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IS THERE A TRANSFORMATION OF SEX IN FROGS?

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THIS paper is a reply to the recent article of Dr. Emil Witschi which appeared in a late issue of the NATURALIST (Vol. LV, No. 641). Witschi is quite convinced that the problem of sex development and differentiation in frogs has been settled, and that nothing further remains to be said. However, the writer feels that instead of being solved, the time has come for a revision of the entire question of sex development in Anurans, and that the subject is ripe for a reinterpretation upon a more rational basis than that accorded to it heretofore.

The first portion of the paper will be devoted to a brief exposition of the writer's interpretation of sex in frog larvæ based upon data obtained from a study of the bullfrog. The second part of the paper is a reply to certain questions raised by Dr. Witschi.

In larval males of the bullfrog two gonads are formed, just as there are two kidneys formed, a pro-testis or embryonic sex gland destined to degenerate and disappear in ontogenetic development and a definite or functional testis which replaces it. The germinal elements of the pro-testis arise in the entoderm and migrate into the germ ridges early in embryonic life. The cells multiply rapidly and together with the mesodermal elements of the germ glands form paired ridges projecting into the cœlomic cavity. While the tadpole is very immature and has yet a year of larval life before metamorphosing, the

germ cells of the pro-testis undergo a precocious and abortive sexual cycle culminating in degeneration and resorption. Beautiful cysts of spermatocytes are formed, but the first maturation division rarely proceeds past the

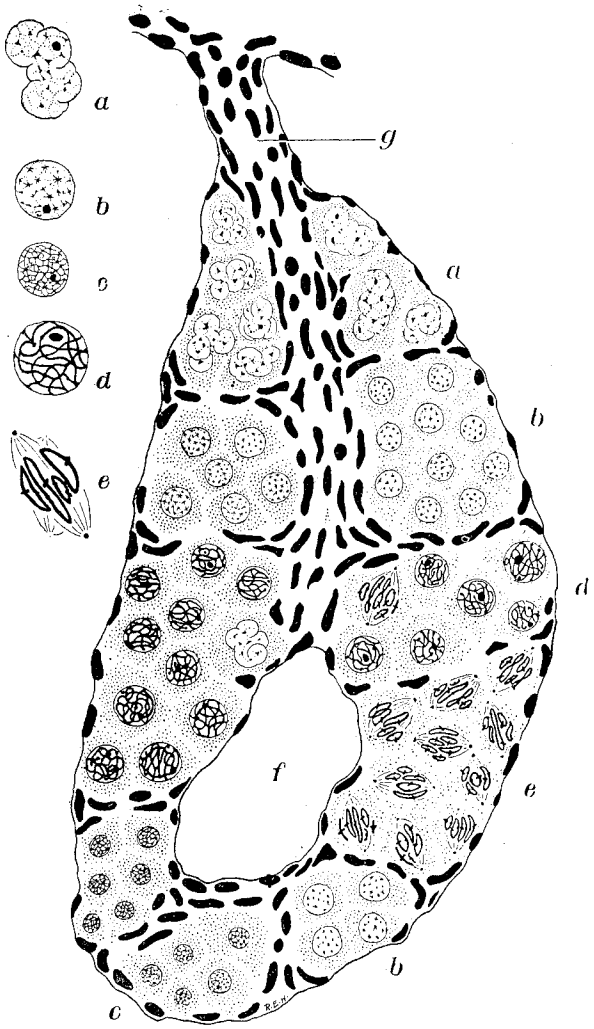

FIG. 1. Transverse section pro-testis *R. catesbeiana* tadpole. Animal has a year of larval life remaining. *A*, Spermatogonia showing nuclear polymorphism due to incomplete fusion chromosomal vesicles; *B*, Final spermatogonia; *C*, Spermatocytes in leptotene stage; *D*, Amphitene and pachytene; *E*, Heterotypic mitosis; *F*, Secondary genital cavity; *G*, Anlage of sex cords of definitive gonad which develops as a core within pro-testis.

anaphase owing to fragmentation of the centrosome and consequent formation of polyasters (Fig. 1). Sometimes aberrant spermatids are formed by suppression of the first and second maturation divisions and growth of axial filaments from the centrosome. Practically all the germ cells of the pro-testis degenerate and disappear while in various stages of maturation—some undergo an oviform type of degeneration, *i.e.,* hypertrophy enormously and take on the superficial characters of oocytes. The oviform type of degeneration, however, is more characteristic of the short larval-lived frogs than of *R. catesbeiana,* for in many animals these large cells appear rarely and in others not at all and this is an important point to keep in mind. This type of degeneration will be discussed in detail in a later paper; suffice to say it gives no clue to the sex of a cell. (See Plates 1 and 2.)

Some cells of the pro-testis fail to take part in the abortive sexual cycle persisting through the phase of maturation and degenerate as spermatogonia. These elements migrate into the sex cords (Fig. 1, *g*) which have formed meantime, and form a core of germinal tissue extending through the center of the pro-testis. This core of tissue plus the sex cords is the anlage of the definitive testis and is quite distinct from the pro-testis, the cells of which are maturating and degenerating, whereas the cells of the forming functional gonad remain as primitive spermatogonia. The definitive testis by rapid growth completely supplants the pro-testis which usually disappears some time before metamorphosis. The functional gonad is generally fully formed at metamorphosis when the larvæ are two years of age. Some tadpoles, but not all, develop ripe spermatozoa in the gonad at metamorphosis due to a second sexual cycle of the germ cells of the definitive gonad. (Swingle, '21, *Jour. Exp. Zool.,* Vol. 32.)

In the frogs with short larval-life the same succession of gonads occurs, but in these forms the developmental processes are greatly accelerated and the pro-testis ma-

turation cycle is cut short by the cells early becoming senescent and undergoing oviform degeneration *i.e.,* hypertrophy to such an extent as to superficially resemble oocytes. This oviform degeneration occurs to an even more marked degree in the progonad of the toad which has a still shorter larval-life, *e.g.,* in Bidder's organ. In male anurans the entire pro-testis or larval gonad is the homologue of the male organ of Bidder in *Bufo.*

The pro-testis of the short larval-lived frogs has been misinterpreted as an ovary owing to the oviform-type of degeneration characteristic of many of its senescent cells, and hence tadpoles are said to develop first as females, fifty per cent. later transforming into males. The normal embryological process by which the definitive testis develops as a central axis through the degenerating pro-testis or larval Bidder's organ, has been described by Witschi as the transformation of female tadpoles into males. In *R. catesbeiana,* where the larval life is prolonged over two years, the true nature of the pro-testis is revealed, for relatively few of the cells are of the oviform type and all transition stages between such cells and normal spermatocytes occur. The evidence presented by this material will be published in due time, and is too clean-cut to admit of any doubt that the entire larval gonad of male anurans is simply an embryonic male sex gland rudiment and not a temporary ovary. Witschi's Fig. 6 (this journal, Vol. LV), which he supposes is an ovary transforming into a testis is simply a transition stage in the development of the definitive testis, and degeneration of the pro-testis or Bidder's organ in a short larval-lived frog. Compare his Fig. 6 with Fig. 1 of this paper and note how the true male character of the cells of the pro-testis comes out in *Rana catesbeiana* tadpoles.

When the facts are considered it is evident that the transitory gonadic rudiment of male frog larvæ is an organ of Bidder which degenerates and is replaced by the definitive gonad. Any one who has studied the oviform-like

cells of the so-called sexually intermediate tadpoles and compared them with the cells of Bidder's organ in male toads, is at once struck by the remarkable similarity in their origin, development, structure and fate in the two groups. They are identical. The crux of the problem is the nature of Bidder's organ in male Bufonidæ and of the oviform-like cells of the pro-testis. The advocates of sex transformation have assumed that such cells are undoubtedly female, but no proof has ever been advanced that they are. Their ultimate fate is the same as that of the first year spermatocytes in the bullfrog tadpole — degeneration (see Plates 1 and 2). The sex-transformationists have been misled by the idea that everything superficially resembling an oocyte is necessarily such, or that any cell in tadpoles and first-year animals undergoing the early growth stages, leptotene, pachytene, etc., is to be regarded as female. These are fallacious criteria. Enormously hypertrophied oocyte-like cells which have passed through the early growth stages and entered the "germinal vesicle" period so characteristic of oocytes, occur as normal features of the male sexual cycle of certain animals, *e.g.*, myriapods (Figs. 5–8). These animals were at first regarded as hermaphrodites by Blackman (1905, *Bull. Mus. Comp. Zool.*, Harvard, Vol. XLVIII, no. 1) who found upon examination, however, that these "oocytes" were in reality spermatocytes of giant proportions, and developed into spermatozoa. The writer has examined some of Professor Blackman's material and the oocyte-like character of the male sex-cells is remarkable. In the material examined these cells practically fill the gonads. Firket, 1920, working on the chick embryo, describes and figures spermatocytes undergoing oviform degeneration, *i.e.*, enlarging to such an extent as to resemble oocytes. There are many other cases reported in the literature. How does Witschi know that the transitory oocyte-like cells he describes in the future male tadpoles or so-called hermaphrodites, are female cells and not senescent organ of Bidder cells occurring

in the course of the abortive and degenerate sexual cycle of an embryonic pro-testis?

The work of Witschi on the problem of sex in anurans can be summarized thus: He has described in great detail and with admirable exactness the process of development of the pro-testis or Bidder's organ in the short larval-lived frogs, its degeneration and final replacement by the definitive gonad. This process he calls transformation of females into males. The experimental investigations of Witschi upon sex transformation by environmental influences consists of this: By means of such agents as heat or cold, etc., he has simply modified the normal course of development of the pro-testis — Bidder's organ, thereby accelerating or delaying the development of the definitive testis. The experimental results show that it is possible to modify the developmental rate of the embryonic testis. Similar experiments carried out with regard to other larval structures would unquestionably give similar developmental modification. Cold hinders metamorphosis and all the normal structural changes metamorphosis implies. All of these environmental influences are interferences with the normal cycle of the gonads, by which the development of the definitive gonad out of the pro-testis is accelerated, retarded, or possibly prevented entirely. The following quotation from Witschi '14, page 10, is significant in this connection:

> Bei seinen Untersuchungen war es Hertwig aufgefallen, das unter dem Einfluss verschiedener Aussenbedingungen sich nicht nur die Geschlechtsziffern, sondern oft auch in ganz auffälliger Weise der Rhythmus, in Welchem die Keimdrüsen und manche andere Organe sich anlegen und entwickeln.

It is probable, judging from certain experiments reported, that the degree of development attained by the larval gonadic rudiment, its position in relation to the definitive gonads, its period of persistence, non-formation in some forms, and such like, may vary in different frog species and is determined by heritable factors. For example, in *Bufo*, the structure persists throughout life in

males, disappears after two years in females, and is anterior to the functional gonads. In frogs it forms the outer husk of the germ gland enclosing the centrally developing functional testis and may or may not show the oviform type of degeneration, *e.g., R. catesbeiana.*

If sex is so labile in tadpoles and young frogs, and females so readily transform into males under environmental stimuli, why is it that such sex reversals do not occur in adult frogs *after the degeneration of the protestis* and the formation of the definitive testis has occurred? All investigators are agreed that the sex ratio of adult frogs of all species reported is approximately 50–50. If environment (ever changing in the same locality, and never the same in different regions), plays such an important sex transforming rôle, why do male tadpoles never transform into females — all investigators agree that they do not. Why do only fifty per cent. of the so-called larval females transform into males if they were not zygotic males from the beginning, and why do not all female frog larvæ transform into males instead of only fifty per cent. if such transformation is possible? Appeal cannot be made to Professor Hertwig's well-known late fertilization experiments because in these experiments the influence of the over-ripeness of the egg upon the zygotic conditions determining sex are unknown. Hormones! To date there is no positive evidence that such secretions have ever actually changed a female germ cell into a functional male germ-cell.

Cases of hermaphroditism in adult frogs are thought by some to furnish evidence of a sex transformation in frogs. However, true hermaphroditism in adult frogs is as rare a phenomenon as it is in mammals when we consider the few recorded cases, and the enormous number of frogs annually dissected the world over. Crew ('21), *Journal of Genetics,* Vol. II, no. 2, has summarized the recorded cases of abnormal sexual organs in frogs and states that there are forty cases. To this number should be added a recent case described in the bullfrog, making

forty-one. Among these forty-one cases, there are but twenty-seven true hermaphrodites. Crew's cases, twenty-one to thirty-three, inclusive, are not hermaphrodites, nor is case thirty-eight, as none of the animals possess ovotestes and some are entirely without gonads. True hermaphroditism in frogs is a permanent and pathological condition, probably due to a mix-up in the genetic constitution of the individual, and is not to be confused with the present problem which has to do with a normal but transitory embryological process.

Much has been written about the marked "sex potencies" of various portions of the gonads in so-called sexually intermediate frogs, *i.e.*, females transforming into males. It is claimed that the outer rind of the gonad exerts a profound female sex influence, while the inner portion exerts a purely male influence. Germ-cells remaining in the outer husk (the main portion of the larval gonad by the way) of the gland are female, those migrating into the central part among the sex cords become male. All such speculations are based upon misinterpretations. The outer portion or husk of the larval male gonad is simply the pro-testis, the cells of which are undergoing a precocious maturation cycle just as they do in the organ of Bidder in *Bufo*, the inner portion or sex cord region is where the definitive gonad begins development and as it spreads and grows the embryonic male gonad degenerates and disappears. *It is in the region of most marked "female" tendencies that the writer finds in the bullfrog entire cysts of unmistakable spermatocytes, and occasional spermatids* (Fig. 1, *e*). In other words, the pro-testis — what Witschi regards as an ovary — can in the bullfrog, where its development is greatly prolonged, give rise to practically mature male sex products. Recently, the writer made an observation of considerable interest. In the degenerating Bidder's organ (pro-testis) of a two-year-old male larva in which formation of the definitive testis had been delayed until metamorphosis and in which the oviform type of degeneration

was the most marked of any animal yet observed, several cysts of unmistakable spermatocytes and spermatids were observed. They arose from the maturating cells of what Witschi regards as the female part of the gonad — in reality the pro-testis, and were of the cell type characteristic of the adult frog. This observation shows two things clearly: (1) *That the direct descendants of the male primordial germ cells (pro-testis elements) can produce practically mature germ cells;* (2) *that the spermatocytes of the structure regarded by the writer as a pro-testis are really male cells, and that the structure in so-called sexually intermediate frogs and tadpoles is in no sense to be regarded as female in character.*

Another point is of interest here — the writer has never observed direct testicular development in *R. catesbeiana,* though it probably occurs in some strains; the indirect method alone has been found, *e.g.,* first a pro-testis is formed which is later supplanted by the definitive gonad. In the bullfrog, which has the longest larval life of any anuran, the pro-testis persists longer than in other forms, sometimes two years before giving place to the definitive gonad. What the writer calls a pro-testis of so-called sexually intermediate tadpoles is according to Witschi a transitory ovary. If this is true why is it that despite its persistence for such a long time, relatively few oocyte-like cells are found in *R. catesbeiana* and in many individuals none, throughout a two-year period, but instead the structure produces spermatocytes and sometimes spermatids? Why is it, if this structure is an ovary in the so-called females that later transform into males, that the shorter the larval life of male anurans, the more the pro-testis in its structure and behavior resembles the Bidder's organ characteristic of male toads, due to rapid oviform degeneration of its cells; the longer the larval life, *e.g., Rana catesbeiana,* the more the germinal elements undergo a normal sexual cycle characteristic of male cells? The answer is, because in forms with extraordinary prolonged larval lives the

true nature of the embryonic male gonad has sufficient time to manifest itself before being supplanted by the definitive gland.

We come now to a discussion of the nature of Bidder's organ in *Bufo*, for this is the classical example of oviform degeneration of racially senescent germ cells. Heretofore, this embryonic sex gland rudiment has been regarded as characteristic of toads, but such is not the case. In frogs the pro-testis or larval gonad is a Bidder's organ, destined to be replaced by the definitive male gonad developing within; in male toad larvæ on the other hand, the functional gonad arises behind the pro-testis or Bidder's organ, consequently this structure persists as a degenerate gonadic rudiment attached to the functional gland.

According to the writer's view, Bidder's organ in *Bufo* is simply a vestigial larval gonad persisting throughout life and has the same sex as the definitive gonad behind it — male in males, female in females. It is just as though the pro-nephros of tadpoles persisted as a nonfunctional and degenerate rudiment at the end of the mesonephros. That many such larval and embryonic rudiments do persist through adult life in various animals is a commonplace of embryology, and their persistence in one species and total disappearance in another related one, is also well known. Bidder's organ in *Bufo* then, is a persisting, in frogs a transitory, embryonic sex gland rudiment, a relic of a phylogenetically earlier sexual condition. The functional gonads are more recently acquired structures (like the larval mesonephros) superimposed upon the older degenerate glands. Briefly stated, the evidence for the view that Bidder's organ is homologous to the pro-testis of frogs and that it is not a rudimentary ovary except in female animals is as follows:

1. The cells of Bidder's organ in *Bufo* are unquestionably germ cells. The gland appears very early in embryonic life (two weeks after hatching) and its cells far

outstrip in development the cells of the definitive gonads located posteriorly.

2. The cells of Bidder's organ extremely early in development undergo a precocious and abortive maturation cycle and become senescent and degenerate oocyte-like structures when the germinal elements of the functional gonads have barely started to multiply to form the definitive glands. This occurs in individuals of both sexes.

3. The larval maturation cycle such as occurs in the bullfrog, and in other anurans, throughout the entire larval gonad is confined to Bidder's organ in *Bufo*, and the changes occurring in this structure do not affect the normal developmental cycle of the definitive germ glands behind.

4. The so-called transformation of female animals into males, claimed by Witschi and others to be the normal course of development in frogs, does not occur in toads. Why? Because in *Bufo*, the definitive gonads are from the beginning located posterior to Bidder's organ, and it is not necessary in order that they may develop that this structure degenerate and disappear as is the case in frogs where the definitive testis starts development as a core within the pro-testis or Bidder's organ, necessitating its complete destruction.

5. Few have ever claimed that sex in toads is labile and easily reversed by environmental influences. Why? Because the sex of the definitive gonads is definitely fixed and clear cut at an early stage of life. The separation of Bidder's organ and the gonads has precluded the possibility of confusing the pro-gonad and the definitive gonad.

6. Bidder's organ is merely a persisting embryonic gonad whose cells have undergone oviform degeneration. It is not a rudimentary ovary except in female animals. This is indicated by its presence in both sexes in toads; its presence in Spengel's case of true hermaphroditism; by the fact that neither in male or female of toads do

its cells develop into true functional eggs; and by its degenerate structure from its inception in both sexes.

In a recent paper (*Zoologischer Anzeiger,* Dec., 1921) Harms describes marked hypertrophy of Bidder's organ following testis removal. He considers that castration of males causes Bidder's organ to develop into an ovary. However, it should be noted that such operated animals with hypertrophied Bidder's organ (ovary according to Harms) retain all their male secondary sex characters, and their normal mating instincts and that these male characters and instincts undergo a normal cyclical development in such induced "females." When Harms removed both testes and Bidder's organ the somatic sex characters and instincts failed to develop, showing clearly that Bidder's organ in male toads acts like a testis in maintaining the secondary sexual characters. This is excellent evidence for the writer's view that in male toads Bidder's organ is simply a persisting embryonic male sex gland rudiment and not an ovary. If it is an ovary why should it develop and maintain the secondary sex characters of the male in absence of the testis?

7. Recent investigators have inclined to the view that this structure is a hermaphrodite gland, *i.e.,* in male toads a rudimentary ovary, in females a rudimentary testis. If this is true then the admission is made that large, senescent, oocyte-like germ cells are not necessarily female cells — the crucial point for which the writer is contending.

8. Bidder's organ in *Bufo* corresponds to the larval gonad of frogs which in these forms disappears in the male and is replaced by the definitive testis. In the case of female anurans so far as the writer is aware no one has carried out a thorough investigation of the germ cycle from larval to fully adult life to see whether or not such a degeneration occurs in the female line. In mammals and birds such degeneration of the female embryonic line of germ-cells is quite well established as the work of Winiwarter, Firket and others shows.

The writer is of the opinion that it is only by adopting the view advanced here regarding the homologous nature of the larval male gonad of frogs, and Bidder's organ in *Bufo*, that the problem of sex differentiation in anurans can be placed upon a rational basis. The theory accords with the embryological facts, covers the experimental finding of Witschi and others, accords with our own cytological data in the bullfrog, accords with the embryonic sexual conditions of other vertebrates, *i.e.*, the degeneration of the embryonic line of germ cells in birds and mammals, and lastly furnishes an explanation of Bidder's organ in *Bufo*.

The key to the puzzle of sex development in frogs is simply this: every cell that superficially resembles an oocyte is not necessarily a female cell especially when occurring in an otherwise male individual, and that the larval male gonad of anurans is an organ of Bidder — a rudimentary embryonic sex gland with the same sex as the definitive gonad arising out of it. Misinterpretation of oviform hypertrophy and degeneration of racially senescent sex cells has rendered chaotic the problem of sex differentiation in anurans (see Plates 1 and 2).

Witschi regards the development of certain somatic sex characters such as the Müllerian ducts as very positive evidence for his theory of sex transformation. He says:

> In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact. After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink but they do not disappear completely, etc., etc.

The following data shows that in reality such so-called parallelism in the behavior of the Müllerian ducts and

the gonads does not exist and that evidence based on such parallelism is worthless.

In the normal males of adult *Rana pipiens* the Müllerian ducts are remarkable for their size and degree of development. They arise as cellular cords in the peritoneum at the time of metamorphosis and only acquire full development long after transformation when they come to resemble to a striking degree the oviducts of females (Fig. 2). In the larva of *R. pipiens* the so-called

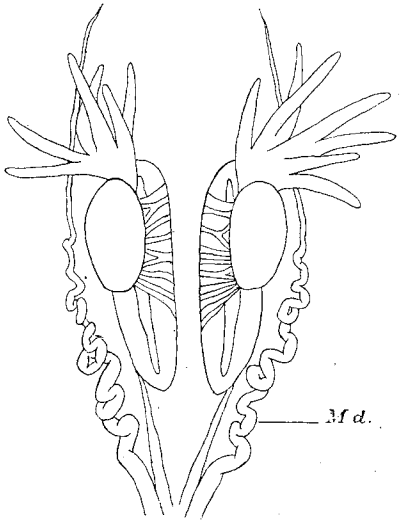

FIG. 2. Urogenital apparatus of adult *Rana pipiens* showing the normal condition of the Müllerian ducts (*md*) in males of this species.

transformation of females into males (degeneration of the pro-testis and formation of the definitive testis) occurs very early in larval life, before the Müllerian ducts appear, and in this species the ducts undergo practically their entire development after the definitive testis has formed. In other words, while subjected to the influence of the fully formed testis and its ripening sex products the ducts undergo the most marked development known in the males of any anuran species. Moreover, in *Rana catesbeiana,* where if we accept Witschi's interpretation of femaleness, the so-called transformation of female in-

dividuals into males is a prolonged process requiring two years, and where the future male larvæ are subjected to the so-called female influence during the entire period, the Müllerian duct does not appear. At metamorphosis when the definitive testes are fully formed and spermatozoa are beginning to appear the cellular cords representing the vestigial Müllerian ducts of the male form but do not develop. If Witschi's interpretation were correct, one would certainly expect to find marked development and hypertrophy of the Müllerian ducts in *R. catesbeiana* because of their being so long exposed to female influence. As a matter of fact, these structures in the male bullfrog are less developed than in other forms.

The same criticism applies to the so-called developmental correlation of the Müllerian duct with the gonad of the same side in cases of lateral hermaphroditism. What Witschi terms lateral hermaphrodites are nothing more than larvæ or young frogs which show the definitive testis developing out of the pro-testis (larval male Bidder's organ) faster on one side than on the other. (See Witschi, AM. NAT., page 533.) In the end such animals develop into definite males with testes symmetrically formed. True lateral hermaphroditism in adult frogs is an exceedingly rare phenomenon. In the writer's material it is rare to find both definitive testes developing out of the pro-testes at the same rate, one gland may be the finished gonad, the other the pro-testis undergoing degeneration. Such larvæ are in no sense to be regarded as lateral hermaphrodites. There is no developmental correlation of the Müllerian ducts with the gonad of the same side in *R. catesbeiana* and *R. pipiens,* because there are no ducts formed until after the definitive testes are formed. Regarding the other somatic sex characters such as seminal vesicles and thumb cushions, it should be pointed out that the thumb pad in *R. catesbeiana* is not formed until after metamorphosis when

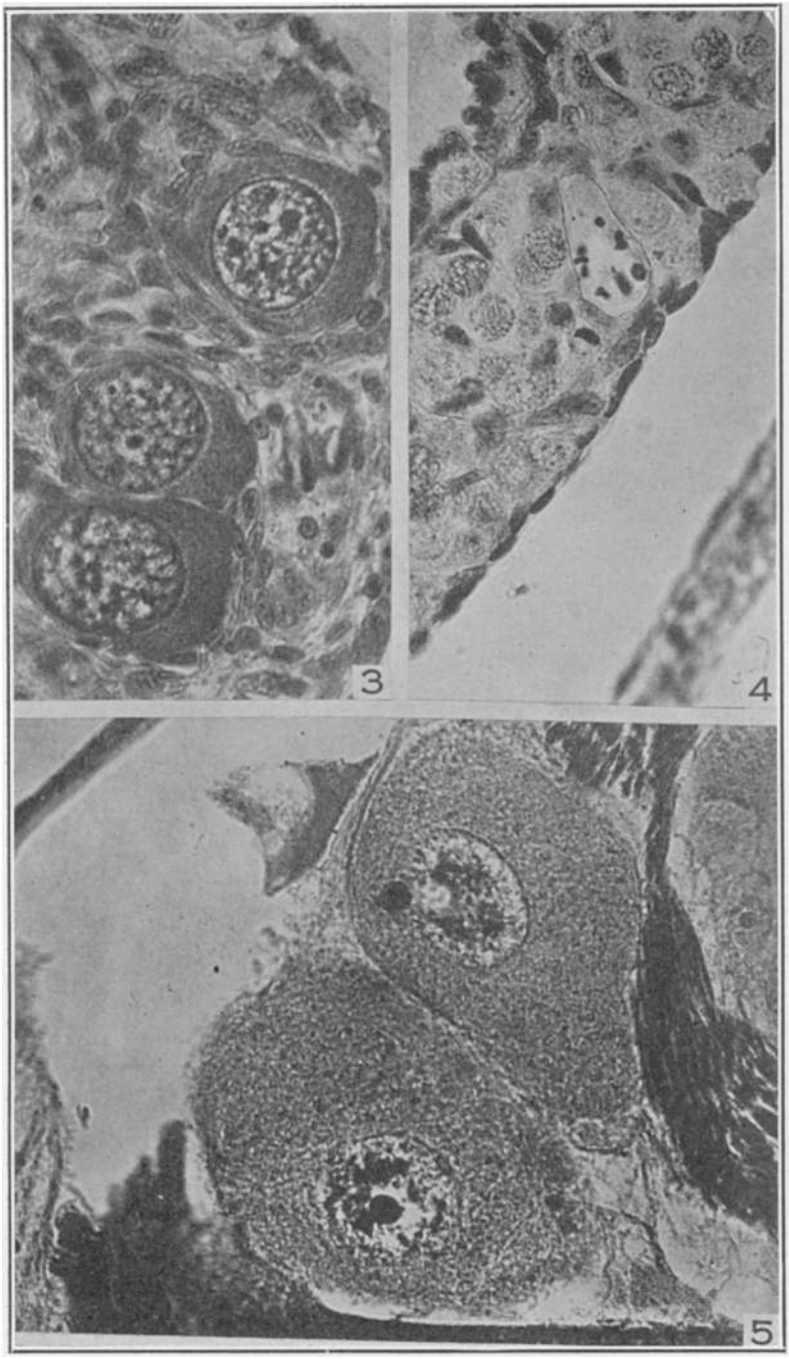

PLATE I

FIG. 3. So-called oocytes occurring in the degenerating pro-testis of larval bullfrogs. These cells according to the writer's view are merely hypertrophied spermatocytes that have undergone oviform degeneration.

FIG. 4. Section of pro-testis male larva before onset of degeneration. At *X* is spermatocyte in prophase. The black bodies are ring tetrads.

FIG. 5. The giant spermatocytes of *Scolopendra Heros* (Chilopoda). These cells form functional spermatozoa and make up the greater part of the testes. Note the "germinal vesicle" condition of the nucleus.

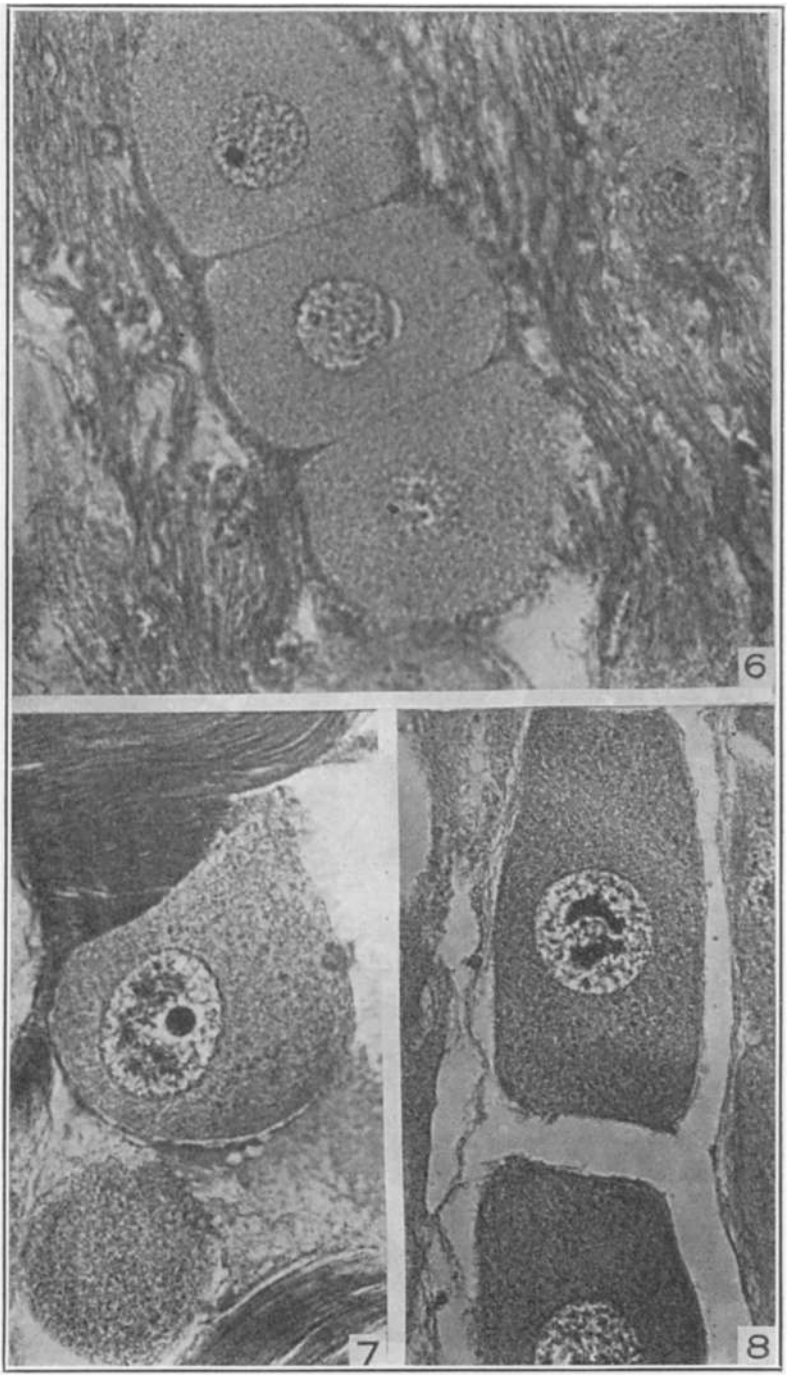

PLATE II

FIG. 6. Spermatocytes of *Scolcpendra.*

FIGS. 7 and 8. Spermatocytes of *Lithobius* (Chilopoda). The resemblance to oocytes in the germinal vesicle stage is remarkable. Sections of the testes look like ovaries.

All photographs on Plates I and II made at a magnification of 500 diameters. No reduction. Figures 5–8 are from Professor Blackman's material.

the fully formed testes are present, and the seminal vesicles are absent or rudimentary in the males of many frog species, and exceedingly well developed in others.

In the few cases reported of true lateral hermaphroditism in adult frogs there is *not always* a developmental correlation of the Müllerian ducts with the gonad of the same side. Crew ('21), (*Journal of Genetics,* Vol. II, no. 2) has summarized the known cases of sexual abnormality in amphibians — see Figs. 7, 8, 9, 12, 14, and 16 of this paper, also the report of cases 21, 22, 23, 24, and 39. These are exceptions to any rule of developmental correlation. In several cases, Figs. 25 and 31, the ducts are quite as well developed in total absence of ovarian tissue as when such is present in large amounts, this, of course, being the normal condition in *Rana pipiens.* Crew also gives a list of frog cases reported where both gonads were entirely missing and yet the Müllerian ducts were well developed in such individuals.

Because of these facts it is fair to conclude that the appeal to the somatic sex characters completely fails as proof of the transformation of female frogs into males.

In closing, it should be pointed out that Witschi has made but one original investigation of sex in anurans (Witschi '14, no. 1). His later papers on the subject contain no new observations or experiments but are purely speculative endeavors to interpret his early work in accordance with Mendelism ('14, no. 2), later ('20, no. 3) in accordance with internal secretions.