absorbed dose per 100 g of body weight, all deficient animals oxidized a greater percentage of the absorbed dose than did the corresponding supplemented animals. The hypothesis that pyridoxine is specifically involved in the conversion of linoleic to arachidonic acid has its origin in observations made by Witten and Holman in 1952 (3). These workers found larger amounts of tetraenes and hexaenes in supplemented when compared to pyridoxine-deficient rats after supplementing a fat-deficient diet with linoleate or linolenate. They suggested that the conversion of linoleate to arachidonate was stimulated by administration of pyridoxine.

Because the polyenoic acid concentrations of tissues from our pyridoxine-deficient rats maintained on a diet containing 20% fat proved to be comparable to those of pyridoxine-supplemented animals, an experiment patterned according to that of Witten and Holman (3) was done. The polyunsaturated fatty acid concentrations of all tissues analyzed were higher in the pyridoxine-deficient rats than in their pair-fed supplemented partners. Although carcases of deficient rats had higher percentages of tetraenes and hexaenes, the total amounts were greater in the

*With the combined values for males and females the difference between deficient and control animals is significant at the 1% level (t = 5.12 with 5 degrees of freedom).
supplemented. However, when calculated as milligrams per g of defatted carcass, the average values for the two groups were similar. The result of this calculation plus the additional information acquired by analyses of individual tissues support the interpretation that the amount of arachidonic acid in those rats was related to the tissue mass and, therefore, to growth. The pyridoxine-deficient rats, retarded in growth, had less tissue mass and smaller amounts of tetraenes than their pair-fed partners. Inasmuch as pyridoxine-supplemented rats contained larger amounts of the saturated plus monoenoic acids than did deficient animals, pyridoxine affected the metabolism of fatty acids in general. This observation is in keeping with the proposal of Carter and Phizackerley (7) that pyridoxine deficiency impairs the conversion of carbohydrate to fat.

If part of the pyridoxine-deficient syndrome results from inadequate levels of arachidonic acid, feeding of this material to deficient animals should alleviate the signs of deficiency. However, Williams and Hincenbergs (8) were unable to alleviate the pyridoxine deficiency syndrome of rats by feeding 100 mg of methyl arachidonate daily for 6 days.

Although it has been assumed that pyridoxine may be involved in the conversion of linoleate to arachidonate, its exact function has not been suggested. Olsen recently emphasized that "Be-type reactions" are not apparent in this conversion (9). A role for pyridoxine in the utilization of arachidonic acid in maintenance of phosphate esterification in vitro was suggested by the work of Tulpule and Williams (10).

Attempts to secure additional information concerning specific effects of pyridoxine deficiency on the metabolism of essential fatty acids involved the use of linoleic acid-1-C^14. No interference of the deficiency with intestinal absorption or oxidation to C^14O_2 of the administered C^14-linoleic acid was observed. The former observation is in keeping with the findings of Carter and Phizackerley (7) that dietary fat absorption was not affected by vitamin B_6 deficiency. C^4 activity was found in hepatic arachidonic acid of deficient animals but in smaller amounts than in supplemented animals. However, the amount of labeled linoleic acid was also smaller in livers of deficient animals, indicating that not only the product but also the labeled precursor was present in smaller amounts. It is, therefore, unlikely that the decreased conversion of linoleic to arachidonic acid was due directly to a lack of pyridoxine.

The C^4 activity of hepatic total fatty acids from pyridoxine-deficient rats also was less than that in supplemented rats. That this might have been the result of relatively greater oxidation of the labeled linoleic acid by the deficient rats is suggested by the fact that they expired as much C^14O_2 as did the supplemented animals despite the smaller size of the deficient animals.

**SUMMARY**

Polysaturated fatty acid concentrations have been determined and the metabolism of linoleic acid-1-C^14 has been studied in pyridoxine-deficient and in pair-fed, pyridoxine-supplemented rats.

Pyridoxine-deficient rats receiving daily 100 mg of linoleic acid as the only dietary fat had slightly higher total amounts and higher concentrations of polyenoic acids than did their pair-fed partners. Pyridoxine-deficient animals on a diet containing 20% fat (hydrogenated cottonseed oil) had higher concentrations but slightly lower total amounts of polyunsaturated fatty acids than did the pair-fed supplemented animals.

When the concentration of tetraenoic acid is calculated as milligrams per g of defatted carcass, the ratios for pyridoxine-deficient and pyridoxine-supplemented rats are similar. These findings suggest that the quantity of tetraenoic acid in the rat is a function of the tissue mass and that pyridoxine is not specifically involved in the conversion of linoleic to arachidonic acid.

Intestinal absorption of linoleic acid-1-C^14 was not impaired in pyridoxine deficiency. The percentage of absorbed C^4 expired as C^14O_2 per 100 g of body weight was uniformly higher in pyridoxine-deficient than in supplemented rats.

Smaller amounts of C^4 were found in pure fractions of both arachidonic and linoleic acids isolated from livers of pyridoxine-deficient than from pair-fed, supplemented animals. Conversion of linoleic to arachidonic acid occurred in deficient animals but the data do not permit a conclusion as to a possible influence of pyridoxine on the rate of conversion.

**Acknowledgments**—It is a pleasure to acknowledge the advice and help of Dr. Charles F. Fiederspiel, Assistant Professor of Biostatistics, with the statistical problems and the help of Dr. William J. Darby, Professor of Biochemistry, with the design of the experiments.

**Addendum**—Since submission of this manuscript, Wakil (11) has published data indicating a possible role for pyridoxine in a mitochondrial enzyme system that effects elongation of long chain fatty acids, and Johnston, Kopaczyk, and Kummerow (12) have reported that little change occurred in fatty acid composition of carcass fat in pyridoxine deficiency unless the rats were also deficient in essential fatty acids.

**REFErences**

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