TESTIS GRAFTS IN A FROG AND THEIR RELATION TO SEXUALITY

BY

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(With Plate VI and Seventeen Text-figures)

There are at present two main questions in regard to testis grafting in the Amphibians, the first inquiring whether the Free-martin effect exists in the animal group or not, and the second inquiring why, when a piece of the testis is engrafted in a castrated male, ova sometimes appear in the regenerated testis graft.

It has generally been believed of Urodelans that the existence of the Free-martin effect is clear as shown in the results obtained from parabiosis and ingrafting experiments made by BURNS (1925, 1928) and HUMPHREY (1927, 1929, 1931). On the other hand, DU BOIS and BEAUMONT (1927) called attention to the fact that in the remainder of the testis of a castrated individual of *Triton cristatus* there were found many regenerated ova.

Regarding Anurans, matters are more complicated than the cases of Urodelans. In 1910 MEYNS published a paper on testis transplantation for *Rana fusca* = *R. temporaria*. According to him, small pieces of testes transplanted in the dorsal lymph sacs of castrated males were found to regenerate, showing multiplication of the germ cells, whereas those engrafted in normal males indicated their degeneration. He pointed out further that among these testis

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grafts a few contained, among regenerating seminiferous tubules, several ova probably newly regenerated. Afterwards (1912) he repeated the experiment on the same species, engrafting undifferentiated gonads of tadpoles on castrated adult frogs and came to a similar conclusion. However, LAUCHE (1915), who transplanted pieces of differentiated testes on young castrated frogs, could not confirm MEYNS' observations on the appearance of the ova. In connection with testis grafting from adult frogs of *R. temporaria* in tadpoles, WITSCHI (1927) came to the conclusion that they neither accelerated the sex-differentiation of the male tadpoles nor tended to inhibit the differentiation of the female tadpoles, and also stated that these grafts regenerate not only in males but also in females. Using the American frog (*Rana pipiens*), DEAL (1930) engrafted a small portion of testis beneath the dorsal skin of the castrated males. He did not find a single ovum in any of the twenty-two grafts. PONSE (1924) and WELTI (1928) observed a fair number of ova in regenerated testicular grafts transplanted in the adult toad (*Bufo vulgaris*). Recently MOSZKOWSKA (1932), studying *Bombinator pachypus*, perceived no ovum in testicular implants in the castrated toads. In regard to the Freemartin effects, WITSCHI's grafting experiment (1927) above mentioned was negative in result, but recently (1927, 1931) he succeeded in the parabiosis experiments to show positive results for *Rana sylvatica*, *R. aurosa*, and *Hyla regilla*.

The work was undertaken at the suggestion of Professor R. GOLDSCHMIDT, director of the Kaiser Wilhelm-Institut für Biologie, Dahlem, and was carried out mostly in his laboratory. I should like to express my cordial thanks to Prof. R. GOLDSCHMIDT for the facilities offered in his well-equipped laboratory which afforded a very pleasant environment for work, and for much precious advice given during this piece of research. I am also much indebted to Dr. K. STERN and Dr. KOLLER for helping me in various ways. Acknowledgments are also due to Mr. H. YAMAGUCHI for his assistance in making the photomicrographs.

Experiments

The frogs used in the experiments were caught in the neighbourhood of Berlin, comprising 174 individuals in all. The operation was begun at the beginning of October, 1930, and finished at the end of February, 1931. After narcotizing by ether, one or both lateral sides of the ventral skin and muscles upon the gonads were cut open to a length of about 10 mm and entire ovaries or whole testes were removed. Expecting the chemical differences and diverse effects of sex-hormones, a small piece of the testis about 2 mm long was transplanted in a) normal males, b) castrated males, c) normal females or d) castrated females on various localities such as, 1) the dorsal lymph sac, 2 the breast muscles, 3) the stomach wall, 4) the mesentery, 5) the eye sockets and 6) the livers. Before applying the grafts to the tissue of the hosts which would come into contact with them, the tissues of the host were destroyed by needles in order to facilitate vascularization. Moreover, the grafts were fastened to the tissue of the host by means of silk thread so as to come into contact with the latter on their cut surface. After that the muscle and the skin incisions caused by the operation were closed with silk thread. The frogs thus operated upon were numbered and placed in a sterilized dried jar placed in a dark room for three or four days. After the wound had healed, they were transferred into the wide common aquarium and remained there usually from two to three weeks, after which the grafts were taken out. These transplants were fixed in Zenker's solution and sectioned serially 8 mm thick, the sections being stained with Iron Haematoxylin. Owing to infection of bacteria and to other unfavourable conditions more than one half of the subjects were killed and, therefore, only 63 of the operated frogs survived for the above mentioned periods.

Grafts at the time of implantation

The process of the spermatogenesis in the frog is usually completed by the middle of September and then the seminiferous tubules

containing the spermatozoa, the spermatogonia and the Sortoli cells continue to remain in a quiescent state till the breeding season next year. As the operations were executed during October–February, the pieces of testes used as grafts were in a quiescent state (Pl. VI, Fig. 1). Near the periphery of the seminiferous tubules are scattered a few spermatogonia generally provided with a nucleus containing

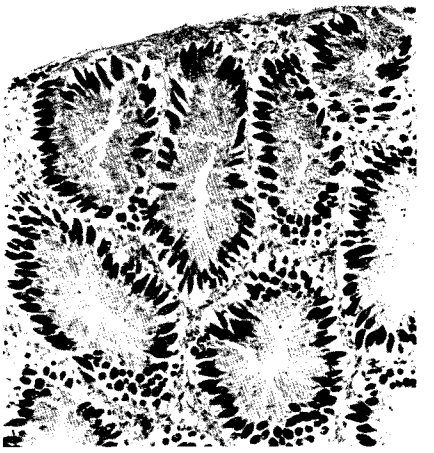

Fig. 1 Section of a normal testis at the time of implantation. ×100

generally one but often two or three nucleoli. No division phase was found in them. The spermatozoa brought together in bundles are radially arranged in the tubules, each spermatozoon having its head near the periphery of the tubule. At the base of each bundle of the spermatozoa there was generally found a Sertoli cell which makes with others of the same kind a sparse circlet on the periphery of the tube, intermingled with the spermatogonia. Outside the tubules there were found only a few interstitial cells and the spaces between these seminiferous tubes were very narrow.

General changes in grafts.

When the transplantation was successful, the wound became better about ten days after the operation, but some subjects were killed owing to bacterial infection. In most cases the transplants

taken out of the hosts became smaller and softer than at the time of engrafting, but some were found to have grown larger. The decrease of the size of ingrafts is due to contraction of the seminiferous tubules on account of destruction of the spermatozoa, while the increase is principally attributed to vigorous proliferation of the interstitial cells which sometimes intermingle with cells of the connective tissue intruded from the host into the ingrafts. The transplants were found generally in broad contact with the tissue of the host, but in some cases they were closely connected with the latter, often making nodules consisting of the connective tissue cells, the spermatogonia and the interstitial cells. The peritoneum of the engrafted testes generally existed except under some unfavourable conditions.

It is generally observed that the regeneration of these grafts is largely owing to sufficient blood supply; their peripheral portion is always in a better state than the central part which receives the least blood supply. As to the process of degeneration of sperm and spermatogonia, my observations generally agree with the description of MEYNS (1910). In those parts which are insufficiently supplied with blood, for the first time sperm bundles gradually become released and indiscriminately entangled. The tail of these decaying spermatozoa is torn to pieces, generally retaining rod-like or coiled forms. After this, their head disintegrates to many granules well stained by Haematoxylin. Following the degeneration of spermatozoa, Sertoli cells and spermatogonia become inactive and gradually destroyed. The residia resulting from the degeneration of these cells are afterwards absorbed by phagocytes and consequently the whole tissue is engulfed into the tissue of the host, when the graft is in close contact with the host (Fig. 7). Eventually a few survived spermatogonia are often found sparsely distributed in the connective tissue of the host, though the interstitial cells are not easily distinguishable from the cells of the connective tissue. When the graft is not well fused with the tissue of the host, the degenerated part is often represented by a fibrous structure without living cells.

When the blood supply is enough to cause regeneration of grafts, an almost similar phenomenon to that of regeneration occurs at first. In this case, however, the spermatogonia and the interstitial cells remain active, though the spermatozoa are, sooner or later, generally ruined. The degeneration of the spermatozoa takes place about ten days after the transplantation, but in some cases they were quite active more than two weeks after the operation (Fig. 6 and Fig. 8). After the degeneration of the spermatozoa the phagocytes clear up these residia and then the lumen of the seminiferous tubules become gradually evacuated and to some extent shrunk. In the meantime the spermatogonia and Sertoli cells gradually increase and consequently fill up the tubules (Pl. VI, Figs. 2, 3 and 4). I have often encountered seminiferous tubules packed with luteal Sertoli cells and spermatogonia (Pl. VI, Fig. 5). When the spermatozoa still remain living, they are often pushed inwards on account of the proliferation of the peripheral spermatogonia. Coincident with the change occurring in the siminiferous tubules, the interstitial cells outside it increase vigorously and the intratubular spaces become gradually broader (Pl. VI, Fig. 2). The process of the change is very similar to that taking place in the normal testis in the spring after the breeding season, as WITSCHI had already pointed out (1927). On account of the short space of time, in most of regenerated graft spermatogonia are found making cysts, but in one graft (No. 63) which resided for thirty days in a castrated male, the first spermatocytes have been regenerated, forming a cyst (Pl. VI, Fig. 6). As to the giant cells occurring in the siminiferous tubule of the graft, WITSCHI stated that "They (germ cells) also are separated from each other in most cases, although germ cells may coalesce and form giant cells. These then are polynucleated, all the more so because each single nucleus divides amitotically into a considerable number of caryomeres." My observations generally agree with him, but these polynucleated giant cells are also formed by multipolar division which has already been reported by LAUCHE (1913) as a pathological phenomenon. These cells become inactive and degenerate before long.

Among the ingrafts available for microscopical investigation, twenty three indicated regeneration, twenty one degeneration and nineteen intermediate conditions. Those engrafted in the stomach wall and the mesentery mostly showed good results, while those in the eye socket usually were in most unfavourable conditions. Transplantations in the liver were nearly negative in result. The ingrafts introduced in the lymph sac and the breast muscles give diverse results. The fact is probably due to external factors which are more influential on those localities than the others. So far as my observations go, the ingrafts were neither affected by the sexuality of hosts nor their condition, whether castrated or non-castrated. The results are summarized in the accompanying table (p. 162–163).

Relations between transplants and the localities in which they were engrafted

a) *Lymph sac.* MEYNS (1910) transplanted testicular pieces of *Rana temporaria* in the lymph sacs of both castrated and normal males. In his view these ingrafts were in quite distinct conditions according to whether they were obtained from the castrated males or the normal males. In the former cases not only spermatogonia and spermatocytes but also in a few of them ova regenerated, while in the latter the testicular pieces degenerated, being represented by a fibrous tissue.

The results obtained in this experiment were very variable; among twelve ingrafts belonging to the group, three were introduced into normal males, four into castrated males, two into normal females, and three into castrated females. The grafts in castrated males were not in exceedingly good condition, but, more or less, show regeneration, with the single exception of No. 99 which exhibits absolute degeneration. In No. 99 the seminiferous tubules degenerated, the spermatozoa broke into numerous minute flecks and short threads, and the spermatogonia and the interstitial cells generally disappeared, though only a few inactive spermatozoa are found in the seminiferous

No. of animal or graft	Age of graft	Sex of host		Condition of host		Graft incorporated in the					
		male	female	normal	castrated	lymph sac	breast muscle	eye socket	stomach wall	mesentery	liver
	days										
27	13	♂			C	!					
30	12		♀		C	!					
31	13	♂			C				+		
32	12		♀		C		!				
47	14	♂			C					+	
52	14	♂			C				⊕		
56	14	♂			C		!				
59	14	♂		N			—				
61	13	♂		N		!!					
62	14	♂			C				⊕		
63	14	♂			C					⊕	
64	14	♂		N				—			
65	14		♀	N		!					
69	14	♂		N					!		
70	14	♂			C					+	
78	14		♀		C				+		
79	14	♂			C			—			
87	15	♂		N		—					
89	14	♂		N		!					
94	14	♂			C						—
97	14	♂			C		—				
99	14	♂			C	—					
100	16	♂			C					+	
101	12	♂			C	!!					
103	14	♂			C						!
105	15	♂		N						+	
106	16	♂			C			—			
109	14	♂			C						!
112	14	♂		N			!				
114	19	♂			C		—				
115	15	♂			C	!!					
121	14		♀		C		+				
122	14	♂		N			!				
125	14	♂		N							!!

Continued

No. of animal or graft	Age of graft	Sex of host		Condition of host		Graft incorporated in the					
		male	female	normal	castrated	lymph sac	breast muscle	eye socket	stomach wall	mesentery	liver
	days										
127	14		♀		C				⊕		
129	14		♀		C	+					
130	14	♂			C		+				
132	14		♀		C						--
134	14		♀	N			—				
136	14	♂		N				—			
139	15		♀		C					+	
140	14	♂		N							!!
141	14	♂			C		—				
142	14		♀		C				⊕		
143	15		♀	N		+					
145	14	♂		N							!!
146	14		♀	N		—					
147	20	♂			C		—				
148	14		♀		C			!			
149	21		♀		C				+		
150	24		♀		C	+					
151	17	♂			C		⊕				
152	14		♀	N				—			
153	14		♀	N			⊕				
154	14		♀	N						+	
155	15		♀	N					!		
157	15		♀		C			—			
160	15		♀		C						—
161	14		♀	N							—
162	14		♀	N							—
163	15		♀		C					+	
164	15		♀		C			—			
169	50	♂		N						+	

The marks + (or ⊕), —, ! (or !!) denote regeneration, degeneration and intermediate condition respecitively, !! and ⊕ indicating better condition than ! and + respectively.

tubules arranged in the peripheral portion of the testis. As the degeneration of the tissue always occurs from the central portion of the ingraft, minute flecks derived from ruined spermatozoa are mostly found there, while in the peripheral portion decaying sperm tails still remain, generally spirally coiled. In this degenerated testis the minute flecks are gradually disappeared, leaving only the hyalin part devoid of living cells. In No. 101 and No. 115 the spermatogonia and interstitial cells are present in both central and the peripheral portions. In the central portion these cells are not active and seem not to be increased; the spermatogonia remain separately and the interstitial cells are small and mostly elliptical in shape, while in the peripheral portion the spermatogonia are increasing, making two or three circlets in the tubules, and the interstitial cells, narrow fusiform in shape, are larger than those of the central. Among the transplants taken from the normal males, No. 87 and No. 89 were on the whole not successful, though some spermatogonia are sparsely found in the connective tissue, making nodules together with the interstitial cells. However, No. 61 presents a better result; the spermatogonia and the interstitial cells are multiplying, and the spermatozoa still partially remain, forming bundles. As regards transplantation in the female hosts, results were more successful than in the male. No. 30 obtained from a castrated female was not in a favourable condition; the seminiferous tubules showing degeneration are filled with cells of the connective tissue in place of the spermatozoa and the spermatogonia, though the latter cells are, more or less, increasing in some limited localities. No. 129 and No. 150 also from castrated females present far better states, with many living spermatozoa, vigorously increasing spermatogonia, and interstitial cells. Out of them No. 150 was in close contact with the muscle of the host and provided with some follicle cells among regenerated spermatogonia. These follicle cells are easily distinguishable from the spermatogonia in having heller plasma, a simple (not lobed) nucleus and a smaller nucleolus of clear outline, while in the spermatogonia the nucleus is generally lobed and the nucleolus is larger and apparently obscure

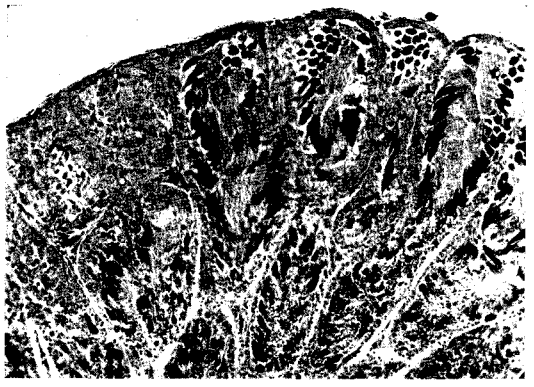

Fig. 2. Section of the graft (No. 151) incorporated with the breast muscles of a castrated male, showing regenerated seminiferous tubules on its free margin. ×100

living spermatozoa, increasing interstitial cells and spermatogonia in proliferation. The follicle cells are also present in No. 143.

b) *Breast muscles.* Fourteen testicular pieces transplanted in the breast muscle were divided into four groups: seven engrafted in castrated males, three in normal males, two in castrated females and two in normal females. Grafts No. 97, No. 114, No. 141 and No. 147, taken from castrated males, show degeneration, having only degenerated remains of spermatozoa but no spermatogonia and interstitial cells. In these ingrafts, however, the seminiferous tubules are mostly degenerated but a few are found to make nodules together with the interstitial cells in the connective tissue. Though No. 146 was negative in result, No. 143 was successful, having in outline. The grafts transplanted in normal females are three in number and different from each other in result. In No. 65

Fig. 3. Section of the graft (No. 151) incorporated with the breast muscles of a castrated male, showing proliferation of spermatogonia and interstitial cells. ×400

ever, the vestiges of seminiferous tubules are indicated by the presence of fragments of broken spermatozoa, since the fragments make elliptical groups, showing, though smaller, rough outlines of the former tubules in the connective matrix which has become thicker than that of the normal testis and lacks living cells. No. 56 shows, for the larger part, a similar phenomenon, but, in the portion attached to the muscles of the host, undergoes regeneration of the tissue, having living spermatogonia which are often in the process of division. No. 130 is more favourable in condition, provided with living spermatozoa and actively increasing interstitial cells. In the seminiferous tubules the remains of degenerated spermatozoa are not yet evacuated, and the spermatogonia, though somewhat increasing, are not so numerous. Differing from the grafts above mentioned, No. 151 also transplanted in a castrated male, shows a satisfactory result. In this graft there can be seen various progressive phenomena of regeneration, having many active spermatozoa, phagocytes in the process of absorbing remains of degenerated spermatozoa, increasing spermatogonia mingled with follicle cells, and vigorously proliferating interstitial cells (Figs. 2, 3 and 4). Among those engrafted in normal males, No. 59 is absolutely negative in regeneration, but No. 112 and No. 122 are provided with a few spermatozoa, increasing spermatogonia and many interstitial cells; the latter cells are actively increasing in the intratubular spaces not only outside the seminiferous tubules containing spermatogonia but also outside the degenerated tubules without living cells. In regard to implantation in normal females, the results are not especially different from the cases above given. In No. 32 the graft is for the most part degenerated but partially furnished with spermatogonia and Sertoli cells, neither of them showing active multiplication. However, No. 121 shows good regeneration. In the graft the spermatozoa are still active, and the spermatogonia and the follicle cells are vigorously increasing, the former often showing the phase of division. On account of exceedingly active multiplication of the interstitial cells the intratubular spaces are luxuriantly packed with these cells. Out of two grafts taken from normal

females, No. 134 shows degeneration of the whole tissue. On the other hand, the graft from No. 153 was extremely successful in transplantation, showing a huge increase of the interstitial cells in widened intratubular spaces and division of the spermatogonia, which, together with the follicle cells, fill up the regenerating seminiferous tubules situated in the peripheral regions. Moreover, active spermatozoa still arranged in bundles are present in fair numbers. As stated above, the results obtained from grafting in the breast muscles are quite different even in the identical condition of the host.

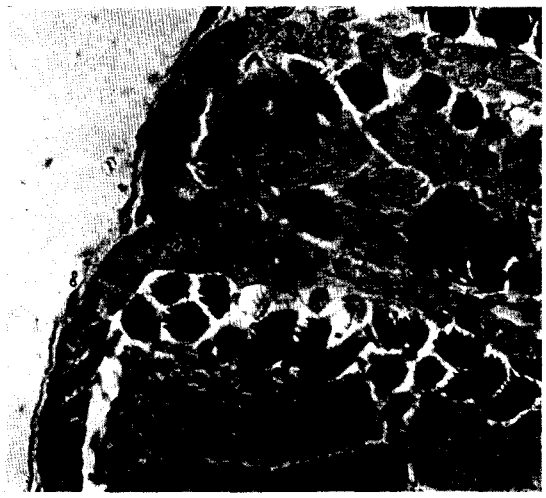

Fig. 4. Section of the graft (No. 151) attached to the breast muscles of a castrated male, showing presence of active spermatozoa and a phase of division of a spermatogonium. ×400

c) *Eye socket.* With regard to transplantation in the eye socket, the results all agree in that they were all (with one exception) altogether negative. Grafts in castrated males (No. 79 and No. 106) have seminiferous tubules containing flecks derived from degenerated spermatozoa, and rarely a few spermatogonia and follicle cells which, from their appearances, seem to be inactive. The seminiferous tubules of the grafts in normal males (No. 64 and No. 136)

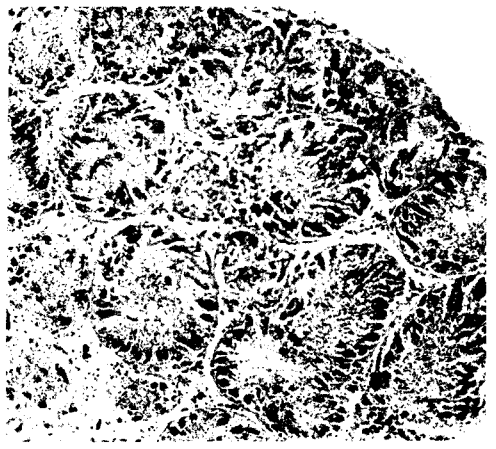

Fig. 5. Section of the graft (No. 106) placed in the eye socket of a castrated male, indicating degeneration of all seminiferous tubules. ×100

are utterly degenerated, pregnant with only minute granula produced by atrophied spermatozoa. Grafts taken from the castrated females (No. 157 and No. 164) exhibit entire degeneration; No. 148 also from a castrated female, though for the most part degenerated, has still living spermatozoa, slightly increasing spermatogonia and follicle cells on the peripheral portion. Transplantion on a normal (No. 152) entirely failed in regeneration.

d) *Stomach wall.* Testicular pieces joined with the stomach wall were mostly in good condition, though variable in extent and in degree of regeneration, always showing regeneration of the seminiferous tubules situated near the tissue of the host. Among those transplanted in castrated males, the graft (No. 31) was closely connected with the stomach wall of the host, cells of the connective tissue of which invade the intratubular spaces of the graft. The spermatozoa are all dead, trace of them remaining as minute flecks, and the spermatogonia and the interstitial cells are increasing especially on the surface attached to the stomach wall. In No. 52 as well as No. 62 the grafts underwent

Fig. 6. Section of the graft (No. 52) connected with the stomach wall of a castrated male, exhibiting regeneration of seminiferous tubules in its free margin and their degeneration in the axial portion. ×100

good regeneration. In these some seminiferous tubules contain living spermatozoa arranged in bundles, while some are already cleared up from them and full of regenerated spermatogonia and follicle cells (Fig. 6). In these grafts the seminiferous tubules became larger on account of the increase of these cells, therefore the interstitial spaces containing long fusiform interstitial cells have been narrowed. The graft taken from the normal male (No. 69) was closely joined with the tissue of the host. From the connective tissue of the host a large number of the mesodern cells are entering into the graft, making several nodules of cells adjacent to the surface fused with the host (Fig. 7). These cells are increasing towards the axial portion of the

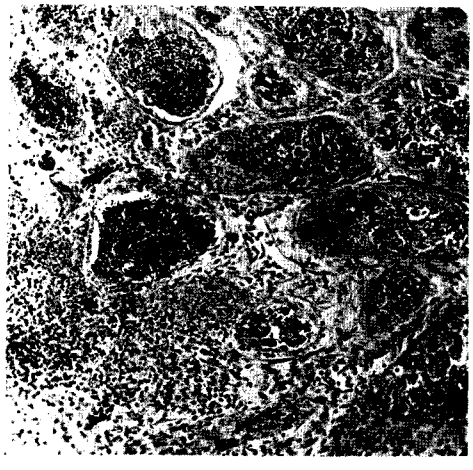

Fig. 7. Section of the graft (No. 69) joined with the stomach wall of a normal male, showing degeneration of seminiferous tubules in the axial portion and increase of interstitial cells. ×100

graft in which the degenerated spermatozoa remain as minute flecks thickly distributed. The spermatogonia and the interstitial cells near the free margin are slightly increasing. All the grafts transplanted in the castrated females (No. 78, No. 127, No. 142, No. 149) show active processes of regeneration. The graft from No. 78 is tightly connected with the tissue of the host, provided with many active spermatozoa, vigorously increasing interstitial cells and seminiferous tubules packed

with luteal Sertoli cells and spermatogonia. The grafts taken from No. 127 and No. 149 are similar in condition, having on the outer periphery seminiferous tubules containing many living spermatozoa, in the axial portion degenerated spermatozoa represented by minute flecks, and on the surface attached to the host many regenerating seminiferous tubules comprising numerous increasing spermatogonia, follicle cells, and interstitial cells which are increasing from the periphery towards the axial part of the these grafts. The graft of No. 142 indicate more pronounced regeneration, showing active spermatogonia and follicle cells: These cells fill the whole lumen of the seminiferous tubules and are increasing in those still containing active spermatozoa (Fig. 8). The intratubular spaces are very narrow and crowded with increasing interstitial cells. The graft from the normal female (No. 155) was, of this series of transplantation the worst in regeneration, but has a few living spermatozoa, interstitial cells, spermatogonia and follicle cells, the latter three increasing only on the peripheral portion.

Fig. 8. Section of the graft (No. 142) incorporated with the stomach wall of a castrated female, exhibiting presence of active spermatozoa and slightly increasing interstitial cells. ×160

e) *Mesentery.* Transplantations on the mesentery were in both sexes, normal and castrated, all successful. Ingrafts taken from castrated males (No. 47, No. 63, No. 70, No. 100) were well wrapped by the mesentery and indicate marked process of regeneration, having many active spermatozoa in bundles, seminiferous tubules containing the spermatogonia and the follicle cells increased, showing phases of division, and proliferating interstitial cells. In these grafts the appearance of the seminiferous tubules evidently differs according to the locality; on the outer area they still contain living sperma-

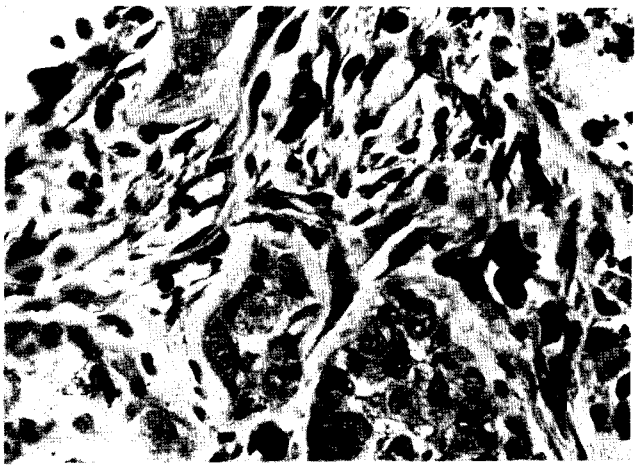

Fig. 9. Section of the graft (No. 70) fused with the mesentery of a castrated male, showing vigorous increase of interstitial cells. ×400

tozoa in bundles, a small number of spermatogonia and follicle cells just in the beginning of proliferation, in the axial portion they have flecks of degenerated spermatozoa, a few spermatogonia, and follicle cells in a state identical with that of the quiescent period, and in the portion united with the mesentery they contain proliferating spermatogonia and follicle cells instead of remains of the spermatozoa, which are utterly absorbed or of which slight trolls still remain. In these seminiferous tubules luteal Sertoli cells are accumulated together with the spermatogonia and follicle cells (Pl. VI, Fig. 5). Out of the four grafts, the one obtained from No. 63 is especially interesting,

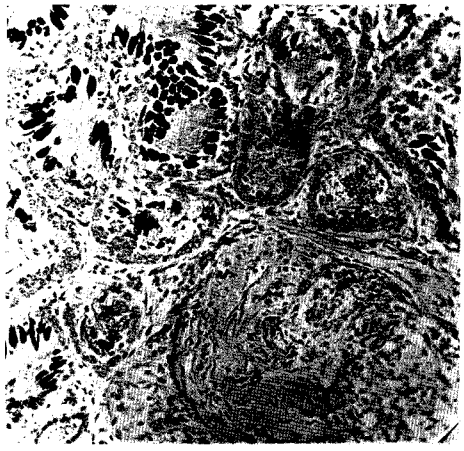

Fig. 10. Section of the graft (No. 63) joined with the mesentery of a castrated male, showing regenerated spermatogonia and interstitial cells, making nodules in the axial portion. ×100

present a cluster of cells consisting of interstitial cells, mesoderm cells, follicle cells and probably germ cells (Fig. 11). As to ingraftings in normal males (No. 105 and No. 169) transplants taken from them indicate good growth, furnished with many regenerated seminiferous tubules with increasing spermatogonia and follicle cells. There remain in No. 105 some living spermatozoa and ruminants of them (Fig. 13), but in No. 169, which resided for fifty days in the host, their remains have been already absorbed, leaving only seminiferous tubules in the because in its axial portion, in which degenerated spermatozoa have been mostly absorbed, mesoderm cells are increasing, forming nodules as shown in Fig. 10. Moreover, in seminiferous tubules situated on the peripheral edge of the graft are regenerated the first spermatocytes grouping in a cyst (Pl. VI, Fig. 6). On the peripheral portion, in which the tubules with their inclusions have been already degenerated and absorbed, is

Fig. 11. Section of the graft (No. 63) joined with the mesentery of a castrated male, showing a part of connective tissue containing interstitial cells and spermatogonia separately distributed. ×400

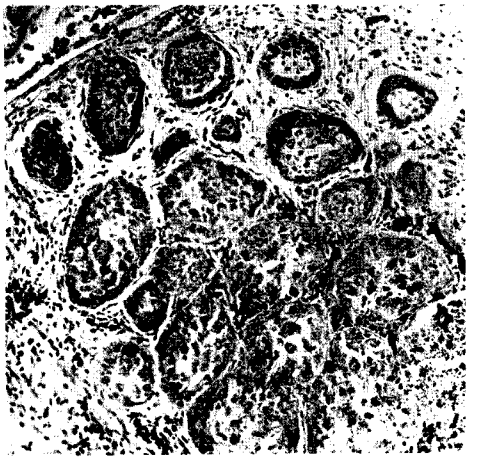

Fig. 12. Section of the graft (No. 169) attached to the mesentery of a normal male, indicating regenerated seminiferous tubules and increase of interstitial cells. ×100

process of regeneration, with proliferating spermatogonia, and follicle cells (Fig. 12 and Pl. VI, Fig. 2). The interstitial cells are vigorously increasing. Those engrafted in castrated females (No. 139 and No. 163) undergo regeneration, having active spermatozoa in bundles on the outer portion, degenerated spermatozoa in the axial part and regenerating seminiferous tubules near the area connected with the host (Fig. 14 and Pl. VI, Fig. 3). The interstitial cells are increasing in the narrow intratubular spaces. The graft taken from the normal female (No. 154) is generally similar to those transplanted in the castrated females (Fig. 15 and Pl. VI, Fig. 4).

f) *Liver.* According to WITSCHI (1927) the grafts joined with the liver of tadpoles were rather unfavourable in condition, as all these grafts showed regressive changes and nearly complete disappearance of the germ cells. In my experiment transplantations in this locality were generally unsuccessful in both sexes and also in castrated and normal individuals. The grafts in castrated males (No. 94, No. 103 and No. 109) were found to be similar in the degree of degeneration,

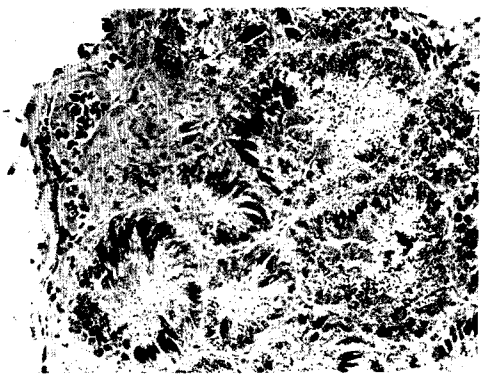

Fig. 13. Section of the graft (No. 105) incorporated with the mesentery of a normal male, exhibiting regenerated seminiferous tubules in the free edge. ×100

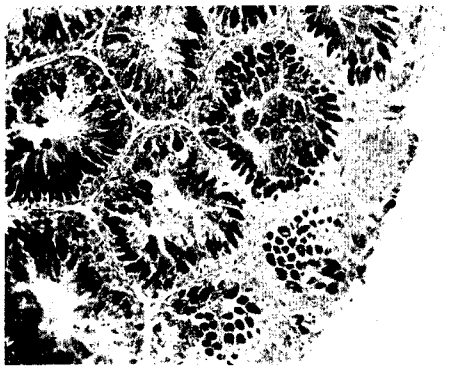

Fig. 14. Section of the graft (No. 169) connected with the mesentery of a castrated female, showing regeneration of seminiferous tubules situated in the peripheral portion. ×100

only provided with ruined seminiferous tubules and intratubular spaces without interstitial cells. Only in limited portions of their peripheral regions is there observable a slight increase of the spermatogonia and the follicle cells. Those engrafted in normal males (No. 125, No. 140 and No. 145) were somewnat better in condition than those transplanted in the castrated, being in possession of some living spermatozoa and in, more or less, advanced degree of regeneration. The transplants in castrated females (No. 132 and No. 160) indicate largely degeneration of the seminiferous tubules, with no living spermatozoa, but a few spermatogonia together with follicle cells, both of which do not exhibit proliferation (Fig. 16). In them the interstitial cells have disappeared. The testicular grafts made in normal females (No. 161 and No. 162) were both degenerated, the one taken from No. 162 having somewhat increasing spermatogonia in limited portions of the periphery (Fig. 17).

Conclusion

Judging from the results obtained in this experiment, it can be concluded that, so far as the testicular graft is resident during the

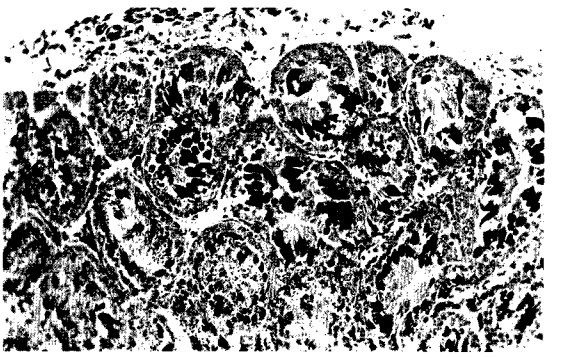

Fig. 15. Section of the graft (No. 154) joined with the mesentery of a normal female, showing regeneration of seminiferous tubules and increase of interstitial cells. ×100

Fig. 16. Section of the graft (No. 160) connected with the liver of a castrated female, showing some living spermatozoa but no sign of proliferation of spermatogonia. ×100

short limited periods in the host, there is no distinct effect of the sexual hormones of the host upon the graft. WITSCHI (1927) stated that the testicular graft from the adult transplanted in the tadpole of *Rana temporaria* regenerated independent of the sex of the tadpole. From these results it is noticeable that the Freemartin effect in the Anurans is not easily observable by the testicular transplantation. MEYNS (1910) pointed out that the testicular grafts transplanted in the lymph sac in normal male of the frog degenerated, while those in the same locality of castrated males showed regeneration. In my experiment there can be no distinct divergence between ingrafting in the normal and that in the castrated individuals. My grafts in the lymph sac gave very different results, some regenerated and some degenerated, as stated above. Moreover, MEYNS' experiment seems to be based on insufficient few data, and his conclusion seems to be somewhat premature. As to locality in which the transplantation takes place, some differences can be seen in regard to the result; in the eye-socket absolute degeneration of the graft, in the liver mostly degeneration,

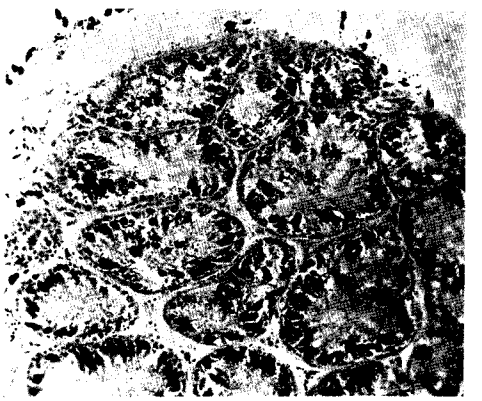

Fig. 17. Section of the graft (No. 161) fused with the liver of a normal female, showing degeneration of seminiferous tubules. ×100

in the lymph sac and the breast muscles varying results, and in the stomach wall and the mesentery generally regeneration. The regeneration of the graft is mainly due to adequate blood supply and consequently to good connection with the tissue of the host.

Though there are often found some large spermatogonia which will probably be degenerated after multipolar division, no real ovum could be found in the regenerated grafts. It is highly probable that the ova observed by MEYNS in the regenerated testicular grafts in *Rana temporaria* are derived from ova *a priori* existing in the tissue at the time of implantation, because the existence of ova in the normal testis of the adult frog, though rare, is already recorded by MAKINO (1931) on the Japanese race of the species to say nothing of the testes of young individuals belonging to the undifferenciated race of WITSCHI. Furthermore, the ova reported by PONSE (1924) in the regenerated testicular graft of *Bufo vulgaris* in the castrated males are probably derived from the ova already in existence at the time of ingrafting, since STOHLER (1928) pointed out the frequent occurrence of ova in the testis of the normal male of the toad. In all events it seems to be highly important to investigate the problem of whether these ova in the testis undergo the regular ovogenesis.

Summary

1) The testicular pieces of the adult frog are engrafted in the lymph sac, the breast muscles, the eye socket, the stomach wall, the mesentery, and the liver of the normal or castrated adults of both sexes.

2) Regeneration of the engrafted testes shows no distinct difference according to the sexuality or the condition, whether normal or castrated, of the host.

3) The grafts are well regenerated in the stomach wall and the mesentery, but degenerated in the eye socket and the liver. Those engrafted in the lymph sac and the breast muscles are very different

in results, some regenerated and some degenerated, probably being subjected to external influences.

4) No ovum is found in regenerated testicular grafts.

5) The ova hitherto described as regenerated in the transplanted testes are probably derived from ova existing in the graft at the time of transplantation.

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Plate VI

Explanation of Plate VI

Fig. 1. Section of a normal testis at the time of implantation. ×400

Fig. 2. Section of the graft (No. 169) fused with the mesentery of a normal male, showing a regenerated seminiferous tubule and increase of interstitial cells. ×400

Fig. 3. Section of the graft (No. 163) connected with the mesentery of a castrated female, showing regeneration of a seminiferous tubule in which residia of the spermatozoa still exist. ×400

Fig. 4. Section of the graft (No. 154) connected with the mesentery of a normal female, showing regeneration of seminiferous tubules. ×400

Fig. 5. Section of the graft (No. 63) joined with the mesentery of a castrated male, showing a seminiferous tubule containing luteal Sertoli cells. ×400

Fig. 6. Section of the graft (No. 63) joined with the mesentery of a castrated male, showing primary spermatocytes in a regenerated seminiferous tubule. ×400

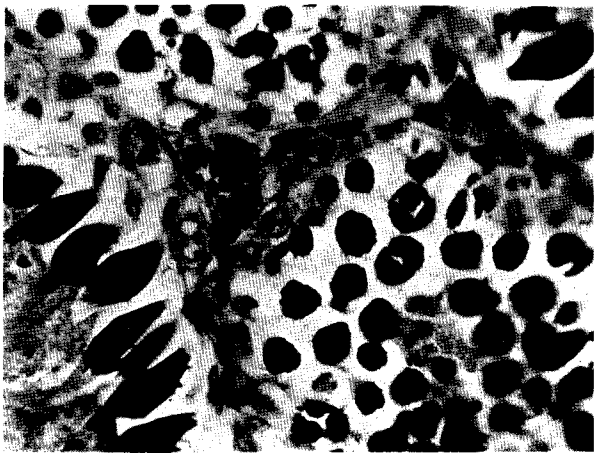

Fig. 1.

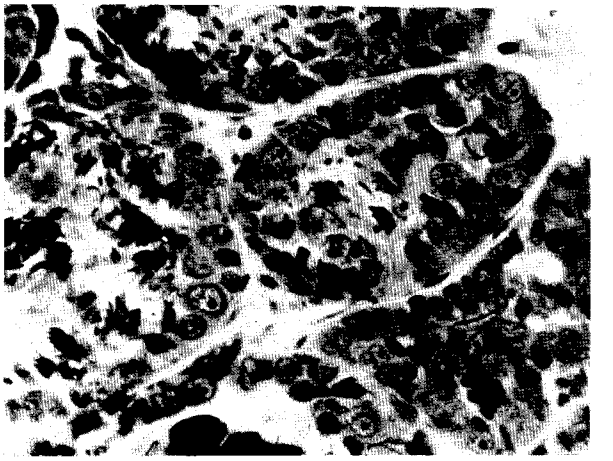

Fig. 4.

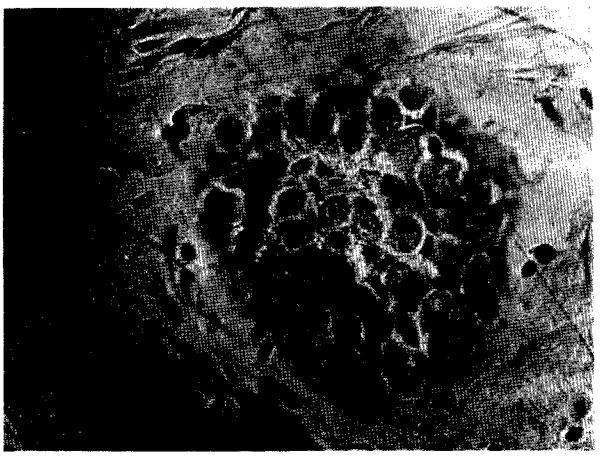

Fig. 2.

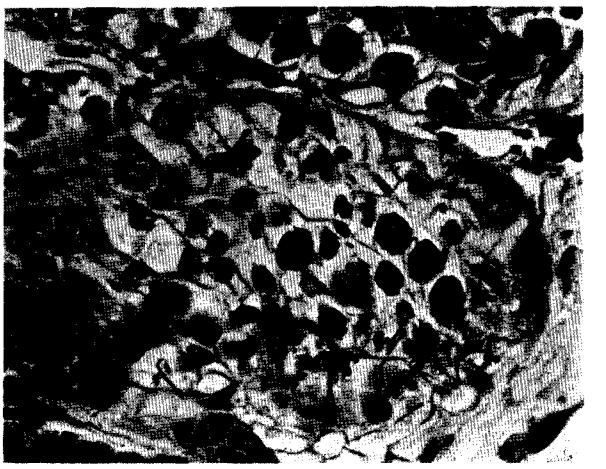

Fig. 5.

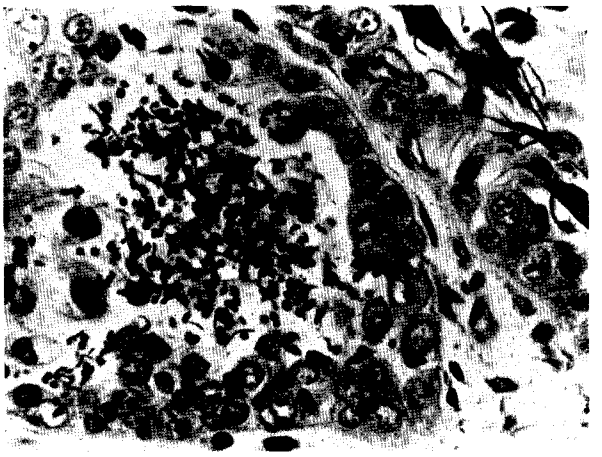

Fig. 3.

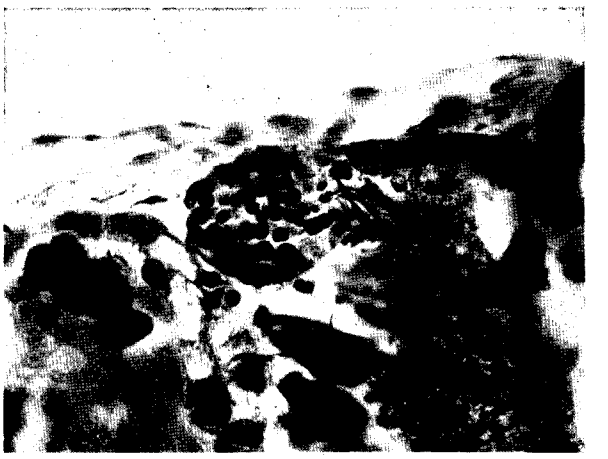

Fig. 6.

T. Uchida: Testis Grafts in a Frog and their Relation to Sexuality

H. Yamaguchi photo.