Nutritional Factors in Hemodynamics

II. Hypertension during Pteroylglutamic Acid Administration in Albino Rats

By Richard E. Lee, M.D., Ph.D., Sachiko Tanaka, B.A., and Elizabeth A. Holze, B.A.

Albino rats treated with pteroylglutamic acid (PGA) developed a significant increase in blood pressure, as compared with litter mate controls of the same sex. The hypertension was associated with wedge-shaped fibroblastic renal cortical lesions. Factors possibly important etiologically in the elevation of blood pressure are discussed in relation to certain of the metabolic roles of pteroylglutamic acid.

An interrelationship between certain of the actions of ascorbic acid and of pteroylglutamic acid (PGA) has been reported recently by several laboratories. The "tyrosyluria" present in vitamin C deficient guinea pigs can be abolished by the parenteral administration of pteroylglutamic acid, although the deficiency of vitamin C and the subsequent development of scurvy is not ameliorated.

Massive doses of this vitamin will similarly inhibit the anticipated "tyrosyluria" of scorbutic infants. In addition, young monkeys on a synthetic diet devoid of pteroylglutamic acid and of ascorbic acid develop a megaloblastic anemia which responds either to pteroylglutamic acid or to ascorbic acid administration.

It has been established previously that vitamin C has an important role in maintaining vasoccompensatory processes in the peripheral circulation. The evidence outlined briefly above suggested that metabolic association of ascorbic acid and pteroylglutamic acid might conceivably extend into hemodynamic mechanisms. For this reason, studies have been initiated concerning blood pressure levels, tissue vitamin C concentrations and certain peripheral vascular reactions in animals fed synthetic diets with and without pteroylglutamic acid supplementation. It is the purpose of this report to describe a blood pressure elevation with renal lesions that accompanies administration of pteroylglutamic acid to albino rats.

METHODS

Twenty-seven albino rats of the strain developed in this laboratory were individually housed at age 21 days and fed a synthetic diet devoid of pteroylglutamic acid. They were maintained on this diet for a total period of six weeks (to age 9 weeks). Seventeen were given intraperitoneal injections of a suspension of pteroylglutamic acid, 1.0 mg. per cc. distilled water per 100 Gm. body weight, three times weekly. The material was made up freshly immediately prior to each injection. The remaining 10 animals received no supplementation. After six weeks on this diet, 48 hours following the last dose of pteroylglutamic acid, the carotid blood pressure of each rat was recorded on a mercury manometer for a minimum period of six minutes. General anesthesia was provided by sodium pentobarbital, 3.5 mg. per 100 Gm. body weight. Blood pressures were similarly measured in 22 additional albino rats of the same strain receiving a regular laboratory pellet diet without additional pteroylglutamic acid. Observations on each animal receiving the injections were compared with those on litter mate controls of the

* The diet consisted of:

<table>
<thead>
<tr>
<th>Supplemented Diet</th>
<th>Supplements 1% Mg./100 Gm. diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>18% Thiamine</td>
</tr>
<tr>
<td>Lard.</td>
<td>8% Riboflavin</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>49% Pyridoxine</td>
</tr>
<tr>
<td>Sucrose</td>
<td>14% Pantotenate</td>
</tr>
<tr>
<td>Agar.</td>
<td>2% Nicotinamide</td>
</tr>
<tr>
<td>Salt.</td>
<td>4% Choline Chloride.</td>
</tr>
<tr>
<td>CLO.</td>
<td>1% Inositol</td>
</tr>
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</table>
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same sex receiving either the synthetic diet or the laboratory pellet diet. Individuals were selected whose average daily food consumption and body weights were as nearly comparable as possible. At

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>No. of animals for B.P. Determinations</th>
<th>Mean B.P. at 6 weeks (aged 9 wks.)</th>
<th>Standard Error (B.P.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synthetic Diet</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>4</td>
<td>140</td>
<td>4.7</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>145</td>
<td></td>
</tr>
<tr>
<td>Synthetic diet plus 1 mg. PGA/100 Gm. 3X weekly intraperitoneally</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>168</td>
<td>5.04</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>175</td>
<td></td>
</tr>
<tr>
<td>Stock Pellet diet</td>
<td></td>
<td>145</td>
<td>2.88</td>
</tr>
<tr>
<td>Male</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

The blood pressures of the rats are recorded on table 1. After six weeks of treatment, the rats receiving pteroylglutamic acid had uniformly developed a significant hypertension (table 1). There were no differences in the blood pressures of the two control groups. The concentrations of ascorbic acid in the adrenal glands and in the kidneys were not influenced by the administration of pteroylglutamic acid. Liver vitamin C was considerably elevated in all rats on the synthetic diet; with moderately but not significantly greater amounts in livers of animals given pteroylglutamic acid (table 2). Also of interest were differences observed between the sexes with regard to tissue levels of ascorbic acid. This finding, not related to the increased blood pressure associated with injections of pteroylglutamic acid, is under further study.

Although the kidneys of treated rats were equal in weight to those of controls, the renal

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Number of Animals</th>
<th>Ascorbic Acid (µg./Gm.)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock Pellet diet</td>
<td>26 (15 males, 11 females)</td>
<td>Kidney: 174 S.E.-9.6</td>
<td>Liver: 270 S.E.-14.6</td>
</tr>
<tr>
<td>Synthetic diet</td>
<td>16 (9 males, 7 females)</td>
<td>Kidney: 159 S.E.-9.76</td>
<td>Liver: 345 S.E.-24.3</td>
</tr>
<tr>
<td>Synthetic diet plus 1.0 mg. of PGA/100 Gm. body wt. 3X weekly, intraperitoneally</td>
<td>19 (9 males, 10 females)</td>
<td>Kidney: 149 S.E.-6.0</td>
<td>Liver: 359 S.E.-9.7</td>
</tr>
</tbody>
</table>

* Statistical examination of these data reveals that the high concentration of liver ascorbic acid in the rats receiving PGA is of no significance when compared with either of the two control groups. There is no significance in the differences shown in adrenal or kidney vitamin C. Animals on the synthetic diet had a relatively low kidney ascorbic acid concentration, regardless of whether or not the animals received PGA.

cortex surface in 15 of the 17 animals receiving pteroylglutamic acid was dotted with whitish, slightly contracted scars of from 0.5 to 2.0 mm. in diameter. These areas extended 1.0 to 3.0 mm. into the cortical parenchyma as small wedge-shaped lesions containing nu-
merous fibroblasts. Cells of the proximal and distal convoluted tubules within and immediately adjacent to these regions were usually moderately shrunken, with pyknotic nuclei and eosinophilic cytoplasm. The remainder of the renal cortex and the medulla, despite the number and extent of cortical scars, compared favorably with that of the control animals. Sections of the kidneys from one rat treated with pteroylglutamic acid showed rare atrophic glomeruli, but the other animals failed to demonstrate this change. Occasional dilatation of the convoluted tubules bordering the scarred portions was observed in approximately 50 per cent of the animals, but casts in the renal tubules were not observed.

**DISCUSSION**

The tubular damage observed in these animals generally agrees with that previously described by others in kidneys of guinea pigs and rats treated with pteroylglutamic acid. Numerous casts, largely of pteroylglutamic acid or derivatives, with resulting tubule dilatation, were prominent features of the renal pathology noted by others, but casts were not found in the kidney tubules in the present study. This conceivably results in part at least from the fact that the dose of pteroylglutamic acid employed was significantly lower than that employed by other workers, and the material was administered three times weekly rather than daily as was the custom in other surveys.

It has been reported that an acute and transient rise in blood pressure occurred immediately following a single intravenous injection of pteroylglutamic acid (5.0 to 100.0 mg. per kilogram) in cats, dogs and in one rabbit. Within 30 minutes, however, the "blood pressure was always normal." This finding contrasts with the rise in blood pressure accompanying relatively long term intraperitoneal administration of pteroylglutamic acid to rats at low dosage levels, for in this latter instance pressures were recorded 48 hours after the last intraperitoneal injection. Moreover, renal lesions following single doses of pteroylglutamic acid were not described.

The mechanism by which a significant hypertension is obtained in rats treated with pteroylglutamic acid is at present obscure. Its association with renal lesions possibly suggests a renal factor comparable to that brought about by interference with renal blood flow, as with renal artery clamps, perinephric packs, or renal ligation. An excess of dietary pteroylglutamic acid, however, is associated with a reduced activity of liver d-amino acid oxidase and xanthine oxidase in the chick, and diminished xanthine oxidase activity in the rat. Liver choline oxidase in the rat may also be altered by an increase in dietary pteroylglutamic acid. It is conceivable that large amounts of parenteral pteroylglutamic acid might similarly interfere with the activity of certain renal or other enzyme systems that normally may inactivate endogenous hypertensive factors, and in this manner induce a sustained blood pressure elevation. Another possible explanation of the hypertension is suggested by recent demonstrations that pteroylglutamic acid is imminently concerned in transmethylation and the synthesis of choline and methionine. In addition, under certain specific experimental conditions, the administration of pteroylglutamic acid accelerates methylation of a toxic substance and results in a consequent loss of available methyl groups from the body. In these circumstances, the development of a yellow, fat-infiltrated liver and a reduced growth rate suggests a choline deficiency. This possible antilipotrophic nature of pteroylglutamic acid under induced conditions is of interest. In the present study, pteroylglutamic acid may have accelerated transmethylation in the body to a degree sufficient to induce a significant over-all deficiency of accessible methyl groups. The onset of hypertension subsequent to a brief transient period of choline deficiency in early life is well established in rats. By producing a relative insufficiency of choline, or other methyl donor substances, administration of pteroylglutamic acid may result in hypertension perhaps by a metabolic action less direct than specific enzyme inhibition.
SUMMARY AND CONCLUSIONS

1. Blood pressure levels and tissue vitamin C concentrations have been determined in rats of our laboratory strain treated with pteroylglutamic acid (PGA).

2. Administration of pteroylglutamic acid for six weeks was accompanied by a significant blood pressure elevation.

3. In comparison with the control animals, the treated animals showed no differences in kidney weight or in the concentration of ascorbic acid in the kidney, liver and adrenal gland. Injections of pteroylglutamic acid were associated with numerous wedge-shaped fibroblastic lesions of the renal cortex.

4. The possible mechanisms by which excess pteroylglutamic acid might produce hypertension are discussed in relation to the metabolic roles of the vitamin.

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