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UNIVERSITY OF IOWA STUDIES IN NATURAL HISTORY

PROFESSOR BOHUMIL SHIMEK, PH.D., Editor

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CONTENTS

THE ENDOSPERM OF UTRICULARIA

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Studies related to seed production remain among the fundamental lines of botanical investigation. The potential value of such information in relation to plant breeding and crop production calls for critical and, if need be, repeated investigation of all problems that may shed any light on the structures and activities related to reproduction in higher plants. Interest is stimulated also because of insufficient information concerning some of the events taking place in the embryo-sac, and uncertainty as to the precise function of some of the parts concerned.

The general uniformity of events centering about seed production in Angiosperms is undoubtedly significant, though capable of various interpretations. The practically uniform behavior of both the male and female gametophytes in such different groups as Monocotyledons and Dicotyledons still seeks explanation. These divisions of the great Angiosperm group are at present widely divergent in many respects, yet the structures immediately related to their reproduction are almost identical. It may be, as would at first appear, that such conditions point to a common past and suggest that adaptation is as yet incomplete. It is hardly conceivable though that any known antecedent structure so resembled the eight-nucleate embryo-sac as to dominate its present behavior. If inherited tendencies prevail why not a larger development within the nucellus as in Gymnosperms? On the other hand why is it not reduced to simply an egg?

There must, then, be hindering factors on the one hand to inhibit marked development of the female gametophyte of the Angiosperm. On the other hand further reduction seems to be inconsistent with its successful operation; accessory parts seem necessary aids to fertilization and nutrition of the early stages of the embryo. Constancy of the gametophyte is a com-

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promise between these opposing tendencies and one comes to feel that it is held rather sharply in check by environing circumstances. That it tries frequently to escape its bonds is evidenced by the numerous departures from the typical story. There are recorded observations of unusual behavior by each of the seven nuclei associated with the egg, and there are various modifications of the gametophyte as a whole. Granted favorable opportunity one might expect to find it taking on highly modified forms.

In the Bladderwort (*Utricularia*) an unusual combination of parts is found within the ovary, and associated with it are striking specializations of both the female gametophyte and its successor in place, the endosperm. The protruding embryo-sac of *Utricularia* has long been known, as it was figured by Mertz (7) twenty years ago. Similarly, Kamienski (5) long since outlined quite fully the remarkable behavior of the endosperm in this genus. Lang (6) finds in the closely related *Polypompholyx* a series of events parallel to those in *Utricularia,* yet displaying interesting points of difference of peculiar significance in this connection. The present writers have undertaken this study feeling that the unusual behavior of these structures merited fuller investigation and perhaps warranted further conclusions.

Living vegetatively as a submersed aquatic, *Utricularia* has long been of interest because of the peculiar bladders produced upon its leaves. These organs, which function as floats and insect traps, have suggested both the common name of the plant and the scientific name of the genus. While belonging to the limited group of seed plants that flourish vegetatively beneath the surface of the water, its flowers are brought to the surface of the water for pollination, and pollen transfer is accomplished in the air by insects.

Utricularia vulgaris americana Gray thrives in our Iowa lakes and ponds. In the vicinity of the Iowa Lakeside Laboratory on the Okoboji lakes in northwestern Iowa the smaller bodies of water are often yellow with its bloom. The flower stalk begins its development beneath the surface but later pushes up out of the water to a height of four to fifteen inches, bearing a raceme of beautiful flowers. As the period of flowering passes, the fertile stalk is gradually submerged, either sinking slowly or tipping over bodily into the water. The seeds thus carry out most of their development in air, invested by the tough capsule of the ovary wall, but are soon submerged through the sinking of the entire inflorescence.

FLOWERS AND POLLINATION

The flowers are bright yellow in color and offer many specializations for cross pollination. They are distinctly zygomorphic, with a strongly lipped corolla bearing the stamens, a tubular style, and a lipped stigma with numerous stigmatic hairs beneath the upper lip. Insect visitors seem to be responsible for pollination, and the writers could find no evidence of cleistogamy in this species. Flowers opening in hooded bags which prevented the visits of insects, were uniformly sterile.

While insects are numerous about the flowers of *Utricularia* most of them merely perch upon some part of the inflorescence. The damsel flies, for example, regularly use the flower stalks as places of temporary rest from flight. The only insect observed to enter the corolla was a small buff-colored fly, the species of which was not determined. These insects are numerous about the beds and were repeatedly observed to enter the closed throat of the corolla, disappearing within the flower and, after an interval of nearly a minute, to emerge and wing their way to other blossoms of the kind. Fertilization seems to be regularly accomplished and of the hundreds of ovaries sectioned few showed sterile ovules. Abortive embryos are not uncommon but this condition seems to be due mainly to crowding and possibly to loss of nutritive connection through partial displacement, rather than to failure of pollination.

Much of the material for this study was killed in one per cent chrom-acetic acid, run through the alcohols and imbedded in paraffin. The sides of the ovaries were clipped so as to invite the free penetration of reagents into the cavity of the ovary. For critical study of fertilization and early post-fertilization stages, material was killed in Flemming's stronger solution. This was incomparably better, and practically all the figures were drawn from material fixed in this way.

PLACENTA AND OVULES

The globular free central placenta rising from the base of the flower is covered with ovules (*Fig. 1*). The number as counted in seven flowers ranged from 175 to 250 with an average of 227. Of these seven flowers, five showed abortive ovules, twenty-eight of them being recognized, all told, but of these, nineteen were in one flower.

The ovules are anatropous and each possesses a single massive integument which arches over and comes into contact with the placenta (*Fig. 6*). At first rather delicate (*Fig. 2*), the integument thickens and soon, as seen in sections, becomes similar to the opposing funicular part, each being hemispherical, with the embryo-sac as a central axis between these halves (*Fig. 11*).

These spherical ovules are tightly packed together, completely covering the free central placenta and thus appearing as a globular mass which, however, is closely invested externally at all stages by the ovary wall.

In the growth following fertilization the ovules become sharply angled together. The seeds are commonly six sided (*Fig. 17*) and are clearly flattened dorsiventrally, due to compression between the placenta and the investing ovary wall (*Fig. 10*).

The funiculus, early massive and typical of this type of ovule (*Fig. 6*), diminishes relatively during the development of the seed and finally consists of merely a slender rudimentary structure (*Fig. 16*). No vascular elements are developed in the funiculus, or in any other part of the ovule, though they are soon prominent in the placenta as discussed below. It should be kept in mind that the funiculus of the ovule of *Utricularia vulgaris* thus early aborts and that the embryo is nourished through another structure, the micropylar haustorium, the developing seed meanwhile being held in position by the pressure of surrounding parts.

The tissues of the free central placenta undergo considerable specialization. A tree-like vascular supply is developed which sends a branch to a point near the base of each ovule. The tip of this branch is directed towards the funiculus but stops alongside the micropylar haustorium (*Fig. 1*).

That portion of the placenta immediately beneath the ovule begins modification before the megaspores are formed. An ovoid mass of tissue, involving a thousand or more cells, begins to stain more deeply and soon becomes sharply delimited from the surrounding tissue, the cells differing both in size and shape as well as staining reaction (*Fig. 11*).

This mass of tissue may include several thousand cells destined to receive the micropylar haustorium and is thus clearly marked out long before fertilization has taken place. Its later behavior will be taken up with discussions of the female gametophyte and the endosperm. The cells adjacent to this nutritive tissue specialize along very different lines, especially do those on the funicular side become large and greatly elongated (*Fig. 16*).

FEMALE GAMETOPHYTE

The female gametophyte developes in the usual manner from the innermost of four megaspores that lie in linear series (*Fig. 3*). These are bounded externally by a thin nucellus consisting of a single layer of cells. Before the first division of the dominant megaspore the nucellus begins to break down at the tip (*Fig. 4*) and soon disappears entirely, leaving the naked female gametophyte protruding from the ovule (*Fig. 5*).

Meanwhile the single massive integument has arched over and pushed down parallel with the funiculus, and by the time the embryo-sac has reached the two-nucleate stage the integument touches the placenta at the base of the ovule (*Fig. 5*). But the female gametophyte, as noted above, has escaped confining structures at this end; it quickly makes contact with the placenta and begins digesting its way into this tissue (*Fig. 6*).

It follows, therefore, that no micropyle is really developed since the gametophyte, after the megaspore stage, is never completely enclosed. A pseudo-micropyle is formed with contact between integument and placenta, but sections at right angles show that the female gametophyte has lobes on either side reaching out into the ovarian chamber (*Fig. 9*). These protrusions of the embryo-sac are later encountered by the pollen tubes moving downward over the surface of the placenta among the ovules.

The embryo-sac at maturity is narrowly triangular in form (*Fig. 7*), the feeble antipodals occupying the narrower end. The egg and broad synergid cysts are anchored to the funicular side of the embryo-sac and are soon left far above its tip as the protoplasm pushes past the egg apparatus into the haustorium.

The polars meet a little above the egg, flatten together somewhat, and seem to remain in that relation until the pollen tube enters the ovule (*Fig. 7*). Proof of this is obtained from flowers in which only a part of the ovules have been fertilized. Intermixed with ovules containing embryos are others that failed to receive pollen tubes, and in these the polars are joined but not fused. Their union seems to be regularly completed, however, before the sperm reaches them.

The antipodals are never prominent and enter into early decline (*Fig. 7*). At maturity they are small and feebly staining nuclei that occupy the tip of the embryo-sac. With the entrance of the aggressive endosperm nuclei into this region they disappear entirely, so that the antipodal haustorium, later developed, is wholly an endosperm structure.

Cells of the ovary adjacent to the embryo-sac take on marked changes during its development and constitute the so-called "tapetum" of various authors. The cells elongate at right angles to the longer dimension of the embryo-sac, and in the outer part of the ovule are twice the diameter of the embryo-sac in length (*Fig. 9*). They seem to be equally prominent on the funicular and integument sides of the gametophyte.

MALE GAMETOPHYTE

The pollen grain measures 26x30 microns, and has its surface covered by a series of encrusted ridges giving it the appearance of a summer squash (*Fig. 15*). The microspores adhere in sticky masses, and in pollination by the visiting flies are rubbed off against the stigmatic hairs which line the inner surface of the upper lip of the stigma (*Fig. 1*). Upon germination the pollen tubes pass upward among the stigmatic hairs and enter the tissues of the upper lip of the stigma. From there they enter the back wall of the style and pass obliquely through it, following no fixed path until they enter the stylar canal.

The tubes strike the stylar canal at various heights above the ovary, and all seem ultimately to reach it, though some enter it very close to the base (*Fig. 1*).

The pollen tubes enter the ovary as a rope which quickly separates into its individual strands as the pollen tubes scatter among the ovules. The tubes are large and conspicuous, stain brilliantly, and so show conspicuously in sections. They creep along the placental surface where some of them encounter the protruding lobes of the female gametophyte (*Fig. 9*). This presents a type of conjugation unusual in Angiosperms; two naked protoplasmic masses come together outside the ovule when the pollen tube meets a lobe of the female gametophyte in the free space of the ovary.

The membrane investing the pollen tube seems to hold for some time after entering the female gametophyte, and all the evidence indicates that the tip of the tube follows its customary route to one of the synergids. In this instance the synergid is submerged within the cytoplasm of the embryo-sac and lies well towards the dorsal side, so that the pollen tube has to take a rather indirect course to reach it. The proof here is unusually strong that the synergid exercises a chemo-directive influence on the pollen tube, which in this instance could more easily have reached the egg directly. The gorged synergids stain brilliantly with safranin for some time, but the reaction grows fainter until by the time the embryo is well started the dilated synergid with the same stain appears as a grayish mass near the base of the suspensor. In *Polypompholyx* where similar conditions occur Lang (6) could not trace the pollen tube to the synergids.

The micropylar end of the embryo-sac often contains a large amount of material that seems to have come from supernumerary pollen tubes that possibly may have later discharged their contents into the embryo-sac (*Figs. 8, 9*). One of the writers (8) had previously noted in *Elodea* that the first and second tubes to enter the ovule terminated in the two synergids, and that if an additional tube entered, this discharged directly into the embryo-sac. In some cases all nuclei of the female gametophyte were obscured by this strongly staining mass that seemed to have come from the pollen tubes. This behavior is highly suggestive of the condition frequently observed in *Utricularia vulgaris.*

FERTILIZATION

Fertilization and secondary fertilization were noted in many ovules. The union of the sperm with the fusing polars is promptly achieved and the primary endosperm nucleus has usually passed to the telophase of its first division before the fusion of egg and sperm is completed. The whole series of events moreover moves rapidly. The upper ovules, nearer the place of exit of the pollen tubes from the stylar canal, have developed their first cells of endosperm before eggs in lower ovules have been fertilized. There is thus presented a wide range of stages in each ovary, though the distance from the top to base of placenta at this age is less than two millimeters. In other words, fertilization, double fertilization, and the first division of the primary endosperm nucleus may take place while the pollen tubes travel a distance of two millimeters. It might be, of course, that the earlier tubes are all taken by the upper ovules, but the method of pollination and the mass of pollen tubes implies excess numbers of tubes.

The cyst containing the fusing egg and sperm is generally more or less completely enwrapped by the gorged synergids, which are opaque with any protoplasmic stain (*Figs. 8, 9*). The second sperm uniting with the fused polars lies near the egg-cyst (*Fig. 8*), within the protoplasm of the embryo-sac.

THE ENDOSPERM

The primary endosperm nucleus divides soon after triple fusion and before egg and sperm have completely united (*Fig. 9*). Following this first division, a diaphragm is laid down across the embryo-sac separating the two daughter nuclei. The endosperm now displays great aggressiveness and with further divisions soon pushes its way out of both ends of the embryo-sac.

The innermost of the initial pair of endorperm nuclei divides repeatedly, with cleavage of the protoplast and development of cell walls (*Fig. 12*). The endosperm presses sharply into the antipodal end of the sac, and the tissue bordering this region soon disintegrates, so that presently two large endosperm cells come to lie side by side in a caecum protruding beyond the original limits of the embryo-sac (*Fig. 12*). Meanwhile the antipodal nuclei have disappeared entirely as the aggressive endosperm shoved past their original location.

The antipodal haustorium rapidly digests its way through all intervening cells and mushrooms out against the epidermis of the ovule (*Fig. 11*). The cells of this limited layer seem to be weakened under the attack, and the epidermis covering the haustorium becomes thinner than elsewhere. The epidermal cells of this region shrivel in preservatives and the riper seeds commonly show an opening through the outer investment at this point (*Fig. 10*).

The antipodal haustorium persists until the embryo is far advanced. It gradually flattens as the seed matures, and at a late stage shows only as a collapsed cap at the end of the embryo. Its nuclei become enormously enlarged, measuring 19x12 micra. Neither of its nuclei seems ever to divide.

It seems likely that the massive epidermis of these ovules functions both mechanically and to conduct materials. Its large cells show much starch, suggesting nutritive significance. The behavior of the antipodal haustorium might be explained as an attack on foods stored in the epidermis or being conducted through the cells of this layer.

MICROPYLAR HAUSTORIUM

The micropylar haustorium as noted above begins much earlier than the antipodal outgrowth, which is a post-fertilization development. The encroachment upon the tissue of the placenta noted above is launched by the protoplasm of the female gametophyte. Previous to fertilization it may have penetrated to a depth of two layers of cells, though no nuclei are thrown into the lobe at this stage (*Fig. 8*).

Early in the development of the endosperm two of its nuclei slide past the embryo and distended synergids, and enter this pouch (*Fig. 12*). Their arrival starts the second phase in the development of this haustorium, which then penetrates farther into the placenta and broadens into a rounded structure. A membrane for a time surrounds the haustorium, separating its protoplasm from the adjacent placental tissue (*Figs. 11-14*).

Digestion of the special receptive placental tissue continues until most of its walls have broken down, leaving a cavity filled with a coenocytic mass. Commonly a small volume of the receptive tissue is left on the funicular side with walls intact. With the disappearance of walls from this portion of the placental tissue the nuclei are set free in the common cytoplasm and present a wide range of sizes and shapes. They may enlarge somewhat, and apparently some of them unite to form tuber-like nuclei many times their normal size (*Fig. 14*).

Midway in the embryo development one or both of the endosperm nuclei of the micropylar haustorium may be seen apparently breaking through the limiting membrane. These endosperm nuclei, measuring 28x15 micra, are very different from those of the placental tissue being larger and having great splotches of darkly staining chromatin. They develop lobes which may in turn become dissociated amitotically into separate masses. These with the placental nuclei lying in the fluids of the haustorium, offer a most peculiar assemblage of nuclear structures (*Fig. 14*).

At a later stage the haustorium shrivels, leaving an empty pit in the placenta and also a companion cavity in the base of the seed. As suggested by Merz (7) the endosperm adjacent to the haustorium forms a plug which closes this opening into the seed. The placenta, after seeds are shed, is pitted all over its surface, marking the positions of the haustoria.

The endosperm about the seed offers no peculiarities. It forms a spindle-shaped mass which entirely surrounds the embryo except at the base of the long and slender suspensor, which remains until late in the development anchored to the funicular wall of the embryo-sac.

THE MATURE SEED

During later development of the embryo the seeds are pressed together in a compact layer between placenta and ovary wall. Under dissection of living material it was noted that the seeds are much more firmly united to each other than to the placenta, and the whole cap of seeds might be lifted off like a ripe raspberry. This only confirms their dependence upon the micropylar haustorium for nutrition and their practical abandonment of funicular connection with the ovary.

DISCUSSION

In the introduction to this paper brief reference was made to the general uniformity of events transpiring within the embryo-sac of the Angiosperm. In *Utricularia* the relation of the ovules to adjacent parts is such as to make possible departures from the typical story, and morphological investigations reveal unusual behavior of both the female gametophyte and the endosperm. While these pecularities differ only in degree from many others frequently noted elsewhere, their prominent expression in this plant is made possible through the relations here existing between ovule, placenta, and ovary wall. Had not the developing seeds been held firmly in place, independent of their own attachments, there could have been no micropylar haustorium of such pronounced type. Under usual conditions such outgrowths are impossible, and even their initiation is generally inhibited.

In considering departures from the typical embryo-sac story these haustorial developments, either micropylar or antipodal, constitute those most frequently encountered. They have generally been interpreted, and probably correctly, as nutritive devices of one sort or another. In the large, they represent efforts on the part of the contained embryo-sac nuclei to express themselves in more marked way than is typically the case. While most of these emergent structures are abortive, some of them proliferate considerable distances through contiguous tissues and constitute striking outgrowths from the embryo-sac.

Without summarizing here the extensive and well known literature on this subject, one might cite typical papers in this field. Billings (2, 3) has made a careful study of nutritive outgrowths from embryo-sacs, particularly in the Labiatæ, and his figures reveal a striking series in this group; Chamberlain (4) found unusual development of the antipodals in certain of the Compositae; Balicka-Iwanowska (1) investigated numerous of the Scrophulariaceæ, recording several cases comparable to that found in *Utricularia,* particularly in *Scrophularia vernalis* which has both micropylar and antipodal outgrowths; while Merz (7) and Kamienski (5) worked on various species of *Utricularia,* outlining quite fully the story discussed in detail above. Of special interest is the paper by Lang (6), which resulted in shifting *Polypompholyx* and *Byblis* from the Droseraceæ to the Lentibulariaceæ. Many of the figures of *Polypompholyx* in this paper are very similar to conditions found in *Utricularia vulgaris.*

Antipodal outgrowths may be quite large, especially in the case of those ovules with massive integument, and some of them may attain to remarkable length and complexity. The antipodal region borders on a mass of tissue, merging into the funiculus, and makes connection quite directly with the major vascular supply of the ovule. This invites specialization in this direction and the question might well be considered as to why such haustoria are not more common and more highly specialized than they seem to be. In *Utricularia* the antipodal haustorium is post-fertilization in development and is purely an endosperm outgrowth, seemingly to tap the foods stored or transported in the epidermis of the ovule. Its importance diminishes relatively as embryo development proceeds, its work being supplemented and later supplanted by the haustorium at the opposite end of the ovule.

In the closely related *Polypompholyx,* Lang (6), finds that the female gametophyte begins an antipodal haustorium which, as in the case of *Utricularia,* is later markedly developed by the endosperm, two cells of which dominate its growth. In *Polypompholyx,* however, there is developed an extensive nutritive tissue into which the haustorium enters so that it does not reach the epidermis of the ovule. The antipodal haustorium in this form is larger and functions longer than in *Utricularia.*

While micropylar haustoria are not uncommon, these in general are smaller and less highly specialized. The explanation for their failure is not far to seek, since it follows from the relations of the embryo-sac to adjacent parts in this direction.

The micropylar haustorium, in contrast to that of the antipodal end, usually finds no receptive tissue before it, so there is little to encourage prolongation in that direction. Outside the thin zone of the nucellus lies the sterile micropyle or the fused integuments. Only in the case of anatropous ovules is there nutritive invitation beyond the integument, where further elongation might tap the placental tissues. Mere inversion of the ovule is not sufficient, however, to insure suitable conditions for the development of a haustorium at the micropylar end. It is important that there be contact with the placenta so that any protruding part may not enter a space system if extended beyond the micropyle. Further, there must be no serious growth movements, preliminary to, or especially after fertilization to alter the relations of these adjacent parts which are merely pressed together and are not organically connected.

Utricularia offers favorable opportunity, therefore, for basilar outgrowth from the embryo-sac, and both the female gametophyte and the endosperm participate in its development. After mid phase in embryo development it seems to be the only channel for transfer of food to the ovule.

There is no development of vascular tissue in the funiculus, which gradually is subordinated until later it becomes a mere rudiment. The seeds are scarcely attached as they approach maturity, and may be lifted off from the receptacle in a cohering layer, so feebly do their funiculi support them.

Utricularia thus presents an anomalous situation in that the embryo is nourished throughout its development by haustorial outgrowths. The fact that the developing seeds are held firmly in place between ovary wall and placenta, while lateral displacement is prevented by mutual pressure of the ovules, permits diminished emphasis upon funicular attachment and favors direct transfer of materials by means of the micropylar haustorium. The not infrequent abortions are probably due to displacement of ovules thus severing the soft suctorial organs. In any event the specializations here noted suggest that with greater liberty the female gametophyte and endosperm might offer in general greater variety of form and behavior.

Such views are fortified by comparison of *Utricularia* with conditions reported in *Polypompolyx* by Lang (6). Developments are similar except that the receptive tissue into which the micropylar haustorium enters is located in a hump on the funiculus of the ovule instead of in the placenta. The outgrowth is dominated by the endosperm and it displays characters similar to that described above for the Bladderwort, except that it never attains the prominence shown in *Utricularia,* and is less conspicuous than the antipodal haustorium of the same ovule. Since both masses of receptive tissue are within the ovule in *Polypompholyx,* it must use its funiculus in the regular way and so is debarred from the unusual achievement of *Utricularia* which attacks the placenta directly.

The writers of this paper would like to emphasize the view that events taking place within the pistil of the Angiosperm are so closely inter-dependent that these relations dominate the gametophytes. They prevent, on one hand, enlargement of the female gametophyte in any such degree as marks the Gymnosperms, since fertilization is not long delayed. Perhaps these same conditions also operate in some way to inhibit reduction of the embryo-sac to the theoretically possible limit of a single egg, by demanding coöperating cells and nuclei. The male gametophyte, as represented by the pollen tube, presents a similarly specialized mechanism. It must, with the female gametophyte, follow a precise program involving both time and space relations which permit of limited variation.

In other words, the make up of the ovule and its contents in relation to the pollen tube habit outweighs all other factors and invites to a peculiar type of specialization. The limitations imposed by the associated parts are very definite, and despite the most marked contrasts in size, habitat, floral and vegetative development, as well as divergent kinship between the various groups of Angiosperms, the behavior of the male and female gametophytes displays a remarkable uniformity. This constancy then represents a survival of structures and habits best fitted to the exacting type of siphonogamy found in this great group, rather than a stage of incomplete reduction or evolution.

SUMMARY

1. The inverted ovules of *Utricularia vulgaris* are crowded

together on a free central placenta and are tightly invested by the ovary wall.

2. The female gametophyte, arising from the innermost megaspore, early digests the nucellus and protrudes from the ovule. It is never enclosed by the integument.

3. This micropylar haustorium invades the tissue of the placenta which in anticipation of such intrusion has developed a mass of nutritive tissue opposite the micropyle of the ovule.

4. The pollen tubes creeping down over the surface of the placenta encounter the protruding lobes of the female gametophyte, and pass through their cytoplasm to the synergids.

5. Fertilization and double fertilization take place in practically all of the ovules, few aborting at this early stage. The primary endosperm nucleus divides before union of egg and sperm is completed.

6. The antipodals are very small and inconspicuous, and disappear soon after fertilization.

7. The endosperm early in its development pushes two cells past the feeble antipodals and develops a haustorium which penetrates to the epidermis of the ovule against which it flattens out. It persists until the seed is nearly mature and seems to take food from the heavy epidermal layer of the ovule.

8. Similarly two endosperm nuclei pass by the egg apparatus and enter the micropylar haustorium, which then energetically attacks the receptive tissue of the placenta.

9. The funiculus of the ovule does not develop after fertilization, and rapid growth of other parts leaves it as a rudimentary structure which remains without vascular tissue and becomes practically functionless.

10. Within the dome shaped placenta vascular strands are freely developed with branches which end near the bases of the ovules. Each strand is directed towards the base of a funiculus, but terminates alongside the mass of ''receptive'' tissue.

11. The micropylar haustorium remains prominent during seed development, digests most of the receptive tissue, and becomes the medium through which food is transferred to the embryo. Adjacent cells of the placenta are elongated radially and seem to conduct food to the haustorium.

12. The seeds during later development are practically with-

out attachment and are held in place by the pressure of surrounding parts,—the placenta, ovary wall and adjacent ovules, —and each is nourished directly by means of its micropylar absorbing organ.

13. Their unusual behavior in *Utricularia* and similar forms suggests that the female gametophyte and endosperm, if given favorable opportunity, may assume more marked development than is typically displayed.

14. It seems probable that the constancy of events within the ovule of the Angiosperm is correlated with its structure and the type of siphonogamy found in this group. The gametophytes must follow a precise program and post-fertilization activities are under ordinary conditions largely under the control of the ovule.

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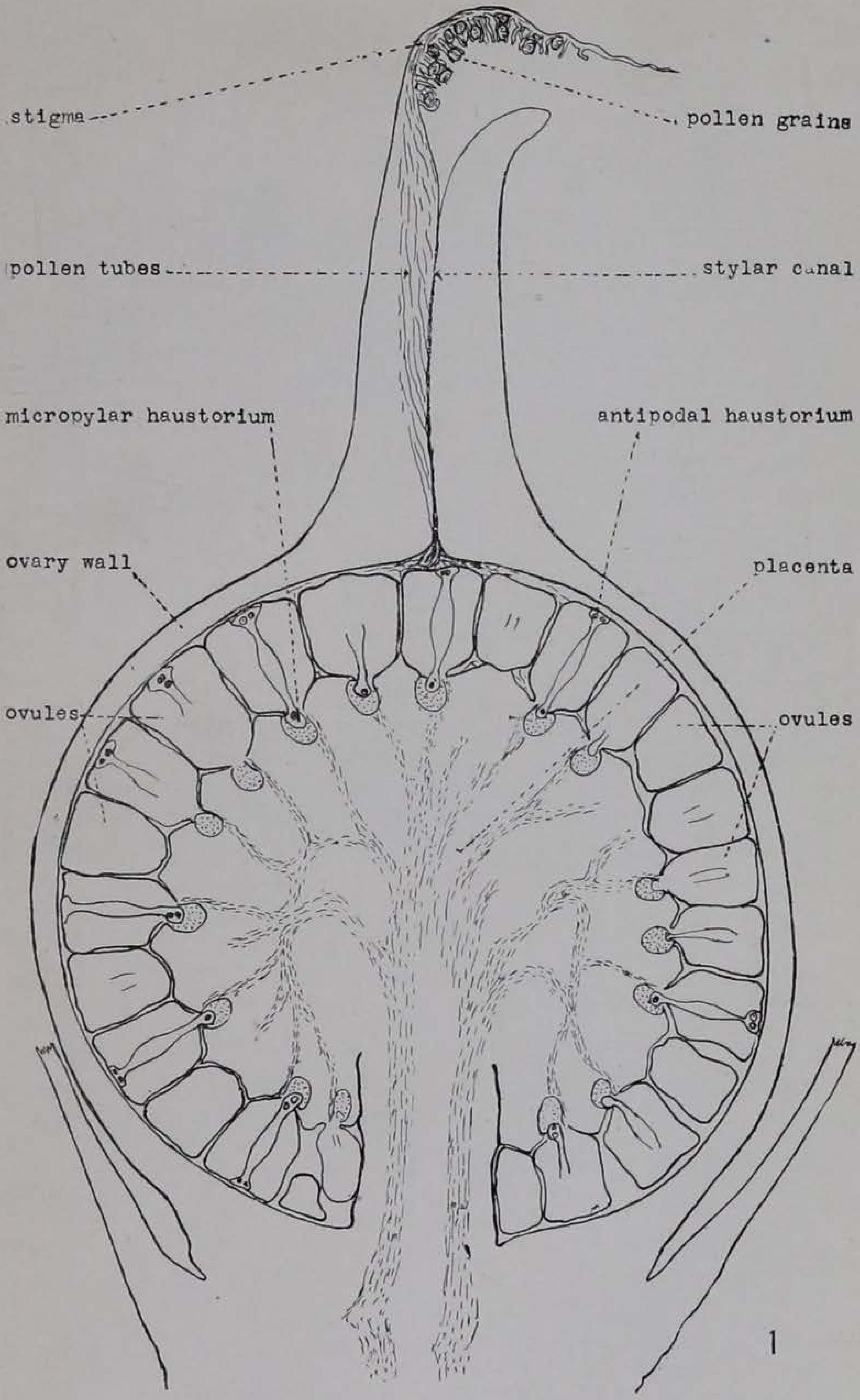

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DESCRIPTION OF PLATES

The following abbreviations are used:
a, receptive portion of placenta; *b*, antipodal haustorium; *c*, embryo; *e*, endosperm; *f*, micropylar haustorium; *g*, egg; *h*, polars; *j*, antipodals; *k*, synergid; *m*, nuclei of micropylar haustorium; *n*, integument; *o*, funiculus; *t*, pollen tube; *v*, placenta; *x*, sperm

PLATE I

Fig. 1. Longitudinal section through pistil showing relative size and arrangement of parts soon after fertilization

PLATE II

Fig. 2. Early stage showing spore-mother-cell, and outlining below the "receptive" portion of the placenta later to receive the basilar haustorium

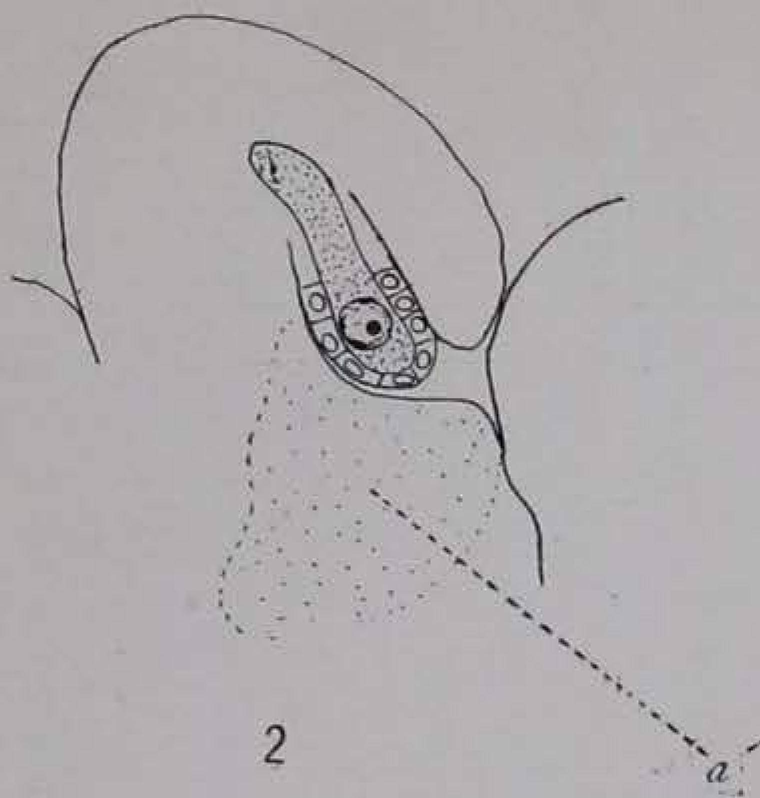
Fig. 3. Stage showing the four megaspores

Fig. 4. Dominant megaspore with other megaspores and nucellar tissue breaking down

Fig. 5. Two-celled gametophyte; nucellus digested, and micropylar haustorium entering placenta

Fig. 6. Early post-fertilization, showing two-celled endosperm, micropylar haustorium, and receptive tissue

PLATE II



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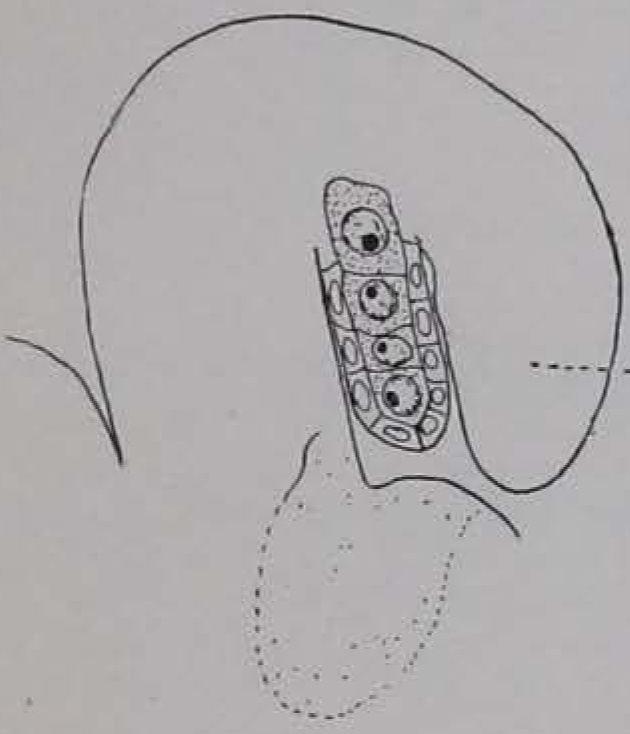

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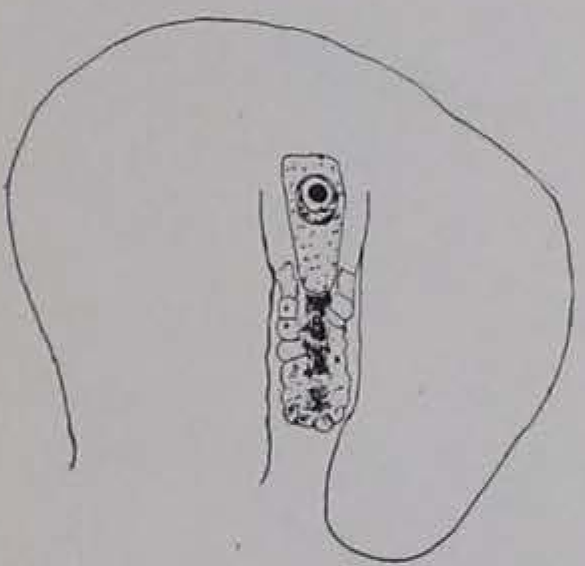

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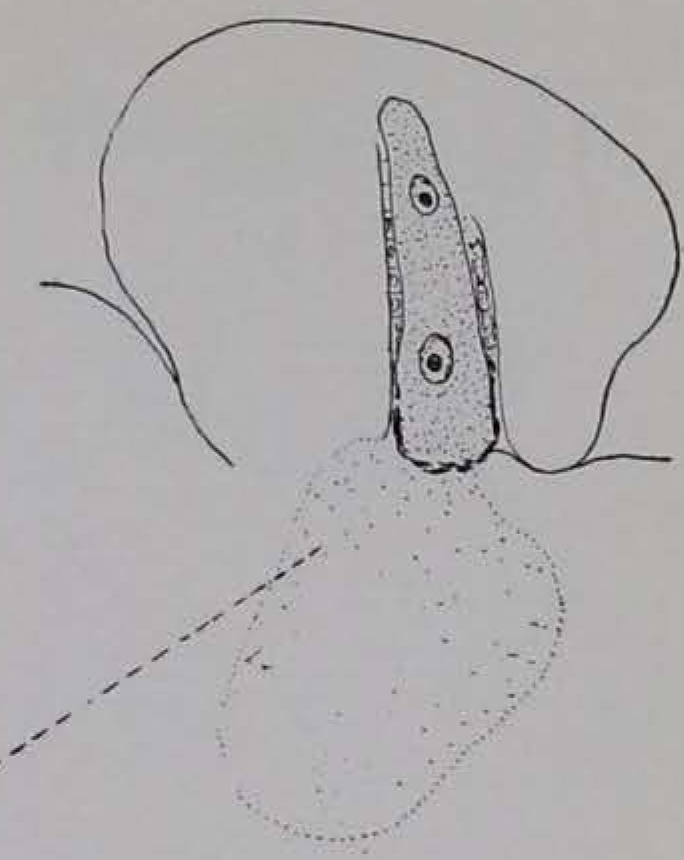

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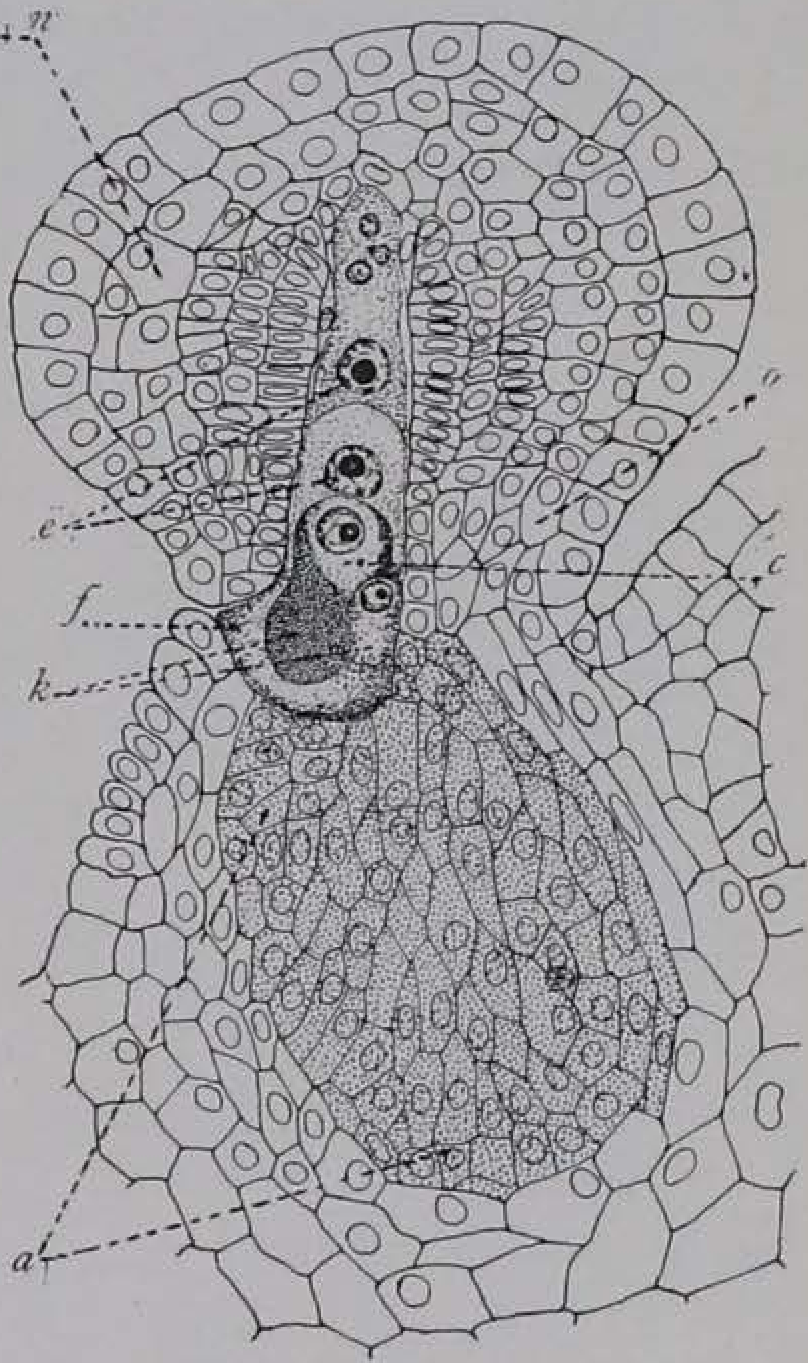


PLATE III

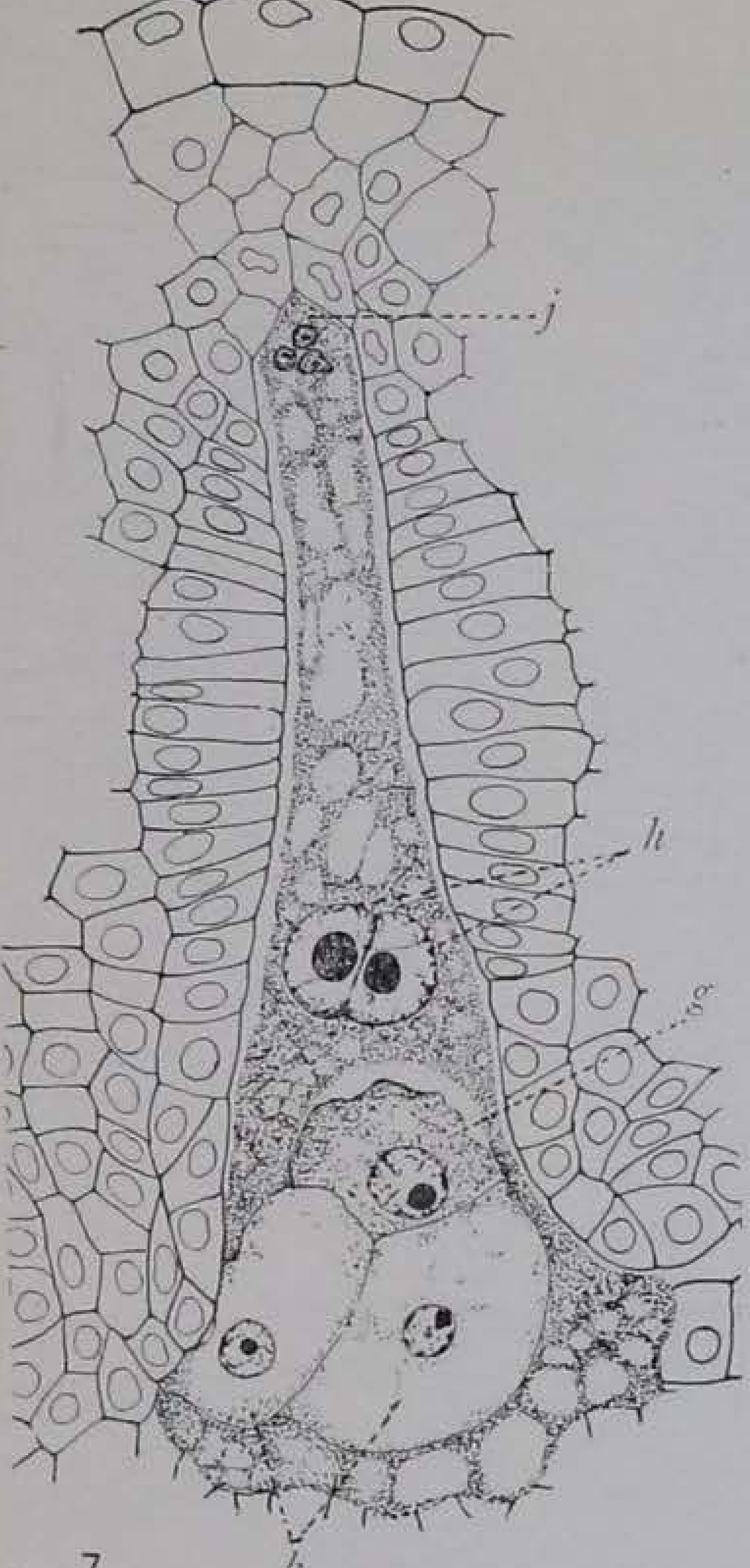
Fig. 7. Mature female gametophyte

Fig. 8. Fertilization and double fertilization; synergid gorged with pollen tube contents

Fig. 9. Pollen tube joined to lobe of the micropylar haustorium; primary endosperm nucleus dividing

Fig. 10. Mature seed, showing its angled form due to pressure during development

PLATE III



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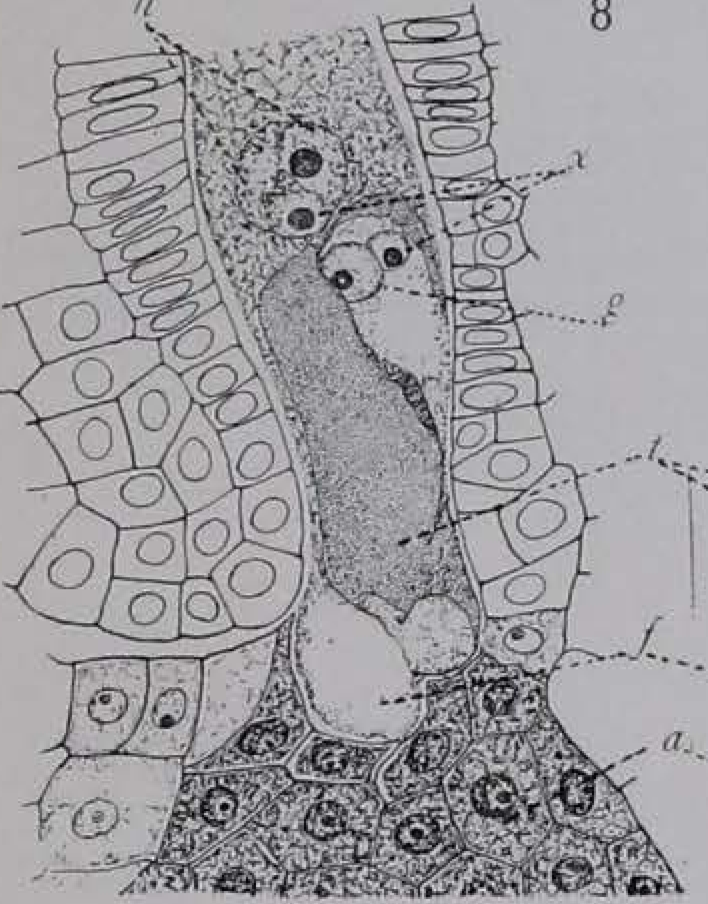

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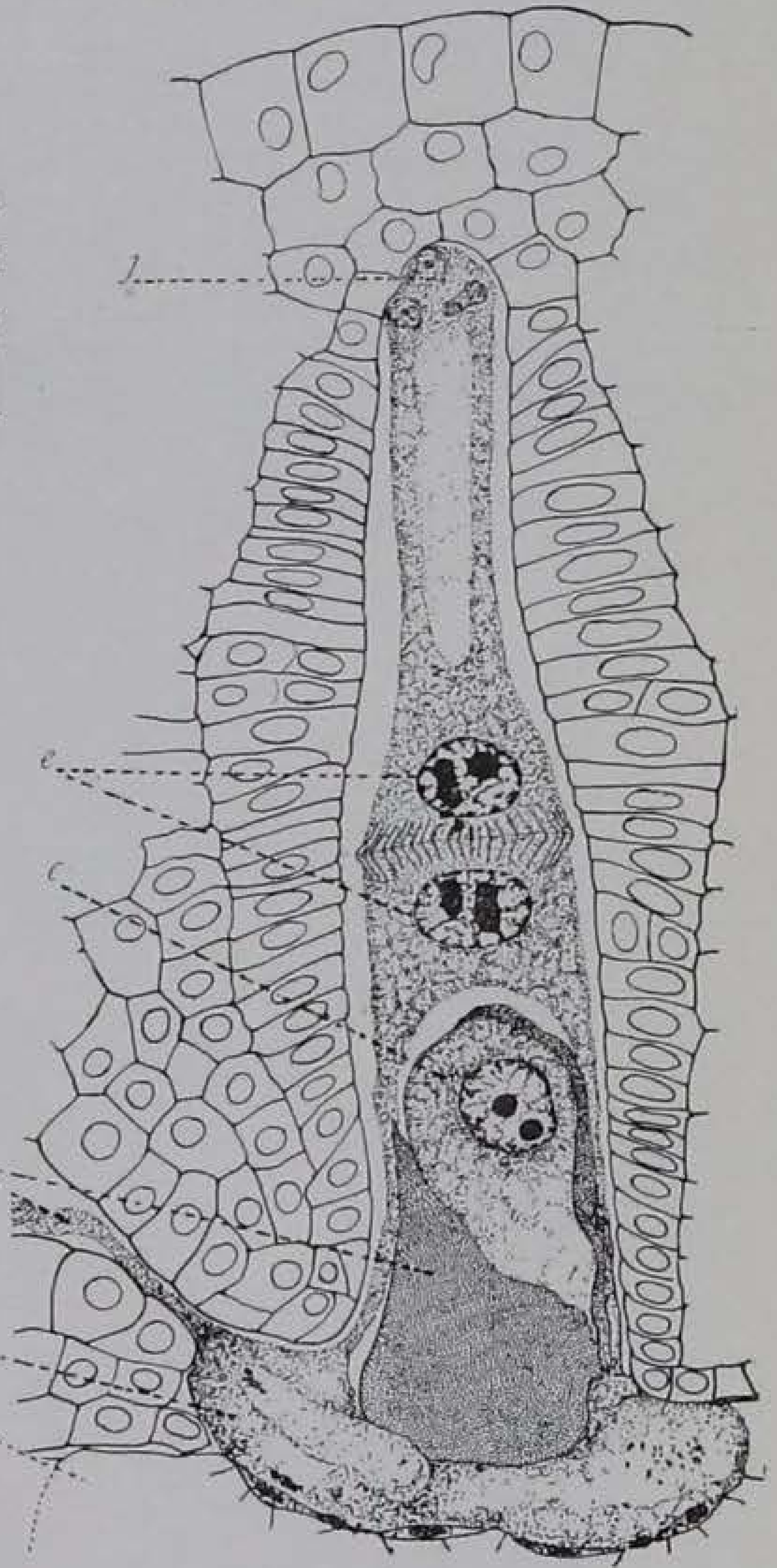

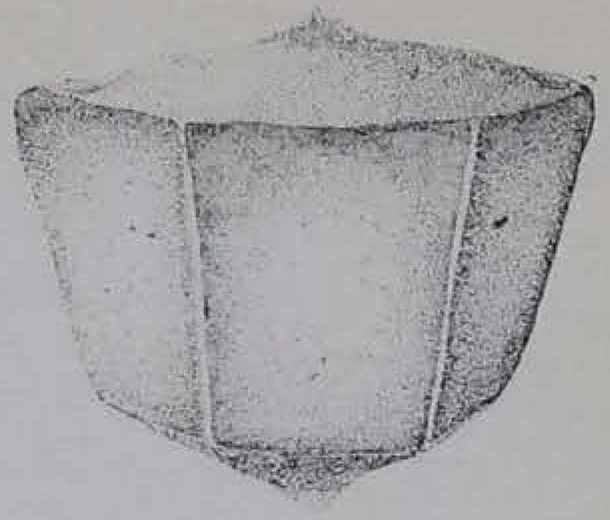

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PLATE IV

Fig. 11. Longitudinal section through ovule showing embryo, haustoria, receptive tissue, and diminutive funiculus

PLATE IV

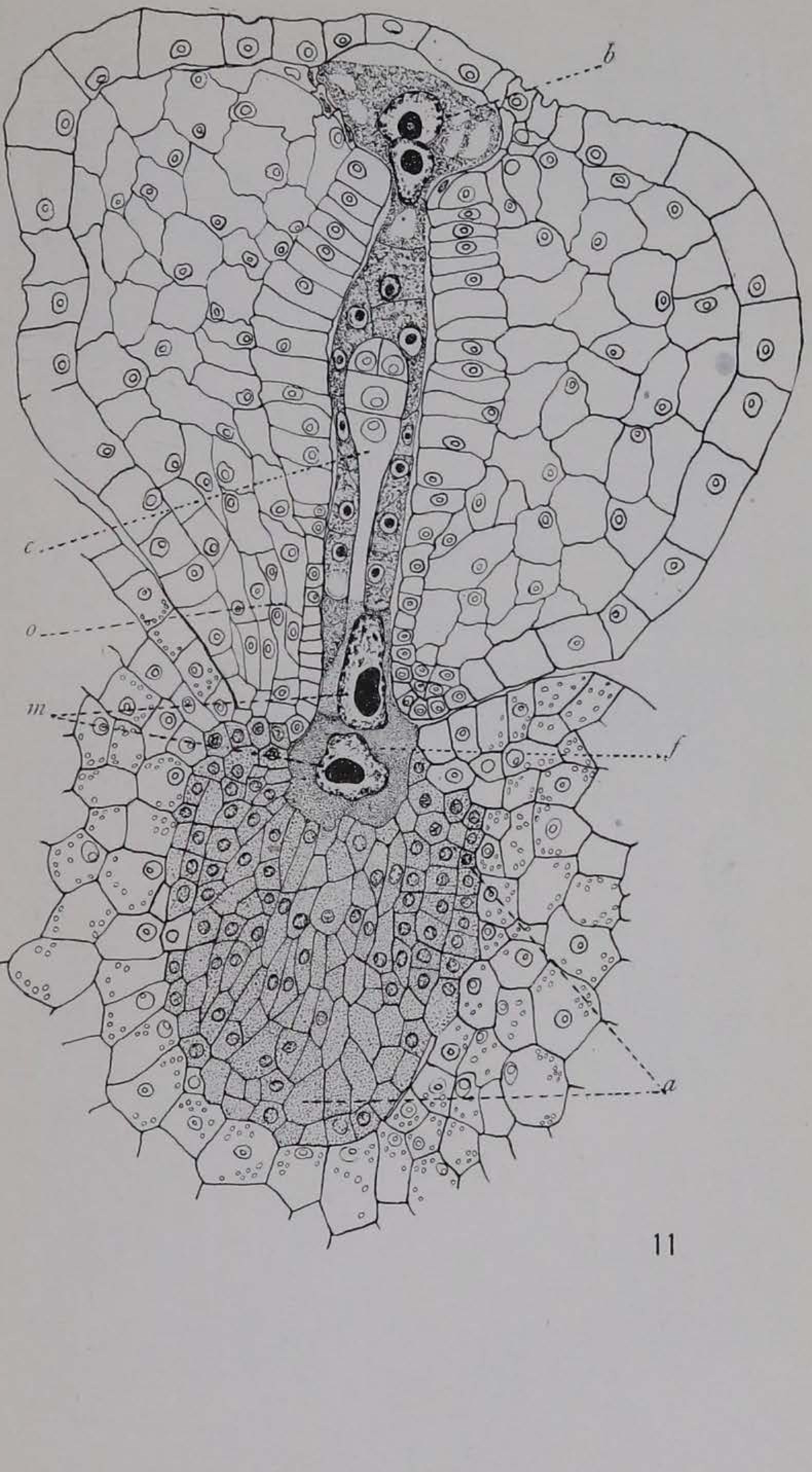

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PLATE V

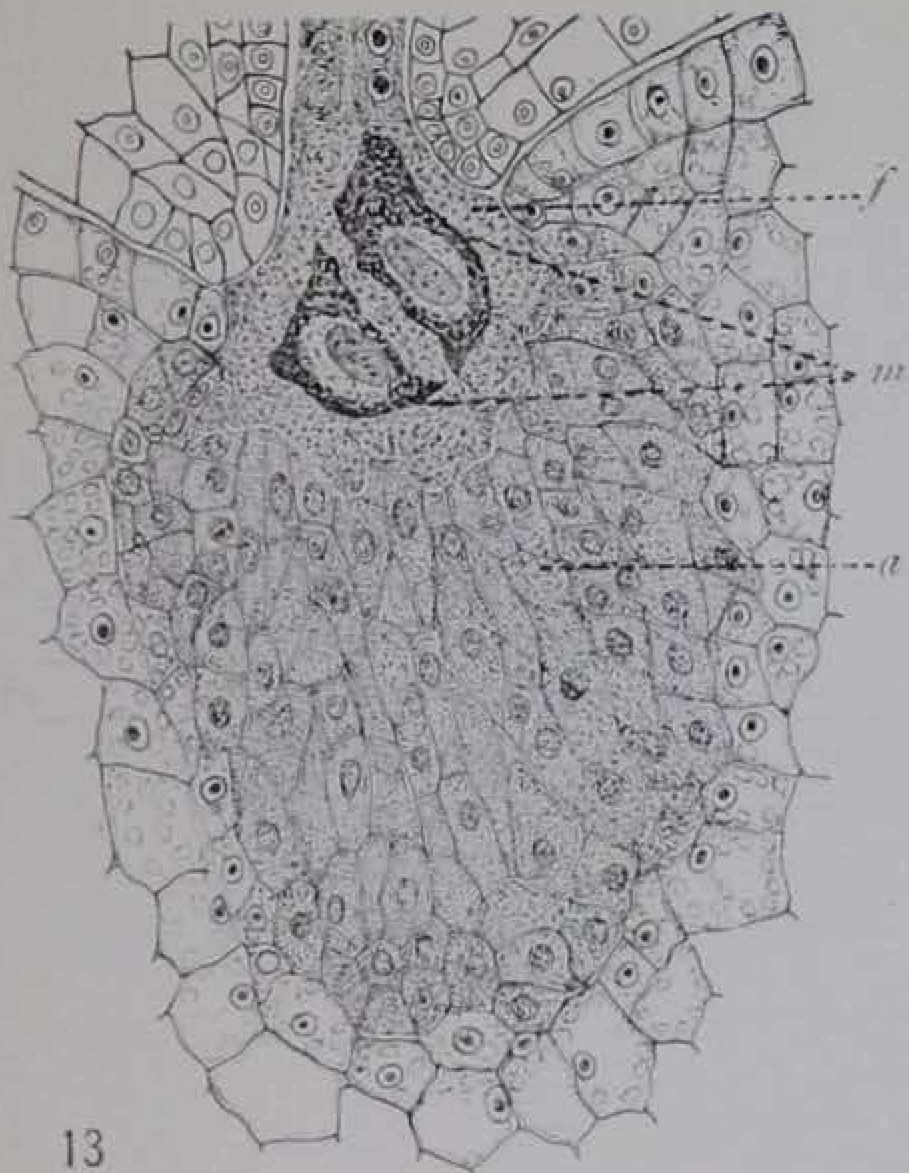
Fig. 12. Slightly younger stage than the preceding, with antipodal haustorium relatively farther advanced than the basilar outgrowth

Fig. 13. Showing invasion of the receptive tissue by the micropylar haustorium with its prominent endosperm nuclei

Fig. 14. Later stage in which the cell walls have disappeared from the receptive tissue, and showing the peculiar nuclei lying in the cavity; the endosperm nuclei are the more darkly staining

Fig. 15. Pollen grain

PLATE V



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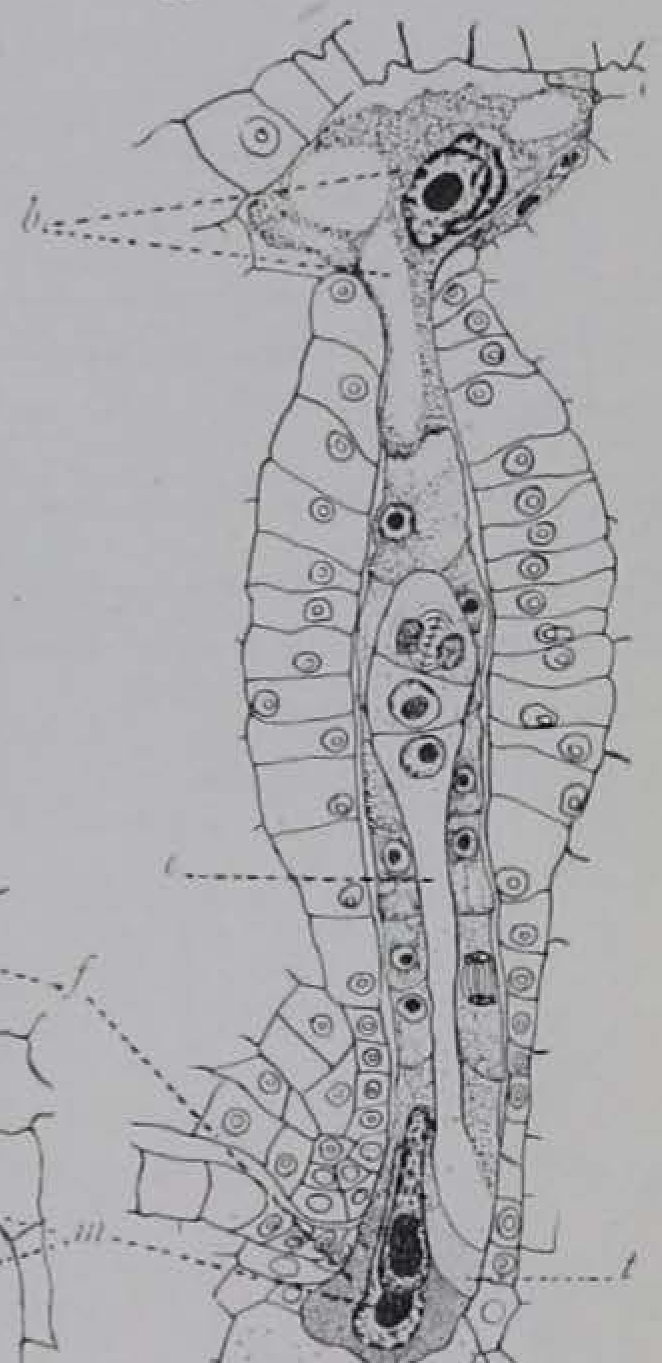

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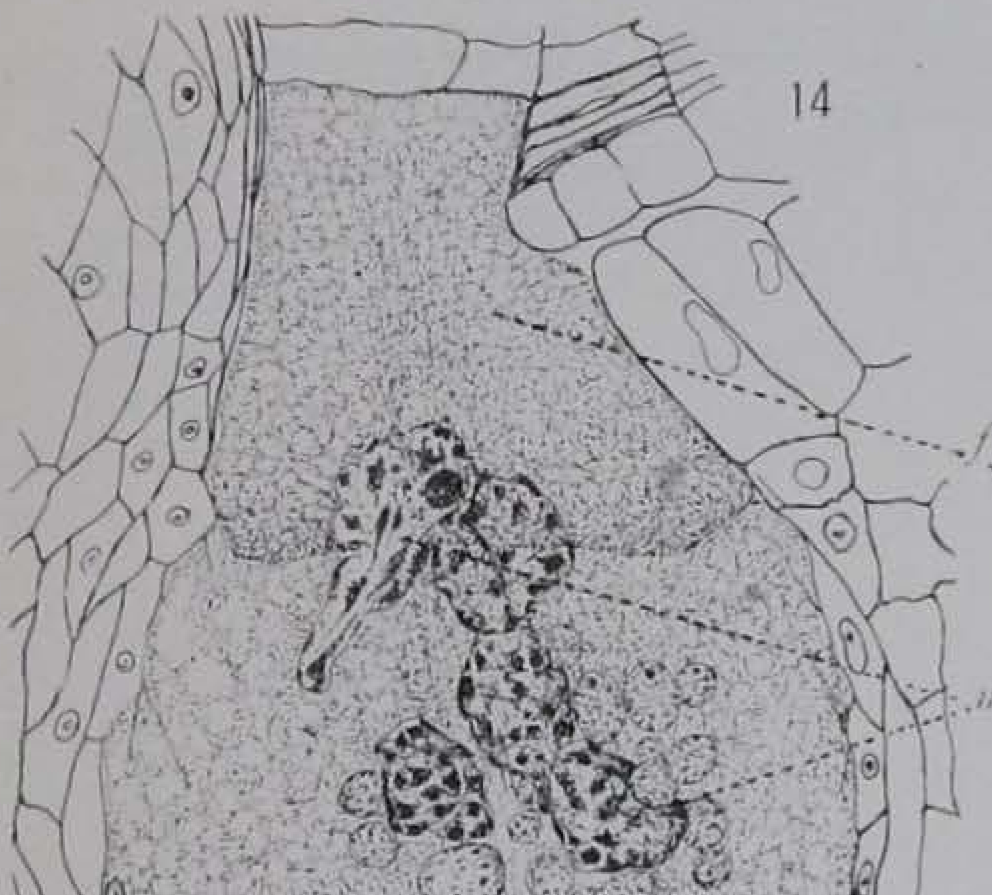
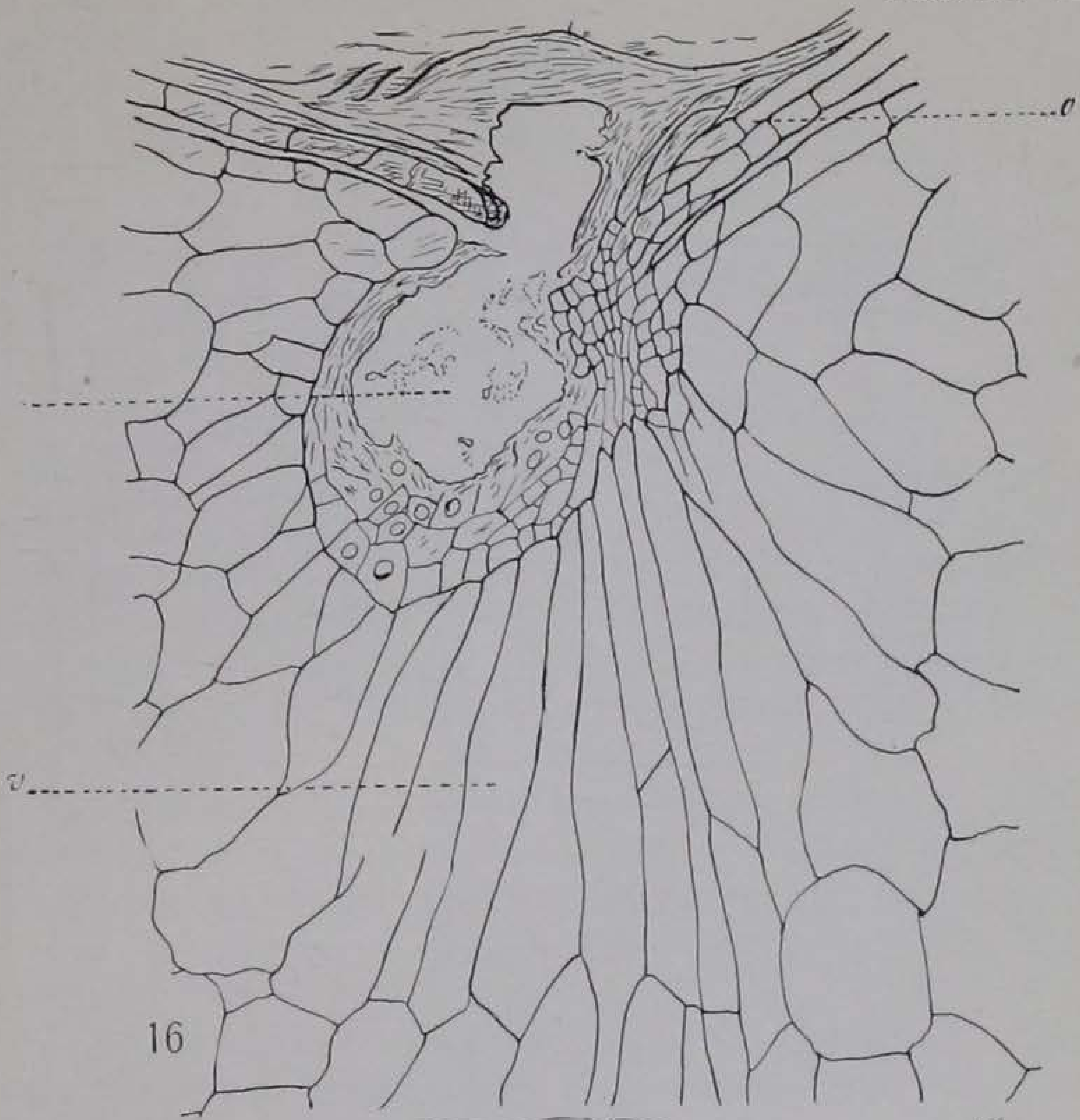


PLATE VI

Fig. 16. Placental tissue near base of a nearly ripe seed

Fig. 17. Showing arrangement of the seeds covering the central placenta, after ovary wall has been removed

PLATE VI



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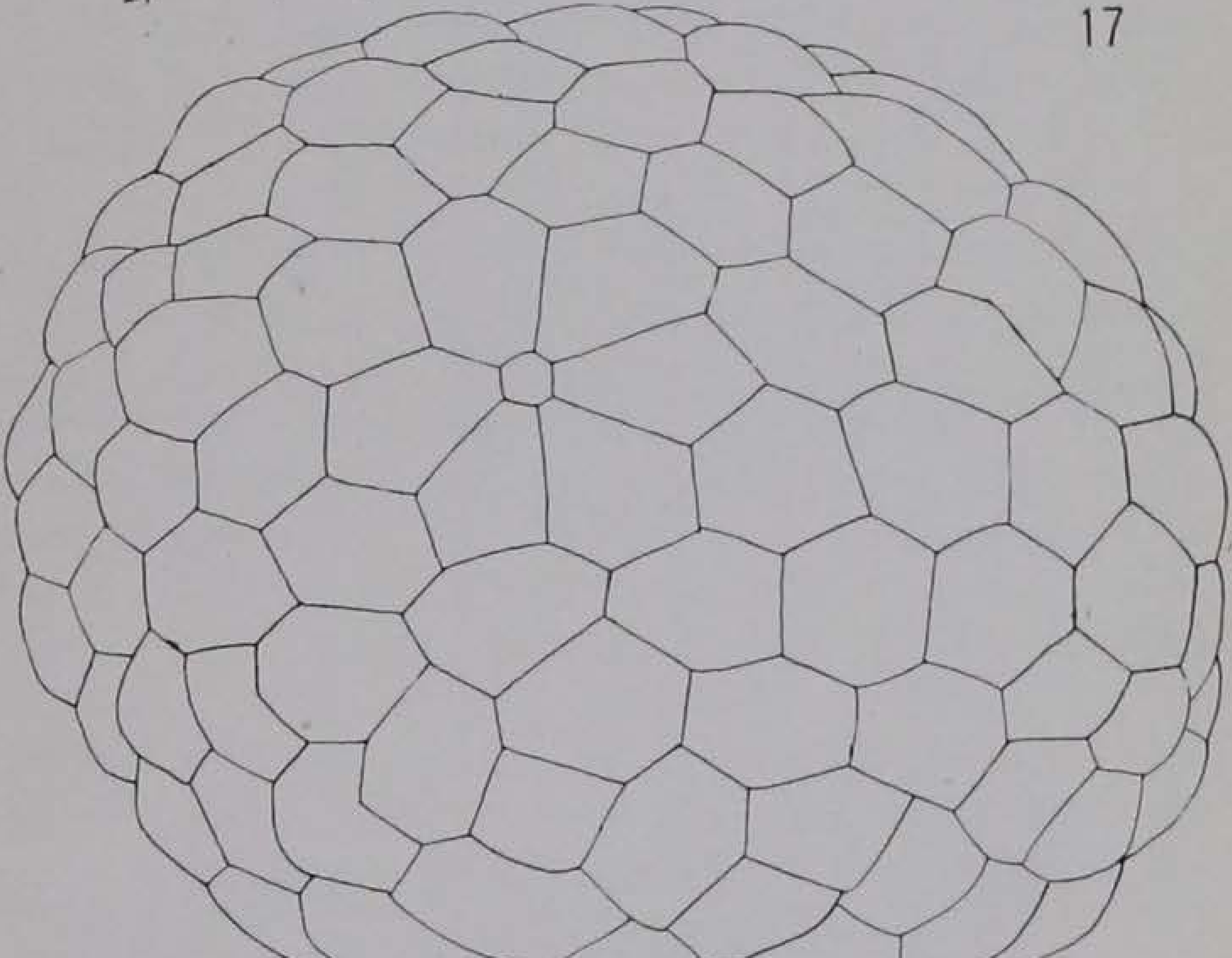

THE AMPHIBIANS AND REPTILES OF DICKINSON COUNTY, IOWA

By Frank N. Blanchard

Department of Zoology, University of Michigan

The period from June 22 to July 31, 1920, was spent by the writer at the Iowa Lakeside Laboratory on Lake Okoboji in Dickinson County, northwestern Iowa, for the purpose of collecting a representative series for the Museum of Zoology of the University of Michigan. Collecting was done in the townships of Lakeville, Spirit Lake, Center Grove, Okoboji, and Diamond Lake.

For their many kindnesses throughout his study, the writer wishes to express his deep appreciation to Professor Robert B. Wylie, Director of the Lakeside Laboratory, and to Professor Frank A. Stromsten of the Department of Zoology of the University of Iowa.

The region is one of terminal moraine topography, dating from the last, or Wisconsin, ice sheet. The central portion of the county is the lake region of Iowa. Here are the three large lakes, East and West Okoboji and Spirit Lakes, besides many smaller ones of all sizes. West of the lakes, and flowing in general from north to south, lies the Little Sioux River, a tributary of the Missouri. The steeper shores of the lakes and of the Little Sioux are generally covered with trees, sometimes pure stands of bur oak (*Quercus macrocarpa*), at other times bur oak accompanied by basswood, ash and elm, but in all other places a natural growth of trees is lacking. Undrained depressions, or sloughs, of all sizes are common everywhere, but their number is being fast diminished by the digging of ditches and the placing of tile drains. Nearly all of the land is either under cultivation or devoted to pasturage, but occasional fields of unaltered prairie and natural woodland still remain. Their number and area are however so small that vertebrate forms dependent upon natural conditions for their existence have been

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nearly or quite exterminated. It is highly important that faunistic studies be undertaken here, and throughout our country, at as early a date as possible if we are to have any record of the composition and distribution of our native fauna, and if we are to deal intelligently with its preservation. For an excellent account of the vegetation of the region see Shimek, 1915, and for a discussion of prairie habitats applicable to this locality, see Ruthven, 1910.

The present study shows the natural reptile-amphibian fauna to be rather poor in number of forms, and to be rapidly growing much poorer. Even those species that find the region naturally highly favorable are maintaining a losing struggle against cultivation of the land and persecution.

As already pointed out by Ruthven (1910, 202) the reptile-amphibian fauna is made up of both eastern and western forms. Those characteristic of the eastern forests are, as would be expected, but scantily represented. There is definite record for only one forest amphibian, *Hyla versicolor versicolor*. Of snakes preferring the forests, *Storeria occipito-maculata* is rare here, and *Diadophis arnyi* has been taken in Plymouth County and may yet be found in this vicinity. *Thamnophis sirtalis parietalis* appears to have greater preference for the woods than for the open prairies, and is moderately common. As is well known, its close relative, *T. sirtalis sirtalis,* has found the eastern deciduous forest region a highly favorable habitat.

Eastern aquatic and land forms are represented by *Bufo americanus, Acris gryllus, Rana pipiens,* and *Liopeltis vernalis* (the latter not yet on record for this county but undoubtedly present.)

Of strictly western forms there are *Pleistodon septentrionalis, Chrysemys marginata bellii, Thamnophis radix, Heterodon nasicus,* and three which doubtless occur here but have not yet been taken, *Pituophis sayi, Lampropeltis triangulum syspila* and *Sistrurus catenatus catenatus.*

For several species of snakes this region appears to be close to, or just beyond, the northern limit of their distribution. It is probable that *Liopeltis vernalis, Diadophis arnyi, Elaphe vulpina, Pituophis sayi,* and *Lampropeltis triangulum syspila,* will yet be found in the county, while the following forms, well-known from a little farther south, are not expected: *Coluber constrictor flaviventris, Lampropeltis getulus holbrooki, Storeria dekayi, Thamnophis proximus,* and *Natrix sipedon sipedon.*

The following amphibians and reptiles were taken by the writer (unless otherwise stated) in 1920 in Dickinson County, Iowa, and are deposited in the Museum of Zoology of the University of Michigan at Ann Arbor.

Necturus maculosus (Rafinesque).—Mud Puppy.—One specimen was seined from a pond near Hottes Lake in Spirit Lake Township on August 7 by Drs. Kuntz and Thomas. This specimen was shipped to me by Professor Stromsten who declares there can be no doubt about its being a *Necturus,* but was spoiled and destroyed before I was able to see it. It is probable that another specimen was taken in the outlet from Spirit Lake a year earlier, but this also was eventually lost.

Ambystoma tigrinum (Green).—Tiger Salamander.—Common throughout the region. One adult was taken in a damp cess-pool on the Laboratory grounds on July 5. Larvae in all stages were plentiful in several of the small ponds in pastures and fields, and at least one specimen was found in nearly every pond seined. In a very small pasture pond by the side of the road north of the Laboratory 160 specimens were taken with a seine in less than an hour. In this as in other ponds where they were common, they were found to be much more plentiful towards the center of the pond, away from grass and algae.

Bufo americanus Holbrook.—American Toad.—Common but not abundant in the county. Eight adults were taken, four near the Laboratory, one on the north shore of West Lake Okoboji, one on the shore of Upper Gar Lake, and two on the banks of the Little Sioux River. Young individuals were found transforming in a marsh near the shore of East Lake Okoboji on July 8, and several others, about a centimeter and a half in length, on the grassy mud-flats of Lower Gar Lake on July 17.

Pseudacris triseriata (Wied).—Swamp Tree Frog.—Two adults were found, one on a rainy day, July 7, in the tall grass of a wet meadow between Miller's and Emerson's Bays, West Lake Okoboji, and the other in the sparse undergrowth of an oak woods between Robinson and Marble Lakes in Spirit Lake Township on July 15. In one specimen the heel when extended forward reaches the anterior border of the tympanum; in the other the posterior border.

Hyla versicolor versicolor Le Conte.—Tree Frog.—A single adult was found under the loose dry bark of a dead oak in the dense woods along the Little Sioux River in Okoboji Township, June 20. Five young ones, four with tails and one without, were found while seining a woods pond covered with duckweed between Marble and Hottes Lakes in Spirit Lake Township on July 28. Other records for this county are: Center Lake region, July 22, 1918, and Lake Okoboji, July 1914, T. C. Stephens (Ruthven, 1919, 2).

Acris gryllus (Le Conte).—Cricket Frog.—Common but not abundant. It was found in nearly all suitable localities; the muddy shores of the Little Sioux River in Okoboji and Lakeville Townships, open shores along the canal and on Miller's Bay in Lakeville Township, open borders of small ponds or sloughs, and along Lower Gar Lake in Center Grove Township.

Rana pipiens Schreber.—Leopard Frog.—This is the widespread and abundant amphibian of the region. Specimens were taken in all the townships visited. It occurs near all the lakes, sloughs, and streams, and is common in tall, and sometimes in short grass, on the prairies and uplands far from water. It is rare, however, in the woods. Young frogs, just metamorphosed, were exceedingly numerous on July 14 and 15 in Spirit Lake Township, between Marble and Hottes Lakes, near their breeding ponds, but even two weeks later tadpoles with only the hind legs developed were still plentiful in the ponds. Several individuals, both young and old adults, were found with no spots on the body and those of the legs much reduced.

Plestiodon septentrionalis Baird.—Skink.—Not common. A single specimen was taken on the Laboratory grounds on June 25. It was first found on Lake Okoboji by Dr. T. C. Stephens in July, 1914.

Heterodon nasicus Baird & Girard.—Western Hog-nosed Snake; Spreading Adder; Blow-Snake.—Apparently rare in the county. One specimen was found by Professor Stromsten, on

July 23, on the bank of the Little Sioux, where the river flows through a pasture two miles west of the Laboratory.

Its description is as follows: Ventrals, 145; caudals, 37; upper labials, 8; lower labials, 10; dorsal scale formula, 23-21-19; 40 dorsal spots on body, 11 on tail; two rows of lateral spots; total length, 594 mm., tail length, 88 mm., tail divided by total length, .148; accessory scales separating the two prefrontals, the prefrontal from the frontal, and the internasals from the rostral; female.

Storeria occipito-maculata (Storer)—Red-bellied Snake.—This snake is occasionally seen in the county. A specimen was taken at Lake Okoboji, June 20, 1917, by Dr. T. C. Stephens.

Thamnophis sirtalis parietalis Say.—Red-sided Garter Snake.—Common, but much less so than *T. radix.* Eight specimens, representing the townships of Lakeville, Okoboji, Center Grove, and Spirit Lake, were secured and these were all found not far from woods.

Thamnophis radix (Baird and Girard).—Common Garter Snake.—This is the common land reptile of the region. It was found in all the townships and in all habitats visited. It was, however, only rarely met with in the woods, but on the prairie in tall or short grass, along fences and roadsides, near marshes, sloughs, and lake shores, it is frequently seen. It will readily take to water when disturbed along the shore of a pond, and one young individual was met with at least a hundred feet from the shore of Miller's Bay, swimming toward the open lake.

Chelydra serpentina (Linné). Snapping Turtle.—Common in all the lakes, sloughs, and rivers in the region. Specimens obtained represent Lakeville, Center Grove, and Spirit Lake Townships. A nest containing nineteen eggs was found near the lake shore in the field north of the Laboratory on July 1, by Professor F. A. Stromsten. The nest had been opened by some animal and two or three eggs taken out and destroyed. It was very fresh, perhaps not more than a day old. A specimen found killed on the shore of Hottes Lake had a carapace 38 centimeters long.

Emys blandingii (Holbrook).—A large female of this species was captured in a bayou of the Little Sioux River directly west of the town of Spirit Lake, and was given by Mr. Frank P. Hopkins of the boat-landing at Spirit Lake to Professor Keyes of the Lakeside Laboratory in the summer of 1921. The specimen was sent to the writer by Professor Stromsten. Its carapace is approximately 23 cm. long, and 15.5 cm. in greatest width, and the turtle is about 10 cm. high. This appears to be the most western point recorded for this species, and the first record for Iowa.

Terrapene ornata (Agassiz).—Box Turtle.—Professor Stromsten reports finding, in a ravine near the Laboratory, a box turtle which he referred to this species. It was found August 17, 1916, dissected and destroyed. As Professor Stromsten could hardly mistake a box tortoise, his report deserves consideration. However, this form is undoubtedly very rare in the region.

Chrysemys marginata bellii (Gray).—Bell's Painted Turtle.—Abundant in all lakes, sloughs, and rivers in the region. One turtle was discovered completing nest building on the grassy lawn in front of the Laboratory on June 26, about an hour or more before sunset. Thirteen eggs were dug from the nest. Another turtle was found completing its nest at the edge of the pasture north of the Laboratory about ten feet from the lake shore. This nest contained twelve eggs. In each case the earth about and over the eggs was well wetted. Another nest found a few days later very near this one contained eleven eggs. Near the small lakes west of Spirit Lake seven apparently complete sets of eggs were recovered containing, respectively, 5, 6, 6, 7, 7, 9, and 12 eggs. Many nests were discovered pilfered by some animal, with all of the eggs destroyed and the shells scattered about in the vicinity .

Amyda spinifera (LeSueur).—Soft Shelled Turtle.—Evidently common in the Little Sioux River. One adult was taken in the Township of Okoboji on the bank of the Little Sioux on July 3 in the middle of the afternoon. It was beginning to prepare a nest about twelve feet from the water on a sunny bank, sloping at an angle of about 30 degrees. The impression of the body on the ground showed that the turtle was facing the river, and that the two hind feet were used in digging the double burrow. The latter was already well under way, as it was 4 inches in greatest length and from 1½ to 2 inches deep.

KEY TO ADULT AMPHIBIANS (SALAMANDERS, FROGS, AND TOADS) LIKELY TO BE FOUND IN DICKINSON COUNTY, IOWA

a1. Tail present; hind legs not elongated for jumping.
  b1. Four toes on hind foot; external gills present throughout life............ *Necturus maculosus* (Rafinesque).
  b2. Five toes on hind foot; no external gills on adult; dark brown or black, variously spotted with yellow. *Ambystoma tigrinum* (Green).
a2. Tail absent; hind legs elongated for jumping.
  c1. Skin dry and warty..*Bufo americanus* Holbrook.
  c2. Skin moist and not warty.
    d1. A pair of dorso-lateral ridges; no sucking disks on toes. Back with roundish black spots edged with white.. *Rana pipiens* Schreber.
    d2 No dorso-lateral ridges; sucking disks on toes present, even if small.
      e1. Belly smooth; upper surfaces somewhat roughened; snout pointed; sucking disks minute; size 1 to 1 1-4 inches............ *Acris Gryllus* (Le Conte).
      e2. Belly granular; upper surfaces smooth.
        f1. Sucking disks large; a broad whitish band from eye to angle of mouth; size over 1 inch. *Hyla versicolor versicolor* Le Conte.
        f2. Sucking disks minute; no broad band from eye to angle of mouth; on sides of body two dark bands separated by a lighter one, the lower band passing forward through the eye to the snout *Pseudacris triseriata* (Wied).

KEY TO REPTILES (LIZARDS, SNAKES, AND TURTLES) LIKELY TO BE FOUND IN DICKINSON COUNTY, IOWA

a1. Legs present.
  b1. No broad dorsal shield; body elongate, covered with smooth scales throughout...*Pleistodon septentrionalis* Baird.
  b2. A broad dorsal shield, or carapace, present; body short and broad.
    c1. Carapace flexible at the edges.
      d1. Front margin of carapace with conical tubercles....................... *Amyda spinifera* (LeSueur).
      d2. Front margin of carapace smooth......*Amyda mutica* (LeSueur).
    c2. Carapace not flexible at the edges.
      e1. Ventral shield, or plastron, very narrow, exposing the soft parts..*Chelydra serpentina* (Linné).
      e2. Ventral shield or plastron broad.
        f1. Parts of plastron immovably attached to each other and to carapace; throat with black stripes.. *Chrysemys marginata bellii* (Gray).
        f2. Plastron with a transverse hinge, and movably attached to carapace, throat yellow without black stripes.
          ff1. Upper jaw notched in front; carapace long and depressed *Emys blandingii* (Holbrook).
          ff2. Upper jaw produced downward at symphysis to form a hook; carapace short and high.. *Terrapene ornata* (Agassiz).
a2. Legs absent.
  g1. No anterior fangs in upper jaw; no rattle on end of tail.
    h1. Anal plate entire (i. e. not divided).

i^1. Body scales smooth (i. e. without median keels). Body with broad dorsal blotches of red bordered with black and separated by bands of whitish or yellow.
Lampropeltis triangulum syspila (Cope).

i^2. Body scales each with a median lengthwise keel.

j^1. Body scales in 27 to 33 rows; rostral plate conspicuously enlarged and partially separating the two scales behind it; pattern of many large spots................*Pituophis sayi* (Schlegel).

j^2. Body scales in 19 to 21 rows; rostral normal; pattern of lengthwise stripes.

k^1. Lateral stripe extending upon the fourth row of dorsal scales anteriorly (counting from the belly); scales in 21 rows........................*Thamnophis radix* (Baird & Girard).

k^2. Lateral stripe not extending upon the fourth row of dorsal scales anteriorly; dorsal scales in 19 rows....................
Thamnophis sirtalis parietalis (Say).

h^2. Anal plate divided.

l^1. Dorsal scales keeled.

m^1. Rostral normal,—not enlarged and recurved; body slender.

n^1. Scales in 25 to 27 rows, only faintly keeled; pattern of large spots; size large......*Elaphe vulpina* (Baird & Girard).

n^2. Scales in 15 to 17 rows, distinctly keeled; pattern not of large spots; size small.

o^1. Scales in 17 rows; a single scale in front of the eye; belly whitish........................*Storeria dekayi* (Holbrook).

o^2. Scales in 15 rows; two small scales in front of the eye; belly reddish.............*Storeria occipito-maculata* (Storer).

m^2. Rostral enlarged and recurved; body stout........................
Heterodon nasicus Baird & Girard.

l^2. Dorsal scales smooth (not keeled).

p^1. Dorsal scales in 15 rows; bright green above, belly light, unspotted....................................*Liopeltis vernalis* (Harlan).

p^2. Dorsal scales in 17 rows; ashy to brownish black above, belly yellow or reddish with small black spots........................
Diadophis punctatus arnyi (Kennicott).

g^2. Anterior fangs present in upper jaw; rattle on end of tail; large plates on top of head *Sistrurus catenatus catenatus* (Rafinesque).

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THE LEARNING OF A SIMPLE MAZE BY THE LARVA OF AMBYSTOMA TIGRINUM (GREEN)

By Albert Kuntz
St. Louis University School of Medicine

One of the methods commonly employed in the study of the behavior of lower vertebrates in attempts to determine whether they possess associative memory, or the capacity to learn by repeated experience, involves setting before them simple tasks which they are able to accomplish without too great effort, providing the proper stimulus, and noting the time and effort required to accomplish the tasks in repeated trials. One of the most convenient devices for this purpose is a simple maze; i. e., a more or less tortuous path along which the animal may be induced to travel. If it has associative memory it will be able to reach a given objective in such a path in less time and with less effort after the task has been accomplished repeatedly than in the initial trial. It will also be able to retain the simple habit thus acquired.

Studies of this character on fish carried out by Triplett (1901), Thorndike (1911), Goldsmith (1914), and Churchill (1916) indicate that these animals are capable of forming simple habits which they retain for some time. Studies on the behavior of Batrachians by Abbott (1894) led him to conclude that the "wits of the frog were too limited to be demonstrated." On the other hand, experimental studies on the behavior of frogs and toads by Knauer (1875), Jourdain (1900), and Yerkes (1903) indicate that these animals are capable of forming simple associations and of acquiring simple habits which may be retained for relatively long intervals of time.

Although the Amphibian brain represents a higher degree of organization than the fish brain, the nervous mechanism of Amphibia like *Ambystoma*, by reason of their mode of life, is less constantly and less intensely stimulated by the environment than that of the fish. Being cryptic in their habits their survival

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depends less on their capacity to escape their enemies by immediate effort than on the protection afforded by the environment. Consequently there is *a priori* no ground for the assumption that these animals possess associative memory or the capacity to learn by repeated experience to a greater degree than certain of the fish, even though the latter possess a relatively less complex nervous mechanism.

Neither is there any ground for the assumption that the Urodela and Anura possess associative memory in like degree. Furthermore, the animals used in this study had not yet attained their adult condition. Consequently, direct comparison of their achievements with those of the animals used in the studies referred to above would have little significance.

One of the most important factors in studies of this character is the stimulus employed. It must be one which will insure more or less continuous effort on the part of the animal until the object is reached. Some of the lower vertebrates, when hungry, are stimulated strongly by the presence of food. Such animals, under proper conditions, make a more or less continuous effort to find the way through a simple maze in order to secure food. In such cases food is a very satisfactory stimulus. The larvae of *Ambystoma* are not sufficiently strongly stimulated by the presence of food, even when hungry, to insure a sustained effort to secure it. However, they react strongly to intense light. When exposed to direct sunlight they seek any available shaded area. They are not always as persistent in their efforts to escape the direct rays of the sun as the experimenter might wish. Nevertheless, direct sunlight probably is the best stimulus available for studies of this character on the larvae of *Ambystoma*.

A simple maze (fig. 1), similar to those used by Thorndike and Churchill, was arranged in an aquarium (62x21x23 cm.) with metal walls. In the bottom of the aquarium was a bed of sand approximately 4 cm. in depth. The water over the sand was approximately 6 cm. in depth. The aquarium was placed in direct sunlight so that the entire area of the water except that which fell within the shadow of the end wall toward the sun was exposed to the sun's rays.

Animals which react negatively to intense light commonly retreat from the source of the light. In order to determine whether the larvae of *Ambystoma* would find the shaded area at the end of the aquarium toward the sun as readily as they would a shaded area at the opposite end, individual animals were placed in a transverse position at the center of the aquarium so that they were free either to turn toward or away from the area shaded by the end wall of the aquarium which was toward the sun. The first animal used in this experiment turned toward the shaded area eight times in the first ten trials, and without exception in the next ten trials. This record is typical of the records made by all the animals used in this experiment.

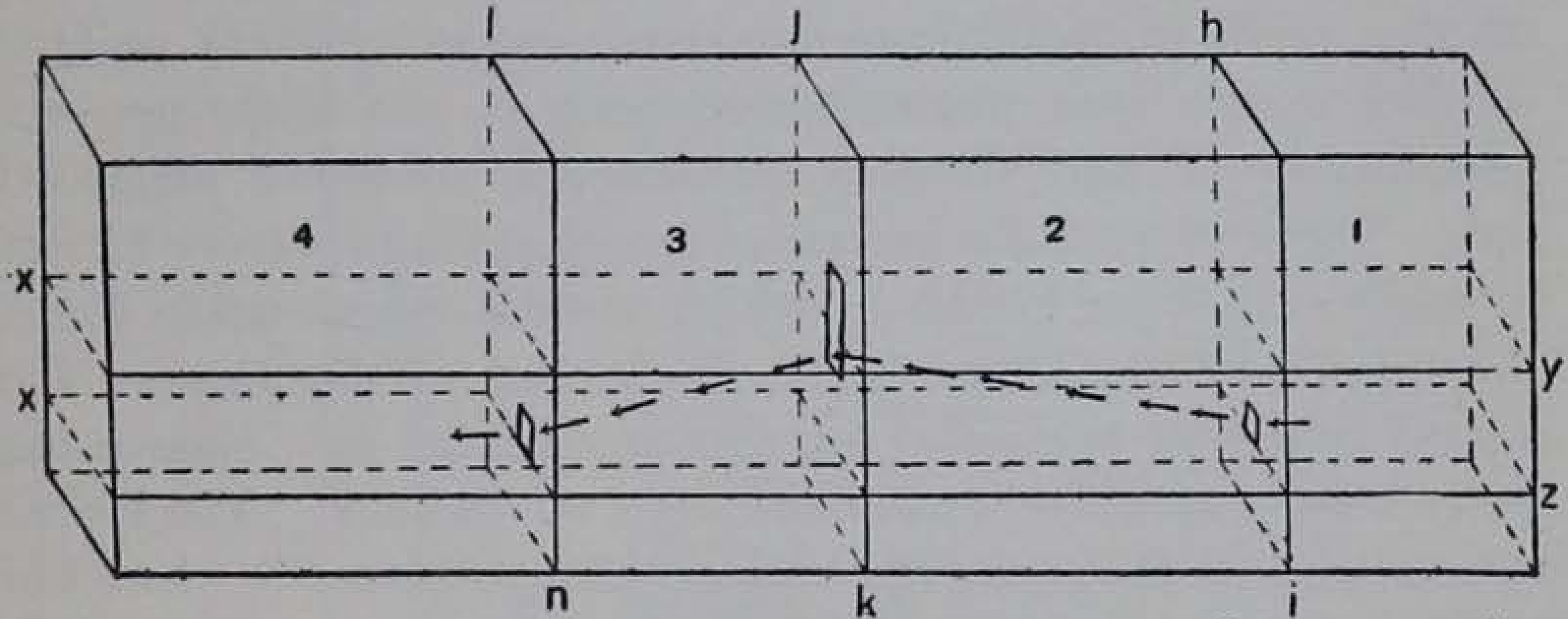

Fig. 1. Diagram of maze with compartments 1, 2, 3, and 4. xz, surface of sand; xy, surface of water; hi, jk, ln, partitions with apertures. Arrows indicate the shortest route through the maze.

Two glass plates 10 cm. wide were now set up near the middle of the aquarium parallel with each other and about 2 cm. apart at right angles to, and in contact with, one of the lateral walls. An animal placed between these glass plates with its head toward the wall would have to move backward before it would be free to turn toward either end of the aquarium. This arrangement would eliminate any directive influence due to manipulation which might have been present in the previous experiment. One of the animals used in the previous experiment when subjected to a series of trials in this manner turned toward the shaded end of the aquarium without exception in the first ten trials. The glass plates were now placed in contact with the opposite wall of the aquarium in the same manner. The same animal was placed between them with its head toward the wall. Whereas in the previous series of trials it was necessary, in order to approach the shaded area, to turn to the left, it was now necessary to turn to the right. Nevertheless, the animal turned toward the shaded area without exception in ten successive trials.

The above experiment indicates clearly that the larvae of *Ambystoma* are capable of finding a shaded area at the end of the aquarium toward the sun quite as readily as at the opposite end; consequently, the maze was arranged in this manner. For the first series of four larvae two partitions of ordinary painted window screen were placed transversely in the aquarium, approximately 20 and 40 cm., respectively, from the unshaded end. The first partition contained a rectangular aperture 2.5 cm. in width, the lower border of which stood approximately 4 cm. above the sand and 2 cm. below the surface of the water. The second partition contained an aperture of 2.5 cm. square, the lower border of which was at the level of the sand. The larvae were placed in the aquarium without the partitions in place until they became accustomed to seeking the shaded area. The partitions were then put in place. Each of the animals in turn was placed in the compartment at the unshaded end of the aquarium and the time noted until it had passed through the apertures in the partitions and reached the shaded area. Each animal was given three successive trials. These larvae were not again placed in the maze until two days later.. Each animal was again placed in the aquarium without the partitions in place and permitted to seek the shaded area three times. The partitions were then replaced and each animal was given three trials to reach the shaded area by passing through the apertures. These exepriments were repeated on five successive days. On the third day one of the larvae had become sluggish by reason of its approaching metamorphosis and was discarded. After this the series included only three animals. Curve AB, fig. 2, is based on the averages of all the trials of all the animals in the series. The ordinates indicate the time in seconds required for the animal to pass from the compartment at the unshaded end of the aquarium through the apertures in the partitions to the shaded area at the opposite end. The abscissae indicate the days on which the trials were made. This curve drops rapidly from 206.5, which is the average of the first series of trials, to

99 which is the average of the third series, although two days intervened between the first and second series of trials. After this the average time interval remains fairly constant until the sixth series of trials which was made on the seventh day, when the experiment was discontinued by reason of the sluggishness of the remaining larvae due to their approaching metamorphosis.

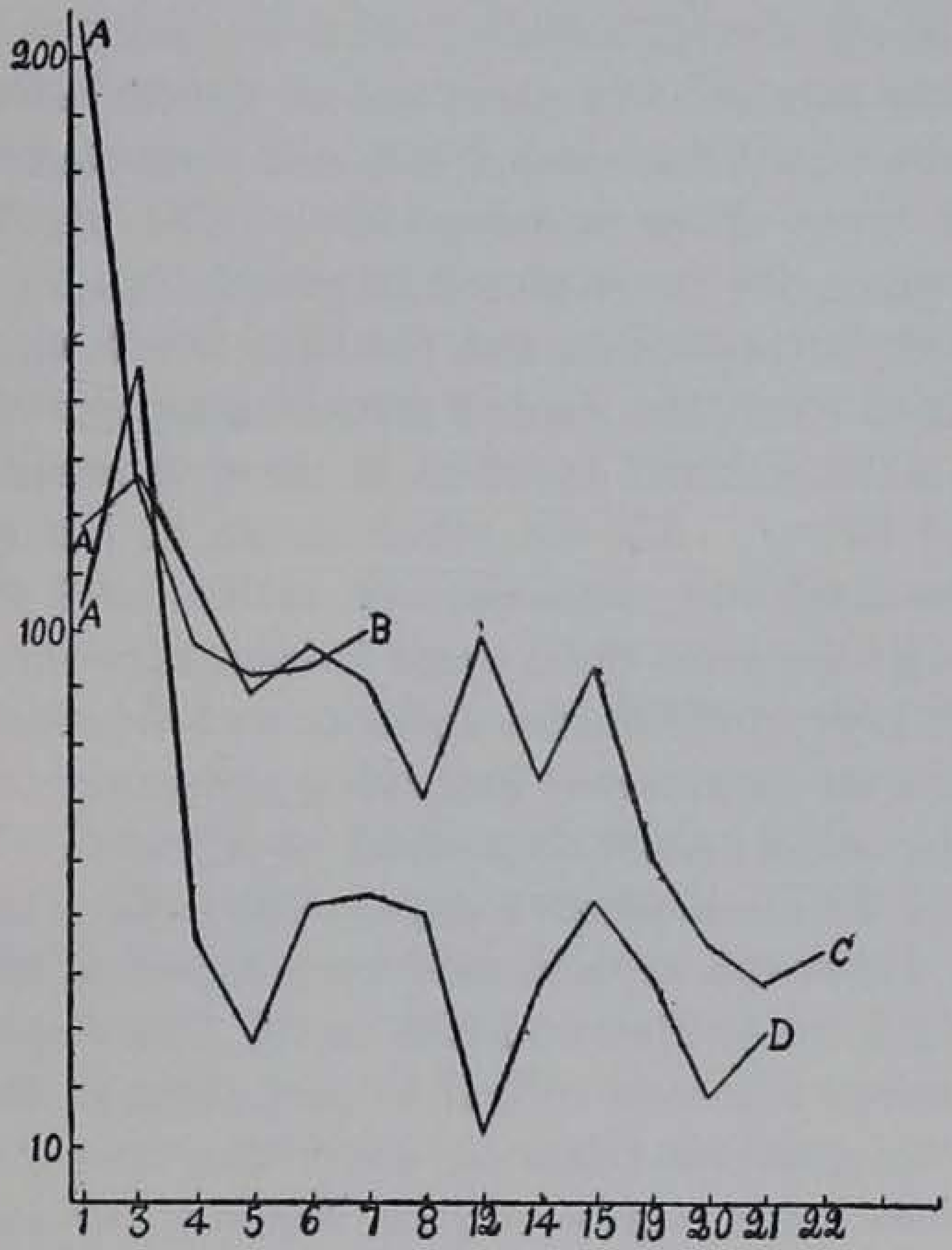

Fig. 2. A B, Curve based on the averages of three trials in successive experiments of all the animals in the first series; A C, Curve based on the averages of the three trials in successive experiments of all the animals in the second series; A D, Curve based on the averages of three trials in successive experiments of larva No. 2 in the second series. The ordinates indicate the time in seconds; the abscissae, the days on which the trials were made.

The second series consisted of six larvae. For this series three partitions of screen were used in the aquarium as illustrated in fig 1. The first (hi), with an aperture at the bottom 2.5 cm. square, and 8.5 cm. from one end, was placed approximately 10 cm. from the unshaded end. The second (jk), with an aperture at the top 2.5 cm. wide and 6 cm. from the other end, so placed that its lower border was 2 cm. below the surface of the water, was placed approximately 15 cm. from the first. The third partition (ln), with an aperture at the bottom 2.5 cm. square and near the middle, was placed just far enough from the end wall of the aquarium toward the sun to include all of the shaded area.

Without the partitions in place one of the larvae was placed in the aquarium at the unshaded end and permitted to seek the shaded area three times in succession. The partitions were then put in place, the larva placed in compartment 1 at the unshaded end of the aquarium, and the time noted which was required for it to reach the shaded area in compartment 4, after it had passed through the aperture in the first partition. This was repeated twice. All the other larvae in the series were treated in the same way; consequently, each animal was allowed to pass through the maze three times in each experiment. These animals were kept in individual aquaria, and individual records were kept for all of them. Following the initial experiment two days intervened before they could be repeated. They were then repeated on six successive days. Three days again intervened after which the experiments were repeated on the 12th, 14th, 15th, 19th, 20th, 21st, and 22d days. The record includes the time required for each animal to pass through the maze in each of three successive trials on every day on which the experiments were made. However, on the 8th day larva No. 5 became sluggish by reason of its approaching metamorphosis and was discarded. On the 19th day larva No. 1 also became sluggish and was discarded. Consequently, after the 8th day the series includes only five, and after the 19th day, only four animals. Curve AC, fig. 2, represents the averages of the three trials of all the animals in the series on the days on which the experiments were made. It indicates a decided drop from the initial interval of 118.3 sec. to 89.9 sec. on the 5th day. After this there is considerable variation in the time interval required, but it does not again rise higher than 99.1 sec. After the 15th day there is again a decided drop until a low point of 39.1 is reached on the 21st day, although three days intervened after the 15th day until the next series of trials was made.

Curve AD, fig. 2, illustrates the record made by a single individual (No. 2 in the second series). From the average initial interval of 105.7 sec. the curve rises to 146 for the second series of trials, but then drops rapidly to 29.3 for the fourth series, and does not again rise above 54 during the successive series of trials and terminates at 30 for the 13th series of trials on the 21st day following the initial series.

These several curves indicate that the late larvae of *Ambystoma tigrinum* are able, by repetition, to form simple habits. The most rapid progress is indicated early in the experiment. The curves drop rapidly until a more or less constant level is reached. Curve AC indicates a second drop following the 15th day; however, the experiment was not continued long enough to determine whether or not a new low level would have been established.

After the 8th day the animals were not again subjected to experiment until the 12th day. The average interval required to pass through the maze on the 12th day was 28.6 seconds longer than the average for the four days preceding the 8th day. Again the experiment was interrupted from the 15th to the 19th day. The average interval on the 19th day was 33.1 seconds shorter than the average interval on the 15th day, and 22.8 seconds shorter than the average for the 14th and 15th days. Therefore, the record affords no evidence that after the first low level was established the animals were less able to accomplish the task set before them after a period of rest of four days than they were at the beginning of that period. Obviously the simple habits formed were retained during this short interval.

Direct observations indicate that the animals gradually became more and more familiar with the maze during the progress of the experiment, and were able to pass through it with appreciably less effort than in the initial trials. Whereas early in the experiment they usually felt their way along the walls of the aquarium and the partitions and passed through the apertures only when they were found in this manner, later some of the animals not infrequently passed through the apertures without feeling their way along the partitions. The aperture in the middle partition, which was 4 cm. above the level of the sand, was found with greater difficulty than the ones in the first and third partitions which were at the level of the sand, because the animals habitually swam near the bottom. There is no evidence that these larvae recognized the apertures by the sense of sight until they were in close proximity to them. Direct observations indicate rather that the animals which did sometimes pass through the apertures without feeling their way along the partitions retained an impression of the general location of the apertures which they had gained by previous experience. The record also indicates that the second and third trials usually resulted in a successful passing through the maze in less time than the first. Curves I, II and III, fig. 3, are based on the average of the first, second and third trials respectively, of all the animals in the second series. While these curves show large variations from day to day, the general level of the second is materially lower than that of the first, and the

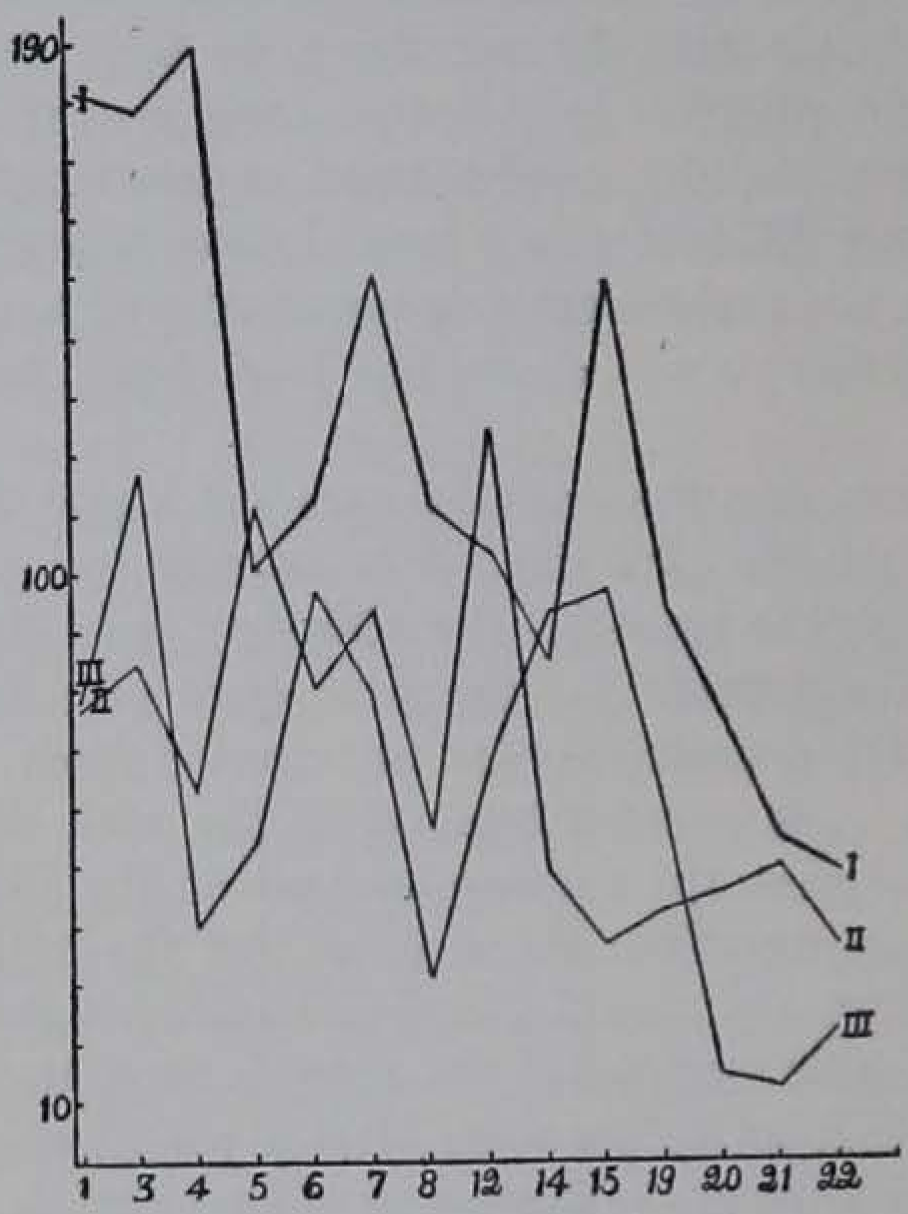

Fig. 3. I, II, and III, curves based on the first, second, and third trials, respectively, of all the animals in the second series. The ordinates indicate the time in seconds; the abscissae, the days on which the trials were made.

general level of the third is somewhat lower than that of the second. The average interval for the first trials of all the animals in ten successive series of trials is 136.2 seconds; while that for the second trials is 80.1 seconds; and that for the third trials is 75.9 seconds. Obviously, the advantage of a second trial immediately following a first is much greater than the advantage of a series of trials following a series on the preceding day.

The results of this study justify the conclusion that the late larvae of *Ambystoma tigrinum* are capable, in at least a low degree, of learning by repetition. The running of a simple maze is accomplished in less time and with appreciably less effort in a second and a third trial than in the first trial immediately preceding. The repeated running of the maze at daily or longer intervals results in the acquisition of simple habits which are retained at least for a short time.

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METAMORPHIC CHANGES IN THE DIGESTIVE SYSTEM IN RANA PIPIENS AND AMBYSTOMA TIGRINUM

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INTRODUCTION

The changes involved in the metamorphosis of the frog are in general well known. The resorption of the tadpole's tail, the gradual elongation of the hind limbs, the emergence of the fore limbs, and the initiation of pulmonary respiration are familiar phenomena. Less conspicuous, though quite as important, are the changes which take place in the digestive tube as the young animal, which during its larval life was essentially herbivorous, becomes prepared for a carnivorous diet. These and other internal changes, and the period of fasting which is coincident with them, also involve material reduction in the size and weight of the body.

Metamorphic changes are less extensive in the Urodela than in the Anura. However, the more important changes which occur in the two orders are similar in character. The resorption of the fin-folds and the gills and the initiation of pulmonary respiration in the Urodela are accompanied by important changes in the digestive tube (although the larvae are not herbivorous) and other internal organs which, with the coincident period of fasting, result in a reduction in the size and weight of the body which is only less marked than in the Anura.

Data regarding the function of the leucocytes in the resorption of tissue and the histological changes involved (Barfurth '87, Griffiths '94, Noetzel '95), as well as quantitative observations on the rate and extent of reduction in the size and weight of the body of the tadpole of the frog during metamorphosis (Schaper '02), are available. The more important histological changes involved in the reduction in the length of the digestive

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tube in tadpoles of the frog have been described (Ratner '91). Data regarding the external changes involved in the metamorphosis of *Ambystoma tigrinum* are also available (Chauvin '76, Powers '03). However, a search of the literature reveals no extensive quantitative data on the extent of the changes undergone by the digestive tube and other internal organs either in an Anuran or an Urodelan species.

The present paper sets forth the results of quantitative observations on the reduction in the length of the digestive tube and associated internal changes in correlation with the reduction in the total weight of the body, and the coincident period of fasting during metamorphosis in *Rana pipiens* and *Ambystoma tigrinum.* It also includes a brief consideration of the histological changes in the stomach and intestine which are correlated with the reduction in the length of the digestive tube.

The greater part of this work was carried out at the Iowa Lakeside Laboratory during the summer of 1921. The writer desires to acknowledge his indebtedness to Prof. R. B. Wylie, Director, for the privileges of the Laboratory and for his interest in furthering the work.

QUANTITATIVE DATA REGARDING METAMORPHIC CHANGES

The animals used in this study were collected in the vicinity of the Laboratory during late July and August. Tadpoles representing various stages of development and young frogs were present in abundance. The tadpoles which were still most immature weighed 3.5 to 4.5 grams and represented a stage of development in which the hind limbs were still functionless and had attained a length of not over 5 mm. Those which had attained their maximum larval size weighed 6.5 to 7.5 grams. The larvae of *Ambystoma* also were present in abundance. Those used in this study were relatively small, but quite uniform. Those which were least advanced in their development had attained a length of 9 to 10 cm. and a weight of 5 to 6 grams. Those which had attained their maximum larval size were 11.5 to 12.5 cm. in length and weighed approximately 12 grams. Larvae which were undergoing metamorphosis were present in considerable abundance. Young adults were taken only in small numbers, but many of the larvae completed their metamorphosis in the laboratory.

When collections were brought into the laboratory the animals were separated into groups representing successive stages of development. The larvae and young adults of *Rana* were arranged in nine groups representing as many stages of development, which may be indicated as follows:

Stage 1. Fore limbs concealed, hind limbs not over 5 mm. in length.
Stage 2. Fore limbs concealed, hind limbs 10 to 15 mm. in length.
Stage 3. Fore limbs concealed, hind limbs 30 mm. or over in length.
Stage 4. Fore limbs free, tail not reduced.
Stage 5. Tail reduced to approximately one half maximum size.
Stage 6. Tail almost completely resorbed.
Stage 7. Small frogs just emerged from the water.
Stage 8. Young frogs which had just resumed feeding.
Stage 9. Young frogs actively feeding.

The larvae and young adults of *Ambystoma* were also arranged in nine groups representing as many successive stages of development. The earlier stages in this series can not be characterized as definitely as those in the series of *Rana*. The successive stages may be indicated as follows:

Stage 1. Larvae 9 to 10 cm. in length; weight 5 to 6 grams.
Stage 2. Larvae 10 to 11 cm. in length; weight 7 to 8 grams.
Stage 3. Larvae of maximum size, gills not reduced.
Stage 4. Larvae with resorption of gills and changes in coloration initiated.
Stage 5. Larvae with gills not over half their maximum length, changes in coloration advanced.
Stage 6. Larvae with gills almost completely resorbed.
Stage 7. Metamorphosis apparently complete.
Stage 8. Young adults about to resume feeding.
Stage 9. Young adults actively feeding.

The curves in the accompanying figures are based on the averages of weight determinations and measurements made on specimens selected from the groups representing the successive stages of development. Two series of groups of five spcimens each were used in the case of each species.

Curve A B. fig. 1, illustrates the changes in the total weight of the larvae of *Rana pipiens* as they approach and pass through the period of metamorphosis. It indicates a relatively rapid increase in weight until the maximum larval weight is attained, then an abrupt decrease from an average maximum of 6.8 to an average minimum of 2.9 grams, or a total reduction of 57.3 per cent. This curve corresponds very closely to Schaper's ('02) curve which illustrates the changes in the total weight of *Rana fusca* during the corresponding period. Curve AE, fig. 2, illustrates the changes in the total length of the stomach and intestine during the same period. This curve also shows a rapid rise until the digestive tube has attained its maximum length; then an abrupt decline. It indicates a reduction from an average maximum of 51 to an average minimum of 6 cm., or a total reduction of 88.2 per cent.

Curve AF, fig. 2, illustrates the changes in the length of the stomach alone. This curve drops abruptly until its low point is reached only a little later than the high point in curve AE. It indicates a reduction from an average maximum of 11 mm. to an average minimum of 5 mm., or a total reduction of 54.5 per cent, which occurs somewhat earlier than the reduction in the length of the intestine and is followed immediately by rapid growth both in length and capacity. Curve AC, fig. 1, illustrates the changes in the total weight of the stomach and intestine with contents. This curve is based on the average weight determinations multiplied by 5. It shows a rapid rise until the digestive tube has attained its maximum length; then an abrupt decline. It indicates a reduction in weight from an average maximum of 1.4 to an average minimum of .1 gram, or a total reduction of 92.8 per cent. Curve AD, fig. 1, illustrates the corresponding changes in the weight of the liver. It also is based on the average weight determinations multiplied by 5. This curve indicates a reduction in the weight of the liver from an average maximum of 0.3 to an average minimum of .06 gram, or a total reduction of 80 per cent. The pancreas also undergoes material reduction in weight which is initiated somewhat earlier than the reduction in the length of the digestive tube and the weight of the liver. It also undergoes changes in form and in its position with respect to the liver and the duodenum. By reason of the small size of the pancreas no attempt was made to illustrate its changes by means of curves.

The curves representing respectively the length of the stomach and intestine and the total weight of these organs with contents in the larvae of *Rana pipiens* reach their highest point at approximately the same time. The curve representing the total weight of the animals reaches its highest point a little later. Obviously, the larvae cease to feed before resorption of the tail is initiated. The period of fasting continues until the metamorphic changes are complete and the little frogs have emerged from the water. When the larvae cease feeding the stomach and intestine are well filled. The ingested material, therefore, accounts for the greater part of the total weight of these organs with their contents when the curve reaches the highest point. As the metamorphic changes advance the contents of the digestive tube are gradually eliminated until the residue left in the large intestine becomes almost negligible. Therefore, the lowest point in the curve represents approximately the weight of the stomach and the intestine without contents. Many of the little frogs which were collected in the grass after they had emerged from the water were still without food in their stomachs. Obviously, feeding is not resumed until at least a short time after the little frogs emerge. Larvae which had attained their maximum size when they were brought into the laboratory, and in which resorption of the tail was initiated soon after, were completely metamorphosed nine to ten days later. Therefore, it may be assumed that the period of fasting is of at least ten days' duration. This is probably a fairly accurate estimate of the interval required for the completion of the metamorphic changes in this species. It corresponds closely with the interval required by the metamorphic changes in *R. fusca* as determined by Schaper's observations on larvae which were reared in the laboratory.

The liver does not reach its maximum weight until the resorption of the tail and the digestive tube are well advanced. It then decreases rapidly until it reaches the minimum weight about the time metamorphosis is completed.

Curve A′B′, fig. 1, illustrates the changes in the total weight of the larvae of *Ambystoma tigrinum* as they approach and pass through the period of their metamorphosis. This curve shows a rapid rise until its highest point is reached; then an abrupt decline. It indicates a reduction in weight from an average maximum of 12.2 to an average minimum of 8.7 grams, or a total reduction of 28.7 per cent. Curve A′E′, fig. 2, illustrates the changes in the total length of the stomach and intestine during the same period. It also shows a rapid rise until its highest point is reached; then an abrupt decline. It indicates a reduction in length from an average maximum of 22.3 to an average minimum of 12.1 cm, or a total reduction of 45.8 per cent. Curve A′F′, fig. 2, illustrates the changes in the length of the stomach alone. Like the preceding curve, it shows a rapid rise until its highest point is reached; then an abrupt decline. It indicates a reduction in length from an average maximum of 31 mm. to an average minimum of 14 mm., or a total reduction of 54.8 per cent. Curve A′C′, fig. 1, illustrates the corresponding changes in the weight of the stomach and intestine with contents. It is based on the averages of the weight determinations multiplied by 5. It indicates a reduction from an average maximum of 1.02 to an average minimum of .32 grams, or a total reduction of 68.6 per cent. Curve A′D′, fig. 1, illustrates the changes in the weight of the liver during the period of metamorphosis. It also is based on the averages of the weight determinations multiplied by 5. This curve indicates a gradual increase in the weight of the liver throughout the period of metamorphosis. The pancreas in the larvae of *Ambystoma* is relatively small and, like the liver, probably undergoes no reduction in weight during metamorphosis.

The curves representing the total body weight and the weight of the stomach and intestine in the larvae of *Ambystoma tigrinum*, with contents, reach their highest point at the same time. The curve representing the length of the stomach and intestine reaches its highest point a little later. Obviously, the larvae of this species cease feeding before the digestive tube has attained its maximum length. At this time the stomach and intestine are well filled. As the metamorphic changes advance, the contents of the digestive tube are gradually eliminated until the residue of the undigested matter becomes almost negligible. The major portion of the weight indicated by the high point in this curve is made up by the ingested matter. The weight indicated by the low point is approximately the weight of the stomach and intestine without contents.

The period of fasting continues until metamorphosis is complete. Larvae in which metamorphic changes were obviously initiated, but whose fins and gills were still of approximately maximum size when they were brought into the laboratory, emerged as young adults and resumed feeding nine days later. This is probably a fair estimate of the interval required for the completion of a metamorphosis in the animals under observation. *Ambystoma tigrinum* is an exceedingly variable species. While the Ambystoma population of any given kettle-hole was relatively uniform, specimens taken from various kettle-holes in the vicinity showed wide variations in size. While it is quite probable that metamorphosis requires no longer interval in the larger than in the small varieties, the data at hand do not justify the conclusion that the interval of approximately nine days here indicated is the average interval for the species. Powers' ('03) observations indicate an interval considerably shorter than nine days.

The morphological changes involved are far less extensive in *Ambystoma* than in *Rana*. The difference in the extent of reduction in the length of the digestive tube and the changes in the size of the liver in these two types of Amphibia, doubtless are correlated with their habits of feeding. The larvae of *Rana* are primarily herbivorous while those of *Ambystoma* are essentially carnivorous. The relatively enormous length of the digestive tube in the larvae of *Rana* is an adaptation correlated with their herbivorous diet. The great reduction in the length of the digestive tube during metamorphosis is an essential part of the adjustment of the little frog to a carnivorous diet. The lesser reduction in the length of the digestive tube during metamorphosis in *Ambystoma*, doubtless must be explained on the same basis. While the larvae of *Ambystoma* are essentially carnivorous they are not strictly limited in their diet. Those used in this study were taken in a habitat in which there was very little vegetation. Their food consisted primarily of aquatic insects and insect larvae. However, the contents of the digestive tube included some mud. Larvae of *Ambystoma* taken in another habitat in which algae were growing in abundance were found to be feeding on these plants extensively. Their stomach contents consisted almost exclusively of Entomostraca and minute algae. Doubtless, the diet of the adults of *Ambystoma tigrinum* is much more strictly carnivorous. Therefore, the reduction in the length of the digestive tube during metamorphosis is probably an essential part of the adjustment of the animals to the more restricted carnivorous diet. The relatively smaller size of the liver in *Ambystoma* than in *Rana* during larval life is probably correlated with the lower carbohydrate content in the diet. Inasmuch as the liver does not assume larger proportions in the larvae of this species, an actual reduction in the size of this organ need not take place during metamorphosis.

HISTOLOGICAL CHANGES IN THE STOMACH AND INTESTINE DURING METAMORPHOSIS

The histological structure of the digestive tube of certain types of Amphibia, especially the frog, is adequately described. The histological changes in the stomach and intestine of *Rana temporaria* which are correlated with the reduction in the length of the digestive tube during metamorphosis, were set forth by Ratner ('91). The purpose of the present histological study is to extend our knowledge of the structural changes in the stomach and intestine which are correlated with the reduction in the length of the digestive tube during metamorphosis in *Rana*, and to compare with these changes the corresponding structural changes in the stomach and intestine of an Urodelan species in which the reduction in the length of the digestive tube during metamorphosis is less extensive than in the Anura.

The accompanying microphotographs illustrating the histological structure of the stomach and intestine were all taken under the same magnification, viz., 165 diameters. If the digestive tube had been equally distended in every case the thickness of the corresponding layers in the successive stages could be compared directly. Inasmuch as the digestive tube was still well filled with ingested material during the two earlier stages of *Rana pipiens* illustrated (figs. 3, 4, 8, 9), it may be assumed that in these cases the corresponding portions of the digestive tubes were distended to approximately the same degree. On the other hand, the digestive tube was practically empty during the two later stages of *Rana* illustrated (figs. 5, 6, 10, 11). Consequently, the corresponding portions were contracted to approximately the same degree. Therefore, in comparing the thickness of the several layers of the wall of the digestive tube in the earlier and later stages, allowance must be made for the difference in the state of contraction. The sections of the intestine of the larvae of *Ambystoma tigrinum* illustrated (figs. 12, 13) were taken from portions of the intestine which were contracted in approximately the same degree. Consequently, the corresponding layers in the two stages illustrated may be compared directly.

The reduction in the length and capacity of the stomach during metamorphosis in *Rana* is less extensive than that of the intestine. Nevertheless, the structural changes in the stomach are well marked. In tadpoles which are still growing and whose digestive tube has not yet reached its maximum length, the walls of the stomach and intestine remain relatively thin and delicate. As pointed out in an earlier section of this paper the reduction in length occurs somewhat earlier in the stomach than in the intestine. The gastric glands arise earliest at the pyloric end and gradually advance toward the cardiac end of the stomach. Glands are not present in the most anterior portion of the stomach until shortly before this organ has attained its maximum larval length, which occurs in tadpoles with hind limbs 5 to 15 mm. in length. Figure 3 is a microphotographic reproduction of a portion of a transverse section of the stomach of a tadpole in which the process of reduction in the length of the stomach was already initiated. The serosa and muscularis are very thin. Longitudinal muscle fibers are not apparent. The muscularis is, therefore, represented by relatively few circular fibers. The deep portions of the gastric glands which are still loosely aggregated come into very close proximity with the muscularis. The submucosa is represented by a small amount of loose connective tissue which lies between the glands. As the process of reduction advances the serosa and muscularis of the stomach become appreciably thicker. A subserous layer of connective tissue makes its appearance, but longitudinal muscle fibers are still absent. The existence of longitudinal muscle in the stomach of the frog has been denied by certain observers (Valatour; P. Schultze). On the other hand, Ratner states that few longitudinal muscle fibers are present even during the earlier metamorphic changes. The present study warrants the conclusion that longitudinal muscle fibers are present in the muscularis of the stomach during metamorphosis. The submucous connective tissue also becomes more abundant and the gastric glands become arranged more compactly. An advanced stage in the progress of these changes is illustrated microphotographically in figure 4, which is taken from a transverse section of the stomach of a tadpole in which this organ had almost reached its maximum size during metamorphosis. Figure 5 illustrates microphotographically a portion of a transverse section of the stomach of a tadpole in which the growth of this organ after reduction was initiated. All the layers except the mucosa are relatively thicker than in the preceding stage. The muscularis is now represented by a thick layer of compactly arranged fibers. The submucosa consists of a well defined layer of connective tissue between the muscularis and the deep portions of the gastric glands. The latter are short and more compactly arranged than in the preceding stages. Figure 6 illustrates microphotographically a portion of a transverse section of the stomach of a young frog recently emerged from the water. The muscularis is not proportionately thicker than in the preceding stage. The submucosa is somewhat thicker and more fibrous. The gastric glands are quite fully developed. In short, the stomach now shows the histological structure of the stomach of the adult frog.

The small intestine of the frog tadpole is arranged in a characteristic double spiral coil which, while the tadpole is growing and actively feeding, causes marked distension of the abdomen. As the tadpole undergoes metamorphosis and the intestine becomes shorter, the spiral coil becomes smaller and finally gives way to the arrangement of the small intestine in the adult frog. Several stages in the reduction of the intestinal coil are illustrated photographically in figure 7. Until the process of shortening sets in, the wall of the intestine is extremely thin and fragile. It consists of little more than the thin serous and muscular layers and the mucous epithelium which, while the intestine is moderately filled with ingested material, is closely applied to the muscularis with but little connective tissue intervening. This condition is illustrated microphotographically in figure 8 which is taken from a transverse section of the small intestine of a tadpole in which approximately 50 per cent of the reduction in the length of the digestive tube had taken place. The intestinal wall is now appreciably thicker than in the preceding stage. The added thickness is made up largely of the increase in the thickness of the muscularis and the submucosa. As the shortening of the digestive tube progresses the intestinal wall becomes thicker, and all the tissues become more compact. Figure 10 illustrates a portion of a transverse section of the small intestine of a tadpole in which the digestive tube had reached its minimum length during metamorphosis. The small intestine is now practically without food content and its caliber is greatly reduced by reason of the contraction of the circular muscles. Consequently, the mucosa and submucosa are thrown into large longitudinal folds. The thickness of the several layers, as indicated by this and the preceding figure, may not be compared directly by reason of the difference in the caliber of the intestine. Nevertheless, figure 10 indicates a relatively enormous increase in the thickness of all the layers except the mucosa. As the metamorphic changes advance to completion and the little frog resumes feeding the muscularis and the submucosa become relatively somewhat thinner and the mucosa is thrown into numerous folds. As illustrated in figure 11, which is taken from a transverse section of the small intestine of a young frog about the time feeding is normally resumed, the histological structure is now essentially that of the small intestine of the adult frog.

The most marked changes in the histological structure of the stomach and intestine in *Rana* during metamorphosis, as noted above, consist in the increased thickness of the several layers, especially the muscularis and submucosa, the more compact arrangement of the gastric glands and the folding of the intestinal epithelium. These changes are coincident with the reduction in the length and caliber of the digestive tube and are accomplished in a relatively short time. Obviously, the increase in the thickness of the several layers in the walls of the stomach and intestine is not accomplished by prolification of the tissue elements, but by rearrangement of those already present. As pointed out by Ratner, mitotic figures occur only rarely in these tissues during the period of metamorphosis. Furthermore, the increase in the volume of the tissue per unit length of the tube occurs so rapidly that it could not be accounted for by the prolification of cellular elements alone. Neither is there any evidence of degeneration or destruction of tissue in the walls of the digestive tube while the process of shortening is going on, as would necessarily be the case if the tissue elements did not become rearranged and aggregated. By what mechanical process the shortening of the tube and the rearrangement of the tissue elements is accomplished is not clear. As suggested by Ratner, the aggregation of the muscle tissue is the important factor. The subserosa and submucosa necessarily become thicker and more compact in response to the changes in the muscularis. Likewise the epithelium becomes more compact and, with the submucosa, is thrown into numerous folds as the tube is reduced in length and caliber.

The subserous and submucous connective tissue shows more evidence of normal growth changes during the metamorphosis than any of the other tissues in the walls of the stomach and intestine. At the beginning of metamorphosis the connective tissue in these layers is not only very meager but also contains very few fibers. As metamorphosis advances, this connective tissue becomes more fibrous and the number of cellular elements is materially increased. After the metamorphic changes are completed and the digestive tube resumes growth this connective tissue soon assumes the character of the connective tissue in the digestive tube of the adult frog. The difference in the submucosa of the stomach during the later stages of metamorphosis and in the recently emerged frog is well illustrated in figures 5 and 6.

The histological changes in the stomach and intestine of *R. pipiens* during metamorphosis, as described in this paper, conform in general to the corresponding changes in *R. temporaria* as described by Ratner.

As indicated in an earlier section of this paper the reduction in the length of the digestive tube during metamorphosis is much less extensive in the Urodela than in the Anura. Consequently, the changes in the histological structure of the stomach and the intestine which are correlated and coincident with the reduction in the length of the digestive tube, though similar in character, are less extensive in *Ambystoma tigrinum* than in *Rana pipiens*. The walls of the stomach and intestine in the larvae of the former are never as thin and fragile as in those of the latter species at the corresponding stage of development. As the larvae of *Ambystoma* undergo metamorphosis and the digestive tube becomes shorter, the walls of the stomach and intestine also increase somewhat in thickness. However, the changes in the layers are much less marked than the corresponding changes in the larvae of *Rana*. Figure 12 illustrates microphotographically a portion of a transverse section of the small intestine of a larva of *Ambystoma* in which the reduction in the length of the digestive tube was well advanced. Figure 13 illustrates a portion of a transverse section of the small intestine of a young adult *Ambystoma* which had not yet resumed feeding. The intestine was contracted to approximately the same degree in both cases and the sections were taken at corresponding levels. The difference in the thickness of the corresponding layers in these two stages is unimportant. Obviously, the tissues are much more compact in the latter than in the former. The submucosa is still very loose and contains relatively few fibers in the former, whereas in the latter it is more compact and more fibrous. Furthermore, during the later stages of metamorphosis the mucosa is thrown into a few large folds, while after metamorphosis the folds of the mucosa are smaller, more definite and more numerous. The structure illustrated in figure 13 is essentially the structure of the intestine of the adult. Inasmuch as the histological changes in the stomach and intestine of *A. tigrinum* during metamorphosis, though less extensive, are similar in character to the corresponding changes in *R. pipiens* a more detailed description of them at this time would be superfluous. There is no more evidence that the increase in the thickness of the several layers in the walls of the stomach and intestine during metamorphosis involves active cell proliferation in *Ambystoma* than in *Rana*. On the other hand, all the facts observed indicate that the increase in thickness is accomplished by rearrangement and aggregation of the elements already present.

SUMMARY

The period of metamorphosis and the coincident period of fasting occupy approximately ten days in *Rana pipiens* and nine days or less in *Ambystoma tigrinum.*

Quantitative data presented indicate the following changes during metamorphosis:

	R. pipiens	A. tigrinum
Average reduction in total weight	57.3 per cent	28.7per cent
Average reduction in length of stomach and intestine	82.2 per cent	45.8 per cent
Average reduction in length of stomach	54.5 per cent	54.8 per cent
Average reduction in weight of stomach and intestine with contents	92.8 per cent	68.6 per cent
Average reduction in weight of liver	80 per cent	none

The more important histological changes in the stomach and intestine during metamorphosis consist in increasing thickness of the several layers and more compact aggregation of the tissue elements as the reduction in the length of the digestive tube takes place. The increase in the thickness of the several layers does not involve active cell proliferation, but is accomplished by rearrangement and aggregation of the elements already present.

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PLATE I

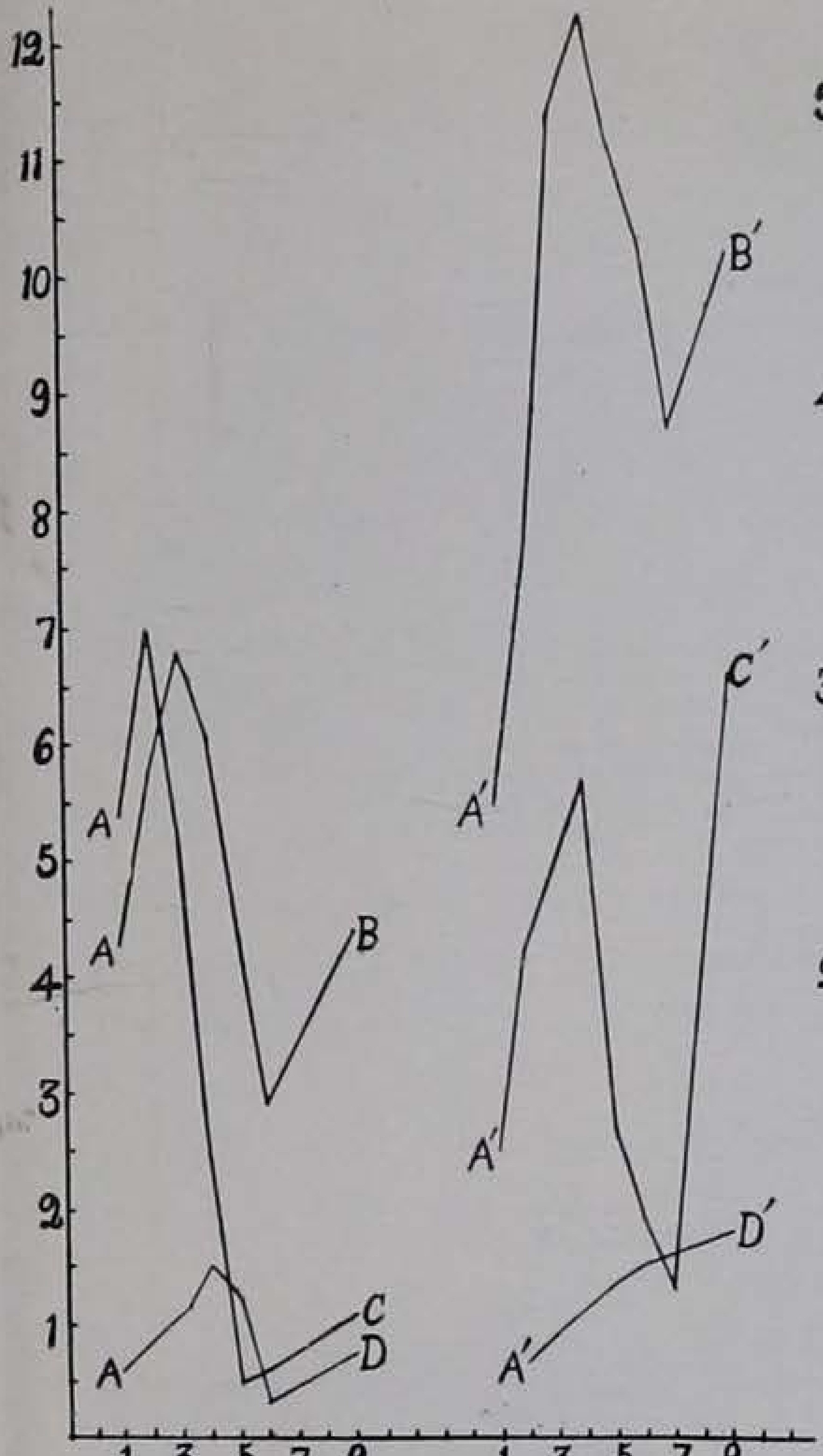


Fig. 1

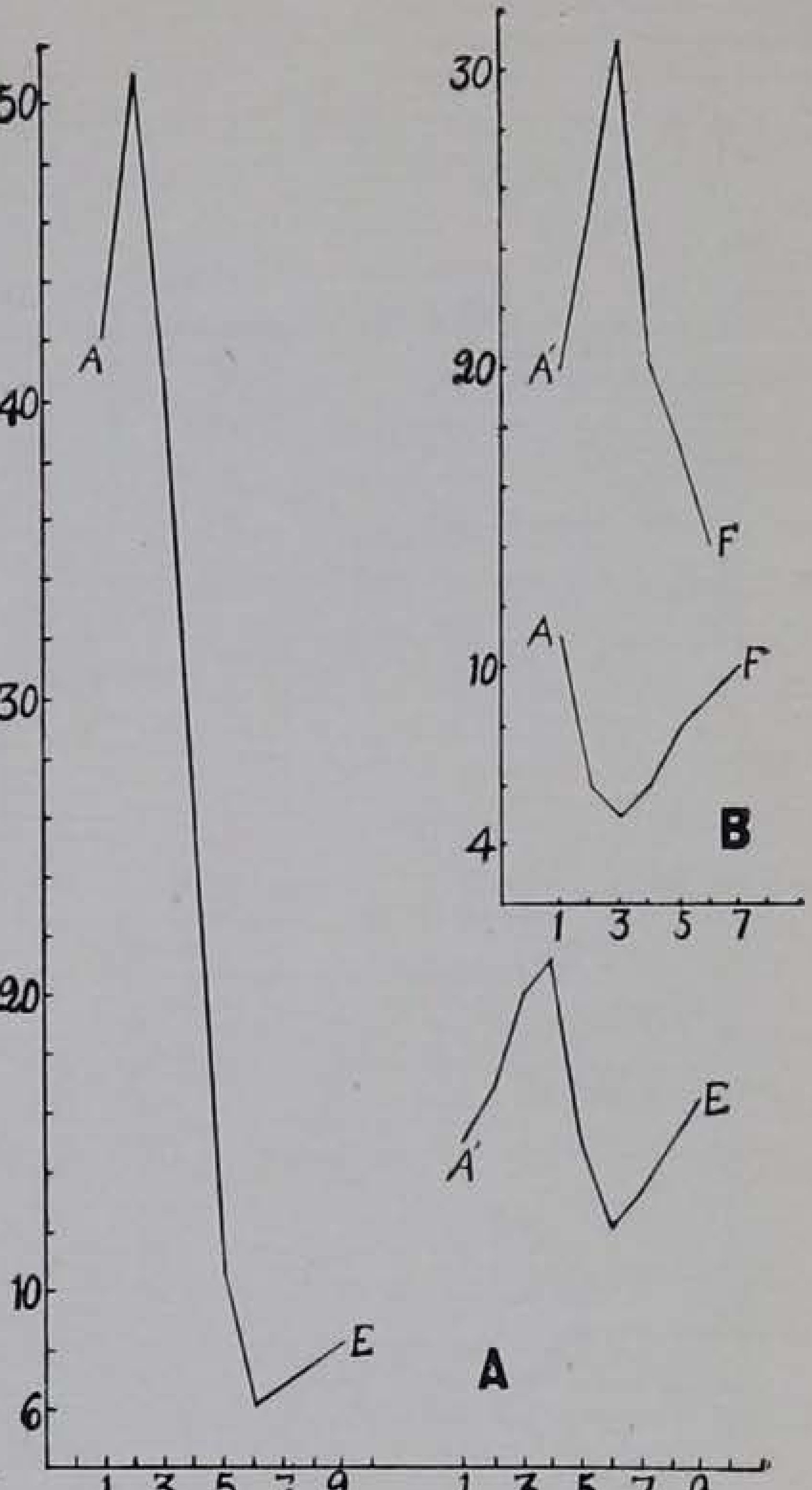


Fig. 2

Fig. 1. Curves illustrating changes in weight during metamorphosis. The ordinates indicate weight in grams; the abscissae, stages in development as defined in the text. AB, Curve of total weight of *Rana pipiens;* A'B', Curve of total weight of *Ambystoma tigrinum;* AC, Curve of weight of stomach and intestine with contents in *R. pipiens;* A'C', Curve of weight of stomach and intestine with contents in *A. tigrinum; AD,* Curve of weight of liver in *R, pipiens;* A'D', Curve of weight of liver in *A. tigrinum*

Fig. 2. Curves illustrating changes in length of stomach and intestine during metamorphosis. The ordinates indicate length in cm. in A. and in mm. in B; the abscissae, stages in development as defined in the text. AE, Curve of length of stomach and intestine in *R. pipiens;* A'E', Curve of length of stomach and intestine in *A. tigrinum;* AF, Curve of length of stomach in *R. pipiens;* A'F', Curve of length of stomach in *A. tigrinum*

PLATE II

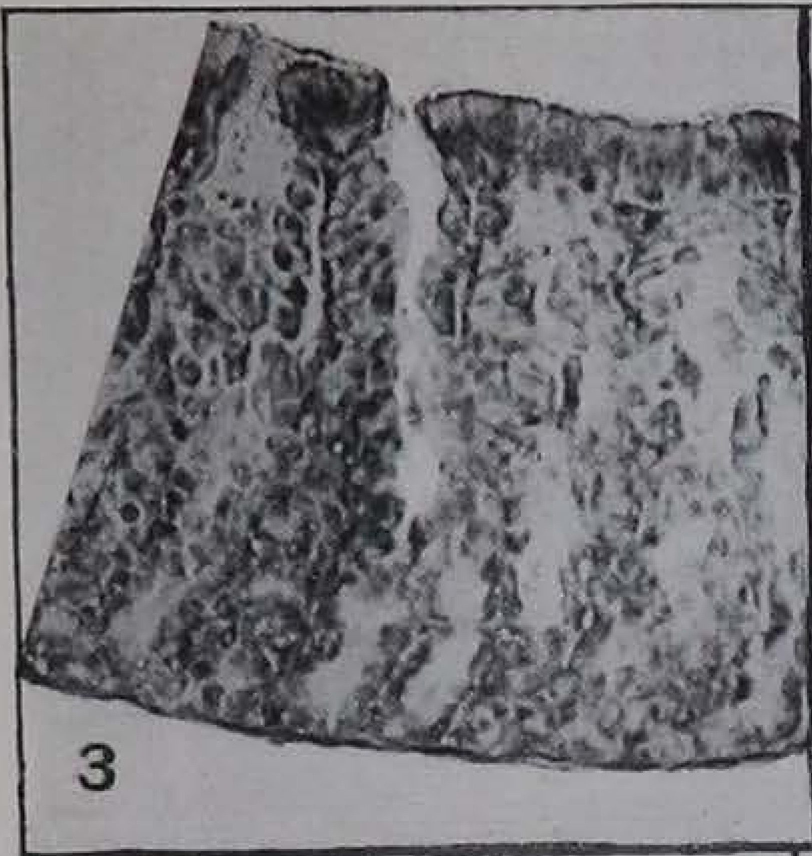
Fig. 3. Microphotograph (x165) from a transverse section of the stomach of a tadpole in which the reduction in the length of this organ was initiated

Fig. 4. Microphotograph (x165) from a transverse section of the stomach of a tadpole in which the reduction in the length of this organ was well advanced

Fig. 5. Microphotograph (x165) from a transverse section of the stomach of a tadpole in which the growth of this organ after reduction was initiated

Fig. 6. Microphotograph (x165) from a transverse section of the stomach of a young frog about the time feeding is resumed

PLATE II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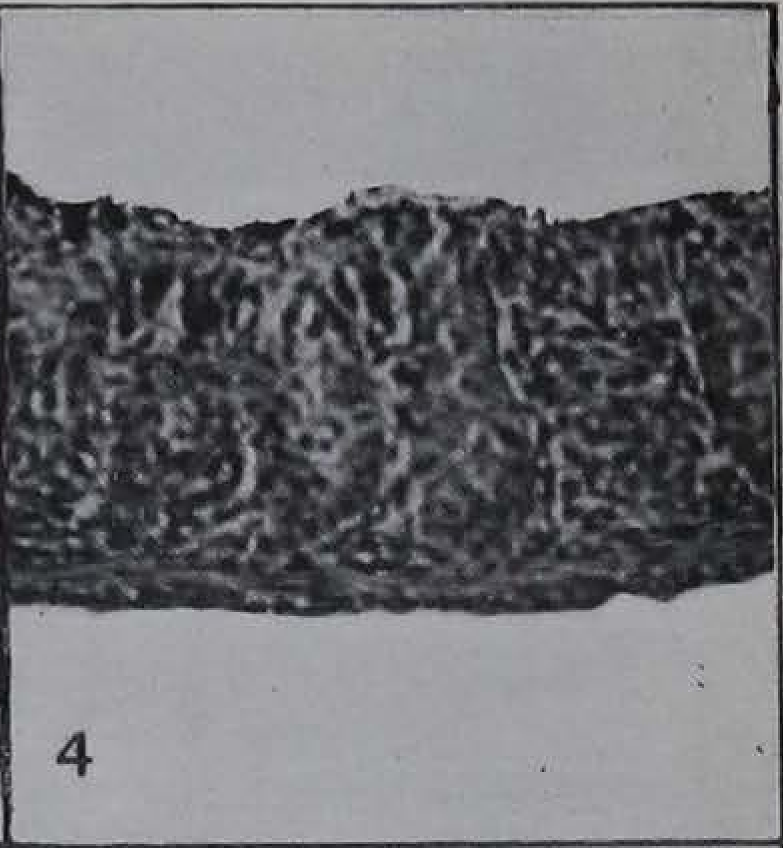
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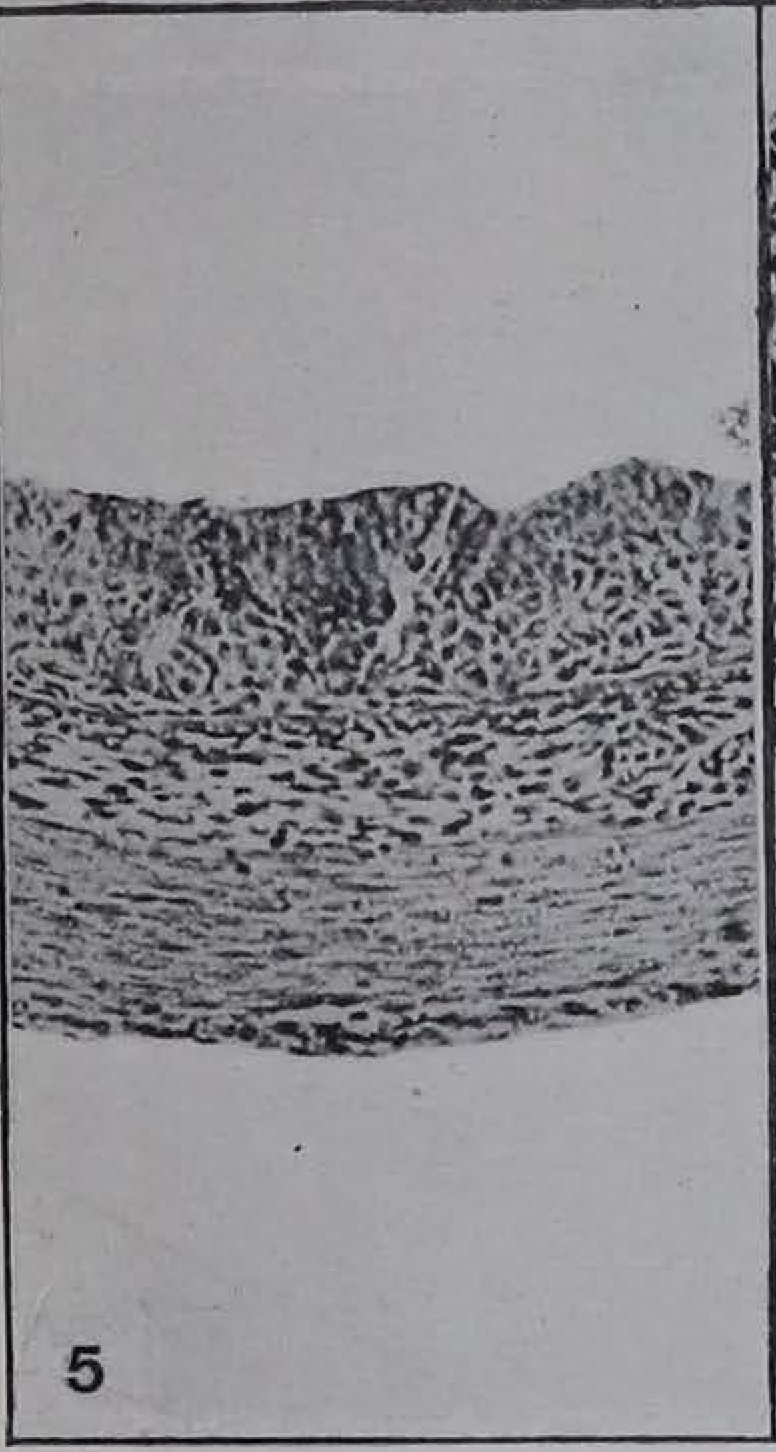
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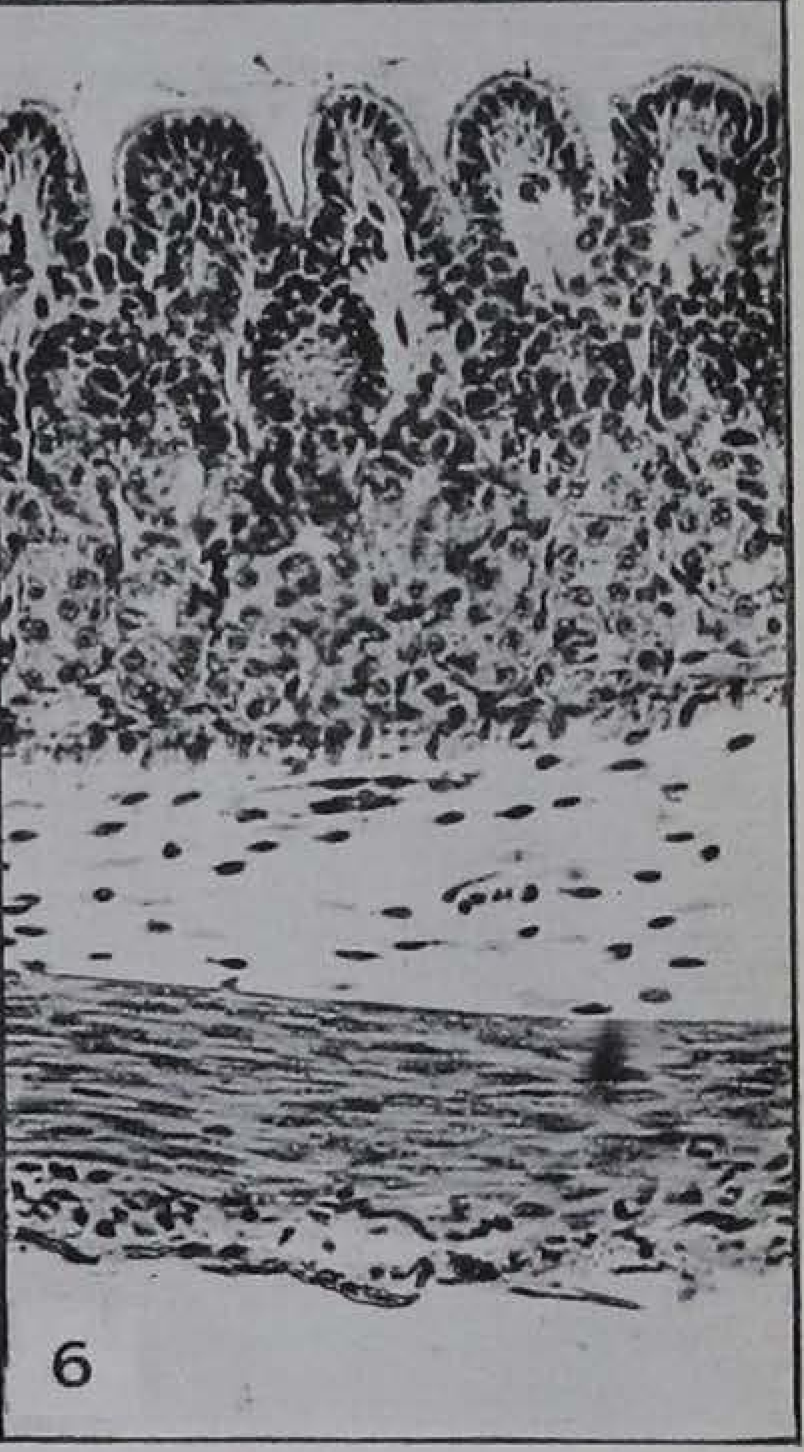
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PLATE III

Fig. 7. Photograph illustrating successive stages in the reduction of the intestinal spiral coil in tadpoles during metamorphosis

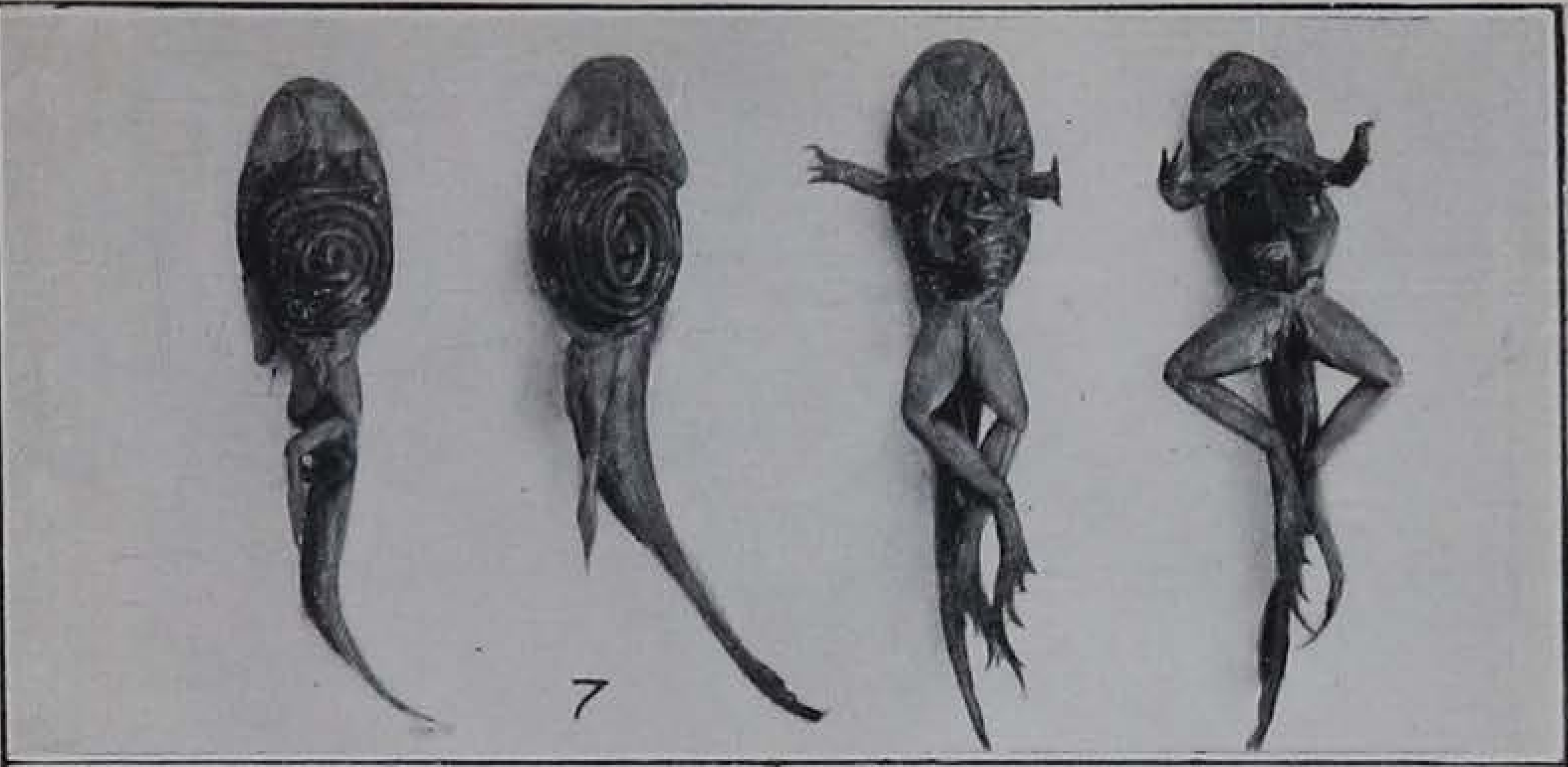
Fig. 8. Microphotograph (x165) from a transverse section of the small intestine of a tadpole in which the digestive tube had nearly reached its maximum length

Fig. 9. Microphotograph (x165) from a transverse section of the small intestine of a tadpole in which approximately 50 per cent of the reduction in the length of the digestive tube had taken place

Fig. 10. Microphotograph (x165) from a transverse section of the small intestine of a tadpole in which the digestive tube had practically reached its minimum length during metamorphosis

Fig. 11. Microphotograph (x165) from a transverse section of the small intestine of a young frog about the time feeding is resumed

PLATE III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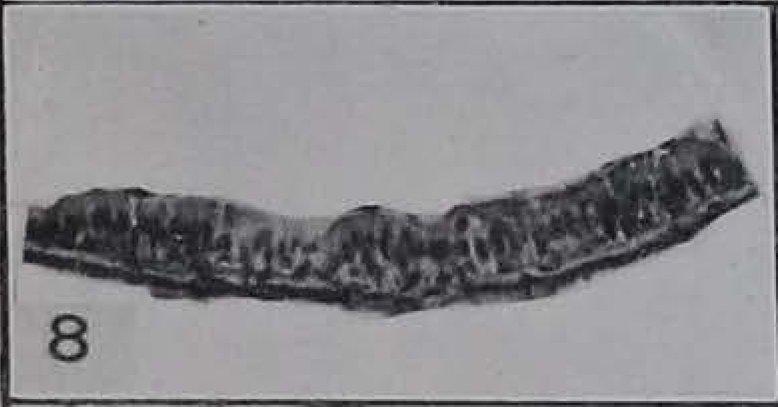
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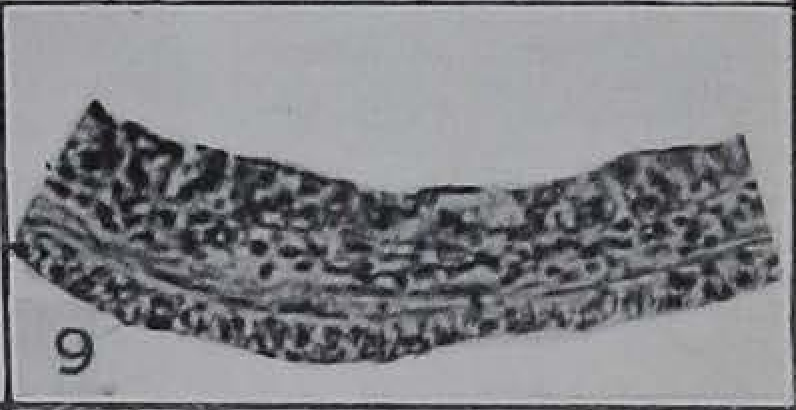
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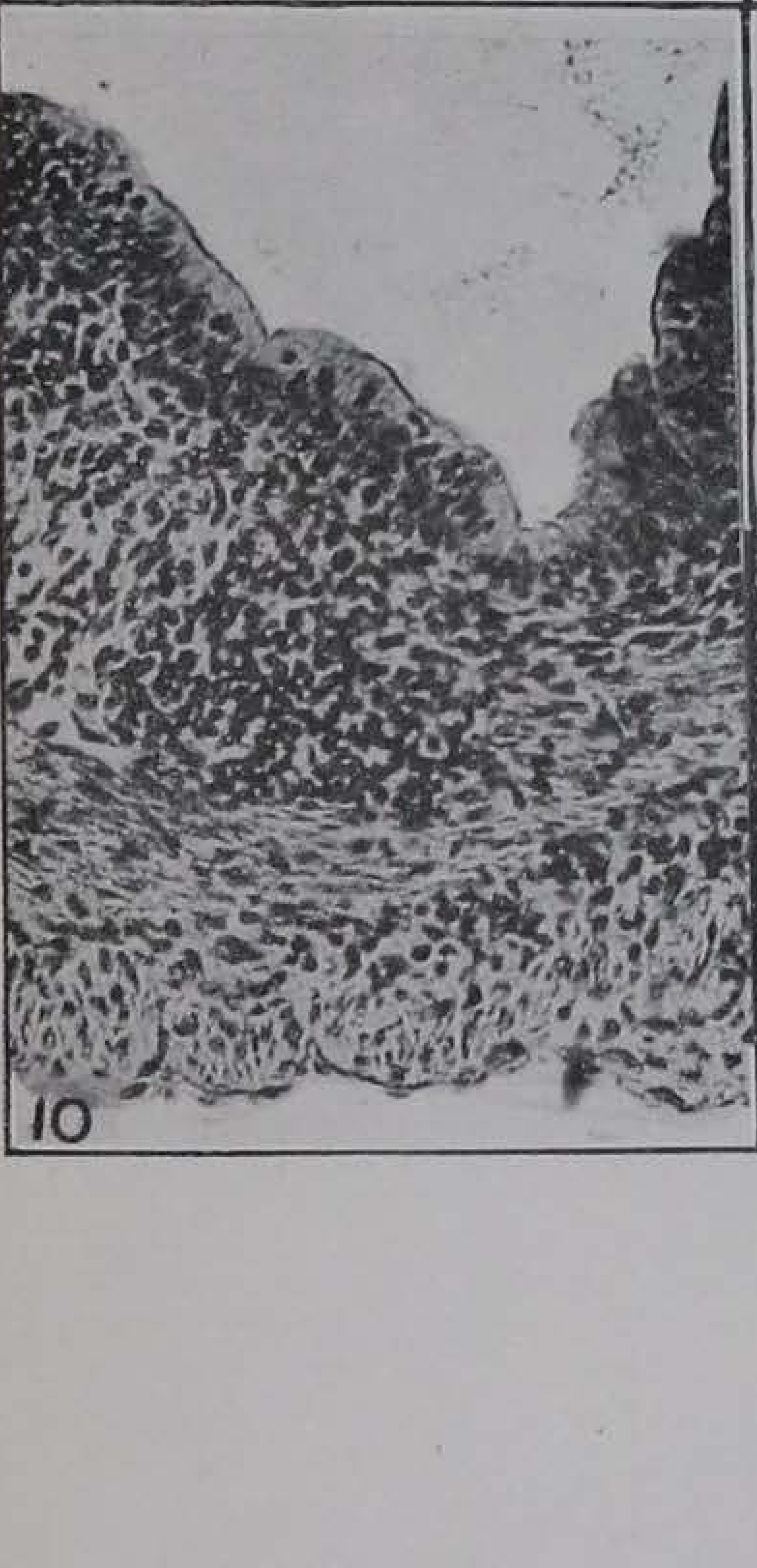
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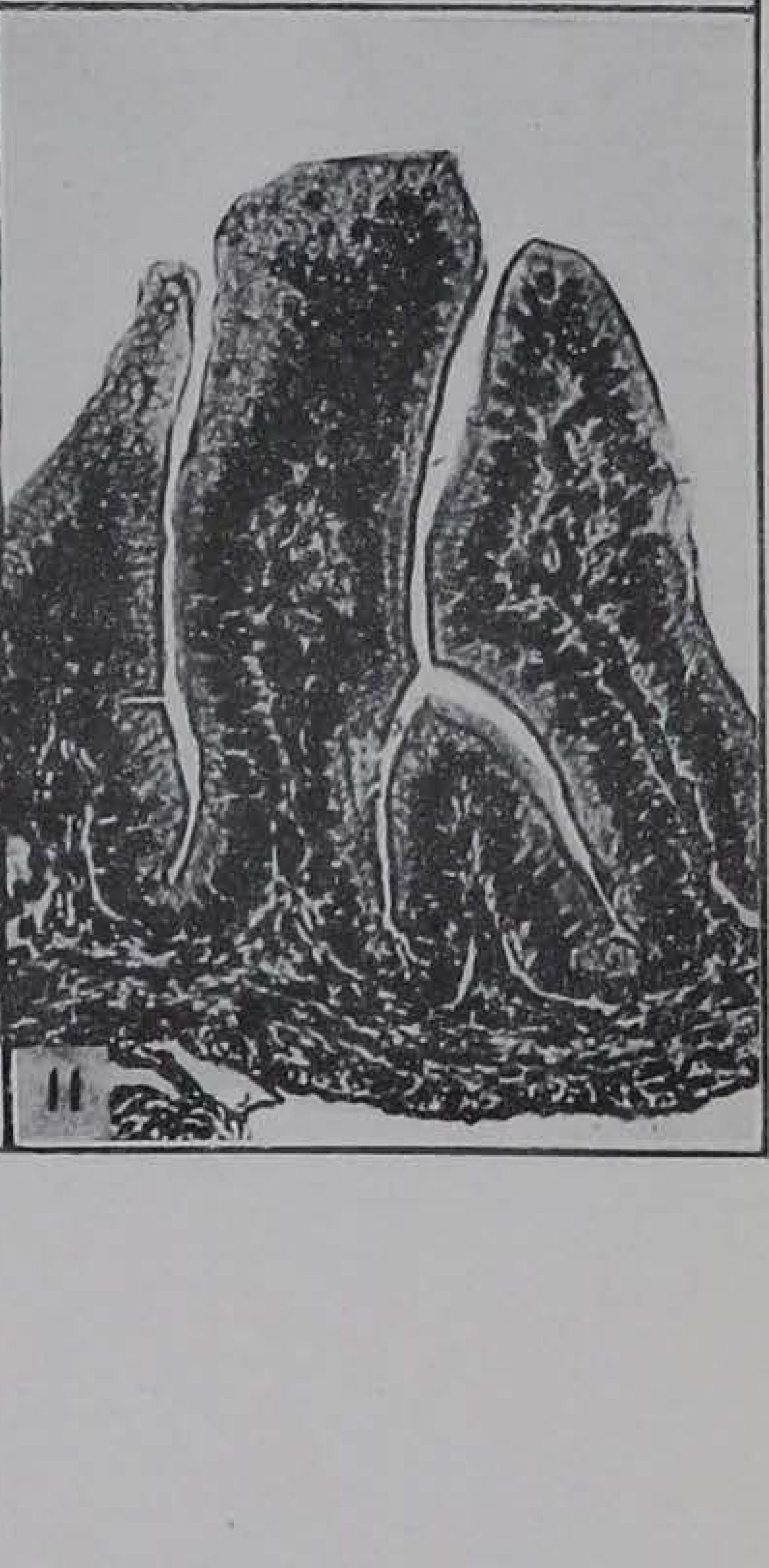
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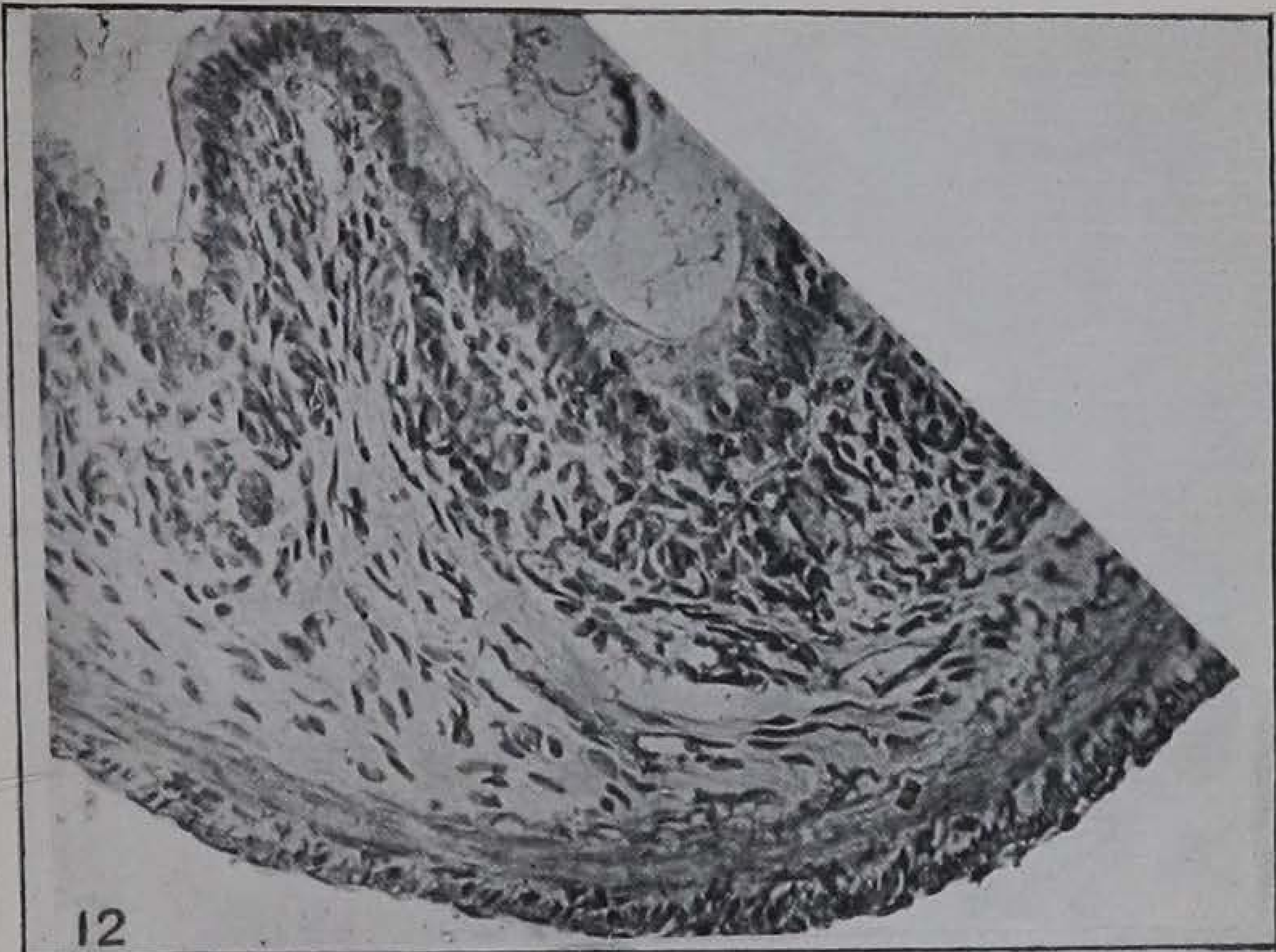
PLATE IV

Fig. 12. Microphotograph (x165) from a transverse section of the small intestine of a larva of *Ambystoma* in which the reduction in the length of the digestive tube was well advanced

Fig. 13. Microphotograph (x165) from a transverse section of the small intestine of a young adult *Ambystoma* before feeding was resumed

PLATE IV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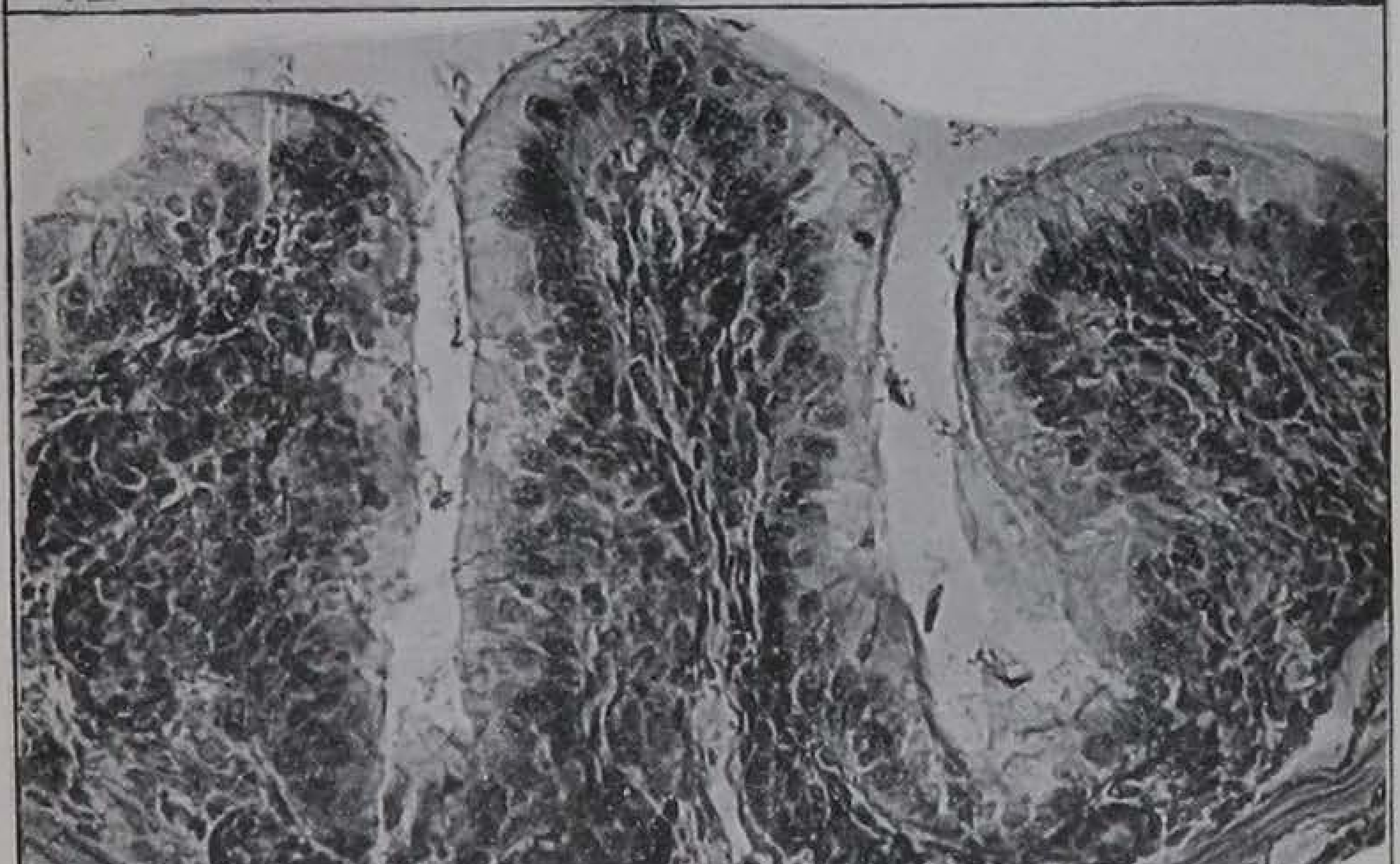
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THE FEEDING REACTIONS OF AMBYSTOMA TIGRINUM (GREEN)

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The following experimental study is based on a large number of late larvae and young adults of *Ambystoma tigrinum*. The majority of the animals used were taken in an upland kettle-hole in which they were present in great abundance. They were relatively small, measuring 8 to 10 cm. in length and weighing 10 to 12 grams. The majority of the animals taken in this habitat were late larvae. The rest were young adults many of which had scarcely completed their metamorphosis. The other animals used were taken in a larger kettle-hole lying somewhat lower. The species was less abundant in this habitat, and the individuals were much larger, measuring 18 to 20 cm. in length and weighing approximately 40 grams. The majority of these animals also were late larvae, the rest were young adults which had scarcely completed their metamorphosis. All the animals used were collected during the latter half of July and August. Many of the animals taken as larvae completed their metamorphosis while under observation in the laboratory.

This study was carried out at the Iowa Lakeside Laboratory during the season of 1920. The writers desire to acknowledge their indebtedness to Prof. R. B. Wylie, Director, for the opportunity of working at the Laboratory and for his interest in furthering the work.

Larvae. All the larvae which had not yet entered the final phase of their metamorphosis when they were taken were well fed and manifested little or no interest in food which was presented to them. Some would accept and eat somewhat reluctantly bits of meat which were held before them, others would pay no attention to food. After being kept in an aquarium without food for several days they manifested hunger by seizing and eating bits of meat which were held before them, or

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Contributions from the Iowa Lakeside Laboratory, No. 53

by following bits of meat or other small objects which were moved about in the aquarium.

A study of the behavior of the hungry animals in the presence of food or other objects which stimulate their interest, reveals at least two distinct reactions. They first approach, then seize or attempt to seize an object. If the object is within easy reach an attempt may be made to seize it without any apparent preceding movement. In his study of the feeding reactions of the spotted newt (*Diemyctylus viridescens*) Copeland ('13)[1] described an "approaching," a "nosing," and a "seizing" reaction. Following Copeland, we will designate the movement toward the object the "approaching reaction" and the attempt to seize it the "seizing reaction." We were unable to recognize any reaction of the larvae of *Ambystoma tigrinum* which corresponds to the "nosing reaction" of *Diemyctylus viridescens* described by Copeland.

When a bit of meat held with forceps or probe, or suspended by a thread, is moved about in the water in the presence of the hungry animals it soon attracts their attention. As soon as one of the animals discovers and pursues it, others near by are attracted and pursue it also. If any of them approach it sufficiently closely they seize or attempt to seize it. If a small inedible object is presented in the same way, the hungry animals react essentially in the same manner. Obviously, the stimuli which play the major part in these responses are received through the sense of sight.

When a bit of meat is held quietly in the water, care being taken that the animals do not observe the movements involved in bringing it into position, the hungry larvae respond less promptly. However, in a relatively short time one or more of the animals are attracted, approach, and seize or attempt to seize it. It is not apparent in many instances whether the meat is detected by the olfactory sense or by the sense of sight. The normal movements of the animals in the aquarium may bring the meat into the field of vision of some of them before they are attracted by its odor. As soon as one or more of the animals approach the meat others are attracted by their move-

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[1] Copeland, M. The olfactory reactions of the spotted newt, *Diemyctylus viridescens* (Rafinesque)—Jour. An. Behavior 3: 260-273. 1913.

ments. On the other hand, it is quite apparent in many instances that the animals respond before the meat is detected by sight. A small inedible object held quietly in the water may be approached as promptly as a bit of meat if in the normal movements of the animals it falls within their range of vision. They approach and may even seize it, but do not manifest as great interest in it as in an edible object. Obviously the discrimination between edible and inedible objects involves the olfactory sense.

These general observations suggest that the sense of sight plays a very important part in the approaching reaction, and that not uncommonly this reaction is followed more or less spontaneously by the seizing reaction. The determination of the relative importance of the sense of sight and the olfactory sense in the feeding reactions requires more exact experimental data. The results of experiments designed to throw some light on this problem are recorded below.

Twenty-two hungry larvae were placed in an aquarium (18 x 28 inches) containing water three inches in depth over a bed of sand. A bit of meat was held quietly near the center of the aquarium and approximately one inch below the surface of the water until it was seized. Each animal which seized a bit of meat was removed from the aquarium before the next trial was made in order that the same animal might not seize more than one bit of meat. Twenty trials resulted in an average interval of 60.5 seconds during which a bit of meat was held in position before it was seized. Several hours later, with all of the animals in the aquarium, bits of meat were offered in the same manner, but none of the animals were removed during the entire series of trials. Under these conditions twenty-four trials resulted in an average interval of 27.1 seconds during which a bit of meat was held in position before it was seized. In this experiment some of the animals responded much more quickly than others, and seized more than one bit of meat. Indeed some of them responded more promptly and more vigorously after having seized a bit of meat than when they were attracted by the meat for the first time. A bit of meat suspended by a thread was now moved slowly to and fro along the midline of the aquarium approximately one inch below the surface of the water. Twenty-five trials resulted in an average interval of 15.2 seconds from the time the meat was lowered into the water until it was seized.

Obviously, in the above experiments the food was detected by the sense of sight. Doubtless the detection of food in the water by the olfactory sense would require longer time intervals. The results indicate that the animals respond more promptly after savory food has been tasted than in the initial trials. They also indicate that these animals become interested in and approach moving objects much more promptly than objects which are not in motion.

In another experiment with the same animals in the aquarium, a bit of meat was dropped on the sand at the bottom. Fifteen trials resulted in an average interval of 3 minutes and 18 seconds during which the meat lay on the sand before it was seized and eaten. In some instances the normal movements of the animals about the aquarium brought some of them so close to the meat that it could be detected by sight. More frequently, however, the behavior of the animals indicated that they were stimulated by the presence of the meat before it was seen by any of them. When the results of this experiment are compared with the results of the experiments cited above, it becomes apparent that these animals find objects suspended or moving in the water much more readily than objects lying on the bottom. This fact, as well as the observed behavior of the animals, suggests that the olfactory sense plays a much more important part in the finding of food when it is in contact with the bottom than when it is suspended or moving in the water.

As determined by examination of their stomach contents, the normal food supply of the larvae of *Ambystoma tigrinum* in the habitat in which the animals under observation were taken, consisted largely of Entomostraca and minute algae. These organisms are everywhere present in the water and are not secured by active pursuit. On the other hand, the larvae of *Ambystoma* feed also on aquatic insects and insect larvae. The latter are found primarily on the bottom. In view of these facts the longer interval required to find food on the bottom of the aquarium than food suspended or moving in the water, can not be accounted for by the normal feeding habits of the animals, but suggests that the finding of food on the bottom depends primarily on the olfactory sense.

An attempt was now made to compare the reactions of these animals to food and odorless inedible objects when the difference between them could not be detected by the sense of sight. Two packets of gauze, one of which contained raw meat, the other cotton, were suspended in the water a few inches apart. During the first interval of ten minutes the packet containing the meat was approached and seized or nibbled 17 times; the packet containing the cotton was approached 11 times, but was not seized or nibbled. During a second ten minute interval the packet containing the meat was seized or nibbled 21 times, but the packet containing the cotton was approached a few times, but was not seized or nibbled. Two packets, each of which contained only cotton, were now suspended in the water for ten minutes. The animals paid little attention to them. They were approached a few times but neither of them was seized or nibbled. The responses elicited by the packets containing raw meat and cotton, respectively, indicate quite clearly that while, under the conditions of the experiment, sight played the more important part in the approaching reaction, the seizing reaction was determined primarily by the olfactory sense except when it followed the approaching reaction more or less spontaneously.

In order to eliminate the sense of sight five animals, all of which manifested hunger, were subjected to an operation in which the eyes were destroyed. One hour later they all seized and ate crayfish's meat which was held before them. They were then placed in a circular aquarium 10 inches in diameter containing water 3 inches in depth. Crayfish's meat was dropped on the bottom at the center of the aquarium and the time which elapsed until it was seized was recorded. Ten trials resulted in an average interval of 84 seconds. On the following day crayfish's meat was again dropped on the bottom of the aquarium in the same manner. In this instance five trials resulted in an average interval of 26.4 seconds during which the meat lay on the bottom of the aquarium before being seized.

Five normal animals were now placed in the circular aquarium and offered crayfish's meat in the same manner. Five trials resulted in an average interval of 6 minutes and 31 seconds during which the meat lay on the bottom of the aquarium before it was seized and eaten.

On the following day the five blinded animals were again placed in the circular aquarium in water 3 inches in depth. Crayfish's meat was held by means of a pair of forceps just beneath the surface of the water near the center of the aquarium. The animals showed by their general behavior that they recognized the presence of food and apparently searched for it. However, none of them succeeded in finding it during an interval of ten minutes. At the close of this interval a bit of crayfish's meat dropped on the bottom at the center of the aquarium was seized in 25 seconds.

The results of the above experiment show clearly that while the normal animals discover food suspended in the water more readily than food lying on the bottom, the blinded animals discover food lying on the bottom more readily than food suspended in the water. As pointed out above, the normal animals detect food suspended or moving in the water largely by the sense of sight. The blinded animals rarely moved away from the walls or floor of the aquarium while searching for food. They apparently depended on contact for guidance in their movements. They could readily find food on the floor of the aquarium, being attracted by its odor; however, they could not find food suspended in the water because they groped along the walls of the aquarium. The more strongly they were stimulated by the odor of the food the more vigorous became their movements; consequently, the more closely they clung to the walls of the aquarium.

In order to test the olfactory sense still further these blinded animals were offered pledgets of cotton some of which were soaked in water, others in a watery extract of crayfish's meat. They manifested no interest in the pledgets of cotton soaked in water; however, when the pledgets of cotton soaked in the extract of crayfish's meat were presented they were promptly seized and in some instances swallowed. In some instances an attempt to swallow the cotton failed, and it was rejected after several movements of the jaws. After having seized several bits of cotton soaked in the extract of crayfish's meat two of the animals somewhat reluctantly seized pledgets of plain cotton which were brought in contact with their mouths, but made no attempt to swallow them. The results of these experiments show clearly that in the absence of sight these animals were guided in their feeding reactions by the olfactory sense.

An attempt was now made to eliminate the sense of smell by filling the nares loosely with cotton. A more drastic operation on these small animals, e. g., severing the olfactory nerves, did not seem advisable. The nares of five animals were filled loosely with cotton. One hour later they all appeared quite normal and were leisurely moving about the aquarium. Crayfish's meat dropped on the floor of the aquarium apparently was not noticed by them, neither would they seize bits of crayfish's meat held before them. On the following day they again refused to accept bits of crayfish's meat held before them. The cotton was now extracted from the nares of three of the animals. The nasal epithelium was inflamed and somewhat œdematous. Crayfish's meat held before them was again refused. On the following day one of the animals from the nares of which the cotton had been extracted seized and ate a bit of the crayfish's meat, but the others still manifested no interest in food held before them. These animals manifested less interest in food during the entire series of experiments than normal hungry animals would in inedible objects presented to them in the same manner. We are of the opinion that the sense of smell was effectively eliminated by filling the nares loosely with cotton, but that the animals were somewhat discomfited by the inflammation of the nasal epithelium which followed and that the olfactory sense was not restored following the extraction of the cotton until the inflammation subsided. Obviously their failure to accept food held before them was in part determined by their discomfort. More crucial experiments designed to eliminate the sense of smell in young adults will be discussed presently.

Young adults. As pointed out above, the larvae of *Ambystoma tigrinum* cease feeding as they approach the final phase of their metamorphosis, and do not resume feeding until their metamorphosis is completed; consequently the majority of the young adults taken as well as those which emerged from the larval stage in the laboratory manifested no interest in food for some time. Even after they manifested hunger by seizing and eating bits of meat or other food held before them the young adults manifested less eagerness for food and less regularity in their feeding reactions than did the larvae.

On July 26, a large number of late larvae and young adults of the larger type were brought into the laboratory. Twenty animals, including young adults and larvae which had almost attained the adult condition, were retained for experimental study. During several days following July 26 none of them manifested any interest in food. On August 3 nearly all of them manifested hunger by promptly seizing and eating crayfish's meat held before them or of approaching bits of it held some distance from them.

Like the late larvae, the young adults demonstrate an "approaching" and a "seizing" reaction. Sometimes they exhibit a type of reaction which might be interpreted as the "nosing" reaction described by Copeland in *Diemyctylus viridescens.* More commonly, however, the seizing reaction follows the approaching reaction without an intervening pause. On the other hand, many of the animals would at times attempt to seize objects held close to them without first manifesting an approaching reaction even though they would not approach an object held a short distance from them. When the seizing reaction is not preceded by the approaching reaction an initial response similar to the nosing reaction may be observed in some instances, while in others the seizing reaction is apparently the initial response.

Obviously the sense of sight is an important factor in these reactions; however, that the olfactory sense also plays an important part in the feeding reactions is demonstrated by the following observations. A packet of gauze containing a bit of crayfish's meat and another containing a small piece of rock were suspended by threads in the presence of several of these hungry animals. They promptly seized the packet containing the crayfish's meat as often as it was presented, but made no attempt to seize the other packet. Two packets of gauze, one of which contained frog's meat, the other dry cotton, were now suspended by threads in the presence of the same animals. They promptly seized the packet containing the frog's meat as often as it was presented, but did not seize the other packet. Obviously the capacity to discriminate between the edible and inedible objects in these experiments involved the olfactory sense.

It may be noted in passing that these animals do not always discriminate thus clearly between edible and inedible objects. Some of them did at times approach and seize inedible objects which emitted no odor. After repeated attempts following the presentation of food a few animals were induced to seize pledgets of cotton. In at least one instance such a pledget of cotton was also swallowed.

In order to determine more accurately the relative importance of the sense of sight and the olfactory sense in the feeding reactions several hungry animals were rendered sightless by enucleation of their eyes, while several others were subjected to an operation under ether anesthesia in which the anterior portion of the cranial cavity was laid open and the olfactory nerves were severed. The former operation caused the animals little apparent discomfort. The latter was more severe; however, all but one of the animals recovered promptly. Both the de-eyed animals and those which had recovered after resection of the olfactory nerves took food on the day following the operations and were not less active than the unoperated animals.

When small pieces of crayfish's meat were held before the de-eyed animals they promptly seized and ate them. They even followed pieces of crayfish's meat moved before them. When inedible objects which emitted no odor were held before them they did not react unless the objects were in actual contact with them. In such instances they would sometimes attempt to seize them just as normal animals sometimes attempt to seize inedible objects which come close to or in contact with them. The de-eyed animals reacted essentially in the same manner on successive days as long as they were kept under observation. Obviously their ability to discriminate between edible and inedible objects involved the olfactory sense.

When pieces of crayfish's meat or other food were held before the animals whose olfactory nerves were severed, they were promptly seized and eaten. When inedible objects were presented in the same manner they were seized just as promptly.

If they could be swallowed without difficulty they were promptly swallowed, otherwise they were discarded after an attempt to swallow them. These animals would seize and swallow pledgets of cotton quite as promptly as pieces of meat regardless of whether or not the pieces of meat and the pledgets of cotton were concealed in packets which were similar in appearance. Obviously these animals did not discriminate between food and inedible objects. Inasmuch as the de-eyed animals did discriminate between edible and inedible objects the conclusion that such discrimination is accomplished primarily by the olfactory sense is warranted.

Attempts to demonstrate a sense of taste in these animals resulted negatively. Obviously it would be quite impossible to differentiate between the manifestations of an olfactory and a gustatory sense in normal animals. However, if animals in which the olfactory nerves are severed should react to the fluid extracts of food introduced into their mouths such reactions might be interpreted as the manifestations of a gustatory sense. Accordingly aqueous extracts of frog's meat and crayfish's meat were introduced into the mouths of the animals whose olfactory nerves were severed, but no reactions were elicited.

The results of the foregoing experiments on *Ambystoma tigrinum*, in so far as they bear upon the relative importance of the sense of sight and the olfactory sense in the feeding reactions, agree in general with the results of Copeland's experiments on *Diemyctylus viridescens.*

SUMMARY

The typical response of the larvae of *Ambystoma tigrinum* to the presence of food consists in an "approaching" and a "seizing" reaction. Young adults frequently exhibit also a "nosing" reaction. The "approaching" reaction is commonly a visual response. The "nosing" and "seizing" reactions, except when the latter follows the "approaching" reaction more or less spontaneously, involve the olfactory sense.

Moving objects are detected and pursued more promptly than objects which are not in motion.

In the absence of sight food is detected by the sense of smell. Discrimination between edible and inedible objects also depends on the olfactory sense.

RESPONSES OF THE DE-EYED LARVAE OF AMBYSTOMA TIGRINUM (GREEN) TO SOLID BODIES

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While studying the feeding reactions of de-eyed larvae of *Ambystoma tigrinum*, a characteristic reaction was observed when the forceps were brought near the head in the water when no food was present. As the forceps approached from the side the animal would turn its head in that direction. It would then resume a position of rest or move slowly backward or forward. Sometimes it would attempt to seize the forceps as soon as their presence was detected.

In the further study of these responses the forceps which were used for handling food were discarded and a clean glass rod and other clean objects were used. A number of larvae which had been de-eyed three days previously, were placed in a shallow circular aquarium either singly or in groups of two or three and allowed to remain undisturbed until they became relatively quiet before observations were begun. As the glass rod was slowly and carefully brought near the head from either side the animal responded by turning its head toward the rod when it was still 5 mm. or farther distant. Sometimes the reaction occurred when the rod was still 20 mm., or farther, from the head. When the rod approached from above, the head was elevated; when it approached from below, the head was depressed. The reaction was essentially the same whether the rod approached the head at the level of the base of the gills or nearer the anterior end. In some instances the movement of the head toward the rod was the only response elicited; in other instances, after turning the head toward the rod, the animal would swim slowly backward or forward. Occasionally it would attempt to seize the rod as soon as the head was turned toward it. All the larvae used in the initial experiments responded to the near

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approach of the glass rod to the head essentially in the same manner, though not with equal promptness and precision. Neither could this response be elicited when the animals were reacting to other stimuli.

Further experiments were made in an attempt to discover whether similar responses could be elicited by bringing the glass rod into proximity with other parts of the body. The majority of these experiments resulted negatively. However, several of the larvae sometimes reacted in a very definite manner when the glass rod approached the lateral surface of the anterior portion of the trunk. The initial response consisted in the contraction of the segmental muscles of the trunk on the side stimulated, which resulted in an appreciable curvature of the body. Following this the animal would swim slowly forward. The response could not be elicited except when the animal was relatively undisturbed by other stimuli. Neither was it entirely constant under these conditions. The area just posterior to the base of the gills proved to be the most sensitive area of the trunk. When the response was elicited by the near approach of the glass rod to this area not only did the characteristic contraction of the segmental muscles take place, but the gills were also adducted. All attempts to elicit a response to the near approach of the glass rod to the tail resulted negatively.

The glass rod used in the above experiments was 4 mm. in diameter and rounded at the end. Clean glass tubes of smaller diameter were used with essentially the same results. When a galvanized steel wire 2 mm. in diameter was used the animals responded somewhat more vigorously than when the glass rod was used. Apparently the galvanized wire afforded a somewhat stronger stimulus than the glass rod. Other objects used as stimulating agents were a dissecting needle, a fine steel wire, a bone needle holder 6 mm. in diameter, rods of pine wood 6 mm. or less in diameter, and pieces of soft rubber tubing. The animals responded to the near approach of all these objects essentially in the same manner, but somewhat less vigorously to the bone, wood, and rubber objects than to the glass rod.

Five of the de-eyed larvae used in the initial experiments were kept in the laboratory and subjected to further experimentation at irregular intervals. Whenever the experiments were made under favorable conditions the larvae responded to the near approach of the glass rod, or other solid bodies, in the characteristic manner. In the course of a week four of these larvae entered the final phase of their metamorphosis. As they became less active and sought to leave the water they no longer responded to the near approach of solid bodies. The remaining de-eyed larva was kept under observation in the laboratory for a period of three weeks. It did not fail to respond in the characteristic manner to the near approach of the glass rod or other solid objects, whenever the tests were made in the absence of conflicting stimuli. Other larvae, taken from another pond and de-eyed later, responded in the same manner to the near approach of solid objects one hour after the eyes were removed as well as on successive days following the operation. All attempts to elicit responses of the same type from young adults and larvae which were passing through the final phase of their metamorphosis resulted negatively.

When a solid body approaches the head of a normal seeing larva in the water the reaction which takes place, doubtless, is mediated through the sense of sight. If the animal is hungry it may attempt to seize an approaching glass rod as it would a bit of food. On the other hand, if no feeding reaction is elicited the response which takes place is usually an effort to avoid contact with the object. Doubtless the normal seeing larvae have the same capacity to detect the presence of solid bodies in close proximity with their skin by some sense other than sight as the de-eyed larvae, but stimuli received through the eyes result in motor responses more promptly than those received from nearby solid bodies by receptors in the integument.

Larvae with only one eye removed responded essentially in the same manner as the de-eyed larvae when a solid body approached the head from the eyeless side in such a manner that its presence could not be detected by the intact eye. No response was elicited from these larvae by the near approach of a solid body to the lateral surfaces of the trunk, or any part of the body except the head.

A comparison of the responses of the de-eyed larvae of *Ambystoma tigrinum*, described above, with the responses of certain blinded fishes to the near approach of solid bodies in the water, is not without interest. Parker and Van Hausen ('17) described the responses of the catfish (*Amiurus nebulosis*) to metallic and non-metallic rods. The only non-metallic rod to the near approach of which these fishes responded when deprived of their sight in the experiments of Parker and Van Hausen, was a rod of cedar wood. This response, as suggested by these authors, was mediated through the sense of smell. Crozier ('18) described the responses of the de-eyed hamlet (*Epinephelus striatus*) to the near approach of rods of various metals and woods as well as such miscellaneous substances as glass, hard and soft rubber, porcelain, hard paraffin, sandstone, and compressed carbon.

According to Parker and Van Hausen the blindfolded catfishes usually responded to a metallic rod approaching them in the water by swimming away when it was still some centimeters distant. However, if but a small portion of the metal was in contact with the water they sometimes responded by moving toward the rod and even nibbling at it. According to Crozier, when a glass rod approaches the head of a de-eyed hamlet to within 4.5 or 5 cm. "the fish bends in the opposite direction and swims slowly backward; or it may back deliberately away for 10 or 15 cm., then abruptly turn away from the side stimulated and assume a position at right angles to that held before being stimulated." He noted also that when the rod approached the caudal peduncle the first response of the tail was to bend toward the opposite side. The initial reaction in these responses as in the characteristic responses of the catfishes to the near approach of a metallic rod is an avoiding reaction; consequently it is negative in character. On the other hand, the initial reaction in the typical response of the de-eyed larvae of *Ambystoma tigrinum* to the near approach of a solid body to the head is a movement toward that body; consequently, it is positive in character. Parker and Van Hausen have demonstrated conclusively that the responses of the blinded catfishes to the near approach of metallic rods in the experiments described by them were stimulated by electrical currents generated on the rods in contact with the water. They have shown also that a positive reaction, i. e., a movement toward the rod, occurs only when the stimulus is very weak. Inasmuch as the de-eyed hamlets in Crozier's experiments, like the de-eyed larvae of *Ambystoma tigrinum* in the experiments described in this paper, responded not only to metallic rods but also to non-metallic bodies on which an appreciable electrical current could not be generated by contact with water, these responses can not be the results of electrical stimulation. As suggested by Crozier, mechanical deformations in the water probably constitute the stimulus involved. The de-eyed hamlets used in Crozier's experiments responded to the approach of solid bodies at greater distances and more vigorously than the larvae of *Ambystoma tigrinum* used in the experiments described in this paper. Furthermore, the initial reaction in the characteristic responses of the former was negative, while that of the latter was positive in character. Doubtless this difference is due to the fact that the receptors involved were stimulated more strongly in the hamlets than in the larvae of *Ambystoma*. Obviously the form of sensitivity involved, which is epicritic in character, is less highly developed in *Ambystoma tigrinum* than in fishes like *Epinephelus striatus*.

The above experiments were carried out at the Iowa Lakeside Laboratory during the summer of 1920. The writers desire to express their indebtedness to Professor R. B. Wylie, Director, for the opportunity of working at the Laboratory and for his interest in furthering the work.

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NEST DIGGING AND EGG LAYING HABITS OF BELL'S TURTLE

CRYSEMYS MARGINATA BELLII (GRAY)

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During the summer of 1921 some forty or fifty Bell's painted turtles dug their nests on the side of a small hill just north of the Iowa Lakeside Laboratory. This afforded an excellent opportunity for the study of the nest-digging and egg-laying habits of this species which is so abundant around the Okoboji lakes. When the turtle is digging her nest or depositing her eggs she is not easily frightened, so that it is possible to get very close to the animal and with a flash light held within an inch or so of the body every movement may be easily studied. On several occasions a number of the students at the Laboratory were able to watch the entire process from the time the turtle landed on the shore until she returned to the water again. The following description taken from my field notes of June 30, 1921, on nest 40 is typical.

A painted turtle measuring about six inches in length appeared at the water's edge at about 8:00 P. M., about sixty feet from where the writer was waiting. She followed an old cowpath up the rather steep slope of the hill for a distance of about thirty feet and then turned at right angles to the path and came directly towards me. After she had traveled for perhaps fifteen feet she stopped and dug into the hard dry clay, first with the right hind foot and then with the left, making the dust fly for a considerable distance. It was only twice, once for the right and then for the left foot, that I could see the dust fly although the animal still continued to dig. She began digging her nest at 8:17 P. M. I gradually approached to within five or six feet of the turtle. This disturbed her slightly, but she soon resumed her operations. At first she inserted one foot into the depression she had started and made four or five dig-

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ging or scraping motions much as a person would in trying to dig a hole in the ground with his finger nails. She then lifted the dirt out and pushed it as far back from the hole as she could, at the same time shifted her body so that the cloaca was directly over the pit and softened the hard clay with water from her bladders. The water was squirted into the pit with considerable force. Then the body was moved further so that the other foot could be thrust into the hole and she continued to dig as before. This was kept up, first with one foot, then softening the dirt, then with the other foot, for some time. This continuous wetting of the dirt soon made it of about the same consistency as thick cream, at which time she discontinued wetting it. As the dirt began to get dryer she commenced to enlarge the diameter of the hole at the bottom so as to make the nest flask-shaped, the neck being slightly larger than the leg and the spherical body as large as the reach of the leg would permit. In digging the body of the nest the turtle would scrape the sides with her claws three or four times, then press the dirt against the pad of her foot with her claws and carry it out by the handful. It was deposited near the edge of the opening and then pushed out so that the dryer dirt was left near the opening while the soft mud was pushed to the periphery. It took about one hour and thirty minutes for this turtle to complete the digging of her nest from the time she began.

At 9:47 the turtle began to deposit her eggs. First she inserted her right hind foot into the nest, moved over so that the cloaca was directly over the opening, and then removed her leg from the nest. The egg was deposited so that it rested slightly on the edge of the opening, and gently slipped into the nest. Immediately the turtle inserted her right foot again and apparently placed the egg to one side of the nest. In about another minute a second egg was deposited and placed in the same manner as the first. It took about one-half second for the egg to pass out of the body, and seemed to involve no undue effort on the part of the turtle. The third egg appeared in about thirty seconds, and thirty seconds later, the fourth. The fifth egg did not appear until a minute and a half later, although two unsuccessful efforts were made at the regular intervals of half a minute. The sixth egg appeared on schedule time in thirty seconds. The sixth was the last one laid at this time, but evidently the effort had been made to lay eight or nine. After each egg was laid it was arranged in the nest by the right hind foot. The process of egg-laying was completed at 9:52, so that the entire process took about five minutes and at intervals of about thirty seconds for each egg.

Immediately the turtle began pulling dirt in from the edges, the dryer dirt going in first. This was pressed down by the hind foot, first dirt from one side and then the other was pulled in, each foot being used alternately. She seemed to press the dirt down with her knuckles much as we might with a loosely closed fist. The wet dirt was the last to be pulled on the nest. The softer mud on top was thoroughly kneaded by the knuckles, and flattened and packed by rubbing the plastron over it. Dry dirt was then scratched in, and grass roots were either accidentally or purposely kneaded in as though transplanted. It almost seemed to the observer that the turtle made a special effort to reach far out for bits of grass and debris to help conceal her nest. When this was completed the turtle appeared startled at our flash light for the first time. It was as though she had awakened from a trance. She stretched out her neck, looked around for an instant and then hurried away towards the lake. It was 10:23 P. M. when she left the nest, so that the entire process of nest-digging and egg-laying took over two hours. She worked quite vigorously most of the time, but towards the end she appeared tired and needed to rest frequently. On another occasion we found that during the last fifteen or twenty minutes of work in concealing the nest the turtle at first worked pretty regularly for about fifty seconds and rested ten to twelve seconds, and that later the periods of work were shortened and the rest periods became longer. At no time during the procedure of nest-digging and egg-laying did the turtle seem much disturbed by the presence of three or four observers, even when the flash light was held directly in front of her.

In the case of one turtle, Professor Larrabee of Yankton held his flashlight in front of her after she had left the nest to return to the lake. This seemed to produce a sort of hypnotic effect, for she would follow this light whichever direction it was moved, up hill or down hill, faster or slower.* If the light was hidden she would start for the lake, but when the light was produced again she would follow it. However, when she was allowed to get within a few feet of the water the light no longer had any fascination for her; she hurried away into the water and disappeared from view.

The number of eggs laid in one clutch varied from six to thirteen. The eggs are oblong, measuring from 1.5-2 cm. in the short diameter to 2.5-3 cm. in length, and weigh from 7-12 grams. When first laid they are somewhat translucent and pinkish but later become white and opaque, less turgid and somewhat more brittle. The nests are dug out and the eggs eaten by some night prowler. One night after a rain some twenty out of forty nests were robbed, even though some of the nests had been protected by covering them with flat stones. The writer has not yet been able to determine exactly the identity of the culprit, but on one hillside he counted more than seventy nests that had so been robbed, and on another a hundred ten nests were dug up and shells strewn around.

The writer wishes to acknowledge the assistance of Miss Pauline Kimball, Miss Esther Lusted, and Professor Austin P. Larrabee in taking notes and watching the time.

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* At times when she was following the light rapidly up hill she appeared to be panting.