A number of reports have been made on the development of transplanted tooth germs in vivo. These studies have made major contributions to estimate the objective of developing and illustrate the growth processes of such anlagen under various environmental conditions.

Several investigators have already presented that the effect of estrogen upon the skeletal system of experimental animals have been confined to observations in the long bones (GARDNER, GAUNT, DAY & FOLLIS, REED, SILBERBERG, SIMPSON, SUTRO, URIST, WENTWORTH etc.) and that of a growth inhibitor of male experimental animals.

There is the suggestion that they stunt an activity of pituitary growth hormone but the mechanism of action of estrogen is not explicitly known. It has been noted that castration in the female found in greater body growth and in size of more increased various organ than would otherwise occur (GAUNT 1954).

FLEMING (1955, 1956) mentioned on the biological activity of embryonic tissue transplants such as tooth germs which become a functional part of the host through connective tissue attachments and vascular-formation in ovariectomized female guinea pig. He also considered that intraocular transplants of tooth germs in guinea pigs might be influenced by estrogens as he obtained larger grafts in ovariectomized than in intact hosts but obtained a higher per-centrage of takes in intact hosts.

GLICKMAN and SHKIAR (1954) reported in the effect of the systemic administration of estrogen upon the alveolar bone because of their earlier finding that, when administered with cortisone, estrogen appeared to stimulate bone formation which compensated for the osteoporosis of alveolar bone induced when the former drug was administered alone. They also stated that resulted in increased endosteal

* Work supported in part by United States Public Health Service Grant D-275 and by United States Public Health Service Senior Research Fellowship SF 223, and at Dept. of Anatomy, University of Texas Dental Branch, Houston, Texas, U.S.A.

This report was presented at the Congress of Japanese Stomatological Society in Sendai in May, 1960.
bone formation in the alveolar bone, when administered with estrogen to the animals (SHKLAR and GLICKMAN 1956).

On the other hand, GLASSTONE (1936) first contributed to find on the ability of tooth germs to organ culture in vitro. More recent researchers (DAY and FOLLIS 1941) have been successfully cultured these such as fetal tooth germs which are a mixed organ composed of ectodermal and mesodermal derivatives. Recently, LEFKOWITZ (1958) presented an effective method for organ culture of tooth germs in vitro, and also suggested the possibility of storage by a useful method for transplanting back to animals.

In connection with these studies, the developmental process of transplants to be functional parts of host animals may have difference between estrogen treated and castrated mice. In addition, it is first investigation to make observations on the appearance and behavior of developing tooth germs which are subcutaneously transplanted into animals previously cultured in vitro, and also an interesting observation is to compare the tooth germ grafts which have been existed in the spheres of hormonal influence with the tooth germ grafts, previously cultured in vitro, which have no hormonal effects. The present report concerns the effect of ovariectomy and of exogenous estrogen on isologous transplants of tooth germs in female mice both with and without previous culture as entire organs.

I. Observations.

Part 1.

1. Materials and Methods.

The host animals were Strong A female mice, from two to three months old, and weighing approximately 20g which were anesthetized with intraperitoneal injections of fresh nembutal. Seventy-nine hosts were divided into three groups treated as follows.

Group 1. Castrated. Host mice were shaved in the costovertebral areas and the ovaries were exposed and excised. Uterine horns were tied off with black silk before closing the incisions by michel wound clips. Seven days were allowed for recovery before transplants were made to axillae of these female mice.

Group 2. Estrogen treated intact. In a number of another series, the intact hosts were given intraperitoneal injections of 0.625mg of estrogen (Ovocylin in oil) once a week for seven weeks.

Group 3. Castrated estrogen treated. These hosts which were ovariectomized a week ago were given estrogen as same method as the above. All animals of each group were weighed once a week before time of treatment.

Dissection of the tooth germs.

The three hundred and ninetyseven tooth germs used were first molars derived from 17-day fetal Strong A mice. At this time, they were not yet calcified and could be satisfactorily removed from the surrounding connective tissues individually. The techniques of dissection of tooth germs have previously been described by YOSHIOKA and GONZALES (1959). The fetuses were removed from the mother and placed
in a sterile petri dish containing TYRODE's solution. The head was removed from the fetus and the mandible separated from the rest of the head. Then with the aid of a dissecting microscope, the first molars along with part of the maxilla or mandible were removed and placed in TYRODE's solution.

Transplantation.

An in vivo method was used to test the viability of the tooth germs, according to the procedure described by YOSHIOKA, by subcutaneous transplantations to the axilla of the mice. This was accomplished by making an incision on the back of the mouse and inserting the transplants. The tissue was deposited as far as possible from the skin incision. Michel wound clips were used to close the incision. These transplants were harvested 40 days later.

2. Results.

All seventy nine female mice as a host animals were weighed once a week for 6 weeks. The average weight of 25 castrated mice was 21.52 g at the time of transplantation of tooth germs and gained 1.98 g. The body weight of 27 estrogen treated intacted hosts gained 2.5 g from the average of 19.6 g. In castrated estrogen treated group, the average of nine animals was 19.83 g and increased 1.72 g. Eighteen intact hosts of 20.6 g average gained 2.28 g. The histological appearance of first molar at time of transplantation is shown in previous work, and the tooth germ contained no dentin or enamel.

1. Castrated group (Fig. 1).

Fig. 1. To show enamel epithelium layer, dentinal tubules and pulpal vessels in axilla transplant in untreated castrated mouse after 40 days. Donor tissue, first molar tooth germ, 17 day mouse embryo. ×80

Key to Figures: OE oral epithelium, OEE outer enamel epithelium, IEE inner enamel epithelium, SR stellate reticulum, UO undifferentiated odontoblasts, DP dental pulp, AB alveolar bone, E enamel, D dentin, O odontoblasts, PD predentin, BV blood vessels, P pulp, DEJ dentinoenamel junction, REE remained enamel epithelium, OG outgrowth.
Fifty one tooth germs were transplanted to the axillae of young adult female mice and 43 of these showed normal development (Table 1).

Table 1. Incidence of developed tooth germs and transplantation takes and location.

<table>
<thead>
<tr>
<th></th>
<th>Number of transfers</th>
<th>Number of developed</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrate treated</td>
<td>51</td>
<td>43</td>
<td>84</td>
</tr>
<tr>
<td>Estrogen intact</td>
<td>73</td>
<td>66</td>
<td>91</td>
</tr>
<tr>
<td>Castrated estrogen treated</td>
<td>36</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>Intact control</td>
<td>32</td>
<td>30</td>
<td>94</td>
</tr>
</tbody>
</table>

Table 2 shows the statistics of these studies with the histological quality and size of recovered transplants of each group.

Table 2. Statistics of the studies.

<table>
<thead>
<tr>
<th>Castrated takes</th>
<th>Estrogen takes</th>
<th>Castrated estrogen takes</th>
<th>Intact control takes</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++ 27</td>
<td>+++ 52</td>
<td>+++ 18</td>
<td>+++ 24</td>
</tr>
<tr>
<td>++ 8</td>
<td>++ 8</td>
<td>++ 6</td>
<td>++ 5</td>
</tr>
<tr>
<td>+ 8</td>
<td>+ 6</td>
<td>+ 6</td>
<td>+ 1</td>
</tr>
<tr>
<td>− 8</td>
<td>− 7</td>
<td>− 6</td>
<td>− 2</td>
</tr>
</tbody>
</table>

Total 51         Total 73       Total 36         Total 32

+++ Good growth, ++ fairly good, + poor growth, − no growth.

2. Estrogen treated intact group (Fig. 2, 3).

Fig. 2. To show dentinal tubules and pulpal blood vessels in axilla transplant in estrogen treated intact mouse after 40 days. Host animal received 7 intraperitoneal 0.625 mg injections of estrogen. Donor tissue from same litter mates as Fig. 1 and the same type of tooth germ. ×8

Fig. 3. Higher magnification of Fig. 2 to show revision of dentin, predentin and pulpal area. There is an active blood supply. ×320
Seventy three transplants were made, 66 of in this group showed excellent development (Table 1). The size of transplants in this group was not significantly different from those in the intact group. Histologically the transplants in this group were most complete in enamel and dentin formation. Pulpal areas showed a greater number of blood vessels than was found in other groups and the vessels were large and heavily engorged with red blood cells. The dentin formation presented the regular appearance of tubular dentin.

3. Castrated estrogen treated group (Fig. 4).

Out of 36 tooth germs transplanted to the axilla 30 appeared to take. The percentage of the growing number of the transplants was quite similar to that in the castrated group but the size of those transplants was larger than castrated group.

4. Intact control group (Fig. 5).

This group showed growth of 30 out of 32 transplants. Those transplants (histologically) were similar to those in the estrogen-intact group except for a lower number of blood vessels in pulpal areas.

In all of groups, but particularly estrogen intact and intact control groups, there was a heavy connective tissue capsule about the transplant and the number of vessels evident indicated an adequate blood supply.
24 W. YOSHIOKA:

Part 2.

1. Materials and Methods.

Dissection of tooth germ. The method was the same as that used in Part 1. Organ culture of tooth germs. Two hundred and five explants derived from 17-day mouse fetuses were cultivated in PUCK's N16* tissue culture medium containing 30% calf serum.

Dissected explants were rinsed in TYRODE's solution for a few minutes. Twenty tooth germs were placed into a 60 mm petri dish containing 10 cc of the tissue culture medium. The pH of the medium was adjusted to 7.6. These petri dishes were

Fig. 6. To show dentin and pulpal area in axilla transplant, previously cultured 8 days in vitro, in untreated castrated mouse after 40 days. Donor tissue from the same as Fig. 1. ×80

Fig. 7. To show dentin and pulpal area in axilla transplant previously cultured 8 days in vitro, in estrogen treated intact mouse after 40 days. Donor tissue from the same as Fig. 1. ×80.

* PUCK's N16 Medium:
1. Calf serum (30%) 30 ml/l.
2. Saline F 300 ml/l.
3. Double distilled H2O 400 ml.
4. Solution N16.

Phenol red 0.024 g, L-arginine HCl 0.750 g, L-histidine HCl 0.750 g, L-lysine HCl 1.60 g, L-tryptophan 0.400 g, B-phenyl-L-alanine 0.500 g, L-methionine 0.500 g, L-threonine 0.750 g, L-leucine 0.500 g, DL-isoleucine 0.500 g, DL-valine 1.00 g, L-glutamic acid 1.50 g, L-aspartic 0.600 g, L-proline 0.500 g, glycine 2.00 g, Penicillin 0.2×10⁶ U, Streptomycin 0.2 gm, Glucose 0.770 gm, NaHCO₃ 0.840 gm, pH 7.6.
placed in a wide mouth jar containing 5% CO₂ in air and the bottle was placed in
the incubator at 37°C. Explants were allowed to remain in the incubator for 3, 5,
6, 8 and 10 days of cultivation. The explants were photographed 3, 6, 8 and 10 days
after explantation. On the 7th day, the medium was changed and at that time tooth
germs were rinsed with TYRODE’s solution but the outgrowth was not dissected
from the explant.

1. Thirty-one explants were cultivated for three days and then transplanted
subcutaneously to host animals.

2. Thirty-seven explants were cultivated for five days.

3. Eighteen explants were cultivated for six days.

4. Twenty-two explants were cultivated for eight days.

5. Ninety-nine explants were cultivated for 10 days.

The medium was changed in groups 4 and 5 on the 7th day.

Transplantation. The explants in all groups were transplanted to both axillae of
host mice by the same technique as the described in Part 1. Two or three explants
together with the zones of outgrowth were transplanted to each axilla. Animals
were sacrificed at the end of 40 days and transplants recovered. All recovered trans-

Fig. 8. To show dentin and pulpal area in axilla transplant, previously cultured 10 days in vitro,
in estrogen treated castrated mouse after 40 days. This transplant was not yet completely formed
as the tooth the same as Fig. 1. ×80

Fig. 9. To show enamel epithelium, dentin and pulpal area in axilla transplant, previously
cultured 10 days in vitro, in intact control mouse after 40 days. This also was not yet com-
pletely formed as the tooth but still growing. Donor tissue from the same as Fig. 1. ×80
plants were fixed in 10 percent formalin, decalcified with 6 percent hydrochloric acid, sectioned and stained with haematoxylin and eosin. The animals in the castrated group were checked to be certain that none of the ovary had been missed in the original operation.

2. Results.

Of the 205 tooth germs that were explanted as organ cultures, 67 showed signs of differentiation and growth after transfer in vivo. The cultivated explants were divided into five groups at the 3rd, 5th, 6th, 8th and 10th days.

![Fig. 10. To show the tooth germ at the time of dessection from 17 day mouse embryo. Donor tissue was the same tooth germ with Fig. 1. ∼32](image)

Table 3. Incidence of developed tooth germs and transplantation takes and location, previously cultured in vitro.

<table>
<thead>
<tr>
<th></th>
<th>Number of transfers</th>
<th>Number of developed</th>
<th>Per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Castrate treated</td>
<td>75</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Estrogen intact</td>
<td>73</td>
<td>27</td>
<td>37</td>
</tr>
<tr>
<td>Castrated estrogen treated</td>
<td>8</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Intact control</td>
<td>49</td>
<td>17</td>
<td>35</td>
</tr>
</tbody>
</table>

In table 3 and 4, each group is listed together with the relative size of recovered transplants which were cultivated in vitro before transplanted in host animals. The connective tissue capsule about these transplants was very slight as compared with uncultivated transplants and the color was whitish. There seemed to be a
Studies on the Isologous Transplantation of Tooth Germs in Mice. II.

Table 4. Statistics of the studies.

<table>
<thead>
<tr>
<th>Castrated takes</th>
<th>Estrogen takes</th>
<th>Castrated estrogen takes</th>
<th>Intact control takes</th>
</tr>
</thead>
<tbody>
<tr>
<td>+++ 8</td>
<td>+++ 14</td>
<td>+++ 0</td>
<td>+++ 3</td>
</tr>
<tr>
<td>++ 5</td>
<td>++ 8</td>
<td>++ 0</td>
<td>++ 4</td>
</tr>
<tr>
<td>+ 8</td>
<td>+ 6</td>
<td>+ 2</td>
<td>+ 10</td>
</tr>
<tr>
<td>– 54</td>
<td>– 45</td>
<td>– 6</td>
<td>– 22</td>
</tr>
<tr>
<td>Total 75</td>
<td>Total 73</td>
<td>Total 8</td>
<td>Total 49</td>
</tr>
</tbody>
</table>

+++ good growth, ++ fairly good, + poor growth, – no growth.

lowering of antagonistic responses by the hosts to transfers.

1. 3 days cultivation.

A total of 31 explants were made, 21 of which were successful (Table 3).

Both epithelial and fibroblastic cells proliferated and these arose from the periphery of the explant. Epithelial outgrowth still survived this period cultivation. Fibroblasts survived for a longer period of time. The outgrowth settled to the grass interface. Little reduction in size of the explant was noted at this time (Fig. 11):×

Fig. 11. Photograph of living tooth germ cultured 3 days in medium containing 30% calf serum. Donor tissue was the same tooth germ with Fig. 1. Photographs of tooth germs in the small petri dish at the following intervals. ×32
2. 5 days cultivation.

Out of 37 explants transplanted to the axilla of hosts, 8 appeared to take. The outgrowth rapidly proliferated and the area was occupied by fibroblasts and stellate reticulum cells. Survival improved but the explant was gradually getting flat. Some of peripheral outgrowth showed degeneration and necrosis. The pH was adjusted to 7.6 originally but at this time the pH changed and dropped to below 7.0 as indicated by the color of phenol red changing to yellowish.

3. 6 days cultivation.

A total of 18 explants were made and none of them showed survival. The degeneration and the necrosis of outgrowth cells increased more than at 5 days cultivation although some fibroblasts and reticulum cells lay scattered on the outside of outgrowth. In these period of cultivation, odontoblasts were dotted in the area of the outgrowth. The explant still improved for getting flat on the surface of glass.

4. 8 days cultivation.

A total of 22 explants were transplanted to host mice, 7 of these appeared to take. Since the explant was refed with fresh medium at 7 days period of cultivation, the degenerating and necrotic cells of outgrowth were reduced and the area of enamel dentin junction was visible caused by the explant flatten.
Fig. 14. To show living tooth germ cultured 8 days in medium containing 30% calf serum, medium changed after 7 days. To show dentinoenamel junction and outgrowth cells scattered over. Donor tissue was the same with Fig. 1. ×32

Fig. 15. Photograph of living tooth germ cultured 10 days in medium containing 30% calf serum. To show great deal of outgrowth, dentino-enamel junction clearly and explant was flattened along the surface of glass. Donor tissue was same with Fig. 1. ×32

5. **10 days cultivation.**

Table 5.

<table>
<thead>
<tr>
<th>Refeeding medium</th>
<th>Castrate</th>
<th>Estrogen</th>
<th>Ca. Es.</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Days)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>100%</td>
<td>90%</td>
<td>80%</td>
<td>70%</td>
</tr>
</tbody>
</table>
Table 6. Incidence of Developed Tooth Germs in Mice by Using Organ Culture Method in Vitro.

<table>
<thead>
<tr>
<th>Location</th>
<th>Period</th>
<th>3 Days</th>
<th>5 Days</th>
<th>6 Days</th>
<th>8 Days</th>
<th>10 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of T.</td>
<td>Takes</td>
<td>No. of T.</td>
<td>Takes</td>
<td>No. of T.</td>
<td>Takes</td>
</tr>
<tr>
<td>Castrated</td>
<td>11</td>
<td>6</td>
<td>17</td>
<td>3</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>55%</td>
<td>18%</td>
<td>0%</td>
<td>33%</td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td>Estrogen intact</td>
<td>12</td>
<td>10</td>
<td>16</td>
<td>4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>83%</td>
<td>25%</td>
<td>0%</td>
<td>33%</td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>Castrated estrogen</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>63%</td>
<td>25%</td>
<td>0%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No. of T.: Number of Transplants.

Out of 97 explants transplanted to hosts 32 were successful. The outgrowth tremendously proliferated.

As shown in Table 5 and 6, the percentage of growing tooth germs which were cultured in vitro went down to zero percent on the 6th day but after refeeding on the 7th day the percentage of those growing rose again to 30% or 40%.

II. Discussion.

The major subject in this present study was the influence of hormonal treatment on isologous transplants of tooth germs in mice and on transplanted tooth germs which had previously been cultivated in vitro.

Several investigator have reported that the injection of estrogens inhibited or reduced the rate of increases in body weight. There is general agreement that large amounts of estrogens inhibit or stunt growth when injected over prolonged period although few investigations have been made on skeletal growth (GARDNER and PFEIFFER 1943).

In the present work, there was slightly difference among those groups in the percentage of tooth germ transplants showing better growth of estrogen injected intact group than castrate treated group both with and without previous culture in vitro (Table 1 and 3). It may be possible that the stimulation of tooth formation of transplants which occurred during five weeks in the host animals was found in the effect of estrogens. However, the effect of estrogens injected for longer periods than 50 days was not determined. SHKLAR and GLICKMAN (1956) reported the results in increased endosteal bone formation in the alveolar bone of mice by injections for a period of five weeks. Injections of estrogens for a ten week period resulted...
in a reduction in cellularity of the periodontal membrane and an inhibition of bone formation along the periosteal surfaces of alveolar bone.

This SHKIAR's work seems to be confirmed the almost similar results with the present work.

There was no significant difference in the size of the developed transplants among these groups but there were changes in the dentin of the estrogen treated intact group. Dentinal tubules were always clear in this group compared to others. When estrogen was injected, there was a remarkable increase of blood vessels within the pulpal area. This is quite similar to the previous study of FLEMING (1956). It has been reported by GARDNER (1940). that estrogens elicit hyperemia of certain body tissues and FLEMING explained that the formation of mineralized tissues by tooth germ transplants was not entirely dependent on the quantity of blood supply.

A comparison of the percentage of takes of direct transplants and of tooth germs previously cultured in vitro showed a ratio of approximately five to two. It was shown that tooth germs previously cultured in vitro for a limited time still have vitality for normal growth.

Both environment of the tissue culture medium and the host-transplant relationship in animals for each group affected the tooth germ transplants. This was indicated by the fact that the differentiation of the transplanted takes in vitro was almost similar to the takes which were directly transplanted to animals without in vitro method (Table 3). In table 5 the percentage of takes showed descent to the 6th day and then little elevated to the 10th day. Presumably this may be caused by the pH changing, waste or toxic substance which occurs from outgrowth cells, which undergo necrosis, at day by day and these explant cells may be damaged. Therefore, after refeeding medium on the 7th day, it seems to be released and the percentage of takes of transplants elevated again. On the 6th day there were no takes, but this is probably because an insufficient number of specimens were transplanted at this study. Because even on the 6th day, explants were able to keep vitality for developing from the point of view which explants recovered after refeeding. This study indicates that the percentage of takes may be increased by changing the culture medium within the 3th day.

The amount of the explant matrix formed after 8 days of culture corresponds to the amount of pre-enamel matrix and predentin which occurs before these tissues are calcified in vivo (Fig. 14). LEFKOWITZ and SWAYNE (1958) have observed that the transplantation of organ cultured tooth germs at intervals was successful and profitable and showed the possibility of dissection of the outgrowth which would otherwise undergo necrosis. The results of the present work with respect to the influence of necrotic outgrowth cells on growth of the explant itself may be in agreement with the previous study.

From this present observation, there are many suggestions concerning the effect of hormonal activity on the transplants and these previously cultured in vitro.
III. Summary.

1. Estrogen treated animals showed an exceptionally well developed blood supply in the pulpal area, but no other significant differences were observed between hormone treated and untreated animals.

2. Tooth germs grown in vitro for periods of up to 10 days were able to establish and were verified to still keep the vitality for normally growing in host animals.

Acknowledgments. I, author, wish to express my sincere thanks to Professor BABA of the Department of Pathology, Osaka Municipal University Medical School and to Dean Dr. I. NAGAI, Osaka University Dental School for their helpful suggestions to this study and to former Professor A. KIRSCHBAUM and Professor H. BROWNING for making it possible to work at the University of Texas Dental Branch. I wish to acknowledge to Dr. F. GONZALES of the University of Texas Dental Branch, who was most helpful in discussing many details.
間発育率の比較では共にほぼ同様の割合差が認められた。

Estrogen の骨格系に及ぼす影響はその発育を抑制するという報告が多い。しかし実験において直接的に、または組織培養を経て完にてホルモン無影響状態にて植かれた胎生歯牙を間接的に動物へ移植して estrogen が移植歯牙に及ぼす影響を観察し、それが組織に対し発育抑制の効果を示さず、むしろ極く僅少であるがその発育率を高めたいという結果を得た。これは勿論 estrogen の投与期間、量等により異なった結果を示すものと考えられる。各群の組織像においては著明な差を認めないが、estrogen 注射群では特に明瞭な象牙質細管形成が見られ、これは活潑な組織の発育を示すと共に他群に比し長期にわたり発育を続けるということが歯槽血管形成の旺盛さにより観察し得た。又直接移植及び組織培養後、間接移植歯発育歯牙と共に上記の所見を呈したが、その発育率の比は 5 対 2 の割合であった。これは培養後移植歯牙では発育エネルギーの低下を示したものであるが、とくに一例でもその発育を示すならば、10日間にわたる長期の体外培養歯牙においても移植後、なお生発育力を失わないことを意味するものである。他方移植時施肥総合してホルモンの影響下にあった直接移植歯牙と培養中ホルモン無影響状態にあった間接移植歯牙に対する estrogen の効果は殆んど大差なく、組織像で後者は僅かに発育の未熟な像を呈していた。組織培養中、歯芽は時間の経過と共に多くの out growth を培養器表面上に沿って増殖させ、従って歯芽そのものは flat 状を呈し培養 8日目にて容易に dentinoenamel junction を観察出来た。培養 6日目全群の歯牙発育率は 0 ％を示しているが、それはこの時期になると out growth 外郭細胞群の一部が壊死に陥り老廃物及び寄生物質を培養液中に放出、或いは又組織新陳代謝の結果培養液の pH を著しく変化させ多くの細胞に障害を与えるか、これらを死に至らしめる結果と考えられる。培養 7日目には新鮮培養液を refeed した為、その後の組織は再生し発育上昇を示している。以上の如く本研究においてマウス胎生歯牙の長期外培養後なお、その生育力を維持し、且つ estrogen 投与が移植歯牙の発育を抑制することなく、むしろ僅少ながら発育促進を示したという事実は今後この方面の研究に有力な手掛りを与え、且つ重要な基礎を作り得たと確信する。

 References.

