SKIN GRAFTS IN DONRYU RATS WITH AND WITHOUT ACQUIRED RESISTANCE TO YOSHIDA SARCOMA AND ASCITES HEPATOMAS*1

(Plates I~III)

Motoi ISHIDATE, Jr.*2
(Sasaki Institute)

Synopsis

1) A pedicle cross-skin grafting combined with a short-term parabiosis in normal Donryu rats was 92% successful over 100 days. Similar result was obtained in M520/N(F88) rats, a highly inbred American strain, which served as control. Thus, the genetic homogeneity of the Donryu rats was proved to be very high.

2) The Donryu rats have an extremely high susceptibility to Yoshida sarcoma or ascites hepatomas which originated in these rats. However, they show resistance to tumors after various immunization treatments. All of the cross-skin grafts between these resistance-acquired and normal Donryu rats were rejected by each side of their recipients within 100 days. The fact seemed to be dependent on different compatibility between the donor and host, assuming that histocompatibility system of the resistance-acquired rats has been modified through the acquisition of resistance to the tumors.

3) The Donryu rats, in which the grafts from the parabiosed donors resistant to a tumor were rejected, showed resistance to further challenge of the same tumor. A possible mechanism for this fact was discussed from an immunogenetical viewpoint, and transfer of the resistance during the parabiosis seemed to be a main causal factor.

INTRODUCTION

The fate of tissue grafts depends on an immunogenetic compatibility between the graft and the host. In mice, there have been found dominant histocompatibility genes that control "takes" of allogeneic grafts of both normal and neoplastic tissues, and much data are accumulating on the immunogenetics of cancer. In rats, however, only a few reports have been made on such an immunogenetic analysis of cancer transplantation, probably because of an insufficient supply of highly inbred animals. Bodgen and Aptekman pointed out that a hemmagglutinogen, designated R-1 by them, present in normal tissues of the rat determines the growth of tumor inoculum. Takeda and Aizawa indicated another histocompatibility system of the rat, R-x, which could be shared by both normal and cancerous cells of the rat. They stated that Yoshida ascites sarcoma or some transplant-strains of the ascites hepatoma in rats and Donryu rats were R-x positive.

*1 Major points of this paper were presented at the General Meetings of the Japanese Cancer Association, 1964 and 1965. This work was supported by a Grant-in-Aid for Fundamental Scientific Research from the Ministry of Education.

*2 Present address: Cancer Institute, Nishisugamo 2-chome, Toshima-ku, Tokyo (石間 基).
In Japan, the Donryu rats are now in wide use as recipients for various rat tumors. They are noted for an extremely high susceptibility to the Yoshida sarcoma\textsuperscript{11, 18, 22} and several ascites hepatomas.\textsuperscript{15, 23} However, their genetical characteristics have never been described.

The present author had a particular interest in their characteristics of high tumor-susceptibility from an immunogenetic view-point. The present study deals, first, with a study of the genetic homogeneity of the Donryu rats by means of skin grafting, second, the failure of skin-grafting between the Donryu rats with and without acquired resistance to the transplantation of rat ascites tumors, and third, susceptibility or resistance to the tumors in the Donryu rats in which the grafts failed.

**MATERIALS AND METHODS**

1) **Experimental Animals used**

**Donryu rats:** These rats were obtained originally from a pair of Japanese albino rats, through continuous sister-brother mating for more than 20 generations. The animals used in this study were supplied from the Central Laboratories for Experimental Animals, Tokyo. They are random bred within the strain for a few generations after continuous sister-brother inbreeding. When they are injected with $10^3$ or more cells of the Yoshida sarcoma or some ascites hepatomas of the rat, 100% of the animals are killed by the growth of these inoculated tumor cells.

**M520/N rats:** An inbred American strain, M520/N(F-88), obtained from the National Institutes of Health, U.S.A., were used.

**Osawa rats:** Japanese random-bred albino rats from the Osawa Animal Farm, Tokyo, were used also for the control experiment. The origin of these rats is unknown. They had been random bred for 12 years within members originally from 26 albino rats.

Throughout the present experiments, the animals of both sexes weighing 80~100 g were employed. They were housed in metal cages with saw-dust bedding. Semisynthetic cube diet, CE-2, and water were given *ad libitum* with supplemental greens.

<table>
<thead>
<tr>
<th>Tumor strain</th>
<th>Origination Year</th>
<th>Host</th>
<th>Intraperitoneal transplantation(^a) Host</th>
<th>Lethal take (%)</th>
<th>Survival days(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yoshida sarcoma</td>
<td>1943</td>
<td>Japanese albino rat (♀)</td>
<td>Donryu rat</td>
<td>100</td>
<td>8 ± 1</td>
</tr>
<tr>
<td>Ascites hepatoma, AH-66F</td>
<td>1956</td>
<td>Osaka rat (♀)</td>
<td>Donryu rat</td>
<td>100</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Ascites hepatoma, AH-122B</td>
<td>1961</td>
<td>Donryu rat (♀)</td>
<td>Donryu rat</td>
<td>100</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Ascites hepatoma, AH-64B</td>
<td>1964</td>
<td>Donryu rat (♀)</td>
<td>Donryu rat</td>
<td>100</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Ascites hepatoma, AH-64E</td>
<td>1964</td>
<td>Donryu rat (♀)</td>
<td>Donryu rat</td>
<td>100</td>
<td>15 ± 2</td>
</tr>
</tbody>
</table>

\(^a\) Ten most recent generations \hspace{0.5cm} \(^b\) Mean ± S.D.
2) **Ascites Tumors used**

The Yoshida sarcoma and 4 rat ascites hepatomas, AH–66F, AH–122B, AH–64B, and AH–64E, were employed. The original tumor of Yoshida sarcoma developed in a male Japanese albino rat in 1943. The ascites hepatoma AH–66F was derived from a hepatoma originating in an Osawa rat in 1956. The AH–122B is a tumor derived from a hepatoma originating in a male Donryu rat in 1961. The AH–64B and AH–64E were derived from different hepatomas originating in a female Donryu rat in 1964.

All these ascites tumors (Photos 8~11) have been kept by serial intraperitoneal transplantation in the Donryu rats. The time of establishment of these tumors, strain of the rat in which their original tumors had developed, rate of lethal takes, and survival time of the Donryu hosts are shown in Table I.

3) **Induction of Resistance to Tumors in Donryu Rats**

Normal female Donryu rats, 6~8 weeks of age, were immunized by injecting either attenuated tumor cells or lymphoid cells from animals which had acquired resistance to the tumor.

For the preparation of the attenuated tumor cells, ascites from the animals bearing the Yoshida sarcoma or ascites hepatoma was pooled and then washed twice with physiological saline, through repeated centrifugation. The sedimented tumor cells, $10^8$ as estimated, were suspended in Nitromin (nitrogen mustard N-oxide) (Yoshitomi Pharm. Ind., Ltd.) dissolved in physiological saline and kept for 30 mins. at 37°. The concentration of the Nitromin solution was 10 $\gamma$/ml for the Yoshida sarcoma and AH–66F, and 30 $\gamma$/ml for other ascites hepatomas. About $4 \times 10^7$ tumor cells were injected intradermally into normal Donryu rats. A single injection was sufficient in the case of Yoshida sarcoma, but 3 or more injections during 4 weeks were required in the case of the ascites hepatoma, in order to induce a marked resistance in the rats against the corresponding tumors. In each case, the injection of Nitromin-treated tumor cells was made at four separate sites in the same individual to produce an effective immunization. When, on rare occasion, the cells grew, forming a solid tumor nodule, the nodule was surgically removed within 2 weeks after the injection.

For the lymphoid cells, the peritoneal fluid, spleen, and/or thymic cells were taken from the Donryu rats which had acquired resistance to Yoshida sarcoma or AH–66F. Approximately $10^7$ of these cells were injected intraperitoneally into normal Donryu rats. Two weeks later, all the injected rats were challenged with $10^6$ viable cells of the same tumor, in order to detect whether they had acquired resistance. The Donryu rats which had acquired resistance by the above methods, will be designated simply as R-rats hereinafter in this paper.

4) **Free-skin Grafting**

Intra-strain skin grafting was performed between randomly selected members of normal Donryu rats. Full thickness, fitted grafts were made, following the technique of Billingham and Medawar.2

About 20 $\times$ 30 mm of the dorsal skin was cut off with a sharp curved scissors and the adhering fascial tissue was snipped away from its raw surface in a sterile, wet petri dish. The graft bed was prepared on the back of the recipient animal, about 15 $\times$ 20 mm in size, by removing both the epidermis and half of the dermal tissue. Care was taken to leave intact the panniculus carnosus and vessels running over it. An antibiotic
Mycillin (Kyowa Hakko Kogyo Co., Ltd.) was spread over the bed. The graft was placed in the bed, rotated 180° to make the hair of the graft lie in the direction opposite to that of the host body, and then held in place with continuous sutures (Photo 2). After that, the graft was dressed with a few sheets of sterile dry gauze and fixed with adhesive plaster tapes. Some of the recipient animals received a skin graft of their own tissue as an autologous control. The operation was carried out in a chamber provided with an ozon lamp inside (Photo 1). A schematic representation of the above grafting procedures is given in Fig. 1.

All the skin grafts were examined every 2 days. The dressing was removed on the 7th postoperative day. The period from grafting to the first evidence of crust formation in more than half of the graft was taken as the survival time of the graft. The observation was continued for more than 100 postoperative days.

5) **Pedicle Cross-skin Grafting in Combination with Parabiosis**

Animals of the same age, sex, and body weight were prepared in combinations of both normal to normal and normal to R-rats. Under Nembutal anesthesia the abdomen of each partner was clipped on the opposite side and rubbed with a depilatory cream. Then the skin of that area was sterilized with 70% ethanol and a Mercurochrome solution. As shown in Fig. 2, approximately 20 × 30 mm of a horseshoe-shaped pedicle of the skin was cut from each of the partners in the opposite direction from one another. The skins were cross-united on the back of the partners to join the pair in parabiosis. After 6 or 7 days, the two parabionts were separated so that the exchanged cross-skin graft was left on each partner. Then, a continuous suture was placed along the cut-edge of the separated grafts, and all the animals were injected with Mycillin, without any postoperative dressings being applied.

All the grafts were examined every other day until the 30th day after the separation and every 7 days thereafter. Some of the grafts were submitted to biopsy for histological studies when necessary. When the entire part of the epithelium of the graft became
necrotic its date was recorded as the end-point of graft survival. The grafts were observed for more than 100 postoperative days. The parabionts which accidentally became separated during the parabiotic periods were discarded.

6) Parabiosis without Pedicle Skin Grafting

An orthodox parabiotic union according to the technique described by Harris was achieved without the pedicle skin grafting between intact and R-rats or members of intact animals using those of the same age, sex, and similar body weight. As shown in Fig. 3, the abdominal wall was incised on the lateral side of each animal, and all four cut-edges were drawn together with one continuous suture in the same way as in the free-skin grafting. A pair of parabionts were fixed with strings of adhesive plaster tapes at their abdomen, hind legs of the opposite side, and tails. On the 3rd or the 6th day after the parabiosis, each of parabionts was separated under ether anesthesia, and the

![Parabiosis](image1)

![Cross-skin graft](image2)

Fig. 2. Cross-skin grafting

Pedicle graft from each parabiont, A and B, 20 × 30 mm each in size, is rotated 180° and joined with discontinuous sutures.

![Parabiosis without pedicle cross-skin grafting](image3)

Fig. 3. Parabiosis without pedicle cross-skin grafting

The abdominal wall is incised and four cut-edges are drawn together with a continuous suture in a combination of normal (A) and tumor-resistant (B) Donryu rats. The skin and muscle are joined with discontinuous sutures.
abdominal wall of each animal was closed with a continuous suture, while their skin and muscle were closed with several Michel’s clips. No postoperative dressing was made.

7) **Angiography in Parabionts without Pedicle Skin Grafting**

According to a modified technique of Eichwald and others,4) angiography was performed using both parabiotic combinations of the normal to normal or the normal to R-rats, in order to detect the transmissibility of circulating blood between the parabionts. One rat of a united pair was injected with 0.5 ml of 70% of Pyraceton (Daiichi Pharmaceut. Co., Ltd.), a contrast medium, in the tail vein at various intervals following the parabiotic operation, in order to demonstrate the shadow of the bladder of the partner on X-ray film.

8) **Transplantation of Tumors to Animals separated after Short-term Parabiosis**

Six days after parabiosis without skin grafts, the parabionts were surgically separated. Two weeks after the separation, each of the separated animals was inoculated intraperitoneally with $10^5$ tumor cells as follows: Each individual from the pair of the normal to R-rats was challenged with the same tumor as used for the previous immunization of the latter. Each animal from the pair of normal rats was challenged with each tumor strain exactly in the same way as the control. Furthermore, new Donryu rats were submitted to sham-operation without parabiosis and then inoculated with the same tumor cells as an additional control.

Proliferation of the inoculated tumor cells was checked on Giemsa-stained smear preparations at 4-day intervals. Successful takes of the tumors were determined when the host animals died of accumulated tumor ascites and/or nodular growths in the abdominal cavity. Animals in which the tumor cells did not proliferate and which survived without any signs of tumor growth were observed at least two months after the transplantation. Among these, the animals which showed resistance to Yoshida sarcoma and survived, were further transplanted with $10^7$ cells of other ascites tumors, in order to examine the nature of that resistance, to determine whether or not it was specific for the Yoshida sarcoma cells.

**Results**

1) **Free-skin Grafts exchanged between Members of Normal Donryu Rats**

Full thickness, free-skin grafts were exchanged between randomly selected members of normal Donryu rats using 23 males and 21 females. Sixteen of the 23 males received, simultaneously, autografts in exactly the same way. As shown in Table II, nearly half of the grafts failed to take. The results are summarized in Table II.

<table>
<thead>
<tr>
<th>Graft</th>
<th>No. of animals</th>
<th>Sex Recipient</th>
<th>Sex Donor</th>
<th>Survival days of graft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1~14 (%)</td>
</tr>
<tr>
<td>Intra-stain graft</td>
<td>23</td>
<td>♂</td>
<td>♂</td>
<td>52</td>
</tr>
<tr>
<td>Autograft</td>
<td>16</td>
<td>♂</td>
<td>♂</td>
<td>13</td>
</tr>
</tbody>
</table>

a) Incomplete takes

b) Complete takes
of the intra-strain grafts were rejected by the hosts of each sex within 2 weeks, and only 13% of the males and 25% of the females had long-surviving intra-strain grafts of more than 100 days, indicated as "complete takes" in the Table. As for the autografts, 13% were rejected within 2 weeks and 50% survived more than 30 days, but they were finally rejected within 100 days ("incomplete takes"). Only the remaining 37% of the autografts survived more than 100 days.

In some cases, the animals which rejected the intra-strain grafts within 2 weeks were again given grafts from the same donors. Some of these secondary grafts survived for more than 100 days. This means that the survival time of the free-skin grafts was greatly dependent upon the technical conditions of the operative procedure.

2) Intra-strain Cross-skin Grafts combined with Short-term Parabiosis in Donryu, M520/N, and Osawa Rats

The pedicle cross-skin grafting combined with various short periods of parabiosis was carried between randomly selected members of the same sex, age, and body weight within each rat strain.

In the case of intra-strain cross-skin grafts combined with 7-day parabiosis, only 3% of the Donryu grafts were rejected within 10 days, but 92% of them survived longer than 100 postoperative days, as shown in Table III. The M520/N grafts also showed as high as 95% long survival, while the Osawa’s grafts had a lower frequency of long survival and more than half of the grafts were rejected within 30 days. These results suggest that the Donryu rats are just as homogeneous as the highly inbred strain of M520/N. However, the Osawa rats are not uniform ones from a genetic point of view.

Table III. Results of Intra-strain Cross-skin Grafting combined with 7-Day Parabiosis in Donryu, M520/N, and Osawa Rats

<table>
<thead>
<tr>
<th>Strain of rat</th>
<th>No. of animals</th>
<th>Sex Recipient</th>
<th>Sex Donor</th>
<th>Survival days of graft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1~10 (％)</td>
</tr>
<tr>
<td>Donryu</td>
<td>16</td>
<td>＃</td>
<td>＃</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>：</td>
<td>Ａ</td>
<td>0</td>
</tr>
<tr>
<td>M520/N</td>
<td>20</td>
<td>Ａ</td>
<td>Ａ</td>
<td>0</td>
</tr>
<tr>
<td>Osawa</td>
<td>17</td>
<td>Ａ</td>
<td>Ａ</td>
<td>35</td>
</tr>
</tbody>
</table>

The intra-strain grafting in Donryu rats was also carried out between different sexes. As seen from Table IV, the grafts between male and female rats also showed as high a percentage of long survivals as those exchanged between the same sexes.

When the duration of parabiosis was limited to only 3 days, 50% of the Donryu grafts were rejected as early as in free-skin grafting, while with 5-day parabiosis the grafts showed an intermediate length of survival (Table V).

Table IV. Results of Intra-strain Cross-skin Grafting combined with 7-Day Parabiosis between Donryu Rats of Different Sexes

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>No. of animals</th>
<th>Survival days of graft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1~10 (％)</td>
</tr>
<tr>
<td>＃</td>
<td>Ａ</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Ａ</td>
<td>＃</td>
<td>12</td>
<td>0</td>
</tr>
</tbody>
</table>
3) **Inter-strain Cross-skin Grafts exchanged between Donryu and M520/N or Osawa Rats**

In each combination of the Donryu to M520/N or the Donryu to Osawa rats, all the grafts were rejected by the hosts of each side within 10 days, as shown in Table VI.

4) **Pathomorphological Findings of the Intra- and Inter-strain Skin Grafts**

When the donor was incompatible with the recipient animal, the graft was rejected very rapidly because of prompt interruption of the blood supply after separating the parabiotic union. In such a case, even when the pedicled skin appeared quite healthy and firmly connected with the recipient bed at the time of separation, the graft changed to a reddish brown color and swelled up, and then showed signs of necrosis within 10 days. Histopathologically, an intense inflammatory reaction proceeded in and around the graft and bed, but no vascularizations were seen (Photo 5). In other cases, the graft looked rather healthy during the first 2 weeks, but later it gradually became swollen. Hairs of the graft were diminished and the epithelium of the graft was replaced by ingrowing epithelium of the host animal. Finally, the graft shrank in size and lost its elasticity, gradually taking on an appearance of a smooth surfaced scab-like mass, composed of a piece of dead residue of the graft epithelium within 30 days. The histological examination of the graft at this stage showed a complete replacement by granulation tissues from the host with a thin epidermis and poorly developed appendages.

On the other hand, when the graft was compatible with the recipient animals a sign of the necrotic or inflammatory changes was extremely slight and the hair of the graft continued to grow in quite a different direction (Photo 3). Neither involution of the appendages of dermis nor any suppression of their development was evident. The junction between the graft and host skin edge was well organized by granulation tissue with much vascularization (Photo 4).
5) Pedicle Cross-skin Grafts exchanged between the Normal Donryu Rats and R-rats

Although most intra-strain grafts survived more than 100 days, the grafts exchanged between the normal Donryu rats and various R-rats were rejected within 100 days by almost all the hosts. Detailed data are shown in Table VII. As seen from the table, the

Table VII. Results of Cross-skin Grafting combined with 6-Day Parabiosis between Normal and Tumor-resistant Donryu Rats

<table>
<thead>
<tr>
<th>Rats resistant to tumors (agent used for induction of resistance)</th>
<th>Recipient (before parabiosis)</th>
<th>Donor of rats</th>
<th>Survival days of graft</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1~10</td>
<td>11~30</td>
</tr>
<tr>
<td>YS-resistant (Nitromin-treated cells)</td>
<td>Normal</td>
<td>Resist</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>21</td>
</tr>
<tr>
<td>YS-resistant (Spleen cells from YS-resistant rat)</td>
<td>Normal</td>
<td>Resist</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>8</td>
</tr>
<tr>
<td>YS-resistant (Thymic cells from YS-resistant rat)</td>
<td>Normal</td>
<td>Resist</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>9</td>
</tr>
<tr>
<td>YS-resistant (Peritoneal cells from YS-resistant)</td>
<td>Normal</td>
<td>Resist</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>17</td>
</tr>
<tr>
<td>AH-66F-resistant (Spleen cells from AH-66F-resistant)</td>
<td>Normal</td>
<td>Resist</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>10</td>
</tr>
<tr>
<td>AH-122B-resistant (Nitromin-treated AH-122B cells)</td>
<td>Normal</td>
<td>Resist</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>Normal</td>
<td>15</td>
</tr>
</tbody>
</table>

YS = Yoshida sarcoma  AH = Ascites hepatoma

results varied a little with different R-rats employed in the experiment. Within 10 postoperative days the animals which were normal before the parabiosis rejected 18% of the grafts from the R-rats which had been made resistant to the Yoshida sarcoma by treatment with attenuated Yoshida sarcoma cells. The remaining 72% of the grafts from these R-rats were rejected within 100 days. Conversely, the R-rats rejected 91% of the grafts from the “normal” donors within 100 days.

Similar phenomena were also observed in the case of another kind of R-rats. In the R-rats which had been immunized with spleen, thymus, or ascites cells from the Yoshida sarcoma-resistant animals, 38, 56, or 59%, respectively, of the grafts from the normal donors failed to take and became necrotic within 30 days, while 57, 83, or 51% of the grafts from these R-rats were also rejected, respectively, within 30 days by normal recipients of the each group. In addition, 40 or 75% of the grafts from the normal donors were rejected by the R-rats which had been immunized with spleen cells from AH-66F-resistant animals or those immunized with attenuated AH-122B cells. In these cases, more than 70% of the R-rat grafts on the normal recipients were also rejected within 30 days.

Histologically, the process of these graft-rejections took place rather gradually in each parabiotic partner. Such a delayed type of reaction in the graft rejection was also ob-
served when the Donryu rats were previously immunized with spleen cells of normal Osawa rats. As shown in Table VIII, 50% of the grafts from these immunized donors were rejected within 30 days by normal recipients, while 65% of the grafts from the normal donors were also rejected within 30 days by the immunized recipients. Table IX shows the result of skin grafting exchanged between the R-rats resistant to Yoshida sarcoma; 58% of the grafts were rejected within 30 days, while only 17% of those survived more than 100 days.

It may be worthy to note, however, that most of the grafts exchanged between normal Donryu rats were not rejected within 30 days even when one of parabiotic partners had been inoculated with lymphoid cells from other intact animals of the same strain.

Table VIII. Results of Cross-skin Grafting combined with 6-Day Parabiosis between Normal Donryu Rats and Those immunized with Spleen Cells of Osawa Rats

<table>
<thead>
<tr>
<th>Recipient</th>
<th>Donor</th>
<th>No. of animals</th>
<th>Survival days of graft (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Immunized</td>
<td>9</td>
<td>1~10 &lt; 1%</td>
</tr>
<tr>
<td>Immunized</td>
<td>Normal</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Table IX. Result of Cross-skin Grafting combined with 6-Day Parabiosis between Donryu Rats Resistant to Yoshida Sarcoma

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Sex</th>
<th>Survival days of graft (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>♂</td>
<td>1~10</td>
</tr>
<tr>
<td>25%</td>
<td>33%</td>
<td>25%</td>
</tr>
</tbody>
</table>

6) Angiography

Angiographic demonstration of the bladder of parabionts was performed by injection of a contrast medium, Pyraceton, into one of the parabionts at 2-day intervals. This was done using the union between the normal Donryu rats or the normal and R-rat resistant to Yoshida sarcoma cells. When the Pyraceton was injected intravenously to any of the united normal partner after 2 or more parabiotic days, the bladder of the injected animal was demonstrated in a clear shadow on X-ray film after 20 minutes. The bladder of the partner parabiont was shadowed first after 1.5 hours (Photo 6). These facts suggest that the circulating blood of the injected rat was transmitted to its partner. However, the angiographic shadowing of the bladder of R-rats was unsuccessful during parabiosis in almost all cases of the union between the normal and R-rats, when the Pyraceton was injected to the normal partner.

7) Transplantation of Tumors to Rats separated after Short-term Parabiosis

Both in the intact and afore-mentioned sham-operated Donryu rats, 100% takes of the Yoshida sarcoma occurred, and most hosts bearing the tumor died within 22 days with a slight difference of their survivals, as shown in Fig. 4 and also in Table X. The Yoshida sarcoma showed lethal takes in 72% of animals separated from parabiosis between the normal Donryu rats. No significant differences were detected in the survival time of the hosts in the 2 groups of 3-day and 6-day parabiosis.

In animals separated from the normal to R-rat union, however, Yoshida sarcoma showed a very low percentage of lethal takes. As seen in Fig. 5, when the R-rats which
were immunized by attenuated Yoshida sarcoma cells were used in this parabiosis the percentage was less than 5% in the separated R-rats, while it was 38% in their partners, at the end of 30 days. A significant difference exists between the above two separated groups in their susceptibility or resistance to the Yoshida sarcoma.

In addition, animals immunized with either the spleen or peritoneal fluid cells of other R-rats were also employed for parabiotic union with normal Donryu rats. In the animals which were normal before the parabiosis and which had been separated from the spleen-treated R-rats, lethal take of Yoshida sarcoma was 45%, while in those from the peritoneal cell-treated R-rats it was 60%. These percentages are slightly higher than those in rats of the corresponding side separated from the union of normal Donryu rats and the R-rats immunized with the attenuated Yoshida sarcoma, but significantly lower than those in the animals from the union of normal Donryu rats.

Table X. Lethal Take of Tumors in Donryu Rats separated from 6-Day Parabiosis and in Sham-operated and Intact Donryu Rats after Intraperitoneal Transplantation with $10^5$ Cells

<table>
<thead>
<tr>
<th>Transplanted into</th>
<th>Separated from parabiosis with</th>
<th>No. of rats</th>
<th>Yoshida sarcoma</th>
<th>Lethal take (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Normal</td>
<td>76</td>
<td>72</td>
<td>not tested</td>
</tr>
<tr>
<td>Normal</td>
<td>Resistant</td>
<td>117</td>
<td>38</td>
<td>71</td>
</tr>
<tr>
<td>Resistant</td>
<td>Normal</td>
<td>108</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Sham-operated</td>
<td></td>
<td>95</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Intact</td>
<td></td>
<td>81</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Fig. 4. Survival curves of animals separated from 3- or 6-day parabiosis of normal Donryu rats and controls, intact and sham-operated, after intraperitoneal transplantation with $10^5$ cells of Yoshida sarcoma

were immunized by attenuated Yoshida sarcoma cells were used in this parabiosis the percentage was less than 5% in the separated R-rats, while it was 38% in their partners, at the end of 30 days. A significant difference exists between the above two separated groups in their susceptibility or resistance to the Yoshida sarcoma.

In addition, animals immunized with either the spleen or peritoneal fluid cells of other R-rats were also employed for parabiotic union with normal Donryu rats. In the animals which were normal before the parabiosis and which had been separated from the spleen-treated R-rats, lethal take of Yoshida sarcoma was 45%, while in those from the peritoneal cell-treated R-rats it was 60%. These percentages are slightly higher than those in rats of the corresponding side separated from the union of normal Donryu rats and the R-rats immunized with the attenuated Yoshida sarcoma, but significantly lower than those in the animals from the union of normal Donryu rats.
Fig. 5. Survival curves of animals separated from 6-day parabiosis of either normal or normal and Yoshida Sarcoma resistant Donryu rats after transplantation of $10^5$ cells of Yoshida sarcoma.

--- Rats, Yoshida sarcoma-resistant originally (51 rats, ♀)
--- Rats, normal originally, united with the Yoshida sarcoma-resistant (29 rats, ♀)
--- Rats, normal originally, united with the normal (32 rats, ♀)

The resistant rats are those prepared by treatment with attenuated Yoshida sarcoma cells.

Fig. 6. Survival curves of animals separated from 6-day parabiosis of normal and Yoshida sarcoma-resistant Donryu rats after transplantation of $10^5$ cells of Yoshida sarcoma.

--- Rats, Yoshida sarcoma-resistant originally, peritoneal fluid cell-treated (19 rats, ♀)
--- Rats, Yoshida sarcoma-resistant originally, spleen cell-treated (11 rats, ♀)
--- Rats, normal originally, united with the peritoneal fluid cell-treated and Yoshida sarcoma-resistant (19 rats, ♀)
--- Rats, normal originally, united with the spleen cell-treated and Yoshida sarcoma-resistant (11 rats, ♀)

The resistant rats are those prepared by treatments with either peritoneal fluid cells or spleen cells from Yoshida sarcoma resistant rats.

Fig. 6. Survival curves of animals separated from 6-day parabiosis of normal and Yoshida sarcoma-resistant Donryu rats.
On the other hand, the Yoshida sarcoma showed only 20% lethal takes in the separated spleen-treated R-rats, while only 5% in the remaining peritoneal cell-treated R-rats. The percentage of takes in the former is 4 times higher than that in the separated R-rats which had been immunized with the attenuated tumor (compare Figs. 5 and 6).

In other words, the effect of R-rats on the normal parabiotic partners was correlated with the decreased susceptibility or increased resistance in the partners to the transplantability of Yoshida sarcoma. It is likely that the degree of susceptibility or resistance in the partners is dependent on that of the united R-rats.

When the R-rats resistant to ascites hepatomas, AH–122B, AH–64B, and AH–64E, of Donryu rat-origin were used in place of the Yoshida sarcoma-resistant R-rats, similar correlations were also observed. There were some differences, however, in the decreased susceptibility of both corresponding partners from the combinations of normal rats, or normal and R-rats depending on the different tumor strains employed. Detailed data are indicated in Figs. 7-9 and in Table X.

In a further experiment, Yoshida sarcoma was transplanted intraperitoneally into corresponding partners at an interval of 1, 2, or 4 weeks after the separation of parabiosis with R-rats. This resulted in 33, 38, or 55% lethal takes, respectively. The more prolonged the interval was after separation of the parabiosis, the more susceptible the animals were to the tumor.

An additional experiment was performed as follows. Ten million Yoshida sarcoma cells were inoculated subcutaneously into each parabiont of the combination between normal and R-rats, during or after parabiosis. A solid tumor developed only in the normal partner, but the rate of lethal growth was only 14% or less. This percentage...
Fig. 8. Survival curves of animals separated from 6-day parabiosis of either normal or normal and AH-64B-resistant, and sham-operated Donryu rats after transplantation of $10^5$ cells of AH-64B

The resistant rats are those prepared by treatment with attenuated AH-64B cells.

Fig. 9. Survival curves of animals separated from 6-day parabiosis of either normal or normal and AH-64E-resistant, as well as sham-operated Donryu rats after transplantation of $10^5$ cells of AH-64E

The resistant rats are those prepared by treatment with attenuated AH-64E cells.
was far lower than that of the control of normal to normal parabiosis, as seen from Table XI. This table also shows an average diameter of the tumors measured on the 10th day after transplantation. When the transplantation was done at an earlier time after the parabiotic operation the largest tumor was observed.

Table XI. Result of Subcutaneous Transplantation of Yoshida Sarcoma (4×10^7 cells) in Donryu Rats after Short-term Parabiosis with Animals Resistant to Yoshida Sarcoma

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Parabiotic partner</th>
<th>Parabiotic duration (days)</th>
<th>Days transplanted</th>
<th>Diameter of tumor nodules developed (mm)</th>
<th>Lethal take (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>YS-resistant</td>
<td>3</td>
<td>2nd parabiotic day</td>
<td>12±1</td>
<td>14</td>
</tr>
<tr>
<td>9</td>
<td>YS-resistant</td>
<td>7</td>
<td>2nd parabiotic day</td>
<td>7±2</td>
<td>11</td>
</tr>
<tr>
<td>8</td>
<td>YS-resistant</td>
<td>7</td>
<td>5th parabiotic day</td>
<td>7±3</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>YS-resistant</td>
<td>7</td>
<td>7th parabiotic day</td>
<td>4±2</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Normal</td>
<td>7</td>
<td>7th post-parabiotic day</td>
<td>8±2</td>
<td>38</td>
</tr>
<tr>
<td>9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>11±2</td>
<td>89</td>
</tr>
</tbody>
</table>

* Measured 10 days after transplantation, mean ± S.D.
YS=Yoshida sarcoma

As mentioned above, Yoshida sarcoma was rejected by the normal parabionts after separation from the union with the Yoshida sarcoma-resistant R-rats. To these animals, ascites hepatomas, AH-122B or AH-64B, and 3 variant sublines of the Yoshida sarcoma, LY-7, FY, or PY-2, were transplanted. As seen from Table XII, the host animals showed high susceptibility to these tumors. This may mean that the animals have no detectable cross-resistance to the tumors other than Yoshida sarcoma.

Table XII. Results of Transplantation of Ascites Hepatomas and Variant Sublines of Yoshida Sarcoma in Donryu Rats which acquired Resistance to Yoshida Sarcoma through Parabiosis with Yoshida Sarcoma-resistant Donryu Rats

<table>
<thead>
<tr>
<th>Tumor strain transplanted</th>
<th>No. of cells inoculated</th>
<th>No. of rats</th>
<th>Sex</th>
<th>Lethal take (%)</th>
<th>Survival days</th>
</tr>
</thead>
<tbody>
<tr>
<td>AH-122B</td>
<td>10^7</td>
<td>21</td>
<td>♀</td>
<td>94</td>
<td>24</td>
</tr>
<tr>
<td>AH-64B</td>
<td>10^7</td>
<td>12</td>
<td>♀</td>
<td>100</td>
<td>13</td>
</tr>
<tr>
<td>LY-7 (YS-variant)[40]</td>
<td>10^7</td>
<td>18</td>
<td>♀</td>
<td>100</td>
<td>41</td>
</tr>
<tr>
<td>FY (YS-variant)[19]</td>
<td>10^7</td>
<td>13</td>
<td>♀</td>
<td>85</td>
<td>14</td>
</tr>
<tr>
<td>PY-2 (YS-variant)[19]</td>
<td>10^7</td>
<td>25</td>
<td>♀</td>
<td>92</td>
<td>7</td>
</tr>
</tbody>
</table>

YS=Yoshida sarcoma

**DISCUSSION**

1) **Genetic Homogeneity of Donryu Rats, as revealed by Skin Grafting**

Silvers' view[19] is that a simple and reliable method for checking the genetic homogeneity of an animal stock, as regards histocompatibility genes, is an intra-strain skin
grafting among members of the stock. The Donryu rats, concerned in this study, are
Japanese albino rats of a closed colony which have been bred by sister-brother mating
for over 20 generations. Therefore, they can be accepted as an inbred strain of rat from
a theoretical viewpoint of genetics. However, the uniformity of these animals has never
been demonstrated by a reliable method.

The author performed, first, intra-strain skin grafting of the Donryu rats by the
technique of free-skin grafting, and second, pedicle cross-skin grafting combined with
short-term parabiosis. The result, however, showed a methodological superiority of the
latter in which 92% of the exchanged Donryu grafts survived more than 100 days in a
healthy condition.

Compared with the result of the free-skin grafting method, a question would arise
whether the results of parabiosis are correlated with the high percentage of long survival
of the grafts. Nakić and others\textsuperscript{13,14,16} reported that the survival time of skin allografts
could be prolonged by parabiosis of their hosts. In the Donryu rats, however, the free-
skin grafts of more than 100 survival days, i.e. "complete takes", were successful in only
20% in both intact and parabiosed animals. This suggests that the parabiosis does
not necessarily cause the prolongation of cross-skin grafts. The highly successful takes
of the grafts may be dependent on a sufficient blood supply from the donors and/or
an adaptation of the grafts on their beds during the parabiosis, rather than immunological
tolerance or neutralization induced between the parabionts.

Attention should be drawn to the fact that the Donryu rats have shown nearly the
same compatibility as the highly inbred M520/N rats (F–88). Therefore, the Donryu
rats can be accepted as a genetically rather homogeneous strain of the rat, as indicated
by the skin grafting technique. On the other hand, the Osawa rats have shown a hetero-
genecity, as revealed by only 47% "complete takes" of the grafts.

It may be worthwhile to add that in the Donryu rats the male skin grafts showed also
"complete takes" in the female recipients and vice versa. This suggests that the male
isoantigens\textsuperscript{7,25} play little or no part in the skin grafting of the Donryu rats.

2) Susceptibility or Resistance of Donryu Rats to Yoshida Sarcoma and
Ascites Hepatoma

Although the Yoshida sarcoma and the ascites hepatoma, AH–66F, are not tumors
of Donryu-rat origin, they killed all the Donryu animals when transplanted intraperito-
neally with $10^3$ or more tumor cells.\textsuperscript{23} According to Isaka,\textsuperscript{8} transplantation of only
a single cell of Yoshida sarcoma or AH–66F to Donryu rats was quite successful, and the
lethal take was 84% or 70%, respectively. Sato \textit{et al.}\textsuperscript{17} reported a high percentage of
lethal take of the Yoshida sarcoma, 86%, in the M520/N hosts, which were inoculated
with $2 \times 10^7$ to $10^8$ cells. As mentioned by Klein,\textsuperscript{12} when a large number of tumor
cells are being inoculated into an incompatible host, any antigenic differences between
the tumor and host resulting in unsuccessful growth of the tumor may become subliminal
and obscure. These observations suggest that the high susceptibility of the Donryu rats
to the tumors may depend not only on the genetic compatibility between the hosts
and tumors, but also on the number of tumor cells inoculated or antigenic changes of
the tumor cells during repeated transfers over a long period of time.
As for the ascites hepatomas of Donryu-rat origin, AH-122B, AH-64B, and AH-64E, all these tumors showed a high degree of compatibility with the Donryu rats, as revealed by 100% of lethal takes.

Despite the high susceptibility of the Donryu rats to these tumors, the animals could acquire resistance against the tumors by various methods of immunization. The Yoshida sarcoma was able to produce a greater transplantation immunity than the ascites hepatomas of Donryu-rat origin.

3) Unsuccessful “Take” of the Cross-skin Grafts exchanged between Normal and R-rats

Skin grafting from normal donors to R-rat recipients was unsuccessful. This suggests that the histocompatibility system of the recipient has been already modified by immunization with the tumor and/or lymphoid cells. Unknown weak histocompatibility genes were possibly activated in the R-rats by immunization, although they could be inactivated or tolerated in normal recipients during the short-term parabiosis with normal Donryu rats.

The skin-grafts exchanged between the R-rats were also unsuccessful. This fact was quite unexpected, and its mechanism is unknown. There might be a slight difference in the modified histocompatibility system of the R-rats, even though they showed resistance acquired against a tumor by the immunization exactly in the same way. Possibly, the animals could distinguish these slight differences by the skin grafting.

The recipients which had been originally normal but rejected the skin grafts from the R-rats showed a marked resistance to further challenge of $10^8$ cells of the same tumor as used for preparing the R-rats. This might be dependent on the effect of transfer of the resistance from R-rats to normal recipients during parabiosis, rather than an unspecified interference by the necrosis in the grafts which were being rejected.

4) Possible Transfer of Tumor-resistance from R-rats to Normal Recipients by Means of Short-term Parabiosis without Pedicle Skin Grafting

The possibility that the tumor-resistance is transferred by only a short-term parabiosis is supported by the following facts. The animals that acquired resistance to a tumor by parabiosis was confirmed to be rather specific for the tumor strains with which the R-rats had been immunized.

Bichel and Holm-Jensen suggested that the immunity was not transferred in xenogeneic parabions, but did in allogeneic parabions. Falls and Kirschbaum reported that the acquired resistance to an allogeneic tumor in inbred mice was transmitted between isogeneic partners in parabiosis. However, the present study demonstrated the successful transfer of acquired resistance to tumors by short-term parabiosis, even with the ascites hepatomas of Donryu-rat origin and Donryu rats, which are highly homogeneous and isogeneic to these tumors. In addition, the transfer could be readily accomplished through the transmission of body fluid rather than blood exchange between the parabions.

All of these results indicate that the resistance to tumors, either acquired or transferred in Donryu rats, causes unsuccessful skin grafting because an insufficient anastomosis developed between the graft and its recipient. However, the mechanisms of the acquisition of resistance against tumors and intra-strain skin grafts in the Donryu rats are still open to further immunoserological studies.
The author is indebted to Prof. Tomizo Yoshida, the Director of this Institute, for his helpful advice and criticism, to Dr. Hidehiko Isaka of Sasaki Institute, and Prof. Hidematsu Hirai of Hokkaido University, Sapporo, for their encouragement, and to Miss Keiko Inadome for technical assistance throughout the present work.

(Received May 13, 1966)

REFERENCES

6) Harris, M., *ibid.*, 3, 546 (1943).
8) Isaka, H., personal communication.
9) Ishidate, M., *et al., This Journal*, 56, 13 (1965).
10) Ishidate, M., Jr., *ibid.,* 57, 413 (1966).
EXPLANATION OF PLATES I~III

Photo 1. The operation box for skin grafting, in which an ozon lamp is furnished.

Photo 2. The skin graft fitted on the prepared bed, rotated 180° in position.

Photo 3. The successful "take" of a pedicle cross-skin graft, with healthy hairs pointed in a different direction. 120 days after the separation of parabiosis.

Photo 4. A histological picture of the junction between the graft and host skin in a successful case. The epidermis in part of the junction is continuous but slightly thickened. The dermis is replaced by a collagen tissue in which small vessels are intact but no necrotic changes can be seen in neighboring appendages. The 6th parabiotic day of normal rats in parabiosis. Azan staining.

Photo 5. A histological picture of the junction between the graft from Yoshida sarcoma-resistant donor and its originally normal recipient. The epidermis is necrotic and transformed partially into a scab-like mass. The dermis is replaced by fibrous tissue accompanied by an intense inflammatory reaction. The involution of its appendages or suppression of their development is marked. The 6th parabiotic day. Hematoxylin-Eosin staining.

Photo 6. Angiography of a pair of normal rats in parabiosis. A parabiont (right) received Pyraceton in its tail vein. The bladders of both parabionts are shadowed very clearly.

Photo 7. Angiography of a pair in parabiosis of normal and Yoshida sarcoma-resistant rats. No transmission of Pyraceton can be seen (compare with Photo 6).


Photo 9. A general view of ascites hepatoma, AH-122B. Phase-contrast, 7×40.

Photo 10. A general view of ascites hepatoma, AH-64B. Phase-contrast, 7×40.

Photo 11. A general view of ascites hepatoma, AH-64E. Phase-contrast, 7×40.