Gonadotropic Potency of the Hypophysis in the Polyunsaturated Fatty Acid Deficient Rat

By

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Burr and Burr (1) reported that a deficiency of polyunsaturated fatty acids such as linoleic acid or linolenic acid results in the dysfunction of reproduction in rats. In the absence of these fatty acids there is degeneration or retrogradation in the testes of male rats; in female rats ovulation and fertilization are comparatively impaired and also there is death and resorption of the fetus. Thereafter, many same observations have been made in mice (2) and dogs (3), and it is well known that this polyunsaturated fatty acid is important for the reproduction. Actually it has been fairly cleared on its chemical formula, deficiency symptoms, physiological action, possibility of its synthesis in the animal body, absorption, storage, utilization, excretion and metabolism, using rats chiefly. But we have as yet very little information as to what is involved in the detailed investigation of the mechanism of its physiological action except the reports of Holmen et al (4-8), and its nature is still a matter of speculation. In earlier papers of this series (9) on the detailed biochemical investigation of the dysfunction of reproduction in the polyunsaturated fatty acid deficient rats several reports were made on the changes of lipids in the reproductive endocrine glands.

It is assumed that all gonadal functions are taken place under the control of the anterior hypophysis in mammals. So, it may throw some light on the mechanism of the action of the polyunsaturated fatty acid to investigate the gonadal function in its deficient rats which have shown the dysfunction of reproduction. This paper deals primary with the gonadotropic potency of the anterior hypophysis in the polyunsaturated fatty acid deficient rats.

METHOD AND RESULTS

The test animals used in this study were both male and female Wistar strain rats at the age of 65 days. The polyunsaturated fatty acid-deficient diet consisted of 25% autoclaved egg albumin, 20% fat mixture (Palmitate 30, Stearate 30, and oleinate 40), 50% sucrose, 4% salt mixture, and 1% vitamins. The vitamins were mixed with the diet. Each kilogram of diet contained the following quantities of vitamins; thiamine hydrochloride 3.0 mg, riboflavin 2.5 mg, pyridoxine 2.0 mg, niacin 2.5 mg, pantothenic acid 20 mg, inositol 1.0 mg, para-amino benzoic acid 500 mg, biotin 0.5 mg, folic acid 2.0 mg, choline 1.0 mg, vitamin B_{12} 100 microgramm, vitamin A acetate 150 mg, calciferol 70 microgramm, and alpha-tocopherol 50 mg. The individual rats fed with the
complete diet were given daily oral supplements of 100 mg linoleic acid and 100 mg linolenic acid above the deficient diet. Each rat of the control group and the deficient group was fed for 80 days.

The animals of both groups were sacrificed on the 81th day of the experiment with decapitation under ether narcosis, immediately were dissected; then each pituitary was brought out, poured into acetone and kept in the refrigerator. This pituitary in acetone was dried at low temperature (less than 10°C) under the decreased pressure and ground up. The pituitary dried powder was placed into 3.5 cc. of 0.9% saline aqueous solution. To investigate the gonadotropin content of the hypophysis this donor pituitary solution was injected to the hypophysectomized immature female rat. The Wistar strain immature female rats were used as this assay animal. These rats were fed at the almost same conditions in our laboratory. The number of the donor rat hypophysis given to one recipient immature rat is three. Each rat of assay animals was hypophysectomized at the age of 25 days by the method of Homma (10), and the injection of saline solution of dried hypophyseal powder was set in 48 hours later from the operation. At evening of the first day once subcutaneous injection of 0.5 cc. of the donor hypophyseal preparation was made intraperitoneally per one recipient immature rat, then daily 1 cc. in 0.5 cc. injections at morning and evening for 3 successive days; total amount of injection per one recipient rat was 3.5 cc. At morning after the last injec-

<table>
<thead>
<tr>
<th>No. of rats</th>
<th>Initial B.W. (g)</th>
<th>Final B.W. (g)</th>
<th>Testes</th>
<th>Accessory organs*</th>
<th>Adrenals</th>
<th>Pituitary</th>
<th>Assay</th>
<th>No. of hypophysectom. rats</th>
<th>No. of hypophysis per rat</th>
<th>Ovaries</th>
<th>Uteri</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>11</td>
<td>85</td>
<td>192</td>
<td>2080±174</td>
<td>537±63</td>
<td>33.5±4.1</td>
<td>7.6±0.6</td>
<td>3</td>
<td>3</td>
<td>115±12</td>
<td>89±11</td>
</tr>
<tr>
<td>Deficiency</td>
<td>14</td>
<td>91</td>
<td>125</td>
<td>1517±151</td>
<td>118±24</td>
<td>31.4±3.5</td>
<td>7.1±0.9</td>
<td>3</td>
<td>3</td>
<td>108±13</td>
<td>90±9</td>
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<tr>
<td>Female</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>13</td>
<td>80</td>
<td>169</td>
<td>51.5±5.2</td>
<td>248±19</td>
<td>43.7±4.0</td>
<td>9.3±0.8</td>
<td>3</td>
<td>3</td>
<td>88±9</td>
<td>83±10</td>
</tr>
<tr>
<td>Deficiency</td>
<td>15</td>
<td>84</td>
<td>128</td>
<td>38.8±3.3</td>
<td>198±16</td>
<td>25.1±2.7</td>
<td>8.9±0.9</td>
<td>3</td>
<td>3</td>
<td>87±11</td>
<td>81±12</td>
</tr>
</tbody>
</table>

*Accessory organs indicate the seminal vesicles and the prostate glands.
tion recipients rats were killed, ovaries and uteri were weighed and the macroscopic sights of the ovaries (follicles and corpus luteums) were also observed.

These results are shown in the next table. It is readily seen that in either the control rat or in the polyunsaturated fatty acid deficient rat, the donor rat pituitary increases the weight of the ovary and the uterus and develops the follicle and the corpus luteum of the ovary in the hypophysectomized immature recipient rat. And there seldom is the detectable differences of gonadotropic potency of the anterior hypophysis between the control group and the deficient group. And moreover, it seems to be noticeable that both the absolute value and the value of a ratio to body weight of the adrenal and hypophyseal weight in the female rat are greater than the male rat in both experimental groups.

**DISCUSSION AND SUMMARY**

Nelson et al (11) have reported that there is a defect of the secretion of anterior hypophyseal hormone in the pyridoxine deficient rat. But from the author's experiment it seems quite evident that the gonadotropic potency of the anterior hypophysis in the polyunsaturated fatty acid deficient rat is normal as compared with the normal rat. Namely, the gonadotropic activity of the anterior hypophysis of the rat does not be impaired by the polyunsaturated fatty acid deficiency.

It is assumed that the gonadotropins produced and secreted in the anterior hypophysis stands in the highest place among the hormones related to the animal reproduction. Eventually it may be suggested that the reproductive dysfunction caused by the polyunsaturated fatty acid deficiency in the rat is not primary but secondary or accessory phenomenon. Mason (12) has also pointed the same suggestion on the meaning of the physiological action of vitamin E which has been assumed to have a close connection with animal reproduction. From this point it is accordingly conceivable that polyunsaturated fatty acid and vitamin E meet on a common ground each other for the reproduction.

From the table especially the weight of suprarenal body shows the different value between male and female rats. That the function of suprarenal body (especially cortex) of normal male rats differs from the one of female rats was already suggested by Simpson et al (13), and also author (14) has obtained the same results on the content of adrenal cholesterol. And Greenberg (15) reported that the required amount for polyunsaturated fatty acid in female rats is smaller than in male rats. From these observations it seems to be able to ascribe to Simpson's suggestion or to Greenberg's report that the adrenal weight of the polyunsaturated fatty acid deficient female rats also is greater than male rats.

The above results are summarized as follows; the gonadotropic potency of the polyunsaturated fatty acid deficient rat remains unchanged as with normal rats.
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