Studies on the Sexuality of Amphibia. III. Sex-transformation in *Hynobius retardatus* by the Function of High Temperature.[1)]

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With Plate V

Among the vertebrates the Amphibians are especially known as a group possessing prominent amphisexual tendencies. The sex of Amphibians is phenotypically determined by the dominant sexual inductor based on the genetic constitution. During the development prior to the sex differentiation, however, the sexual balance due to the reactions of the two inductors is apt to be lost on account of environmental conditions. In 1929 Witschi reared tadpoles of *Rana sylvatica* after the sex differentiation at a high temperature (32°) and observed that the cortical inductor in larval females was destroyed, while the medullary inductor remained unharmed. According to Piquet's experiment (1930) on *Rana temporaria* and *Bufo vulgaris*, the sexes of these Anurans metamorphosed from tadpoles reared at a moderate temperature (20° C) were approximately equal in number, but the tadpoles raised grown at a relatively low temperature (10° C) metamorphosed with the majority of female individuals while from the tadpoles reared at a high temperature (25° C) more males were obtained than females. The outcome of the present writer's herein reported experiment on the larval gonads of the semi-differentiated Urodelans before the sex differentiation was generally in harmony with the results of these former experiments. The investigation has been carried out through the financial aid of the Imperial Adademy since the spring of 1935. The writer is under very gr:at obligation to Mr. K. Hanaoka for his several forms of kind assitance and to Mr. H. Yamaguchi for the photomicrographic illustrations.

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Experiment

Salamander larvae used in this experiment were mostly reared from eggs collected, shortly after they were laid, in the environs of Sapporo. Several series of the larvae varying in size were reared at a high temperature (30° C) and after various durations of time as shown in Table I they were fixed and sectioned. The autopsy was always made before the completion of the metamorphosis. To avoid the putrification of water the larvae were by feeding transferred into other petry-dishes for an hour. Some larvae were directly put in water of 30° C, but some were first reared in water of 20° C, the temperature of which was then raised by one degree daily up to 30° C. All the experimental animals were more or less retarded in development in comparison with the controls. The larvae used in this experiment were in the following stages at the time of rise of temperature.

A-group. Body length 37–40 mm, from the snout to the anus 20–21.5 mm long. Fore legs 3.5–4 mm, with four digits. Hind legs 1.5–3 mm, with rudimentary digits.

B-group. Body length 22–23 mm. Fore legs 1.5 mm, with rudimentary digits. Hind legs rudimentary.

C-group. Body length 47 mm, from the snout to the anus 27 mm long. Fore legs 7 mm, with four digits. Hind legs 7 mm, with five digits. Tail and other characteristics still in the larval condition.

D-group. Body length 21 mm, from the snout to the anus 12 mm. Fore legs 2 mm, with rudimentary digits. Hind legs rudimentary.

E-group. Body length 36 mm, from the snout to the anus 21 mm. Fore legs 5 mm, with four digits. Hind legs 3 mm, with four rudimentary digits.

F-group. Body length 28 mm, from the snout to the anus 15 mm long. Fore legs 5 mm, with three rudimentary digits. Hind legs 1 mm, with rudimentary digits.

G-group. Body length 34 mm, from the snout to the anus 19 mm long. Fore legs 5 mm, with four digits. Hind legs 4 mm, with four digits.

H-group. Body length 32 mm, from the snout to the anus 19 mm long. Fore legs 4 mm, with three digits. Hind legs 2.5 mm, with rudimentary digits.

L. M. N-groups. Body length 23-24 mm. In this stage the gonads were beginning to make their appearance.

Having examined the gonads of the 79 experimental animals, it was found that the mode of transformation of gonads is somewhat different from that seen in the gonads affected by the testicular grafts, described in the proceeding paper and, though different in degree in individuals, the similar process always occurs. The modification of gonads in the salamander larvae used in this experiment generally lies in the degeneration of the cortical germ layer and in the subsequent growth of interstitial cells towards the medulla.

When high temperature was applied to the young larvae with rudimentary gonads and these larvae were fixed in the indifferent stage, one can perceive only degeneration of cortical germ cells and slight immigration of interstitial cells (Fig. 1). In the experimental gonads in a slightly later stage the rudimentary ovarian cavity is often observable (Fig. 2). In the gonads fixed in the ova ian stage the process of the transformation is clearly displayed. In a slightly affected female gonad cortical germ cells, especially those situated in the proximal portion have been degenerated and the ovarian cavity is slightly narrowed on account of the increase of interstitial cells (Fig. 3). In a more advanced stage (Fig. 4) several degenerated cortical germ cells are distinctly observable and the ovarian cavity is loosely occluded with the immigrated interstitial cells. In the gonads illustrated by Figs. 5 & 6 the ovarian cavity is almost packed with the immigrating interstitial cells. In Fig. 6 exuberant ingrowth of the medullary portion is obvious. In these gonads the portion in which the cortical germ cells have been atrophied is first represented by a seemingly vesicular and sparse connective tissue as shown in Figs. 4 and 6, but is gradually replaced by proliferated interstitial cells. The gonads just referred to, though largely modified in the cortical germ layer and in the ovarian cavity, are still in a female condition.

When the process of the modification proceeds further, the cortical germ layer is utterly atrophied and in the medullary portion rete-cords and sex-cords gradually make their appearance. The gonad pictured in Fig. 7 containing sex-cords and rete-cords, and lacking cortical germ cells is obviously of a male condition. But in the gonad are found some small cavities which can be inferred to be remnants of the ovarian cavity. On the other hand, the gonad represented by Fig. 8 has a well-developed medullary portion composed of rete-cords and sex-cords and no indication of the ovarian cavity, but the cortical germ cells are arranged in a loose row on the periphery. This gonad offers a clear evidence that the gonad undergoes transformation from the female to the male condition. The gonad shown in Fig. 9 is a solid masculinized one, containing a meagre number of germ cells. On the terminal crest of this gonad, however, a peripheral germ cell is present. This germ cell is probably a remnant of the cortical germ layer and of female character. The gonad of Fig. 11 is nearly the normal male gonad

TABLE I

No. of gonads	Body length at the beginning of the experiment	Duration of subjection to high temperature	Body length at autopsy	Condition of gonads
A I	37-40 mm	30/V—9/VI & 9/VI—16/VI	52 mm	DI
A II	37-40 mm	30/V—9/VI & 9/VI—21/VI	46 mm	♀(DI)
A III a	37-40 mm	30/V—9/VI & 9/VI—24/VI	43 mm	♀(Di)
A III b	37-40 mm	30/V—9/VI & 9/VI—24/VI	41 mm	♀(di)
A III c	37-40 mm	30/V—9/VI & 9/VI—24/VI	45 mm	♀(DI)
A III d	37-40 mm	30/V—9/VI & 9/VI—24/VI	49 mm	♀(DI)
A III e	37-40 mm	30/V—9/VI & 9/VI—24/VI	44 mm	♀(DI)
A IV a	37-40 mm	30/V—9/VI & 9/VI—1/VII	47 mm	♀(di)
A IV b	37-40 mm	30/V—9/VI & 9/VI—1/VII	49 mm	♀(Di)
A IV c	37-40 mm	30/V—9/VI & 9/VI—1/VII	50 mm	♀(di)
A IV d	37-40 mm	30/V—9/VI & 9/VI—1/VII	54 mm	♀(dI)
A V a	37-40 mm	30/V—9/VI & 9/VI—8/VII	53 mm	♂(s)
A V b	37-40 mm	30/V—9/VI & 9/VI—8/VII	47 mm	♂(s)
A V c	37-40 mm	30/V—9/VI & 9/VI—8/VII	42 mm	♀(di)
A V d	37-40 mm	30/V—9/VI & 9/VI—8/VII	45 mm	♀(di)
A VI a	37-40 mm	30/V—9/VI & 9/VI—11/VII	49 mm	♀(dI)
A VI b	37-40 mm	30/V—9/VI & 9/VI—11/VII	45 mm	♂
A VII a	37-40 mm	30/V—9/VI & 9/VI—15/VII	47 mm	♂(O)
A VII b	37-40 mm	30/V—9/VI & 9/VI—15/VII	46 mm	♀(dI)
A VII c	37-40 mm	30/V—9/VI & 9/VI—15/VII	50 mm	♀(di)
A VII d	37-40 mm	30/V—9/VI & 9/VI—15/VII	53 mm	♀(dI)

TABLE I (*Continued*)

No. of gonads	Body length at the beginning of the experiment	Duration of subjection to high temperature	Body length at autopsy	Condition of gonads
A VIII a	37–40 mm	30/V–9/VI & 9/VI–22/VII	50 mm	♀(d)
A VIII b	37–40 mm	30/V–9/VI & 9/VI–22/VII	55 mm	♂(O)
B I a	22–23 mm	30/V–9/VI & 9/VI–24/VI	26 mm	Di
B I b	22–23 mm	30/V–9/VI & 9/VI–24/VI	26 mm	DI
B I c	22–23 mm	30/V–9/VI & 9/VI–24/VI	26 mm	dI
B I d	22–23 mm	30/V–9/VI & 9/VI–24/VI	26 mm	dI
B II a	22–23 mm	30/V–9/VI & 9/VI–1/VII	28 mm	dI
B II b	22–23 mm	30/V–9/VI & 9/VI–1/VII	28 mm	DI
B II c	22–23 mm	30/V–9/VI & 9/VI–1/VII	28 mm	♀(D)
B II d	22–23 mm	30/V–9/VI & 9/VI–1/VII	25 mm	dI
B II e	22–33 mm	30/V–9/VI & 9/VI–1/VII	25 mm	dIO
C I a	47 mm	25/VI–8/VII	50 mm	♀(D)
C II a	47 mm	25/VI–2/VIII	51.5 mm	♀(D)
D I a	21 mm	25/VI–24/VII	24 mm	DI
D I b	21 mm	25/VI–24/VII	24 mm	dIO
D I c	21 mm	25/VI–24/VII	24 mm	DiO
E I a	36 mm	1/VII–20/VII	39 mm	Di
E II a	36 mm	1/VII–6/VIII	48 mm	♂
E II b	36 mm	1/VII–6/VIII	46 mm	♀(Di)
E II c	36 mm	1/VII–6/VIII	36 mm	♀(Di)
F I a	28 mm	1/VII–15/VII	32 mm	DI
F I b	28 mm	1/VII–15/VII	27 mm	DO
F I c	28 mm	1/VII–15/VII	24.5 mm	di
F I d	28 mm	1/VII–15/VII	30 mm	DI
G I a	34 mm	8/VII–30/VII	37 mm	♀(Di)
G I b	34 mm	8/VII–30/VII	42 mm	♂(♀)
G I c	34 mm	8/VII–30/VII	36 mm	♀
G I d	34 mm	8/VII–30/VII	42 mm	♂(♀)

TABLE I (*Continued*)

No. of gonads	Body length at the beginning of the experiment	Duration of subjection to high temperature	Body length at autopsy	Condition of gonads
G I e	34 mm	8/VII—30/VII	43 mm	♂
H I a	32 mm	8/VII—6/VIII	34 mm	di
H I b	32 mm	8/VII—6/VIII	34 mm	DI
H I c	32 mm	8/VII—6/VIII	30 mm	DI
H I d	32 mm	8/VII—6/VIII	30 mm	di
L I a	23-24 mm	1/VI—19/VI & 19/VI—21/VI	45 mm	♀(dI)
L II a	23-24 mm	1/VI—19/VI & 19/VI—25/VII	47 mm	♀(dI)
M I a	23-24 mm	1/VI—19/VI & 19/VI—1/VIII	45 mm	♀(d)
M I b	23-24 mm	1/VI—19/VI & 19/VI—1/VIII	47 mm	♀(Di)
M I c	23-24 mm	1/VI—19/VI & 19/VI—1/VIII	43 mm	♀(Di)
M II a	23-24 mm	1/VI—19/VI & 19/VI—6/VIII	43 mm	♀(dI)
M III a	23-24 mm	1/VI—19/VI & 19/VI—9/VIII	52 mm	♀(Di)
M III b	23-24 mm	1/VI—19/VI & 19/VI—9/VIII	45 mm	♀(di)
M III c	23-24 mm	1/VI—19/VI & 19/VI—9/VIII	46 mm	♂(s)
N I a	23-24 mm	1/VI—19/VI & 19/VI—22/VIII	48 mm	♀(dI)
N I b	23-24 mm	1/VI—19/VI & 19/VI—22/VIII	40 mm	♀(DI)
N II a	23-24 mm	1/VI—1 /VI & 19/VI—26/III	44 mm	♀(Di)
N II b	23-24 mm	1/VI—19/VI & 19/VI—26/VIII	41.5 mm	♀(DI)
N II c	23-24 mm	1/VI—19/VI & 19/VI—26/VIII	46 mm	DIO
N III a	23-24 mm	1/VI—19/VI & 19/VI—29/VIII	54 mm	♀(di)
N III b	23-24 mm	1/VI—19/VI & 19/VI—29/VIII	57 mm	♀(dI)
N III c	23-24 mm	1/VI—19/VI & 19/VI—29/VIII	53 mm	♀(DI)
N III d	23-24 mm	1/VI—19/VI & 19/VI—29/VIII	54 mm	♀(DI)

TABLE I (*Continued*)

No. of gonads	Body length at the beginning of the experiment	Duration of subjection to high temperature	Body length at autopsy	Condition of gonads
N III e	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	50 mm	♀(dI)
N III f	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	46 mm	♀(Di)
N III g	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	47 mm	♀(dI)
N III h	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	44 mm	♀(Di)
N III i	23-24 mm	1/VI–19/VI & 1 /VI–29/VIII	42 mm	♀(D)
N III j	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	40 mm	♀(Di)
N III k	23-24 mm	1/VI–19/VI & 19/VI–29/VIII	42 mm	♀(di)

Explanations of abbreviations of Table I.

D germ cells absolutely degenerated, D germ cells greatly degenerated, d germ cells more or less degenerated, I with exuberant growth of interstitial cells into the medulla, i interstitial cells proliferating, s with small number of germ cells, o with remnants of ovarian cavity, ♂(♀) male gonad with female features.

N.B. In the groups; A, B, L, M, N, the lavae were first put in water of 20°C and the temperature of water was raised by one degree daily up to 30°C.

having distinct sex-cords. Fig. 12 is the picture of a better organized male gonad. In the gonad illustrated by Fig. 10 the germ cells are hardly visible but the interstitial cells are proliferating making nodules here and there, thus causing a state of cell-anarchy. This gonad has probably been regenerated from a degenerated gonad, but no germ cells have been developed in it.

Gonads subjected to high temperature in the ovarian stage are shown in Figs. 13 & 14. In Fig. 13 cortical germ cells have been utterly degenerated while the interstitial cells surrounding the ovarian cavity and located on the periphery are all inactive. The gonad represented by Fig. 14 has several inactive germ cells but interstitial cells are also inactive. The common features observable in these gonads are the degeneration of cortical germ cells, the presence of the distinct ovarian cavity, and non-proliferation of interstitial cells. These gonads are degenerated female ones but have no male characteristics. They recall the gonad of *Rana sylvatica* in Witschi's experiment (Pl. 2, fig. 7). In the Table I have been summarised the results of this experiment.

Discussion

As is shown in the table the gonads of the experimental salamanders are mostly in stages of transformation from female to male gonads as is seen in Figs. 4–6. This is because at the autopsy all the larvae were in the stages before the metamorphosis, and the gonads therefore were mostly in the later ovarian stage. There are, of course, some distinct male and female gonads. In these gonads, the male ones are not different from those in the control animals, though largely delayed in development, while gonads of the female type including some male gonads in the ovarian stage, are always more or less affected. In addition there are some active gonads of the male type, which are still furnished with remnant characteristics of the female, such as the ovarian cavity and cortical germ layer. Judging from the active medulla and the degenerating cortex, these gonads can undoubtedly be inferred to be female gonads in process of transformation into male ones. Though the experiment was interrupted by the metamorphosis[1], during which the salamanders are liable to die, it is probable that the female features will gradually fade out in these larvae, if the high temperature be maintained further. But it can not be difinitely concluded that the male features will gradually develop in all the larvae at the high temperature. Considerations of this point will be discussed below.

The writer suggested in his proceeding paper that the effect of testicular grafts upon the gonads of the larval hosts is divergent according to the genetical constitution of the latter. In conformity with that idea the results obtained in this high temperature experiment are different in degree even in the similar environments. The correlation between the medullary growth and the degeneration of the peripheral cortical layer varies in the larvae of the same series; in some gonads, in which the cortical germ cells were still partially active, the interstitial cells were immigrating towards the medulla, while in other gonads, in which the cortical germ cells were already degenerated, no

1) After the metamorphosis the species lives on land.

proliferation of the interstitial cells was observed. These gonads seem to have a genetical constitution inclining to the female and are probably represented by the gonads pictured by Fig. 13 and Witschi's Fig. 7, Pl. 2. There are some gonads which have been slightly modified by heat treatment but still are distinctly ovarian in structure. These gonads seem to have relatively strong resistance againt heat influence. The gonads, in which the medullary cells are liable to be proliferated and to immigrate toward the medulla, despite the presence of cortical germ cells, are surmised to have male tendencies in the genetical constitution.

Comparing with gonads under the effect of testicular grafts, the gonads influenced by the high temperature nearly coincide in the process of transformation as stated above. The special cases found in the former gonads; as the free-martin condition, appearance of ova probably in a pathologically accelerated maturation, and the mosaic hermaphrodite gonads, were not encountered in the temperature experiment.

It is surmised by the writer that the high temperature causes the degeneration of the cortex (female inductor) but does not directly activate the medullary growth (male inductor). In this experiment the degeneration of the cortex always occurs first and subsequently the medullary growth takes place. Furthermore, there were found some gonads in which the cortex was utterly degenerated but no multiplication of the interstitial cells was observable. Regarding the degeneration of the female germ cells Witschi pointed out in his paper that the oocytes and ova became degenerated but the primary oogonia could resist against heat and even multiply by mitotic divisions. Further, he is of the opinion that some oogonia enter the newly formed seminiferous tubules and transform into spermatogonia. In the present writer's experiment mitotic division of the germ cells could be observed not infrequently and germ cells were often engulfed by sex cords towards the medulla but the germ cells thus incorporated with sex-cords were found generally to become degenerated.

Summary

1. The larvae of the semi-differentiated salamander in several series of different stages of development were reared at a high temperature (30° C).

2. The gonads of these larvae are found, with a few exceptions, all to undergo transformation from the female to the male features, though different in degree.

3. The transformation always commences with the degeneration of the cortical germ layer and is followed by the subsequent medullary growth of interstitial cells.

4. Differences of grades of the transformation under the same environmental condition seem to be due to the genetical constitution of individuals.

5. Modifications of these gonads are not so diverse as in the gonads influenced by the testicular grafts.

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PLATE V

Explanation of Plate V

All × 300

Fig. 1. Section through the middle part of an affected gonad in the indifferent stage (B II a); cortical cells slightly degenerated and interstitial cells more or less proliferated.

Fig. 2. Section through the middle part of an affected gonad in the later indifferent stage (B I b), showing degeneration of cortical germ cells, medullary growth of interstitial cells and a small ovarian cavity.

Fig. 3. Cross section of a slightly affected female gonad (A VIII a); cortical germ cells slightly degenerated and ovarian cavity more or less narrowed. Proliferation of interstitial cells not prominent.

Fig. 4. Section through the middle portion of an affected female gonad (A VI a), showing degeneration of cortical germ cells and multiplication of interstitial cells. Ovarian cavity gradually being obliterated.

Fig. 5. Section through the middle portion of an affected female gonad (A VII d) slightly more advanced in stage than the gonad in Fig. 4. Ovarian cavity nearly occluded.

Fig. 6. Section through the middle portion of an affected female gonad (A IV c) showing exuberant growth of interstitial cells towards the medulla.

Fig. 7. Section through a nearly masculinized gonad (A VIII b) containing rete cords and lacking cortical germ cells. Rudiments of the ovarian cavity still observable.

Fig. 8. Section through a masculinized gonad (G I b) with the well-developed medullary part but still having cortical germ cells which are inactive and arranged in a row on the periphery.

Fig. 9. Section through a masculinized solid gonad (A VI b) with a cortical germ cell in the terminal end.

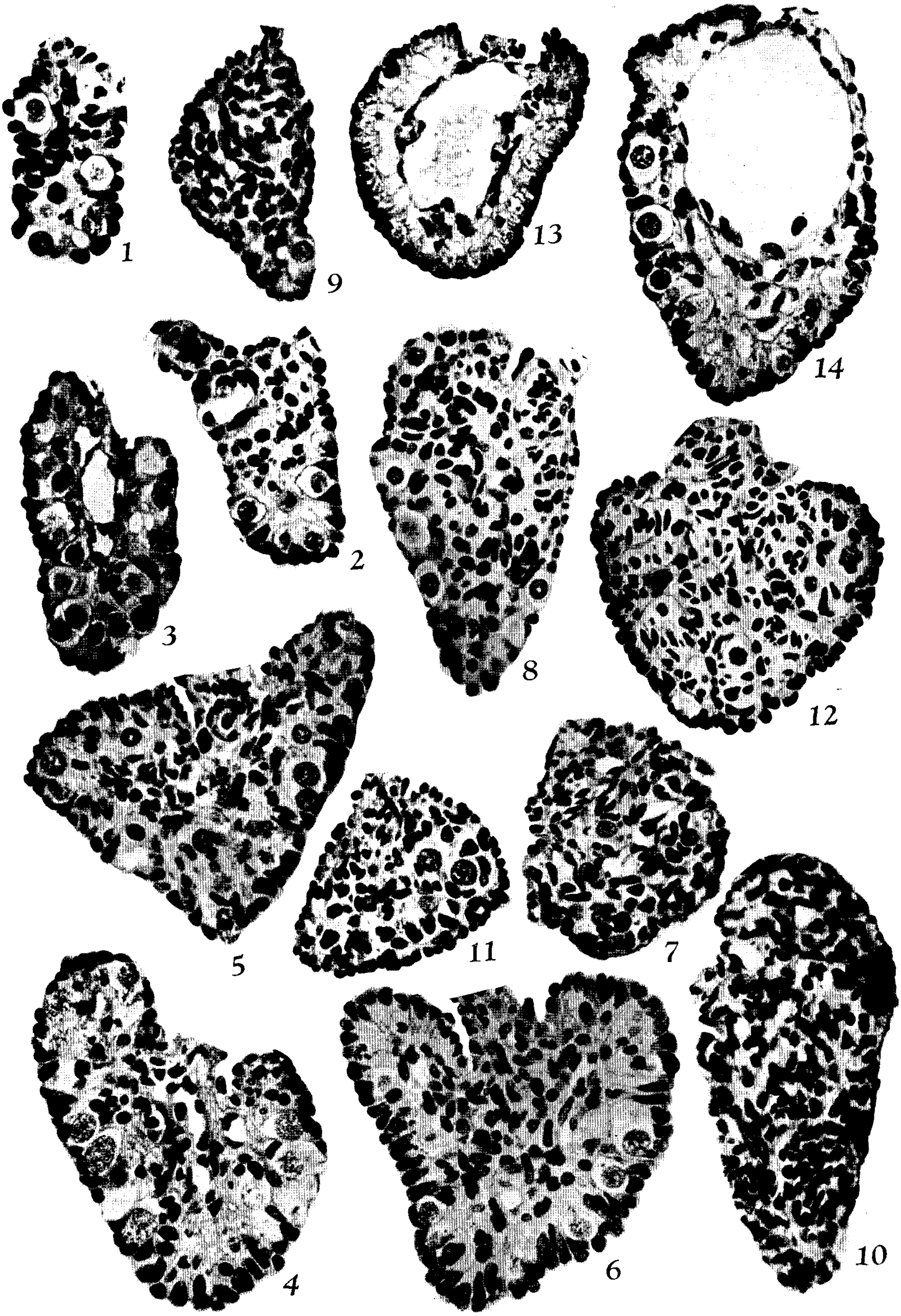
Fig. 10. Section through a masculinized gonad (A VII a) showing a cell-anarchy. Germ cells hardly observable in this gonad.

Fig. 11. Section through a masculine gonad (E II a) containing rete- and sex-cords.

Fig. 12. Section through the middle portion of a well-formed masculine gonad (G I d) containing rete- and sex-cords.

Fig. 13. Section through the middle portion of a degenerated female gonad (C I a) having a distinct ovarian cavity but no germ cells. Interstitial cells in this gonad scarce and inactive.

Fig. 14. Section through the middle portion of a degenerated female gonad (C II a) with a clear ovarian cavity and a few inactive cortical germ cells. Interstitial cells inactive and showing no multiplication at all.



H. Yamaguchi photo.

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