Sexual Induction in a Sexually Differentiated Salamander by the Implantation of Testis and Hypophysis[1)]

By

Tohru Uchida and Kin-Ichiro Hanaoka

Zoological Institute, Faculty of Science, Hokkaidô Imperial University, Sapporo

(With 4 Textfigures and 2 Plates)

In 1937 and 1939 the writers separately reported on the sexual induction in a sexually semidifferentiated salamander, *Hynobius retardatus* in Sapporo. This paper is a report on the similar experiment on a sexually differentiated salamander, *Hynobius lichenatus* in Aomori. Comparing the result of this paper with those of the previous experiments, it could be found that there exist some distinct differences in the similar experiments for these two races. The results obtained in these experiments will be summarized and discussed on in another paper on the reciprocal testicular transplantation between the two salamanders. The expense of this investigation was partly defrayed from the Scientific Research Expenditure of the Department of Education.

Normal Development of the gonads of *Hynobius lichenatus*

In *Hynobius lichenatus* the appearance of gonadal buds takes place in larvae of about 20 mm. The germ cells are seen in larvae more than 20 mm in length. As the larvae grow further, the germ cells increase gradually and rete cells immigrate from the hilium to the gonad in large numbers. The germ cells are eventually moved toward the peripheral, particularly distal, portion of the gonads in the larvae of a length of approximately 35 mm. The gonads in this

1) Contribution No. 167 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

stage is still sexually neutral, because the medullary part are packed with many rete cells (Fig. 1, Pl. II). When the larvae reach approximately 40 mm long, the sex-differentiation begins; in the male immigration of rete cells to the medullary portion is followed by the subsequent development of sex-cords in the medulla and also by the degeneration of cortical germ layer, whereas in the female medullary rete cells gradually degenerate from the distal end and finally the ovarian cavity becomes formed; thenceforth, the cortical germ layer becomes thick on account of the increase of germ cells which occurs at first in distal portion and gradually over the whole periphery. When the larvae reach 50 mm, the sex-differentiation is generally completed. The male gonad is solid, packed with interstitial cells and sex-cords and, lacks cortical layer. The female gonad is provided with cortical germ cells arranged in three or four layers and with the distinct ovarian cavity (Fig. 2, Pl. II). The sex-differentiation of the present species is in marked contrast to that of *H. retardatus*. In these two salamanders the gonads begin to appear in larvae, approximately 20 mm long and thereafter the gonads retain the neutral condition which lasts to the larvae, about 40 mm long. After this stage the gonads of *H. lichenatus* directly differentiate either toward the male or toward the female, whereas the gonads of *H. retardatus* take at first the ovarian form, with a well developed ovarian cavity, and then about a half of them undergoes the sex-reversal toward the male. Therefore, the final sex differentiation is first observable in the gonads of the larvae during metamorphosis.

Experiment I (Testis-grafting)

The operation of testis grafting was performed in larvae, approximately 20 mm long, in which the primitive gonads began to appear. The first autopsy was executed three weeks after the operation and thenceforward 3–5 experimental animals were examined every week. Out of the animals used in this experiment 46 healthy specimens were obtained. On account of the operation the growth of the salamander larvae was more or less arrested especially in younger stage. The larvae at the first autopsy were about 24 mm long and still had the primitive gonads. The primitive gonads are furnished with one or two germ cells in the median sections, and can hardly be distinguishable from those of the control animals. The effect of

testicular pieces is first observable in the gonads of larvae, about 30 mm in the whole length, of 4 weeks after the operation. For the convenient sake the experimental animals will be separately described in the following 4 groups according to the period of rearing as, 4–6 weeks, 7–9 weeks, 10–11 weeks and 12–15 weeks after the operation.

During 4–6 weeks since the operation, the gonads of the experimental larvae were all more or less affected, though very diverse in phase. Some gonads as in Fig. 3, Pl. II. have cortical germ cells, sparsely arranged and now on the spot of elimination, and a germ cell in the medulla of the proximal part. But most of these gonads are apparently of male type. It must be pronouncedly noted that especially in younger gonads all the germ cells (3–4 in number) are grouped in the medullary centre as shown in Figs. 4, 5, Pl. II and they are relatively large. On account of the testicular implantation two effects are clearly observable: the first is the elimination of germ cells and the second is the shifting of germ cells toward the medulla.

In the larvae 7–9 weeks after the operation gonads are mostly of rather male type with solid medullary part containing many rete cells and a few germ cells. Out of 12 gonads belonging to this group 9 are distinctly male, though most of them are small and contain a few germ cells in the medulla. The remaining 3 gonads are all affected females. In these female gonads the cortical part is generally more or less degenerated and cortical germ cells, if present, are scarce in number and sparsely arranged. Elimination of cortical germ cells often occurs in the gonads of this series. Some gonads belonging to this group are of typically male type as shown in Fig. 6, Pl. II, giving a figure of the testis in which remnants of cortical epitherial cells are now in elimination. The gonad expressed by Fig. 7, Pl. II is of the female type but the elimination of cortical germ cells takes place in the terminal portion and owing to immigration of sex-cords the distribution of germ cells are strongly distorted. The gonad illustrated by Figs. 8 & 9, Pl. II is the one of the most female structure; the cortical germ cells, though partially degenerated, are typically arranged. The medullar part of this gonad, however, is nearly packed with rete cells containing a few germ cells. In the control animals during these stages the gonads are still in the state of neutral phase.

Out of the larvae examined 10–11 weeks after the operation 3 were males and 3 were affected females. The gonads belonging to

the male are all small, containing germ cells of rather small numbers, which are seen to have the tendency to shift toward the medullary portion. Sex cords are not well-developed in these gonads. Out of them two have no trace of ovarian indication but a single one is bordered with slight remnants of cortical elements in the anterior portion. The affected ovaries, though different in degree, have all cortical germ cells which are more or less degenerated and reduced in number, and the ovarian cavity generally narrowed. These modifications do not occur synchronously. In the gonad expressed by Fig. 10, Pl. II the cortical part is largely degenerated and contains only few number of inactive cortical germ cells but the ovarian cavity is not so narrowed. The caudal part of this gonad still retains fair numbers of medullary rete cells, while the anterior part has no germ cell but only scanty interstitial cells which are sparsely scattered in the hyalin tissue. On the other hand, the gonad shown in Fig. 11, Pl. II has many active cortical germ cells, though containing some just before elimination, and the ovarian cavity reduced in size owing to the immigration of germ cells. In other sections the ovarian cavity is more reduced and on account of the solid medulla some sections apparently display the figures of the testis but for the typical arrangement of cortical cells.

The next group of the experimental larvae were at the autopsy 12–15 weeks after the operation and covers the individuals before and after the metamorphosis. The experimentals belonging to this group are 14 in number, including 6 metamorphosed ones. Out of them 11 are the females and 3 are the males. The male gonads of this group are better developed and larger than those of control animals (Fig. 12, Pl. II). One of these male gonads is distinctly of the male type, displaying sex-cord formation, but contains several cavities which are probable to be remnants of ovarian cavities (Fig. 13, Pl. II). The female gonads of this group are all affected and provided with a rather small ovarian cavity. The most remarkable feature of these gonads lies in the occurrence of large oocytes in them. Such large oocytes are not found in the gonads of control animals immediately after the metamorphosis. They are exceedingly large and equal in size to those found in the normal gonads of one-yeared salamanders. The acceleration of the growth of these ovicells is clearly due to the implanted testicular pieces. These ovicells are generally situated on the inner wall of the ovaries as shown in Fig.

14, Pl. III. As the ovarian cavity is generally narrowed, some ovaries are nearly or completely solid with these large ovicells (Figs. 15–16, Pl. III). The gonad illustrated by Fig. 17, Pl. III is the extreme case of these solid ovaries. The gonad of this type is probable to be transformed into the mozaic gonad, when the elimination of germ cells and the formation of sex-cords suitably take place in it. The figure (Fig. 18, Pl. III) shows a sagittal section of a deformed ovary. The characteristics of this gonad lies in the reduction of the ovarian cavity and solidness of the distal half, which shows some resemblances to the testis. The gonad shown in Fig. 19, III is another form of affected ovaries. The gonad is ovarian in structure but contains no large oocytes. The ovarian cavity is nearly occluded by the ingrowth of interstitial cells mingled with scarce germ cells. The gonads above mentioned are all affected ovaries and undergoing the sex-modification toward the male.

So far as the writers have examined, there could be found neither sterile nor "free-martin gonads" which occurred very frequently in the former experiment on *H. retardatus*. In the "free-martin gonad" the germ cells are generally pushed toward the distal margin of the gonads and are gradually eliminated. It is very peculiar in *H. lichenatus* that the young gonad affected by the testicular implant has a few germ cells in tiers in the medulla. The effect of the testicular implants causes the elimination of germ cells but also the shifting of them toward the medullar portion all the stages throughout. Acceleration of growth of oocytes seems to be more pronounced in *H. lichenatus* than in *H. retardatus*. Reduction of the ovarian cavity and the ingrowth of the rete cells in the medulla are distinctly observable as in the case of *H. retardatus*.

When compared the results of this experiment with the controls, the following facts can be recognized: 1) immigration of germ cells to the medulla is seen in larvae, 20–30 mm long, 2) differentiation of the sexes is accelerated and occurs from larvae, about 30 mm, 3) elimination of cortical germ cells (especially in the female) is observable throughout the whole stage to the sex-differentiation, 4) development of the male gonads is accelerated, 5) in the female gonads the ovarian cavity becomes reduced, and germ cells partly become degenerated, are partly eliminated or are accelerated in development to form large ovicells.

Experiment II (Testis and hypophysis grafting)

The pieces of hypophysis and adult testis were transplanted on May 18 into the larvae of *Hynobius lichenatus* in which the gonads had already appeared and probably were in the neutral stage. At the autopsy the larvae were generally 40–55 mm in length and 22 healthy examples were secured. These larvae were fixed in the following period; 4 specimens in 30 days after the operation, 5 specimens in 46–52 days after the operation and 13 specimens in 55–68 days after the operation respectively. Most of the larvae had already completed metamorphosis.

Out of the 22 experimental animals 12 were males. The testes of the males were relatively well advanced in stage, containing well marked sex cords and larger than the normal gonads, but otherwise normal (Fig. 21, Pl. III).

The gonads of the females were found to be all modified more or less, though different in degree. The two females alone are only slightly affected; they have the cortical germ layer which is not regressed, but the ovarian cavity which is narrowed in size. The modifications lie in the degeneration of the cortical germ layer and in the development of medullary part. These modifications generally occur synchronously but sometimes quite separately. The degeneration of the cortical germ cells generally seems to be due to the elimination of germ cells. The medullary part is gradually packed with interestitial cells and subsequently becomes to contain sex-cords. The figures (Figs. 20 & 22, Pl. III) are the typical affected gonads. The cortical germ layer in Fig. 20 displays elimination of germ cells and is slightly degenerated, while the ovarian cavity is obliterated by immigrant rete cells containing germ cells. The similar phase is seen in other several gonads. In Fig. 22 the degeneration of the cortical layer is more conspicuous, though germ cells are still present in a coarse layer. The ovarian cavity of the gonad is almost occluded by the vigorous immigration of the rete cells having germ cells. In some gonads, however, the two modifications do not appear together. For example Textfig. 1 illustrates the female gonad in which the ovarian cavity becomes narrowed with some immigrating interstitial cells (involving no germ cells in all sections) but the cortical germ cells are normal and well-developed. In the other gonad (Textfig. 2) the cortical germ layer has been fairly degenerated but the ovarian

cavity is still present, immigration of interstitial cells is scarcely observable. Judging from the figure, the cortical germ cells had not been eliminated but degenerated in situ. This figure reminds the degenerated gonad upon the high temperature experiment. The figures (Textfigs. 3–4) made from different sections of a gonad are interesting. The gonad has the marked cortical germ layer, but the ovarian cavity is nearly packed by exuberant immigration of rete cells which sometimes form sex cords. In the figure (Textfig. 3) the cortical germ layer is distinct, but in the figure (Textfig. 4) the germ cells are partly in elimination and some shift toward the medullary portion, thus the figure more or less resembles the section of testis.

Explanation of Textfigures. In all transplanted testis and hypophysis.

1. Affected ovary of salamander already metamorphosed (No. 23 II), 53 mm long. ×200. The ovary is nearly normal with developed cortical germ layer but the ovarian cavity is nearly reduced.

2. Affected ovary of the larva (No. 8 II), 55 mm long. ×200. Fairly degenerated.

3. Affected ovary of the larva (No. 6 III), 47 m long. ×200. Cortical germ layer largely degenrated and ovarian cavity loosely packed with interstitial cells.

4. Another section of the same gonad, showing male gonad in the proximal half. ×200.

The female gonads of this experiment bear some resemblances to those of high temperature experiment, especially in the gonads with the cortical germ layer and medullary interstitial core and in the degenerating ovary (Fig. 13, Pl. 5, 1937), but seem to be different in the following points. In the gonads here considered the elimination of cortical germ cells is easily observable, while in the gonads of the high temperature experiment cortical germ cells generally degenerate in situ and elimination was hardly visible.

Hanaoka (1939) reported that implantation of hypophysis only causes the ingrowth of rete cells to the medulla in the semidifferentiate male of *H. retardatus*. In the present experiment the medullary ingrowth of rete cells is far more remarkable than in the Experiment I (Testis-grafting). Moreover, it is very noticeable that no large oocytes could be seen in these gonads, though frequently observable in the Experiment I.

Literature

HANAOKA, K. I. 1939. Modification of sex in a salamander *Hynobius retardatus* induced by the implantation of testis and hypophysis. Jap. Jour. Zool., vol. 8, no. 3, pp. 255–264, pls. 33–37.

UCHIDA, T. 1937. Studies on the sexuality of Amphibia. II. Sexual induction in a sexually semidifferentiated salamander. Jour. Fac. Sci., Hokkaido Imp. Univ., ser. 6, Zool., vol. 6, no. 1, pp. 35–58, pls. 2–4.

————. 1937. Studies on the sexuality of Amphibia. III. Sex-transformation in *Hynobius retardatus* by the function of high temperature. Ditto, pp. 59–68, pl. 5.

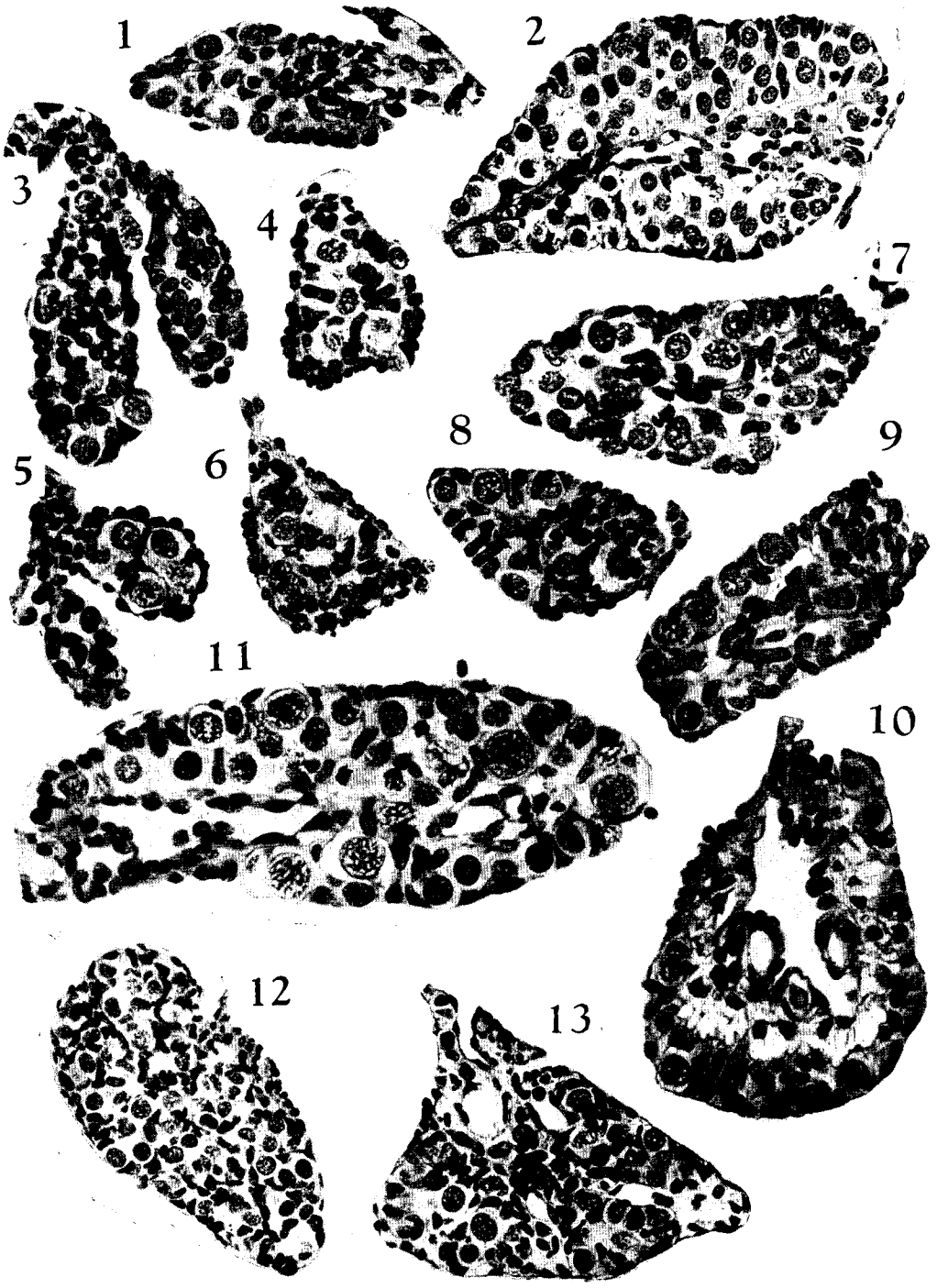
Explanation for Pl. II

1. The neutral gonad of the control larva of *Hynobius lichenatus* (No. 15 B IV), 41 mm in the whole length. ×250.
2. The normal ovary of the control larva (No. 19 E), 48 mm long. ×200.
3. Affected gonad of the larva (No. 14 III), 29 mm long, showing elimination of cortical germ cells. ×250.
4. Affected gonad of the larva (No. 10 I), 32 mm long, showing elimination and medullary immigration of germ cells. ×250.
5. Affected gonad of the larva (No. 19 I), 28 mm long, indicating medullary immigration of germ cells. ×250.
6. Affected gonad of the larva (No. 39 II), 42 mm long, already showing the appearance of typical testis. ×250.
7. Affected gonad of the larva (No. 34 II), 38 mm long. The female gonad is distorted in form, illustrating apparently the resemblance to testis. ×250.

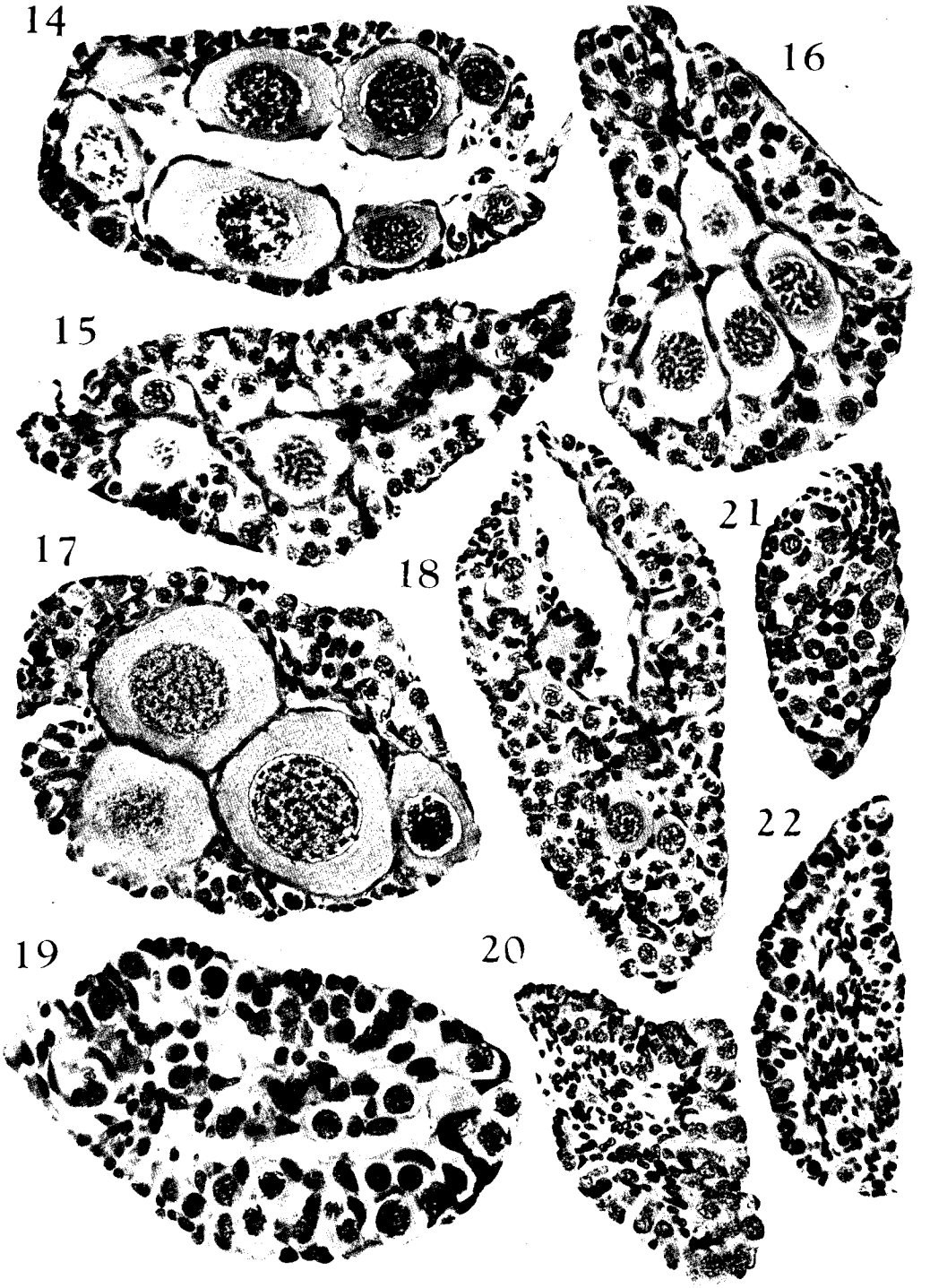
8. Affected gonad of the larva (No. 38 III), 42 mm long. The gonad, female in nature, has cortical germ cells before elimination and medullary germ cells. ×250.
9. Another section of the gonad (Fig. 8), showing the violent ingrowth of sex cords to the medulla. ×250.
10. Affected gonad of the larva (No. 56 III), 47 mm long. The female gonad is largely degenerated. ×250.
11. Affected gonad of the larva (No. 58 IV), 40 mm long. The ovarian cavity of the female gonad is reduced on account of the medullary immigration. ×250.
12. Well developed testis of the salamander, after metamorphosis (No. 66 II), 50 mm long. ×200.
13. Testis of the salamander after metamorphosis, with several cavities (No. 76 VI), 49 mm long. ×200.

Explanation for Pl. III

14. Affected ovary of the salamander after metamorphosis (No. 85 IV), 52 mm long. ×200. Germ cells well activated.
15. Affected ovary of the salamander after metamorphosis (No. 83 II), 43 mm long. ×200. Ovarian cavity obliterated.
16. Another section of Fig. 15.
17. Affected ovary of the salamander after metamorphosis (No. 68 II), 49 mm long. ×20. This ovary seems to be closely related to the mosaic gonad.
18. Affected ovary of the salamander after metamorphosis (No. 78 IV), 47 mm long. ×150. The distal half of the this gonad is masculinized.
19. Affected ovary of the salamander after metamorphosis (No. 71 III), 49 mm long. ×250. The ovarian cavity of this gonad is partially packed with rete cells containing germ cells.
20. Affected ovary of the salamander after metamorphosis (No. 17 III), 49 mm by grafting of testis and hypophysis. ×200 long. The cortical germ layer is slightly eliminated and degenerated, and the ovarian cavity is loosely packed with interstitial cells.
21. Activated testis of the salamander already metamorphosed (No. 18 II), 48 mm long.
22. Affected ovary of the salamander already metamorphosed (No. 9 IV), 60 mm long. ×200. The cortical germ layer is partially degenerated and the ovarian cavity is loosely packed with interstitial cells.



T. Uchida and K. I. Hanaoka: Sexual induction in a sexually differentiated salamander.



T. Uchida and K. I. Hanaoka: Sexual induction in a sexually differentiated salamander.

Sexual Induction in a Sexually Differentiated Salamander by the Implantation of Testis and Hypophysis[1)]

By

Tohru Uchida and Kin-Ichiro Hanaoka

Zoological Institute, Faculty of Science, Hokkaidô Imperial University, Sapporo

(With 4 Textfigures and 2 Plates)

In 1937 and 1939 the writers separately reported on the sexual induction in a sexually semidifferentiated salamander, *Hynobius retardatus* in Sapporo. This paper is a report on the similar experiment on a sexually differentiated salamander, *Hynobius lichenatus* in Aomori. Comparing the result of this paper with those of the previous experiments, it could be found that there exist some distinct differences in the similar experiments for these two races. The results obtained in these experiments will be summarized and discussed on in another paper on the reciprocal testicular transplantation between the two salamanders. The expense of this investigation was partly defrayed from the Scientific Research Expenditure of the Department of Education.

Normal Development of the gonads of *Hynobius lichenatus*

In *Hynobius lichenatus* the appearance of gonadal buds takes place in larvae of about 20 mm. The germ cells are seen in larvae more than 20 mm in length. As the larvae grow further, the germ cells increase gradually and rete cells immigrate from the hilium to the gonad in large numbers. The germ cells are eventually moved toward the peripheral, particularly distal, portion of the gonads in the larvae of a length of approximately 35 mm. The gonads in this

1) Contribution No. 167 from the Zoological Institute, Faculty of Science, Hokkaido Imperial University, Sapporo.

stage is still sexually neutral, because the medullary part are packed with many rete cells (Fig. 1, Pl. II). When the larvae reach approximately 40 mm long, the sex-differentiation begins; in the male immigration of rete cells to the medullary portion is followed by the subsequent development of sex-cords in the medulla and also by the degeneration of cortical germ layer, whereas in the female medullary rete cells gradually degenerate from the distal end and finally the ovarian cavity becomes formed; thenceforth, the cortical germ layer becomes thick on account of the increase of germ cells which occurs at first in distal portion and gradually over the whole periphery. When the larvae reach 50 mm, the sex-differentiation is generally completed. The male gonad is solid, packed with interstitial cells and sex-cords and, lacks cortical layer. The female gonad is provided with cortical germ cells arranged in three or four layers and with the distinct ovarian cavity (Fig. 2, Pl. II). The sex-differentiation of the present species is in marked contrast to that of *H. retardatus*. In these two salamanders the gonads begin to appear in larvae, approximately 20 mm long and thereafter the gonads retain the neutral condition which lasts to the larvae, about 40 mm long. After this stage the gonads of *H. lichenatus* directly differentiate either toward the male or toward the female, whereas the gonads of *H. retardatus* take at first the ovarian form, with a well developed ovarian cavity, and then about a half of them undergoes the sex-reversal toward the male. Therefore, the final sex differentiation is first observable in the gonads of the larvae during metamorphosis.

Experiment I (Testis-grafting)

The operation of testis grafting was performed in larvae, approximately 20 mm long, in which the primitive gonads began to appear. The first autopsy was executed three weeks after the operation and thenceforward 3–5 experimental animals were examined every week. Out of the animals used in this experiment 46 healthy specimens were obtained. On account of the operation the growth of the salamander larvae was more or less arrested especially in younger stage. The larvae at the first autopsy were about 24 mm long and still had the primitive gonads. The primitive gonads are furnished with one or two germ cells in the median sections, and can hardly be distinguishable from those of the control animals. The effect of

testicular pieces is first observable in the gonads of larvae, about 30 mm in the whole length, of 4 weeks after the operation. For the convenient sake the experimental animals will be separately described in the following 4 groups according to the period of rearing as, 4–6 weeks, 7–9 weeks, 10–11 weeks and 12–15 weeks after the operation.

During 4–6 weeks since the operation, the gonads of the experimental larvae were all more or less affected, though very diverse in phase. Some gonads as in Fig. 3, Pl. II. have cortical germ cells, sparsely arranged and now on the spot of elimination, and a germ cell in the medulla of the proximal part. But most of these gonads are apparently of male type. It must be pronouncedly noted that especially in younger gonads all the germ cells (3–4 in number) are grouped in the medullary centre as shown in Figs. 4, 5, Pl. II and they are relatively large. On account of the testicular implantation two effects are clearly observable: the first is the elimination of germ cells and the second is the shifting of germ cells toward the medulla.

In the larvae 7–9 weeks after the operation gonads are mostly of rather male type with solid medullary part containing many rete cells and a few germ cells. Out of 12 gonads belonging to this group 9 are distinctly male, though most of them are small and contain a few germ cells in the medulla. The remaining 3 gonads are all affected females. In these female gonads the cortical part is generally more or less degenerated and cortical germ cells, if present, are scarce in number and sparsely arranged. Elimination of cortical germ cells often occurs in the gonads of this series. Some gonads belonging to this group are of typically male type as shown in Fig. 6, Pl. II, giving a figure of the testis in which remnants of cortical epitherial cells are now in elimination. The gonad expressed by Fig. 7, Pl. II is of the female type but the elimination of cortical germ cells takes place in the terminal portion and owing to immigration of sex-cords the distribution of germ cells are strongly distorted. The gonad illustrated by Figs. 8 & 9, Pl. II is the one of the most female structure; the cortical germ cells, though partially degenerated, are typically arranged. The medullar part of this gonad, however, is nearly packed with rete cells containing a few germ cells. In the control animals during these stages the gonads are still in the state of neutral phase.

Out of the larvae examined 10–11 weeks after the operation 3 were males and 3 were affected females. The gonads belonging to

the male are all small, containing germ cells of rather small numbers, which are seen to have the tendency to shift toward the medullary portion. Sex cords are not well-developed in these gonads. Out of them two have no trace of ovarian indication but a single one is bordered with slight remnants of cortical elements in the anterior portion. The affected ovaries, though different in degree, have all cortical germ cells which are more or less degenerated and reduced in number, and the ovarian cavity generally narrowed. These modifications do not occur synchronously. In the gonad expressed by Fig. 10, Pl. II the cortical part is largely degenerated and contains only few number of inactive cortical germ cells but the ovarian cavity is not so narrowed. The caudal part of this gonad still retains fair numbers of medullary rete cells, while the anterior part has no germ cell but only scanty interstitial cells which are sparsely scattered in the hyalin tissue. On the other hand, the gonad shown in Fig. 11, Pl. II has many active cortical germ cells, though containing some just before elimination, and the ovarian cavity reduced in size owing to the immigration of germ cells. In other sections the ovarian cavity is more reduced and on account of the solid medulla some sections apparently display the figures of the testis but for the typical arrangement of cortical cells.

The next group of the experimental larvae were at the autopsy 12–15 weeks after the operation and covers the individuals before and after the metamorphosis. The experimentals belonging to this group are 14 in number, including 6 metamorphosed ones. Out of them 11 are the females and 3 are the males. The male gonads of this group are better developed and larger than those of control animals (Fig. 12, Pl. II). One of these male gonads is distinctly of the male type, displaying sex-cord formation, but contains several cavities which are probable to be remnants of ovarian cavities (Fig. 13, Pl. II). The female gonads of this group are all affected and provided with a rather small ovarian cavity. The most remarkable feature of these gonads lies in the occurrence of large oocytes in them. Such large oocytes are not found in the gonads of control animals immediately after the metamorphosis. They are exceedingly large and equal in size to those found in the normal gonads of one-yeared salamanders. The acceleration of the growth of these ovicells is clearly due to the implanted testicular pieces. These ovicells are generally situated on the inner wall of the ovaries as shown in Fig.

14, Pl. III. As the ovarian cavity is generally narrowed, some ovaries are nearly or completely solid with these large ovicells (Figs. 15–16, Pl. III). The gonad illustrated by Fig. 17, Pl. III is the extreme case of these solid ovaries. The gonad of this type is probable to be transformed into the mozaic gonad, when the elimination of germ cells and the formation of sex-cords suitably take place in it. The figure (Fig. 18, Pl. III) shows a sagittal section of a deformed ovary. The characteristics of this gonad lies in the reduction of the ovarian cavity and solidness of the distal half, which shows some resemblances to the testis. The gonad shown in Fig. 19, III is another form of affected ovaries. The gonad is ovarian in structure but contains no large oocytes. The ovarian cavity is nearly occluded by the ingrowth of interstitial cells mingled with scarce germ cells. The gonads above mentioned are all affected ovaries and undergoing the sex-modification toward the male.

So far as the writers have examined, there could be found neither sterile nor "free-martin gonads" which occurred very frequently in the former experiment on *H. retardatus*. In the "free-martin gonad" the germ cells are generally pushed toward the distal margin of the gonads and are gradually eliminated. It is very peculiar in *H. lichenatus* that the young gonad affected by the testicular implant has a few germ cells in tiers in the medulla. The effect of the testicular implants causes the elimination of germ cells but also the shifting of them toward the medullar portion all the stages throughout. Acceleration of growth of oocytes seems to be more pronounced in *H. lichenatus* than in *H. retardatus*. Reduction of the ovarian cavity and the ingrowth of the rete cells in the medulla are distinctly observable as in the case of *H. retardatus*.

When compared the results of this experiment with the controls, the following facts can be recognized: 1) immigration of germ cells to the medulla is seen in larvae, 20–30 mm long, 2) differentiation of the sexes is accelerated and occurs from larvae, about 30 mm, 3) elimination of cortical germ cells (especially in the female) is observable throughout the whole stage to the sex-differentiation, 4) development of the male gonads is accelerated, 5) in the female gonads the ovarian cavity becomes reduced, and germ cells partly become degenerated, are partly eliminated or are accelerated in development to form large ovicells.

Experiment II (Testis and hypophysis grafting)

The pieces of hypophysis and adult testis were transplanted on May 18 into the larvae of *Hynobius lichenatus* in which the gonads had already appeared and probably were in the neutral stage. At the autopsy the larvae were generally 40–55 mm in length and 22 healthy examples were secured. These larvae were fixed in the following period; 4 specimens in 30 days after the operation, 5 specimens in 46–52 days after the operation and 13 specimens in 55–68 days after the operation respectively. Most of the larvae had already completed metamorphosis.

Out of the 22 experimental animals 12 were males. The testes of the males were relatively well advanced in stage, containing well marked sex cords and larger than the normal gonads, but otherwise normal (Fig. 21, Pl. III).

The gonads of the females were found to be all modified more or less, though different in degree. The two females alone are only slightly affected; they have the cortical germ layer which is not regressed, but the ovarian cavity which is narrowed in size. The modifications lie in the degeneration of the cortical germ layer and in the development of medullary part. These modifications generally occur synchronously but sometimes quite separately. The degeneration of the cortical germ cells generally seems to be due to the elimination of germ cells. The medullary part is gradually packed with interestitial cells and subsequently becomes to contain sex-cords. The figures (Figs. 20 & 22, Pl. III) are the typical affected gonads. The cortical germ layer in Fig. 20 displays elimination of germ cells and is slightly degenerated, while the ovarian cavity is obliterated by immigrant rete cells containing germ cells. The similar phase is seen in other several gonads. In Fig. 22 the degeneration of the cortical layer is more conspicuous, though germ cells are still present in a coarse layer. The ovarian cavity of the gonad is almost occluded by the vigorous immigration of the rete cells having germ cells. In some gonads, however, the two modifications do not appear together. For example Textfig. 1 illustrates the female gonad in which the ovarian cavity becomes narrowed with some immigrating interstitial cells (involving no germ cells in all sections) but the cortical germ cells are normal and well-developed. In the other gonad (Textfig. 2) the cortical germ layer has been fairly degenerated but the ovarian

cavity is still present, immigration of interstitial cells is scarcely observable. Judging from the figure, the cortical germ cells had not been eliminated but degenerated in situ. This figure reminds the degenerated gonad upon the high temperature experiment. The figures (Textfigs. 3–4) made from different sections of a gonad are interesting. The gonad has the marked cortical germ layer, but the ovarian cavity is nearly packed by exuberant immigration of rete cells which sometimes form sex cords. In the figure (Textfig. 3) the cortical germ layer is distinct, but in the figure (Textfig. 4) the germ cells are partly in elimination and some shift toward the medullary portion, thus the figure more or less resembles the section of testis.

Explanation of Textfigures. In all transplanted testis and hypophysis.

1. Affected ovary of salamander already metamorphosed (No. 23 II), 53 mm long. ×200. The ovary is nearly normal with developed cortical germ layer but the ovarian cavity is nearly reduced.

2. Affected ovary of the larva (No. 8 II), 55 mm long. ×200. Fairly degenerated.

3. Affected ovary of the larva (No. 6 III), 47 m long. ×200. Cortical germ layer largely degenrated and ovarian cavity loosely packed with interstitial cells.

4. Another section of the same gonad, showing male gonad in the proximal half. ×200.

The female gonads of this experiment bear some resemblances to those of high temperature experiment, especially in the gonads with the cortical germ layer and medullary interstitial core and in the degenerating ovary (Fig. 13, Pl. 5, 1937), but seem to be different in the following points. In the gonads here considered the elimination of cortical germ cells is easily observable, while in the gonads of the high temperature experiment cortical germ cells generally degenerate in situ and elimination was hardly visible.

Hanaoka (1939) reported that implantation of hypophysis only causes the ingrowth of rete cells to the medulla in the semidifferentiate male of *H. retardatus*. In the present experiment the medullary ingrowth of rete cells is far more remarkable than in the Experiment I (Testis-grafting). Moreover, it is very noticeable that no large oocytes could be seen in these gonads, though frequently observable in the Experiment I.

Literature

HANAOKA, K. I. 1939. Modification of sex in a salamander *Hynobius retardatus* induced by the implantation of testis and hypophysis. Jap. Jour. Zool., vol. 8, no. 3, pp. 255–264, pls. 33–37.

UCHIDA, T. 1937. Studies on the sexuality of Amphibia. II. Sexual induction in a sexually semidifferentiated salamander. Jour. Fac. Sci., Hokkaido Imp. Univ., ser. 6, Zool., vol. 6, no. 1, pp. 35–58, pls. 2–4.

————. 1937. Studies on the sexuality of Amphibia. III. Sex-transformation in *Hynobius retardatus* by the function of high temperature. Ditto, pp. 59–68, pl. 5.

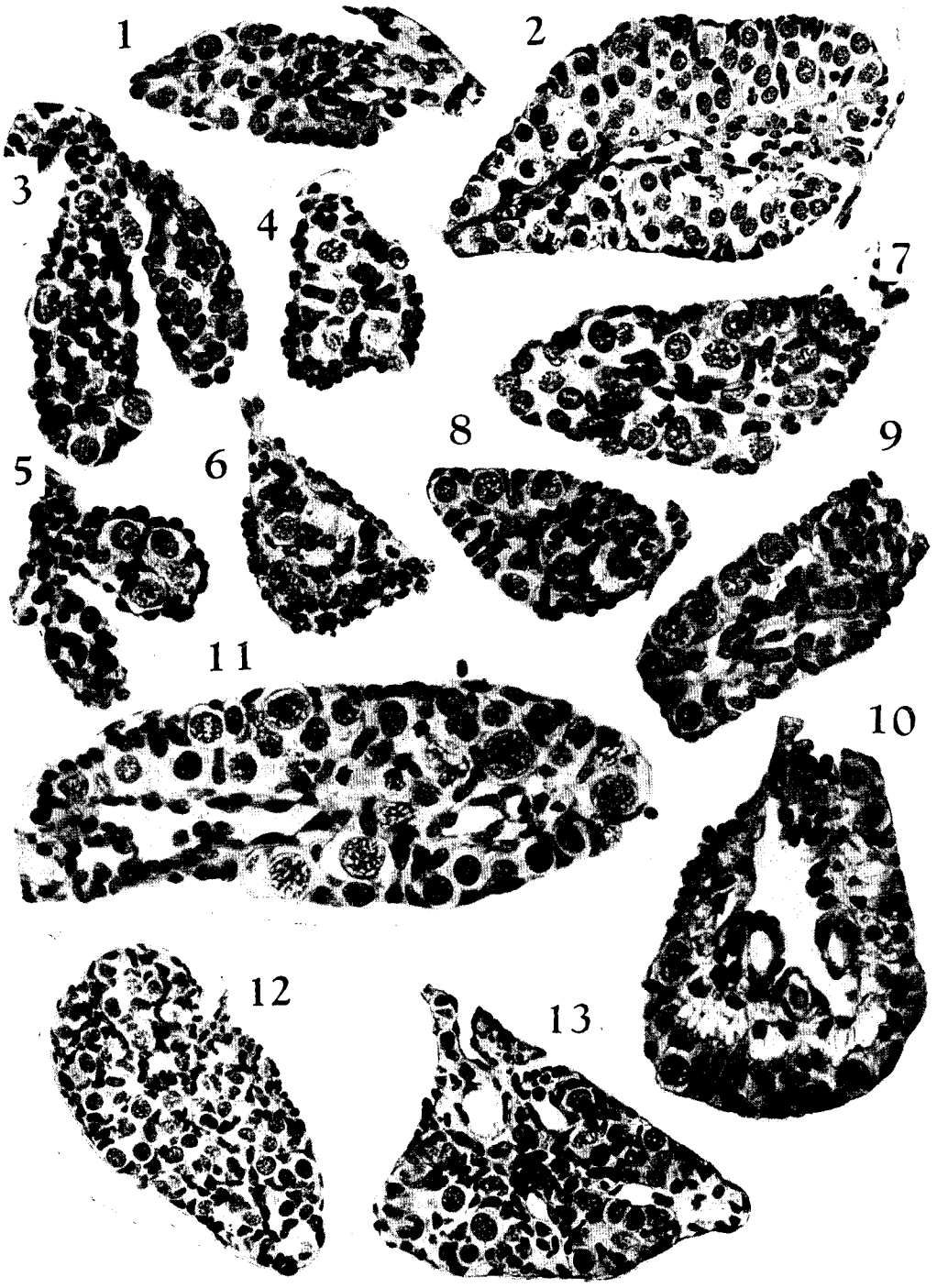
Explanation for Pl. II

1. The neutral gonad of the control larva of *Hynobius lichenatus* (No. 15 B IV), 41 mm in the whole length. ×250.
2. The normal ovary of the control larva (No. 19 E), 48 mm long. ×200.
3. Affected gonad of the larva (No. 14 III), 29 mm long, showing elimination of cortical germ cells. ×250.
4. Affected gonad of the larva (No. 10 I), 32 mm long, showing elimination and medullary immigration of germ cells. ×250.
5. Affected gonad of the larva (No. 19 I), 28 mm long, indicating medullary immigration of germ cells. ×250.
6. Affected gonad of the larva (No. 39 II), 42 mm long, already showing the appearance of typical testis. ×250.
7. Affected gonad of the larva (No. 34 II), 38 mm long. The female gonad is distorted in form, illustrating apparently the resemblance to testis. ×250.

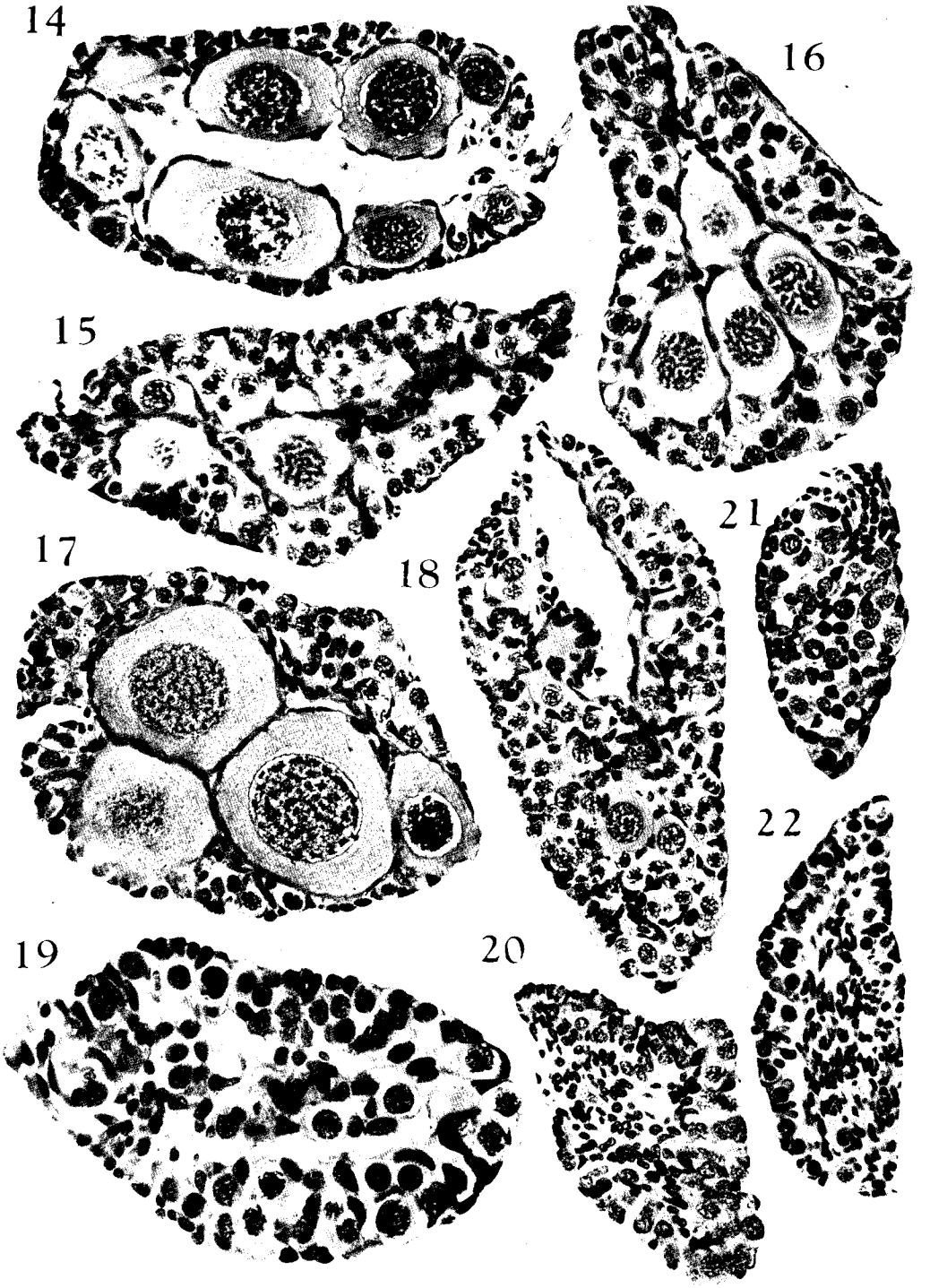
8. Affected gonad of the larva (No. 38 III), 42 mm long. The gonad, female in nature, has cortical germ cells before elimination and medullary germ cells. ×250.
9. Another section of the gonad (Fig. 8), showing the violent ingrowth of sex cords to the medulla. ×250.
10. Affected gonad of the larva (No. 56 III), 47 mm long. The female gonad is largely degenerated. ×250.
11. Affected gonad of the larva (No. 58 IV), 40 mm long. The ovarian cavity of the female gonad is reduced on account of the medullary immigration. ×250.
12. Well developed testis of the salamander, after metamorphosis (No. 66 II), 50 mm long. ×200.
13. Testis of the salamander after metamorphosis, with several cavities (No. 76 VI), 49 mm long. ×200.

Explanation for Pl. III

14. Affected ovary of the salamander after metamorphosis (No. 85 IV), 52 mm long. ×200. Germ cells well activated.
15. Affected ovary of the salamander after metamorphosis (No. 83 II), 43 mm long. ×200. Ovarian cavity obliterated.
16. Another section of Fig. 15.
17. Affected ovary of the salamander after metamorphosis (No. 68 II), 49 mm long. ×20. This ovary seems to be closely related to the mosaic gonad.
18. Affected ovary of the salamander after metamorphosis (No. 78 IV), 47 mm long. ×150. The distal half of the this gonad is masculinized.
19. Affected ovary of the salamander after metamorphosis (No. 71 III), 49 mm long. ×250. The ovarian cavity of this gonad is partially packed with rete cells containing germ cells.
20. Affected ovary of the salamander after metamorphosis (No. 17 III), 49 mm by grafting of testis and hypophysis. ×200 long. The cortical germ layer is slightly eliminated and degenerated, and the ovarian cavity is loosely packed with interstitial cells.
21. Activated testis of the salamander already metamorphosed (No. 18 II), 48 mm long.
22. Affected ovary of the salamander already metamorphosed (No. 9 IV), 60 mm long. ×200. The cortical germ layer is partially degenerated and the ovarian cavity is loosely packed with interstitial cells.



T. Uchida and K. I. Hanaoka: Sexual induction in a sexually differentiated salamander.



T. Uchida and K. I. Hanaoka: Sexual induction in a sexually differentiated salamander.