16. **Spontaneous Occurrence of Congenital Malformations and Mortality in Prenatal Inbred Rats**

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The protection of human beings from hazards of teratogenic, mutagenic and carcinogenic actions by environmental substances has recently been becoming a serious matter around us. The need of well-established laboratory animals for carrying out the experiments of protection has greatly increased. The establishment of experimental animal strains with genetic homogeneity, as well as the breeding of mutant strains bearing genetic characters are seriously requested in embryologic, teratogenic, mutagenic and other related research fields. In this paper I wish to furnish some critical data particularly on spontaneous congenital malformations and prenatal mortality, derived from breeding experiments with certain inbred rat strains.

Materials and methods. Animals herein dealt with were rats (*Rattus norvegicus*) of eight inbred strains which were maintained in the Experimental Animal Laboratory of the Hokkaido University (substrain symbol: Hok, Fasting and Staats 1973). The maintenance of each strain was carried out by means of full brother-sister mating under conditions controlled by an air-conditioner (23±1°C room temperature; 65±5% humidity). Animals were fed on NMF or CMF pellets (Oriental Yeast Co. Ltd., Tokyo), giving fresh tap water *ad libitum*. Descriptions on the origin of these inbred rats were partially given in the Standardized Nomenclature for Inbred Strains of Rats, Fourth Listing (Festing and Staats, 1973). Their brief notes are given below:

1) ACI/NHok (A×C 9935) : Curtis and Dunning, Columbia University, 1926; to Heston, 1945, at F$_{30}$; to N, 1950, at F$_{41}$; to Thi; to Hok, 1967, at F$_{87}$.
2) BUF/NHok (Buffalo) : Heston, 1946, from Buffalo stock of Morris; to N, 1950, at F$_{10}$; to Hok, 1956.
3) F344/NHok (Fischer 344) : Curtis, Columbia University,

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1920; to Heston, 1948; to N, 1950, at F$_{45}$; to Hok, 1956.


5) Kyoto-notched: Bearing "notched" character isolated by Nakata from wild rat at Kyoto; to Hok, 1960, inbreeding started by Makino, Hokkaido University, maintaining the black notched character from hybrids between WKA/Hok and Nakata's rat.

6) TO/Hok (Tokyo): From a breeder, Tokyo; to Hok, 1952, inbreeding started by Makino, Hokkaido University.

7) W/Hok (Wistar): Wistar Institute to Tokyo University 1938; to Hok, 1944, inbreeding started by Makino, Hokkaido University.

8) WKA/Hok (Wistar-King A): King to Aptekman, Wistar Institute; to Hok, 1953, at F$_{148}$.

Estrous virgin female rats, aged 90 to 120 days and inspected by means of vaginal smears, were caged overnight with males of the same strain. The day on which a vaginal plug was observed was considered as day 0 of gestation. All females were killed on day 20 of gestation in order to examine the condition and development of fetuses in utero. The fetuses were recorded as being alive or dead. Prenatal death was divided into early (embryonic resorption sites) and late (placental remnants and macerated fetuses) categories. All living fetuses were examined for the occurrence of gross external malformation under a dissecting microscope, and subsequently preserved in 90 percent alcohol. The fixed specimens were examined for grossly visceral anomalies.

Results and discussion. The results of the present prenatal observations in eight inbred rat strains are given in Table I in a form of summary. A total of 213 dams produced 1747 implantations, including 1555 living fetuses (89.0%) and 192 prenatal losses (11.0%) at early (7.5%) and late (3.5%) stage of gestation. Mean litter sizes of implantations which served as an index of fertility ranged from 4.4 for the Kyoto-notched strain to 10.0 for the F344/NHok strain, and those of living fetuses showed a range from 4.0 for the Kyoto-notched strain to 9.8 for the F344/NHok strain. Prenatal mortality varied according to the difference of strain. It was highest in the BUF/NHok (29.1%), while lowest in the F344/NHok strain (2.4%). The frequency of early death was significantly higher than that of late death in most strains here concerned. Robinson (1965) mentioned some evidence giving the high percentage of embryonic or fetal loss probably derived from the transmission of a probable reciprocal translocation, based on the data presented by Long and Evans (1922) and Tyler and Chapman (1948). The F344/
NHok strain gave the largest litter size for living fetuses, together with the largest implantation and the lowest prenatal mortality. However, the number of living fetuses in the BUF/NHok strain showed a remarkable decrease on account of their high mortality. Sato and Ohzu (1963) presented some postnatal data on the same inbred rat strains as those employed for the present study, and described that the mean litter size at birth was the largest in the F344/NHok strain among the 9 rat strains studied, and that postnatal mortality was relatively high in the BUF/NHok and F344/NHok strains. The F344/NHok strain, therefore, proves useful as laboratory rats because of their high fertility, if the breeding would carefully be carried out in suckling periods.

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Table I. Summary of prenatal data obtained in eight inbred rat strains

<table>
<thead>
<tr>
<th>Rat strain</th>
<th>Dam</th>
<th>Implantation (a)</th>
<th>Prenatal mortality early (b)</th>
<th>late (b)</th>
<th>Living fetus (a)</th>
<th>Malformed fetus (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACI/NHok</td>
<td>23</td>
<td>159 (6.9)</td>
<td>17 (10.7)</td>
<td>1 (0.6)</td>
<td>141 (6.1)</td>
<td>14 (9.9)</td>
</tr>
<tr>
<td>BUF/NHok</td>
<td>22</td>
<td>203 (9.2)</td>
<td>36 (17.7)</td>
<td>23 (11.3)</td>
<td>144 (6.5)</td>
<td>0</td>
</tr>
<tr>
<td>F344/NHok</td>
<td>21</td>
<td>210 (10.0)</td>
<td>5 (2.4)</td>
<td>0</td>
<td>205 (9.8)</td>
<td>0</td>
</tr>
<tr>
<td>Kyoto-notched</td>
<td>20</td>
<td>88 (4.4)</td>
<td>2 (2.3)</td>
<td>6 (6.8)</td>
<td>80 (4.0)</td>
<td>0</td>
</tr>
<tr>
<td>Long-Evans</td>
<td>18</td>
<td>114 (6.3)</td>
<td>7 (6.1)</td>
<td>13 (11.4)</td>
<td>94 (5.2)</td>
<td>4 (4.3)</td>
</tr>
<tr>
<td>TO/Hok</td>
<td>21</td>
<td>160 (7.6)</td>
<td>3 (1.9)</td>
<td>4 (2.5)</td>
<td>153 (7.3)</td>
<td>0</td>
</tr>
<tr>
<td>W/Hok</td>
<td>23</td>
<td>199 (8.7)</td>
<td>9 (4.5)</td>
<td>5 (2.5)</td>
<td>185 (8.0)</td>
<td>1 (0.5)</td>
</tr>
<tr>
<td>WKA/Hok</td>
<td>65</td>
<td>614 (9.5)</td>
<td>52 (8.5)</td>
<td>9 (1.5)</td>
<td>553 (8.5)</td>
<td>11 (2.0)</td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
<td>1747 (8.2)</td>
<td>131 (7.5)</td>
<td>61 (3.5)</td>
<td>1555 (7.3)</td>
<td>30 (1.9)</td>
</tr>
</tbody>
</table>

(a) Mean value, (b) % for implantation, (c) % for living fetus.
1: anomaly of urogenital organ, 2: cleft palate, 3: clubfoot, and polydactyly.

Spontaneous malformations occurring in rat fetuses appeared in 4 of 8 strains in the present study. They showed 4 types involving cleft palate, clubfoot, polydactyly, and abnormal urogenital organ. The total incidence of abnormal fetuses was 1.9%, or 30 out of 1555 living fetuses, in all strains concerned here. Kalter (1968) and Palmer (1968) noted a seldom occurrence of congenital malformations in rats in contrast to other laboratory animals such as mice and rabbits. A recent study has resulted in that only 3 of 5766 fetuses of Sprague-Dawley rats were malformed (Mizutani et al. 1969), while another report showed the frequency of 1.5% in the study of 548 fetuses of Sprague-Dawley (Hasegawa and Noguchi 1971). Thus, the frequency and types of malformations differed from those obtained in the present study, due probably to the limited observations with non-inbred rats. The results of the present survey indicated that fetuses with cleft palate appeared at 4.3% of 94 living fetuses in the Long-Evans strain, while it was 0.5% of 185 living
fetuses in the W/Hok strain. Further, the frequency of external malformations of clubfoot and polydactyly was 2.0% of 553 living fetuses in the WKA/Hok strain. All the malformed fetuses were found to have unilateral or bilateral clubfoot, leaving only one showing hindfoot polydactyly. Clubfoot was rather frequent in female fetuses (8 females: 3 males), and further on left side of fetuses (6 on left: 4 on right: 1 on both), though the difference was insignificant. The abnormality of the urogenital organ occurring in ACI/NHok rats was agenesis of kidney combined with agenesis or hyperplasia of uterus appearing on the same side in 5 female fetuses (5.4%), and atrophy of testis and seminal vesicle on the same side in 9 male fetuses (11.9%). These anomalies occurred unilaterally on the right side of 11 fetuses and on the left side of 3 fetuses. The difference in the incidence of this anomaly was insignificant as to the sex or the side distinction of fetuses. Prenatal urogenital malformations of the ACI rats were reported by Fujikura (1970) and postnatal ones by Suzuki (1974). While the data in the latter report were limited, there is some similarity in the incidence of the malformations between the Fujikura's and my reports. Two abnormal characters of urogenital organ in the ACI/NHok strain and clubfoot in the WKA/Hok strain were also detected in my postnatal survey (unpublished). The aim of this study is to provide some data that can contribute to the establishment of mutant strains as animal models resembling human malformations.

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References