Masculinization by Testicular Transplantation in Spayed Female *Triturus pyrrhogaster* with Remarks on Testis-ova and the Modification of Lateral-line Organs[1)]

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(With Plates VI-VII, 1 Table, and 11 Textfigures)

Introduction

The present paper deals with the masculinization effects which have been secured in the spayed female of *Triturus pyrrhogaster* by means of grafting of the testicular tissue of the same species. Experimental work of this nature in urodeles designed to test the existence of a close correlation between sexual characters including all the somatic differences between the two sexes and gonadal secretion has already been carried out by du Beaumont. His full results were published in 1929, providing the first conclusive evidence of this correlation in *Triturus cristatus*. In the same year, Noble and Pope showed in the more specialized *Desmognathus fuscus fuscus* that transplantation of the testis into the adult female after spaying results in the development of pelvic, abdominal, and cloacal glands characteristic of the male, and also in the replacement of the female teeth by the male ones. Adams ('30) working on *Triturus viridescens* was successful in that hypertrophy of the rudimentary Müllerian ducts of the castrated males occurred under the influence of the grafted ovarian tissue. Obviously, the evidence in favour of feminization effects of the ovarian tissue will contribute to the present inquiry with reciprocal benefit. The male somatic characteristics which could be made to arise in the spayed female body of this newt are to be

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found in the following structures: the integument and its glands, the tail, the accessory sex-apparatus, the collecting ducts of the lumbar kidney, and the cloaca where the development of three sets of glands of the male (pelvic, cloacal, and abdominal) and the cloacal papilla are remarkable. Thus when the experiment was successful, the masculinization effects of the testicular tissue could be demonstrated almost altogether satisfactorily.

In 1936, Dawson proved a surprising dependence of the lateral-line sense organs in *Triturus viridescens* on endocrine factors and he has been the primary exponent of the view that conditions of the lateral-line organs of *T. viridescens* in different modes of living are under the control of an anterior pituitary-thyroid mechanism. In this connection, the regular occurrence of enlarged lateral-line organs which rise above the surface of the general epidermis of the masculinized females of this experiment deserves special mention. Now, an endocrine (gonadal) influence may be responsible for hypertrophy and hyperplasia of the organs which are under the control of the grafted testis instead of the normal ovaries.

Among 7 testicular grafts which were growing healthily in the host body and exerted masculinizing influence upon it, the testis-ovum within the seminiferous tubule was found in 6 cases. In both cases found in No. 17 and No. 18 masculinized females, that part of oviform transformations of the male germ cells predominates the area in which normal spermatogenesis can be secured. Although proper interpretation of the nature of these cells and the significance of their appearance is not an easy task, it is felt that a brief description of the morphological process should be made. The author wishes to express his appreciation to Prof. T. Uchida for his interest and very helpful criticism.

Material and Methods

Adult females of *Triturus pyrrhogaster* were used for this experiment. First of all, the removal of both ovaries with fat-bodies from the animals, 52, in all, was performed in January and in March, 1934. Among them, 34 were submitted to the second operation for testicular grafting in January and March of the next year, 302 to 444 days after being spayed. The animals throughout the course of the

experiment were kept in separate glass dishes containing water and were fed with meat and liver.

Before operating, the animals were anesthetised in 0.2% solution of chloretone. A small longitudinal slit was made on the right side of the posterior ventral surface of the body. Through this slit a small testicular piece, consisting of gonia and cytes, from the adult male of the same species was fixed with silk thread to the mesovarium which was left in place at the time of ovariotomy (26 cases), or to the cut-surface of the right oviduct which was leveled with the mesovarian site (8 cases).

All of the experimental animals were completely safe from any unfavourable effects of the operations. After a fairly long period, in the autumn of 1935, some of the animals were gradually acquiring male characteristics. After masculinization with all its changes in the external features had been secured, the animals were dissected and fixed *in toto* in Bouin's fluid and the grafted testis, the urinogenital system, the cloaca, and the lateral-line sense organs were later removed for microscopic study. The stains used were Delafield's hematoxylin and eosin.

Experimental Results

As stated above, 34 spayed females were used as the animals to receive testicular transplantation. After spaying from 302 to 444 days passed before they were transplanted with a testicular piece. Whether or not the lapse of such long periods of time between the removal of the ovaries and the testicular transplantation was instrumental in securing the decisive results of masculinization in this experiment, was not determined here.

Six of the animals were killed within 83 days after the grafting of testicular piece. Without taking into account the effects which might be expected later upon the host body, the conditions of the engrafted pieces in these cases were examined. Thirteen of the animals died at intervals of from 195 to 408 days after grafting. In some of them, at autopsy, was found a small amount of testicular tissue that still remained. But in others it had already been absorbed. Henceforth the remaining ones (15 in number) will be considered (Table I). The most successful cases in this experiment are found in animals Nos. 17, 18, 19, 20, 32, and 47. The animal No. 16 is next

TABLE I

Specimen No.	Site of graft	Duration of experiment		Observation	
		Days after spaying, date of grafting in parentheses	Days after grafting, date of dissection in parentheses	Effect on sexual characters	Development of testis-ova in graft
No. 16	Oviduct	443 (28/III/'35)	328 (19/II/'36)	Masculinization with all its changes, but nuptial coloration was not developed	Groups of testis-ova in seminiferous tubules
No. 17	,,	443 (28/III/'35)	454 (24/VI/'36)	Masculinization with all its changes	Predominating growth of testis-ova
No. 18	,,	444 (29/III/'35)	312 (4/II/'36)	,,	,,
No. 19	,,	444 (29/III/'35)	346 (9/III/'36)	,,	Single testis-ovum
No. 20	,,	444 (29/III/'35)	194 (9/X/'35)	,,	Did not occur
No. 27	Mesovarium	304 (21/I/'35)	304 (21/XI/'35)	No apparent influence	,,
No. 32	,,	304 (22/I/'35)	399 (25/II/'36)	Masculinization with all its changes	Groups of testis-ova in seminiferous tubules, more than No. 16
No. 36	,,	303 (23/I/'35)	526 (2/VII/'36)	Only slight indications of masculinization	Did not occur
No. 39	,,	303 (23/I/'35)	408 (6/III/'36)	No apparent influence	(Graft is absorbed)
No 41	,,	302 (23/I/'35)	422 (20/III/'36)	,,	(,,)
No. 43	,,	305 (26/I/'35)	419 (20/III/'36)	,,	Did not occur
No. 46	,,	304 (26/I/'35)	389 (19/II/'36)	,,	(Graft is absorbed)
No. 47	,,	304 (26/I/'35)	479 (19/V/'36)	Masculinization with all its changes	*Single testis-ovum
No. 51	,,	304 (26/I/'35)	418 (19/III/'36)	No apparent influence	(Graft is absorbed)
No. 52	,,	304 (26/I/'35)	537 (16/VII/'36)	,,	(,,)

* The fixation of this graft resulted in failure. Only a part of it was available for microscopic study.

to them in the degree of masculinization. In these cases the type of the tail ordinarily characterising the male is striking, and the rest of the experimentals (respective number is 27, 36, 39, 41, 43, 46, 51, and 52) in which no apparent changes in the tail have been secured, are considered as 'negative response'. At the time of autopsy, a thorough search was made for any remnant of ovarian tissue which might have been left behind and regenerated. None of the tissue was found in any of the masculinized individuals (7 in number). Among 8 individuals which were classed as 'negative response', No. 43 and No. 46 animals contained some ovarian tissues. In No. 43, a testicular nodule was also present, but in No. 46 it had been completely degenerated and not a trace of grafted tissue was found at the time of autopsy. Three animals (numbers 39, 51, and 52) were those in that the grafts had been consumed. In every case of the remaining 3 animals (numbers 27, 36 and 41) which belonged to the group of 'negative response', however, a grafted testicular piece of considerable size was found.

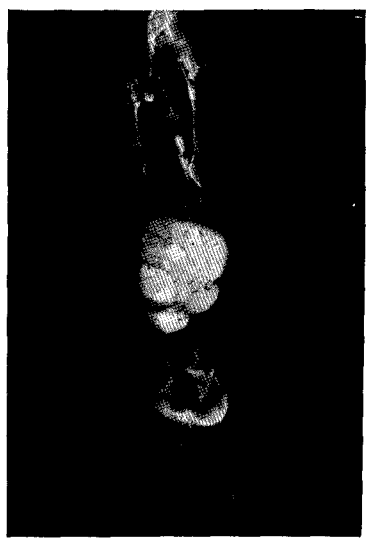
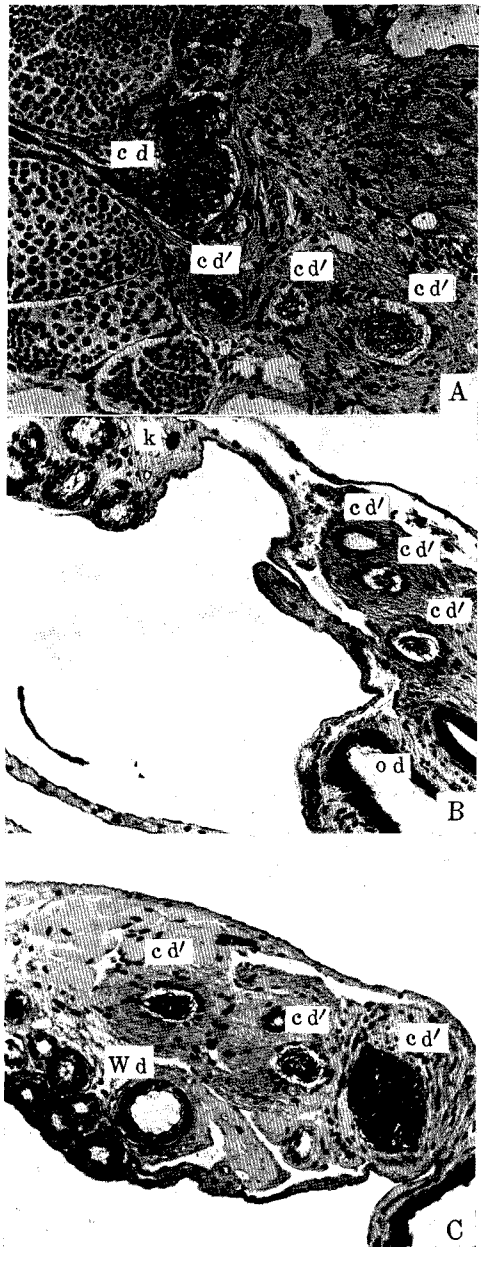


Fig. 1. Photograph showing a regenerated testicular graft in No. 17 masculinized female. ×1.5, about.

In those animals in which the distinct symptoms of masculinization have developed, the testicular pieces usually become well-vascularized and develop up to the average size of a testis of this species or even surpass it. Some are ovoid with smooth contour, and others exhibit an irregular lobulation (Fig. 1). No matter whether ovoid or lobulated in shape, the internal organization of the recovered testis has shown a certain regularity in the formation of seminiferous tubules in which the germ cells are well maintained, and the collecting ducts filled with spermatozoa have developed. Moreover, in one case (No. 19), the collecting ducts pass out of the graft as small ducts which are filled with spermatozoa and extend posteriorly until

they taper off near the dorsal surface of the genital kidney (Fig. 2, A, B, and C).



Although the number of animals is rather insufficient for any quantitative treatment, it is to be noted that the experiments are successful in all of 5 cases in which the grafts have united with the oviduct and have remained for periods of 194 to 454 days, whereas out of 10 grafts of the mesovarian site only 2 flourishing ones have produced the distinct changes in the host body.

Fig. 2. A. Photomicrograph of a portion of a section through regenerated testicular graft in No. 19. Some of the adjacent connective tissue which is growing at junction of the graft with the oviduct is included in the section. The collecting ducts passing out of the graft into the connective tissue are shown. B. Photomicrograph of a portion of a cross-section through the kidney and oviduct of right side posterior to the site of the graft. Note three cross-sections of the ducts extending into peritoneal tissue dorsal to the oviduct, towards the kidney. C. Successive cross-section posterior to B, showing the ducts are still more closely approaching to the kidney of right side. Hypertrophied Wolffian duct cut across is shown. The masses of spermatozoa in the ducts appear dark in these photomicrographs. × 80, cd, collecting duct in the graft; cd′, collecting duct outside of the graft; k, kidney; od, oviduct; Wd, Wolffian duct.

1. *External appearance of the masculinized females*

For an example of the masculinized female, the illustration of No. 17 animal in natural colours is given in Plate VII. The masculinization effects of the graft stand out more clearly in the tail of the male structure with distinct nuptial coloration than in other parts such as the enlarged parotoid glands and the cloaca which has swollen. The animal illustrated has the following history. On January 9, 1934 the ovaries as well as the fat-bodies were removed on both sides. On March 28, 1935, it underwent the second operation and a small testicular piece was placed in the abdominal cavity united with the oviduct of right side. About seven months later, in

Fig. 3. Photograph showing transformation of the tail into male shape in No. 19, lateral view. Note well-developed dorsal and lateral fins and degenerating portion hanging down at the sharp point. Hypertrophied lateral-line organs are indicated by white spots in tail as well as in trunk. ×4/5.

October, the external appearance in general was much like that of a male. The development of the nuptial coloration induced by the graft was at its height in February, 1936. But even at that time it was barely evident on the other parts of the body except the tail. When it was killed on June 24, the tail had lost its blue shade, but black spots which stand in a line on both sides, a pattern of the male, were maintained.

Concerning the two remarkable points, the transformation of the tail into male shape and the appearance of the nuptial coloration, the most successful cases of this experiment (6 in number) are found to be uniform. The remaining one (No. 16) of the masculinized group was to be distinguished from the above 6 for the lack of the

nuptial coloration. In general, a survey of the observations made for the external changes which determine the male characters showed the following facts. The tail becomes wide and thin on account of the development of the dorsal and ventral fins. As a result of the changes which have been made in the whole structures of the tail, the tissues of the terminal end which are hanging down at the sharp point of the tail show a pigment degeneration (Fig. 3). The head and tail skins become soft and smooth suggestive of those of the male, but in the trunk the coarse female skin is retained. Moreover, the tail skin is conspicuous for the appearance of the nuptial coloration. The parotoid glands behind the eyes enlarge greatly and the dorso-lateral and ventro-lateral ridges of dermal glands become distinct. The cloacal region swells up to a certain extent and the slight development of the hair-like appendages on each side of the posterior end of the vent is discernible.

2. *Internal appearance of the masculinized females at the end of the experiment*

The accessory sexual apparatus and the collecting ducts of the kidney. At the time of the grafting, the oviducts of the spayed females which were used as recipient animals, had decreased very much in size and the oviduct tissues showed degenerative changes to a considerable extent. The final condition of the oviducts of highly masculinized females, when killed, revealed the fact that this degeneration had not made any progress in degree worth mentioning during the course of the grafting experiment. Most impaired was the mucous membrane which lost its numerous longitudinal folds. In transection the duct, therefore, appears to have a thickened muscular layer (Fig. 4).

The Wolffian ducts, which in the female sex serve simply as the urinary duct, assume some considerable size, though not up to the male standard. The minute structure of these ducts, on microscopical examination, shows much resemblance to that of the male Wolffian (urinogenital) ducts (compare Figs. 4 and 5). The collecting ducts of the lumbar kidney also become hypertrophied. It will be noticed that the ducts not only underwent enlargement, but they also developed secretory activity characteristic of the male (Fig. 4). In every case of the masculinized females, the Wolffian ducts as well

as the collecting ducts of the lumbar kidney were of equal development on both sides of the body.

The cloacal region. The cloaca of the adult male *Triturus pyrrhogaster* is lined with three sets of glands. The pelvic glands lying in the roof and upper portion of the sides of the cloaca empty into it at the tips of the low papillae there. The cloacal glands cover the greater part of the cloacal wall and the papillae traversed by these

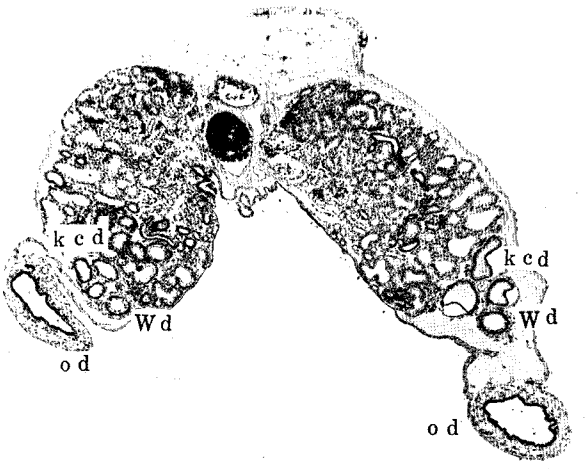


Fig. 4. Photomicrograph of a cross-section through lumbar kidney of No. 20, showing hypertrophied collecting ducts which develop secretory activity, hypertrophied Wolffian ducts, and degenerating oviducts. kcd, collecting duct of lumbar kidney; od, oviduct; Wd, Wolffian duct. ×20, about.

glands form the villous pad on either side of the cloaca. The abdominal is in position of the posterior-dorsal corner of the cloaca. Some cloacal glands extend behind the abdominal and occupy the extreme posterior-ventral angle of the cloaca. In Delafield's hematoxylin-eosin slides, the secretion fixed in the lumen of the tubule of the pelvic gland stains a vivid pink. The secretions of the cloacal and the abdominal glands stain a pale purple and a faint pink respectively. Thus, these three kinds of glands are distinguishable by the

character of their secretions in the histological sections. Secretion of some considerable amount is also found in the cloacal chamber. It stains a vivid pink.

Within the cloaca of the female *T. pyrrhogaster*, there are no glandular structures such as found in the male, except the spermatheca which have a glandular form buried in the dorsal and lateral walls of the cloaca (Fig. 6).

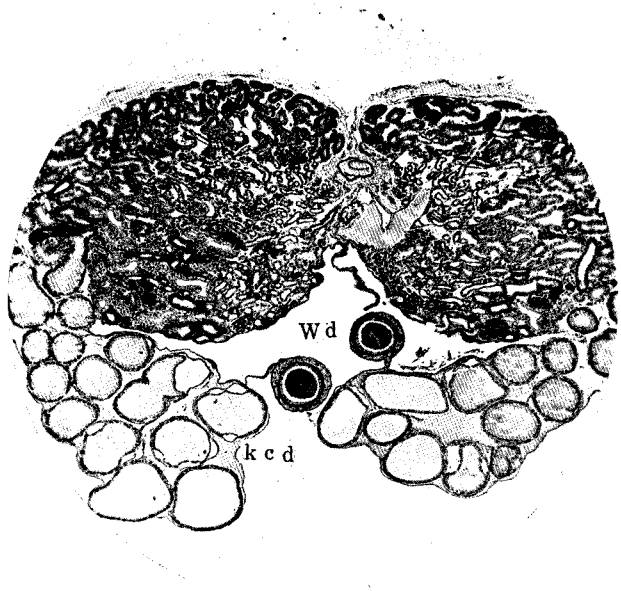


Fig. 5. Photomicrograph of a cross-section through lumbar kidney of a normal male, showing collecting ducts and Wolffian ducts in which masses of spermatozoa appear dark. For lettering see Fig. 4. ×20, about.

In the cloaca of the highly masculinized females of this experiment, all three sets of glands of the male are well formed (Figs. 7, 8, and 9). The pelvic glands are the most developed. They differ from the typical glands in that the tubules show considerable irregularities in size and the lumen of the tubule appears larger. The cloacal glands are found to be moderately well-developed but they appear quite similar to those found in the typical male in the position where they arise as well as in the structure and the secretory activity of the gland-epithelium. The abdominal glands, on the other hand, are

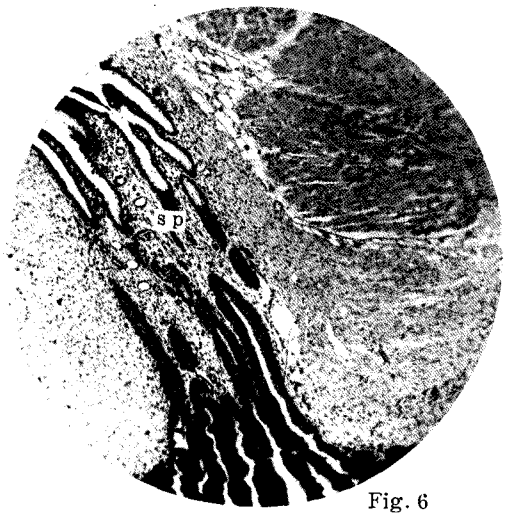


Fig. 6

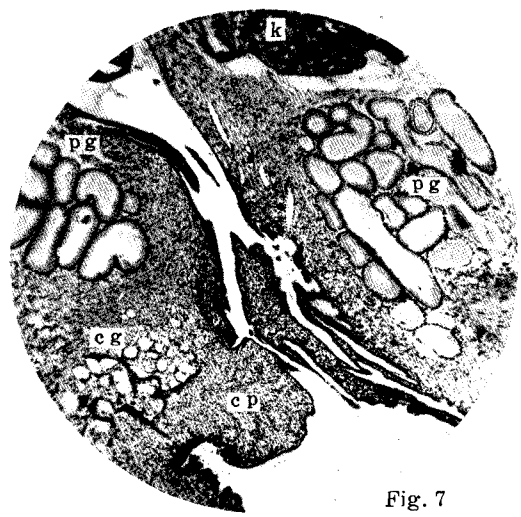


Fig. 7

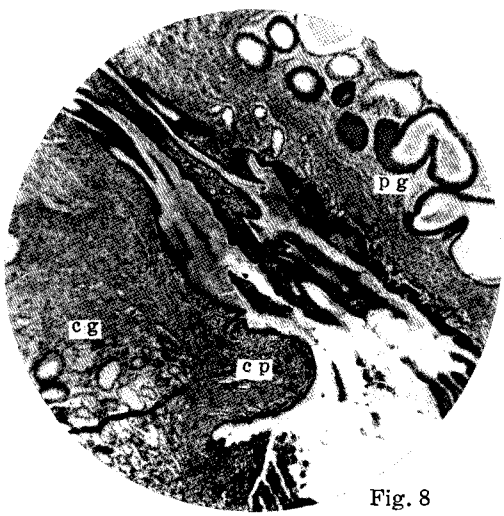


Fig. 8

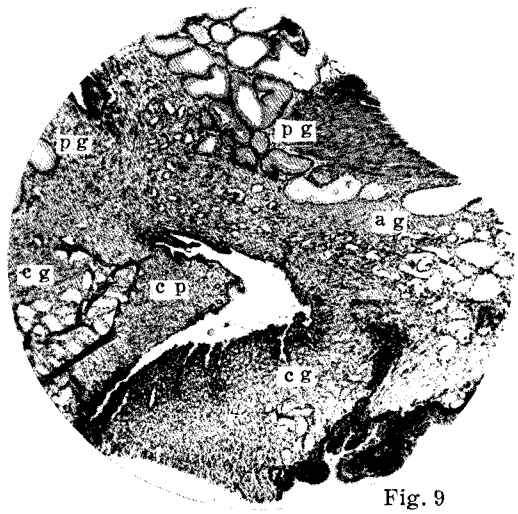


Fig. 9

Fig. 6. Photomicrograph of a portion of a longitudinal section through the cloaca of a normal female, showing the absence of glands, but the presence of the spermathecal tubules. sp, spermatheca. ×18.

Fig. 7. Photomicrograph of a portion of a longitudinal section through the cloaca of No. 17 masculinized female. Pelvic and cloacal glands and also the cloacal papilla are well formed. The abdominal gland is not seen. cg, cloacal gland; cp, cloacal papilla; k, kidney; pg, pelvic gland. ×18.

Fig. 8. The development of the glands and the cloacal papilla in No. 32 masculinized female is shown. The abdominal gland is not seen. Note the secretion in the cloacal chamber. For lettering see Fig. 7. ×18.

Fig. 9. Another section of the cloaca of No. 17, showing pelvic, cloacal and abdominal glands, and the cloacal papilla. ag, abdominal gland. ×18.

the least developed. A secretion found in the lumen of the tubule is meagre and it stains feebly in eosin. The epithelial cells with a clear cytoplasm are low (Fig. 9). A considerable amount of secretory products which stain a vivid pink becomes evident in the cloacal chamber which has now been lined with rich glands.

Besides the male glandular equipment of the cloaca, the cloacal papilla of the male becomes developed under the influence of the grafted testis. The most developed condition of this structure in which the sign of taking its definite form may already be recognized, is shown in Fig. 7 from No. 17 animal, one of the highly masculinized females in the present series.

The occurrence of the testis-ova in the testicular graft which has caused masculinization

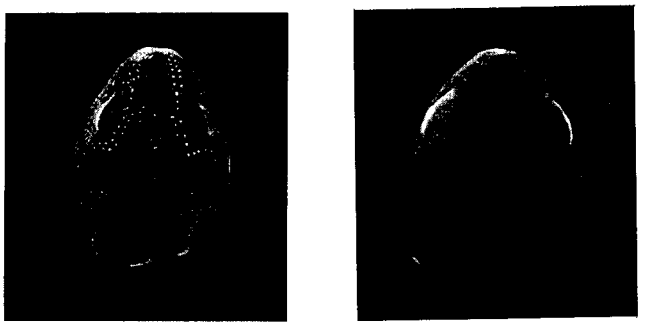
As stated above, in every masculinized female, when killed, a grafted testicular piece was always found to be well-regenerated. In these grafted testes, the testis-ova inside the seminiferous tubules are frequently found (Table I). A testis found well-regenerated in No. 17 animal will serve as representative; in it the seminiferous tubules filled with many testis-ova of approximately uniform in size occupy two-thirds of the whole mass while the remaining one-third of it is normal as judged from its cellular picture (Pl. VI, Fig. A). In the region where the whole of the germ cells has been transformed into the testis-ova, the seminiferous tubules are closely packed with these cells and distend. An investment of connective tissue which separates each of the distended tubules from its neighbouring ones appears very thin. It is the usual arrangement of the testis-ova in a seminiferous tubule that the larger ones occupy the peripheral region and centrally to them are the smaller ones (Pl. VI, Fig. B). Many of such seminiferous tubules filled with the testis-ova lack any of the typical male germ cells. But some are rarely found in which large primary spermatogonia enclosed in their follicle cells remain. All of the testis-ova found in groups are naked lacking the follicular envelope. But a single testis-ovum with an envelope of cells of epithelial type is also met with, as shown in one of them in Fig. D, Pl. VI, though it can not be considered as a structure which will give origin to the true granulosa of the egg. All of the testis-ova are found inside the seminiferous tubules while those embedded in the

connective tissue between the tubules are not seen. In the structure of the germinal vesicle, the testis-ovum closely resembles the typical ovocyte of small size. Though a testis-ovum may increase in size considerably, the process of forming the yolk is not ascertained in the cytoplasm. The present writer is sceptical of any possibility of the further development of these testis-ova, because the process of degeneration prevails particularly large-sized ones and the sign of liquefaction is already seen in the cytoplasm (Fig. C, Pl. VI). Germ cells which have failed to develop into the definite shape of the testis-ovum show pycnosis. These are seen between the growing testis-ova or engulfed in the cytoplasm (Fig. E, Pl. VI).

On the other hand, the earlier history of the germinal vesicle of the testis-ovum is uncertain. Hence, its true nature is obscure to the writer. In a certain tubule which is rarely to be met with, germ cells in various stages of enlargement are found. Some of them have a nucleus in which the chromatin masses towards one side (Fig. F, Pl. VI). But it is by no means certain that in the oogenesis of the testis-ova the synaptic phenomena will occur normally.

The lateral-line sense organs of the masculinized females

Besides the male features, the skin of the masculinized females is remarkable for the presence of the hypertrophied lateral-line



A B

Fig. 10. A, Untouched photograph of the head of No. 16 masculinized female fixed in Bouin's fluid, showing the distribution of the hypertrophied lateral-line organs. Note the smooth skin characteristic of the male. × 2. B, Head of a normal female which served as a control. The lateral-line organs are not discernible. ×2.

organs. The distribution of the organs in the skin is indicated by well-defined white spots which are easily visible to the naked eye in the intact animal (compare A and B in Fig. 10). The head possesses a full complement of the hypertrophied organs while only dorsal branches in which the organs are arranged singly or in pairs in a row become distinct on both sides of the trunk and tail (Fig. 3). An instance of these lateral-line organs is shown in Fig. 11 from a section passing vertically through the epidermis of the tail. The entire organ rises toward the surface; so that the free end of the organ is slightly above the general epidermal surface. A hyperplasia is brought about in the sensory cells grouped in the central portion of the organ as well as in the interstitial cells extending around and among the sensory cells.

Fig. 11. Photomicrograph of an organ from the tail of No. 20 masculinized female. The organ is enlarged and the free end rises above the surface of the epidermis. Both interstitial supporting and sensory cells show hypertrophy and hyperplasia. i, interstitial supporting cell; s, sensory cell. ×380.

It should be emphasized that all the members of the masculinized group, 7 in number, have shown this excessive development of the lateral-line organs. Hence it seems permissible to conclude that the hypertrophy of the lateral-line organs is brought about by the grafted testis, being parallel with other changes in the general epidermis which can be classed as the masculinization effects.

Summary

1. The masculinizing action of the testicle in urodelan amphibians was demonstrated using the common Japanese newt, *Triturus pyrrhogaster* by means of homoplastic transplantation of a testicular piece into the adult female after about one year's rearing under spayed condition.

2. The grafted testicular piece which had become well-vascularized and had developed up to the normal standard of a testis caused the development of a full complement of male somatic characters in the spayed female. Especially there should be noted, the transformation of the tail into the male shape with distinct nuptial coloration, the hypertrophy of the rudimentary Wolffian ducts, the development of the male glandular equipment of the cloaca, and the appearance of the cloacal papilla characteristic of the male.

3. The hypertrophied collecting ducts in the lumbar kidney develop secretory activity characteristic of the male. In the cloacal chamber, the walls of which are now lined with rich glands (the pelvic, cloacal, and abdominal), a considerable amount of secretory products is found, which stain a vivid pink in hematoxylin-eosin preparation.

4. At autopsy after 194 to 537 days, 6 among 7 of the grafted testes which had exerted the masculinizing influence, contained the testis-ovum within the seminiferous tubule. In both testes found in No. 17 and No. 18 masculinized females, as could be seen from serial sections, the region of the atypical oogenesis was predominating over that of the normal spermatogenesis. Some detailed descriptions of this peculiar morphological process are given.

5. All the members of the masculinized group of this experiment showed hypertrophy of the lateral-line organs. That a hyperplasia was brought about in the sensory and interstitial cells of the organ was shown by examination of sections.

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Explanation of Plate VI

All figures are photomicrographs

A. Section through the region of testis-ova in regenerated graft in No. 17 masculinized female. × 10.

B. Arrangement of testis-ova in a seminiferous tubule: from the graft of No. 17. × 100.

C. A large-sized testis-ovum, but liquefaction in the cortical cytoplasm is apparent: from the graft of No. 17. × 200.

D. Solitary testis-ovum with an envelope of cells of epithelial type: from the graft of No. 17. × 200.

E. Section of seminiferous tubules filled with testis-ova. The small dark cells in and between testis-ova are pycnotic germ cells which have failed to develop: from the graft of No. 17. × 100.

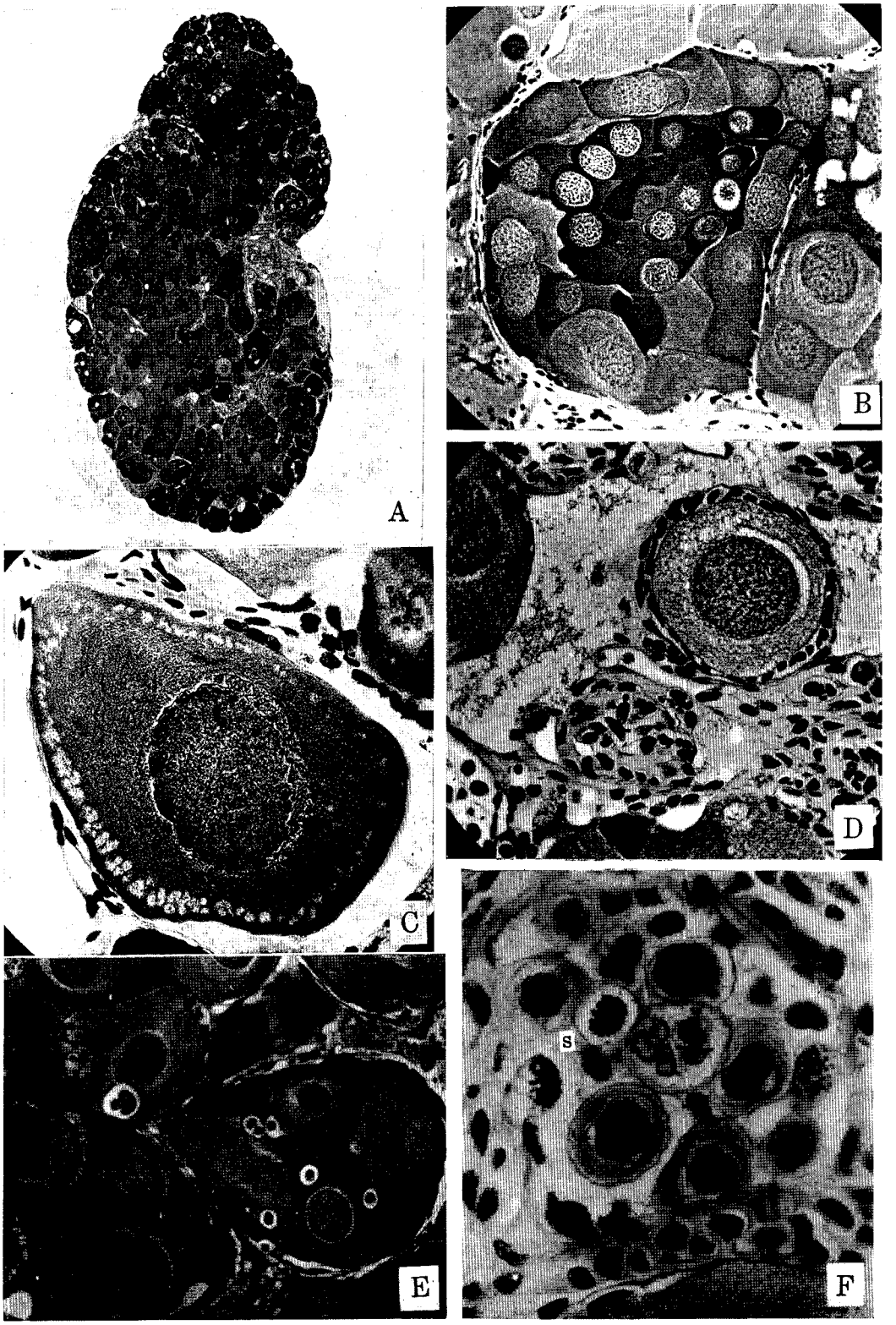
F. Seminiferous tubule of rare occurrence, showing germ cells in various stages of enlargement. Spermatogonium in synizesis (s) is seen: from the graft of No. 18. × 360.

Explanation of Plate VII

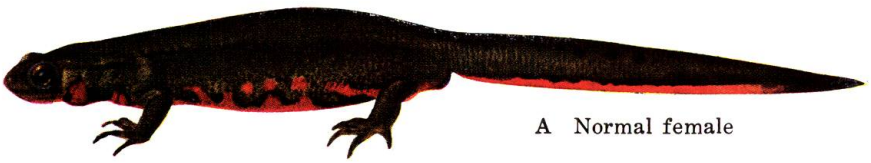
A. Normal female (natural size).

B. Normal male (natural size).

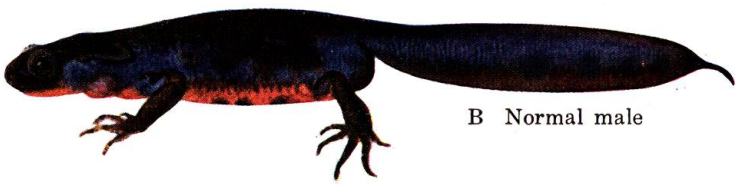
C. Masculinized female (No. 17, natural size).



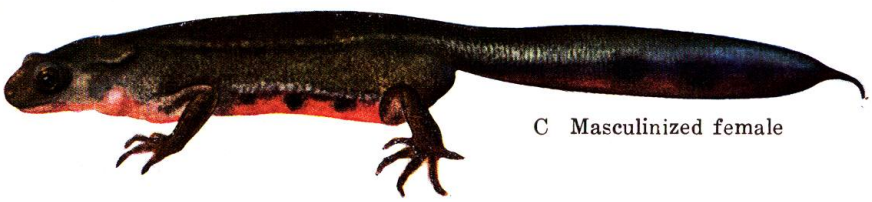
A. Ichikawa: Masculinization by Testicular Transplantation



A Normal female



B Normal male



C Masculinized female

A. Ichikawa: Masculinization by Testicular Transplantation