THE EFFECT OF INANITION UPON THE DEVELOPMENT OF THE GONADS AND GERM CELLS OF LARVAL FROGS.

by

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INTRODUCTION.

The following experiment was undertaken at the suggestion of Dr. B. M. Allen, of Kansas University, under whose supervision and güidance the work was carried to completion.

The problem, stated briefly, was to determine the effect of prolonged starvation upon the development of the germ gland anlage, and germ cells of frog larvae. The view is now current among biologists, that the germ cells of animals are more or less autonomous of the soma, and that the latter serves merely as a vehicle or carrier of the racial substance and source of its nourishment. According to this view, the nutritional relation existing between germ cell and soma is somewhat similar to the relation of a parasite to its host. Now, when we take into consideration the fact, that as a rule, germinal cells possess much more deutoplasm than soma cells, and correlate it with germinal independence of soma influences, one is led to believe that perhaps, interference with the normal processes of growth, nutrition and differentiation of the soma might not greatly affect the normal developmental cycle of the sex cells, or of the gonads. It was with the end in view of testing the validity of this possibility that the following experiment was undertaken.

LITERATURE.

There is a great mass of literature dealing with the general subject of inanition, and its effects upon growth, weight of organs, in the higher animals, but so far as the writer of this paper knows, there is but one brief mention of the effects of starvation upon the gonads and germ cells of larval frogs. This reference is a brief record by Nussbaum, of some experiments he performed in the late 70's. In view of the relation between his work and my own, I shall quote him verbatim:

"Die sogleich zu beschreibenden Veränderungen der primären Anlage sind durchaus typische, indem sie in derselben Reihenfolge an einer sehr grossen Zahl von Exemplaren in zwei aufeinander folgenden Jahren beobachtet wurden. Allgemein gültige Angaben über zeitliches Auftreten, sowie präcise Bestimmungen der Beziehungen zum Erscheinen gewisser anderer Anlagen, beispielsweise der Beine, lassen sich jedoch nicht machen. Man muss von Tag zu Tag aus einer reichen Brut mehrere Exemplare untersuchen und wird alsdann die Entwicklung der Organe in der Folge sich abspielen sehen, wie sie unten wird geschildert werden. Zum Beweise wie trüglich äussere Merkmale führe ich Folgendes an. Im Winter 1877/78 zog ich zwei Bruten getrennt, von denen die eine gut, die andere nur kümmerlich sich nähren konnte. Untersuchte ich nun die gut genährten, deren Hinterbeine schon die Anlage der Zehen zeigten, so fand sich in den Geschlectsdrüsen dasselbe Stadium wie in den Schlecht genährten, obwohl die letzteren nichts weiter als jene weisslichen Höckerchen zur Seite des Afters, die erste nur mit der Loupe sichtbare Anlage der Hinterbeine, aufwiesen. Man kann also nich mit Sicherheit bestimmen, welcher Zustand der Geschlechtsdrüsen bei diesem oder jenem Entwicklungsgrade der Larve wird gefunden werden; man ist dagegen wohl im Stande anzugeben, welche Veränderung einem bestimmten Zustande voraufgeht oder folgen wird. Zugleich zeigt aber auch der obige Versuch, welche grosse Rolle in der thierischen Oekonomie die Geschlectsorgane spielen: das Individuum verkümmert wegen mangelnder Ernährung; die Geschlechtsdrüsen entwickeln sich weiter."

As was stated before, a search thru the literature dealing with inanition, since the time Nussbaum performed his experiment, has failed to reveal any further work done along this particular line.

One investigator, Mead ('00) working with star-fish, obtained great size differences between his fed and unfed animals. He concluded from the results obtained, that the difference in growth of different individuals is due to the variations in the amount of food supply. This author also finds that sexual maturity in the starfish is correlated with the attainment of a certain size.

Jackson ('15), finds that in young rats held at constant body weight by underfeeding, there is an increase in the weight of the testes, but that in adult rats, the testes apparently lose weight in about the same proportion as the entire body.

In case of the ovaries of young rats, three to ten weeks of age, held at constant body weight, there is a considerable decrease in weight (27% in absolute weight). The author remarks however, that the ovary is an extremely variable organ, and difficult to dissect out with accuracy.

Another investigator, Hatai ('15) used a chemically defective (lipoid-free) diet upon albino rats, and found a marked atrophy of the testes in those animals whose growth had been retarded by the lipoid-free ration.

Falck (54) experimented with dogs, and found that the relative (percentage) weight of the testes in these animals is unaffected by inanition.

Voit, ('66) found that there is a relative decrease in the weight of the testes of the cat when subjected to inanition.

Grandis observed in fasting pigeons, that spermatogenasis ceased a few days after the beginning of the inanition experiment. He found that the sperm cells already formed soon died and were reabsorbed, as was also the greater portion of the seminiferous tubules. Some cells in the walls of the tubes persisted however and after completion of the fast and renewed feeding, these remaining cells gave rise to new sexual elements. This author expresses the opinion that testes cells assume the character of embryonic cells during periods of inanition.

Semonowitch starved a series of rabbits and guinea pigs and found, upon examination of the testes, a parenchymatous modification (weak swelling and granular degeneration) which was associated with fatty degeneration, vacuolization of the cells and chromatolysis. These degenerative changes, however, appeared irregularly in the starved animals. Functional spermatozoa were found in the seminiferous tubules. All of the degenerative changes vanish, the author states, upon renewed feeding.

Cordés found that in conditions of chronic illness many which produce cachectia there is an increase of interstitial tissue in the testes, and thickening of the walls of the seminiferous tubules. In twenty one cases, examined by him without cachectia, spermatogenesis had ceased. In very emaciated cases he found cessation of spermatogenesis.

Contrary to the findings of most of the investigators, recorded above, Traina maintains that the changes which the testes of starved animals suffer, are by no means marked. Even where there is a loss of weight as high as 20-25%, spermatogenesis is maintained in adult animals. This author states that only when the loss of weight becomes as high as 30 to 35%, does spermatogenesis cease, but that even then one finds division figures in the spermatogonia and spermatocytes.

Van Hansemann observed that in hibernating murmeltieren spermatogenesis ceases, and that the instertial cells mostly disappear. In non-hibernating animals, such as the ober (otter), on the other hand, the interstitial substance of the testes is very abundant.

In many of the investigations just quoted, no results of microscopic examination of the gonads and germ cells are reported and in others, where such examinat ions were made the results conflict ~~conflicted~~. This rather extensive review of the literature, was thought justifiable in view of the discrepency of the results obtained by the various investigators.

MATERIAL AND OBSERVATIONS.

Throughout the present experiment, the larvae of Rana pipiens were used. The larvae were reared from the egg in the laboratory, in order that the exact age of each tadpole could be determined.

The eggs were collected the 3rd of April, 1915, from a string of shallow pools along the Santa Fe railroad tracks near Lawrence, Kansas. When brought to the laboratory, many of the eggs were in fairly late segmentation stages. April 7th, the eggs, still enclosed within their envelopes, were transferred to finger bowls, two inches deep, by eight in circumference. Twenty eggs were placed in each bowl and left to develop; ordinary tap-water was used to cover them and was changed daily.

Two groups of eggs were started the same day, the larvae developing from one culture, were destined to serve as future controls for the other group.. Both series of eggs were kept under uniform conditions of light and temperature throughout the experiment. The eggs developed rapidly, and when the young larvae had escaped from their gelatinous envelopes and developed to the free feeding stage, the animals of the culture intended for controls were each day fed fresh water algae.

The growth of both controls and starved larvae, ran parallel as regards size, for about eight days, i.e., until April 18th. The increase in size of the unfed tadpoles was probably due to the consumption of the surplus yolk material held in reserve within the body cells. From April 18th on, however, the algae-fed tadpoles increased rapidly in size, as compared to the starved animals, and by April 25th, were twice the size of the unfed larvae. See fig 1 and 2.

On May 6rd, ten larvae of both the control and starvation cultured were killed, and preserved for microscopic examination of the germ cells, by fixation in Flemming's solution. The control animals at this time, measured 32 m.m. in length, whereas the starved tadpoles averaged but 11 m.m., thus showing a length difference in the animals of the two series, of 21 m.m. The controls when killed, revealed indications of limb development, the limb buds of the posterior extremities were clearly discernable. A careful examination of all the starved larvae failed to show any signs whatever of limb development.

The gonads of the two series of larvae were sectioned; a thickness of 7 u was found preferable. Haidenhein's iron-alum hematoxylin stain was used exclusively. A counter stain of Congo red was also employed, but equally satisfactory results were obtained without it, in the younger larvae.

Microscopic examination of the germ anlage of the two series, showed that the gross bodily differences in size between fed and unfed larvae did not extend to size and developmental differences in the germ cells, for the sex cells of both control and starved tadpoles were in the primitive, sexually un-differentiated state, and apparently of the same size and number. The nuclear structure, and ratio of nucleus to cytoplasm were found to be the same. Yolk spherules were plentiful in the cytoplasm of the germ cells of both series.

The structure of the germ anlagen, however, revealed considerably more differentiation in the controls than in the starved larvae. The gonads of the control animals were much larger than the germ anlagen in the unfed tadpoles; the increased size being due chiefly to a proliferation of mesodermal cells into the anlage. Such cells probably originate from the nephrogenous tissue in the region of the mesonephras. These mesenchyme, or sex-cord cells, had migrated between the germ cells, completely investing some of them in clumps of three and four, by a sheath, no doubt secreted by the cells themselves, thus forming the so-called cell nests.

The germ anlage of the starved animals presented none of these changes, but instead, remained a simple grape-like cluster of sex cells, surrounded by a thin, tenuous investment of epithelium. No proliferation of mesenchyme cells into the gland had occurred at this stage.

Sexual differentiation had not taken ;place in either fed or unfed larvae.

At this stage of the work, considerable difficulty was experienced in determining the sex of the young larvae. A condition of pseudohermaphroditism is the rule rather than the exception in the larvae of Rana pipens. Male and femaleness in these animals are conditions, hard for the investigator to differentiate clearly. Although there is quite a mass of literature upon the question of sex differentiation in the anurans, each investigator who has dealt with the problem seems to have promulgated a new sex criterion. I have found that only one or two of the many morphological features of the gonads advocated as sex criteria by the various authors, are trustworthy, when applied to very immature gonads.

For several of the earlier series of animals sectioned, the criterion for sex differentiation was that worked out by Kuschakewitch for Rana esculenta. This author claims that the only sure criterion of sex, in the particular species of frog he used, consists of a fundamental difference in the genital cords of the potential males and females. In the females, the sex cords remain sterile, i.e., without germ cells until sexual maturity. In males, on the other hand, the genital cords play an important role in the formation of germinal tissue, and an animal is to be considered a male so soon as its genital cords produce germ cells. In other words, germ cells must arise in the endothelium of the germ anlagen before the tadpole can be regarded as a male.

In the present work, when the attempt was made to differentiate sex in the larvae by the method of Kuschakewitch, it soon became apparent, that, although this author's sex criterion may be true of Rana esculenta, it is not satisfactory for Rana pipens, for several of the anlagen examined, undoubtedly of the female sex because of the large number of oöcytes within the gland, contained sex cells in the endothelium, or sex cord tissue. Also the acceptance of this author's sex criterion, necessitates the acceptance of his view that germ cells may arise by direct transformation from sex-cord cells, a view not generally concurred in by most investigators. Because of these facts, Kurschakewitchs' sex criterion was therefore abandoned, and sex differentiation was determined according to the criteria advanced by King ('08) for Bufo, and confirmed by Witchi ('15) for Rana temporaria.

Both of these authors worked with young larvae, and found that in the germ anlagen of those animals destined to develop into males, the germ cells are scattered move evenly throughout the gland. Conversely, in the immature ovary, the germinal cells remain near the periphery, which is usually much thicker than in males.

The next group of tadpoles ~~were~~ killed May 12th, and sectioned. The nine day interval of starvation intervening between this series, and that of May 3rd, had sufficed to bring about striking differences between fed and unfed larvae. The controls averaged 47 m.m. in length, the largest animal of the lot attained the length of 52 m.m. All of the controls showed development of the hind limbs into their two primary divisions; also the toe points had differentiated. When compared with the controls, the growth of the starved series of larvae was found to be inhibited, for the average length of the animals of this series was but 13 m.m., exactly the same length, as the tadpoles killed nine days previous. No indications of limb development were observed.

Microscopically, the difference in the germ anlagen of the controls and starved animals were very great. The germ cells of the unfed tadpoles presented few developmental changes from those of the larvae killed May 3rd. The yolk spherules, so abundant in the cytoplasm of the earlier series, had in large ~~part~~ disappeared. The number of sex cells, however, had nearly doubled. This is clearly shown in Table 6 where the number of germ cells of the May 3rd starvation animals is compared with the number in the May 12 unfed series.

The figures in this table, and also in the others, not only for the germ cell counts, but for the measurements of the gonads, sex cells and their nuclei, are based upon counts and measurements of the cells and anlagen of eight representative transverse sections of the gonad, thru different regions. The extreme anterior region of the glands which usually contains but one or two germ cells ~~was~~ were discarded. The germ cell number in each of the eight sections is given, but the figures for the length and width of the anlagen, germ cells and nuclei are averages. In measuring the length of the transverse sections the mesorchium of the anlagen were not included in the measurements. All measurements were made with a micrometer.

The structure of the anlagen of the May 12th starvation larvae had undergone no observable changes, and remained a simple cluster of cells. Sexual differentiation had not occurred.

When contrasted with the anlagen of the starved animals, the gonads of the controls of the May 12th group, presented marked developmental changes. The number of germ cells had increased, there being almost twice as many in the anlagen of the control animals as in the glands of the unfed larvae of the same age (See table 2). This increase in the germ cell number had greatly augmented the size of the gonad, especially its length (transverse section) standing in the relation of 3:1, to the gland of the starved larvae. Many mesenchyme cells had proliferated into the anlagen, forming an endothelium lining a well developed secondary genital space, and partitioning the ~~sex~~ germ cells off into groups or nests.

The germ cells presented striking nuclear changes, consisting in a rearrangement of the chromatic material into the characteristic configurations of presynaptic maturation stages. No yolk granules were present. Sexual differentiation had occurred in all of the controls examined.

I have spoken of the formation of a secondary genital space in the anlage, and in the future frequent reference will be made to the primary and secondary sexual spaces or cavities. A word of explanation might not be inappropriate as these terms have been adopted from Kuschakewitch and Witchi. These authors explain the formation of genital spaces somewhat as follows: in the development of the germ glands of frog larvae, two kinds of cavities appear -- the primary and secondary cavities. The primary spaces are simply those cavities between the germ cells, which have never been obliterated by the migration of the germ cells into the gland. These primary spaces later become filled with embryonic connective tissue which still later is directly transformed into the difinitive connective tissue of the gonad.

The secondary genital space is the lumen formed within the mass of mesenchyme or sex cord cells, which proliferates into the germ gland.

On May 25th, another lot of larvae from both control and starvation cultures were killed and sectioned. The unfed animals averaged 15 m.m., showing an increased length of 2 m.m. over the starved animals killed May 12th. This increase in length of the unfed larvae was probably due to the fact that until a few days previous to the date of killing (May 25th) the larvae had withstood the deleterious effects of prolonged hunger very well; there was little mortality, and little disposition on the part of the surviving tadpoles to feed upon their dead companions. About May 20th, the mortality among the animals of the unfed culture greatly increased, until it became difficult to keep the containers free from dead larvae. The live tadpoles, if left unmolested would eagerly feed upon these. None of the starved animals revealed indications of limb buds.

The controls for this culture averaged 56 m.m. All of the larvae possessed will developed hind limbs.

The structure of the germ cells and anlagen of the two series of animals, when examined microscopically, presented interesting contrasts. The sex cells of the unfed larvae retained the primitive undifferentiated character, and their size and number had not increased in the seventeen day interval from those animals killed May 15th, but had increased in number considerably as compared with the starved animals of May 6th. (See table 1. No nuclear changes had occurred. The anlagen remained undeveloped, consisting of a cluster of germ cells surrounded by a thin layer of epithelium. Sexual differentiation had not occurred.

The nuclear material in the sex cells of the controls for this series of starved larvae were ~~all~~ in late synaptene, and many in post synaptene (contraction) stages. Also many division figures were found. The germ cells were larger than those of the animals. (See table 3).

The germ anlagen of the controls were in advanced developmental stages, and when compared with the anlagen of the unfed animals of the same age, the difference in the size of the glands of the two series of tadpoles was striking (see table 3). The anlagen of the control larvae were three times longer and more than twice as broad as the gland of the unfed animals and containedfive times as many germ cells. (Measurements computed from transverse sections).

The next series of tadpoles ~~were~~ was killed June 1st. The unfed animals were, at this time, very sluggish, swimming about only when disturbed. The skin over the abdomen appeared transparent, and thru it, the shrivelled intestines were plainly visible. The mortality of the larvae of the starvation culture became so great at this time, it was necessary to feed the tadpoles a few algae fronds in order to prolong the experiment.

The starved animals measured 15 m.m., and a careful examination failed to reveal limb buds in any of them. As contrasted with the unfed larvae, the controls were rapidly metamorphosing. They averaged 62 m.m. in length, and had well developed fore and hind legs. The tails of many of the controls were frayed along the edges, showing indications of atrophy and resorption. The typical frog mouth was rapidly developing.

Examination of the sectional material revealed little, or no change in the germ cells and anlagen of the starved tadpoles, other than those already recorded for the earlier lots of May ~~3~~6rd, 1~~2~~5th and 2~~0~~5th. The germ dells were still in the primordial indifferent state, and showed no nuclear changes indicative of maturation. The gonads of these animals remained undeveloped, both as regards size and internal structure. In one respect, though, the anlage of these animals differed from those of all previous series of unfed larvae, in that a few migratory mesenchyme cells were found scattered thru the germ gland.

The nuclei of the germ cells of the controls were in late synaptic stages. The number of germ calls had greatly increased (see table 4).

The gonads of the controls revealed considerable developmental change when compared with those of the starved animals. The anlagen contained large secondary genital cavities lined with endothelium. The sex cords were well developed, and the gross size of the gland was much larger in the controls than in the unfed tadpoles (see table 4). Sexual differentiation had occured in all of the fed larvae examined.

From June 1st, on, the control animals began to metamorphose rapidly, and by June 17th, the change from the larval to the adult state was complete, in most of the larvae, except for a rudimentary tail. This was completely absorbed in all of the animals by June 22nd. Owing to the difficulty in keeping the young frogs, and the pressure of the other work, the control animals for the starved series were killed June 29th.

Those sectioned for microscopic examination proved to be females; the germ cells were full grown oöcytes.

The germ anlage of these young frogs had not, as yet, developed into ovaries or testes. This transformation probably does not occur until several weeks after metamorphosis is complete.

The next group of starved larvae was killed July 8th, one month and eight days after the last series of June 1st were killed. The animals were fed a few whisps of algae five days before the date of killing. At the time of death, the larvae averaged but 16 m.m. in length, and showed no indications of limb buds, whereas the controls for this group of tadpoles had completely metamorphosed by June 22nd, seventeen days previous.

The germ cells of this series of starved larvae, were for the most part, still of the primitive undifferentiated type. About one tenth of the total number of sex cells in each cross section of the gland examined, were entering early maturation stages (pacytene). The total number of germ cells within the anlage, had increased somewhat, when compared with the number found in the germ anlage of the June 1st starvation larvae. (See table 6).

The gonads of this unfed series of tadpoles were undeveloped, although the number of migratory sex-cord cells within the gland had increased somewhat when compared with the glands of previous series. Correlated with this increase in the number of interstitial and germinal cells, there was an increase in the size of the germ gland, (See table 6), an increase considerably greater than any other of the starvation series revealed. None of the criteria for differentiating the sexes were observable in the gonads of this series.

It seems anomalous that one tenth of the total number of germ cells contained within the anlage of this series of larvae were, in regard to development, far in advance of the gonad itself. For, as was stated, before, many of the germ cells were entering the early maturation stages, whereas, the anlage remained a simple cluster of cells, and had undergone no differentiation whatever. In all of the larvae heretofore examined, differentiation of the germ cells from the primordial, indifferent type, to the maturation stage was correlated with development and differentiation of the germ anlage; the two conditions were intimately related.

It seems probable, judging from the results of examination of this series of larvae, that starvation inhibits the onset of the maturation processes of the germ cells for a definite time only, beyond which, its effects are not sufficiently potent to overcome the inherent tendencies of the sex cells to undergo differentiation. More extended work along this line is needed.

I succeeded in keeping three tadpoles of the starvation culture, alive until August 19th, fifty-five days after the controls had metamorphosed, by feeding them a few bits of finely crumbled bread every five days. This meagre diet, while sufficient to keep the animals alive, was not enough to permit growth. The animals swam about very feebly, and then only when disturbed. Most of the time the starved tadpoles lay upon the bottom of the container. Only a few times were these animals observed to rise to the surface of the water for air.

These three tadpoles very likely could have been kept alive by underfeeding, for an indefinite time, but unfortunately they were killed by accident, and at a time when it was impossible to preserve them for microscopic examination. At the time of death, they were minute tadpoles 16 mm. in length, and showed no indication whatever of limb development when examined with a hand lens.

DISCUSSION.

The experiments quoted fail to confirm the findings of Nussbaum ('80), in fact, give results diametrically opposed to his. Where he found that starvation does not effect in any way the gonads and germ cells of frog larvae, the results obtained in the present experiment, justifies the conclusion that total inanition not only prevents the formation of the germ anlage of frog larvae, but inhibits the onset of the maturation stages of the germ cell cycle. and proceeds no further It is possible that the species of frog with which Nussbaum worked, reacts differently to the effects of starvation than does Rana pipens; or more probably he used old larvae, for it seems anomalous that forms as closely related as Rana Fusca and Rana pipens should vary so widely in their capacities to grown and develop under adverse environmental conditions.

In view of Mead's ('00) work with starfish, in which he found that sex is correlated with the attainment of a certain size (50 m.m.) in these animals, the results obtained for the starved larvae of Rana pipens, are interesting. None of the small unfed animals differentiated sexually. In the controls, sexual differentiation was first observed when the tadpoles averaged 47 m.m. in length. Possibly it occurred earlier when the larvae were smaller. No examination to test this point was made.

The observation, that many of the germ cells of the unfed larvae, starved from the time of their emergence from the egg capsule (April 10) until July 8th, were entering early maturation stages, despite the fact that the development of the germ anlage had been completely inhibited, seems to show that the germ cells possess certain capacities for physiological recuperation from nutritional disturbances, independent of the soma,

SUMMARY AND CONCLUSION.

I. Total starvation inhibits indefinitely, the growth and metamorphosis of larval frogs.

II. Prevents the development of the germ glands and delays any increase in the number of germ cells ~~and~~ interstitial cells.

III. Starvation greatly retards the normal course of development of the germ cells.

IV. Prevents the onset of sexual differentiation

I take this opportunity of acknowledging my indebtedness to Dr. B. M. Allen for many helpful suggestions, and for the interest he has shown in the work.

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Table 1.

May 3rd Starvation larvae.

Germ Gland		Germ Cell		Cell No.	Nuclei		Sex
W	L	W	L		W	L	
32	37	8	12	10.	8	10.5	Undifferentiated.

May 3rd Control.

W	L	W	L		W	L	
48	87	8	12	~~28~~ 24.	8.	11	Undifferentiated.

Table 2.

May 12th Starvation.

Germ Gland		Germ Cell		Cell No.	Nuclei		Sex
W	L	W	L		W	L	
41.8	48.8	8	13	16	7	11.5	Undifferentiated.

May 12th Control.

W	L	W	L		W	L	
58.4	133	8.8	13.5	38	8.5	9	Differentiated.

(W - The figures under this letter are averages computed from eight represenative transfer sections. The widest part of the gland was measured.)

Table 3.

May 20th Starvation.

Germ Gland		Germ Cell		Cell No.	Nuclei		Sex
W	L	W	L		W	L	
30	36	7	11.5	9.	6.5	7	Undifferentiated

May 20th Control.

W	L	W	L		W	L	
81.5	142	9	10	52	7	7	Differentiated.

Table 4

June 1st Starvation.

Germ Gland		Germ Cell		Cell No.	Nuclei		Sex
W	L	W	L		W	L	
37.5	42	8.5	10.5	15	6.5	6.5	Undifferentiated.

June 1st Control.

W	L	W	L		W	L	
122.6	95	8.5	11	57 ~~40~~	6.5	7	Differentiated.

Table 5.
Starvation Series.

July 8th Starvation.

Germ Gland		Germ Cell		Cell No.	Nuclei		Sex.
W	L	W	L		W	L	
39	41	9	10.5	20	6	7.5	Undifferentiated.

June 17th Control.

W	L	W	L		W	L	
460	694	52.5	66	56	34	38	Differentiated.

Table 6.

	Germ Gland		Germ Cell		Cell No.	Nuclei		Sex.
	W	L	W	L		W	L	
May 3	32	37	8	12	9.	8.	10.5	Undifferentiated.
May 12	41.8	48.8	8	13	16	7	11.5	Undifferentiated.
May 20	30	36	7	11.5	14	6.5	7	Undifferentiated.
June 1	37.5	42	8.5	10.5	15	6.5	6.5	Undifferentiated.
July 8	39	41	9	10.5	20	6	7.5	Undifferentiated.

Table 7
Control Series.

	Germ Gland		Germ Cell		Cell No.	Nuclei		Sex.
	W	L	W	L		W	L	
May 3	48	87	8	12	19.5	8.5	11	Undifferentiated.
May 12	58.4	133	8.8	13.5	38	8.5	9	Differentiated.
May 20	81.5	142	9	10	52	7	7	Differentiated.
June 1	122.6	95	8.5	11	40	6.5	7	Differentiated.
June 17	460	694	52.5	66	56	34	38	Differentiated.

PLATE I.

All diagrams made with Abbe camera lucida and drawn to scale.

Fig. 1 Transverse section through gonad of larvae starved April 11 to May 3. X 660 Mag.
- a. Germ cell.
- b. Follicle cell.
- c. Yolk spherules.
- d. Polymorphic nucleus.

Fig. 2 Transverse section through gonads of control of May 3 starvation.larvae. X 660 Mag.
- a. Germ cell.
- b. Sex cord cells.
- c. Secondary genital cavity.
- d. Mesorchium.
- x. Germ cell in division.

Fig. 3 Transverse section through the gonad of larvae starved from April 11, until May 12. X 660 Mag.
- a. Germ cell.
- b. Follicle cell.
- c. Primary genital space.

Fig. 4 Cross section through gonad of control of starvation larvae killed May 12. X 660 Mag.
- a. Germ cell.
- b. Sex cord cells.
- c. Primary genital space.
- X. Germ cells in late synaptene stage,

PLATE II.

Fig. 5 Transverse section through gonad of larvae starved from April 11 to May 20. X 660 Mag.

a. Germ cell.
b. Follicle cell.
c. Mesorchium.
d. Primary genital space.

Fig. 6 Transverse section through the gonad of control of starvation larvae killed May 20. X 660 Mag.

a. Germ cell.
b. Sex cord cell.
c. Mesorchium.
d. Primary genital space.
X. Germ cells in diplotene stage
t. Nuclei of germ cells in pacytene stage,

Fig. 7 Cross section through the gonad of larvae starved from April 11 to June 1. X 660 Mag.

a. Germ cell.
b. Sex cord cell.
c. Primary genital space.

Fig. 8 Cross section through the gonad of control for starvation larvae killed June 1. X 660 Mag.

a. Germ cell region.
b. Sex cord cell.
c. Primary genital space.
t. Nuclei germ cells pacytene stage
X. Nuclei of germ cells in synaptene stage.

PLATE III.

Fig. 9 Singe Oöcytes from gonad of young frog killed June 17 X 660 Mag.

Fig. 10 Cross section through the gonad of larvae starved from April 11 to July 8. X 660 Mag.

Fig. 11 Cross section of the ovary of young frog killed June 17. X 70 Mag.

Fig. 12 Cross section through the gonad of larvae starved from April 11 until July 8. X 70 Mag.

Figures 11 and 12 drawn with low power magnification,

The larvae from which Figure 12 was taken was twenty-one days older than the animal from which Figure 11 was taken.

Fig. 13 Single germ cell from gonad of larvae starved from April 11 until July 8 X 660 Mag.

Fig. 14 Single germ cell from ovary of young frog twenty one days younger than animal from which figure 13 was taken. X 660 Mag.

Figures 13 and 14 drawn to the same magnification.

FIGURE 15 Starvation of May 12th compared with control of May 12th